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Regular Article

Preparation of photo-responsive poly(ethylene glycol) microparticles and their influence on cell viability

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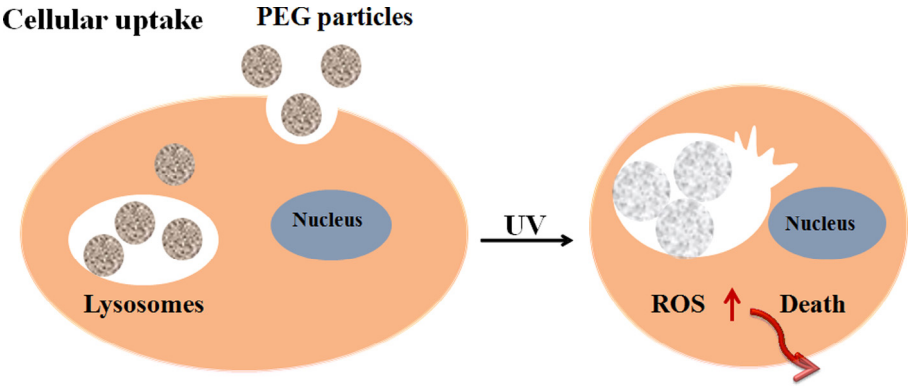
GRAPHICAL ABSTRACT



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ABSTRACT

Intelligent colloidal particles have been widely used as carriers for delivery of bioactive molecules due to the ability of controlled release. However, attention is mainly paid to the effects of their payloads, whereas the impacts of carriers are largely ignored. In this study, photo-responsive polyethylene glycol (PEG) microparticles were fabricated by using 8-arm-PEG with terminal amine groups (8-arm-PEG-NH_2) and a photo-cleavable cross-linker. Due to the cleavable C—O bond in the cross-linker, under UV irradiation the PEG particles could be decomposed gradually, leading to particle swelling and eventual disappearance. The PEG particles could be internalized by smooth muscle cells and HepG2 cells, and located in lysosomes. Their intracellular photo-response induced significant decrease of cell viability and increase of reactive oxygen species level.

1. Introduction

Stimuli-responsive colloidal particles have been widely used as carriers for delivery of different substances. To achieve multifunctions and better performance, many efforts have been paid to design particles with quick response, specific surface and suitable biocompatibility [1–3]. Stimuli-responsiveness can make the regulation of materials easier and more accurate. In the past decades, colloidal particles with responses to different stimuli such as pH, redox, and enzymes have been developed by incorporating various functional components into their structures or utilizing the unique property of some materials [4–6]. In particular, the particles with photo-response have gained much attention because

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photo-control is a minimally invasive manner with real-time spatiotemporal precision [7–9]. However, the major attention is paid to the effects of released bioactive molecules, whereas the influence of changes in carriers' physicochemical properties during the response is largely ignored.

As a material with excellent biocompatibility, poly(ethylene glycol) (PEG) can reduce the immune response efficiently, enhance the stability of particles, reduce the possibility of being cleared, and realize the enrichment of particles in lesion location [10,11]. Thus, PEG has been widely used for the fabrication of particles and hydrogels [12–16]. For example, redox-sensitive PEG-polypeptide nanoporous particles were fabricated to deliver a small interfering RNA sequence to achieve the survivin silencing in prostate cancer cells [15]. Although PEG-based colloidal particles have been intensively used to delivery substances to cells, there is little research concerning the influence of PEG particles' intracellular response on cell viability alone. ortho-nitrobenzyl derivatives, first reported by Schofield [17] and co-workers, are a kind of photo-cleavable molecules. In these derivatives, the chemical bond formed by carbon atom which connects to benzene ring with nitrogen, oxygen or sulfur atoms can be cleaved upon UV irradiation [8,18,19] via an intramolecular rearrangement process [20,21]. So far, hydrogels and planar films based on ortho-nitrobenzyl derivatives have shown the excellent ability for photo-control of cell behaviors [19,22]. However, the colloidal particles composed of the ortho-nitrobenzyl derivatives are rarely reported with respect to their intracellular response [8]. Herein, the photo-cleavable cross-linker based on ortho-nitrobenzyl derivatives with an optimized structure, which can be decomposed under mild conditions, was synthesized. Photo-responsive PEG particles were prepared via 8-arm-PEG with terminal amine groups (8-arm-PEG-NH_2) adsorption into porous $CaCO_3$ particles and cross-linked by the photo-cleavable cross-linker (Scheme 1A and B). The physicochemical properties and photo-responsive behaviors of PEG particles were characterized. The influence of intracellular response of the PEG particles on cell viability, cell spreading and intracellular reactive oxygen species (ROS) generation was investigated by using smooth muscle cells (SMCs) and HepG2 cells (Scheme 1C).

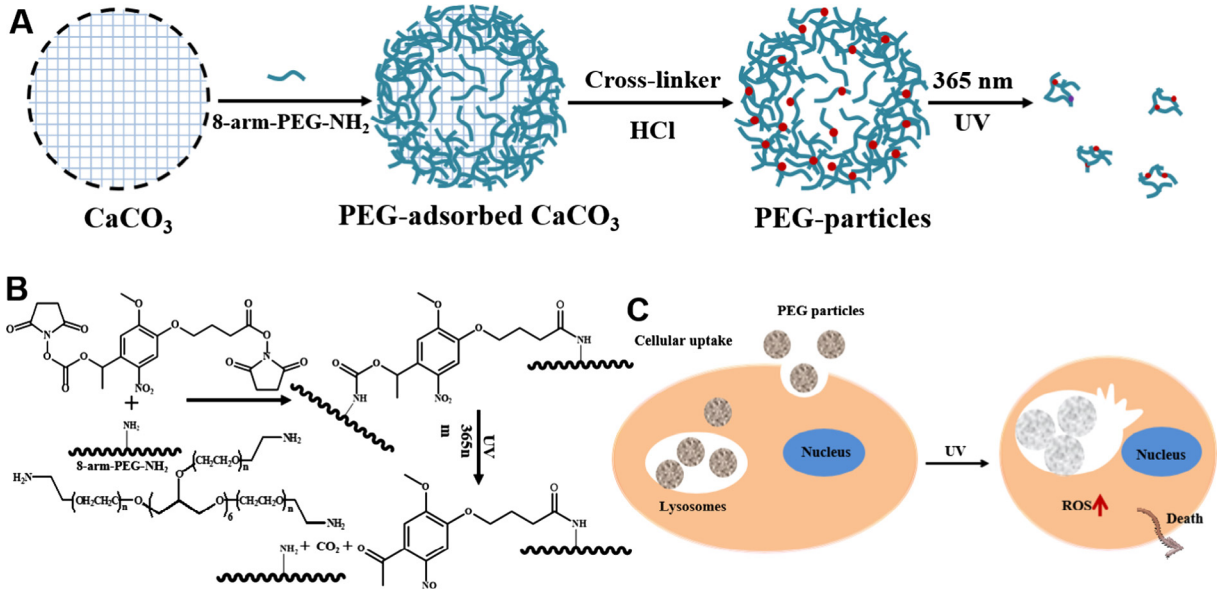
2. Experimental section

2.1. Materials

Rhodamine B isothiocyanate (RBITC) was purchased from Sigma-Aldrich. 8-arm-PEG with terminal amine groups (8-arm-PEG-NH_2, Mw 5000) was purchased from Jenkem Technology Co., Ltd. LysoTracker® Green was purchased from Invitrogen. Reactive Oxygen Species Assay Kit (containing 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA, 10 mM) and Rosup 50 mg mL^{-1}) was purchased from Beyotime Biotechnology. Sodium carbonate anhydrous (Na_2CO_3), calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$), *N*, *N*-dimethylformamide (DMF), ethyl acetate, petroleum ether 60–90 °C, dimethylsulfoxide (DMSO) and hydrochloric acid were purchased from Sinopharm Chemical Reagent Co., Ltd. The water used in all experiments was prepared via a Millipore Milli-Q purification system and had a resistivity higher than 18 MΩ cm^{-1}. $CaCO_3$ particles were prepared by mixing Na_2CO_3 and $Ca(NO_3)_2$ solutions according to literatures [23,24].

2.2. Fabrication and characterization of PEG microparticles

The as-prepared $CaCO_3$ particles (100 mg) were incubated in 10 mL 8-arm-PEG-NH_2 ethanol solution (10 mg mL^{-1}). After mild agitation at room temperature overnight, the sample was centrifuged and washed three times with ethanol. Then, the PEG-adsorbed $CaCO_3$ particles were dispersed in DMSO solution containing photo-cleavable cross-linker (See supporting information for synthesis details). After 12 h reaction in dark, the sample was centrifuged and washed three times with DMSO. For better observation, the particles were labeled with RBITC as well. In brief, after cross-linking, the particles were incubated in RBITC solution (1 mg mL^{-1}, DMSO), and the mixture was maintained in dark overnight under shaking. Finally, the as-prepared particles were washed with DMSO to remove free RBITC, and then incubated in 0.1 M HCl solution for 15 min under shaking to remove the $CaCO_3$ template. The obtained PEG microparticles were further washed with water until neutral pH was reached, and finally dispersed in water and kept in dark at 4 °C.



Scheme 1. Schematic illustrations showing the fabrication and decomposition process of PEG particles (A), cross-linking of 8-arm-PEG-NH_2 and the following photocleavage reaction (B), and cellular uptake of PEG particles and the influence of their intracellular response on cell viability (C).