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Synthesis and characterization of Fe_3O_4-MPTMS-PLGA nanocomposites for anticancer drug loading and release studies

Ceren Atila Dinçer[a], Nuray Yildiz[a], Ayşe Karakeçili[a], Nihal Aydoğan[b] and Ayla Çalimli[a]

[a]Department of Chemical Engineering, Ankara University, Ankara, Turkey; [b]Department of Chemical Engineering, Hacettepe University, Ankara, Turkey

ABSTRACT

Magnetic nanocomposites (Fe_3O_4-MPTMS-PLGA) were synthesized by single oil emulsion method and characterized by transmission electron microscopy (TEM), X-Ray diffraction (XRD), and vibrating sample magnetometer (VSM). Particle size of nanocomposites was between 117 nm and 246 nm. High performance liquid chromatography (HPLC) was used to investigate drug loading (paclitaxel, PTX) and release from Fe_3O_4-MPTMS-PLGA-PTX nanocomposites. The percentages of drug loading and encapsulation efficiency onto nanocomposites were found as 7.35 and 68.58, respectively. Cytotoxities of free anticancer drug and anticancer drug-loaded nanocomposites were determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. *In vitro* cell culture studies indicated that Fe_3O_4-MPTMS-PLGA-PTX had significant toxicity on MG-63 cancer cells.

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KEYWORDS
Fe_3O_4 nanoparticles; silane compounds; magnetic polymer nanocomposites; anticancer drug

Introduction

The anticancer drugs can be delivered in the body via two types of mechanisms, including active and passive targeting. Magnetic-targeted drug delivery is one of the active targeting method which has a great importance of decreasing high regional concentration, its side effects, and preventing adverse effects to other cells in the body (Barreto et al. 2011, Sun et al. 2014). For this purpose, superparamagnetic nanoparticles being useful for bioapplications can be coated by natural and synthetic polymers such as gelatin (Gaihre et al. 2009), chitosan (Zhu et al. 2009), dextran (Tassa et al. 2011), polyvinyl alcohol (PVA) (Kayal and Ramanujan 2010), polyethylene glycol (PEG) (Butterworth et al. 2001), poly(L-lactic acid) (PLLA) (Hu et al. 2006), and poly(DL-lactide-co-glycolide) (PLGA) (Jia et al. 2012) commonly. Some compounds such as silanes and oleic acid can be used to modify superparamagnetic nanoparticles for increasing the coating efficiency and stabilization (Chomoucka et al. 2010, Wu et al. 2008). There are many studies on the synthesis of magnetic polymer nanocomposites and anticancer drug loading on this structure in the literature. Lv et al. synthesized methoxy PEG-PLGA nanocomposites and loaded the chemotherapeutic agent evodiamine by solvent evaporation technique (Lv et al. 2013). Cho et al. investigated Fe_3O_4 (magnetite)-quantum dot-PLGA nanocomposites as nanocarrier system of anticancer drug paclitaxel (taxol) (Cho et al. 2010). Cheng et al. coated Fe_3O_4 nanoparticles by the fluorescent labeling molecules of fluorescein isothiocyanate (FITC)-modified gelatin to investigate prodrug platinum (IV) delivery and magnetic resonance image (MRI) applications (Cheng et al. 2014). Dilnawaz et al. used glycerol monooleate-coated magnetic nanoparticles as hydrophobic anticancer drugs (paclitaxel, rapamycin alone, or combination) carriers. *In vitro* cell culture studies indicated an enhanced uptake of magnetic nanoparticles in human breast carcinoma cell line (MCF-7) (Dilnawaz et al. 2010). Akbarzadeh et al. synthesized superparamagnetic iron oxide-PLGA-PEG nanocomposites and encapsulated anticancer drug doxorubicin hydrochloride into this structure. It was concluded that magnetic nanoparticles can be used for medical applications (Akbarzadeh et al. 2012).

In this study, surface modified with 3-(trimethoxysilyl)-propyl methacrylate (MPTMS) superparamagnetic nanoparticles were coated by PLGA polymer in order to synthesize magnetic nanocomposites. To our knowledge, there is no study in the literature anticancer drug loading and release studies of Fe_3O_4-MPTMS-PLGA nanocomposites. Poly(DL-lactide-co-glycolide) (PLGA) is among the widely used biodegradable polymers for bioapplications because metabolite monomers lactic acid and glycolic acid are obtained after its hydrolysis (Danhier et al. 2012). For the drug loading and releasing experiments paclitaxel (taxol, PTX) (Figure 1) anticancer agent which can be used for breast, lung, ovarian, skin, head, and neck cancers was selected.

Paclitaxel was first isolated from the bark of the northwestern pacific yew tree *Taxus brevifolia* in 1971. This anticancer drug is white crystalline powder and poorly soluble in water (Yildiz et al. 2007, Schleich et al. 2013).

CONTACT Nuray Yildiz nyildiz@eng.ankara.edu.tr Department of Chemical Engineering, Ankara University, Tandoğan, Ankara 06100, Turkey

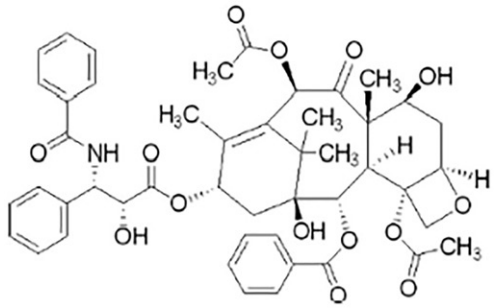

Figure 1. Chemical structure of paclitaxel (taxol) (Koneracka et al. 2008).

Experimental

Material

Iron (II) chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$, 99%), iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$, 97%), sodium hydroxide (NaOH, $\geq$ 98%), hydrochloric acid (HCl, 37%), toluene (HPLC grade) were purchased from Sigma-Aldrich. Deionized water was used for the synthesis of magnetic nanoparticles. 3-(Trimethoxysilyl)propyl methacrylate, (MPTMS, $\geq$ 98%, Sigma-Aldrich), was used for the surface modification of Fe_3O_4 nanoparticles. Poly(DL-lactide-co-glycolide) (PLGA 50:50, M_w 30,000–60,000, Sigma-Aldrich), poly(vinyl alcohol) (PVA, M_w 13,000–23,000, 98% hydrolized, Sigma-Aldrich), dichloromethane (DCM, Sigma-Aldrich), phosphate buffer saline (PBS, Sigma-Aldrich), tween 80 (Merck), and paclitaxel ($\geq$% 98, Sigma-Aldrich) were used for synthesizing magnetic polymer nanocomposites, drug loading, and release studies. Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), trypsin-EDTA, and penicillin–streptomycin, 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma-Aldrich.

Synthesis of Fe_3O_4-MPTMS-PLGA nanocomposites

Synthesis and surface modification of Fe_3O_4 magnetic nanoparticles were realized based on our previous study (Atila-Dinçer et al. 2014). Briefly, Fe_3O_4 nanoparticles were synthesized by co-precipitation method of the iron salts (Fe^{2+}/Fe^{3+}=0.5) in alkaline medium at 80 °C under inert atmosphere. To modify the surface of Fe_3O_4 nanoparticles, excess amount of silane compounds 3-(trimethoxysilyl)propyl methacrylate were dispersed in toluene under inert atmosphere, and then the solution was stirred at room temperature for 24 h. Magnetic nanoparticles were collected by centrifugation and dried under vacuum.

Fe_3O_4-MPTMS-PLGA nanocomposites were prepared by single oil in water emulsion method (o/w) with slight modifications (Ashjari et al. 2011, Tansik et al. 2014). Surface-modified Fe_3O_4 nanoparticles were dispersed in DCM using an ultrasonic probe, and the dispersion was mixed with an organic solution of PLGA in DCM in an ice bath (Fe_3O_4-MPTMS/PLGA ratio 1/1–1/20). This organic mixture was immediately added into an aqueous PVA solution (1–3% w/v, 4–15 ml) and homogenized at 20,000 rpm for 30 s. The single oil in water emulsion was diluted in PVA solution (0.3–1% w/v, 50–75 ml). The organic phase was removed by mechanical stirring at room temperature overnight. Fe_3O_4-MPTMS-PLGA nanocomposites were dried by freeze-drying for 48 h and stored at +4 °C.

Synthesis of drug-loaded Fe_3O_4-MPTMS-PLGA nanocomposites

To obtain drug-loaded Fe_3O_4-MPTMS-PLGA nanocomposites, the similar emulsion method was used. The only difference of this method was addition of the anticancer drug (PTX) into the PLGA solution. The drug loading and encapsulation efficiency were determined by following equations (Papadimitriou and Bikiaris 2009):

$$\text{Drug loading } (\%) = \frac{\text{weight of encapsulated drug in nanocomposite}}{\text{weight of } Fe_3O_4 \text{ - PLGA - drug nanocomposite}} \times 100$$

$$\text{Encapsulation efficiency } (\%) = \frac{\text{weight of drug in nanocomposite}}{\text{weight of initial amount of drug}} \times 100$$

Particle characterization

The particle size of the magnetic nanoparticles was determined by transmission electron microscopy (TEM, FEI Tecnai G2) operating at 120 kV. The samples for TEM analysis were prepared on a copper grid covered with carbon. Magnetic nanoparticles were also analyzed by X-Ray diffraction (XRD, Rigaku Ultima-IV). Vibrating sample magnetometer (VSM, Cryogenic Limited PPMS) was used in order to determine magnetic behavior of nanoparticles.

In vitro *drug release from* Fe_3O_4-MPTMS-PLGA nanocomposites

The *in vitro* release of PTX from Fe_3O_4-MPTMS-PLGA nanocomposites was carried out in PBS medium (phosphate-buffered saline, pH 7.4 and pH 5.5) containing 1% (w/v) Tween 80. About 10 mg of paclitaxel-loaded magnetic nanoparticles was dispersed in PBS solution (10 ml), then the suspensions were placed in an orbital shaker at 150 rpm and 37 °C. At selected time intervals, the mixture was centrifuged at 10,000 rpm for 10 min, and the supernatants were kept for the analysis. The supernatants were extracted firstly with DCM and then with acetonitrile:water (50:50). Evaporation of DCM was realized under nitrogen flow. The precipitated magnetic nanoparticles were resuspended in fresh PBS medium (Mu and Feng 2001). The drug content and encapsulation efficiency of magnetic nanoparticles and *in vitro* drug release form the Fe_3O_4-MPTMS-PLGA-PTX were analyzed by HPLC method at 227 nm. A reverse-phase Inertsil ODS-3 column (250 × 4.6 mm, pore size 5 μm) was used. The mobile phase consisted of asetonitril:water (50:50) and flow rate, temperature, enjection volume were 1.5 ml/min, 40 °C, and 20 μl, respectively (Mu and Feng 2001, Pandit and Dash 2011).

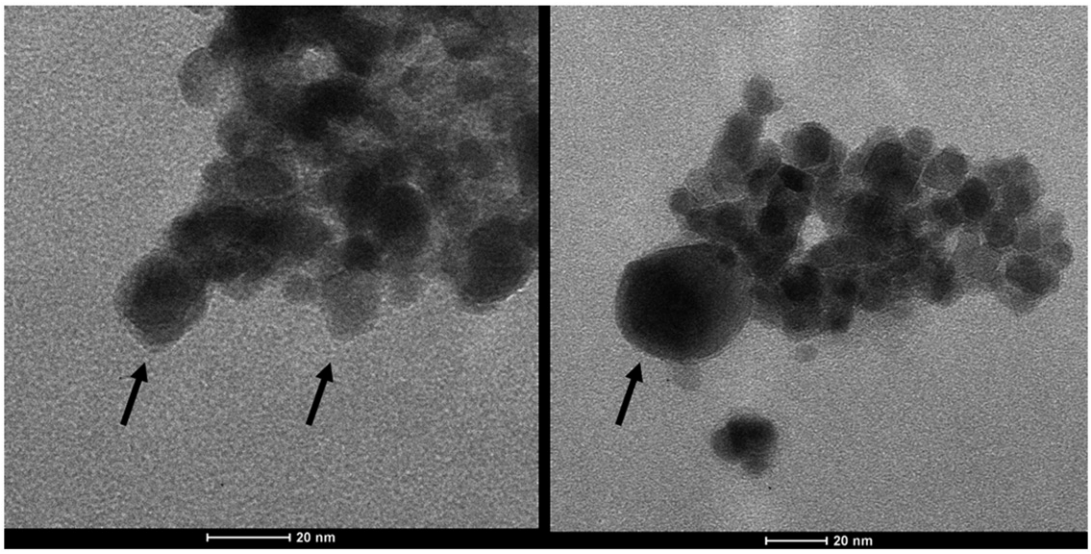

Figure 2. TEM images of the Fe_3O_4-MPTMS-PLGA (1/10 ratio) nanocomposites.

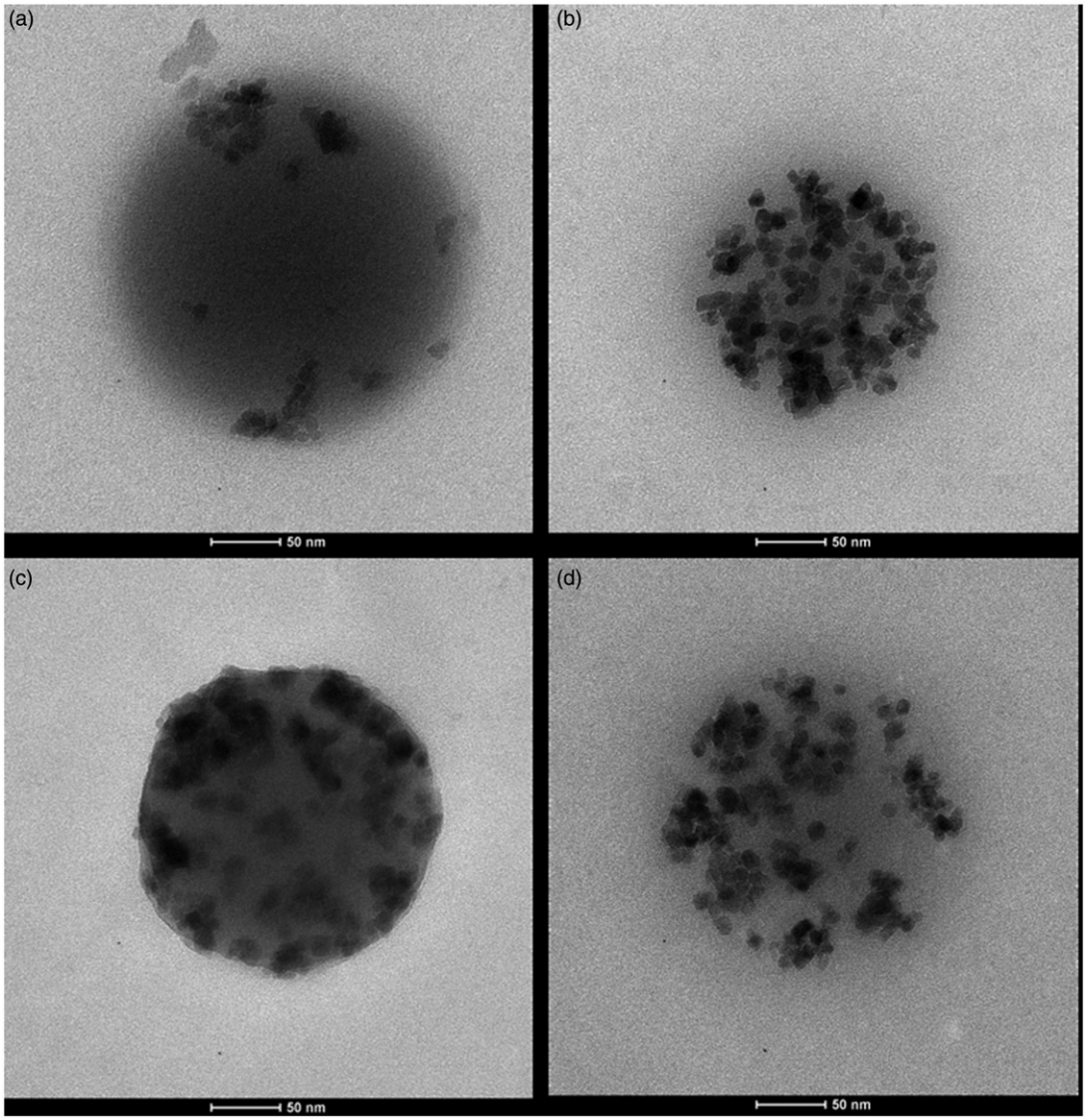

Figure 3. TEM images of the (a–b) Fe_3O_4-MPTMS-PLGA nanocomposites (c-d) drug (paclitaxel)-loaded Fe_3O_4-MPTMS-PLGA nanocomposites.

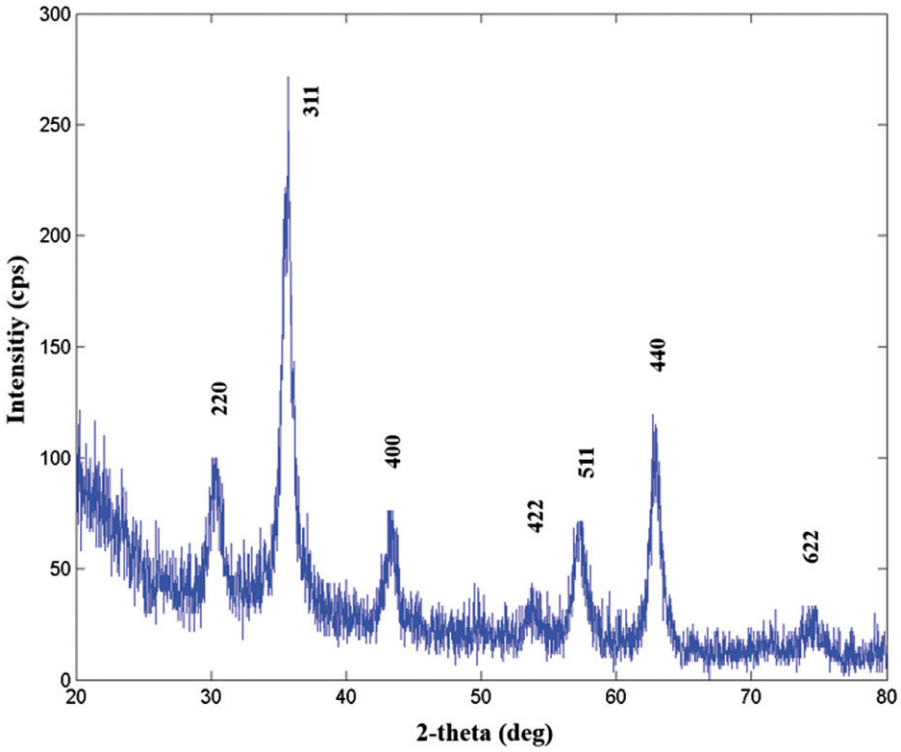

Figure 4. XRD patterns of Fe_3O_4-MPTMS-PLGA nanocomposites.

In vitro *cytotoxicity studies*

The cytotoxicity studies were carried out by 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. MG-63 osteosarcoma cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin mixture. The cells were grown in ambient conditions of 5% CO_2 at 37 °C in 24-well plates until they reached confluency. The media were replaced with fresh media containing free Paclitaxel or Fe_3O_4-MPTMS-PLGA-Paclitaxel with different drug concentrations (2, 10, 20 μg/ml). The control group of cells were cultured on tissue culture polystyrene (TCPS) 24-well plates. After 72 h, supernatants were removed, and cells were incubated with DMEM containing MTT for 3 h. After MTT solution was removal, isopropanol:HCL (0.1 N) was added to dissolve the formazan crystals. Absorbance was measured at 570 nm using a microplate reader (BioTek μQuant) with a reference wavelength of 630 nm (Karakeçili and Arikan 2012, Wang et al. 2015).

Statistical analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) using the software GraphPad InStat to determine statistical significance ($P < .05$). All data are expressed as means ± standard deviation (SD).

Results and discussion

TEM analysis

The mean particle size of Fe_3O_4 and Fe_3O_4-MPTMS magnetic nanoparticles was 8.8 ± 1.8 nm and 8.7 ± 1.8 nm, respectively

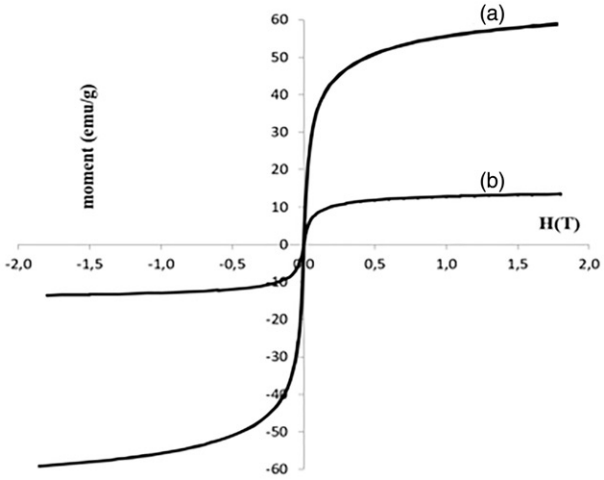

Figure 5. Magnetization curves of (a) Fe_3O_4-MPTMS nanoparticles (b) Fe_3O_4-MPTMS PLGA nanocomposites.

as previously reported (Atila-Dinçer et al. 2014). TEM images of Fe_3O_4-MPTMS-PLGA nanocomposites synthesized by o/w method (Fe_3O_4-MPTMS/PLGA ratio 1/10; 3% w/v, 4 ml and 0.3% w/v, 50 ml PVA solutions) are shown in Figure 2 with 1–2 nm polymer coating thickness. Magnetic nanocomposites having 1/15 and 1/20 of Fe_3O_4-MPTMS/PLGA ratio were ineffective due to excess amount of PLGA polymer. Due to lower polymer coating thickness, drug loading and release were not realized using nanocomposites synthesized at these conditions.

Figure 3 represents TEM images of the Fe_3O_4-MPTMS-PLGA nanocomposites (Figure 3(a,b)) and the drug-loaded Fe_3O_4-MPTMS-PLGA nanocomposites (Figure 3(c,d)) synthesized by o/w method (Fe_3O_4-MPTMS/PLGA ratio 1/1; 1% w/v, 15 ml and 1% w/v, 75 ml PVA solutions). TEM images showed

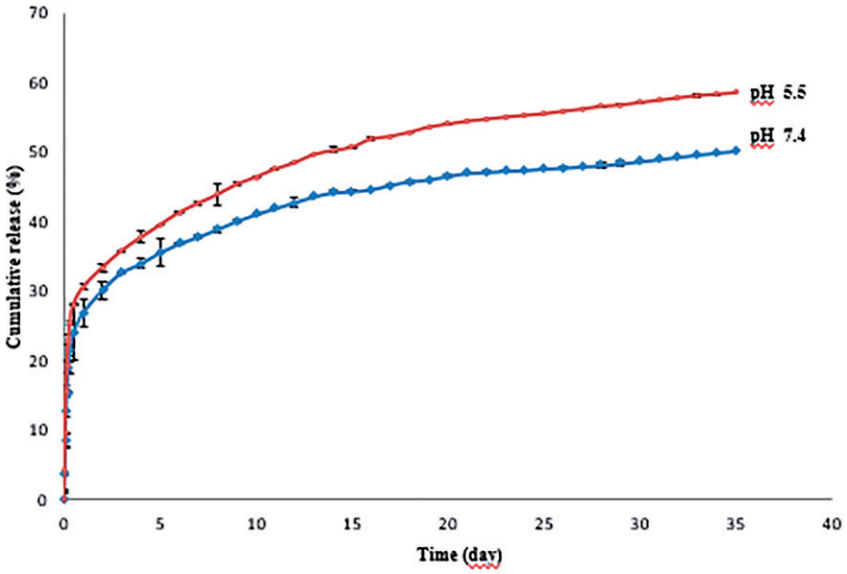

Figure 6. *In vitro* release of PTX in PBS at different pH values.

that Fe_3O_4-MPTMS nanoparticles were successfully embedded into the PLGA matrix. These nanocomposites were selected for the drug loading studies due to effective embedding with low agglomeration of the surface-modified magnetic nanoparticles inside the polymer matrix and high production yield. The particle size distribution of Fe_3O_4-MPTMS-PLGA and Fe_3O_4-MPTMS-PLGA-PTX nanocomposites was 117–246 nm and 120–248 nm, respectively. TEM images of the magnetic nanocomposites were compatible with those in the literature (Lee et al. 2005, Wassel et al. 2007, Kim et al. 2008).

XRD analysis

Fe_3O_4 and surface-modified nanoparticles had cubic spinel structure with XRD characteristic peaks at (220), (311), (400), (422), (511), (440), (622), as described in our previous work (Atila-Dinçer et al. 2014). Figure 4 shows the XRD pattern of Fe_3O_4-MPTMS-PLGA nanocomposites. The pattern of the nanocomposites indicated that the PLGA coating did not cause any change on the crystalline structure of Fe_3O_4. Similar results for the Fe_3O_4-PLGA nanocomposites synthesized by emulsion method were reported in the literature (Yang et al. 2014, Zhou et al. 2008). The crystallite size of magnetic nanoparticles in the polymer matrix was calculated by using Scherrer equation (Cengiz et al. 2008) as 3.4–11.3 nm for the characteristic planes which were compatible with the TEM results (Atila-Dinçer et al. 2014).

VSM analysis

The saturation magnetization of Fe_3O_4 and Fe_3O_4-MPTMS nanoparticles was obtained as 58.87 emu/g and 58.49 emu/g, respectively by VSM as stated in our previous study (Atila-Dinçer et al. 2014). Figure 5 shows the magnetization curve of the Fe_3O_4-MPTMS-PLGA nanocomposites in comparison to the Fe_3O_4-MPTMS curve. VSM curves with no coercivity indicated that polymer coating reduced magnetic properties of Fe_3O_4-MPTMS nanoparticles but did not affect the superparamagnetic behavior of the nanostructure. The low saturation magnetization of Fe_3O_4-MPTMS-PLGA nanocomposites (13.4 emu/g) was compatible with the literature (Liu et al. 2007, Sun et al. 2012, Rajan et al. 2014).

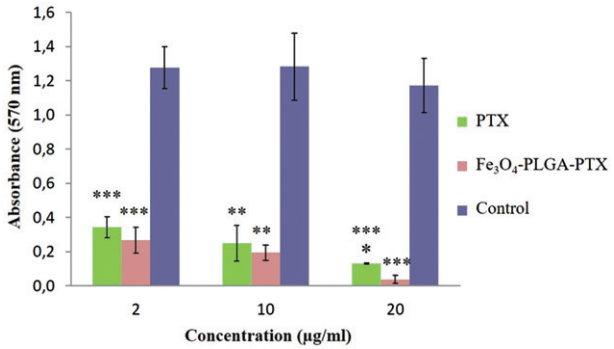

Figure 7. MTT results of the cytotoxicity test for MG-63 osteosarcoma cells. $^*P < .05$, $^{**}P < .01$, $^{***}P < .001$ significance levels according to control.

In vitro *drug loading and release studies*

Fe_3O_4-MPTMS-PLGA-PTX nanocomposites synthesized by o/w method (Fe_3O_4-MPTMS/PLGA ratio 1/1; 1% w/v, 15 ml and 1% w/v, 75 ml PVA solutions) were used for *in vitro* drug loading and releasing studies because of high production yield. The percentage of paclitaxel drug loading and encapsulation efficiency was determined as 7.35% and 68.58%, respectively. The percentage of drug loading was found higher than literature (Schleich et al. 2013). The drug release profiles of paclitaxel from magnetic nanocomposites are given in Figure 6. During the first 24 h, which is called burst release, 26.8% (pH 7.4) and 30.6% (pH 5.5) of paclitaxel were released in PBS medium at different pH values. The burst release was probably related to the excess drug particles released rapidly from matrix into buffer in the first few hours (Li et al. 2011). After 24 h, paclitaxel release was slower. After 35 days, the percentages of cumulative release of the anticancer drug were

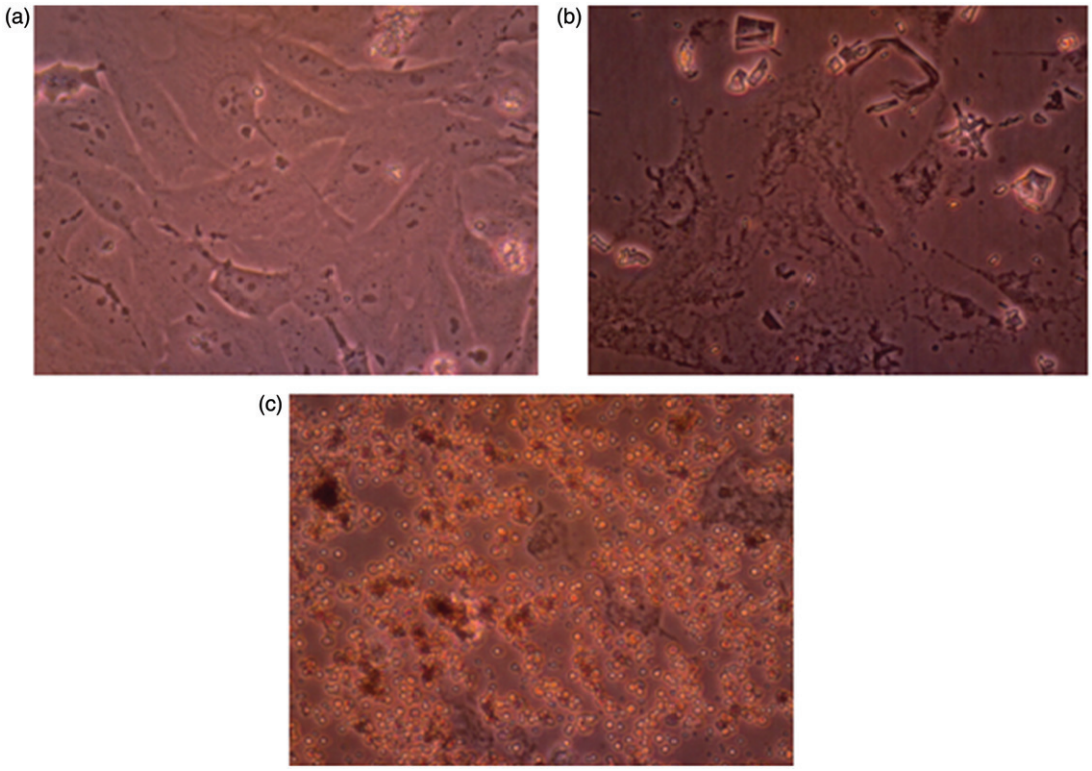

Figure 8. The optical microscope images of (a) control (b) free PTX (c) Fe_3O_4-MPTMS -PLGA-PTX (20 μg/ml, 72 h) on MG-63 cells.

determined as 50.3% and 58.63% at pH 7.4 and pH 5.5, respectively. The higher percentage of drug release at pH 5.5 is necessary for cancerous area in the body.

In vitro *cytotoxicity studies*

The cytotoxicity of free PTX and Fe_3O_4-MPTMS-PLGA-PTX nanocomposites was determined by the MTT test in MG-63 osteosarcoma cells after 72 h incubation. The absorbance values of the PTX and Fe_3O_4-MPTMS-PLGA-PTX nanocomposites for MG-63 osteosarcoma cells are given in Figure 7 in comparison to control groups.

As shown in Figure 7, increasing the drug concentration from 2 to 20 μg/ml caused a decrease in the absorbance values in the presence of free PTX and Fe_3O_4-MPTMS-PLGA-PTX. Fe_3O_4-MPTMS-PLGA-PTX nanocomposites showed higher cytotoxicity than free PTX at all drug concentrations (2, 10, 20 μg/ml) on the MG-63 cells. The absorbance values of free PTX and Fe_3O_4-MPTMS-PLGA-PTX ($P < .001$ at 2 and 20 μg/ml; $P < .01$ at 10 μg/ml) groups were significantly lower than control groups. The results indicated that controlled drug delivery was more effective than free drug on the cell death of the cancer cells. The optical microscope images of different experimental groups supported the graphical results. Increase in the drug concentration (2, 10, 20 μg/ml) led to a significant decrease in viable MG-63 cell density. The effect of free PTX and Fe_3O_4-MPTMS-PLGA-PTX (20 μg/ml drug concentration) on MG-63 cells in comparison with the control group is given in Figure 8. The original morphology seen in the control group of the MG-63 cells (Figure 8(a)) were damaged in the presence of anticancer drug (Figure 8(b)) and anticancer drug-loaded nanocomposites (Figure 8(c)) (Schleich et al. 2013, Cui et al. 2013, Abulateefeh et al. 2013).

Conclusion

In this study, Fe_3O_4-MPTMS-PLGA nanocomposites were successfully synthesized by single oil in water (o/w) emulsion method. The particle size of superparamagnetic nanocomposites was determined between 117 and 246 nm. The crystal structure of Fe_3O_4-MPTMS nanoparticles did not change by polymer coating. The anticancer drug paclitaxel was loaded on the magnetic polymeric nanocomposites with 7.35 drug loading and 68.58 encapsulation efficiency percentages. Fe_3O_4-MPTMS-PLGA-PTX nanocomposites had significant toxicity on the cancer cells. The results showed that superparamagnetic polymeric nanostructures could be used for targeted drug delivery.

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Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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