Micron 92 (2017) 25–31

Contents lists available at ScienceDirect

Micron

journal homepage: www.elsevier.com/locate/micron

Tutorial

Functionalized nanoparticles enable tracking the rapid entry and release of doxorubicin in human pancreatic cancer cells

Maheshika P. Arachchige[a], Suvra S. Laha[b], Akshata R. Naik[b], Kenneth T. Lewis[b], Ratna Naik[a], Bhanu P. Jena[a,b,*]

[a] Department of Physics & Astronomy, Wayne State University, Detroit, MI 48201, USA
[b] Department of Physiology, Wayne State University School of Medicine, Detroit, MI 48201, USA

ARTICLE INFO

Article history:
Received 13 October 2016
Received in revised form 24 October 2016
Accepted 25 October 2016
Available online 29 October 2016

Keywords:
Pancreatic cancer
SPIO nano particles
Drug delivery
Doxorubicin

ABSTRACT

Efficient drug delivery is critical to therapy. Using electron microscopy, X-ray, and light microscopy, we have characterized functionalized superparamagnetic iron oxide (SPIO) nanoparticles, and determined their ability for rapid entry and release of the cancer drug doxorubicin in human pancreatic cancer cells. Dextran-coated SPIO nanoparticle ferrofluid, functionalized with the red-autofluorescing doxorubicin and the green-fluorescent dye fluorescein isothiocyanate as a reporter, enables tracking the intracellular nanoparticle transport and drug release. This engineered nanoparticle enables a >20 fold rapid entry and release of the drug in human pancreatic cancer cells, holding therapeutic potential as an advanced drug delivery and imaging platform. The low extracellular pH of most tumors precluding the entry of a number of weakly basic drugs such as doxorubicin, conferring drug resistance, can now be overcome.

1. Introduction

Low extracellular pH of most tumors, preclude the entry of a number of weakly basic drugs, conferring drug resistance. To overcome this resistance, superparamagnetic iron oxide (SPIO) nanoparticles (NPs) that enter cells through endocytosis, possess low toxicity, excellent biodegradability, produced inexpensively and rapidly, capable of magnetic resonance imaging (MRI) and therapeutic hyperthermia, have been engineered in the current study for rapid entry and release of the cancer drug Doxorubicin (DOX) in human pancreatic cancer cells. The novel 0.5–1.0 nm dextran functionalization of SPIO NPs to obtain a ferrofluid, and the chemical linking of a reporter fluorescent dye fluorescein isothiocyanate (FITC) and the red-autofluorescing cancer drug DOX, enables tracking the rapid intracellular entry, transport, and the release and localization of the drug to the target organelle, holding therapeutic potential as an advanced drug delivery platform. The unique properties of nanoparticles (NPs) have been extensively exploited due to the broad spectrum of their use ranging from applications in electronic materials to medical therapy (Davis and Shin, 2008). In the last decade, gold (Chithrani et al., 2006; Huang et al., 2006), silver (Foldbjerg et al., 2011; Jeyaraj et al., 2013) and magnetic NPs (Pankhurst et al., 2003) especially superparamagnetic iron oxide (SPIO), have emerged as potential candidates for the diagnosis and treatment of various forms of cancer, due in-part to their low toxicity, biodegradability, drug delivery, magnetic resonance imaging (MRI), hyperthermia, and its inexpensive and rapid synthesis (Pankhurst et al., 2003). The interaction of these NPs with cancer cells continues to be studied for understanding their usage in drug delivery and therapy (Dam et al., 2012; Veiseh et al., 2010; Verma and Stellacci, 2010). The SPIO NPs exhibiting unique superparamagnetic properties (no magnetic hysteresis) combined with biocompatibility are ideal for use in nanomedicine (Gupta and Gupta, 2005; Peng et al., 2008; Petri-Fink et al., 2005; Thorek et al., 2006). Surface functionalization of these SPIO NPs with anti-cancer drugs have been conducted in recent years to determine their efficacy in cancer treatment (Berry and Curtis, 2003), however the major issue has been the entry of a number of weakly basic cancer drugs, among them Doxorubicin (DOX), conferring drug resistance (Gerweck and Seetharaman, 1996; Stubbs et al., 2000). Studies report that Polyethylene Glycol (PEG)-functionalized porous silica shell on DOX-conjugated Fe_3O_4 nanoparticle cores (Chen et al., 2010), Poly(amidoamine) or PAMAM-coated Fe_3O_4 NPs-DOX complex (Nigam et al., 2014), and DOX-loaded Fe_3O_4 NPs modified with PLGA-PEG copolymers (Akbarzadeh et al., 2012), hold potential promise in cancer therapy. Pancreatic cancer, among the most aggressive forms of cancer, is a leading cause of mortality (Siegel

* Corresponding author at: George E. Palade University Professor, Distinguished Professor, Department of Physiology, School of Medicine, Department of Chemical Engineering and Materials Science, College of Engineering, Department of Physics and Astronomy, College of Liberal Arts and Sciences, Wayne State University, 5245 Scott Hall, 540 E. Canfield, Detroit, MI 48201, USA.
E-mail address: bjena@med.wayne.edu (B.P. Jena).

http://dx.doi.org/10.1016/j.micron.2016.10.005
0968-4328/

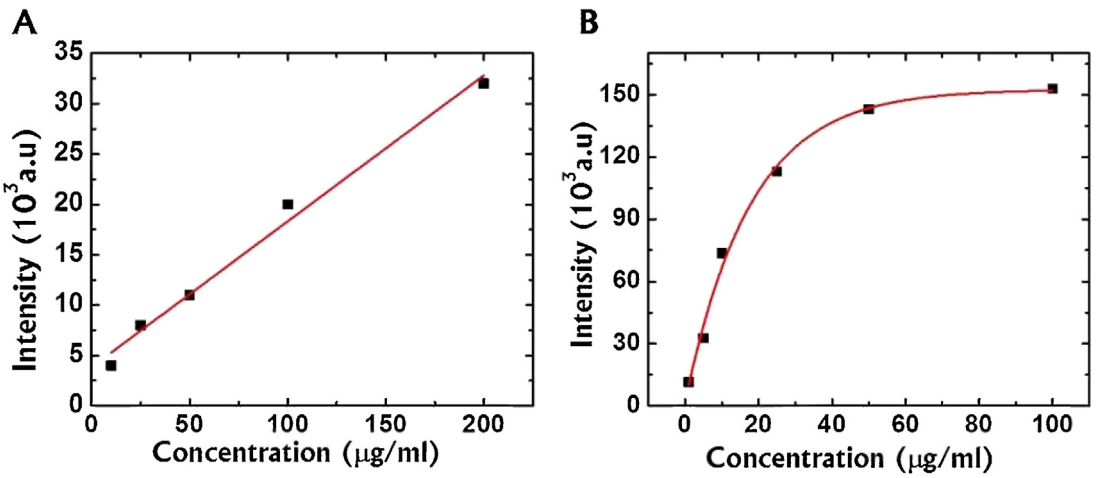

Fig. 1. Calibration curves of (A) FITC and (B) doxorubicin (DOX) to estimate the drug/dye quantification incorporated with the NP conjugate.

et al., 2015). Although DOX-conjugated NPs have been used as drug carrier and delivery platform especially in treating human breast cancers (Brigger et al., 2012), their use in the treatment of human pancreatic cancer cells has been little explored. This necessitated a detailed understanding of the entry, intracellular distribution, and drug release of SPIO-drug functionalized NPs in human pancreatic cancer cells, which is the subject of the current study. In this paper, we report the rapid synthesis of 10 nm SPIO-dextran ferrofluid, with the novel conjugation of drug (DOX) and a fluorescent tracker (FITC) using minor modification of a published EDC/NHS procedure (Young et al., 2009), as well as the demonstration of its rapid entry, subcellular drug release and accumulation in the target organelle in human pancreatic cancer MIA PaCa-2 cells. This unique drug-dye dual conjugate has enabled the tracking of SPIO NPs entry into cells and the subcellular release and distribution of the drug, with drug accumulation in the nucleus (target organelle) and little or no FITC in the organelle. Our study further demonstrates that the rate of intracellular entry of the drug is >20 fold greater when conjugated to the SPIO NPs, as opposed to when free.

2. Materials and methods

2.1. Synthesis of SPIO NPs

An aqueous solution of $FeCl_2{\cdot}4H_2O$ and $FeCl_3{\cdot}6H_2O$ in a molar ratio of 1:2 was mixed in a beaker. A 2 M NH_4OH solution was added into the mixture at once, under continuous magnetic stirring at 700 rpm. Upon addition of the base, a brown color precipitate was formed which gradually turned black, indicating the formation of iron oxide (Fe_3O_4) NPs. The NPs were then isolated with a magnet and washed several times with deionized double distilled water until a neutral pH was achieved. The synthesized NPs were used for further surface functionalization while a small fraction was dried to obtain a powder for use in structural and magnetic characterizations.

2.2. Synthesis of dextran-coated SPIO NPs

Equivalent masses of SPIO NPs and dextran (MW: 15–20 kDa) were dispersed in similar volumes of 0.5 M NaOH separately. The dextran solution was kept under continuous sonication while the suspension containing the NPs was gradually added drop-wise. The resultant mixture was further sonicated for 24 h to obtain a well-dispersed ferrofluid.

2.3. Structural and magnetic characterizations of synthesized SPIO NPs

The crystalline nature of the synthesized SPIO powder NPs was confirmed using a Rigaku MiniFlex 600 x-ray diffractometer, generating Cu Kα radiation at a wavelength of 1.54 Å. For determining the shape, size, and detailed morphology of both bare and dextran coated SPIO NPs, a JEOL-2010 FasTEM transmission electron microscope (TEM) operated at 200 kV was employed, with the NPs sonicated in alcohol before placing them on a carbon coated copper grid for obtaining high resolution images. The *dc* magnetic properties of these bare superparamagnetic NPs were investigated by a Quantum Design physical property measurement system (PPMS). For conducting the magnetic measurements, approximately 30 mg of the nanoparticle sample was mounted in a gelatin capsule packed with cotton (to restrict the motion of the particles).

2.4. Crosslinking of dextran coated SPIO NPs

The solution containing 1 ml of dextran-coated NPs, 5 ml of 5 M NaOH, 2 ml of deionized water and 2 ml of ECH was prepared. This mixture was then incubated at room temperature under continuous shaking to promote the interaction between the aqueous and the organic phases. Following 24 h of incubation, the black colloidal suspension was dialyzed several times against deionized water to remove any excess ECH and residual ions.

2.5. Carboxymethylation of crosslinked SPIO NPs (CMD-SPIO)

10 ml of the crosslinked SPIO NPs having a concentration of 4 mg/ml was adjusted to pH 11 using 0.1 M NaOH under continuous stirring for 1 h at room temperature while purging with N_2 gas. 100 mg of monochloroacetic acid (MCA) was then slowly added to the solution and the mixture was heated at 60 °C in a water bath and under N_2 atmosphere. The reaction mixture was then dialyzed against deionized water to obtain the CMD-SPIO.

2.6. Double labeling of dextran-SPIO NPs with DOX and FITC (SPIO-DOX/FITC)

The DOX and FITC were attached to the CMD-SPIO NPs through EDC/NHS chemistry. 10 mg of EDC and 6 mg of NHS was added to 2.5 ml CMD-SPIO NPs suspension and stirred for 15 min at room temperature to activate the carboxyl groups. 1 μmol of DOX was added to this mixture and subjected to stirring for 10 min, followed by the addition of 1 ml Ethylene diamine hydrochloride (EDA). The