Original Article

Synthesis of porous nanoparticles and their use as a cytotoxic agent

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Summary:

Background: In this study, a targeted drug delivery system has been developed based on nanoporous particles covered with cadmium sulfide and loaded with 5-flourouracil. The nanoparticles loaded drug exerts a cooperative antineoplastic activity.

Methods: Iron oxide nanoparticlers were synthesized and functionalized with cadmium and loaded with 5-flourouracil. Different techniques were used for characterization such as infrared (IR) and ultraviolet (UV) measurements. A cell culture test was done in order to investigate the effect of drug loaded on nanoparticles.

Results: The development of nanoparticles loaded anticancer agent for possible application in targeted treatment of cancer where the drug release is triggered by externally applied UV irradiation. In addition, the treatment of cancer cells with drug loaded magnetic material leads to a decrease in viability of the cells due to the activity of cadmium nanoparticles. Upon exposure to low power UV light (365 nm) the loaded 5-flourouracil is released which induces additional decrease in viability of Cervical carcinoma cells HeLa.

Conclusions: In summary, an anticancer drug delivery system based on nanoporous material loaded 5-fluorouracil has been developed. The cooperative anticancer effect of nanoparticles and loaded 5-flourouracil was observed.

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Introduction:

In recent decades, the application of nanotechnology in medicine (so called nanomedicine) has attracted much attention. Various nanomaterials have been widely used in nanomedicine as potential therapeutic agents for cancer imaging and diagnostic treatment [1]. Compared to traditional organic lipids or polymerbased nanoparticles, the inorganic counterparts exhibit unique properties such as inertness, stability, and ease of functionalization [2]. Specifically, nanoporous particles with high surface areas, tunable pore structures as well as particle sizes, and controllable surface chemistry have attracted enormous research interest in various bio-applications, including cell imaging, diagnosis and bioanalysis, and drug/gene delivery [3]. For efficient cancer treatments, it has been well documented that a targeted delivery is vital because most anticancer drugs distribute throughout the body and can be harmful to healthy cells [4].To minimize the side effects, it would be desirable to specifically increase the anticancer drug concentration at the target sites. Nanoparticles with small sizes (1–200 nm) preferably accumulate at tumor sites caused by the enhanced permeability and retention effect, also called passive targeting [5]. The abundant silanol groups (Si–OH) in nanoporous materials facilitate the modification of the above active moieties, thereby achieving active targeting to specific cancer cells [6]. The active targeting action together with the passive targeting effect will further enhance the cellular uptake of particles in defective cells, leading to a significant improvement in cancer therapy [7]. In the present work, nanoporous particles covered with cadmium have been synthesized as drug nanocarriers. Cadmium containing nanoparticles are known to inhibit DNA repair and to cause free radical-induced DNA damage, mitochondrial damage, induction of apoptosis, and disruption of intracellular calcium signaling [8]. 5-fluorouracil (5-FU), a pyrimidine analog, is one of the broad spectrum anticancer drugs used in the treatment of malignancies like gastrointestinal tumors (mainly colon and rectum) and breast cancer. Since 5-FU interferes with DNA synthesis, it principally acts as a thymidylate synthase inhibitor. However, short half-life, wide distribution, and various side effects limit its medical applicability [9]. To overcome the above-mentioned limitations, an ample number of studies has been carried out on sustained drug delivery systems for 5-fluorouracil.

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Patients and Methods

Tetraethylorthosilicate (TEOS), n-cetyltrimethylammonium bromide (CTAB), 3- isocyanatopropyltriethoxysilane, 5,5-dithiobis (2-nitrobenzoic acid) and 5-flourouracil were obtained from Sigma-Aldrich company and used as received.

Preparation silica nanoparticles: Iron oxide Fe_3O_4 nanoparticles (300.0 mg) were sonicated for 12 h in a solution of CTAB (2.0 g, 5.5 mmol) and NaOH (7.0 mL, 2 M) in distilled water

(480.0 mL) [8]. The suspension was then heated to 80 C under mechanical stirring followed by rapid injection of TEOS (10.0 mL, 44.3 mmol). After an additional 2 hours of stirring, the material was filtered, washed with water and methanol and air dried. Finally iron oxide silica particles were obtained upon removal of surfactant molecules by refluxing with ethanol twice. Synthesis of cadmium nanoparticles Cadmium acetate and 2-nitro-5-mercaptobenzyl alcohol, were then mixed under vigorous stirring and the solution of sodium sulfide (Na_2S) was added dropwise into the mixture while the whole solution was stirred and protected from light. Addition of sodium sulfide was completed after 40 minutes and the solution was stirred for an additional 10 minutes. Solvent was then evaporated and acetone was added to the oily residue until cadmium sulfide nanoparticles precipitated. The product was centrifuged, washed with acetone and dried in oven. Yield: 0.3 g. The 5-fluorouracil was loaded into the particles under vigorous stirring. Loading of the drug was determined by the UV absorption measurements of residual 5-fluorouracil from the filtrate solution. The final loaded amount was determined to be 50 mg of drug per g of nanoparticles [9].

Cell cultures:

Cell cultures were cultured on 12 well plates with Dubelcco's Modified Eagle Medium obtained from Sigma-Aldrich plus 5 % horse serum, L-alanyl-L-glutamine, gentamicin, and penicillin/streptomycin. The medium was then replaced by suspensions of prepared materials at specific concentrations (0 mg ml^{-1} for CTRL (control), 10 mg mL^{-1} and 25 mg ml^{-1} for CdF (particles with drug and 25 mg ml^{-1} for CdM (particles without drug) in a fresh medium without added serum. Serum was not used in this case in order to increase the endocytosis efficiency of the materials. The cells were then protected from light and placed back into the incubator for an additional five hours. The suspensions/solutions were removed; the cells were washed with PBS buffer. Cervical carcinoma cells HeLa was gratefully obtained from Dublin University and experiments were done in Iraqi Center for Research on Cancer and Medical Genetics, The University of Mustansiriyah. All the experiments were performed in triplicate for each group. The statistical significance between the two groups was analyzed by t-tests using the software in GraphPad Prism. The bar graphs in the figures represent (the standard error of the mean i.e. the spread of the mean of a sample of the values would have if you kept taking samples) of the two groups (± SEM). The differences were considered to be significant if $p < 0.05$, the p value was calculated based on t ratio, which is the difference between sample means divided by the standard error of the difference.

Results:

The infrared (IR) spectrum of cadmium sulfide nanoparticles is dominated by the presence of acetate ions which precipitated with cadmium silica nanoparticles. Strong carboxylate stretch vibrations are evident at 1575 cm^{-1} and 1417 cm^{-1} and characteristic (COO^-) at 671 cm^{-1} (Fig. 1).

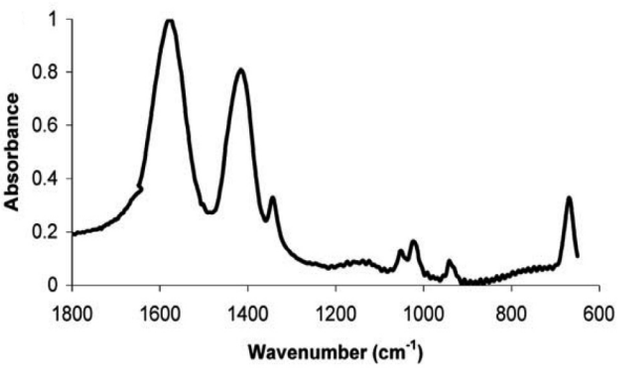

Figure 1: IR spectra of nanoporous material loaded drug

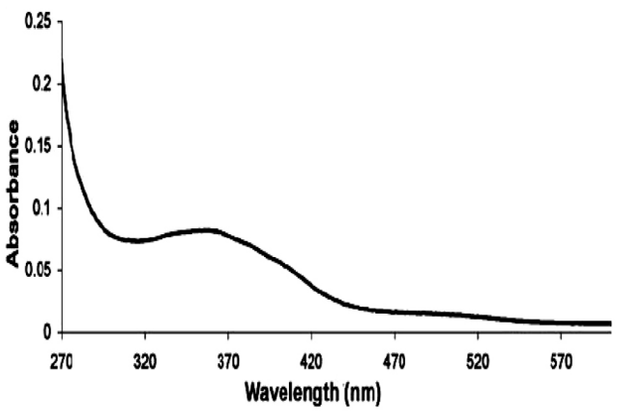

Figure 2: UV spectra of nanoporous material loaded drug

The quantum dot nature of the synthesized nanoparticles was revealed by UV-VIS absorbance (Figure 2). UV-VIS absorbance revealed the absorption band of cadmium sulfide nanoparticles with the maximum of the band centered at 360 nm. The diameter of cadmium sulfide nanoparticles was calculated to be 3.25 nm.

In order to test the applicability of drug delivery system for drug delivery to cancer cells, the viability of cervical carcinoma cells HeLa in the presence of (CdF) and (CdM) material without (Dark) and with exposure (Irr) to low power UV lamp was monitored. The viability of treated cells was normalized to non-treated, control cells (Ctrl). As depicted in Fig. 3a, in the case of the treatment with 10 mg ml^{-1} dosage of the materials, CdF-treated cells which were kept in the dark

(Dark- CdF) exhibited no influence on culture cells HeLa viability. However, if the treated cells were exposed to UV irradiation for 30 minutes, the number of viable cells decreased to 84% (Irr- CdF) ($p < 0.05$).

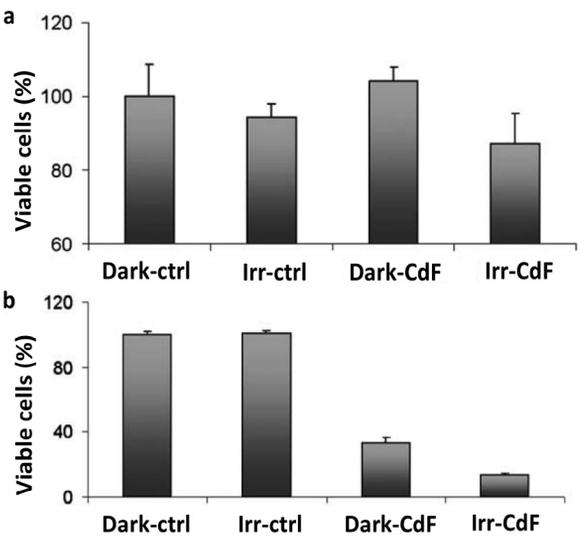

Figure 3: Number of viable culture cells upon treatment with: (a) 10 mg ml^{-1} of CdF and (b) 25 mg ml^{-1} of CdF. The cell cultures was continually kept in the dark (Dark- CdF); and upon exposure to 30 min irradiation with a low power UV lamp.

The Dark-CdF and Irr-CdF samples could be established if the treatment concentration was increased to 25 mg ml^{-1} (Fig. 3b). However, it can be noted that the CdF material at the 25 mg ml^{-1} dosage caused decrease in viability of the cells even if the treated cells were kept in the dark at all times. The viability of the cells for the Dark-CdF sample decreased to 33.4%, while the viability of the Irr-CdF sample was 13.7%. This influence of CdF on the viability of cancer cells without exposure to UV light can be ascribed to the cytotoxicity of covering cadmium sulfide nanoparticles. Still, exposure of the CdF sample to UV irradiation (Irr- CdF) clearly led to an additional decrease in the viability of HeLa cells, which is ascribed to the toxicity of the released 5-fluorouracil. As a control experiment, the cytotoxicity of the empty shell material was tested, i.e. the magnetic material with attached cadmium sulfide but without loaded drug, after UV irradiation under the same experimental conditions (Fig. 4).

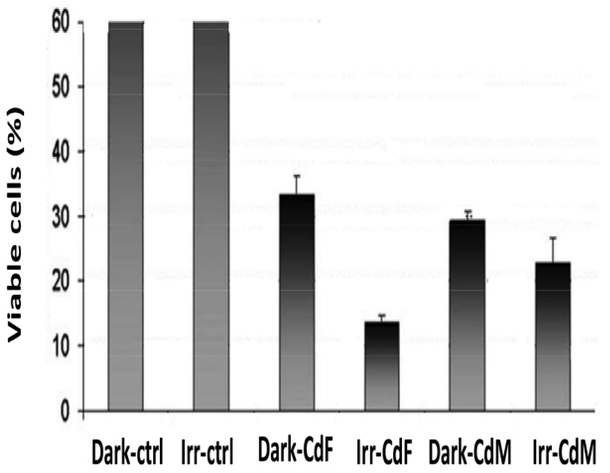

Figure 4: Comparison of cytotoxicity of treatment with 25 mg mL-1 of 5-Fluorouracil loaded into the nanoparticles (CdF) and empty nanoparticles (CdM) when exposed to 30 minutes of UV irradiation (irr) or kept in the dark (Dark). Viability values are adjusted to the Dark-Ctrl sample.

As can be seen, there is no increase in the toxicity of cadmium sulfide nanoparticles upon exposure to UV light (Irr-CdM). That was due to the working principle of the prepared drug delivery system, which may affected by UV light irradiation due to the breaking of the carboxylic linkage and subsequent release of functionalized cadmium nanoparticles and 5-flurouracil ($p > 0.05$ for samples Dark- CdM and Irr- CdM) [10]. In addition, from the same figure the cytotoxicity of cadmium sulfide nanoparticles is indeed responsible for the low viability measured in the sample Dark-CdF because similar viability values were found in the sample Dark-CdM ($p > 0.05$).

Discussion:

The results showed that the cadmium sulfide containing materials were not cytotoxic at a 10 mg ml^{-1} dosage. Hence, the materials may be applicable at a lower than 25 mg ml^{-1} dosage which would minimize the harmful effects on healthy tissues and yet enhanced permeability and retention (EPR) effect and application of nanoparticles loaded drug targeting could concentrate the materials in tumor tissues in order to induce cytotoxic effects on cancer cells selectively [10]. The decrease in viability of culture cells in the case of the Irr- CdF sample in comparison to Irr- CdM ($p < 0.05$) arises from the cytotoxic effect of 5-Fluorouracil, which is released from the material upon exposure to UV irradiation due to the effect of that radiation on drug delivery system. Hence, the nanoparticles loaded drug and cadmium sulfide could exhibit cooperative anticancer effect on HeLa cells. This due to the above discussed construct can be effectively used

for combination therapy of cancer because: (a) as discussed in introduction of materials are typically capable of entering the cells through endocytosis and selectively accumulating in tumor tissues due to EPR (permeability and retention effect) [12, 13], (b) the constructed drug loaded on nanoparticles which allows targeting of the cancer tissue with an externally applied effect [14,15]. Also nanopartilcles with small sizes (1-200 nm) preferably accumulate at tumor sites caused by enhanced permeability and retention effect, also called passive targeting [14].

Conclusion:

In summary, an anticancer drug delivery system based on nanoporous material loaded 5-fluorouracil has been developed. The cooperative anticancer effect of nanoparticles and loaded 5-flouruoracil was observed upon treatment of cell cultures in vitro with 25 mg ml^{-1} concentration. The constructed nanodevice may be applicable for selective, targeted combination therapy of cancer.

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