A Photo-Responsive Porphyrin-Mn@Choles Complex for Bacteria Treatment

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Abstract
Biocompatible photo-driven producers of singlet oxygen can inhibit the growth of drug-resistant bacteria and tumors. In order to develop bacteria targeting generator of singlet oxygen for tumor and bacterial treatment, a metal porphyrin liposome (Phy–Mn–Ls) was prepared by the metal coordination reaction and self-assembly of porphyrin compounds with bacteria targeting polymer (HS–PEG–chol). The photo-driven production of $^{1}\text{O}_2$, binding with bovine serum protein (BSA) and lipase, toxicity to MCF-7 breast cancer cells and inhibitory effect on the growth of Escherichia coli have been investigated. Fluorescence analysis results show that Phy–Mn–Ls can bind to lipase, and it shows less effect on the conformation of BSA and is low cytotoxicity without irradiation. In particular, the good biocompatibility made Phy–Mn–Ls exhibit good photosensitive antibacterial activity and anti-tumor properties. The results demonstrate that the coordination of HS–PEG–chol with metal-phorphrin coordination is an effective way to develop bacteria targeting nano-complexes (Phy–Mn–Ls) for lipase affinity and photodriven bacteria treatment.

Keywords Mn complex · Antimicrobial activity · Porphyrin · Protein interaction

1 Introduction
The drug resistant bacteria and the effect of bactericides to environment have caused a public healthy issue [1, 2]. It is necessary to develop new bactericides for the treatment of pathogen infection [3, 4]. Photo-irriated reactive species (such as $^{1}\text{O}_2$), have been reported as effective photo-driven therapy agents [5, 6]. Singlet oxygen and other reactive oxygen species (ROS) can damage protein or cause the oxidation of intracellular thiols in bacteria, which could reduce the resistance of bacteria to drug [7]. Porphrins and borondipyrromethene fluorophore (BODIPY) derivatives were reported as $^{1}\text{O}_2$ generators for photodynamic therapy [8–10]. Photoactive Mn(II) complexes can be $^{1}\text{O}_2$ generators for the cancer cell treatment by attenuating the cell energy metabolism [11]. Metal porphyrins were reported as mimics of PSII (photosynthesis II) for the oxygen generation [12–15]. Oxygen and $^{1}\text{O}_2$ can attenuate the cell metabolism. Attenuating the cell metabolism is also a promising strategy for pathogen infection [16]. Bacteria have low metabolic activity and are less sensitive to antibacterial compounds, while bacteria show strong response to nanoparticles because of their good cell membrane penetration [17–19]. Porphyrin could assemble with cholesterol-linked polyethylene glycol polymer (Chol–PEG) resulting bacteria surface targeting nanoparticles [20, 21]. Therefore, Chol-PEG conjugated porphyrin might be targeting inhibitors of bacteria. However, it is a challenge to make photosensitive porphrins be reversible affinity to protein and non-toxic to cells in black. To develop safer nano-generator of $^{1}\text{O}_2$ for bacteria treatment, thiol polyethylene glycol cholesterol (HS–PEG–chol) coordinated and assembled with Mn(II) complex of phorphrin (TAPP, Scheme 1) resulting a photodriven nano-generator (Phy–Mn–Ls) of $^{1}\text{O}_2$ for cancer and bacteria treatment.

2 Experimental

2.1 Chemicals and Instruments
1,3-Diphenyl isobenzofuran (DPBF), thiol polyethylene glycol cholesterol (HS–PEG–chol, Mw. 3400), MnCl$_2$ and dichloromethane (DCM), bovine serum protein (BSA),
pancreatic lipase (PL), were obtained from DeWei Chemicals (Zhenjiang, China) Co., Ltd. 2-Chloro-N-(4-(10,15,20-triphenylporphyrin-5-yl)phenyl)acetamide (TAPP) was synthesized according to a reported publication [22]. Fluorescence spectra were taken on a fluorescence spectrophotometer (VARIAN, USA), (slit width = 5 nm). The UV-vis spectra were measured on a UV-2450 spectrophotometer. FT-IR spectra were recorded by a Nicolet Nexus 470 FT-IR spectrophotometer. SEM images came from a scanning electron microscope (S5-550 from Shimadzu, Japan). NMR spectra were recorded on a Bruker AVANCE II 400 MHz spectrometer.

### 2.2 Synthesis of Mn Complex of 2-Chloro-N-(4-(10,15,20-triphenylporphyrin-5-yl)phenyl)acetamide Thiol Polyethylene Glycol Cholesterol (Phy–Mn–Ls)

TAPP (100 mg, 0.12 mmol) was added into the ethanol solution (20 mL) of MnCl₂ (23 mg, 0.12 mmol). After the mixture was stirred at 80 °C for 1 h HS–PEG–chol (20 mg) was added, and then the mixture was stirred at 80 °C for 4 h. After cooled to room temperature, the solution was purified by cellulose membrane (5000) dialysis in DMF and water, respectively. The Phy–Mn–Ls (80 mg) was obtained after concentration and dried in vacuum, yield, 60%.

### 2.3 Interaction with Protein and Lipase

The interaction between Phy–Mn–Ls and BSA or pancreatic lipase (PL) was determined on CARY Eclipse fluorescence spectrophotometer. Firstly, BSA or PL solution (0.5 mg/mL) in PBS buffer (20 mM, pH 7.0) was prepared. Different volume of Phy–Mn–Ls in water (100 µg/mL) was mixed with proteins. After have shaken for 5 min, the emission spectra (λ_{ex} = 290 nm; λ_{em} = 340 nm) were determined.

### 2.4 Singlet Oxygen Determination

1,3-Diphenyl isobenzofuran (DPBF) (100 µM, 15 mL) and Phy-Mn-Ls (2 µM, 15 mL) in water were mixed; the absorption change of DPBF at 415 nm was recorded on UV-vis spectrometer every 1 min under irradiation with red LED light (4 W).

### 2.5 Antibacterial

The effect of Phy–Mn–Ls on *E. coli* and *S. aureus* was investigated. The single colony of bacteria was inoculated in LB broth medium (3 mL), and cultured in a vibration incubator (180 RPM) at 37 °C under aerobic condition. Bacterial growth was evaluated by measuring absorbance at 600 nm (OD₆₀₀). Bacteria were washed with PBS (phosphate buffered saline) and collected by centrifugation (10 min, ~ 2000 g/m) for 10 min. Then the bacteria were diluted by PBS solution to 1 × 10⁵ CFU/mL, all instruments were sterilized before use.

### 2.6 MTT Assay

The anticancer property of Phy–Mn–Ls was evaluated by measuring the inhibition of samples on the proliferation of MCF-7 cancer cells under normal culture conditions. Cells (4 x 10⁴ per milliliter) were incubated into sterile 96-well plates for 24 h. Phy–Mn–Ls (100 µg/L) was diluted with culture solution to different concentrations and then was incubated with MCF-7 cells for 24 h. Cell viability was evaluated by recording the absorption at 570 nm (MTT assay). The test was repeated for three times.
3 Results and Discussions

3.1 Characterization of Phy–Mn–Ls

TAPP was synthesized as reported method [22]. NMR spectrum confirms the structure of TAPP (Fig. S1). In the mass spectrum of TAPP, the main peak at \( m/z = 706.61 \) (100) corresponds to the positive TAPP [(TAPP)+] in ES-MS condition (Fig. S2). TAPP coordinated with MnCl₂ resulting TAPP:MnCl, in which Mn–Cl can be replaced by HS–PEG–chol producing Phy–Mn–Ls (Scheme 1). In the ES-MS spectra of TAPP:MnCl in methanol, the \( m/z = 758.60 \) (100) corresponds to the specie [TAPP:Mn]⁺, indicating the 1:1 ratio of TAPP and Mn in TAPP:MnCl (Fig. S3). The ESR data confirm the existence of Mn(II) ion in Phy–Mn–Ls (Fig. 1) [23]. The intermolecular forces between TAPP and HS–PEG–chol makes the Phy–Mn–Ls self-assembled into tube-shaped nano-complex (Fig. 1d). The UV–Vis absorption of Phy–Mn–Ls in the range of 400–450 nm shows a hypochromic effect compared to TAPP. The absorption at ca. 420 nm (Soret band) and 510–650 nm (Q bands) of Phy–Mn–Ls indicates the existence of tetraaryporphyrin [24]. The coordination of Mn(II) with TAPP resulted in the enhancement of absorption at 475 nm [13, 22]. The content of Mn complex of TAPP in Phy–Mn–Ls was evaluated to be 14.5% according to the absorption of TAPP MnCl at 475 nm (Fig. S4). In IR spectra, the peak at 2933–2978 cm⁻¹, 3033 cm⁻¹, 2500 cm⁻¹ indicate the presence of H–C, H–C=, H–S groups in Phy–Mn–Ls.

3.2 Interaction with Lipase and BSA

There are high level of lipase or fat acid synthase in most bacteria or cancer cells. The interaction of Phy–Mn–Ls with lipase was investigated by measuring the fluorescence emission change of lipase at 348 nm. The addition of Phy–Mn–Ls to lipase resulted in the decrease of fluorescence intensity at 348 nm (Fig. 2), indicating a high affinity with lipase. The deceased fluorescence emission showed that the Phy–Mn–Ls can interacted with both lipase and BSA. The affinity of Phy–Mn–Ls to BSA is a litter stronger than lipase (Fig. 3b and c). Do the affinity of compounds with proteins change the conformation? The effect of Phy–Mn–Ls on the conformation of BSA was evaluated by Circular dichromatography (CD). Results show that the Phy–Mn–Ls changed the band intensity of the BSA at 195 nm and 210 nm, indicating the decreased level of α-helix BSA [25]. The spectra of BSA and BSA/Phy–Mn–Ls are similar indicating the weak effect for Phy–Mn–Ls on the conformation of BSA (Fig. 3). The higher affinity and weak conformation change indicate protein can be good carrier of Phy–Mn–Ls.

Fig. 1 a UV absorption spectra of TAPP (red) and Phy–Mn–Ls (black) (0.1 mg/mL, H₂O), b Infrared spectra of Phy-Mn-Ls (red) and TAPP (blue), c ESR spectrum of Phy–Mn–Ls at 298 K, d SEM diagram of Phy–Mn–Ls (Color figure online)

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3.3 Photodriven Generation of $^1\text{O}_2$

DPBF can combine singlet oxygen with the absorption change at 415 nm [18]. The peak of DPBF decreased at 415 nm under red LED light irradiation (Fig. 4a), indicating the production of singlet oxygen. Singlet oxygen (can reduce the probability of the drug resistance and kill microbial cells [26]. Metalloporphyrin complexes have been reported as good photosensitizer (PS) for photodriven intracellular $^1\text{O}_2$ generation [27]. Therefore, Phy–Mn–Ls is a photo-driven singlet oxygen generator for potential photo-dynamic therapy.

3.4 MTT Assay and Antibacteria Property

The cytotoxicity of Phy–Mn–Ls was studied by measuring the cell viability of compounds treated MCF-7 cells using the MTT assay. Under red LED light irradiation, Phy–Mn–Ls did alter the viability of MCF-7 after 24 h treatment. The IC$_{50}$ of Phy–Mn–Ls is 1.674 µg/mL. In contrast, Phy–Mn–Ls shows no toxicity to cells without irradiation (Fig. 5), indicating Phy–Mn–Ls is a safer photodynamic agent [27]. The higher the concentration of the compound the more it inhibits cell proliferation, which proves that the Phy–Mn–Ls is a concentration dependent photo-responsive anticancer agent.

The effect of Phy–Mn–Ls on the inhibition of E. coli was investigated (Fig. 6). Phy–Mn–Ls inhibited the bacteria proliferation under light irradiation; but the antibacterial potency of Phy–Mn–Ls was weak without irradiation which indicated that the Phy–Mn–Ls was a LED light responsive antibacterial agent. In the absence of Phy–Mn–Ls, LED light has little inhibition effect on bacterial growth. Therefore, Phy–Mn–Ls interact with lipase and produce singlet oxygen
to inhibit bacteria under irradiation (Scheme 2). Metallophyrin complexes have been reported as photosensitizers (PS) for bacteria treatment [27, 28]. The receptor of cholesterol is highly expressed in most bacteria [21]. Phy–Mn–Ls is a potential bacteria surface targeting nanoparticles for bacteria photodynamic inactivation.

4 Conclusions

A biocompatible nanocomplex (Phy–Mn–Ls) was synthesized and used as a photodynamic agent for both cancer and bacteria treatment. The phorphyrin and cholesterol groups in Phy–Mn–Ls make it can bind with lipase, which are highly expressed in most bacteria. The Phy–Mn–Ls can produce $^1$O$_2$ under red LED light irradiation. Meanwhile, Phy–Mn–Ls show less effect on the conformation of BSA indicating BSA can be carrier of Phy–Mn–Ls. In particular, Phy–Mn–Ls show good inhibition on the proliferation of both MCF-7 cancer cells and E. coli bacteria under irradiation, while it is less toxicity to cells without irradiation. Results indicate that the Phy–Mn–Ls is an environmental friendly photosensitive agent for bacteria treatment.

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