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Fabrication and characterization of dual acting oleyl chitosan functionalised iron oxide/gold hybrid nanoparticles for MRI and CT imaging

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ABSTRACT

Bionanocomposites fabricated using metal nanoparticles serve a wide range of biomedical applications viz., site targeted drug delivery, imaging etc. Theranostics emerge as an important field of science, which focuses on the use of single entity for both disease diagnosis and treatment. The present work aimed at designing a multifunctional nanocomposite comprising of iron/gold hybrid nanoparticles, coated with oleyl chitosan and conjugated with methotrexate. The HR-TEM images revealed the spherical nature of the composite, while it's nontoxic and biocompatible property was proved by the MTT assay in NIH 3T3 cells and hemolysis assay. Though the VSM results exhibited the magnetic property, the MRI phantom images and X-ray contrast images demonstrated the potential of the composite to be used as contrast agent. Thus the prepared nanocomposite possess good cytocompatibility, magnetic property and also high X-ray attenuation, wherein it could serve as a novel platform for both MRI and CT diagnosis, as well as drug conjugation could aid in targeted drug delivery.

1. Introduction

Nanocomposites comprising of different components, have stirred great interest in the field of nanomedicine because of their diverse physicochemical properties. Nanoparticles are widely used for diagnostics and therapeutics owing to their unique capabilities and few side-effects. Due to this, recently nanoparticles are highly exploited for their theranostic potential as well, which aims at simultaneous detection and treatment of the disease using a single agent [1]. Among nanoparticles, superparamagnetic iron oxide nanoparticles (IONPs) offer high potential for several biomedical applications, such as magnetic resonance imaging (MRI), hyperthermia treatment, tissue engineering and as drug delivery systems, due to their biocompatibility, surface architecture and easy conjugation with targeting ligands [2,3].

Magnetic resonance imaging (MRI) is a potent, non-invasive, three dimensional technique used in disease diagnosis. It provides high spatial resolution with three-dimensional anatomic details devoid of instigating any harmful side effects in patients. The conventionally used gadolinium based contrast agents in MRI are reported to be toxic in nature and induces nephrogenic systemic fibrosis in patients with renal diseases [4]. Superparamagnetic iron oxide nanoparticles are used as T2 contrast agents in MRI owing to their better resolution than gadolinium based contrast agents [5]. However in many instances MRI alone doesn't work as the sole methodology of diagnosis, wherein the need of computed tomography (CT) arises. CT is also yet another diagnostic imaging modality which is non-invasive and exhibits fast scanning speed [6]. Among the noble metals (silver, gold and platinum), gold nanoparticles play a pivotal role in biomedical applications viz., in bioimaging, biosensors, diagnostics etc. The use of gold nanoparticles as a contrast agent for computed tomography (CT) is slowly gaining momentum owing to its remarkable properties including nonreactivity, high X-ray absorption coefficient, low cytotoxicity, tailored surface chemistry, excellent biocompatibility, and unique surface plasmon resonance [7]. Hence development of iron/gold hybrid nanoparticles could serve as dual modality contrast agents which could be used for both MRI and CT diagnosis [8,9].

Though the biomedical applications of IONPs are promising, their successful application depends on the surface modification of IONPs, because bare IONPs have poor colloidal stability and they are usually opsonised and sequestered by the reticuloendothelial system, especially by the Kupffer cells in the liver [10]. Hence, in order to overcome this limitation, stabilizers such as polyethylene glycol, dextran, chitosan etc. are used to enhance the dispersibility of nanoparticles in aqueous medium. Chitosan is a natural non-toxic biopolymer, derived from the partial deacetylation of chitin and it consists of repeating units of

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glucosamine and *N*-acetyl glucosamine. Its unique characteristics such as biocompatibility, biodegradability, rigid linear molecular structure etc. make it as an ideal candidate for medical applications [11]. Though chitosan is soluble in aqueous solution of acids, it doesn't have amphiphilic property. Hence surface modification of chitosan with long chain fatty acids viz., oleic acid, lineoleic acid, palmitic acid etc. can introduce hydrophobic groups into chitosan and form amphiphilic chitosan polymers. Chitosan conjugated with fatty acid moiety aids in the formation of nanomicelles in aqueous solution, with a hydrophobic core-hydrophilic shell structure. The hydrophobic core structure provides an amenable environment to load the drugs, while the shell structure offers colloidal stability of the particles [12,13].

There are several reports regarding the synthesis of iron oxide nanoparticles using inorganic sources, but this study reports the use of goat blood as the starting material for the synthesis of IONPs. The synthesised IONPs, if coupled with gold nanoparticles to form hybrid constructs, could be used for both MRI and CT diagnosis. In order to enhance the stability and dispersibility of the hybrid nanoparticles in aqueous medium, it will be encapsulated using oleyl chitosan. This functionalised hybrid nanoparticles will be conjugated with the anti-cancer drug methotrexate (MTX). Since, MTX is a folic acid analogue; it binds with the folate receptors expressed on tumor cells and inhibits dihydrofolate reductase, an enzyme critical for cell survival and division. Hence MTX displays dual function as targeting ligand and as a therapeutic agent [14].

Thus the objective of the current study is to synthesize a multifunctional nanocomposite comprising of iron/gold nanohybrid particles coated with oleyl chitosan and conjugated with methotrexate. This nanocomposite will be evaluated for its physico-chemical properties and biocompatibility in vitro. In addition the suitability of the nanocomposite for use in MRI and CT as contrast agent will also be determined.

2. Materials and methods

2.1. Materials

Goat blood was hygienically collected from a nearby slaughter house at Chennai. All the chemicals used were of analytical grade and were purchased from Sigma Aldrich Chemicals Pvt. Ltd. (USA) and Himedia, Pvt. Ltd., India. Fetal bovine serum (FBS) was purchased from Gibco. Water used for the experiments were prepared using Milli-Q purification system.

2.2. Synthesis of oleyl-chitosan (OC)

Oleic acid conjugated chitosan (oleyl-chitosan) was synthesised according to Lee et al. [13]. Chitosan (100 mg) was dissolved in 50 mL acetic acid solution (1% v/v), and 8 mg oleic acid was dissolved in 10 mL ethanol. The two solutions were mixed at 80 °C under constant stirring. 27.4 mg of EDC (1-[3-(dimethylamino) propyl]-3-ethylcarbodiimide hydrochloride) was added to this reaction mixture to start the coupling reaction, which was carried out for 6 h at 80 °C under continuous stirring. The final solution was dialyzed against a 10% (v/v) ethanol solution using a dialysis membrane (MWCO: 12 kDa, Sigma) for three days. To remove ethanol from the product, dialysis was performed against distilled water for 24 h. Finally, the dialyzed product was lyophilized.

2.3. Synthesis of iron oxide nanoparticles (IONPs)

IONPs were isolated from goat blood according to Periyathambi et al. [15] with slight modifications. In brief, the collected goat blood was mechanically stirred using a glass rod for 15 min continuously to isolate the fibrin. The defibrinated blood was centrifuged at 10,000 rpm for 20 min and the supernatant (serum portion) was discarded. Red blood cells (RBCs) collected at the bottom of the tube was removed, washed with water for 10 times and stored at 4 °C. The inorganic part, iron oxide was prepared by incinerating the RBCs at 800 °C. The residue (IONPs) obtained was dissolved in 5 N HCl and the undissolved materials settled at the bottom were discarded. This $FeCl_2$ solution was treated with ammonium hydroxide and heated at 200 °C for 2 h. Dark black colored precipitate (IONPs) formed when the pH was increased to 11. This precipitate was stirred vigorously at 60 °C for 30 min under nitrogen atmosphere. The resulting IONPs were washed thoroughly several times with ethanol/water (1:1) using magnet and dried at 100 °C.

2.4. Synthesis of iron oxide/gold hybrid nanoparticles

Iron oxide/gold hybrid nanoparticles were synthesised using DMSA (meso-2,3-dimercaptosuccinic acid) according to Zhao et al. [9]. 40 mg DMSA was added to 40 mL deionized (DI) water and the pH of the solution was adjusted to 3.0–3.5 with HCl. After addition of IONPs suspension, the pH of the solution was again adjusted to 4.0 with HCl and it was allowed to react for 1 h under nitrogen atmosphere with continuous stirring. The resulting DMSA modified IONPs were collected with magnet and washed with DI water several times and it was finally dispersed in DI water prior to further reaction. To 5 mL of DMSA modified IONPs, $HAuCl_4$ solution was added and allowed to react for 30 min under continuous stirring. The color of the solution changed from black to brownish. Samples were harvested with magnet, washed with DI water and dried at 60 °C.

2.5. Synthesis of oleyl-chitosan coated iron oxide/gold hybrid nanoparticles

Iron oxide/gold hybrid nanoparticles were functionalised with oleyl chitosan according to Lee et al. [13] with modifications. 10 mg of oleyl-chitosan was dissolved in 10 mL 0.1 M acetic acid under constant stirring. The solution was sonicated at 100 W for 5 min. Iron oxide/gold hybrid nanoparticles were dispersed in 500 μL of chloroform and this was added to the oleyl-chitosan solution. This mixture was sonicated at 100 W for 10 min. The reaction was allowed to proceed for 12 h at room temperature, in order to evaporate the organic solvent and the pH of the solution was adjusted to 7.0 with 0.1 N NaOH solution. Samples were harvested with magnet, washed with DI water and dried at 60 °C.

2.6. Synthesis of methotrexate conjugated, oleyl-chitosan coated iron oxide/gold hybrid nanoparticles (nanocomposite)

Methotrexate (MTX) was conjugated to the nanoparticles using EDC/NHS (N-hydroxysuccinimide) chemistry. Briefly, 5 mg of MTX in 10 mL of MES (2-(N-morpholino)ethanesulfonic acid) buffer (45 mM, pH 6.5) was prepared. To this 192 mg of EDC (0.1 M) and 115 mg of NHS (0.1 M) was added and the reaction was allowed for continuous stirring for 12 h at room temperature. 10 mL of nanoparticles (2 mg/mL) was added to the above reaction mixture and the reaction was carried out in the dark for 12 h. Then the MTX-conjugated nanoparticles were washed thoroughly with MES buffer using magnet. The product was transferred to the dialysis bag and filtered through a 0.22 μm membrane to remove large aggregates, followed by freeze drying to obtain dried nanoparticles.

2.7. Characterization

2.7.1. Fourier transform infra-red spectroscopy

Nicolet 360 Fourier transform infra-red (FT-IR) spectrophotometer was used to determine the functional groups present in nanoparticles. 2–5 mg of each sample was mixed with 500 mg of KBr and the spectra were obtained within the frequency range of 4000–500 cm^{-1}.