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Neodymium doped hydroxyapatite theranostic nanoplatforms for colon specific drug delivery applications

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ABSTRACT

Theranostic nanoplatforms integrate therapeutic payloads with diagnostic agents, and help monitor therapeutic response. In this regard, stimuli responsive nanoplatforms further favour combinatorial therapeutic approach that can considerably improve efficacy and specificity of treatment. Herein, we present the engineering of a smart theranostic nanoplatform based on neodymium doped hydroxyapatite (HAN). The presence of neodymium endows the HAN nanoplatforms with near-infrared fluorescence capability. These HAN nanoparticles were then subsequently modified with alginic acid (HANA) to confer pH responsiveness to the synthesized nanoplatforms delivering them to the colon after oral administration. These nanoplatforms possessing optimum size, needle shaped morphology and negative zeta potential, are conducive to cellular internalization. On excitation at 410 nm they exhibit near infrared emission at 670 nm unraveling their theranostic capabilities. Cytotoxic effects systematically assessed using MTT and live dead assays reveal excellent viability. Raman microscopic imaging technique used to visualize uptake in HeLa cells demonstrate increased uptake from 4 to 16 h, with growing cluster size and localization in the cytoplasm. Moreover the concomitant presence of alginic acid manifested advantages of augmented loading and pH dependent release profiles of the model drug, 4 acetyl salicylic acid (4ASA). We could thus establish a theranostic system for early tumour detection, targeted tumour therapy and monitoring of colon cancer that can be administered via the oral route.

1. Introduction

The field of nanotechnology holds incredible potential in the diagnosis and treatment of disparate diseases. Diagnosis and therapy can be effectively combined in "theranostic" based systems to interact with cancer cells to combat diseases at the molecular level; and considerably improve specificity and efficacy of cancer treatment. This integration of therapeutic payloads with diagnostic agents, into nanoplatforms is termed the "theranostics" [1,2]. These theranostic nanoplatforms emphasize on diagnosis and delivery of drugs and help monitor therapeutic response and are expected to usher in a new paradigm for treatment regime. In this regard, quantum dots [3], carbon nanotubes [4], iron oxide nanoparticles [5], liposomes [6], gold nanoparticles [7] and silica nanoparticles [8] are considered as exceptional candidates for nanoparticle based theranostics. Despite their promises, these systems are associated with drawbacks that include toxicity of quantum dots, non biodegradable nature of carbon nanotubes and undesirable size of silica nanoplatforms to mention a few [2]. Diverse strategies are being envisaged to address these limitations and to develop more efficient theranostic nanoplatforms.

The last few years have witnessed the use of lanthanide-doped nanoparticles as alternatives to conventional systems for biological imaging applications [9]. Lanthanide based imaging offers excellent characteristics like large Stokes shift, long lifetime, excellent quantum yield, and good stability [10]. In this context, it is worth noting that hydroxyapatite (HA) based nanoparticles are particularly useful as nanoplatforms for effective lanthanide doping. HA, the dominant component present in natural bone mineral, possess

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exemplary properties like facile functionalisation, biocompatibility, bioactivity and efficient biodegradability in biological milieu [11,12]. More specifically, HA nanoparticles bequeathed with a strong tendency for ionic substitution readily replace their calcium ions leading to substitution with variant lanthanide elements [13]. The luminous intensity of these doped HA nanoparticles depends on the crystallinity of the host material and the concentration of the doping agent added. Several research groups have reported luminous lanthanide/HA compounds incorporated with payloads like cisplatin [14], nile red [15] and ceramide [16]. Europium doped apatite displayed stable red luminescence as an effective biological probe [17]. HA doped with gadolinium and europium demonstrated significant fluorescence [18] and provide simultaneous therapeutics and fluorescence imaging guidance. In this context, fluorescence imaging guidance in the near-infrared (NIR) region is a unique strategy for visualizing morphological details in tissue. This is ascribed to the innate low absorption of tissues in the NIR region. NIR fluorescence imaging permits deeper tissue penetration with high spatial and temporal resolution, lesser auto fluorescence and high sensitivity [19]. Emerging strategies like up conversion luminescence of rare earth materials address these benefits and are expected to be more viable as luminescent NIR fluorescent imaging moieties [20].

Stimuli responsive nanoparticles are yet another fascinating concept, which has evolved to include combinatorial therapeutic approach for sustained delivery and co-delivery of drugs. Nanoparticles equipped with different functionalities can respond to different stimuli and the most commonly encountered are pH, temperature and ionic strength [21]. In this work we engineered biocompatible alginic acid modified HA nanoplatforms to respond to environmental changes in pH. Alginic acid, an anionic polysaccharide is a linear block copolymer of α-L-guluronate and β-D-mannuronate connected by 1,4-glycoside linkage [22]. A variety of alginic acid based products in the form of beads, scaffolds, microcapsules and nanocapsules have been investigated as cell and drug carriers [23–25]. Moreover alginic acid when used in conjunction with ceramic like materials, exhibit superior properties such as enhanced biomineralisation capability, higher stiffness and increase in surface adsorption of proteins coupled with lower degradation characteristics owing to the synergistic effects of ceramic and polymer [22]. These systems can overcome drawbacks like burst release and uncontrolled swelling, and provide better controlled drug release systems as evidenced in chitosan grafted poly acrylic acid/sodium alginate beads, employed for the sustained release of diclofenac [26].

We have previously demonstrated a facile co-precipitation method for the preparation of multifunctional neodymium-doped HA–cyclodextrin nanoparticle complexes that demonstrate preferential affinity for albumin adsorption [27]. In this study we explore the possibility of preparing stimuli responsive near infrared luminescent HA nanoplatforms primarily employing neodymium as the luminescent moiety. The presence of neodymium endows the nanoplatforms with near-infrared fluorescence ability. These nanoplatforms were then subsequently functionalized with alginic acid using APTS and EDC mediated chemistry to confer pH responsiveness to the synthesized systems delivering the model drug 4ASA to the colon after oral administration. These nanoplatforms thus facilitate simultaneous imaging of colon cancer cells and stimuli responsive drug delivery.

2. Materials and methods

The chemicals calcium chloride, disodium hydrogen phosphate, trisodium citrate, aminopropyltriethoxysilane (APTS), 1-ethyl-3-(-3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), alginic acid (A7003; Formula wt: 176.2 g/mol) and sodium bisulphate were from Sigma (Sigma, Bangalore, India). neodymium carbonate was obtained from Merck (Bangalore, India) and converted to neodymium chloride by dissolving with hydrochloric acid followed by evaporation of the solvent. Sodium dihydrogen phosphate and disodium hydrogen phosphate obtained from Merck chemicals were utilized for buffer preparations (pH 3, 5, 7.4, 8 and 9). 4-amino salicylic acid USP (4ASA) was obtained as a gift from Biochemical & Synthetic Products Ltd., Hyderabad, India.

3. Methods

In a typical process, 1.8 g of calcium chloride and 0.5 g of the as prepared neodymium chloride was dissolved in 500 ml water. Tri sodium citrate was then added to adjust the pH of this solution to around 8. Then 10 g of disodium hydrogen phosphate was dissolved in 500 ml of water. Subsequently the phosphate solution was added drop wise to the former solution with continuous stirring for 24 h. The milky suspension was filtered, washed several times with water, decanted and lyophilized to obtain a fine powder. The as synthesized neodymium doped HA nanoparticles has herein after been abbreviated as HAN in the test. The modification of the HAN nanoparticles by alginic acid was facilitated by adapting APTS mediated functionalisation and EDC chemistry. 250 mg of HAN was uniformly dispersed in 200 ml water/ethanol mixtures to give a homogenous solution. Subsequently, amino group was introduced on its surface by mixing 10 ml of APTS and 5 ml of water under continuous stirring overnight. These HAN-APTS nanoparticles were then filtered, dried and finally coupled with alginic acid utilizing EDC technique to obtain the HANA nanoplatforms.

Preliminary studies were undertaken to select the amount of alginic acid required for effective coupling with the HAN nanoparticles. We selected the mass based on the size and morphology of the engineered HANA nanoplatforms. Particle size and shape affect cellular internalization and biodistribution of nanoparticles. We selected that particular concentration of alginic acid which when coupled with HAN nanoparticles yielded needle shaped nanoplatforms with average particle size of 80–100 nm. For the optimized composition, 125 mg of alginic acid was initially dissolved in water. To this, EDC (100 mg) was added and the reaction was allowed to proceed overnight to activate the COOH groups. 250 mg of HAN-APTS nanoparticles was later added to the above solution and stirred for 14 h. The final product obtained was washed, filtered and dried and coded as HANA nanoplatforms for further characterization studies.

3.1. Characterizations

Fourier transform infrared (FTIR) spectra were obtained on a Jasco, FT/IR-4200 spectrophotometer to determine the chemical composition of the nanoplatforms. The samples were placed in between KBr pellets and spectra obtained in the region 400–4000 cm^{-1}.Phase analysis of the nanoplatforms was determined by X-ray diffraction (XRD) pattern recorded on a Bruker AXS, X-ray diffractometer equipped with CuKα radiation with tube voltage 40 kV and 35 mA of tube current. Similarly, Scherrer formula and least square fit method were utilized to evaluate average crystalline size and cell parameters respectively. The elemental composition of the nanoplatforms was determined by energy dispersive X-ray analysis (EDAX) using environmental scanning electron microscopy (FEI, Quanta 200, USA). Thermal behaviour of the nanoplatforms was determined by differential scanning colorimetry (Q20, TA Instruments) using a sample mass of 5 mg at a heating rate of 10 °C under a nitrogen stream. Samples were sealed in a platinum pan for analysis with an empty pan as ref-