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Iron nanoparticles from animal blood for cellular imaging and targeted delivery for cancer treatment

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ABSTRACT

Background: Iron nanoparticles (INPs) are usually prepared from inorganic sources, but we have prepared it from goat blood using incineration method. These INPs are then coated with chitosan (C) and coupled with folic acid (F) to form bionanocomposite for folate receptors.
Methods: The bionanocomposite was characterized for its physicochemical properties and cancer cell targeting studies using Fourier transform infrared spectroscopy, transmission electron microscopy, Zeta potential analysis, scanning electron microscopy–energy dispersive X-ray spectroscopy and magnetic resonance imaging analyses.
Results: The results have shown that the particle size of the INP-CF was found to be 80–300 nm and confirmed the presence of chitosan and folic acid in the bionanocomposite. Cancer and normal mouse embryonic cell line study confirmed the internalization of INP-CF and this phenomenon was also supported by physicochemical studies.
Conclusion: Thus, nanobiocomposite prepared using natural sources as a raw material will be beneficial compared to commercially available synthetic sources and can be used as receptor targeting agent for cancer treatment. This nanobiocomposite when coupled with substances such as monoclonal antibodies might act as a theranostic nanoagent for cancer therapy in the years to come.
General significance: The prepared novel nanobiocomposite containing INPs isolated from natural source may be used as multifunctional agent due its paramagnetic property apart from its drug delivery effect.

1. Introduction

Nowadays, nanoparticles are being extensively used as carriers for drugs, proteins, vaccines and genes due to their smaller size when compared to the micrometer-sized mammalian cells [1]. Cancer nanotechnology is an interdisciplinary field which provides a wide range of potential application such as molecular imaging, molecular diagnosis and targeted therapy [2–5]. Among the commercially available nanoparticles, magnetic nanoparticle plays a versatile role in the field of oncology for both diagnosis and treatment of cancer. Apart from this, it acts as a theranostic agent to solve the complexity of oncology related disorders [6]. The main focuses of these applications are to destroy selectively disseminated tumor cells while sparing the normal tissues [7]. The knowledge of cellular interaction of nanoparticles with normal and cancer cells in vitro helps in the development of improved diagnostics and better treatment methods for imaging and targeted drug delivery [8]. Several methods have been adapted for preparing magnetic nanoparticles in various forms such as liposomes, solid lipid nanoparticles, dendrimers, nanocomposites, nanobrushes, nanotubes, micelles, nanogels and nanorods for biomedical applications [9,10]. Recently, a thermo-sensitive magnetic polymer based hybrid nanogel has been reported which finds its wide role in a variety of magnetic based applications [11].

Breast cancer, the most frequently diagnosed cancer affecting women as well as the second leading cause of cancer death requires early detection [12,13]. A wide variety of breast cancer cell lines such as BT-20, MDA-MB-231, MDA-MB-435, MDA-MB-468, MCF-7, SkBr 3, T47D and ZR75.1 [14] have been used as an experimental tool for targeted delivery and imaging. Normal mouse embryonic fibroblast cells such as NIH 3T3 which have low expression of folate receptors can be used as a negative control [15,16]. Magnetic Resonance Imaging (MRI) is one of the non-invasive techniques used to visually track magnetic nanoparticles in vivo and in vitro [17]. Magnetic nanoparticles due to their non-invasive character help to improve the contrast of MRI by providing changes in longitudinal (T1-recovery) and transverse

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relaxation (T2 decay). Among these two relaxations the T2-weighted images (T2*W) are preferably useful for diagnosing the internal injuries and cancer lesions [18–20]. Folic acid, an essential water soluble vitamin, is used as a target ligand for folate receptor which is over-expressed in human cancer cells such as ovarian, breast and prostate cancers while only minimally distributed in normal tissues [16,21]. Chitosan, a natural polycationic biopolymer is biodegradable, biocompatible and non-toxic polymeric material which has been particularly selected as a potential carrier for drugs [22,23]. Though magnetic nanoparticles are coated with biopolymers such as starch and collagen, chitosan coated magnetic nanoparticle finds its vital role in biomedical field and cell separation techniques [24]. Many studies were conducted to test the toxicity of Gadolinium (Gd)-based MRI contrast agent and results have shown that Gd could not be completely cleared from the body even after 2 weeks, thus increasing its toxic effect in the organs such as liver, kidney etc [25–27].

Considering the above aspects, targeted nanocarriers have been developed for cancer imaging, diagnosis and therapy. In this study, we have developed a folate conjugated chitosan coated magnetic bionanocomposite which can be detected by MRI. This magnetic bionanocomposite may be an economical alternative to the commercially available toxic MRI contrast agents and provide high resolution images to detect the cancer cells (MCF-7 — human breast adenocarcinoma).

2. Materials and methods

2.1. Materials and reagents

Chitosan and folic acid were purchased from Sigma-Aldrich St. Louis, MO, USA; goat blood was collected from nearby municipal slaughter house; MCF-7 and NIH 3T3 cell lines were purchased from the National Centre for Cell Science Pune; all other chemicals used were of analytical grade.

2.2. Preparation of INPs from goat blood

1 liter of goat blood was collected and mechanically stirred using a glass rod for 15 min continuously to isolate the fibrin. The defibrinated blood was centrifuged at 10,000 rpm (7155 g) for 20 min; the supernatant (serum portion) was discarded and the RBC collected at bottom of the tube was removed, washed with water for 10 times and stored at 4 °C. The RBC was incinerated in a silica crucible using a muffle furnace at 800 °C for 2 h. After cooling the residue (INPs) were collected and stored in a glass container. The preparation and characterization of INPs from blood were reported in our earlier publication and the experiment was designed with the approval of Institutional Ethical Committee [28].

2.3. Preparation of folate conjugated INP bionanocomposite

50 mg of INPs was dissolved in 500 μl of 6 N HCl and this INP solution was treated with 200 μl of 0.5% chitosan solution, vortexed and incubated for 1 h at 25 °C with frequent vortexing for every 15 min. The varying concentration of folic acid 1–5 mg was prepared and checked for maximum loading with INP-C using UV–vis spectroscopy. Finally the INP-C was then treated with 400 μl (4 mg) of folic acid solution which shows maximum loading and incubated at 25 °C for 2 h with frequent vortexing. The folate conjugated INP-C (INP-CF) was then precipitated by raising the pH to 7 using 1 M NaOH solution. The precipitate was then separated by centrifuging at 10,000 rpm (7155 g) for 10 min, washed with 500 μl of distilled water and dried at 37 °C (INP-CF). The sterilization of INP-CF was done by exposing to UV radiation for 2 h.

2.4. Characterization

Fourier transform infrared (FT-IR) analysis of the samples was recorded on a Nicolet 360 FT-IR spectroscope using KBr pellet. The size and morphology of the bionanocomposite were investigated using Tecnai 10, Philips Transmission electron microscope at an accelerating voltage of 80 kV. The zeta potential of the uncoated and coated nanoparticles was analyzed using laser light scattering technique by Malvern Zetasizer (v2; SL.No. MAL1066495), UK. The MCF-7 cells were cultured to carry out in vitro experiments such as cellular uptake of Fe through folate receptor mediated endocytosis, superficial morphological observations using phase contrast microscopy, the cytoplasmic distribution using SEM–EDX and MRI studies.

2.5. Cell culturing

The MCF-7 and NIH 3T3 cells were grown in folate-free Dulbecco Modified Eagles Medium (FFDMEM) containing 10% fetal calf serum, 100 units/mL penicillin, 100 μg/mL streptomycin and 4 mM L-glutamine, incubated at 37 °C in a 5% CO_2/95% air humidified atmosphere.

2.6. In vitro MRI imaging, SEM–EDX and phase contrast microscopy studies

The MCF-7 cells were grown for 24 h in FFDMEM, followed by the addition of INP, INP-C, and INP-CF solutions containing varying concentrations of Fe 50 μl (25 μg), 100 μl (50 μg), 150 μl (75 μg), 200 μl (100 μg) and 250 μl (125 μg). After 24 h incubation, (untreated and treated cells with: INP, INP-C, INP-CF were incubated at 37 °C and one set of INP-CF was incubated at 4 °C). A negative control was conducted with NIH 3T3 cells using INP-CF solution containing varying concentrations of Fe, which was then incubated at 37 °C for 24 h. The cells were viewed under phase contrast microscope and lifted using Saline:Trypsin:Versene solution; centrifuged at 4000 rpm (2862 g) for 5 min and was washed thrice with 200 μL of phosphate buffered saline. Then each pellet was suspended in 500 μL of 1% agarose solution followed by the measurement of spin–spin relaxation time T2*W MR images for each sample using 1.5 T Avanto high field magnetic resonance image analyzer Siemens Erlanger, Germany. The imaging parameters were given in our earlier publication [28]. One set of pellets was used for SEM–EDX analysis.

3. Results

3.1. FT-IR analysis

The FT-IR spectra of INP, INP-C and INP-CF are presented in Fig. 1A–C. The FT-IR spectrum of INP (Fig. 1A) exhibits a characteristic peak at 564 cm^{-1} which confirms the presence of iron in oxide form maghemite [γ-Fe_2O_3]; INP-C (Fig. 1B) the characteristic peaks of chitosan that appear at 3038 to 3200 cm^{-1} represent –OH stretching absorption band, peak at 1645 cm^{-1} represents N–H stretching vibration of amide I band, peak at 1003 cm^{-1} represents free primary amino group at C2 position of chitosan molecule, bands at 1404 and 1431 cm^{-1} represent C–O stretching of chitosan; INP-CF (Fig. 1C) characteristic absorption bands of folic acid were observed. Peaks at 1606 cm^{-1} representing the benzene ring absorption, 1674 cm^{-1} representing ester bond and 1484 cm^{-1} showing the hetero ring and conjugated double bond confirm the presence of folic acid.

3.2. TEM analysis

TEM study (Fig. 2A and B) reveals the size and shape of the prepared INP-CF before and after coating. The INP-CF exhibits spherical shaped nanoparticles before coating with chitosan. After coating