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Luteinizing hormone-releasing hormone targeted superparamagnetic gold nanoshells for a combination therapy of hyperthermia and controlled drug delivery

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ABSTRACT

In this, we developed superparamagnetic iron oxide nanoparticles (SPIONs) to be appropriate for the diagnosis and treatment of cancer cells by means of magnetic resonance imaging (MRI) and magnetically controlled hyperthermia/drug delivery (respectively). For the preparation of composite, we started with SPIONs, followed by its coating with gold to form SPIONs@Au, which further conjugated with luteinizing hormone-releasing hormone (LHRH) protein by making use of the cysteamine (Cyst) space linker and finally loaded with 5-Fluororacil (5-Fu) anticancer drug to form SPIONs@Au-Cyst-LHRH_5-Fu composite. Thus formed composite was thoroughly characterized by making use of the instrumental analysis such as HRTEM, EDAX, DLS, TGA, XPS, UV–vis, FTIR, HPLC and SQUID magnetics. We found from the analysis that the particles are spherical in shape, monodispersed with a size distribution of around 6.9 nm in powdered dry form, while in solution phase it is 8.7 nm. The UV–vis, FTIR, and HPLC studies confirmed for the loading of the 5-Fu drug onto the surface of SPIONs core and the maximum amount of drug that got adsorbed to be about 42%. The SQUID magnetic studies provided the information for the superparamagnetic behavior of the drug loaded SPIONs and the saturation magnetization (Ms) values observed to be about 11 emu/g and the blocking temperature (T_B) of 348 K. On testing the particles to see the effects of magnetic fluid hyperthermia (MFH) due to some changes in the solvent medium and oscillating frequency, the material seems to be highly active in aqueous medium and the activity gets increased with respect to the applied frequency of oscillation (430 Hz > 230 Hz > 44 Hz). From the heat release studies, the calculated specific power loss (SPL) values for the SPIONs@Au-Cyst-LHRH_5-Fu composite are at the highest of 1068 W/g in water (430 Hz) vs the least of 68 W/g in toluene (44 Hz). Further, the drug release studies tested under the influence of magnetic field provided the information that the composite released its entire loaded drug following an exposure to the magnetic field (430 Hz over 4 h time), while only 53% (over 5 h) for the controlled measurements of no magnetic field, thereby supporting to have the magnetic field so as to observe the externally controlled drug release effects. Finally, the results of the study provide the information that the SPIONs@Au-Cyst-LHRH_5-Fu composite can be potential for theranostic applications of cancer through the phenomenon of applying for MRI, magnetically controlled hyperthermia and drug delivery externally.

1. Introduction

In recent years, the superparamagnetic iron oxide nanoparticles (SPIONs) explored for various applications in the biomedical sector includes the magnetic resonance imaging (MRI) [1], targeted drug delivery [2], radionuclide therapy [3], hyperthermia [4], and cell sorting & separation [5], to mention some. Since for a majority of these applications, the deciding factor is the biocompatibility and associated toxicity, as the SPIONs of any efficiency level would not survive in the biomedical sector if the material found to compromise on its toxicity behavior. The commonly applied processes to reduce the toxicity of SPIONs include the surface modification by ligands [6], composite formation with biopolymers [7], and core-shell formation [8]. Among those processes, the core-shell formation by making use of gold (Au)/silver (Ag)/silicon (Si) as shell is of high significance as the formed particles maintains enhanced biocompatibility and reduced toxicity, in addition to fine-tuned magnetic properties [9–12]. In addition to the toxicity, the other two commonly associated issues with the SPIONs are the reduction of specific magnetization values and the particles precipitation in the biological environment and all these changes can prolong to the changes in the electronic/oxidation state of iron. Since the naked SPIONs with their

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extremely small sizes (<8 nm) and surface electronic charges are prone to form complexes with the intracellular components and can initiate some unwanted oxidation reactions which further responsible for the generation of reactive oxygen/nitrogen species (ROS/RNS) [6,13]. Hence, by considering the facts about the fundamental properties of iron oxide nanoparticles (NPs), it is highly important to protect the particles surface if the material has to employ for bio-related applications.

The SPIONs when applied for cancer diagnosis and/or treatment purposes, one of the commonly encountered issue is their selective localization within the tumor diseased cells, as the localization into the tissue microenvironment can occur by either size-mediated targeting (passive) or receptor mediated targeting (active). Since the SPIONs which lacks the targeting moiety, the other methods of tumor accumulation includes the direct injection, selective tumor embolization, and passive targeting by means of reticuloendothelial system or the enhanced permeability and retention (EPR) effect [14–17]. It was indicated that with the use of naked SPIONs the treatment options can become more difficult for the tumors which are located deep inside the body (cancers of breast and prostate), in addition to the other limitations like the variations in the degree of tumor vascularization and porosity of tumor vessels with respect to the tumor type and status. Also, the naked SPIONs when injected through the blood stream (passive), there is a chance that the particles can end up in the liver or kidney because of the tissue pathophysiologic characteristics of tumor blood vessels and from there they can initiate systemic toxicity [18–20]. Hence, it is advised to have the SPIONs particles to be conjugated to the suitable targeting ligand so as to direct the particles only to the tumor targeted site, and in addition, the ligand should protect the surface from the undergoing unwanted oxidation reactions which are potential for the release of ROS/RNS.

There have been a number of studies which deal with the individual application of magnetic nanomaterials for as contrast enhancing agents for MRI, hyperthermia, drug delivery, cell sorting etc. [21–25]. However, to the best of our knowledge, there are not many studies to deal with the combined effects of hyperthermia and drug delivery applicable to the cancer diagnosis and treatment. Inspired by our earlier studies for the release of loaded Doxorubicin drug under the influence of magnetic fluid hyperthermia (MFH) [4], here we tested the effects of SPIONs coated with gold, conjugated with luteinizing hormone-releasing hormone (LHRH) and further loaded with 5-Fluoracil (5-Fu) anticancer drug towards hyperthermia-based therapy and hyperthermia-based drug delivery related applications. We expect that the coating of gold will protect the SPIONs core from undergoing oxidation, LHRH is to act as the targeting ligand for the specific delivery of SPIONs to the cancer cells and 5-Fu serves as the anticancer drug agent. For that, we first prepared iron oxide NPs with superparamagnetic behavior (SPIONs) by the decomposition of iron acetyl acetonate at higher temperature conditions and in the presence of oleic acid/oleylamine surfactants. In the following step, the formed SPIONs were coated with gold (to form SPIONs@Au of hydrophobic nature) by the application of the same reaction conditions, which was followed by the bonding/replacement of oleic acid and oleylamine groups with that of cysteamine cross linker to form SPIONs@Au-Cyst NPs of hydrophilic nature. Now the SPIONs@Au-Cyst NPs were used for the conjugation with LHRH targeting agent to form SPIONs@Au-Cyst-LHRH and this reaction was followed by the loading of 5-Fu anticancer drug (SPIONs@Au-Cyst-LHRH_5-Fu). The formation of drug loaded composite, SPIONs@Au-Cyst-LHRH_5-Fu is shown schematically in Fig. 1; the composite was thoroughly characterized for its physicochemical properties by means of UV–vis, FTIR, EDAX, DLS, XPS, TGA, and SQUID magnetic analysis. Further, the effect of applied magnetic field for the release of the 5-Fu loaded drug and towards hyperthermia are studied, in addition to the reaction kinetics for the release of drug from SPIONs due to MFH.

2. Materials and methods

2.1. Synthesis of SPIONs@Au-Cyst-LHRH_5-Fu NPs

For the synthesis of 5-Fu anticancer drug loaded and LHRH targeted SPIONs, we started first with the synthesis of SPIONs by the decomposition of organometallics which further resulted SPIONs@Au-Cyst NPs in the subsequent steps and the detailed synthesis procedure until this step was described elsewhere [4]. In the following step for the formation of SPIONs@Au-Cyst-LHRH NPs from SPIONs@Au-Cyst, about 60 mg of SPIONs@Au-Cyst in 10 mL of distilled water was added dropwise to 40 mg of EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide) in 2 mL of distilled water and sonicated for 30 min. To this, 4 mg of LHRH in 2 mL of distilled water was added dropwise and stirred in a chiller for about 2 h so as to maintain the constant

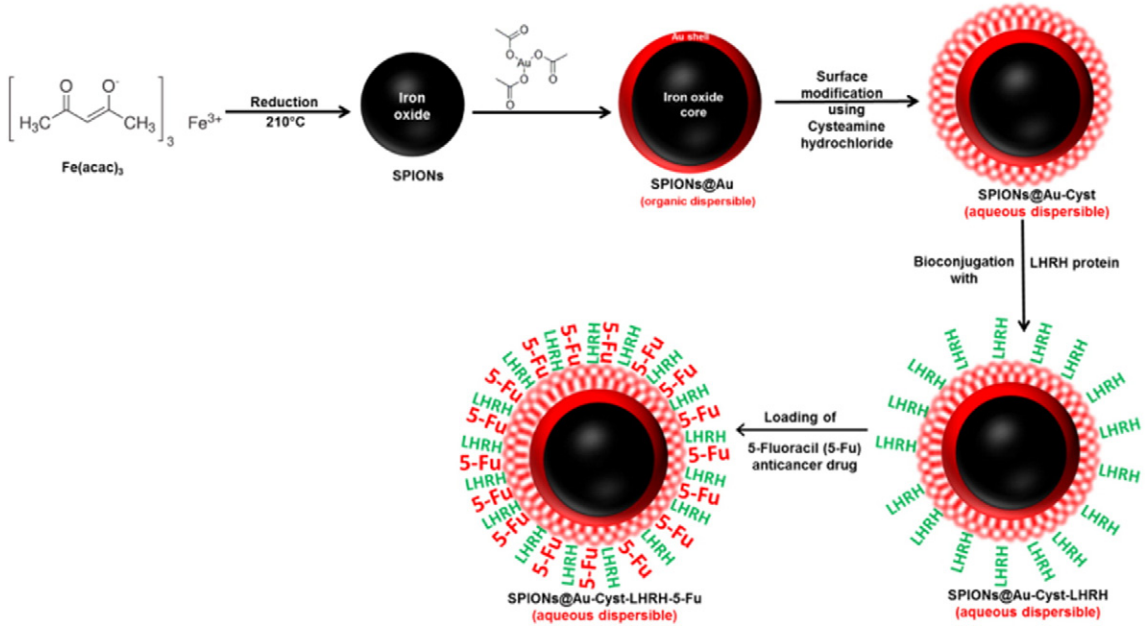

Fig. 1. Schematic representation of the formation of 5-Fu loaded SPIONs@Au particles. From the figure, we first started with the formation of iron oxide NPs (SPIONs), followed by its coating with gold (SPIONs@Au), then surface modification with Cysteamine (SPIONs@Au-Cyst), bioconjugation with LHRH protein (SPIONs@Au-Cyst-LHRH) and finally used for the loading of 5-Fu anti-cancer drug to form SPIONs@Au-Cyst-LHRH_5-Fu.