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Materials Science

Prussian blue nanoparticle-loaded microbubbles for photothermally enhanced gene delivery through ultrasound-targeted microbubble destruction

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Abstract By adsorbing chitosan (CS)-functionalized Prussian blue (PB) nanoparticles (CS/PB NPs) complexing DNA onto the surface of gas encapsulated microbubbles (MBs), a multifunctional gene delivery system of MBs@CS/PB/DNA was fabricated for photothermally enhanced gene transfection through ultrasound-targeted microbubble destruction. CS/PB NPs of (2.69 ± 0.49) nm could complex DNA effectively when the mass ratio was 2:1. It was found that MBs@CS/PB/DNA could enhance ultrasound imaging greatly both in vitro and in vivo. In addition, MBs@CS/PB/DNA could be disrupted by applying a higher-intensity ultrasound irradiation to release CS/PB/DNA, which could effectively transform the near-infrared (NIR) light into heat to assist the uptake of CS/PB/DNA by cells. With the aid of ultrasound irradiation and NIR light irradiation, the gene transfection efficiency was significantly enhanced to (43.08 ± 1.13) %, much higher than polyethylenimine. Moreover, MBs@CS/PB/DNA showed excellent biocompatibility, encouraging the further exploration of MBs@CS/PB/DNA to be a platform for combined ultrasound image, photothermal therapy, drug delivery, and gene therapy.

Keywords Ultrasound imaging · Microbubble · Gene delivery · Prussian blue nanoparticle

1 Introduction

In recent years, gene therapy has progressed rapidly as a revolutionary therapeutic strategy of preclinical and clinical research to treat various types of diseases including tumor diseases [1, 2]. To satisfy the clinical applications of cancer gene therapies, the development of gene delivery systems with high efficiency, safety, and selective targeting ability is highly required for gene therapy because of easy hydrolysis in biological fluids and low cellular uptake efficiency of the nucleic acids with polyanionic nature. So far, a variety of gene delivery vectors have been developed and optimized. Particular research interests have been focused on developing gene delivery vectors which are responsive to external stimuli, such as light, temperature, radiofrequency, magnetic field, and ultrasound. Among of them, light takes more advantage to deliver genes at the desired site at a specific time with high efficiency and minimum adverse effects [3]. In particular, near-infrared (NIR) light has received much attention due to the "water window" (650–900 nm) with minimal absorption by skin and tissue, thus leading to deep tissue penetration with high spatial precision without damaging normal biological tissues [4]. It was reported that polyethylenimine dual-functionalized nanographene oxide could enhance intracellular transportation and the gene transfection efficiency greatly upon irradiation with NIR light [5].

Chitosan (CS), naturally occurring linear cationic polysaccharide with good biocompatibility, has been widely used to modify the nanoparticles for gene delivery, showing high transfection efficiency and little cytotoxicity

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[6, 7]. Prussian blue (PB) has been a drug in clinic for many years for the treatment of radioactive exposure and approved by USA Food and Drug Administration (FDA). Its stability in biological environments and biosafety in the human body have been proved in clinic for several decades. Our group [8] has demonstrated that PB could be a new-generation photothermal ablation agent for cancer photothermal therapy because of its strong absorption in the NIR region and the superior photothermal efficiency. In addition, CS functionalized PB nanoparticles (CS/PB NPs) were fabricated as a photothermal ablation agent to enhance gene transfection efficiency under NIR laser irradiation [9].

Among all the diagnostic imaging techniques, ultrasound imaging has been the most widely used clinical diagnostic imaging modality due to its unique features including real time, low cost, high safety, and ready availability for portable devices [3]. With the use of ultrasound contrast agents (UCAs), such as gas-filled microbubbles (MBs), the resolution and sensitivity of clinical ultrasound imaging can be greatly improved. Moreover, MBs have been developed as novel controlled-release carrier vehicles for targeted gene or drug delivery through ultrasound-targeted microbubble destruction (UTMD) technique [10, 11]. The triggered microvessel ruptures can provide a focal delivery of colloidal particles in a given tissue.

The main obstacles for successful gene therapy are the insufficient delivery of genes to target tissues and the difficulty to monitor gene delivery. The imaging strategies offer us an opportunity to optimize gene therapy by evaluating the effectiveness of gene delivery noninvasively and spatiotemporally. In this work, DNA was absorbed onto CS/PB NPs, and the resulting CS/PB/DNA complex was further loaded onto the surface of gas encapsulated microbubbles ST68 MBs, which were generated from Span 60 and Tween 80 (Fig. 1a). The resulting MBs@CS/PB/DNA showed the outstanding ultrasound imaging capability, which may have the potential to monitor gene delivery. After reaching the targeting sites, the ST68 MBs could release CS/PB/DNA complex to penetrate into the tumor interstitium through UTMD by applying the higher-intensity ultrasound irradiation. Followed by the NIR light irradiation, CS/PB/DNA could enhance gene transfection efficiency greatly (Fig. 1b).

2 Materials and methods

2.1 Preparation of CS/PB/DNA complexes for electrophoresis assay

CS/PB NPs was prepared according to the literature [12]. Assigned amounts of CS/PB NPs in 10 μL deionized water were mixed with 1 μg DNA in 10 μL deionized water at different molar ratios of 0:1, 0.1:1, 0.2:1, 0.5:1, 1:1, 2:1, 4:1, and 8:1, and incubated at room temperature for 20 min. Then CS/PB/DNA complexes were analyzed by 0.8 % agarose gel electrophoresis running in the Tris–EDTA buffer at 120 V for 30 min. The gel was stained with EB and imaged by a gel imaging analysis system.

2.2 Formation of MBs@CS/PB/DNA

ST68 MBs encapsulating perfluoropropane gas were prepared from Span 60 and Tween 80 according to the literature [13]. A total of 0.2 mL MBs@CS/PB/DNA aqueous solution (containing 0.5 mol/L NaCl) was added into 0.2 mL ST68 MBs suspension in the centrifuge tube. The mixture was slightly shaken for 10 min to allow the sufficient adsorption reaction and centrifuged at 500 r/min for 5 min. The obtained MBs@CS/PB/DNA were resuspended and washed by 0.4 mL PBS for three times. A total of 1×10^7/mL MBs@CS/PB/DNA contained 10 mg/L DNA, 20 mg/L CS/PB NPs, and 1×10^7/mL MBs.

2.3 UTMD and ultrasound imaging in vitro and in vivo

An ultrasonic transfection instrument (SonoPore KTAC-4000, NepaGene, Japan) was used to irradiate MBs@CS/PB/DNA solution by ultrasound for 30 s at the power density of 0.8 W/cm^2 to simulate UTMD process in vitro. The sample was then filtered through 0.45-μm filters, and only CS/PB/DNA could pass through. Then, the concentration of Fe was measured by inductively coupled plasma optical emission spectrometry (ICP-OES).

A tube simulating as the blood vessel phantom was immersed in a water tank in which an ultrasound probe was pointing closely at the tube. MBs@CS/PB/DNA was injected into the tube with an inner diameter of 5 mm for ultrasonography in vitro. Ultrasonograms were obtained using a broadband linear array L9-3 transducer (9–3 MHz extended) of the IU22 ultrasound system (Philips Medical Systems, Germany) from the longitudinal cross section of the tube. The pulse inversion harmonic imaging (PIHI) mode with a mechanical index (MI) of 0.06 was applied to acquire contrast-enhanced images.

A New Zealand white rabbit was anesthetized with 2 % w/v pentobarbital sodium (2.0 mL/kg weight). In total, 200 μL MBs@CS/PB/DNA was injected through ear vein and flushed with 1.0 mL saline. The right kidney was imaged using a broadband L9-3 transducer of the IU22 ultrasound system in PIHI mode with MI of 0.06. All applicable institutional and/or national guidelines for the care and use of animals were followed.