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In vivo tumor targeting and anti-tumor effects of 5-fluororacil loaded, folic acid targeted quantum dot system

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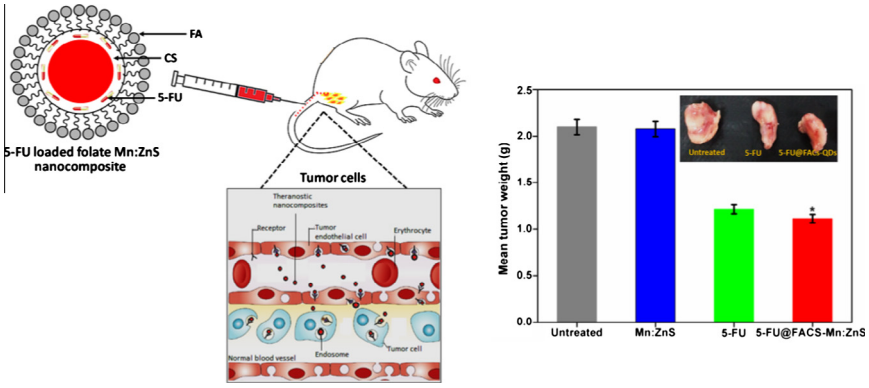
HIGHLIGHTS

- In-depth analysis of targeted quantum dots using many different instrumental methods.
- Stability of colloidal particles was studied using *in vivo* mouse models.
- 5-Fluroracil drug release effects by means of pH and temperature were studied.
- Toxicity and intracellular effects of Mn:ZnS quantum dots were studied *in vitro*.
- *In vivo* tumor targeting effects of folic acid targeted particles were investigated.

GRAPHICAL ABSTRACT

The work discusses about the anti-cancer drug release effects of tumor-bearing mice and the tumor targeted efficiency following the injection of folic acid-bound 5-Fluororacil loaded Mn:ZnS quantum dots.



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ABSTRACT

In this study, we modulated the anti-cancer efficacy of 5-Fluorouracil (5-FU) using a carrier system with enhanced targeting efficacy towards folate receptors (FRs) expressing malignant tissues. The 5-FU drug was loaded onto Mn-ZnS quantum dots (QDs) encapsulated with chitosan (CS) biopolymer and conjugated with folic acid (FA) based on a simple wet chemical method. The formation of 5-FU drug loaded composite was confirmed using Fourier transform infrared spectroscopy (FTIR), thermo gravimetric analysis (TGA) and differential scanning calorimetry (DSC). Furthermore, the *in vivo* biodistribution and tumor targeting specificity of the 5-FU@FACS-Mn:ZnS in the tumor-bearing mice was conducted based on the Zn^{2+} tissue bioaccumulation using inductively coupled plasma (ICP) spectroscopy. In addition to the characterization, the *in vitro* release profile of 5-FU from the conjugates investigated under diffusion controlled method demonstrated a controlled release behaviour as compared against the release behaviour of free 5-FU drug. The as-synthesized 5-FU@FACS-Mn:ZnS nanoparticle (NP) systemically induced higher level of apoptosis in breast cancer cells *in vitro* as compared to cells treated with free 5-FU drug following both cell cycle and annexin assays, respectively. Also, the *in vivo* toxicity assessment of the

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5-FU@FACS-Mn:ZnS NPs as compared to the control did not cause any significant increase in the activities of the liver and kidney function biomarkers, malondialdehyde (MDA) and nitric oxide (NO) levels. However, based on the FA-FRs chemistry, the 5-FU@FACS-Mn:ZnS NPs specifically accumulated in the tumor of the tumor-bearing mice and thus contributed to the smaller tumor size and less event of metastasis was observed in the lungs when compared to the tumor-bearing mice groups treated with the free 5-FU drug. In summary, the results demonstrated that the 5-FU@FACS-Mn:ZnS QDs exhibits selective anti-tumor effect in MDA-MB231 breast cancer cells *in vitro* and 4TI breast cancer cells *in vivo*, providing a blueprint for improving the 5-FU efficacy and tumor targeting specificity with limited systemic toxicity.

1. Introduction

Current research in targeted delivery and imaging through engineered nano-carriers has demonstrated a promising milestone towards achieving specialized and personalized medicine following the exploitation of biomolecules uniquely expressed by cancer cells. This expression is mediated to conveniently transport therapeutics and other modalities like imaging contrast agents into malignant tissues with improved efficiency and limited systemic toxicity [1,2]. One among many strategies employed by researchers towards ameliorating cancer proliferation follows the incorporation of anti-metabolite drugs into the cell cycle in order to inhibit the essential biosynthetic processes of DNA replication [3,4]. Antineoplastic drugs such as 5-Fluorouracil (5-FU), an analogue of uracil with its C-5 substituted with fluorine atom that readily binds to DNA are widely used in chemotherapy for the treatment of malignancy such as breast, colorectal, gastric cancer etc. Intracellularly, 5-FU readily metabolize uradine monophosphate (UMP) and uradine triphosphate (UTP) forming its respective fluorodeoxyuridine that actively disrupt RNA/DNA synthesis and also impairs the normal activity of thymidylate synthase (TS), an essential precursor in DNA synthesis [5,6]. Despite the potent anti-cancer action of 5-FU, a range of toxicity events are also reported [7,8]. In addition to the toxicity profiles, 5-FU impacts on cancer therapy follows a radical killing spree on both normal and malignant tissues [7,9]. With all these observations in mind, we have modulated a 5-FU nanocomposite in order to increase its bioavailability and target specificity by deploying the binding chemistry of specific ingredients that is highly needed in the TS metabolism [6]. One of the essential ingredients needed for the synthesis of TS is FA; a B-vitamin needed by the fast dividing tumor cells for the replication and synthesis of purine and thymidine. This demand is being exploited to channel FA-based therapeutics and other biomolecules mediated by folate receptor (FR) pathways [10,11]. When FA is attached to the carboxylate side through the pendant group, the folates retain their normal receptor-binding affinity and can be internalized by the receptor-mediated endocytosis [12]. The FRs are cysteine-rich cell-surface glycoproteins that bind readily to FA with high affinity under physiological conditions ($K_d = 1 \times 10^{-10}$ M) and in the process mediating its cellular uptake [13,14].

In addition to the ligand-receptors mediation towards enhancing the drug uptake and selectivity, Abdelhamid reported that the coordination of drugs to biological metal ions such as Fe^{2+}, Fe^{3+}, Zn^{2+}, Cu^{2+} etc. could improve the pharmaceutical efficacy as well as fostering stronger interaction with the cellular molecules such as proteins and DNA [15]. Based on this concept, formulation of targeted drug delivery carrier labelled with QDs are widely getting acceptance. The use of low cytotoxic QDs as a suitable material for drug delivery were reported by Xu et al., where they synthesized a fluorescent glycopolypeptide (GP) NPs with Mn-ZnS QDs as the core and glycopolypeptide of dextran-conjugated poly(L-alanine) as shell for the delivery of Ibuprofen drug [16]. The report shows that the GP NPs not only exerts higher loading efficiency but also demonstrated controlled release behaviour. The doped QDs in addition to its application as the drug delivery material was being deployed as the fluorescent contrast agent due to its multi-photon excitations which allows for the non-invasive immobilization during the imaging of cancer cells [17]. Since, the doping of Mn^{2+} enhances the optical and electronic properties of ZnS semiconductor nanocrystals, thereby providing a better alternative to Cd-based QDs owing to its lower toxicity, larger stoke shifts and longer excited state lifetimes [18]. For example, Sharma et al. reported that the chitosan (CS) capped ZnS:Mn QDs could be modulated to emit different colours without varying the size of QDs by simply changing the excitation wavelength of the sample to produce desired characteristic imaging [19]. In another study, the imaging capability of folate based-Mn-ZnS carboxymethyl CS NPs was reported to demonstrate the controlled drug delivery and imaging of MCF-7 breast cancer cells *in vitro* [20]. Similar findings were reported by Aswathy et al. indicate that the folate-based Mn-ZnS encapsulated carboxymethyl cellulose was observed to exhibit stronger fluorescence imaging of MCF-7 cancer cells compared to L929 normal breast cells *in vitro* [21].

In our earlier study [22], we took advantage of the affinity between tumor cell overexpressing FRs and FA-bound chemistry and developed a colloidal Mn^{2+}-doped ZnS QDs system with CS as stabilizer and FA groups as targeting surface ligands. We hypothesized that the formed composite (for simplification, the composite of folic acid-chitosan encapsulated Mn-doped ZnS is termed as FACS-Mn:ZnS) can find applications during the targeted drug delivery and cancer cell imaging. In the study, we reported that the incorporated Mn:ZnS QDs in the carrier system at 15 at. % Mn^{2+} doping concentration emits orange–red fluorescence around 600 nm due to the $4T_1$-$6A_1$ transition of the Mn^{2+} ion. The doping of ZnS with Mn^{2+} impurities significantly enhances the fluorescence efficiency of the QDs towards the cut-off wavelength of the visible region. The assessment of the *in vitro* cell imaging properties of the folate-doped ZnS nanocrystals as investigated under confocal laser scanning microscope demonstrated more pronounced fluorescence in the breast cancer cells (MCF-7 and MDA-MB231) due to the FA-FRs binding chemistry as compared to the normal breast cells (MCF-10). Furthermore, the cell viability and proliferation studies of the FACS-Mn:ZnS by means of MTT assay have demonstrated that the as-synthesized composite did not exhibit any significant toxicity towards the human breast cell line and the breast cancer cell lines up to 500 μg/mL concentration [22].

In continuation to our previous study, we used the same composite (FACS-Mn:ZnS) and utilized the mediating role and binding chemistry of FA-FRs to enhance the intracellular uptake of 5-FU loaded composite as compared to the administration of pure 5-FU anti-cancer drug. The assessment of *in vitro* cellular uptake was conducted by means of cell cycle and Annexin V-FTIC apoptosis assays. In addition, the *in vivo* toxicity and anti-breast tumor effects were also investigated with the view to ascertain the safety and targeting efficacy of the FACS-Mn:ZnS NPs loaded 5-FU drug (termed as 5-FU@FACS-Mn:ZnS). The overall results indicated that the FACS-Mn:ZnS QD system could be effective towards