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Doxorubicin-loaded silicon nanowires for the treatment of drug-resistant cancer cells

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ABSTRACT

Multidrug resistance (MDR) remains a major challenge for cancer treatment thus far. Free doxorubicin (DOX, one of the most widely used chemotherapy agents for cancer treatment) generally features a large value of resistant factor (RF), which is regarded as a significant parameter to assess therapeutic efficiency of cross-resistance. To address this issue, we herein present a kind of silicon nanowires (SiNWs)-based drug nanocarriers (SiNW-DOX), which is high-efficacy for treatment of drug-resistant cancer cells. Typically, drug-resistance cancer cells (e.g., MCF-7/ADR cells) can be significantly inhibited by the SiNWs-based nanocarriers, exhibiting ~10% cell viability during 72-h incubation with the SiNWs-DOX (80 μg mL^{-1} DOX), which is in sharp contrast to free DOX-treated cells preserving ~40% cell viability. Remarkably, the RF value of SiNW-DOX is as low as ~2.0, which is much better than that (~300) of free DOX under the same experiment conditions. To the best of our knowledge, it is the lowest RF value ever reported by nanomaterials-based drug carriers (3.3–24.7).

1. Introduction

Multidrug resistance (MDR), associated with overexpression of P-glycoprotein (P-gp) and lowered accumulation and retention of drugs in tumor cells, remains a huge challenge for cancer treatment thus far [1–3]. To address this issue, drug carriers are required to enhance drug concentration in intracellular region of tumor cells [4–9]. Resistance factor (RF), determined by the ratio of half-maximal inhibitory concentration (IC_{50}) value for the resistant cellular line to that for the sensitive line, is regarded as a significant parameter to assess therapeutic efficiency of cross-resistance [7,10,11]. The value of RF is ideally set as 1, indicating entire reverse of the drug resistance. Free doxorubicin (DOX, one of the most widely used chemotherapy agents for cancer treatment) generally possesses a high RF value, which is due to overexpress of P-gp, a membrane transporter that actively pumps DOX out of the cells [5–7,12]. Notably, nanomaterials (e.g., mesoporous silica nanoparticles (NPs), gold NPs (AuNPs), and iron oxide NPs, etc)-based drug carriers with high drug-loading capacity have recently developed for enhancing the intracellular drug accumulation and reducing the RF value [5–7]. The reason is that while nanomaterials can enter cells by an endocytosis pathway, exocytosis of nanomaterials is independent from the P-gp pathway, suggesting nanomaterials are not the substrate of P-gp [6,13]. As a result, in comparison to that (several tens to hundreds) of free DOX [5–7,12], the RF value of mesoporous silica NPs-, AuNPs-, or iron oxide NPs-based drug nanocarriers can be distinctly reduced to 24.7, 4.67, or 3.3, respectively [5–7]. Nevertheless, drug nanocarriers of lower RF values are still in high demand to meet increasing requirement of drug-resistant cancer treatment.

Silicon nanostructures have shown great promise for myriad biological and biomedical applications because of their unique optical/electronic/mechanical properties and favorable biocompatibility [14–26]. Besides, silicon materials are found to be readily biodegradable and cleared *via* renal clearance from mice with undetectable toxicity [27,28]. Moreover, silicon naturally exists in numerous tissues as a common trace element [27,28]. The above merits have motivated intensive investigation of silicon nanomaterials-based bioapplications, including our recent success in design of silicon nanomaterials-based platform for cancer diagnosis and therapy [20,22,24–26]. Of particular note, one of our latest studies reveals that DOX molecules can readily loaded on silicon nanowires (SiNWs) of large-area porous structures, producing SiNWs-based nanocarriers (SiNW-DOX) with high drug-loading capacity (~20800 mg g^{-1}) [24]. However, it remains

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unknown whether such resultant nanocarriers are available for MDR applications.

In this current work, this kind of SiNWs-based nanocarriers is employed for MDR investigation in a detailed way. Significantly, based on systematic studies of *in vitro* behavior of the SiNWs-based nanocarriers, we demonstrate that such nanocarriers featuring a notably low RF value are highly efficacious for reversing drug resistance. Our results suggest the SiNWs-based nanocarriers as high-performance nanoagents for drug-resistance cancer treatment.

2. Experimental Section

2.1. Materials and devices

Hydrofluoric acid (≥40%), hydrogen peroxide (≥30%), silver nitrate (≥99.8%), and nitric acid (65–68%) are bought from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Silicon wafer (100, phosphate-doped (p-type), 0.01–0.02 Ω sensitivity) is bought from Hefei Kejing Materials Technology Co., Ltd. (China). DOX is purchased from Huafeng United Technology CO., Ltd (Beijing, China). Milli-Q water (Millipore) is employed as the solvent for preparing solutions. UV–vis absorption and photoluminescence (PL) spectra are recorded using a Perkin–Elmer Lambda 750 UV–vis-near-infrared spectrophotometer and a HORIBA JOBTN YVON FLUOROMAX-4 spectrofluorimeter, respectively. The scanning electron microscopy (SEM) and transmission electronic microscopy (TEM)/high-resolution TEM images are captured by Philips CM 200 electron microscope and scanning electron microscopy (FEI Quanta 200F), respectively.

2.2. Synthesis and characterization of SiNWs

Free silicon nanowire (SiNW) arrays are produced *through* an HF-assisted etching method as described elsewhere [16–22,24,25]. In our experiment, Si wafer is firstly treated by ultrasonic treatment for 10 min in acetone solution, followed by washing with Milli-Q water for 3 times. Thereafter, the silicon wafer is immersed in a mixture solution (H_2SO_4 (98%) + H_2O_2 (30%), $v/v = 3:1$) for half of an hour, following by washing Milli-Q water for three times. Afterward, the resultant Si wafer is treated with HF (5%) solution for 30 min, producing the hydrogen-terminated Si wafer (H–Si wafer). The as-prepared H–Si wafer is immediately immersed in a mixture solution ($AgNO_3$ + HF (10%)) with slow stirring for 6 min to produce SiNW arrays on the surface of Si wafer. Ultrasonic treatment is then performed to detach the as-prepared SiNWs, which are collected for drug delivery in following experiments. SEM, TEM and HRTEM images show that the diameter and length of SiNW are about ~100 nm and ~500 nm.

2.3. DOX Loading

To load DOX onto SiNWs, SiNWs (200 μg mL^{-1} dispersed in water) at different pH values is dispersed in various concentration of DOX solution and stirred for 12 h, followed by centrifugation and washing three times with phosphate buffers (PB) to remove the unbound excess DOX and to obtain the drug-loaded SiNWs (SiNW-DOX). The SiNW-DOX are then re-suspended and stored at 4 °C. The DOX loaded onto SiNWs is determined by UV-vis-NIR absorbance spectroscopy measurements by the absorbance peak at 490 nm. The 490 nm absorbance peak with a molar extinction coefficient of 0.762×10^4 $M^{-1}\cdot cm^{-1}$ (calculated from the standard calibration curve, Fig. S1) is used to determine the DOX concentration. Drug loading amount is calculated according to the following equation [24]:

$$\text{Drug loading capacity} = (W_{\text{initial DOX}} - W_{\text{DOX in supernatant}}/(W_{\text{SiNWs}})) \quad (1)$$

where $W_{\text{initial DOx}}$ is the weight of DOX initial added, $W_{\text{DOx in supernatant}}$ is the weight of DOX in supernatant and W_{SiNWs} is the weight of SiNWs.

2.4. DOX Releasing

SiNW-DOX complex prepared in pH = 9 condition are re-suspended in PB solutions at various pH values (5, 7, and 9) for various times (1, 2, 3, 5, 7, 14, and 21 h) at room temperature. DOX detached from the SiNWs surfaces are collected through centrifugation, whose concentration is determined by UV–vis-NIR absorbance spectrum.

2.5. Cell culture

MCF-7 and MCF-7/ADR cell lines, served as drug-sensitive and drug-resistant cancer cells, are cultured in RPMI-1640 medium supplemented with 10% (v/v)

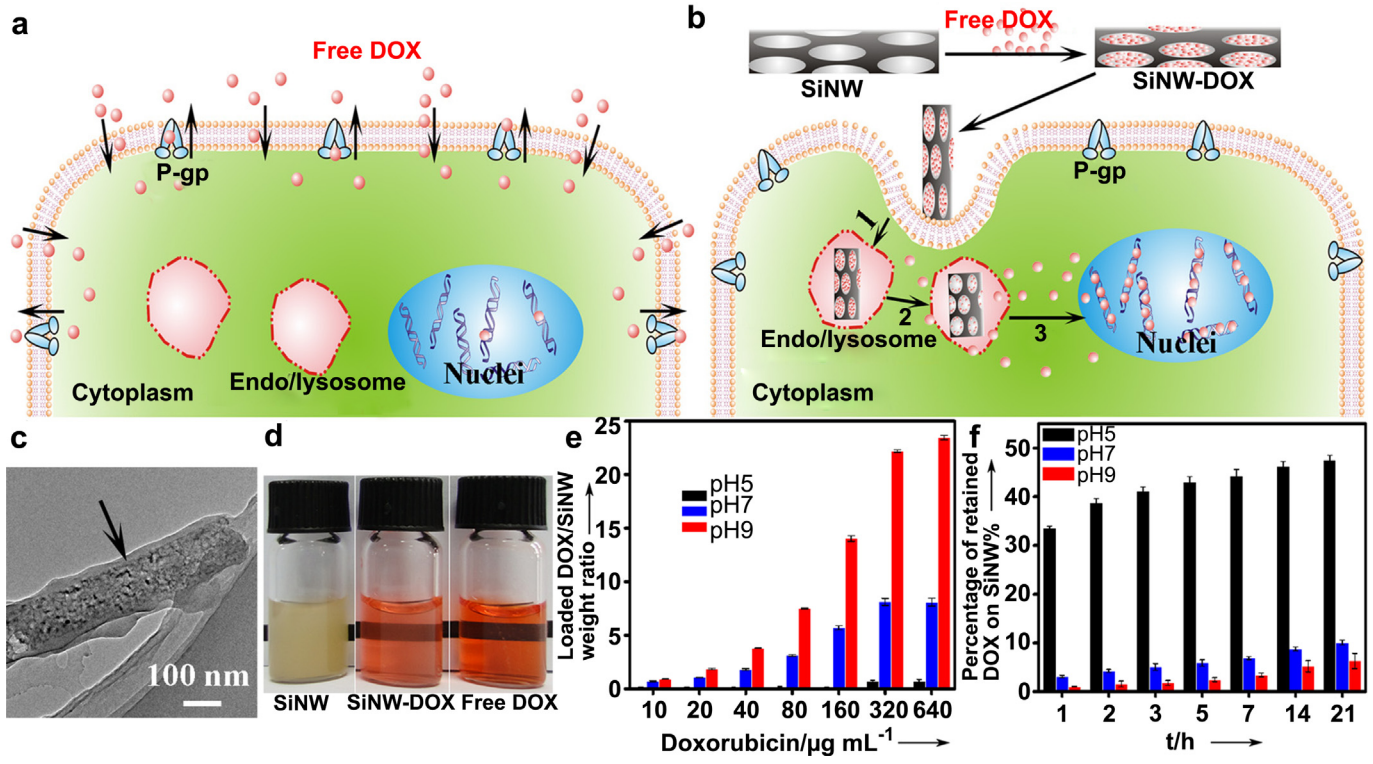

Fig. 1. Schematic illustration of different cellular internalizations, intracellular drug distributions and mechanisms in overcoming the MDR effect of free DOX (a) and SiNW-DOX complex (b). (a) Free DOX diffuses directly through intact cell membranes, and then is quickly pumped out of the MCF-7/ADR cells. (b) After free DOX bounded to the surface of SiNWs, the SiNW-DOX complex enters the MCF-7/ADR cells by endocytosis (Step 1), thereafter DOX is released at endo/lysosomal pH (Step 2) and finally enters into nuclei (Step 3) to overcome drug resistance. (c) A TEM image of the SiNW-DOX complex (loading pH = 9), with an obvious DOX layer on surface of the SiNW (marked by arrow). (d) Photos of SiNWs (left), DOX- SiNWs (middle) and free DOX (right). (e) Weight ratio of the loaded DOX at various concentrations (loading pH = 5, 7 and 9, concentration of SiNW solution: 0.2 mg mL^{-1}). (f) DOX release from SiNWs at three different pH values. Error bars stand for standard deviations of triplicated samples.