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Research paper

PEG functionalized luminescent lipid particles for cellular imaging

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ABSTRACT

We report here the synthesis, characterization and cellular uptake of luminescent micelle-like particles with phospholipid core and non-ionic PEG based surfactant polysorbate 80 shell. The adsorption of polysorbate 80 at the interface of lipid containing microemulsion droplets and its solidification upon removal of solvent leads to anchoring of PEG chain to the lipid particles. Hydrophobic partitioning of luminescent molecules, sodium 3-hydroxynaphthalene-2-carboxylic acid to the phospholipid core offers additional functionality to these particles. Thus, the cooperative assembly of lipid, non-ionic amphiphile and organic luminescent probe leads to the formation of multifunctional biocompatible particles which are useful for simultaneous imaging and therapy.

1. Introduction

Surface functionalized particles are gaining increasing attention in biomedical research owing to their potential applications in areas such as cellular imaging and drug delivery [1,2]. Delivery of active ingredients to the right place at right amount is important for effective therapy and reduced side effects. Interfacial modification of materials with specific functional groups offers attractive ways of targeting active ingredients to the site of interest and hence reduces drug toxicity [3]. Polyethylene glycol (PEG) grafted liposomal carriers have been employed for the passive targeting of anticancer drug, doxorubicin [4]. Some of the shortcomings of liposomal carriers include limited stability in biological fluids, electrolytes, effect of pH, temperature etc. Recently, efforts have been made for the development of alternate nanoparticulate drug delivery carriers formed by solid lipids or lecithins [5–8]. The use of solid lipids instead of liquid lipids is advantageous for the controlled release of drug molecules as drug mobility in a solid lipid is considerably slow. The physical and chemical stability of solid lipid particles in biological fluids are superior to those of liposomes, and at the same time maintains excellent biocompatibility offered by lipids. These particles have a tendency to incorporate both hydrophilic as well as hydrophobic drugs.

Recently, lecithin nanoparticles have been prepared and explored for intravenous injection of docetaxel, a potent anticancer drug [8]. Positively charged lecithin particles were also prepared by using cationic surfactant as a stabilizer for oil in water emulsions [9]. These particles showed excellent biocompatibility and are amenable for binding of DNA and vaccine delivery. Thus, the engineered lecithin or solid lipid particles are emerging as new biocompatible materials for drug delivery. Adding luminescent functionality to the drug delivery carrier is important for cellular imaging, identify location of target carriers in the tissue and in-vivo monitoring of biomolecular interactions [10]. Impregnating phospholipids with fluorescent organic molecules could provide multiple functionalities to these nanoparticles such as imaging and therapy [11]. For instance, highly stable luminescent vesicles have been prepared via spontaneous assembly of dimyristoylpho sphatidylcholine and a fluorescent amphiphile [12].

Size selective preparation of biocompatible particles is another important aspect to achieve selective permeation and accumulation of cancer drugs in tumors [13]. Liposomal and polymeric carriers with sizes less than 100 nm are shown to be highly effective for treating permeable tumors [13–15]. In particular, polymeric micelles of diameter 30 nm could effectively penetrate pancreatic tumors which are otherwise poorly permeable to other sizes [14]. With this objective, we explored the possibility of using lipid solubilized microemulsions as a means to produce multifunctional drug carriers. Here, we report the preparation, characterization and cellular internalization of luminescent phospholipid (lecithin) particles which are surface functionalized with the hydrophilic PEG chains of a non-ionic surfactant, polysorbate 80 (Tween-80). The prepared particles are of sub 100 nm size with narrow polydisper-

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sity, resistant to protein adsorption and shows good cellular uptake.

2. Materials and methods

2.1. Materials

Tween-80 (PEG-80 sorbitan monooleate) was obtained from E. Merck, Mumbai (India). Isopropyl myristate (IPM) was procured from Sisco Research Laboratories, Mumbai. Lipid (phospholipon®90G) was obtained from Lipoid, Germany. Sodium 3-hydroxy naphthalene 2-carboxylate (SHNC) was purchased from S.D. Fine chemicals, Mumbai (India). Bovine serum albumin (BSA) was procured from Sigma-Aldrich, USA. Dulbecco's Modified Eagle Medium (DMEM) and fetal calf serum (FCS) were procured from Invitrogen, USA and Himedia Laboratories, India, respectively. All the chemicals were used as received. Milli Q water from a Millipore system was used to prepare aqueous solutions. Small angle neutron scattering (SANS) samples were prepared in D_2O medium.

2.2. Preparation of lipid particles (LPs) and luminescent lipid particles (LLPs)

Lipid particles were prepared by vacuum evaporation of lipid solubilized microemulsion (LSM) as reported elsewhere [16]. LSM (oil-in-water) was prepared by solubilizing equal weights of FDA approved oil, isopropyl myristate (IPM) and a biocompatible lipid (Phospholipon 90G) in a Tween-80solution. First, equal weights (1.13 wt.%) of phospholipon 90G and IPM were mixed together by vortexing and then, LSM was obtained by microemulsifying the above mixture in 1% aqueous Tween-80 solution (5 ml), such that the lipid to surfactant mass ratio is 0.075. During solvent evaporation of LSM under vacuum, the phospholipid is encapsulated with a protective shell of surfactants to form lipid particles (LPs).

To add luminescent functionality to these LPs, SHNC was incorporated into the lipid via pH induced partitioning. Briefly, 3.5 mM of SHNC was first added to 5 ml of LSM having 1% of Tween-80. Then, this microemulsion was evaporated at pH 1 to obtain the luminescent lipid particles (LLPs). These LLPs were redispersed in 2 ml of water and pH was adjusted to ~5 by dialysis against milli Q water for further use.

2.3. Characterizations

DLS measurements were performed using a Malvern 4800 Autosizer employing a 7132 digital correlator. The light source was a He-Ne laser operated at 632.8 nm with maximum power output of 15 mW. The correlation functions were analyzed by the method of CONTIN analysis. The measurements were carried at a constant angle of 130°. Small angle neutron scattering (SANS) study of LSM was carried out using SANS diffractometer at Dhruva Reactor, Bhabha Atomic Research Centre, Mumbai, India. The diffractometer makes use of a beryllium oxide filtered beam of mean wavelength of 5.2 Å. The angular distribution of the scattered neutrons was recorded using a one-dimensional position-sensitive detector (PSD). The PSD allows simultaneous recording of data over the full Q-range. The samples were held in a quartz sample holder of 0.5 cm thickness. SANS study of LPs was performed using the D11 instrument, Institut Laue–Langevin, Grenoble, France. The mean wavelength of the incident neutron beam was 6 Å with a wavelength resolution of approximately 9%. The scattered neutrons from samples were detected using a 2-dimensional 96 cm × 96 cm ^{3}He detector with a pixel size of 7.5 mm × 7.5 mm. All the measurements were carried out at room temperature and samples were prepared in D_2O. The measured SANS data were corrected and normalized to a cross sectional unit, using standard procedures. The SANS data have been analyzed by comparing the experimental scattering data to appropriate models. Throughout the data analysis corrections were also made for instrumental smearing. The modeled scattering profiles were smeared by the appropriate resolution function to compare with the measured data. The fitted parameters in the analysis were optimized by means of nonlinear least-square fitting program. Zeta potential measurements were made with a Zetasizer nanoseries, Malvern instruments, UK by phase analysis light scattering with applied field strength of 2.5×10^3 V/m. The light source was a He-Ne laser operated at 632.8 nm operating at 4.0 mW. The experiment was performed using a quartz cuvette with 10 mm light pathway at room temperature. Differential scanning calorimetry (DSC) measurements were carried out using a Mettler Toledo (Switzerland) DSC 822, with an empty aluminum pan as a reference. Temperature and enthalpy calibration of the instrument were done, using cyclohexane and indium. The samples (25–30 mg) were hermetically sealed in an aluminum pan. The thermal measurements were carried out in the temperature range of 45–65 °C with a constant heating rate of 5 °C/min. to measure the transition temperature (T_m). The fluorescence measurements of the LLPs were carried out by using a Hitachi F4500 model Fluorescence spectrophotometer. The fluorescence emission was recorded in the range of 380–700 nm with an excitation wave length of 364 nm.

Cellular imaging capability of LLPs was studied by confocal microscopy using mouse skin fibrosarcoma (WEHI-164) cell line (obtained from National Centre for Cell Sciences, Pune, India). Cells were cultured in DMEM supplemented with 10% FCS and antibiotics (100 U ml^{-1} penicillin and 100 μg ml^{-1} streptomycin) in a humidified atmosphere of 5% CO_2 at 37 °C. For confocal imaging, the desired number of cells were seeded in complete DMEM and incubated at culture conditions for overnight, followed by treatment with LLPs. Briefly, the cells (0.5×10^6) were seeded on glass cover slips and cultured overnight. The cells were then treated with LLPs (250 μl) for 3 h at culture conditions, followed by washing with PBS in order to remove the loosely bound particles from cell surface. The cells were fixed with 4% paraformaldehyde at 4 °C for 30 min, followed by washing with PBS. Cells treated with LLPs were imaged using confocal microscope (LS510 Meta, Carl Ziess, Germany). The excitation source used was an UV laser (364 nm) and emission window was set at LP 505 nm.

In order to investigate the stability of LLPs in cellular mimicking environment, we have investigated the release of SHNC from LLPs under a reservoir-sink condition. The LLPs (2 ml) were first put into a dialysis bag and then it was dialyzed against 100 ml of 0.1 M phosphate buffered saline (PBS, pH 7.3) under continuous stirring at 37 °C to mimic the cellular environment. 1 ml of the external medium was withdrawn at fixed interval of time and replaced with fresh PBS to maintain the sink conditions. The amount of SHNC release was determined by measuring the absorbance at a wavelength of 235 nm using JASCO V-650, UV-visible spectrophotometer against the standard plot prepared under similar condition.

3. Results and discussion

Solid lipid particles constitute an important class of biocompatible materials for drug delivery and imaging applications. Lipid particles with low melting points are very often prepared by high temperature emulsification and solidification process. In this process, a microemulsion/emulsion of the molten lipid is prepared in water at high temperature and is cooled to obtain solid lipid particles stabilized by surfactants. In the present study in order to lower the emulsification temperature, the lipid is solubilized in a limited