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Self-assembled nanoparticles of poly(lactide)–Vitamin E TPGS copolymers for oral chemotherapy

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Abstract

Nanoparticles (NPs) of poly(lactide)–Vitamin E TPGS (PLA–TPGS) copolymers were synthesized by a dialysis method in the present study to formulate paclitaxel for oral chemotherapy with Caco-2 cells as an *in vitro* model of the gastrointestinal (GI) drug barrier. The PLA–TPGS NPs were of size 340 nm in diameter with 5.2% drug loading. The drug release kinetics showed a 31% initial burst in the first day, followed by 80% accumulative drug release after 30 days in the PBS buffer at pH 7.4, and the release rate was found lower in simulated gastric and intestinal conditions. The internalization of fluorescent PLA–TPGS NPs by Caco-2 cells was visualized by confocal laser scanning microscopy (CLSM). PLA–TPGS NPs showed significant increase in the cellular uptake by 1.8- and 1.4-fold in comparison with poly(lactide-*co*-glycolide) (PLGA) NPs cultured with HT-29 and Caco-2 cells, respectively, and the cellular uptake efficiency was found affected by the incubation time and the particle concentration in the culture medium. Investigation on HT-29 and Caco-2 cytotoxicity showed advantages of the PLA–TPGS NP formulation versus Taxol®. The IC_{50} of the PLA–TPGS NP formulation with HT-29 cells was found 40% lower than of Taxol® at the same dose of paclitaxel. The results obtained in this research demonstrated feasibility for the PLA–TPGS NPs to be applied for oral delivery of paclitaxel as well as other anticancer drugs.

Keywords: Biodegradable polymers; Cancer; Cancer nanotechnology; GI barrier; Oral drug delivery; Nanomedicine; Taxol®

1. Introduction

Oral chemotherapy (OCT) is an attractive area since it provides great convenience to the patients and improves their quality of life (Liu et al., 1997). OCT can provide continuous exposure of cancer cells to the drug at a sustainable, appropriate concentration level, with which therapeutic index can be improved and the side effects can be reduced (Feng and Chien, 2003). Nevertheless, most anticancer agents such as paclitaxel are not bioavailable (Eiseman et al., 1994; Malingre et al., 2001). Paclitaxel is one of the best of anticancer drugs against a wide spectrum of cancers such as breast cancer, ovarian cancer, colon caner, small and non-small cell lung cancer, and neck cancer (Donehower et al., 1987; Lopes et al., 1993; Panchagnula, 1998; Rowinsky et al., 1992; Wani et al., 1971). It has been found that orally administrated paclitaxel would be eliminated from the first pass extraction by the cytochrome P450 and the drug transport protein P-glycoprotein (P-gp), which are rich in the intestinal epithelium cell layer and other part of the digest system. Such a gastrointestinal (GI) drug barrier can pump the drug out of the intestinal epithelium cells into the intestinal lumen (Sparreboom et al., 1997). Nanoparticles (NPs) of biodegradable polymers for OCT have been under intensive investigation in last decades. NPs can be made of small enough size and desired surface properties by appropriate coating, which give the NPs ability to cross the GI tract (Yoo and Park, 2004). Continuous efforts for OCT by NPs include selecting biodegradable polymers of desired performance (Bonduelle et al., 1996; Feng et al., 2004), making nanoparticles of smaller size, and coating particles with bioadhesive materials such as carbopol, chitosan, gelatin, pectin, alginate, PEG (Feng et al., 2004; Luo et al., 2002). In addition, the presence of some coating material having

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P-gp subduing effects on the particles surface may allow NPs to evade the "watchful eyes" of P-gp.

D-Alpha-tocopheryl polyethylene glycol 1000 succinate (Vitamin E TPGS or simply TPGS) is a water-soluble derivative of natural vitamin E and is synthesized by esterification of D-alpha-tocopheryl acid succinate with polyethylene glycol 1000. It is an effective emulsifier, absorption enhancer and bioavailability promoter (Feng et al., 2004; Bittner et al., 2002; Boudreaux et al., 1993; Chang et al., 1995, 1996; Dintaman and Silverman, 1999; Goldberg et al., 1988; Rege et al., 2002; Sokol et al., 1991). In nanoparticle preparation, TPGS has been found to be an excellent surfactant and additive for nanoparticle formulation of paclitaxel (Feng et al., 2004; Mu and Feng, 2002, 2003a,b; Win and Feng, 2005). TPGS demonstrated inhibition of the P-gp mediated talinolol transport in Caco-2 cells and increased the AUC and C_{max} of oral talinolol by 39% and 100%, respectively (Bogman et al., 2005). Bioavailability was enhanced about 6.3-fold when [C-14]paclitaxel was administrated with TPGS in close comparison with [C-14]paclitaxel administered alone (Varma and Panchagnula, 2005). Ke et al. (2005) also demonstrated that TPGS can be used as a surfactant for oral delivery of moelcular drugs (proteins and peptides). It has been shown that vitamin E TPGS-coated polystyrene NPs could have 1.4-folds higher cellular uptake than that of the PVA-coated NPs and 4–6 times higher cellular uptake than uncoated NPs by using Caco-2 cell line as a model GI drug barrier (Win and Feng, 2005). *In vivo* pharmacokinetics study demonstrated that TPGS-emulsified PLGA nanoparticles can obtain 4.9 times larger of AUC and 2.39 times longer of therapeutic time compared with that of Taxol® (Win and Feng, 2006).

In the process of TPGS-emulsified NPs, however, the TPGS on the NP surface might be desorbed *in vivo* because of their high affinity to the proteins in the blood (Gref et al., 1994). To increase the stability of the TPGS molecules on the nanoparticle surface as well to improve the drug release from the NP matrix, we synthesized novel poly(lactide)–Vitamin E TPGS (PLA–TPGS) copolymers and prepared PLA–TPGS NPs by the solvent extraction/evaporation method for drug delivery (Zhang and Feng, 2006). In the present study, we shall investigate the feasibility to make PLA–TPGS NPs by the dialysis method for paclitaxel formulation for oral chemotherapy with an assumption that PLA–TPGS NPs may escape the drug efflux pump protein P-glycoprotein (P-gp) and thus enhance the oral bioavailability of paclitaxel. The dialysis method can be much simpler than other NP techniques since no sonication or homogenization is needed that would denature molecular drugs. Also, the dialysis method is a self-assemble process with no surfactant/emulsifier/stabilizer required, which can avoid the side effects from surfactants such as PVA.

The drug loaded PLA–TPGS NPs prepared by the dialysis method were then characterized by various state-of-the-art techniques such as the laser scattering for particle size and size distribution, field emission scanning electron microscopy (FESEM) for surface morphology, and differential scanning calorimetry (DSC) for the physical status of the drug in the polymeric matrix. *In vitro* cellular uptake of fluorescent PLA–TPGS NPs was visualized by confocal laser scanning microscopy (CLSM) and quantitatively measured by the microplate reader. *In vitro* HT-29 cancer cell cytotoxicity of paclitaxel formulated in the PLA–TPGS NPs was accessed by the 3-(4,5-cimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), which was made in close comparison with that of Taxol®.

2. Materials and methods

2.1. Materials

Poly(D-L-lactic-*co*-glycolic acid) (PLGA) with L:G molar ratio of 50:50 and M_w of 40,000–75,000, fluorescence marker coumarin-6, propidium iodide (PI), 3-(4,5-cimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), phosphate buffered saline (PBS), MEM medium, penicillin–streptomycin solution, Trypsin–EDTA solution, Triton® X-100 and Hank's balanced salt solution (HBSS) were purchased from Sigma. Fetal bovine serum (FBS) was received from Gibco (Life Technologies, AG, Switzerland). Paclitaxel was purchased from Dabur India Limited, India. PLA–TPGS copolymer was synthesized in our previous work with M_w 12,700 and 12% TPGS content in the copolymer. All reagent water used in the laboratory was pretreated with the Milli-Q Plus System (Millipore Corporation, USA).

2.2. Preparation of paclitaxel-loaded PLA–TPGS nanoparticles

Paclitaxel-loaded PLA–TPGS or PLGA NPs were prepared by the dialysis method. In brief, 5.5 mg paclitaxel and 50 mg PLA–TPGS copolymer were dissolved into 20 ml DMF and then vortexed for 60 s. The solution was dialyzed for 36 h in a dialysis bag (MWCO 3500, Spectrum) in 5 l of water, which was exchanged every 3 h. After dialysis, the suspension was sonicated and filtered through 1.2 μm membrane filter. The suspension was then centrifuged at 11,500 rpm for 20 min and the pellet was resuspended in water. The suspension was freeze-dried for 2 days to get the NP powder.

2.3. Characterization of nanoparticles

2.3.1. Particle size and surface morphology

Particle Size System 90 Plus from Brookhaven Instruments Corporation was used to analyze the size and size distribution of NPs. The dried NPs were dispersed in water and the suspension was filled into an acrylic square cell. A typical result was obtained based on the average from seven runs.

The surface morphology of the NPs was observed by a field emission scanning electron microscopy (FESEM, JEOL, SM-6700F, Japan). Powder samples of the NPs were stuck on metallic studs with double-sided conductive tape and then coated with a platinum layer by the Auto Fine Platinum Coater (JEOL JFC-1300, Tokyo, Japan) for 50 s.

2.3.2. Drug encapsulation efficiency

Paclitaxel entrapped in the PLA–TPGS or PLGA NPs was measured by HPLC (Agilent LC 1100). A 3 mg of the paclitaxel-

loaded nanoparticles were dissolved in 2 ml DCM under vigorous vortexing and extracted by 3 ml mobile phase (50/50, v/v, acetonitrile/water solution). DCM was evaporated at nitrogen atmosphere and a clear solution was then obtained. The solution was transferred to HPLC vials for analysis after filtered through 0.45 μm PVDF syringe filter. Drug encapsulation efficiency was obtained from the weight ratio between the drug entrapped in nanoparticles and that used in the fabrication (Mu and Feng, 2002).

2.3.3. DSC analysis

The differential scanning calorimetry (DSC, Mettler Tolerdo DSC 822e) was used to investigate the physical status of paclitaxel encapsulated in the NP matrix. Purging of the sample was carried out at 40 ml/min of dry nitrogen. The sample was heated up at a rate of 10 °C/min from 0 to 250 °C.

2.3.4. In vitro drug release

To simulate gastrointestinal environment, *in vitro* drug release experiment was carried out in a pH 1.2 or 3.0 buffer solution for the first 1 h to simulate the gastric fluid and subsequently in a pH 6.8 buffer solution to simulate the intestinal fluid (Itoh et al., 2006). Drug release in pH 7.4 PBS buffer was also conducted, which is a usual practice in the literature. A 1 mg of the paclitaxel-loaded PLA–TPGS NPs was dispersed into 2 ml release medium in a centrifuge tube. The tube was put in an orbital water bath shaking at 120 rpm at 37.2 °C. At allocated time intervals, the tube was taken out and centrifuged at a speed of 11,500 rpm for 15 min. The supernatant was transferred into a tube with screw cap, extracted with 1 ml DCM and reconstituted in 1 ml mobile phase. The analysis procedure was similar as the measurement of drug encapsulation efficiency. The pellet was redispersed in a fresh 2 ml release medium and the tube was put back to the shaker for continuous measurement.

2.4. Cell line experiment

2.4.1. Cell culture

In this study, Caco-2 cells (ATCC, VA) of passages between 26 and 31 and HT-29 cells of passages between 20 and 24 were cultured in the DMEM medium supplemented with 10% FBS, 100 mM sodium pyruvate, 1.5 g/l of sodium bicarbonate and 1% penicillin–streptomycin solution, and incubated in SANYO CO_2 incubator at 37 °C in a humidified environment of 5.0% CO_2. The medium was replenished every other day until confluency was reached. The cells were then washed twice with PBS and harvested with 0.125% of Trypsin–EDTA solution. They were seeded at a density of 6.5×10^4 cells/well in 96-well black plates (Costar, Corning, NY) for quantitative cellular uptake analysis by the microplate reader, 96-well transparent plates for cell viability analysis, or in a chambered cover glass system (LAB-TEK, Nalge Nunc, IL) for qualitative cellular uptake investigation by CLSM.

2.4.2. In vitro cellular uptake of nanoparticles

After the cells reached confluency, the cells were equilibrated with HBSS at 37 °C for 1 h and incubated with coumarin-6 loaded NP suspension (100, 250 and 500 μg/ml in HBSS, pH 7.4) at 37 °C for 0.5, 1, 2 and 4 h, respectively. The incubation was terminated by washing the cell monolayer three times with cold PBS and 50 μl of triton, which was then introduced into each well to lyse the cells. The fluorescence intensity of each sample well was measured by microplate reader (GENios, Tecan, Switzerland, λ_{ex} 430 nm and λ_{em} 485 nm). Cellular uptake efficiency was expressed as the percentage of the fluorescence associated with the cells versus that present in the feed solution (Win and Feng, 2005).

For the qualitative study, the cells were incubated with 250 μg/ml coumarin-6 loaded NPs at 37 °C for 2 h and then rinsed with cold PBS for three times and fixed by ethanol for 20 min. After that, the cells were washed two times, followed by counterstaining the nucleus with 30 μl propidium iodide (PI, 20 μg/ml in PBS). The cells were further washed twice with PBS and mounted in the Dako® fluorescent mounting medium (Dako, CA) for investigation by the confocal laser scanning microscope (CLSM) (Zeiss LSM 410, Germany) equipped with an imaging software (Fluoview FV300).

2.4.3. In vitro cytotoxicity of paclitaxel-loaded nanoparticles

The drug-loaded NP suspension of the drug concentration ranged from 0.025 to 25 μg/ml was added to the HT-29 or Caco-2 cells monolayer and the cells were incubated for 1, 2, and 3 days, respectively. Samples of various concentrations were seeded in six wells and the experiment was triply replicated. Cytotoxicity was determined by MTT assay, in which the absorbance was measured at 570 nm by the microplate reader (Genios, Tecan, Männedorf, Switzerland).

2.5. Statistical analysis

Statistical analysis was performed by using the Student's *t*-test with $p < 0.05$ significance. The experimental data are given in the format of mean ± S.D. in the tables and figures.

3. Results and discussion

3.1. Physicochemical properties of nanoparticles

3.1.1. Size, size distribution, and drug encapsulation efficiency

The particles size, size distribution, and drug encapsulation of the PLGA NPs and the PLA–TPGS NPs are presented in Table 1. PLA–TPGS NPs showed smaller particles size with 343 ± 19 nm in diameter and a narrow distribution of 0.06 ± 0.03

Table 1
The characteristics of paclitaxel-loaded PLGA and PLA–TPGS nanoparticles prepared by dialysis method ($n = 3$, $p < 0.05$)

Polymer	Particles size (nm)	Polydispersity	e.e. (%)	Drug loading (%)
PLGA	437 ± 25	0.16 ± 0.02	54.7 ± 6.8	5.5 ± 0.7
PLA–TPGS	343 ± 19	0.06 ± 0.03	51.8 ± 4.9	5.2 ± 0.5

polydispersity compared with PLGA NPs, which were found of particles size 437 ± 25 nm in diameter and 0.16 ± 0.02 polydispersity ($p < 0.05$). The particles size and size distribution could play an important role in determining *in vitro* and *in vivo* drug release kinetics, cellular uptake and biodistribution of the NPs, and thus the therapeutic effects of the drug-loaded NPs (Feng et al., 2004). The NP absorption efficiency to the GI tract has been showed to be strongly affected by the NPs size. It was suggested that large particles (~5 μm) can be taken up via the lymphatics (M cells of Peyer's patches) and small particles (~500 nm) can cross the membrane of epithelial cells through endocytosis (Luo et al., 2002; Savic et al., 2003). The drug loading of the PLA–TPGS NPs prepared in this research was 5.2 ± 0.5%.

3.1.2. Surface morphology of nanoparticles

FESEM was applied to capture high resolution images of the PLA–TPGS NPs. It can be seen from Fig. 1 that the PLA–TPGS NPs were relatively uniform in size and spherical in shape. The particles size measured from the FESEM images were found in good agreement with that obtained by the laser light scattering measurement.

3.1.3. DSC analysis

The physical status of the drug formulated in the PLA–TPGS NPs was compared with that for the pure drug and pure PLGA and PLA–TPGS as shown in Fig. 2. Paclitaxel in its natural state exists as crystals, which are characterized by the high peak at the melting point (216–217 °C). However, when encapsulated in the PLA–TPGS NPs, the peak at its original melting point disappeared. In contrast, a low peak close to 50 °C was detected. It can be surmised that paclitaxel has become amorphous. This is desired as crystalline state of paclitaxel causes phase separation, which would impede the release of the drug from the NPs. Solubility of the drug can also be improved (Feng et al., 2004).

3.2. In vitro investigation

3.2.1. In vitro drug release

Fig. 3 shows the *in vitro* drug release profiles of the paclitaxel-loaded, PLA–TPGS NPs and (b) PLGA NPs in a pH 1.2 (dashed line), 3.0 (dotted line) buffer solution for the first 1 h to simulate the gastric fluid and subsequently in pH 6.8 buffer solution

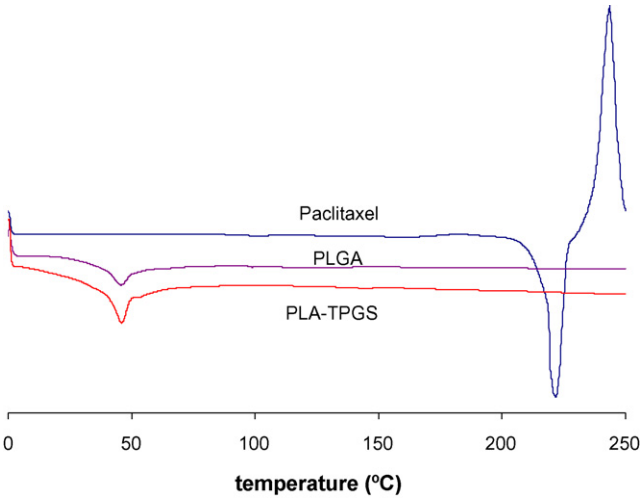

Fig. 2. DSC thermograms of paclitaxel-loaded PLGA and PLA–TPGS nanoparticles.

to simulate the intestinal fluid, and in pH 7.4 PBS buffer (solid line) at 37 °C. The inserts are magnifications of the release from 0 to 1 day, where the measurement was made at 1, 3, 6 and 24 h, respectively. The drug release profiles for the paclitaxel-loaded PLA–TPGS NPs are shown in comparison with that for the paclitaxel-loaded PLGA NPs. We can see from Fig. 3a and b that the *in vitro* drug release is proportional with the pH value. The larger the pH value, the faster the drug release. This can be refereed to the large diffusivity of the drug molecules in a medium of large pH value. It can be seen that paclitaxel-loaded PLGA NPs and PLA–TPGS NPs all displayed an initial burst release up to 24.0 ± 0.6% and 31.1 ± 1.9% in the 1st day and accumulative release of 63.6 ± 2.8% and 80.3 ± 2.2% after 30 days, respectively. The PLA–TPGS NPs showed much faster drug release than PLGA NPs either in ordinary pH 7.4 buffer solution or in simulated gastrointestinal fluid, which may be caused by the greater hydrophilic character and the faster degradation rate of the PLA–TPGS copolymer than those of PLGA. Similar results of hydrophilic composition effect on *in vitro* drug release were reported in the literature (Lin et al., 2003; Ryu et al., 2000). The inserted figures in Fig. 3a and b demonstrate that the drug release was inhibited in lower concentration (pH 1.2 or 3.0) compared with pH 7.4. It may be because that the

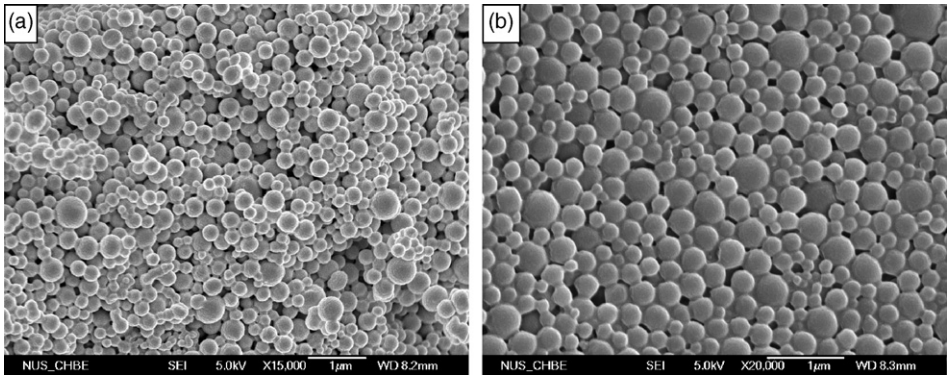

Fig. 1. FESEM images of paclitaxel-loaded (a) PLGA and (b) PLA–TPGS nanoparticles.

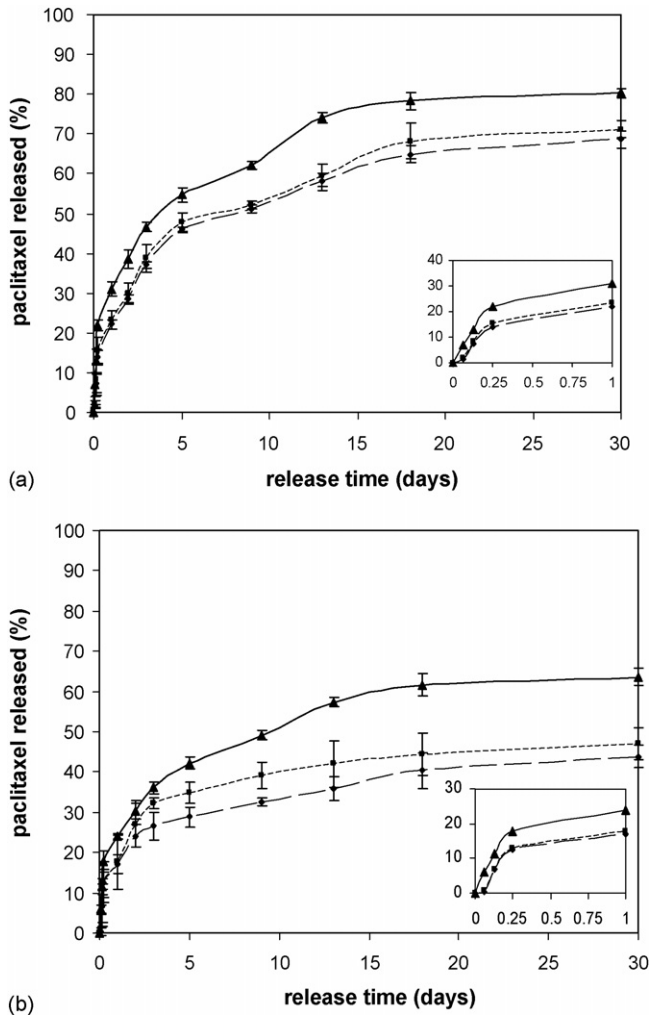

Fig. 3. *In vitro* drug release profiles of the paclitaxel-loaded, (a) PLA–TPGS NPs and (b) PLGA NPs in a pH 1.2 (dashed line), 3.0 (dotted line) buffer solution for the first 1 h to simulate the gastric fluid and subsequently in pH 6.8 buffer solution to simulate the intestinal fluid, and in pH 7.4 PBS buffer (solid line) at 37 °C. The inserts are magnifications of the release from 0 to 1 day, where the measurement was made at 1, 3, 6 and 24 h, respectively. Data represent mean ± S.D., $n = 3$.

swelling and erosion of the polymers were inhibited in lower pH value.

3.2.2. Cellular uptake of nanoparticles

To evaluate the feasibility of the NPs for oral chemotherapy, Caco-2 cells were utilized as an *in vitro* model of the GI drug barrier and HT-29 cells were used as model cancer cells. Caco-2 and HT-29 monolayers are well established *in vitro* models in the literature to evaluate the intestinal permeability and metabolism of drugs (Chang et al., 1996). Fig. 4 shows confocal microscopic images of Caco-2 cells after 2 h incubation with courmarin-6 loaded PLA–TPGS NPs at 37 °C, which were imaged by (a) the combined PI channel and FITC channel, (b) FITC channel, and (c) PI channel, respectively. Coumarin 6, a fluorescence marker, has been widely used as a probe for marking nanoparticles in cellular uptake experiment because of its biocompatibility, high fluorescence activity, low dye loading (<0.5% w/w) and low leaking rate (Win and Feng, 2005; Desai et al., 1997). The intensity of the fluorescence (green, coumarin-6 loaded NPs) closely around the nuclei (red, stained by propidium iodide) indicates that the NPs had been internalized by the cells. The images are made by sectioning function of CLSM, thus can evidence the internalization of the fluorescent nanoparticle.

Fig. 5 shows the effects of the concentration of the fluorescent PLA–TPGS NPs and the incubation time on the cellular uptake efficiency of the NPs by Caco-2 cells, which were made in comparison with fluorescent PLGA NPs. It can be seen from Fig. 5a that the cellular uptake efficiency was expressed as the percentage of the number of the eternalized NPs versus the total number of the fluorescent NPs applied in incubation. Fig. 5a shows the advantages of the PLA–TPGS NPs versus the PLGA NPs in endocytosis by Caco-2 cells. PLA–TPGS NPs were found to have 1.4-, 1.4- and 1.2-fold greater cellular uptake than the PLGA NPs at the particle concentration of 100, 250 and 500 μg/ml, respectively. It can be seen from Fig. 5b that the incubation time is also an important factor to determine the cell uptake efficiency. The longer incubation time, the higher cell uptake efficiency of the NPs could be resulted. The advantages of the PLA–TPGS NPs versus the PLGA NPs were also clearly demonstrated in Fig. 5b. PLA–TPGS NPs enhanced the cell uptake efficiency by about 2-fold for 0.5 h incubation and 1.2-fold for 4 h incubation. These results are consistent with those

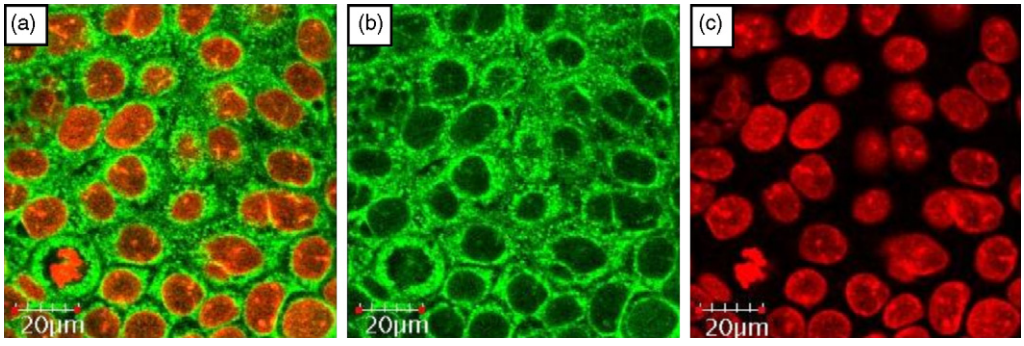

Fig. 4. Confocal microscopic images of Caco-2 cells after 2 h incubation with courmarin-6 loaded PLA–TPGS nanoparticles at 37 °C, which were imaged by (a) the combined PI channel and FITC channel, (b) FITC channel and (c) PI channel, respectively.

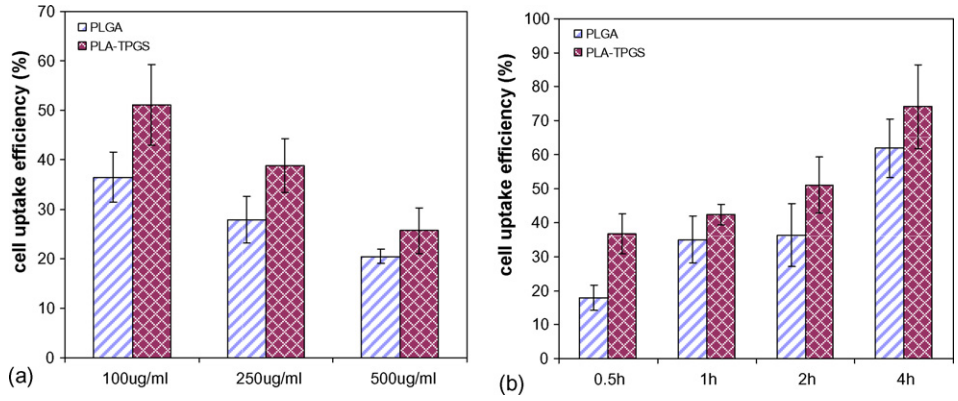

Fig. 5. Effects of (a) the nanoparticles concentration ($n = 6$, $p < 0.05$) and (b) the incubation time at 100 μg/ml nanoparticles concentration on the cell uptake efficiency by Caco-2 cells after 2 h incubation ($n = 6$, $p > 0.05$).

found from the TPGS-coated PLGA NPs in the earlier work of our group, where the TPGS-coated PLGA NPs were found to enhance the Caco-2 cell uptake efficiency by 1.3 times in comparison with the PVA-coated PLGA NPs (Win and Feng, 2005). In comparison with our earlier work on TPGS-emulsified PLGA NPs, PLA–TPGS NPs can increase TPGS absorption stability on the surface of the NPs, which can be controlled by adjusting the TPGS content in the PLA–TPGS copolymers. PLA–TPGS NPs also showed enhancement in the cell uptake efficiency of HT-29 cancer cells compared with PLGA NPs. The cellular uptake efficiency was significantly increased from 32.4 ± 5.8% for PLGA NPs to 57.2 ± 9.0% for PLA–TPGS NPs (data not shown in figure, $p < 0.05$).

3.2.3. Cytotoxicity of nanoparticles

Fig. 6 shows the cytotoxicity of paclitaxel formulated in the PLA–TPGS NPs in comparison with Taxol® incubated with (a) HT-29 and (b) Caco-2 cells, respectively ($n = 6$). To exclude the effects of sample pollution, the drug-loaded NPs were sterilized by gamma-radiation for 72 h. We first confirmed that the placebo PLA–TPGS NPs did not show any detectable cytotoxicity. It can be seen from Fig. 6 that the PLA–TPGS NP formulation of paclitaxel has a comparable cancer cell cytotoxicity with that of Taxol® at all the investigated drug concentration levels. The cell viability was decreased from 45.5 ± 1.7% and 61.4 ± 3.6% for free drug to 38.8 ± 1.7% and 58.9 ± 3.3% for the NPs formulation with 25 μg/ml drug concentration after one day incubation with HT-29 and Caco-2 cells, respectively.

Table 2
IC_{50} values of paclitaxel formulated PLA–TPGS nanoparticles or Taxol® for Caco-2 and HT-29 cells ($n = 3$, $p > 0.05$)

Incubation time (h)	IC_{50} (μg/ml)			
	Caco-2 cells		HT-29 cells	
	NP	Taxol®	NP	Taxol®
24	46.5 ± 18.3	53.0 ± 20.2	3.33 ± 1.24	8.41 ± 2.62
48	17.81 ± 6.21	19.70 ± 4.23	0.144 ± 0.036	0.111 ± 0.042
72	9.47 ± 4.12	10.85 ± 3.21	0.048 ± 0.024	0.025 ± 0.020

IC_{50} values (the drug concentration at which 50% of the incubated cells was deceased) were shown in Table 2. It is clear from the table that the PLA–TPGS NP formulation showed a much lower IC_{50} than Taxol® for HT-29 cells, which is 3.33 ± 1.24 μg/ml for the PLA–TPGS NP formulation compared with 8.41 ± 2.62 μg/ml for Taxol®, after 24 h incubation ($p < 0.05$), a significant decrease of 60.2%. For longer time incubation, the trend still preserved. For IC_{50} of paclitaxel for Caco-2 cells, it can be found than the IC_{50} value was decreased from 19.70 ± 4.23 and 10.85 ± 3.21 μg/ml for Taxol® to 17.81 ± 6.21 and 9.47 ± 4.12 μg/ml for the PLA–TPGS NPs formulation after

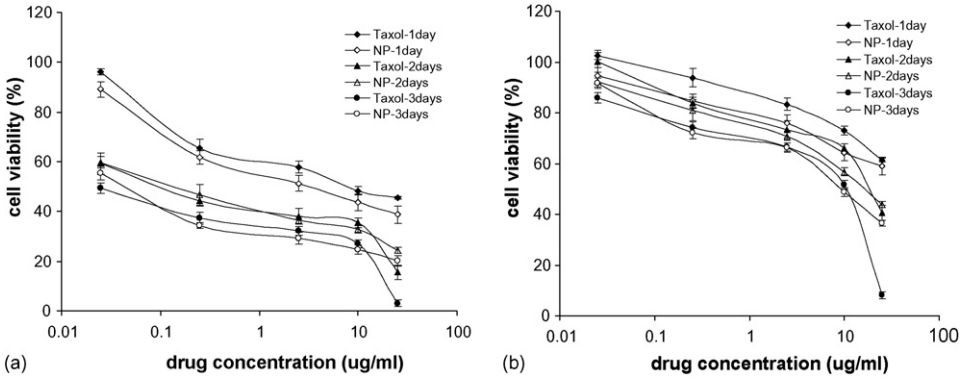

Fig. 6. Cytotoxicity of paclitaxel formulated in PLA–TPGS nanoparticles and Taxol® incubated with (a) HT-29 and (b) Caco-2 cells, respectively ($n = 6$).

48 and 72 h incubation, a 9.59% and 12.7% decrease, respectively ($p > 0.05$). It should be pointed out that such advantages of the PLA–TPGS NP formulation versus Taxol® should be much more significant if the sustainable drug release feature could have been included. It can be seen from Fig. 3b, the drug released from the PLA–TPGS NPs was only $31.1 \pm 1.9\%$, $38.6 \pm 2.2\%$ and $46.6 \pm 1.2\%$ of the encapsulated amount after 24, 48 and 72 h, respectively. The results should have further been corrected by a factor 2 since the drug is released from 0 to the percentage value at the time considered. The formula for such a correction should thus be (corrected value) = (measured value)/(accumulated drug release) × 2 for the cell mortality analysis, and (corrected value) = (measured value) × (accumulated drug release)/2 for the IC_{50} analysis. Since *in vitro* drug release could be very much different from the *in vivo* drug release and it is not feasible to measure the *in vivo* drug release, such a correction is not shown to save journal's space.

4. Conclusion

PLA–TPGS NPs were prepared in this work by the dialysis method, which requires no surfactant/emulsifier/additive involved in the process in comparison with other nanoparticle techniques such as the solvent extraction/evaporation technique that is used most often in the literature. It has been known that chemical surfactants/emulsifiers/additives such as PVA may cause side effects. Paclitaxel-loaded PLA–TPGS NPs were found of size around 343 ± 19 nm in diameter with $5.2 \pm 0.5\%$ drug loading, which showed much faster drug release than the PLGA NPs. Moreover, PLA–TPGS NPs achieved much higher cellular uptake efficiency for Caco-2 cells, which are used as an *in vitro* model of the GI drug barrier for oral chemotherapy, and HT-29 cells, which are used as model cancer cells, in comparison with the PLGA NPs. The drug formulated in the PLA–TPGS NPs also demonstrated much higher *in vitro* cytotoxicity to the HT-29 cancer cells in comparison with Taxol®. Oral chemotherapy by the PLA–TPGS NPs seems feasible.

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