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A versatile, stimulus-responsive nanoparticle-based platform for use in both sonodynamic and photodynamic cancer therapy

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ABSTRACT

A PLGA-based multifunctional biodegradable nanoparticle platform co-harboring hematoporphyrin and indocyanine green has been developed. *In vitro* studies demonstrate ultrasound and light stimulated generation of cytotoxic reactive oxygen species. *In vivo* studies show that the ICG component facilitates nIR fluorescence imaging that demonstrates accumulation of IV- administered nanoparticles in tumours. *In vivo* studies also demonstrate ultrasound- and light-mediated inhibition of tumour growth in animals treated with the platform. Since the platform consists entirely of clinically-approved agents it could find use in sonodynamic- and photodynamic-based therapies for cancer.

Statement of Significance

We describe a biocompatible and biodegradable nanoparticle-based platform for use in sonodynamic and photodynamic therapeutic approaches for the treatment of cancer. The non-toxic nanoparticles produce cytotoxic reactive oxygen species when exposed to ultrasound and/or light at levels that have no impact on tissues. The system is unique in that it is accumulated by tumours within six hours and has the ability to release its sensitising capability while retaining its imaging capability within a therapeutic time frame. The former could enhance dispersion and sensitising capabilities in less permeable tumour tissues and the latter permits the design of therapeutic approaches that minimize collateral damage to normal tissues.

1. Introduction

The search for non-invasive or minimally invasive approaches for the treatment of more deeply-seated cancers has led to the development of therapeutic regimes such as photodynamic therapy (PDT) [1]. The approach is based on the administration of a sensitiser that is taken up by tumours and is subsequently stimulated by light to produce cytotoxic reactive oxygen species (ROS). Although it delivers site-specific therapy, its acceptance in clinical practice as a mainstream cancer treatment modality is hindered, for the most part, by accumulation of sensitisers in skin [2] and the inability of light to penetrate deeply into living tissues [3]. The former necessitates the protection of patients from exposure to direct light and the latter precludes the treatment of inaccessible or more deeply-seated tumours. An alternative emerging approach involves the use of ultrasound to 'activate' the sensitising drug and this is more generally known as sonodynamic therapy (SDT) [4]. Although many of the sensitisers used in PDT can also serve as sonosensitisers, the mechanism by which the sensitiser is activated by ultrasound in SDT is less clear. It is however generally accepted that site-specific toxicity results from the generation of ROS on exposure to ultrasound and a number of hypotheses have been suggested, all of which involve events that derive from ultrasound-induced cavitation [5,6]. This is supported by the observation that when sensitisers are chemically conjugated to the surface of microbubbles, the generation of ROS is enhanced in an acoustic field [7].

Since SDT uses ultrasound to activate the sensitiser, the major perceived clinical benefit is that it may be used to target more deeply-seated lesions since ultrasound can penetrate tissues more easily than light. Because SDT offers the potential to target more deeply seated lesions, accumulating sensitizer solely at the target site becomes essential in order to preclude uptake by non-target tissues and in particular by tissues that interspace the actual target

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and the ultrasound source. Achieving this would also preclude overall patient photosensitization; an adverse effect associated with many sensitisers employed in PDT as mentioned above. One potential means of accumulating sensitizer at the lesion would be to use a nanoparticle platform and exploit the enhanced permeation and retention (EPR) phenomenon exhibited by many solid tumours [8]. Masters et al. have reviewed nanoparticle-based platforms for use in PDT and describe their ability to accumulate sensitizer in target lesions, although the inability of light to penetrate deeply into tissues in PDT remains a challenge [9]. Since tissue penetration is not a challenge for SDT, the use of a nanoparticle-based platform for more site-specific sensitizer delivery would provide significant advantage. Recently You et al. described the use of TiO_2 nanoparticles in SDT demonstrating preferential uptake by tumours and therapeutic efficacy [10]. In addition to aiding the preferential accumulation of sensitiser in target lesions, nanoparticles can also be exploited to deliver multiple functionalities and this approach has been used to incorporate diagnostic as well as therapeutic capabilities in a single formulation. In another recent study, Chen et al. described the use mesoporous silica grown on reduced graphene nanosheet that was capped with rose bengal-conjugated to iron-oxide nanoparticles and demonstrated its use to simultaneously generate cytotoxic ROS and induce hyperthermia in an acoustic field [11]. Here the authors suggested that the graphene sheet provided a heat conducting base that induced hyperthermia and the iron nanoparticles provided seeding of cavitation to enhance ultrasound-mediated ROS generation by rose bengal. The particles could also be used to magnetically target the construct to a specific locus and could provide a means of assessing accumulation at a target site using magnetic resonance imaging (MRI). Although a variety of novel and indeed extremely elegant nanoparticle-based platforms incorporating sensitisers and exhibiting multiple functionalities have recently been suggested for SDT, their potential behavioral characteristics in patients and their clinical acceptability remain unproven.

Interestingly the first studies reporting the phenomenon of SDT exploited hematoporphyrin (HP) as the sensitizer [4]. Although its use as a sensitiser for SDT would be clinically acceptable, since it is a natural porphyrin and indeed derivatives of this are currently used clinically for PDT (Photofrin), its direct clinical exploitation is hindered by its limited solubility in aqueous media and its adverse biodistribution characteristics which result in prolonged skin photosensitization [12,13]. Since it has been suggested that nanotechnology could play a very significant role in the clinical exploitation of porphyrins [14], primarily because it could resolve issues associated with limited solubility and impact favorably on biodistribution, we decided to explore the possibility of using HP as a sensitiser payload in a nanoparticle-based platform. Poly(DL-lactic-co-glycolic acid) (PLGA) was used as a platform polymer because it is biocompatible, biodegradable and is approved by the United States Food and Drug Administration for use in humans [15]. In order to provide an imaging capability in the platform, it was decided to incorporate the cyanine dye, indocyanine green (ICG) into the HP-containing particles because it is a clinically-approved near infra-red (nIR) fluorescence imaging agent that is used to assess vascular patency [16]. Although ICG has also been reported to serve as a sensitizer that responds both to light and ultrasound, it was used in this study at concentrations that were at least 30-fold lower than those required to elicit a therapeutic effect [17]. Here, we describe the physicochemical characterization of this stimulus-responsive platform. Using *in vitro* and *in vivo* target systems, it is shown to exhibit cytotoxic activity in response to light, ultrasound and combinations of both. The diagnostic capability of the platform and its exploitation in therapy design is also described. The potential benefits offered by such a system in the treatment of solid tumours are discussed.

2. Materials and methods

2.1. Preparation and characterization of nanoparticles

50 mg of hematoporphyrin dichloride (HP) (>75%; Sigma Aldrich, UK) and 10 mg of indocyanine green (ICG) (Sigma Aldrich, UK) were dissolved in 3 mL of ethanol. Where HP containing nanoparticles were produced in the absence of ICG, the latter was omitted from this solution. A 100 mg quantity of poly(DL-lactic-co-glycolic acid) (75:25; molecular weight: 66,000–107,000; Sigma Aldrich, UK) was dissolved in 4 mL of acetone. Following dissolution, both solutions were mixed and added dropwise to 40 mL of a vigorously-stirred 1% (w/v) aqueous solution of polyvinyl alcohol (MW 30,000–70,000; 87–90% hydrolyzed; Sigma Aldrich, UK) over a 7 min period. To enhance emulsification an ultrasound probe (6 mm; 20 kHz, Vibra-Cell; Sonics and Materials, Newton, CT, USA) was immersed in the polyvinyl alcohol solution and operated at 91 W (70% of net power output) during addition of the PLGA mixture. The suspension was then stirred for 3 h and nanoparticles were recovered and washed once in distilled water, then in phosphate buffered saline (PBS) and finally in distilled water by centrifugation at 15,000*g* for 30 min. The pellet was then suspended in distilled water and centrifuged at 500*g* for 3 min to remove larger aggregates before finally lyophilizing and storage at −20 °C. To determine the HP and ICG content, 1 mg of nanoparticles was dissolved in acetone and the concentration of HP and ICG was determined using UV/Vis absorption spectrophotometry at 500 nm and 800 nm, respectively. Encapsulation efficiency is expressed as the mass of each payload recovered in the nanoparticle preparation as a % of the mass of each payload initially employed in the preparation. UV/Vis absorbance scans of nanoparticle preparations were performed using dilutions of a 1 mg/mL suspension of each nanoparticle preparation in PBS. In order to examine passive release of payload from the nanoparticles preparations, 1 mL of a 1 mg/mL suspension in PBS was placed inside dialysis tubing (SpectroPor, MW cut-off: 50,000, Spectrum Laboratories, Breda, The Netherlands) and this was then placed in a beaker containing 25 mL of distilled water. The solution outside the tubing was stirred using a magnetic stirrer and samples were harvested at the indicated times. HP and ICG that diffused into the external solution were determined by measuring the absorbance at 500 nm and 800 nm, respectively.

2.2. Determination of nanoparticle size

Dynamic light scattering was employed to determine the diameter of the nanoparticles by analyzing dilutions of a 1 mg/mL suspension of particles in PBS using a Malvern Zetasizer Nano Z system (Malvern Instruments, Worcestershire, UK) and Zetasizer software version 6.12. The diameter of the nanoparticles was also determined using scanning electron microscopy by initially drying a sample of particles onto aluminum stubs, sputter coating with gold/palladium for 3 min at 18 mA and subsequently visualizing under high vacuum mode using a Quanta Environmental scanning electron microscope (FEI, Hillsboro, OR, USA).

2.3. Determination of ROS production

Ultrasound- and light-mediated ROS generation was determined using oxidation of 1,3-diphenylisobenzofuran (DPBF) as described previously [6]. Essentially a nanoparticle preparation (1 mg/mL) was added to a 10 μM solution of DPBF prepared in an ethanol:water (50:50) mixture, aerated for 10 min and exposed to ultrasound at the indicated power densities (expressed as spatial average, temporal peak) for 60 min. A Sonidel SP100 sonopora-