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Photoluminescent and superparamagnetic reduced graphene oxide–iron oxide quantum dots for dual-modality imaging, drug delivery and photothermal therapy

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ABSTRACT

Reduced graphene oxide–iron oxide quantum dots (QDs) with intrinsic photoluminescent and superparamagnetic properties were synthesized through a green, hydrothermal method that simultaneously reduced and shattered graphene nanosheets to form the dots. The structure, morphology, properties and cell viability of these QDs were investigated. The QDs emitted violet light when excited at 320 nm, possessed no residual magnetization upon magnetic hysteresis tests, and had low cytotoxicity to healthy cells at low concentrations. The suitability of the QDs for fluorescent and magnetic resonance dual-modality imaging was shown by *in vitro* imaging with dermal fibroblast cells and T2 relaxation time. A drug could be loaded onto the surface of the QDs, with a loading ratio of drug to QD of 0.31:1. The drug achieved a steady but full release from the QDs over 8 h: these drug-loaded QDs could be manipulated by an external magnetic stimulation for targeted drug delivery. The potential for use as a cancer photothermal therapy was demonstrated by both a rapid, ~50 °C temperature increase by a suspension of 100 μg ml^{-1} of QDs and the photothermal ablation of HeLa cells *in vitro* under near infrared irradiation. The stability of the MGQDs in fetal calf serum was shown to improve when an ionic drug was coated on the surface.

1. Introduction

Biomedical technology is undergoing a period of rapid development, and one of the keys to this advancement is nanotechnology [1]. Graphene, and its chemically oxidized derivative graphene oxide (GO), have been investigated for drug delivery, biosensing, and cancer photothermal therapy as they have a large surface area, ample functional groups on the surface, photothermal properties and/or low cytotoxicity [2,3]. Iron oxide (IO) nanoparticles have also been studied for biomedical purposes [4]. Their superparamagnetic (i.e. they hold no residual magnetic force and can be manipulated by external magnetic fields) behavior allows for them to be used for targeted therapeutic delivery [5]. They can be imaged *in vivo* by magnetic resonance imaging (M.R.I.), enabling the visualisation of tumors and the circulatory system, as well as finding use in magnetic hyperthermia [6,7].

IO can be deposited onto GO to form GO–IO or reduced graphene oxide (rGO)–IO hybrids [8–11] (the chemical deposition may reduce GO to rGO during the synthesis [12]), which can combine the interesting aspects of both individual nanoparticles [8–12]. Previous work [9,12] has shown that graphene–IO nanoparticles can be used for targeted drug delivery, M.R.I., and photothermal therapy; the functional groups of GO or rGO allow for drug loading while the superparamagnetic properties of IO enable the hybrids to be used for targeted drug delivery using an external magnetic stimulus and M.R.I. Both graphene [13] and IO [14] were shown to absorb near infrared (NIR) light, which was converted into heat for photothermal therapy. For instance, GO–IO reduced the cancer cell viability by ~90% by using a 2 W cm^{-2} laser for 5 min [9], and rGO–IO achieved the full ablation of tumours within mice within 24 h using a 0.5 W cm^{-2} laser as the irradiation source [12]. However, no intrinsic photoluminescent

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properties were demonstrated for the graphene–IO nanoparticles reported in the literature [11,15,16]. The fluorescent imaging capability of the rGO–IO nanoparticles were from dyes (polyfluorene [11] and cyanine [12]) bonded to the nanoparticle surface. The photoluminescent properties of the GO–IO were attributed to the bonded doxorubicin, which the GO–IO quenched in comparison to free doxorubicin [9]; these nanoparticles were approximately 50–300 nm in size and did not possess intrinsic photoluminescent properties.

Quantum dots (QDs) emit a luminescence due to quantum confinement effects [17]. Conventionally, QDs were made of compounds like cadmium selenide (CdSe) or cadmium telluride (CdTe), but they were found to be cytotoxic during *in vitro* cell tests due to the photolysis release of cadmium ions and the uncoiling of DNA helical strands [18,19]. This led to the development of biocompatible carbon-based QDs, with QDs derived from carbohydrates [20], nanodiamonds [21], and graphene [22–25]. Graphene QDs (GQDs) can be created by ultrasonication [22], chemical reduction [23], photo-reduction [24], and hydrothermal cutting [25]. These carbon QDs emit the same level of luminescence as the cadmium QDs, but do not carry the same cytotoxic risk. It has been shown that GQDs could enter cells but did not enter the nucleus of stem cells during *in vitro* tests; they maintained a high (80%) viability for neurosphere and pancreas progenitor cells up to 100 μg ml^{-1} after 72 h [26], and carboxylated GQDs caused no acute toxicity to rats after 22 days post injection at a concentration of 10 mg kg^{-1} [27]. Carbon QDs can be excreted rapidly from the body after being administered through intravenous, intramuscular or subcutaneous injections [28].

To the authors' knowledge, biocompatible graphene-based magnetic QDs that are intrinsically both superparamagnetic and photoluminescent have not yet been reported. Quantum dots with magnetic properties (MQDs) can by synthesized by either doping conventional QDs (for example CdSe, or CdTe) with metal elements like manganese [29], nickel [30], or gadolinium [31], or by encasing conventional QDs with IO [32] in silica spheres. These MQDs possess the photoluminescent properties of conventional QDs and the magnetic properties of the doping agent or the encapsulated IO, but they contain cytotoxic compounds such as cadmium selenium or cadmium telluride and are therefore cytotoxic without a biocompatible passivation coating [30]. The aim of this work was to synthesize magnetic graphene–IO quantum dots (herein MGQDs) as a multifunctional nanosystem for fluorescent imaging, M.R.I., targeted drug delivery and photothermal therapy for concurrent detection, monitoring and treatment of diseases. The MGQDs were synthesized by depositing IO on GO surface, followed by autoclaving to reduce GO into rGO and form the MGQDs. Their structure, morphology, cell viability, drug release behavior, fluorescent imaging and M.R.I. capabilities and photothermal properties were assessed.

2. Experimental

2.1. Materials

The following chemicals were used as purchased from Sigma to Aldrich; sulfuric acid (95–98%), hydrogen peroxide (29–32% in water), potassium permanganate (97%), sodium nitrate (>99%), ferrous chloride tetrahydrate (>99%), hydrochloric acid (36.5%), ferric chloride hexahydrate (97%), fluorescein sodium, graphite powder (⩽20 μm), rhodamine B, norharmane, lidocaine hydrochloride (>99%), porcine trypsin (BioReagent), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) solution (1 mg ml^{-1} in phosphate buffered saline (PBS)), and ethylenediaminetetraacetic acid (EDTA, BioReagent). Dulbecco's modified eagle medium (DMEM, 500 ml, Gibco Invitrogen (Paisley, UK)) was used with fetal calf serum (FCS, Advanced Protein Products, Brierley Hill, UK), penicillin (100 units ml^{-1}), streptomycin (100 μg ml^{-1}), and fungizone (630 ng ml^{-1}) from Gibco Invitrogen (Paisley, UK). Isopropanol alcohol (reagent grade) and PBS tablets (pH = 7.4) were acquired from Fisher Scientific UK.

2.2. Preparation of graphene oxide–iron oxide nanoparticles

Graphene oxide was synthesized from a modified Hummers method [33] and GO–IO was prepared according to methods described in the literature with some modifications [8,34]. The freeze-dried GO powder (1 g) was dispersed in 150 ml of distilled water through stirring and sonication for 1 h and the pH of the suspension was raised to pH = 8 with the addition of ammonium hydroxide NH_4OH. Separately, ferrous chloride tetrahydrate $FeCl_2$-$4H_2O$ (5.4 g) and ferric chloride hexahydrate $FeCl_3$-$6H_2O$ (4 g) were dissolved in 135 ml distilled water. The GO suspension and the solution of IO precursors were added together, ammonium hydroxide was added drop wise until a pH = 10 was reached, and then the mixture was stirred for 2 h under a nitrogen atmosphere at ambient temperature. The precipitate, iron oxide coated graphene oxide (GO–IO), was washed with distilled water, ethanol and dichloromethane to remove residual chemicals, separated from unbound, hydrophobic IO precipitate by decanting the GO–IO as the supernatant from an aqueous suspension, before being re-dispersed in distilled water (∼3 mg ml^{-1}) by sonication for 1 h in a Fisherbrand sonication bath (230 V, 50–60 Hz). As a control sample, IO was prepared using the same method but without the addition of GO.

2.3. Preparation of reduced graphene oxide–iron oxide quantum dots

The aqueous GO–IO suspension (∼3 mg ml^{-1}) was treated in a Parr Series 4000 autoclave at 200 °C for 10 h (pressure of boiling water at 201 °C = 1.6 MPa) to generate reduced graphene oxide–iron oxide quantum dots (namely, MGQDs). The suspension was placed in a dialysis bag (Fisher Scientific Biodesign Dialysis tubing, molecular weight cut off = 3.5 kDa) and the residual chemicals were allowed to diffuse into the distilled water surrounding the dialysis bag [25]. The MGQDs were collected in an aqueous suspension from the dialysis bag and lyophilized in a Labconco FreeZone Triad freeze-dryer to be stored as a powder in a desiccator. As a control, GO and IO (both with a raised pH of 8) were autoclaved at 200 °C for 10 h to produce graphene quantum dots and autoclaved iron oxide (A-IO) for a comparison study.

2.4. Drug loading onto quantum dots

Lidocaine hydrochloride (LH) (0.1 mg ml^{-1}) was added to a 100 ml suspension of MGQDs (0.1 mg ml^{-1}) under stirring for 48 h. The suspension was centrifuged at 9000 rpm for 1 h, after which the supernatant (containing unbound LH) was removed and the precipitate was re-dispersed in water. This process was repeated several times to remove all of the unbound LH. The MGQD-LH was then freeze-dried and stored in a desiccator.

2.5. Characterization

Transmission electron microscopy (TEM), energy dispersive X-ray spectroscopy (EDS), and selected area electron diffraction (SAED) were achieved using a Philips Technai T20 electron microscope operating at an accelerating voltage of 200 kV. High resolution TEM (HR-TEM) was carried out using a JEOL 2010F field emission gun TEM operating at an accelerating voltage of 200 kV.