In-vitro evaluation of paclitaxel-loaded MPEG–PLGA nanoparticles on laryngeal cancer cells

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Induction chemotherapy or concurrent chemotherapy with radiotherapy can preserve laryngeal function without a detrimental effect on survival in patients with advanced laryngeal cancer. However, systemic chemotherapy with traditional injection often causes side effects. In this study, methoxy poly(ethylene glycol)–poly(lactide-co-glycolide) (MPEG–PLGA) nanoparticles (NPs) were developed as a carrier for paclitaxel, a potent cytotoxic agent. The NPs were prepared by an emulsification–solvent evaporation method with the particle size and zeta potential around 153.3 ± 41.7 nm and −5.36 mV. Transmission electron microscopy showed that the NPs were homogeneous and spherical in shape. Differential scanning calorimetry and X-ray powder diffractometry did not detect any crystalline drug in the NP samples. High-performance liquid chromatography was used to measure the drug loading, encapsulation efficiency, and in-vitro drug release. The drug loading efficiency was (5.35 ± 0.75)% and encapsulation efficiency was (75.56 ± 2.61)%. In the in-vitro drug release study, paclitaxel was released from the NPs in a slow but time-dependent manner. In-vitro cytotoxicity of the paclitaxel-loaded NPs was investigated by using human laryngeal cancer Hep-2 cells. The anticancer activity of paclitaxel-loaded NPs was comparable with the free paclitaxel. No significant cytotoxicity was observed in blank MPEG–PLGA NPs. Laser scanning confocal microscopy was used to observe the uptake of fluorescent coumarin-6-loaded NPs by Hep-2 cells. We conclude that the formulation of NPs inhibits Hep-2 cell growth to a similar extent as free paclitaxel injection. These results suggest that MPEG–PLGA NPs may have potential as an alternative delivery system for paclitaxel.

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Keywords: laryngeal carcinoma, methoxy poly(ethylene glycol)–poly(lactide-co-glycolide), nanoparticle, paclitaxel

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Introduction

About 500 000 squamous cell carcinomas of the head and neck are newly diagnosed worldwide each year [1]. Laryngeal cancer remains one of the most common cancers in head and neck. Surgery and/or radiotherapy are the mainstay of locoregional treatment [2]. However, despite improved surgical technology and radiotherapy, the long-term prognosis of patients with advanced laryngeal cancer has not markedly improved during the past decades. Since Laccourreye's group [3,4] performed induction chemotherapy in patients with invasive squamous cell carcinoma of the true vocal cord in the early 1980s, many clinical trials have been focused on chemotherapy. In 1991, a prospective, randomized clinical trial by the Veterans Affairs Laryngeal Cancer Study Group was published [5]. In their study, 62% of advanced laryngeal cancer patients treated with chemotherapy and radiation preserved their larynx. No significant differences in survival were found between the chemoradiation group and total laryngectomy followed by radiotherapy group. It has been shown that induction chemotherapy or concurrent chemotherapy and radiotherapy can preserve the laryngeal function without a detrimental effect on survival in patients with advanced laryngeal cancer [6–8]. However, systemic chemotherapy with traditional injection is often toxic and can cause a high incidence of side effects, which deduce the need for an alternate formulation.

Paclitaxel, an extract from the bark of the Pacific yew tree, has been determined as one of the best antineoplastic drugs in the past decades. It has exhibited potent cytotoxic activity against a wide spectrum of solid tumors [9–11]. It has a unique mechanism of action, different from other anticancer drugs, which has traditionally been attributed to its ability to induce stabilization of the cellular microtubule apparatus and therefore interfere with cell division as well as other vital cellular processes [12]. The use of paclitaxel, as a single agent or in combination with other cytotoxic agents and/or radiotherapy, has been successfully tested in patients with head and neck cancers [13–15]. However, it has difficulty in clinical formulation because of the low solubility in water and many other pharmaceutical solvents. Currently,

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paclitaxel is marketed commercially in a formulation containing a solvent system of Cremophor EL and dehydrated ethanol (50 : 50, v/v), which is associated with serious side effects, such as severe hypersensitivity reactions, nephrotoxicity, and neurotoxicity [16,17]. Thus, there is a need for an improved formulation of paclitaxel to provide a better clinical administration.

In 2006, we developed a microsphere system, the paclitaxel-loaded poly(lactide-co-glycolide) (PLGA) microspheres for local administration in a Hep-2 laryngeal squamous cell carcinoma model [18]. We showed that compared with conventional paclitaxel injection, localized delivery of paclitaxel microspheres was more effective in inhibiting tumor growth. In this study, we considered the feasibility of using long-circulating methoxy poly(ethylene glycol)–poly(lactide-co-glycolide) (MPEG–PLGA) nanoparticles (NPs) that can be used as intravenous administration for the passive targeting of paclitaxel to tumor cells.

NPs have been investigated intensively because NPs of biodegradable polymers can provide a method of sustained, controlled, and targeted drug delivery to improve the therapeutic effects and reduce the side effects of formulated drugs [19]. As the cancerous tissues need rapid vascularization to serve the fast-growing cancers, tumor vasculature has been described as 'leaky'. Several tumor models have been reported to have pore cutoff sizes ranging between 0.2 and 1.2 μm [20,21]. The 'leaky' vasculature, coupled with poor lymphatic drainage, allows an enhanced permeation and retention (EPR) effect, which thereby facilitated the extravasation of the NPs into the tumor [22,23]. Therefore, particles adequately small can penetrate tumor tissues. However, NPs can usually be recognized and eliminated by the reticuloendothelial system depending on their sizes and surface characteristics. Coatings of hydrophilic polymers, poly(ethylene glycol) (PEG), on the particles can create a cloud of chains at the surface, which will avoid elimination by the reticuloendothelial system and stay longer in the circulation.

In this study, we prepared MPEG–PLGA NPs by an emulsification–solvent evaporation method. The preparation, characterization, and in-vitro release of the NPs were explored. High-performance liquid chromatography (HPLC) was used to measure the drug loading, encapsulation efficiency, and the in-vitro drug release kinetics. The physical status of the drug incorporated in the NPs was detected by differential scanning calorimetry (DSC) and X-ray powder diffractometry (XRD) measurements. Hep-2 cells were used for in-vitro cell line experiments. The cytotoxicity of paclitaxel-loaded MPEG–PLGA NPs was investigated by using the MTT assay and cellular uptake of fluorescent MPEG–PLGA NPs (coumarin-6-loaded) was imaged by laser scanning confocal microscopy.

Materials and methods

Materials

Paclitaxel was purchased from Tianwei Biotechnology (Shanghai, China). The paclitaxel injection was obtained from Sichuan Taiji Pharmaceutical (Sichuan, China). $MPEG_{3300}$–PLGA (L : G = 50 : 50, Mw of 60 000) was purchased from Daigang Biomaterial (Shandong, China). Polyvinyl alcohol (PVA, Mw = 31 000) was purchased from Fluka (Buchs, Switzerland). Coumarin-6 was purchased from Sigma (St Louis, Missouri, USA). All other chemicals were of HPLC or reagent grade and used without further purification. The human laryngeal cancer Hep-2 cell line was provided by the Cell Bank of the Chinese Institute of Biochemistry and Cell Biology.

Preparation of paclitaxel-loaded MPEG–PLGA NPs

Preparation of paclitaxel-loaded NPs was based on an o/w emulsification–solvent evaporation method. Briefly, 1 mg paclitaxel and 10 mg MPEG–PLGA were dissolved in 1 ml dichloromethane (DCM, Sinopharm Chemical Reagent, Shanghai, China). The organic solution was added dropwise into 15 ml aqueous phase containing 0.2% (w/v) PVA under magnetic stirring for 10 min. Then, the mixture was sonicated in an ice bath for 200 s at 170 W output. The resulting emulsion was then placed on a magnetic stirrer plate and continuously stirred at room temperature for 4 h to evaporate DCM. The NPs were collected by centrifugation (centrifuged at 15 000 rpm for 30 min) and washed three times with distilled water to eliminate free drug and PVA. Then, the NPs were freeze-dried with a vacuum freeze dryer (α 1-2; Martin Christ, Osterode, Germany). The powder was stored at 4°C and was resuspended with deionised water or saline at the time it was used.

Particle size and surface charge measurement

Average size and zeta potential of the drug-loaded NPs were measured by dynamic light scattering technique by Nicomp TM 380ZLS zeta potential/particle sizer (Santa Barbara, California, USA). The dried samples were suspended into deionised water and slightly sonicated before measurement. The particle size was expressed as intensity-weighted mean diameter and measured at least for three times.

Surface morphology

Transmission electron microscopy (TEM) was used to determine the shape and surface morphology of the NPs. A drop of NP suspension was placed on a TEM copper grid coated with carbon film and dried at room temperature. Morphology observation was performed using a Hitachi H-600 TEM (Hitachi, Tokyo, Japan) at 80 kV.

Differential scanning calorimetry

The physical status of the paclitaxel inside the NPs was characterized by DSC thermogram analysis (DSC 204

Phoenix; Netzsch, Bavaria, Germany). Accurately weighed samples were sealed in standard aluminium pans. The samples were purged with pure dry nitrogen at a flow rate of 20 ml/min. A temperature ramp speed was set at 10°C/min.

X-ray powder diffractometry measurement

To determine the existing form of paclitaxel in paclitaxel-loaded NPs, X-ray diffraction spectra of pure paclitaxel, MPEG–PLGA, mixture of paclitaxel and MPEG–PLGA, and paclitaxel-loaded NPs were obtained with D/Max-RB (Rigaku Denki Co. Ltd, Tokyo, Japan) using Ni-filtered Cu–Kα1 radiation. The sample was scanned from 2.5 to 50° at a speed of 4° per minute.

Drug loading and encapsulation efficiency

To determine the paclitaxel entrapped in the NPs, HPLC analysis (Shimadzu, Kyoto, Japan) was performed. A reverse-phase C18 column (200 × 4.6 mm, 5 μm; Dikma, Beijing, China) protected by a C18 guard column (7.5 × 4.6 mm, 5 μm; Dikma) was used. The mobile phase was a mixture of acetonitrile/water [60 : 40, (v/v)] delivered at a flow rate of 1.0 ml/min. The column temperature was maintained at 30°C. Paclitaxel was detected at 227 nm with a wavelength detector. The concentration of paclitaxel was determined from the area correlated with the standard curve, which was linear over the range of standard solutions with a correlation coefficient of $R = 0.9995$. Lyophilized paclitaxel-loaded NPs were weighed and dissolved in DCM. Then, it was evaporated at nitrogen atmosphere and reconstituted at mobile phase of 60 : 40 (v/v) acetonitrile/water solution. The following equations were applied:

$$\text{Drug loading efficiency} = \frac{(\text{amount of drug in NPs})}{(\text{amount of drug} - \text{loaded NPs} \times 100\%)}$$

$$\text{Encapsulation efficiency} = \frac{(\text{actual amount of drug} - \text{loaded in NPs})}{(\text{initial amount of drug} \times 100\%)}$$

In-vitro release study

The release rate of paclitaxel from the NPs was performed as follows: 1 ml paclitaxel-loaded NPs solution with the paclitaxel concentration of 1 mg/ml was incubated in a dialysis bag (cellulose membrane, 10 000 MWCO) and immersed in a centrifuge tube containing 40 ml phosphate buffer solution (PBS, pH 7.4) with 0.1% (w/v) Tween-80. The tube was placed in an orbital shaker water bath, which was maintained at 37°C and shaken horizontally at 100 rpm. At particular time intervals, the total PBS in the tube was poured out as sample for HPLC and 40 ml fresh medium was added. The analysis procedure was the same as described above.

Cell culture

Human laryngeal squamous Hep-2 cells were cultivated in 1640 (Gibco, Invitrogen Corporation, Carlsbad, California, USA) supplemented with 10% bovine serum and 1% penicillin–streptomycin at 37°C in humidified environment of 5% CO_2. The medium was replenished every other day and the cells were subcultured after reaching confluence.

In-vitro cytotoxicity study

Hep-2 cells were seeded in 96-well plates (Costar, Illinois, USA) at the density of 8000 cells/well and incubated to allow cell attachment. Twenty-four hours after planting, the cells were incubated with blank MPEG–PLGA NPs (without paclitaxel), paclitaxel-loaded MPEG–PLGA NPs suspension and paclitaxel injection at various concentrations diluted in 200 μl of culture medium for 24, 48, and 72 h. The final concentration ranged from 1 to 50 nmol/l. The blank MPEG–PLGA NPs were evaluated at the concentration corresponding to drug-loaded NPs. At designated time intervals, 20 μl of MTT (5 mg/ml in PBS, pH 7.4) were added into each well, and the plates were further incubated at 37°C for 4 h. Then, the solution was removed, leaving the precipitate and 150 μl dimethyl sulfoxide was added to the wells and agitated thoroughly to dissolve the crystals. The plates were analyzed by a microplate reader (Power Wave XS; Bio-Tek, Winooski, Vermont, USA) at a wavelength of 490 nm. The cell viability was calculated by the following equation:

$$\text{Cell viability}\,(\%) = \frac{(\text{intensity experiment})}{(\text{intensity control} \times 100\%)}$$

In-vitro cellular uptake of nanoparticles

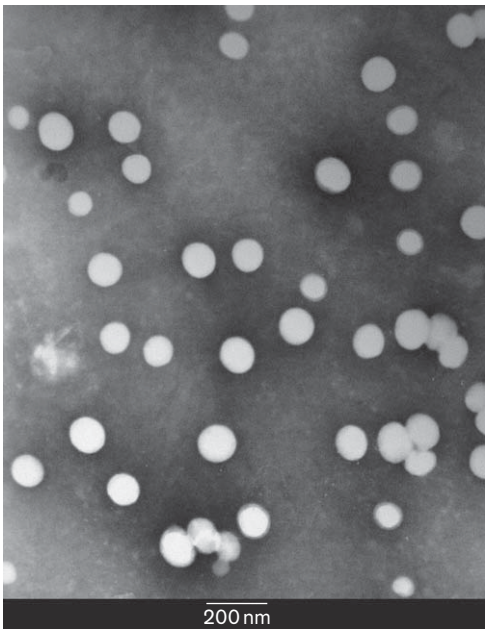
To investigate the cellular uptake of NPs into cells, we synthesized fluorescent MPEG–PLGA NPs. Coumarin-6 at 1% was incorporated into MPEG–PLGA NPs as a fluorescent marker. Then, the NPs were purified by passing through a Sepharose CL-4B column eluted with saline [24]. Hep-2 cells were cultured on coverslips in the culture capsule for 24 h at 37°C. After attachment of the cells, the medium was removed and 1 ml 0.2 mg/ml coumarin-6-loaded MPEG–PLGA NPs was added. Cells were washed four times after incubation for 1 h and then fixed by ethanol for 20 min. The cells were further washed twice with PBS and the nuclei were stained with propidium iodide for 10 min.

Results

Size distribution and surface morphology

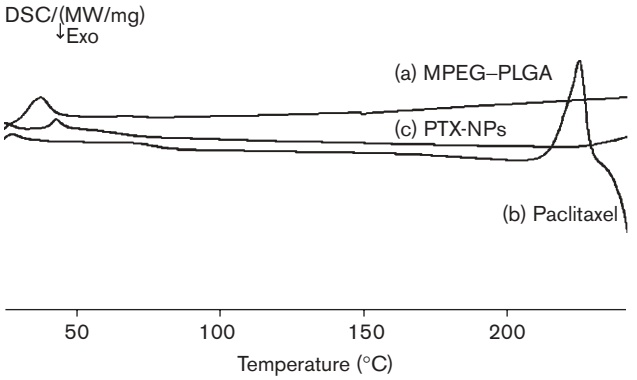
The average size of MPEG–PLGA NPs was 153.3 ± 41.7 nm, polydispersity index = 0.076. NPs exhibited low negative zeta potential values of −5.36 mV, which is consistent with the presence of the PEG corona around the PLGA core of the NPs.

Fig. 1



Transmission electron microscopy image of paclitaxel-loaded MPEG–PLGA NPs.

Fig. 2



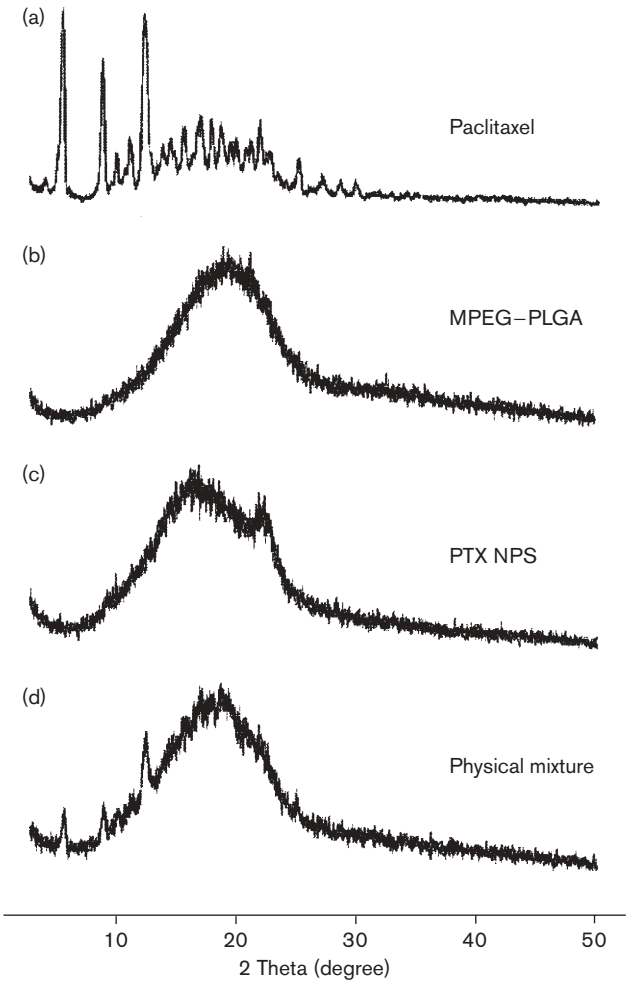
Differential scanning calorimeters (DSC) thermograms of (a) MPEG–PLGA; (b) paclitaxel; (c) PTX-NPs: paclitaxel-loaded MPEG–PLGA NPs.

TEM was used to evaluate the morphology of the paclitaxel-loaded NPs. As shown in Fig. 1, the particles were spherical with size about 150 nm.

Differential scanning calorimetry

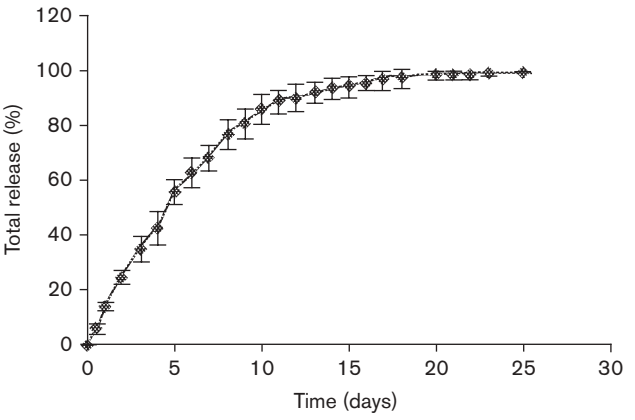
DSC, which provides the physical status of the drug in the polymers, was used to investigate the existing form of paclitaxel in the MPEG–PLGA NPs. The pure drug and pure MPEG–PLGA served as control samples. The pure paclitaxel showed an endothermic peak of melting at

Fig. 3



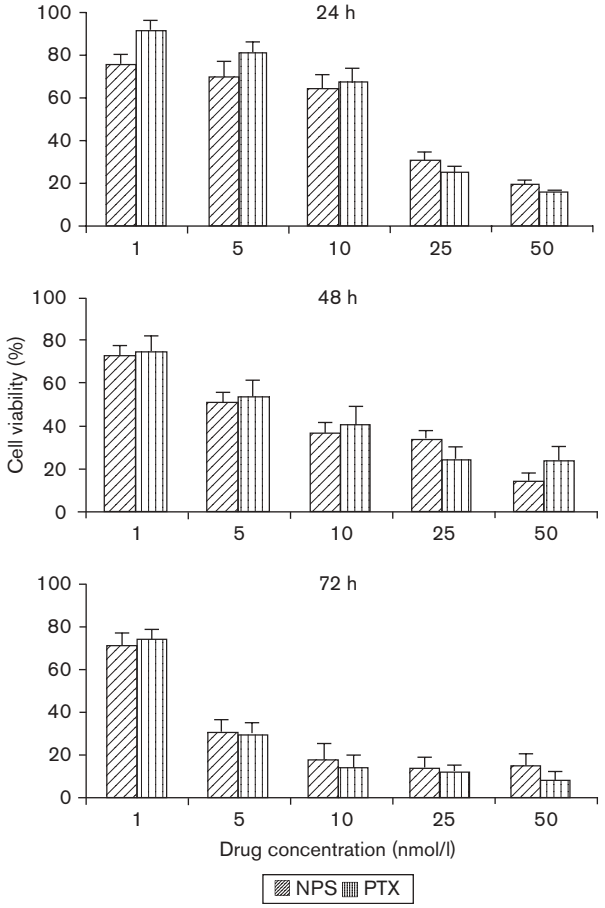
X-ray powder diffractogram (XRD) of (a) paclitaxel; (b) MPEG–PLGA; (c) PTX-NPs: paclitaxel-loaded MPEG–PLGA NPs; (d) physical mixture of paclitaxel and MPEG-PLGA.

Fig. 4



In-vitro drug releases of paclitaxel-loaded MPEG–PLGA NPs. Data present mean ± SD, $n = 3$.

Fig. 5



Cytotoxicity of free paclitaxel and paclitaxel-loaded MPEG–PLGA NPs on Hep-2 cells.

Table 1 IC_{50} of Hep-2 cells incubated with paclitaxel and paclitaxel-loaded MPEG–PLGA NPs

Incubation time (h)	IC_{50}(nmol/l)	
	NPs	Paclitaxel
24	10.39	12.82
48	5.01	5.75
72	2.21	2.34

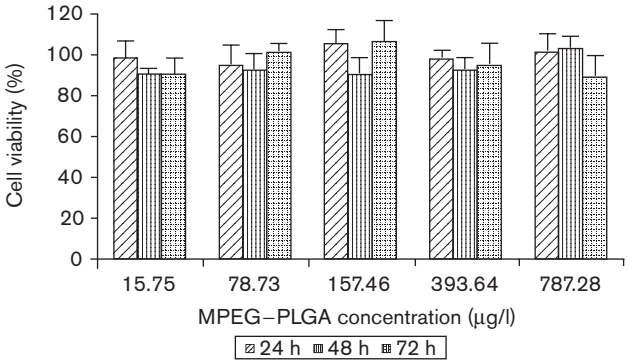
NPs, nanoparticles.

about 225°C (Fig. 2). There was no peak observed at the temperature range of 150–250°C for the paclitaxel-loaded NPs. The DSC study did not detect any crystalline drug in the paclitaxel-loaded NPs samples. It suggested that paclitaxel in the NPs exists in an amorphous state.

X-ray powder diffractometry

X-ray diffraction analysis was also performed to determine whether the entrapped paclitaxel existed in the less water-soluble crystalline state or the more soluble amorphous state. Sharp peaks in XRD indicate a crystal structure. Sharp peaks were shown for free paclitaxel and the mixture of free paclitaxel and MPEG–PLGA (Fig. 3), but not for paclitaxel-loaded MPEG–PLGA NPs. This also indicates that the paclitaxel entrapped in the NPs existed in an amorphous state.

Fig. 6



Hep-2 cell viability after 24 h, 48 h, 72 h treatment with blank MPEG–PLGA NPs.

In-vitro release

The in-vitro drug release of the paclitaxel-loaded MPEG–PLGA NPs in the first 25 days was shown in Fig. 4. The drug was released in diphase. The loaded drug of 6.02% was released in the first 12 h and 13.95% of the loaded drug on the first day. About 86% of the drug was released at an approximately constant rate over a 10 day period. Then, the drug released slowly. Complete drug release took about 25 days.

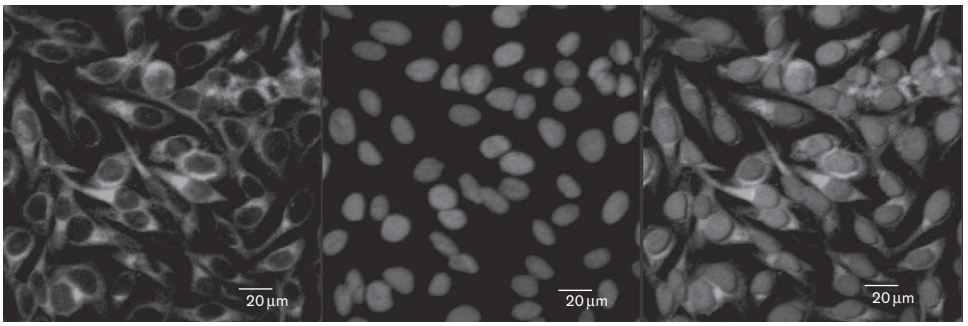
Drug loading and encapsulation efficiency

In this study, HPLC analysis indicated that a drug loading efficiency of $5.35 \pm 0.75\%$ and an encapsulation efficiency of $75.56 \pm 2.61\%$. The encapsulation efficiency was greatly influenced by the drug loading.

In-vitro cytotoxicity study

The in-vitro anticancer cytotoxicity of free paclitaxel, paclitaxel-loaded MPEG–PLGA NPs and blank MPEG–PLGA NPs are shown in Figs 5 and 6. The activity of both free and NPs incorporated paclitaxel increased with increasing paclitaxel concentration and incubation time. The paclitaxel-loaded NPs exhibited comparable in-vitro cytotoxicity with that of free paclitaxel. The differences in anticancer activity between them were low ($P > 0.05$). The IC_{50}, which represents the concentration of a drug that is required for 50% inhibition *in vitro*, are listed in Table 1. The IC_{50} value of NPs was similar to that of free paclitaxel. The blank MPEG–PLGA NPs did not show significant cytotoxicity (Fig. 6).

Fig. 7



Laser scanning confocal microscopic images of Hep-2 cells incubated with coumarin-6-loaded NPs for 1 h. The cells stained by propidium iodide (red) and fluorescent coumarin-6-loaded NPs (green). Bar represents 20 μm.

Cellular uptake

The microscopic image of Hep-2 cells after 2 h incubation with coumarin-6-loaded MPEG–PLGA NPs is shown in Fig. 7, and we can observe the fluorescent green color in the cytoplasm surrounding the red nucleus, which suggested that the fluorescence gathered in the cytoplasm.

Discussion

Laryngeal cancer is one of the most common cancers of the upper aerodigestive tract. Chemotherapy can preserve laryngeal function and improve the quality of life for patients without compromising survival. Concomitant chemotherapy and radiotherapy is the strategy that has proven to be the most effective treatment for organ preservation, unresectable disease, and the postoperative adjuvant treatment for patients with high risk of recurrence [25]. To reduce the side effects caused by traditional systemic chemotherapy, for decades researchers have shown increased interest in developing biodegradable polymeric NPs as effective drug delivery systems. PLGA is a biodegradable and biocompatible polymer and is well tolerated by the body and cells [26].

In this study, we have shown the use of long-circulating MPEG–PLGA NPs as a carrier for passive targeting of paclitaxel to head and neck cancers. We prepared the NPs with the particle size of 153.3 ± 41.7 nm by an emulsification–solvent evaporation method. Particle size plays an important role in determining the drug release behavior of the NPs as well as their fate after administration. Smaller ones tend to accumulate in the tumor sites because of the EPR effect. The particle size in our study is larger than prepared by the nanoprecipitation method [27]. However, our drug loading and encapsulation efficiency is higher. It may be because the nanoprecipitation method is based on the theory that particles form when the organic solvent diffuses to the outer aqueous environment. The faster the diffusion occurs, the smaller the particle is [27,28]. However, the drug may not have enough time to be incorporated into the polymers, which leads to lower drug loading and encapsulation efficiency.

For the in-vitro release study, we added 1 ml of paclitaxel-loaded NPs suspension with the paclitaxel concentration of 1 mg/ml into 40 ml PBS with 0.1% Tween-80. The solubility of paclitaxel in water is quite low (about 0.3 μg/ml). However, the most widely used method for increasing the aqueous paclitaxel solubility is to add surfactants to the aqueous release medium. Therefore, we added 0.1% Tween-80 in release kinetics medium, which was reported to increase the solubility of paclitaxel up to 6.32 μg/ml [29]. We removed the release medium and added fresh medium at each time interval. The purpose was to reduce the influence of the poor water solubility that may limit the release of paclitaxel out of the NPs. The drug diffusion, erosion and swelling of polymer matrix and the degradation of polymer are the main mechanisms for the drug release [30]. Some studies [30,31] have reported that the accumulative drug release from PLGA NPs within 1 month is usually 30–40% or less, which may be too slow to meet the therapeutic needs. Thus, our MPEG–PLGA NPs have an advantage in increasing the rate of drug release. This can be a contributing factor to the PEG content in the material that makes the NPs have greater hydrophilic character, leading to a faster release of the drug. In addition, the more water-soluble amorphous state of paclitaxel within the NPs, which was proved by both DSC and XRD measurements, might also have contributed to the faster drug release rate.

The paclitaxel-loaded MPEG–PLGA NPs exhibited comparable antitumor activity with the free paclitaxel injection. Both formulations inhibited the growth of Hep-2 cells *in vitro* in a dose-dependent and time-dependent manner. For 24 h incubation, the Cremopher EL-based paclitaxel injection showed a slightly higher

IC_{50} value than the NPs, but the difference was not significant. The paclitaxel-loaded NPs and free paclitaxel had similar IC_{50} for 48 and 72 h, which indicated that both formulations showed equivalent cytotoxic activity. However, the drug released *in vitro* from the NPs after 24, 48, and 72 h was only 13.95, 24.48, and 34.54% of the total amount, respectively. The paclitaxel-loaded MPEG–PLGA NPs should be considered more effective. No cytotoxicity of the unloaded NPs was observed even at the highest MPEG–PLGA concentration of 787.28 μg/l (corresponding to 50 nmol/l paclitaxel-loaded NPs). Earlier, Gryparis *et al.* [32] found that blank MPEG–PLGA NPs had low cytotoxicity, which increased with an increasing PLGA/PEG ratio in the MPEG–PLGA copolymer. In their study, more than 600 μg/ml were required to cause higher than 20% cell growth inhibition. The polymer concentration in our study was much lower than their study.

The cytotoxicity of NPs is achieved by the passage of incorporated drug into the cancer cells. The use of fluorescent or radioactively labeled NPs is the most common experimental approach found in literature. The NPs incorporated coumarin-6, which provides a green color and have been widely used as a particle internalization marker [33–35]. Coumarin-6 has been proven to be quite suitable for fluorescence/confocal microscopic observation. The dye has a high fluorescence activity and its release from the PLGA NPs is reported to be quite slow [33]. We used a Sepharose CL-4B column to purify the NPs. The NPs came out of the column first and then the free dye came out. There is controversy about the mechanism of intercellular drug delivery. In our study, the cell cytoplasm was stained with green fluorescence, which is generally interpreted that coumarin-6-loaded NPs were taken up by endocytosis and then the fluorescence released at intracellular locations. However, some literature proposed an alternative view. Pietzonka *et al.* [36] reported that particle uptake is caused by a collision-induced process facilitating the transfer of a lipophilic fluorescent marker by the formation of a complex between the NPs and the biomembranes of the cells, followed by diffusion of the marker within this complex according to the concentration gradient. Xu *et al.* [37] reported that intercellular fluorescence is mainly a result of fluorescence transfer from NPs to the cells rather than uptake of the NPs. In this study, the confocal microscopic image showed that the fluorescence was homogeneously dispersed in the cytoplasm, and only several separate NPs can be found in amplified image. It was possible that the cellular delivery of coumarin-6 could partly be attributed to drug transfer from NPs to cells. It is possible that the paclitaxel-loaded MPEG–PLGA NPs selectively accumulate in tumor tissue by the EPR effect when entering the blood circulation. Then part of the NPs is taken up by tumor cells and part is transfers physically encapsulated drug to cells.

In conclusion, we showed that the NPs can be readily prepared by the emulsification–solvent evaporation method with good reproducibility. The size of paclitaxel-loaded MPEG–PLGA NPs was 153.3 ± 41.7 nm, with the drug loading efficiency and encapsulation efficiency of $5.35 \pm 0.75\%$ and $75.56 \pm 2.61\%$, respectively. The paclitaxel-loaded MPEG–PLGA NPs showed higher or comparable cytotoxicity and IC_{50} value against human laryngeal cancer cell Hep-2 versus the commercial Cremopher EL-based paclitaxel formulation. These results suggest that paclitaxel-loaded MPEG–PLGA NPs are promising for laryngeal cancer and other tumors sensitive to paclitaxel. It may provide a new formulation for chemotherapy.

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