RESEARCH ARTICLE

Synthesis and cytotoxicity evaluation of electrospun PVA magnetic nanofibers containing doxorubicin as targeted nanocarrier for drug delivery

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ABSTRACT

Objective(s): The purpose of this study was preparation and evaluation of PVA-Fe_3O_4 nanofibers as nanocarrier of doxorubicin (DOX) by measuring their drug release together with their in vitro cytotoxicity toward cancer cells at different pH values.

Methods: Fe_3O_4 nanoparticles were synthesized by coprecipitation method. The composite nanofibers of polyvinyl alcohol containing nanoparticles and anticancer drug DOX were fabricated by electrospinning method. The nanostructures were characterized by different techniques. The drug release was investigated by UV-Vis spectrophotometer at different pHs and 37.5˚C.

Results: In vitro drug release experiments show that the doxorubicin release at pH=6.0 is promisingly more and faster than drug release at pH= 7.4. The fitted equation of release curves corresponds to Peppas model. Also, MTT assays indicate that the MNPs-doxorubicin-loaded nanocarrier has cytotoxicity comparable with free drug.

Conclusions: The synthesized nanocarrier was successfully used for the efficient delivery of an anti-cancer drug into the tumor region. The DOX-loaded nanocarrier showed a steady and sustained release profile in vitro up to 72 h. The drug release from nanocarrier was better described using Peppas model.

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INTRODUCTION

Cancer is a group of diseases involving abnormal cell growth that can affect any part of the body and causes a high number of deaths in the world [1]. Doxorubicin is an anthracycline antibiotic with a broad spectrum of antitumor activity, including various solid tumors in humans and animals [2]. These solid tumors are destroyed using conventional chemotherapy, which exhibit this method is not safe and has a low efficiency for patients. Drug delivery systems are useful strategies for administering more performance and safe treatments in real scenarios [3,4].

Among the numerous approaches used for this purpose, targeting based on magnetic nanoparticles, mainly MNPs, is widely considered as a promising targeted delivery system [5]. This is due to its distinct advantages such as well biosafety, easy preparation, the possibility of controlling the nanocarrier characteristics, affordability of needed

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materials and more importantly, the possibility of targeting the interest drugs to the desired location within the host body using an external magnetic field [6,7].

Recently, polymeric nanofibers are known as a novel supports type. The polymers offer unique features for modification of around environment of magnetic nanoparticles and access to the catalytic sites [8-10]. So far, various nanocarriers as composite nanofibers have been synthesized with different methods and used for DOX delivery [11-15]. Among various methods for nanofibers building, electrospinning is a versatile and facile method for production of nanofibers with long uniform structure from organic, inorganic and organic/inorganic hybrid materials with diameters ranging from submicron to nanometers [16]. Electrospinning uses electrical forces for production of fibers from the polymer solution. This electrostatic technique uses a high voltage polymer solution and ejects a liquid jet through a spinneret [17]. Through electrospinning technique, various morphologies structures can be achieved with high porosity, specific surface area, flexibility and stability [18-21]. Polyvinyl alcohol (PVA) is a water-soluble polymer with good biocompatibility [22].

Combining good biocompatibility, flexibility and porous structures of PVA nanofibers, we used this solution to the preparation of composite nanofibers containing MNPs. In this work, the PVA-Fe_3O_4 nanofibers were evaluated as nanocarrier of DOX by measuring their drug release together with their in vitro cytotoxicity toward cancer cells at two different pHs.

MATERIALS AND METHODS

A Sonorex RK255 ultrasonic water bath was used for Fe_3O_4 synthesis. The electrospinning apparatus was purchased from Fanavaran Nano Meghyas (Fnm-ES1000, Tehran, Iran). A Metrohm 827 pH/Ion Meter was used for pH measurements. XRD data was recorded by a Rigaku D-max C III, X-ray diffractometer using Ni-filtered Cu Kα radiation (Tokyo, Japan). A Shimadzu system FT-IR 8400 spectrophotometer using KBr pellets was used to record spectra. A Varian scanning spectrophotometer (CARY 50 Conc) was employed (Agilent, American). The samples were characterized with SEM (Hitachi S-9220) with gold coating.

Iron (III) chloride hexahydrate ($FeCl_3.6H_2O$) and iron (II) sulfate dihydrate ($FeSO_4.2H_2O$) were purchased from Sigma-Aldrich. PVA and ammonium hydroxide (NH_4OH) were purchased from Merck. Anticancer drug of doxorubicin was prepared from Pharmacia Italia S.P.A.

Synthesis of Fe_3O_4 nanoparticles

First, 1.6g $FeSO_4.2H_2O$ and 3.8g $FeCl_3.6H_2O$ were dissolved in 100mL water. 10mL of 20% NH3 solution was slowly added to the solution under ultrasonic waves (100 W/50°C) for 15 min. The whole process was carried out under N2 atmosphere. After a period, a black suspension obtained. The precipitated powders were separated applying an external magnetic field and washed several times with distilled water and ethanol. At last, the Fe_3O_4 magnetic nanoparticles were dried in an oven at 70°C for 12 h. Fe_3O_4 nanoparticles have confirmed with FT-IR, XRD, SEM and VSM.

Preparation of polymer solutions containing Fe_3O_4 nanoparticles and DOX

Polymer solutions for electrospinning process were prepared by dispersing 0.01 g Fe_3O_4 nanoparticles in deionized water and addition to the various concentration of PVA water solution at the room temperature. After shaking at 1500rpm for 1h, these solutions were electrospuned under different conditions. The best polymer solution concentration was selected based on SEM results. Eventually, 3.5 %w/w doxorubicin was added to the polymer solution containing nanoparticles and electrospinning process was carried out in optimum condition for preparation of nanocarrier.

Electrospinning procedure

The composite aqueous solutions with different concentrations were placed in a 5mL syringe attached to a needle with 18 gauge (0.216 mm) diameter. The syringe was fixed at 15cm distance of the collector which was covered with aluminum foil. The high voltage anode was connected to the needle, and the cathode was connected to the collector. By applied voltage of 25±0.1 and solution flow of 0.5 mL h-1, the droplet was disintegrated into fibers and eventually was deposited on the foil.

Conditions for DOX release from the DOX-loaded nanocarrier

For investigation of pH-based tissue targeting,

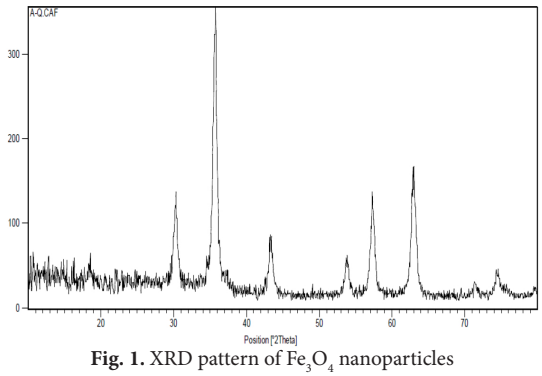

Fig. 1. XRD pattern of Fe_3O_4 nanoparticles

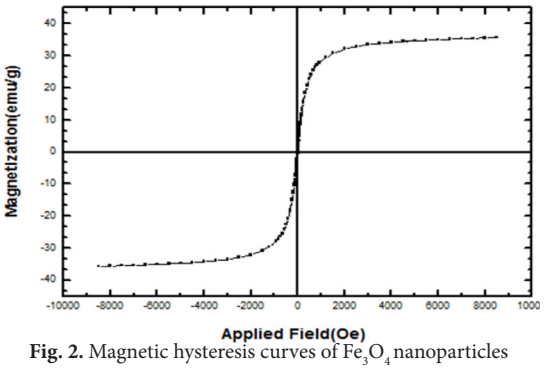

Fig. 2. Magnetic hysteresis curves of Fe_3O_4 nanoparticles

considering the acidic nature of the tumor tissue compared to the healthy tissues. The doxorubicin release from the targeted nanofibers was investigated at two different pH of 7.4 and 6.0 (equal blood and tumor environment) at 37˚C. The DOX-loaded nanocarrier was transferred to a dialysis bag and placed in 20mL of PBS. In each of the selected time intervals, 2mL of the solution was removed, and subjected to UV-Vis assay at 480 nm to determine the doxorubicin content, and the amount of released drug was calculated.

Conditions for cytotoxicity experiment

The MCF-7 cells were cultured in 10% FBS containing DMEM, 25mM glucose, 100µg mL^{-1} streptomycin and 100U mL^{-1} penicillin in an incubator containing 5% CO2 at 37˚C. The growing cells were seeded in a 96 well plate at a density of 5000 and stored for 24 h. The cells were incubated with the nanocarriers containing different concentrations of DOX after 24h. After incubation with various treatments, the viability of cells was investigated using a MTT assay. 20µL MTT (5mg mL−1) was added to each well. After 2h, optical absorption measurements were performed at 490 nm.

RESULTS AND DISCUSSION

Nanostructures Characterization

Fe_3O_4 nanoparticles were characterized by XRD for investigation of crystalline structure. As shown in Fig. 1, the position and relative intensity of the reflection peaks at (220), (311), (400), (422), (511), (440) and (533) demonstrate the cubic structure of Fe_3O_4 (ICSD CARD#01-072-2303). Moreover, the crystaline size of the Fe_3O_4 nanoparticles was calculated 18.0 nm by Debye-Scherrer formula [23].

The magnetic hysteresis curves of the Fe_3O_4 nanoparticles is shown in Fig. 2. In this magnetization curve, hysteresis loop was not observed. Therefore, nanoparticles exhibited typical superparamagnetic behavior. The saturation magnetization (Ms) was 36.0emu g^{-1}, that is enough for biomedical applications.

Effects of concentration on fibers morphology were investigated in the concentration range of 8-14%w/v PVA solutions. At the concentration of lower than 8%w/v and higher than 14%w/v, no continuous fibers was obtained because of lower and higher viscosity of the solution, respectively. Fig. 3 shows the SEM images of PVA nanofibers in the concentration of 10 %w/v containing 0.01g MNPs and 3.5%wt. DOX on aluminum foil. When the concentration is 10w/v%, electrospun nanofibers are smooth and have a bead. The average diameter of nanofibers is 60nm. The concentration of 10%w/v was selected for nanocarrier preparation.

Fig. 4 shows the FT-IR spectra of (a) MNPs, (b) PVA, (c) DOX and (d) nanocarrier containing DOX. The characteristic band of Fe-O at 573cm^{-1} in Fig. 4(a) was indicative of Fe_3O_4. The peaks at 1618 and 3389 cm^{-1} were indicative of bending and stretching vibration of OH. The existence of peaks in 1462 and 2329cm^{-1} (Fig. 4 (b)) is related to stretching and bending vibration of C-H and in 1096 cm^{-1} was related to C-O in PVA. The characteristic bands at 1072, 1615 and 1585cm^{-1} were attributed to the C-O-C, C=O and NH_2 groups of DOX (Fig. 4 (c)). The FTIR spectrum of nanocarrier in Fig. 4 (d) show that the Fe-O, O-H and NH_2 peaks related to Fe_3O_4, PVA and DOX shifted to 610, 3344 and 3303cm^{-1} in nanocarrier. This shows that the DOX was successfully encapsulated.

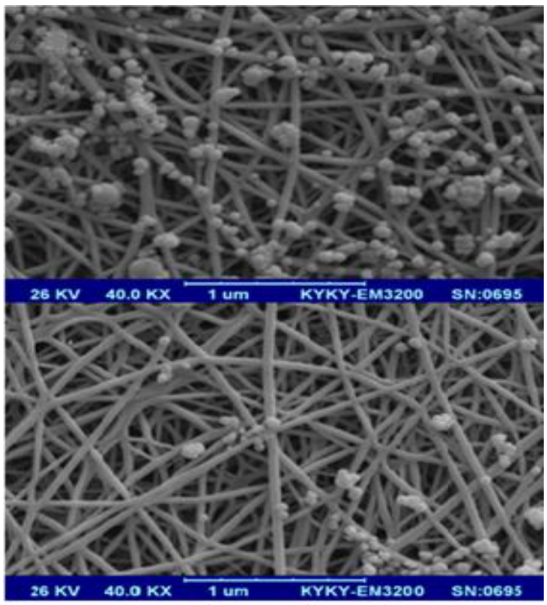

Fig. 3. SEM images of PVA nanofibers in the concentration of 10%w/v containing 0.01g MNPs and 3.5%wt. DOX

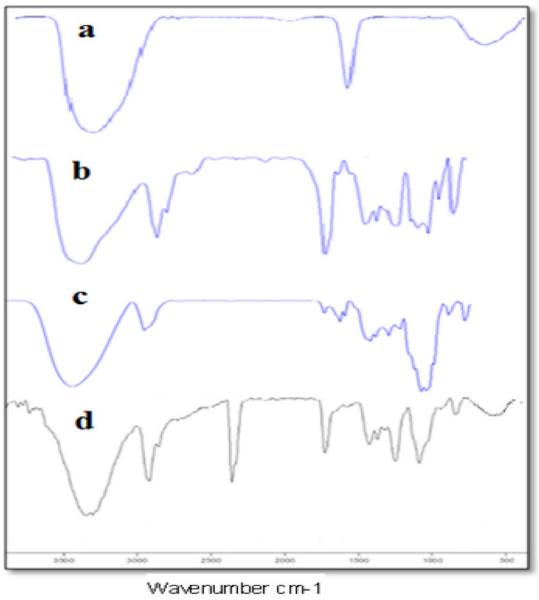

Fig. 4. FT-IR spectra (a) Fe3O4 nanoparticles, (b) PVA, (c) DOX, (d) DOX-loaded nanocarrier

In vitro release of DOX in pH=6 and 7.4

The behavior of DOX-loaded nanocarrier for drug release was investigated in PBS (pH 6) at 37 ˚C. As shown in Fig. 5a, at first, an initial burst of DOX release in during 5h, and then a sustained and slow release were occurred over 3 days. The initial burst maybe attributed to the absorbed DOX molecules on nanocarrier surface. Moreover, the cumulative amount of released DOX from the nanocarrier was 23.3% during 72h.

Fig. 5b shows the release profiles of DOX from nanocarrier in PBS (pH 7.4) at 37˚C. The cumulative release amount of DOX from composite nanofibers was 13.8% after 72h.

The results show that drug release is dependent on the medium pH and releasing time. The drug release was slow and steady with a ratio of 13.8% during 72 h at pH 7.4. The drug release was faster with a ratio of 23.3% during 72 h at lower pH (pH 6.0). The most of DOX remain in the nanocarrier for a significant period at normal conditions (pH 7.4) and reduce the side effects to the normal tissues. Besides, the DOX-loaded nanocarrier has a faster release in tumor environment or inside the endosome and lysosome of tumor cells and lead to the considerable improvement in cancer treatment efficiency [24].

Drug release kinetics

The kinetics of drug release were investigated by fitting various standard models, and mathematical equations such as zero-, first-, Higuchi and Peppas equations were characterized [25]. Table 1. shows the results for calculation and comparison of equations and correlation coefficients. It was clearly observed that drug release from nanocarriers was better described using Peppas model where correlation coefficient was greater than 0.99.

Table 1. Simulated equations and correlation coefficients of release curves for different kinetic models

Kinetic models	Correlation coefficients	Line equations
Zero-order	0.4186	y = 0.0023x + 7.4381
First-order	0.6146	y = 0.0005x + 1.779
Higuchi	0.7500	y = 0.3737x + 0.042
Peppas	0.9959	y = 0.5575x - 0.3614

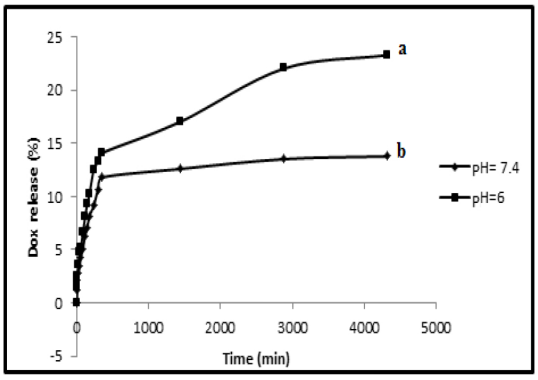

Fig. 5. Release profiles of DOX from nanocarrier in PBS at 37˚C (a) pH=7.4, (b) pH=6.0.

In vitro cytotoxicity study

In vitro antitumor effect of the free DOX and the DOX-loaded nanocarrier was evaluated in MCF-7 cells with the MTT assay. As shown in Fig. 6(a), the free DOX showed cytotoxicity slightly lower than that of DOX-loaded nanocarrier. The lower cytotoxicity value of the free DOX indicates that the free DOX rapidly diffuses into the cells and exerts antitumor activity where as the DOX-loaded nanocarrier exhibits a more sustained and controlled release in the intracellular compartments after cellular internalization. Interestingly, the nanocarrier did not significantly affect on tumor cell growth under the experimental conditions (up to 50 µg/mL; Fig. 6(b)). These results suggest that the nanocarrier are not inherently cytotoxic and cytotoxicity was caused by the DOX. Poly (vinyl alcohol) (PVA) have been approved by the FDA for use in the human body as drug delivery system .SPIONs are also biocompatible and biodegradable. Hence, the biomaterials used in the synthesis of the nanocarrier can be considered nontoxic.

CONCLUSIONS

In this work, we developed a simple method for preparation of electrospun PVA magnetic nanofibers containing doxorubicin as a targeted nanocarrier for drug delivery. The synthesized nanocarrier has been successfully employed for the efficient delivery of an anticancer drug into the tumor region. The DOX-loaded nanocarrier showed a steady and sustained release profile in vitro up to 72 h. DOX release from nanocarrier was better described using Peppas model. All these results together suggest that the DOX-loaded

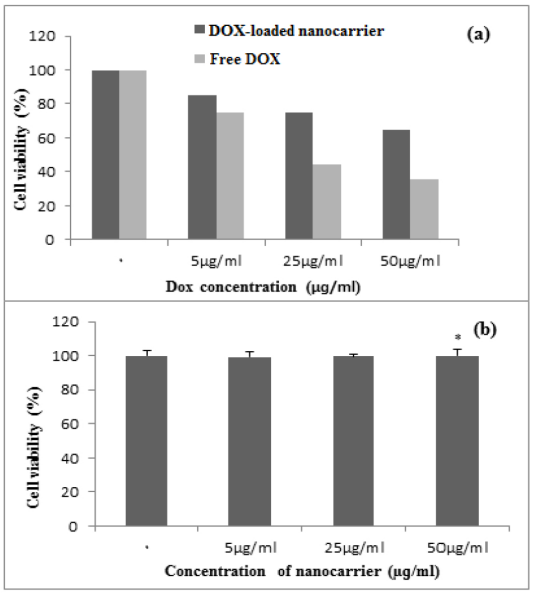

Fig. 6. Survival of MCF-7 cells to (a) free DOX and DOX-loaded nanocarrier with different concentrations of DOX, and (b) nanocarrier. Cell survival was assessed by MTT assay

nanocarrier may serve as a promising magnetic targeting therapy for the treatment of tumor cells.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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