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PEG coated and doxorubicin loaded multimodal Gadolinium oxide nanoparticles for simultaneous drug delivery and imaging applications

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ABSTRACT

We report water-in-oil microemulsion mediated synthesis of PEG[1] coated Gd_2O_3 NPs[2] loaded with fluorescent anti-cancer drug dox[3] for synchronous drug delivery, optical and MR[4] imaging applications. These PEG covered Gd_2O_3 NPs loaded with dox (Gd-PEG-dox NPs) were found to possess spherical morphology with 13 nm size as measured from TEM and the hydrodynamic diameter comes out to be 37 nm as determined from DLS. Fluorescence spectra and fluorescence microscopy images confirmed optical activity of the NPs. The paramagnetic nature of NPs was affirmed by NMR line broadening effect on the spectrum of surrounding water protons. Therefore, these particles can be efficiently used as CA[5] in MR imaging. *In vitro* analysis showed significant cellular uptake of particles by A-549 cells. A pH dependent drug release pattern was observed for the NPs. Cell viability assay performed on A-549, PANC-1 and U-87 cancerous cell lines revealed that Gd-PEG-dox NPs are cytotoxic. On the basis of these observations, it can be concluded that these multi-modal paramagnetic NPs promise potential cancer therapy along with optical and MR imaging applications.

1. Introduction

In biomedical science ample of attention is devoted, over the last few decades, on development of multifunctional NPs which can efficiently act as drug-delivery vehicles in biological system. Substantial efforts have been made to incorporate nanotechnology in drug delivery to have site-specific and sustained delivery of drug molecules. Mostly, efficacy of a drug is hampered by its high toxicity, poor solubility, high dose, non-specific distribution and *in vivo* decomposition (Parveen et al., 2012). Such limitations faced by naked drug are overcome by NPs based drug-delivery system which increases safety of drug molecule against chemical or enzymatic decomposition, amends extended bioavailability of drug to pathological site and allows targeted delivery of drug to disease site (Soppimath et al., 2001; Hamidi et al., 2008; Moghimi et al., 2001). Numerous kinds of nano structures have been used as drug-delivery vehicles namely nanotubes, NPs, nanocapsules, microemulsion and liposomes (Wu et al., 2011). Collaboration of therapy along with imaging in a single entity is an emerging trend in nanomedicine. Such drug delivery system having both therapeutic and diagnostic imaging moieties offer dual advantage of targeted drug delivery and real-time monitoring of drug response through imaging. These therapeutic NPs are called theranostic (therapeutic + diagnostic) NPs (Fang and Zhang, 2010; Lammers et al., 2010).

Chemotherapy remains one of the best treatments for various types of cancer but it is usually hindered by the unfavorable distribution of the drug which results in severe side effects. Therefore, nanosize drug delivery systems carrying therapeutic agent along with diagnostic imaging moiety are desirable (Hong et al., 2008; Shuai et al., 2004). Dox is an anthracycline anti-tumor drug which intercalates DNA and inhibits macromolecular biosynthesis (Benyettou et al., 2015). Upon direct administration of dox in body, it shows low cellular uptake and distributes unevenly resulting in undesirable side-effects and toxicity to normal cells (Arya et al., 2009). This concern led to the development of alternative routes for administering dox like entrapping or attaching it with nano materials. Missirlis et al. (2006) prepared polymeric nanoparticles of PEG and poloxamer 407 encapsulating dox in inverse emulsion as controlled drug

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[1] Polyethylene glycol
[2] Nanoparticles
[3] Doxorubicin
[4] Magnetic resonance
[5] Contrast agent

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delivery system. Furthermore, they showed that encapsulation of drug delayed it degradation significantly. Dreis et al. (2007) prepared dox loaded human serum albumin NPs and cell viability study done on two neuroblastoma cell lines showed that anticancer activity of the NPs was more as compared to dox solution.

Magnetic NPs, such as iron oxide and gadolinium oxide, exhibit potential biomedical applications such as contrast enhancing ability in MR imaging (Corr et al., 2008), in drug targeting (Hans and Lowman, 2002) and hyperthermia (Fortin et al., 2007), to name a few. Particularly, Gadolinium based magnetic NPs have drawn tremendous interest to be used as positive contrast enhancing agent in MRI. Numerous studies have shown that gadolinium oxide (Gd_2O_3) NPs can be efficiently used as CA (Ahmad et al., 2015; Bhakta et al., 2011; Gupta et al., 2012). The major advantage of using paramagnetic metal oxide NP as CA is the high surface to volume ratio which maximizes the interaction of paramagnetic metal ion with water protons giving high longitudinal relaxivities (Faucher et al., 2012; Singh et al., 2014). Maeng et al. (2010) synthesized polymeric NPs made up of folate, poly (ethylene oxide)-trimellitic anhydride chloride-folate, dox and superparamagnetic iron oxide for chemotherapy and MR imaging in liver cancer. They showed that relative tumor volume decreased 2 and 4 fold in comparison to free dox and doxil drug in rat and rabbit models, respectively. Also, NPs showed MR imaging sensitivity which was as good as conventional MR imaging CA Resovist. Fang et al. (2012) prepared superparamagnetic iron oxide NPs and poly (beta-amino ester) copolymer pre-loaded with dox, was assembled to its surface. They reported that these NPs could serve as smart theranostic system for sensitive detection *via* MR imaging and for chemotherapy through controlled drug release. Bridot et al. (2007) synthesized luminescent hybrid NPs which consisted of paramagnetic Gd_2O_3 core and polysiloxane shell which had fluorophores and carboxylated PEG covalently attached to the surface. It was shown that the NPs had higher longitudinal proton relaxivities as compared to positive CAs like Gd-DOTA and are well suited for MR and fluorescence imaging.

Keeping the dual purpose of therapy and imaging in mind, herein, we report synthesis of PEG coated paramagnetic Gd_2O_3 NPs loaded with dox (Gd-PEG-dox) for simultaneous contrast enhancement in MR imaging and drug delivery applications. The Gd_2O_3 nanoscale core was prepared in water-in-oil microemulsion of water/AOT/hexane which was followed by PEG coating and lastly dox loading *via* conjugation on surface. We have chosen PEG as coating material of Gd_2O_3 core because of its biocompatibility (Oyewumi et al., 2004; Zhang et al., 2002), non-toxic nature (Klapshina et al., 2010) and it can be functionalized easily. The ensuing Gd-PEG-dox NPs were subsequently characterized for their size, morphology, composition, surface, crystallinity, magnetic behavior and optical activity. After characterization, *in vitro* biological studies of the NPs were conducted which included their cellular uptake study and cytotoxicity evaluation by SRB cell viability assay on cancerous cells namely A549, PANC-1 and U-87. Such multifunctional NPs are anticipated to be explored profoundly in near future and such efficient systems will alter the way of cancer treatment in future.

2. Experimental section

2.1. Materials

Sodium bis-(2-ethylhexyl)sulfosuccinate (AOT; 96%), hexane, gadolinium nitrate pentahydrate, ethanol and ammonia were purchased from Acrōs Organics (New Jersey, USA), Spectrochem Pvt. Ltd Mumbai (India), Central Drug House(Mumbai, India), Merck (Darmstadt, Germany), and Rankem (Delhi, India), respectively. Doxorubicin hydrochloride and deuterium oxide (D_2O) were procured from Alfa Aesar (Heysham, England). Polyethylene glycol (6000 KD), citric acid anhydrous and sodium phosphate dibasic anhydrous (Na_2HPO_4) were procured from s.d.fine-chem limited, spectrochem pvt. Ltd. (India) and SRL Ltd (India) respectively. Phosphate buffer saline (PBS), Sulforhodamine B (SRB) and Hoechst Stain were incurred from Sigma Aldrich (St Louis, MO). Tris base and paraformaldehyde were purchased from Merck. The lung carcinoma cell line (A-549), pancreatic cancer cell line (PANC-1) and U-87 cell line were procured from National Centre for Cell Sciences, Pune India. All chemicals were used as such without any further purification.

2.2. Synthesis of PEGylated Gd_2O_3 NPs loaded with dox (Gd-PEG-dox NPs).

For the synthesis of Gd-PEG-dox NPs, we prepared microemulsion 'A' by adding 300 μL of aqueous gadolinium nitrate ($Gd(NO_3)_3 \cdot 5H_2O$, 0.1 M) solution to 15 mL of 0.1 M AOT in hexane. Microemulsion 'B' was obtained in the similar manner in which 300 μL of 1 M NH_3 was added to 15 mL of 0.1 M AOT in hexane. Both the microemulsons were stirred till they were optically clear. Molar ratio of water to surfactant (W_o) was maintained at 11.11 in both the microemulsions (Lo'pez-Quintela et al., 2004; Sharma et al., 2003). Now microemulsion 'B' was added dropwise to microemulsion 'A' with constant stirring at room temperature. After complete transfer, the resultant solution was stirred for another 24 h which resulted in the formation of Gd_2O_3 NPs. The ensuing particles were extracted and washed several times with hexane and alcohol. The washed Gd_2O_3 NPs were dispersed in 5 mL double-distilled water and 200 μL of 1% PEG solution was added to it. The solution was further stirred for another 24 h. The PEG coated Gd_2O_3 particles (Gd-PEG) were separated and redispersed in 5 mL DDW to which 40 μL of 0.1% dox solution was added. The solution was stirred for another 48 h which resulted in the formation of dox loaded NPs (Gd-PEG-dox NPs). The resulting Gd-PEG-dox NPs were separated and finally dispersed in 1 mL of DDW for characterization and further use.

2.3. Characterization

2.3.1. Ultraviolet–vis (UV–vis) spectroscopy

UV–vis spectra of all the samples were recorded on Shimadzu-1601 UV–vis spectrophotometer in the range of 190 nm to 1100 nm.

2.3.2. Transmission electron microscopy (TEM)

TEM images of the prepared NPs were taken using TECNAI G^2–30 U TWIN instrument (FEI, Eindhoven, Netherlands) which operates at acceleration voltage of 300 KV. A drop of dilute aqueous NP dispersion was deposited on copper grid. TEM images were taken after completely drying the grid at room temperature.

2.3.3. Dynamic light scattering (DLS)

Hydrodynamic diameter of NPs was determined by DLS analysis performed on Zetasizer Nano ZS-90 analyzer from Malvern having He-Ne laser (λ = 633 nm, power 4 mV) as light source and recorded at a backscattering angle of 90^0 using an avalanche photodiode detector. The hydrodynamic diameter (d) of NPs was determined from diffusion of the NPs using Stokes–Einstein equation.

2.3.4. Fourier transform infrared (FT-IR) spectroscopy

FT-IR spectrums of the samples were taken using RXIFT IR (Perkin Elmer) instrument which has nichrome wire coated with alloy as source. Dry samples were dispersed into KBr powder and pressed into a pellet. The pellets were scanned from 400 cm^{-1} to 4000 cm^{-1} at room temperature.