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Photodynamic therapy evaluation of methoxypolyethyleneglycol-thiol-SPIONs-gold-*meso*-tetrakis(4-hydroxyphenyl)porphyrin conjugate against breast cancer cells

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ABSTRACT

Magnetic field enhanced photodynamic therapy is an effective non-invasive technique for the eradication of cancer diseases. In this report, magnetic field enhancement of the photodynamic therapy (PDT) efficacy of a novel methoxypolyethyleneglycol-thiol-SPIONs-gold-*meso*-tetrakis(4-hydroxyphenyl)porphyrin conjugate (nano-drug) against MCF-7 breast cancer cells was evaluated. The nano-drug exhibited excellent blue and red emissions under suitable ultraviolet (380 nm) and visible (430 nm) excitations and was well taken up by the cells without any significant dark cytotoxicity after 24 h post-incubation. However, after exposure of cells to light for about 15 min, high rate of cell death was observed in a dose-dependent manner. In addition, the cells that were exposed to external magnetic field displayed higher phototoxicity than the non-exposed cells. Altogether, these results suggest that the nano-porphyrin drug system can function as a new promising magnetic-field targeting agent for theranostic photodynamic eradication of cancer diseases.

1. Introduction

Photodynamic therapy (PDT) utilizes reactive oxygen species generated via interaction of non-toxic light with a photosensitizer (PS) and molecular oxygen for the destruction of cancer cells [1,2]. However, the non-directional accumulation of the PS has been a major challenge [3–5], causing normal cells within the vicinity of the tumour to suffer from the toxicity effect of the irradiating light. To circumvent this challenge, many efforts such as receptor-mediated strategy [6] and magnetic targeting [7] are currently being exploited. The receptor-mediated intervention involves the delivery of a PS into tumour via attachment of the PS to a platform that bears certain biomolecules such as carbohydrate [8], antibody [6], folate [9,10] and peptide [11] whose specific receptors are overexpressed on the surface of the tumour [7,12]. These targeting species help to convey the PS into tumour via a host-guest receptor-molecule binding mechanism. On the other hand, magnetic targeting involves the delivery of a PS into tumour via conjugation of the PS to biocompatible superparamagnetic nanomaterials [13]. The latter deliver the PS into tumour via their response towards the direction of an external magnetic field [7,13]. Magnetic field targeting has an advantage of concentrating rapidly the magnetic nanomaterial and its loads within the target environment [14,15] and thus requires relatively lower dose to achieve therapeutic action. The commonest nanomaterials employed for magnetic targeting are functionalized superparamagnetic iron oxide nanoparticles (SPIONs) [7,13,15,16]. These nanomaterials have the advantages of relatively high biocompatibility, functionazable surfaces [14,17–21], low toxicity and efficient superparamagnetism [4,22], the latter being essential for prevention of embolism of the blood vessels [23,24]. Besides, they show inherent theranostic capability by functioning as excellent magnetic resonance imaging contrast agents [19,25] and heat-generating protherapeutics [26]. In addition, due to their intrinsic small sizes, biocompatible polymeric functionalized SPIONs may accumulate in tumour via enhance permeability and retention (EPR) effect [27–29].

Development of theranostic agents capable of simultaneous detection of disease sites and therapeutic functions has been a major focus of many current research efforts [12,30–33]. Porphyrins are one of the recognized theranostic PSs in PDT. They exhibit excellent fluorescence characteristics and high generation of singlet oxygen species [32,34–36]. However, many porphyrins being hydrophobic in nature

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tend to compromise their fluorescence qualities in water due to solvent quenching effect [37], thus limiting their theranostic values. Thus, there is a need for the development of water-soluble nano-porphyrin systems with better fluorescence properties. In addition, like many other PSs, non-directional light-associated toxicity has been one of the major challenges of porphyrin-based PDT. To circumvent this ordeal, magnetic targeting PDT seems to be gaining considerable attention. In this report, the magnetic targeting photodynamic therapeutic efficacy of a novel PEGylated SPIONs-gold-*meso*-tetrakis(4-hydroxyphenyl)porphyrin conjugate (nano-drug), synthesized via a facile green procedure, was evaluated against MCF-7 breast cancer cells. The fluorescence properties of the nano-drug were also probe to see the possibility of it being used as promising photodynamic theranostic agent.

2. Materials and methods

2.1. Materials

All chemicals were purchased from Sigma Aldrich except fetal bovine serum and Tryple™Select which were obtained from Life Technologies, USA. Nano-pure water was used for all aqueous solutions.

2.2. Synthesis of porphyrin nano-drug

The nano-drug was synthesized according to our previous report with slight modifications [38]. The porphyrin was synthesized following modified Rothemund synthesis and purified using silica gel column. Briefly, a given solution containing propionic acid (330 mL) and freshly distilled pyrrole (16 mL) in a 500 mL flask was refluxed at 138 °C for 40 min. Afterward, 4-hydroxybenzaldehyde (7.1727 g) was added slowly and the resulting mixture refluxed for 5 h to obtain a black purple solution with black residue (Note: as soon as the aldehyde was in contact with the hot solution, aggressiveness of boiling occurred turning the solution immediately to red purple violet). The resulting product was cooled to room temperature (2 h, 20 min) and the supernatant decanted into a beaker. This was followed by the addition of deionized water (50 mL) to 25 mL of the supernatant in a separatory funnel (250 mL) to form a grayish solution. At this time, 100 mL of petroleum ether was added to the funnel to obtain two immiscible layers (Top: organic - colourless; Bottom (aqueous - grayish colour). The bottom layer was collected in a beaker. This was transferred back into the separatory funnel and washed with 38 mL of ethyl acetate to form a dark peach layer (pH $<$ 1) on top of a grayish aqueous layer. Afterwards, the organic layer was separated from the aqueous layer, followed by addition of 50 mL hexane to the former to obtain a dark peach precipitate. The precipitate was dried in an oven at 68 °C to obtain a black purple solid. Moreover, a given amount of the latter (0.0473 g) was purified on a silica gel column (10.1 × 140.5 mm), already preloaded with a silica gel slurry (34.6881 g/90 mL elution solvent mixture: ethyl acetate: dichloromethane, ratio 1:2) with a flow rate of 0.4 mL/min). The pure porphyrin was identified using ultraviolet-visible spectrophotometry (UV–Vis), (Fourier Transform infrared spectroscopy (FT-IR) and proton nuclear magnetic resonance spectroscopy (^{1}H NMR) (Fig. S1, Supplementary information).

The SPIONs were synthesized via modification of our previously reported approach [39]. Briefly, a mixture of ferric chloride hexahydrate solution (25 mL, 0.3506 M) and D-(+)-glucose monohydrate solution (0.051 M) in a 100 mL Pyrex beaker was heated from room temperature to 66 °C and maintained at this temperature until the colour of the solution changed from brown to dull yellow-orange. The resulting solution was then cooled for 42 min after which it was added dropwisely to 50 mL of ammonium hydroxide solution (1 M) in a 100 mL Pyrex beaker at 50 ± 2 °C for 21 min. This was followed by further heating at this temperature for another 24 min. Afterward, the as-synthesized SPIONs were washed five times with deionized water (4 × 250 mL; 1 × 75 mL) and magnetically harvested by placing a neodymium-boron iron (Nd-B-Fe) magnet at the side of the beaker, followed by decantation of the supernatant. The obtained gluconic acid capped SPIONs were dried in an oven at 75 ± 1 °C and subsequently stored under ambient condition for further analysis. The gold-coated SPIONs were synthesized by adding 30 mL of SPIONs' solution (0.0007 g/mL) and 20 mL of gold (III) chloride hydrate solution (0.001076 g/mL) in a 100 mL Pyrex beaker and heated at 74 °C without stirring for 7 h. The core-shell nanomaterials precipitated as a reddish-brown network nanostructure. The latter was separated from the supernatant via magnetization using neodymium-boron-iron magnet followed by decantation after washing with deionized water. This was followed by drying in an oven at 60 ± 2 °C and subsequent storage under ambient condition. The SPIONs were covered with gold to passivate their surfaces (Fig. S2, Supplementary information) and to serve as a link between them and the mPEGSH. This is to prevent leaking of iron species and ensure firm attachment of the thiolated polymer to the core-shell matrix respectively.

Finally, the nano-drug was synthesized by combining the SPIONs-gold core-shell with porphyrin. Briefly, aqueous solution (20 mL) containing methoxy polyethylene glycol thiol-6000 (0.06416 g) and gold-coated SPIONs (0.0279 g) were mixed with 60 mL methanolic porphyrin solution (0.0005533 g/mL) for 10 min. This was followed by evaporation of the resulting solution to dryness under ambient condition to obtain the magnetic nano-drug system.

2.3. Dark cytotoxicity evaluation

Breast cancer cells (MCF-7) were seeded at a concentration of 4×10^5 cells/mL in Dulbecco Modified Eagle's Medium (DMEM), supplemented with 10% Fetal Buffer Saline (FBS), penicillin-streptomycin and amphotericin B following standard protocol. The cells were incubated in the dark in a 5% CO_2 incubator (85% humidity) at 37 °C for 4 h. Afterward, the nano-drug was added at different concentrations of 0.5, 1.0 and 2 μg/mL to the designated cell dishes. This was followed by additional incubation for 20 h and subsequently, Trypan Blue cell viability evaluation.

2.4. In vitro uptake and fluorescence studies

MCF-7 cells were grown on glass coverslips in 35 mm culture dishes and subjected to the following two conditions; cells + 1 μg/mL nano-drug and cells + 1 μg/mL nano-drug + magnetic field exposure (68 G). An hour prior to observation, cells in culture dishes were washed thrice with Hank's Balanced Salt Solution (HBSS), fixed with 200 μl of 3.5% (v/v) Paraformaldehyde in DMEM and permeabilized with 200 μl of 0.5% (v/v) TritonX-100 in water. Cells were washed again thrice with HBSS and the coverslips were inverted onto glass microscope slides onto which a 30 μl of 20% Fluoromount™ Aqueous Mounting in water had been added. Coverslip borders were sealed with nail polish and slides were examined using the Carl Zeiss Axio Z1. The 358Ex/461Em filter was used to detect fluorescent probe signal produced from cells that contained the nano-drug and these images were overlaid into simultaneously captured differential interference contrast (DIC) images.

2.5. PDT efficacy of the nano-drug

The media containing the cells and nano-drug were replaced with phosphate buffer saline (PBS) after 24 h dark incubation. This was followed by irradiation with light of 10 J/cm^2 at 673 nm wavelength for 14 min, 51 s. At this time, the PBS was replaced with cell media and the cells were incubated for another 24 h before evaluation of cell viability by Trypan Blue assay.

2.6. Magnetic targeting effect on PDT efficacy

To evaluate the magnetic targeting effects of the nano-drug, two