β -Cyclodextrin Functionalized poly (5-amidoisophthalicacid) Grafted Fe₃O₄ Magnetic Nanoparticles: A Novel Biocompatible Nonocomposite for Targeted Docetaxel Delivery

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Abstract

Thiol-lactam initiated radical polymerization (TLIRP) was successfully employed to prepare poly-*N*-5-acrylamidoisophthalicacid grafted onto Fe₃O₄ magnetic nanoparticles (MNPs@PAIP). β -Cyclodextrin (CD) was then conjugated to the carboxylic groups of the prepared MNPs via carbodiimide activation. Subsequently, tumor-targeting folic acid (FA) was attached to the hydroxyl groups of CD on the surface of the latter MNPs to increase the site-specific intracellular delivery. The prepared MNPs were fully characterized by FTIR, VSM, TGA, XRD, FE-SEM and TEM. Docetaxel (DTX) as hydrophobic anticancer drug was loaded via host-guest inclusion complexation with CD and the release profile of the system was studied at different pH. The effect of MNPs on the cell viability was evaluated for the human embryonic kidney normal cell line (HEK293) as well as HeLa and MDA-MB-231 cancerous cell lines and the results did not show any apparent cytotoxic effect. In comparison, DTX loaded MNPs reduced the growth of HeLa and MDA-MB-231 cells more than free DTX. Intracellular uptake ability of DTX loaded MNPs was also studied using fluorescent microscopy and showed cellular uptake about 90% after 4 h treatment.

Keywords: Cell targeted drug delivery, β -Cyclodextrin, Docetaxel, Superparamagnetic iron oxide.

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1. Introduction

In recent years, magnetic nanoparticles (MNPs) have attracted enormous interest for medical purposes such as nanocarrier for drug delivery and contrast agent in magnetic resonance imaging [1, 2]. Since iron oxide MNPs can be converted to a part of natural iron stores in body like hemoglobin in red blood cells, they have been numerous applied in biomedical applications as biodegradable and non-toxic materials [3]. Because of high surface area and dipole-dipole interaction, iron oxide MNPs are agglomerated and subsequently their particle size is increased [4]. To overcome this drawback, the surface engineering of MNPs is a suitable manner to protect them against agglomeration. This aim is attainable using different polymer and inorganic materials or by chemical grafting of some biomolecules [5-8]. The choice of suitable organic molecules as surface modifier or drug trapping agent are one of the most important step in the designing of new drug delivery systems for obtaining efficient drug loading and release.

Cyclodextrin (CD) which contains a hydrophilic outer surface and a lipophilic central cavity is an interesting organic moiety with ability of different lipophilic drugs trapping via its accommodative hydrophobic cavity [9, 10]. CD modified MNPs form convenient inclusion complexes with drugs that cause to increase drug solubility and stability [11], controlled drug release profile [12], enhanced drug absorption [13], and improved drug permeability across biological barriers [14].

Docetaxel (DTX) is an anti-cancer chemotherapy medicine in the taxane class with low solubility in water. It has been shown that DTX complexation by CD can significantly increase its solubility [15-17] and efficient delivery to tumor position such as prostate, breast, ovarian, lung cancer, and etc. [18-23].

Also, it has been established that target localization of drug delivery system on tumor cells can be improved by different bio-molecules such as folic acid (FA) [24, 25], polysaccharides [26], peptides [27] and monoclonal antibodies [28, 29]. Among all targeting agents, FA as a cancer marker has been found a promising candidate of targeting agents due to its low cost and high stability, simple conjugation with different molecules, and high affinity for the folate receptors on carcinomas [30].

Therefore, spontaneous use of CD and folic acid on the surface of magnetic nanoparticles can provide significant features for designed drug delivery system including noncovalent inclusion complex formation which is performable for a wide variety of lipophilic drug molecules and consequently enhancing their solubility and stabilization as well as providing a magnetically conductible system to the target site and subsequently controlled release of drug [30–32]. According to the above-mentioned topics, in the present study, we aimed to introduce a novel magnetic nanocarrier containing folic acid functionalized CD as a potential and biocompatible targeting drug delivery system for cancer therapy. Moreover, the drug release, cytotoxicity profile and intracellular uptake were investigated in detail.

2. Experiment

2.1. Materials and apparatuses

Ferric chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), ferrous chloride ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$), ammonium hydroxide, 3-mercaptopropyl trimethoxysilane (MPTMS), butyrolactam (BL), triethylamine, folic acid (FA), *N*-hydroxysuccinimide (NHS), *N,N*-dicyclohexylcarbodiimide (DCC), DTX, β -Cyclodextrin (CD), *N,N*-dimethylformamide (DMF), and all solvents were purchased from

Merck chemical corporation. For *in-vitro* cytotoxicity test, the HeLa, MDA-MB-231 and HEK cells were obtained from Pasteur Institute (Tehran, Iran). RPMI 1640, fetal bovine serum (FBS), trypsin were obtained from GIBCO Invitrogen Corporation and also 3-(4, 5 dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) and dimethyl sulfoxide (DMSO) were purchased from Sigma Co. The Fourier transform infrared (FTIR) spectra of nanoparticles were taken with Perkin-Elmer 597 spectrometer using KBr over the range of 4000–400 cm^{-1} .

The surface morphology of MNPs was investigated by using a field emission scanning electron microscopy (FE-SEM, Tescan, Mira LMU). Transmission electron microscopy (TEM) images were recorded using a (Zeiss-EM10C-80 KV) instrument at an accelerating voltage of 200 kV, in which the sample was prepared by drop casting the nanoparticle suspension on a copper grid with carbon film and dried at room temperature (RT). The magnetic properties of magnetic nanoparticles were measured using a vibrating sample magnetometer (VSM) (Meghnatis Kavir Kashan Co, Kashan, Iran) at RT. Thermogravimetric analyses (TGA) were performed using a TGA, Q50 thermogravimetric analyzer. Samples were heated from RT to 800 °C at a rate of 20 °C/min under argon atmosphere. All absorption spectra were carried out using a CECIL spectrophotometer against a solvent blank. Samples were prepared in concentration of 0.01 mg/mL of FA or MNPs in EtOH before measurement. All the quantitative results were expressed as mean $\pm$ standard deviation (SD) of the mean. Statistical analysis was carried out by means of two-way analysis of variance (ANOVA) followed by the Tukey post-test. A *p*-value less than 0.05 were considered statistically significant. The DTX loading contents of MNPs were measured by high performance liquid chromatography (HPLC, CECIL, UK).

2.2. Synthesis of *N*-5-acrylamidoisophthalicacid (AIP)

AIP was prepared according to the previous reported procedure [33]. Typically, 5-aminoisophthalic acid (1 g, 5.52 mmol) was added to a solution of sodium hydroxide (0.66 g, 16.56 mmol) in water (11 mL), and then the mixture was cooled to 0 °C in an ice bath. The excess amount of NaOH solution was served to maintain the pH above 12. Acryloyl chloride (0.62 mL, 6.10 mmol) was added drop-wise over 12 min under vigorously stirring to the later suspension and then the mixture was stirred at 0 °C for 10 min. Concentrated HCl was then added drop-wise to the obtained reaction mixture at 0 °C until the pH was adjusted to 2.6. Finally, the light pink precipitate was filtered, washed with a little ice cold water and dried under vacuum, the reaction afforded 0.76 g (60%) of product as light pink solid: m.p. 267-272 °C [33].

2.3. Synthesis of Fe_3O_4 MNPs

Fe_3O_4 MNPs were synthesized by the chemical co-precipitation method as mentioned in our earlier publication [34].

2.4. Synthesis of thiol functionalized MNPs (MNPs-SH)

The surface of MNPs was modified via the grafting of MPTMS as an organosilane [35]. Briefly, 1.0 g of dried MNPs were well dispersed in 25 mL of toluene by using ultrasonic to produce a homogeneous suspension after two times washing with toluene. After that, 3.0 g of MPTMS was added to the well-dispersed mixture under inert atmosphere and allowed to stir for 24 h at RT. The resulting black precipitate was then collected by an external

magnet and washed three times with toluene to remove unreacted MPTMS. The obtained MNPs-SH was dried under vacuum at 40 °C overnight.

2.5. Synthesis of poly(5-amidoisophthalicacid) grafted Fe₃O₄ MNPs (MNPs@PAIP)

A typical procedure for synthesis of the MNPs@PAIP was as follow: 0.2 g of MNPs-SH were dispersed in 4 mL toluene by sonication for 30 min in a 50 mL round bottom flask. Then, 0.5 g of BL and 2.0 g of AIP were added into the reaction mixture and polymerization was done for 10 h at 90 °C under N₂ atmosphere. The flask was cooled to RT and the reaction mixture was re-dispersed in 5 mL of DMF. The magnetic residual was separated by an external magnet and washed several times with EtOH and deionized water (DW) to remove any free AIP monomer and exclusively obtain PAIP functionalized MNPs. Finally, MNPs@PAIP were obtained as a light brown precipitate after drying in a vacuum oven at 60 °C for 24 h.

2.6. Synthesis of MNPs@PAIP-CD

CD was grafted onto the surface of MNPs@PAIP via esterification applying DCC as activating agents. At first, 0.5 g (0.5 mmol) of DCC and (0.5 mmol) of NHS were added to the well-dispersed mixture of 100 mg of MNPs@PAIP in 3 mL of DMSO and stirred at RT for 3 h. 1.0 g of CD was activated by 0.5 g of KOH in 3 mL of DMSO for 3 h and then added to the latter suspension. The mixture was allowed to stir under nitrogen atmosphere at RT for 24 h. The obtained product was separated by an external magnet, washed three times with DW and dried under vacuum at 60 °C for 24 h.

2.7. Synthesis of MNPs@PAIP-CD-FA

In order to attach FA onto the surface of MNPs@PAIP-CD, folate *N*-hydroxysuccinimide ester (FA-NHS) was initially synthesized according to the reported procedure [36]. 1.0 g of FA, 0.53 mg of NHS and 2.35 mg of DCC were dissolved in 40 mL DMSO (with molar ratio of FA: NHS: DCC = 1:1:2.5). Then, 0.5 mL of triethylamine was added to the solution while solution was stirred in dark at RT. After 24 h, the mixture was filtered to remove the precipitated side-product (dicyclohexylurea). On the other hand, 50 mg of MNPs@PAIP-CD were dispersed in 10 mL DMSO, and a solution of activated FA was then added to the reaction media and stirred at RT for 24 h under nitrogen atmosphere. The MNPs@PAIP-CD-FA were collected using an external magnet, washed three times and dried in vacuum oven.

2.8. Drug loading study

DTX was loaded into the MNPs@PAIP-CD-FA according to the following procedure. Briefly, a solution of 100 mg of DTX in 1 mL of acetonitrile was added to a well-dispersed mixture of MNPs@PAIP-CD-FA (500 mg) in 5 mL of acetonitrile and then stirred at RT for 24 h. The DTX loaded MNPs were magnetically separated and then lyophilized under vacuum. The DTX loading capacity of MNPs@PAIP-CD-FA were analyzed by an indirect method in which the absorbance of extra drug in the supernatant was measured by reverse-phase HPLC, using a C18 column (250mm×2.0mm, 5 μ m pore size) and a UV detector (CECIL, CE 4900, UK). The chromatographic separation was performed with a flow rate of 1 mL/min at RT. The mobile phase was consisted of acetonitrile/water solution (65:35, v/v) and the detection wavelength was 230 nm. The concentration of DTX was calculated

using a standard calibration curve experimentally. The encapsulation efficiency (EE) and drug loading content (DL) were calculated according to the standard curve of DTX in acetonitrile as follows [37]:

$$EE \text{ (wt \%)} = \frac{\text{weight of the DTX in nanoparticles}}{\text{weight of the nanoparticles}} \times 100$$

$$DL \text{ (wt \%)} = \frac{\text{weight of the DTX in nanoparticles}}{\text{weight of the feeding DTX}} \times 100$$

2.9. *In vitro* drug release study

To determine the *in vitro* DTX release, 10 mg of DTX loaded MNPs@PAIP-CD-FA were dispersed in 2.0 mL of release medium (phosphate buffer solution, pH 7.4 and 5.0 containing 0.1% w/v Tween 80). The tube was then incubated at 37 °C with gentle shaking (100 rpm). At different time points, the tube was centrifuged at 13,000 rpm for 5 min and 2.0 mL of medium were taken and replaced with fresh PBS. The concentration of DTX in the release samples was determined by HPLC assay as described above [38].

2.10. *Cell culture*

The human breast cancer cell lines (MDA-MB-231) and human cervical cancer cells (HeLa) were cultured in RPMI-1640 containing 10% FBS (v/v) as well as 1% (v/v) penicillin/streptomycin at 37 °C under moist environment of 95% air and 5% carbon dioxide. All cells used in our experiments were in their exponential growth phase.

2.11. *In vitro* cellular uptake

The HeLa cells were grown in RPMI and treated with 10% FBS and 1% penicillin/streptomycin at 37 °C and 5% CO₂. The cells at density of 5 × 10⁴ per well were plated on 24-well plate and allowed to adhere for 24 h till the cells reached 70%

confluence. The cells were then treated with DTX loaded MNPs@PAIP-CD-FA and incubated for 4 h at 37 °C. Subsequently, the cells were rinsed with PBS three times to remove excess MNPs and fixed with 4% paraformaldehyde for 20 min. Cellular uptake of target MNPs on HeLa cells was studied by invert fluorescent microscope.

2.12. *In vitro* cytotoxicity assay

The cytotoxicity of the DTX loaded MNPs towards MDA-MB-231 and HeLa cells was evaluated by performing methyl thiazolyl tetrazolium (MTT) assays. The cells were seeded into a 24-well cell culture plate at density of 5×10^4 per well in RPMI with 10% FBS and 1% penicillin/streptomycin and incubated overnight at 37 °C. Then, the cells were treated with different concentrations of DTX loaded MNPs@PAIP-CD-FA (100, 200 and 500 µg/mL) for 24, 48, and 72h at 37 °C under 5% CO₂. The culture without any DTX loaded MNPs was used as control group. Thereafter, 20 µL MTT solution (5 mg/mL) was added to each well, and the plates were incubated for an additional 4 h at 37 °C. Then, 1 mL DMSO was added to each well and the plates were agitated for 1 min to dissolve the MTT formazan crystals. The optical density (OD) was measured by a spectrophotometer (CECIL, CE 7500, UK) at the wavelength of 570 nm. The survival rate was calculated by the following equation:

$$\text{Viable rate \%} = \frac{\text{OD}_{(\text{treated})}}{\text{OD}_{(\text{control})}} \times 100$$

Where OD_(treated) is absorbance of the cells incubated with the nanoparticles and OD_(control) is absorbance of the cells in the absence of nanoparticles.

3. Results and discussion

3.1. Synthesis and characterization of multifunctional MNPs

The sequential steps for the preparation of targeted MNPs are shown in **Fig. 1**. Fe_3O_4 MNPs were initially synthesized by co-precipitation method, and then poly-*N*-5-acrylamidoisophthalic acid was grafted on surface of the MNPs via TLIRP. CD was then conjugated to acidic groups of the polymer and finally FA attached to the hydroxyl groups of CD through esterification.

Fig. 1. Schematic presentation for the synthesis of targeted nanocarrier.

The prepared MNPs were characterized by FTIR spectroscopy to confirm the successful surface functionalization. The FTIR spectra of Fe_3O_4 MNPs, MNPs-SH, MNPs@PAIP and MNPs@PAIP-CD were shown in **Fig. 2**. As seen, FTIR analysis of Fe_3O_4 MNPs shows strong band around 580 cm^{-1} corresponding to the Fe–O vibration and a broad band at 3423 cm^{-1} for –OH stretching vibrations of hydroxyl groups on the surface of MNPs (**Fig.**

2a) [39]. The bands of C–H stretching vibrations (2925 cm^{-1} and 2850 cm^{-1}), S–H stretching vibration (2597 cm^{-1}) and the Si–O–H and Si–O–Si groups (1185 cm^{-1} , 1099 cm^{-1} and 942 cm^{-1}) confirm the existence of silane and thiol moieties on the surface of the MNPs-SH (**Fig. 2b**). The appeared band at around 2965 cm^{-1} , 1711 cm^{-1} , 1659 cm^{-1} and 1551 cm^{-1} due to the stretching vibrations of (C–H), (C=O), (N–C=O) and (C=C) can confirm polymerization of PAIP on the surface of MNPs-SH (**Fig.2c**). The FTIR spectrum of $\text{MNP}_5\text{@PAIP-CD}$ shows the bands at around 2900 cm^{-1} with high intensity that has resulted from the increment of CH_2 groups indicating the conjugation of CD to $\text{MNP}_5\text{@PAIP}$ (**Fig.2d**). All FTIR results confirm that surface of MNP_5 has successfully functionalized with CD.

Fig. 2. FT-IR spectra of the prepared (a) MNP_5 , (b) $\text{MNP}_5\text{-SH}$, (c) $\text{MNP}_5\text{@PAIP}$ (d) $\text{MNP}_5\text{@PAIP-CD}$.

The UV–Vis spectroscopy was used to further confirmation of FA on the surface of MNPs@PAIP-CD. As shown in **Fig. 3**, the UV–Vis spectrum of MNPs@PAIP-CD-FA shows three absorption peaks at 234, 282, and 362 nm, due to the presence of FA [40].

Fig. 3. UV–Vis spectra of folic acid (0.01 mg/mL), MNPs@PAIP-CD (0.01 mg/mL) and MNPs@PAIP-CD-FA (0.01 mg/mL) in ethanol.

Surface morphology of the MNPs was studied by the field emission scanning electron microscope (FESEM) and transmission electron microscope (TEM) that have been shown in **Fig. 4**. The FESEM results showed that the prepared magnetic nanoparticles were regular quasi-spherical with relatively uniform size distribution. More importantly, the morphology of the magnetic core (Fe_3O_4) did not change after surface functionalization by polymerization and CD conjugation (**Fig. 4a-b**). TEM image of MNPs@PAIP-CD shows

spherical nanoparticles with a distinct structure. The size of MNPs@PAIP-CD was also analyzed by DLS, in which narrow dispersity was observed (PDI=0.240) for prepared MNPs (**Fig. 4c**).

Fig. 4. (a, b) SEM images of MNPs@PAIP and MNPs@PAIP-CD and TEM (c) images of MNPs@PAIP-CD.

The chemical structure of MNPs, before and after polymerization and CD conjugation were also studied using X-ray diffraction (XRD). All samples showed similar six diffraction peaks at 2θ values of 30.1, 35.4, 43.1, 53.6, 57.1 and 62.7° that can be assigned to the diffractions of (2 2 0), (3 1 1), (4 0 0), (4 2 2), (5 1 1) and (4 4 0), respectively (**Fig. 5**). The reflection peak positions of Fe₃O₄ MNPs were in well agreement with the reported XRD patterns [34, 39 and 41] and indicated that the crystalline structure of Fe₃O₄ was preserved after the polymerization and CD conjugation processes.

Fig. 5. XRD pattern of the MNPs, MNPs@PAIP and MNPs@PAIP-CD.

The magnetic properties of MNPs, MNPs@PAIP, and MNPs@PAIP-CD were evaluated by a vibrating sample magnetometer (VSM) at RT (300 K) (**Fig. 6**). The amounts of specific saturation magnetization (σ_s) for MNPs, MNPs@PAIP and MNPs@PAIP-CD were 60, 25, and 19.5 emu/g, respectively, which suggested the

superparamagnetic nature of the synthesized nanoparticles. The decrease in the amount of the saturation magnetization of modified MNPs can be attributed to the application of organic moieties (PAIP and CD) on the surface of the MNPs. The results are in good harmony with the obtained results of TGA.

Fig. 6. Magnetization curves of (a) MNPs, (b) MNPs@PAIP and (c) MNPs@PAIP-CD; (d) and (e) the magnetic separating of nanocarrier.

TGA analyses were undertaken at the temperature range from RT to 800 °C, at rate of 20 °C/min in argon flow. The TGA curves of MNPs, MNPs-SH, MNPs@PAIP and MNPs@PAIP-CD are shown in **Fig. 7**. The TGA curve of MNPs-SH showed the weight-loss of about 8% resulting from decomposition of propyl thiol groups on the surface of MNPs and removal of adsorbed water (**Fig. 7b**). TGA analysis of MNPs@PAIP showed the weight loss of 50% which indicated the amount of the grafted PAIP (**Fig. 7c**). The comparison between the TGA curves of MNPs@PAIP-CD and MNPs@PAIP revealed a

major decomposition with the weight loss of about 55%, indicating about 5% surface grafting of MNPs@PAIP by CD (**Fig. 7d**).

Fig. 7. TGA analyses of (a) Fe_3O_4 MNPs, (b) MNPs-SH, (c) MNPs@PAIP and (d) MNPs@PAIP-CD.

3.2. DTX encapsulation and release behavior

DTX is one of the most important anticancer drugs with a very low solubility in water. To evaluate the capability and efficacy of synthesized nanoparticles as an anti-cancer carrier, DTX was selected as a model drug. The DTX entrapment efficiency and DTX loading content in the MNPs@PAIP-CD-FA were 75% and 15%, respectively. Different factor can affect the drug release rate including drug solubility, drug diffusion through the nanoparticle matrix, desorption of the surface-bound/adsorbed drug, nanoparticle matrix erosion or degradation, and the combination of erosion and diffusion processes [42]. Hence, polymer composition, molecular weights of the hydrophobic and hydrophilic segments, the concentration and polymer degradation rate control the release kinetics of

DTX from MNPs@PAIP-CD-FA [43]. The cumulative percentages of released DTX from the MNPs@PAIP-CD-FA in 2 mL of PBS solution (pH 7.4) during 100 h are shown in **Fig. 8**. The DTX release pattern display a relatively rapid release phase with about 40% drug cumulative release during first 24 h and followed by a slow and continuous release with about 71.1% drug cumulative release after 100 h in PBS at 37 °C. The initial release (within the first 24 h) could be mainly attributed to the weakly bound or adsorbed drug on the surface of nanoparticles. The second phase with a relatively slow release up to 100 h, could also be attributed to the drug diffusion through the nanoparticles matrix.

Fig.

8. Cumulative drug release profiles of DTX loaded MNPs_s@PAIP-CD-FA at different conditions: (PBS, pH: 7.4 and pH: 5.5 T = 37 °C).

3.3. *In vitro* cytotoxicity studies

Cytotoxicity assay (MTT assay) was applied to distinguish the biocompatibility and the *in vitro* cytotoxicity of the synthesized MNPs. The HEK 293 (Human Embryonic Kidney

293) normal cell line was used to study the cell viability of MNPs. The MNPs@PAIP-CD and MNPs@PAIP-CD-FA showed no significant cytotoxicity on the HEK 293 cells that confirms their biocompatibility at concentrations of 100, 200 and 500 $\mu\text{g/mL}$ for 24 h. As shown in **Fig. 9**, the cellular viability in the presence of MNPs@PAIP-CD and MNPs@PAIP-CD-FA were about 93% and 97% after 24 h, respectively. The results demonstrated that the modified MNPs do not exhibit apparent cytotoxicity. Also, the cytotoxic effect of the DTX loaded MNPs was evaluated on the HeLa and MDA-MB-231 cancer cell lines using cytotoxicity assay. **Figs. 10** and **11** shows that the growth inhibition of HeLa and MDA-MB-231 cells by the free DTX, MNPs@PAIP-CD, MNPs@PAIP-CD-FA, DTX loaded MNPs@PAIP-CD, and DTX loaded MNPs@PAIP-CD-FA at 100, 200 and 500 $\mu\text{g/mL}$ concentrations for 24, 48, and 72 h. The results showed that DTX loaded MNPs@PAIP-CD-FA reduced the growth of HeLa and MDA-MB-231 cells more than free DTX and DTX loaded MNPs@PAIP-CD after 24, 48, and 72 h. This suggests that MNPs@PAIP-CD-FA can increase the targeted delivery of DTX to the cancer cells *via* FA-receptor binding endocytosis.

Fig. 9. Viability of HEK cells treated with MNPs@PAIP-CD-FA and MNPs@PAIP-CD at various concentrations for 24 h.

Fig.10. Viability of MDA cells incubated with synthesized MNPs and free drug for 24,48 and 72 h, at temperature of 37 °C (*p < 0.05, **p < 0.01 and ***p < 0.001).

Fig.11. Viability of HeLa cells incubated with synthesized MNPs and free drug for 24,48 and 72 h, at temperature of 37 °C (*p < 0.05 and **p < 0.01).

3.4. *In vitro* cellular uptake

After successful synthesis and full physical and chemical analyses, intracellular uptake ability of DTX loaded MNPs@PAIP-CD-FA was evaluated. For this purpose, DTX loaded MNPs@PAIP-CD-FA which possessed some free sites, was incubated with rhodamine B to tag the nanocarrier. Then, the HeLa cell line as a FA-receptor expressing cancerous cell line was treated with rhodamine B tagged DTX loaded MNPs@PAIP-CD-FA for investigation of intracellular uptake ability of the MNPs (**Fig. 12**). After 4 h incubation at 37 °C, the cells were evaluated using fluorescent microscopy. The cytoplasm of all HeLa cells was highly fluorescent due to the presence of rhodamine B tagged MNPs. In addition, rhodamine B tagged MNPs demonstrated cellular uptake about 90% after 4 h incubation. These results along with the data of cytotoxicity assays on FA receptors expressing cells showed that the increase of internalization of drug is presumably mediated by FA receptor. In consistent with our results, it has been shown that these multifunctional MNPs could be used for targeting cancerous cells which increase the intracellular uptake through folic acid receptor [44].

Fig. 12. Fluorescence microscopy images of HeLa cells incubated with DTX loaded MNPs@PAIP-CD-FA and MNPs@PAIP-CD for 4 h at 37 °C;(a and b for DTX loaded MNPs@PAIP-CD-FA, c and d for DTX loaded MNPs@PAIP-CD, right images: fluorescence field, left images: bright field).

4. Conclusion

The unique structure of magnetic nanoparticles has made them promising and admirable materials for a wide range of biological applications such as drug and gene delivery. Herein, we have designed, synthesized and characterized a novel and robust magnetically nano-drug delivery system to improve the DTX targeted delivery. The constructed nanocarrier showed proper loading capacity and release profile. Moreover, the cytotoxicity profile and intracellular uptake were clearly showed no apparent cytotoxicity for nanocarrier against normal and cancerous cell lines, nonetheless DTX loaded nanocarrier

showed significant cytotoxicity on both HeLa and MDA-MB-231 cells. Moreover, the presence of FA decreased the cell viability of DTX loaded nanocarriers in comparison with nanocarriers without FA and also increased DTX penetration due to the presence of FA receptors. The proper inherent magnetic properties of the nanocarriers can make them as suitable candidate for theranostic applications after ensuring their MRI imaging abilities.

Acknowledgment

This study was supported by grants from the Research Council of Tehran University of Medical Sciences, Nanomedicine and Tissue Engineering Research Center, Shahid Beheshti University of Medical Sciences and the Iran National Science Foundation (INSF).

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