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Radiosensitizing Silica Nanoparticles encapsulating Docetaxel for Treatment of Prostate Cancer

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Summary

The applications of nanoparticles in oncology include enhanced drug delivery, efficient tumor targeting, treatment monitoring and diagnostics. The ‘theranostic properties’ associated with nanoparticles have shown enhanced delivery of chemotherapeutic drugs with superior imaging capabilities and minimal toxicities. In conventional chemotherapy, only a fraction of the administered drug reaches the tumor site or cancer cells. For successful translation of these formulations, it is imperative to evaluate the design and properties of these nanoparticles. Here, we describe the design of ultra-small silica nanoparticles to encapsulate a radiosensitizing drug for combined chemoradiation therapy. The small size of nanoparticles allows for better dispersion and uptake of the drug within the highly vascularized tumor tissue. Silica nanoparticles are synthesized using an oil-in-water microemulsion method. The microemulsion method provides a robust synthetic route in which the inner hydrophobic core is used to encapsulate chemotherapy drug, docetaxel while the outer hydrophilic region provides dispersibility of the synthesized nanoparticles in an aqueous environment. Docetaxel is commonly used for treatment-resistant or metastatic prostate cancer, and is known to have radiosensitizing properties. Here, we describe a systematic approach for synthesizing these theranostic nanoparticles and preparing them for use in prostate cancer.

Keywords

Silica nanoparticles; docetaxel; drug delivery; microemulsions; prostate cancer; bottom-up synthesis; radiosensitization

1. Introduction

While chemotherapy is frequently used as a first line of treatment, adverse toxicities and low bioavailability associated with the nonspecific systemic drugs often limit treatment timing and dosing in patients (1). To overcome these toxicities and improve circulation time, many researchers are using nanoparticles as a means for delivery(2, 3). Since radiation therapy is used in more than 50% of the cancer patients, the development of nanoparticles formulations

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which can combine the two monotherapies, chemo- and radiation, will not only boost the therapeutic efficacy but also reduce the toxicities associated with monotherapies(4). Currently, docetaxel is given systemically as Taxotere to patients via intravenous infusion once every 2-3 weeks to allow for recovery. Docetaxel is non-cell specific and acts on the microtubules of cells, preventing depolymerization. This traps the cell in G2/M phase, which also results in increased radiosensitizing properties(5). As a result, co-delivery of docetaxel with radiation treatment can prove to be an effective dual treatment in the case of certain cancers. It is believed that this radiosensitization effect can lead to the use of lower doses to achieve synergistic therapeutic effects, while reducing the overall toxicity experienced during the treatment. Here, we propose the use of PEGylated (i.e. polyethylene glycol-functionalized) ultra-small silica nanoparticles to encapsulate docetaxel for combined chemoradiation therapy.

We describe a systematic protocol for synthesis of silica nanoparticles to encapsulate the hydrophobic drug in the core of nanoparticles. Silica nanoparticles can be formulated at various sizes, ranging from 10-250 nm in diameter(6)(7). The core is non-polar and can trap a variety of hydrophobic chemotherapeutics, small molecules, or dyes. The surface of the nanoparticles is modified with PEG and/or targeting ligands for long term circulation and targeted cellular uptake. The PEGylation of nanoparticles minimizes the opsonization process and allows for increased systemic circulation(8). The functional groups on the surface of the nanoparticles can be used for conjugating targeting molecules like peptides, antibodies or aptamers(9). Below, we describe the formulation of docetaxel-loaded ultra-small silica nanoparticles for radiosensitization in prostate cancer. We conjugate a near-infrared fluorophore, Cyanine 7.5, to the silica nanoparticles to track the nanoparticle *in vitro* and *in vivo*. First we describe the fabrication and purification of the nanoparticles, followed by characterization and quantification of drug loaded silica nanoparticles using various instrumentation techniques.

2. Materials

1. Aerosol-OT (AOT) or Sodium Bis (2-Ethylhexyl) Sulfosuccinate
2. Hydrophobic drug or dye (see note 1): Chemotherapeutic drug, docetaxel or fluorophore, Cyanine 7.5 N-hydroxysuccinimide (NHS) ester
3. Triethylamine
4. Silane precursors Vinyltriethoxysilane (VTES) and Aminopropyl triethoxysilane (APTES)
5. 2-[METHOXY(POLYETHYLENEOXY)6-9PROPYL]TRIMETHOXYSILANE, tech-90
6. HPLC grade solvents
7. Dichloromethane
8. Sterile spin filter tubes (100kD MWCO) (Pall Corporation)
9. Cellulose Membrane 14kD cutoff pore size

3. Methods

Carry out all procedures and keep all reagents at room temperature (25°C) unless otherwise noted.

3.1 Modified Cy-7.5

1. Degas 1 mL dimethyl sulfoxide (DMSO) using argon to remove oxygen (~1 minute) and add 1 mL of DMSO to 5 mg of Cy7.5 NHS ester to make 5 mg/mL concentration.
2. Add 20 μL of APTES to the dye solution (the color should now have a slight yellow tint) followed by addition of 20 μL of trimethylamine.
3. Add a small stir bar for stirring the reaction mixture gently and put a septum on the reaction vial. Purge the reaction mixture with argon gas overnight.
4. The final product, Cy 7.5 conjugated to APTES via the amine groups of APTES can be used without further purification. When added to the nanoparticles, the conjugate will polymerize along with VTES to form fluorescent nanoparticles. (see note 2)

3.2 Synthesis of Silica Nanoparticles

1. Prepare 2.2% (w/v) AOT solution in deionized water (see note 3). Weigh 220 mg of the surfactant AOT in a scintillation vial and add 10 ml of deionized water. To ensure accuracy in measurement and quick solvation, break AOT wax into small pieces before weighing.
2. Place a stir-bar in scintillation vial and stir on a magnetic stirrer at approximately 1200 rpm (see note 4). The solution will be become cloudy in appearance as the AOT dissolves.
3. After 15-20 minutes of stirring, add 300 μL of 1-Butanol (see note 4). The solution should become clear upon addition.
4. After 5 minutes, add 75 μL of Docetaxel (10 mg/mL in DMSO) and 50 μL modified Cy7.5 (5 mg/mL in DMSO) (see note 5). Reaction mixture should be protected from light to avoid photo-bleaching using aluminum foil.
5. After 5 min of stirring, add 100 μL of silane precursor, vinyltriethoxysilane (VTES).
6. Stir the reaction mixture for 40 minutes and thereafter add 10 μL of ammonium hydroxide (see note 6).
7. For PEGylation of the nanoparticles, add 10 μL 2-[methoxy(polyethyleneoxy)6-9propyl]trimethoxysilane 10 mins after the addition of ammonium hydroxide.
8. Stir the solution overnight, or for at least 12 hours to allow nanoparticle formation and Ostwald ripening. (see note 7).

9. Remove stir bar and store the nanoparticles at 4 °C if not immediately dialyzed.

3.3 Dialysis

The purpose of dialysis is to remove the surfactant, excess of reactants and any unencapsulated drug/fluorophore molecules from the nanoparticles solution.

1. Cut the dialysis tubing to approximately 5 inches and prepare the tubing as per manufacturer's recommended protocols.
2. Pipette as prepared nanoparticles solution into dialysis tubing while securing the end of the tubes with the dialysis clips.
3. Put the dialysis tubing with sample in a container with 4 L of deionized water.
4. Stir water at roughly 100 rpm. Caution: ensure that the dialysis tubing is stirring slowly and if required adjust speed accordingly.
5. Change deionized water (DI) every 30 minutes for 2 hours, then again after 8 hours. Change again at 24 and 36 hours (see note 8).
6. Remove the sample after 48 hours of dialysis against DI water (see note 9).

3.4 Preparation for high performance liquid chromatography (HPLC) Analysis

Sonicate dialyzed nanoparticle solution for 1 minute to allow for even dispersion of particles.

1. Pipette out 250 μL of the nanoparticles solution into a micro-centrifuge tube. All samples must be taken in triplicate to ensure accuracy.
2. Add 250 μL of dichloromethane and 250 μL HPLC grade methanol to the tube.
3. Sonicate for 45 minutes.
4. Leave micro-centrifuge tubes in fridge at 4°C overnight.
5. Vortex for 10 seconds, then evaporate off the remaining dichloromethane with air.
6. Remove solution and transfer to spin filter centrifuge tubes. Centrifuge the tubes for 30 mins at 16128 rcf using a 100 mm rotor..
7. Remove filtrate from tubes and transfer to HPLC vials.
8. Add 250 μL of mobile phase (65:35 Methanol in HPLC Water) and vortex briefly.
9. Run HPLC via standard protocol (see note 10).

3.5 Dynamic Light Scattering (DLS)

1. Sonicate dialyzed nanoparticle solution for 1 minute to allow for even dispersion of particles.
2. Place 1 mL of nanoparticles to clear 2 mL cuvette. Dilute if necessary.

3. Using 90plus zeta sizer (Brookhaven) instrument perform measurements to determine hydrodynamic diameter of the nanoparticles. (see note 11)

3.6 Transmission Electron Microscopy

1. Sonicate dialyzed nanoparticle solution for 1 minute to allow for even dispersion of particles.
2. The TEM grids were prepared by drop casting the sample (20 μL) dispersion onto an amorphous carbon coated 300 mesh copper grid.
3. Place copper grid on a filter paper to absorb the excess solvent and allow to dry before loading into chamber for TEM analysis using JEOL model JEM- 100CX microscope at an acceleration voltage of 80 kV (see note 12).

4. Notes

1. We have used a hydrophobic drug docetaxel to encapsulate inside silica nanoparticles. Similar synthetic strategies can be adopted to encapsulate other hydrophobic drugs.
2. The conjugation of Cy 7.5 to nanoparticles gives a purple color solution which can be due to the aggregation of dye molecules in the silica matrix whereas when the same fluorophore Cy 7.5 is encapsulated gives a typical green color.
3. The surfactant AOT along with co-surfactant 1-butanol and DMSO when mixed together will create an oil-in-water microemulsion system. A surfactant like AOT highly reduces the interfacial tension at the water/oil interface. In this formulation, water is the bulk phase of the microemulsion, whereas DMSO forms the oil phase in the form nanosize droplets/reactors. The hydrophobic drugs/fluorophores will reside inside these droplets and exchange between the two microemulsion droplets will result in hydrolysis and polymerization of silane precursors (in basic pH) inside these nanosize droplets. This leads to entrapment of hydrophobic molecules in the silica matrix. Reverse micellar approach using a water-in-oil microemulsion system can also be used to synthesize silica nanoparticles. In such an instance, the polar groups are directed into the aqueous core, formulating nanoparticles which will entrap polar drugs/fluorophores(10, 11).
4. The size of the particles can be modulated by many variables including stirring speed of the reaction mixture. The amount of butanol (the oil phase) is a major factor in modulating the particle size. Addition of more butanol will increase the size of the nanoparticles. e.g. for synthesizing 200 nm silica, add 700 μL butanol to the AOT mixture, and for 30 nm silica add 300 μL butanol.
5. Docetaxel and Cy7.5 can be substituted by other hydrophobic drugs or dye. The quantity was determined by our loading efficiency of the drug. The HPLC protocol and mobile phase must be selected accordingly if the drug of choice is not Docetaxel.

6. Addition of ammonium hydroxide results in hydrolysis and polycondensation of vinyltriethoxysilane to vinylpolysiloxane inside the micellar core which leads to formation of small size nanoparticles.

7. Drug/dye encapsulated nanoparticles prepared as above will maintain a translucent appearance and be approximately 30 nm in size. The size of the particles can be checked with dynamic light scattering (DLS) and further confirmed with transmission electron microscopy (30 nm).

8. Dialysis must be done for 48 hours with at least six water changes to ensure removal of excess reagents especially AOT which have known toxicities in both *in vitro* and *in vivo* systems.

9. Store nanoparticles in fridge at 4 °C. It is best to use a new batch of nanoparticles for experiments to limit the amount of drug/dye released from the particles.

10. Dispose of all waste for HPLC and after preparation according to hazardous waste management protocols.

11. Dynamic light scattering (DLS) measurements were performed by using 90Plus zeta sizer (Brookhaven Inc, NY). Zeta potential measurements can be acquired with the same instrument.

12. Transmission Electron Microscopy (TEM) images were obtained using a JEOL model JEM- 100CX microscope at an acceleration voltage of 80 kV.

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