Available online at www.sciencedirect.com
ScienceDirect
International Journal of Pharmaceutics 342 (2007) 208–214
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Poly(D,L-lactide-*co*-glycolide) (PLGA) nanoparticles prepared by high pressure homogenization for paclitaxel chemotherapy

Yuancai Dong [a], Si-Shen Feng [a,b,*]

[a] *Department of Chemical & Biomolecular Engineering, National University of Singapore, Block E5, 02-11, 4 Engineering Drive 4, Singapore 117576, Singapore*
[b] *Division of Bioengineering, Faculty of Engineering, National University of Singapore, Block E5, 02-11, 4 Engineering Drive 4, Singapore 117576, Singapore*

Received 15 February 2007; received in revised form 3 April 2007; accepted 25 April 2007
Available online 6 May 2007

Abstract

High pressure homogenization was employed in the current work to prepare poly(D,L-lactide-*co*-glycolide) (PLGA) nanoparticles (NPs) for controlled release of paclitaxel. The prepared drug-loaded PLGA NPs were found of spherical shape with a size of 200–300 nm. The drug encapsulation efficiency ranged from 34.8 ± 1.6 to $62.6 \pm 7.9\%$ depending on the homogenization pressure and cycles. Paclitaxel was released from the nanoparticles in a biphasic profile with a fast release rate in the first 3 days followed by a slow first-order release. A higher or comparable cytotoxicity against glioma C6 cells was found for the drug formulated in the PLGA NPs in comparison with the free drug Taxol®. Confocal laser scanning microscopy (CLSM) evidenced internalization of the fluorescent coumarin 6-loaded PLGA NPs by the C6 cells. The freeze-dried nanoparticles were found to possess excellent water redispersability. The high pressure homogenization could be applied for large industrial scale production of nanoparticles for drug delivery.

Keywords: Anticancer drugs; Cancer nanotechnology; Chemotherapeutic engineering; Paclitaxel; Nanobiotechnology; Nanomedicine

1. Introduction

Nanoparticles of biodegradable polymers are widely investigated for controlled and targeted delivery of various drugs. Numerous work has demonstrated that such a novel formulation, whether administered by the intravenous or extravascular route, can confer the drug with reduced side effects, extend blood circulation time, and enhance *in vitro*/*in vivo* antitumor efficacy (Gref et al., 1994; Suh et al., 1998; Fernández-Urrusuno et al., 1999; Kim et al., 2001; Redhead et al., 2001; Avgoustakis et al., 2002; Brigger et al., 2002; Fonseca et al., 2002; Cavallaro et al., 2003; Panyam and Labhasetwar, 2003; Miura et al., 2004; Xu et al., 2005). Poly(D,L-lactide-*co*-glycolide) (PLGA) is an excellent synthetic biodegradable copolymer, which has been widely applied to formulate hydrophobic as well as hydrophilic drugs due to its excellent biocompatibility and biodegradability (Jain, 2000). There are several techniques such as the solvent extraction/evaporation method (Feng and Huang, 2001; Mu and Feng, 2002, 2003), the nanoprecipitation method (Dong and Feng, 2004; Govender et al., 1999; Chorny et al., 2002) and the dialysis method (Jeong et al., 2001; Xie and Wang, 2005; Zhang and Feng, 2006), which are used most often in the literature in preparation of therapeutic/imaging agent-loaded PLGA NPs for various biomedical applications. Among them, the solvent extraction/evaporation technique is often adopted due to its good reproducibility, high drug loading capacity, high stability and applicability to both of the hydrophobic (the single emulsion method) and the hydrophilic drugs (the double emulsion method). In a typical single emulsion method, both of the polymer and the drug are dissolved in a water immiscible organic solvent like dichloromethane (DCM), which is then mixed with the water phase containing certain surfactants to form

* Corresponding author at: Department of Chemical & Biomolecular Engineering, Faculty of Engineering, National University of Singapore, Block E5, 02-11, 4 Engineering Drive 4, Singapore 117576, Singapore.
Tel.: +65 6874 3835; fax: +65 6779 1936.
E-mail address: chefss@nus.edu.sg (S.-S. Feng).

0378-5173/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2007.04.031

the primary emulsion. The mixture is further disrupted to fine droplets by some energy input such as sonication, ultra-speed homogenization or high pressure homogenization. The sonication and ultra-speed homogenization are commonly used in the laboratory scale due to their ease of operation. They may not be practical for large industrial scale production. Instead, the high pressure homogenization can be applicable for both of small laboratory scale and large industrial scale productions since it can provide a high throughput and continuous production. Moreover, the high pressure homogenization can produce polymeric nanoparticles with excellent uniformity and dispersability. Indeed, the high pressure homogenization has found wide applications in food, cosmetic and pharmaceutical industries to obtain nanosized emulsions or drug nanocrystals (Muller and Peters, 1998; Krause and Muller, 2001; Jenning et al., 2002; Schultz et al., 2004; Keck and Muller, 2006).

Paclitaxel is a yew tree-derived antineoplastic agent possessing potent activity against a wide range of cancers, especially breast and ovarian cancers (Wani et al., 1971; Schiff et al., 1979; Kohler and Goldspiel, 1994). Since the current commercial formulation of paclitaxel, i.e. Taxol®, contains toxic Cremophor EL, various alternative systems have been developed, which include emulsions, micelles, liposomes, nanoparticles (Meerum Terwogt et al., 1997; Singla et al., 2002). We believe that nanoparticles of biodegradable polymers could be an ideal solution to the poisonous adjuvant problem in formulation of paclitaxel, which can be applied either for intravenous infusion, intratumoral injection, and oral administration (Feng and Chien, 2003; Feng, 2004, 2006; Feng et al., 2004). The purpose of the current work is to investigate the feasibility to prepare drug-loaded PLGA nanoparticles by the high pressure homogenization technique, which will have advantages versus other methods in preparing nanoparticles of higher redispersability and great potentials in promoting large industrial scale production. Effects of the main process parameters related to this technique, i.e. the homogenization pressure and the number of the homogenization cycle, on the nanoparticle characteristics such as the particles size and the drug encapsulation efficiency (EE) were investigated. Field emission scanning electron microscopy (FESEM) was performed to visualize the particles' morphology. The physical state of the drug encapsulated in the polymeric nanoparticles was investigated by differential scanning calorimetry (DSC). High performance liquid chromatography (HPLC) was used to determine the drug loading level in the nanoparticles and the *in vitro* drug release kinetics. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was employed to evaluate the *in vitro* cytotoxicity of the drug formulated in the PLGA NPs with glioma C6 cells as model cancer cells, which was made in close comparison with Taxol®. It was found that the paclitaxel formulated in the PLGA NPs exhibited higher or comparable cytotoxicity compared with the Taxol® at various drug concentrations. Cellular uptake of the fluorescent coumarin 6-loaded PLGA NPs produced by the high pressure homogenization was visualized by confocal laser scanning microscopy (CLSM).

2. Materials and methods

2.1. Materials

PLGA (50:50, Mw 40,000–75,000), polyvinyl alcohol (PVA, Mw 40,000–70,000) and coumarin 6 were purchased from Sigma. Paclitaxel was supplied by Yunnan Hande Biotechnology, Kunming, China. Dichloromethane (DCM) and HPLC grade acetonitrile were from Aldrich.

2.2. Preparation of paclitaxel-loaded PLGA NPs

One hundred and ten milligrams of PLGA and 5% (w/w) paclitaxel were dissolved in 5 ml DCM. After complete dissolution, the drug solution was mixed with 50 ml aqueous PVA (1%) solution to form the primary emulsion, which was then introduced to the high pressure homogenizer (EmulsiFlex™-C5, Avestin Inc., Canada). Homogenization was performed at 86 or 155 MPa for one, two and three cycles, respectively. The processed emulsion was evaporated overnight to remove the DCM and collected by centrifugation at 11,000 rpm for 0.5 h. The samples were washed two times. The dried powder was obtained by freeze-drying the nanosuspension for 48 h. Fluorescent coumarin 6-loaded PLGA NPs were prepared in the same way except that paclitaxel was replaced by 0.5% (w/w) coumarin 6.

2.3. Particles size

Size and size distribution of the prepared PLGA NPs were measured by the dynamic laser light scattering technique (90 Plus Particle Sizer, Brookhaven Instruments Corporation). Before measurement, the particle suspension was appropriately diluted. The reported value was mean ± S.D. ($n = 5$).

2.4. Drug encapsulation efficiency

Drug loading in the polymeric nanoparticles was assessed by HPLC. Briefly, 3 mg dried drug-loaded nanoparticles were dissolved in 1 ml DCM. After solvent evaporation, the residue was reconstituted in 2 ml mobile phase, i.e. the mixture of 50% acetonitrile and 50% water for HPLC analysis (Agilent 1100). The mobile phase was delivered at flow rate of 1 ml/min and paclitaxel was detected at 227 nm. The drug encapsulation efficiency (EE) was expressed as the percentage of the drug amount found in the nanoparticles to the initially feed drug amount. The measurement was done in triplicate.

2.5. Morphology

FESEM (JEOL, JSM-6700F) was used to observe the nanoparticle surface morphology. Before visualization, a diluted nanoparticle suspension was deposited onto the copper grid and coated by platinum for 40 s.

2.6. DSC

The physical state of paclitaxel loaded in the nanoparticles and the pristine paclitaxel was investigated by DSC (DSC 822e, Mettler Toledo, Switzerland) under nitrogen atmosphere at flow rate of 20 ml/min. Ten milligrams of nanoparticles were heated from 20 to 250 °C at speed of 10 °C/min.

2.7. In vitro drug release

Paclitaxel or coumarin 6-loaded PLGA NPs prepared at the homogenization pressure of 86 MPa for one cycle were used for presentation of the *in vitro* drug release, cell viability and cell uptake studies. Briefly, 5 mg freeze-dried drug-loaded nanoparticles were put in a capped tube and suspended in 5 ml phosphate buffer solution (PBS) containing 0.1% Tween 80 to maintain the sink condition. The tubes were placed in the water bath at 37 °C shaking at 120 rpm. At specific intervals, the release medium containing the drug was transferred out and extracted with 1 ml dichloromethane. Five millitres of fresh release medium was added to the test tubes for the continuous release studies. The dichloromethane in the extraction solution was allowed to evaporate completely and the residue was reconstituted in 1 ml 50% Millipore water plus 50% acetonitrile for HPLC analysis as described before. The measurement was done in triplicate.

2.8. Cell viability studies

In a 96-well plate (Nunclon™), glioma C6 cells (American Type Culture Collection) were seeded at a density of 8000 cells/well. The culture medium was Dubelco's modified essential medium (DMEM, Sigma) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin. After 12 h incubation allowing for cells attachment, the medium was removed and the cells were incubated with the Taxol® or the paclitaxel-loaded PLGA NP suspension at paclitaxel concentration of 0.1, 1 and 10 μg/ml, respectively. The drug-loaded PLGA NPs had been sterilized by γ-irradiation before the cell culture experiment. One column, i.e. eight wells, was used for one sample assessment. At specific intervals (24, 48 and 72 h), the supernatant in each well was piped out and the wells were washed twice by cold PBS. Ten microlitres of MTT solution (5 mg/ml in PBS) and 90 μl culture medium were introduced. After 4 h incubation, the precipitates were formed and solubilized by 100 μl isopropanol. The absorbance intensity was measured by the microplate reader (Genios, Tecan, Männedorf, Switzerland) at 570 nm with a reference wavelength of 620 nm. One column cells without drug treatment were used as the control. Cell viability was expressed by the following equation:

$$\text{cell viability}\,(\%) = \frac{\text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

$\text{Abs}_{\text{sample}}$ was the absorbance intensity of the cells treated with Taxol® or paclitaxel-loaded PLGA NPs, while $\text{Abs}_{\text{control}}$ was the absorbance intensity of the cells without drug treatment.

2.9. CLSM

C6 cells were cultured on the chambers (Lab-Tek® Chambered Coverglass System) at a density of 5×10^4 cells/well. On the second day, the medium was piped out and the cells were incubated with 500 μl 0.25 mg/ml coumarin 6-loaded PLGA NPs for 2 h. Upon the removal of the remained nanoparticles and washing the wells thrice with PBS, the cells were fixed by ethanol for 20 min. Subsequently, propidium iodide (PI) was added to stain the nucleus. Finally, the cells were mounted by the mounting medium (DAKO® Fluorescent Mounting Medium) and observed under confocal laser scanning microscopy (Leica TCS SP2) using FITC filter (Ex (λ) 495 nm, Em (λ) 520 nm).

3. Results and discussions

3.1. Size and EE

Paclitaxel-loaded PLGA NPs were prepared in the present study by the high pressure homogenization, which has at least three main advantages in comparison with other technologies: (1) it applies to large industrial scale production of polymeric nanoparticles, (2) it produces uniform nanoparticles, and (3) its products have excellent redispersability, which is especially important for nanoparticles of biomedical applications. In this technology, nanosizing the primary emulsion was accomplished by the high pressure homogenizer, which was a piston-gap type in our study. Its working principle is based on the Bernoulli's law. Cavitation is believed to be the main cause of size reduction. Turbulence, collision and shear process are also responsible (Keck and Muller, 2006). Table 1 shows effects of the homogenization pressure and the number of homogenization cycles on the nanoparticles size and the drug encapsulation efficiency (EE). Under the homogenization pressure of 86 or 155 MPa, the prepared nanoparticles had a size of 200–300 nm. It seems that particles size was not significantly influenced by the homogenization pressure and the number of homogenization cycles. The reason is perhaps, that since the emulsion is a fluid system, a low homogenization pressure (e.g. 86 MPa) and one homogenization cycle is sufficient to nanosize the primary emulsion. In comparison, a higher homogenization pressure and 10–20 cycles are normally needed to obtain solid drug nanocrystals (Keck and Muller, 2006). It was also found that the EE was not significantly influenced by the homogenization pressure, while

Table 1
Size and EE of drug-loaded PLGA NPs produced by the high pressure homogenization

Sample	Homogenization pressure (MPa)	Cycle	Size (nm)	EE (%)
NP1	86	1	305.4 ± 10.6	62.6 ± 7.9
NP2	86	2	261.7 ± 3.3	49.5 ± 2.7
NP3	86	3	269.2 ± 12.2	35.1 ± 3.1
NP4	155	1	304.9 ± 41.0	52.2 ± 4.9
NP5	155	2	266.8 ± 13.6	50.9 ± 6.5
NP6	155	3	245.0 ± 20.4	34.8 ± 1.6

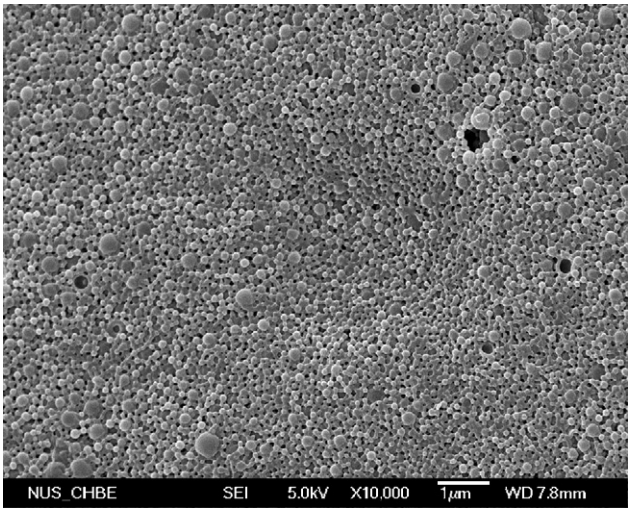


Fig. 1. FESEM image of paclitaxel-loaded PLGA NPs.

increasing the number of homogenization cycles caused more drug loss and therefore a decrease of the EE.

3.2. Morphology

The paclitaxel-loaded PLGA NPs were found spherical in shape as shown in Fig. 1. The size was around 300 nm with good uniformity, which is consistent with the result determined by the laser light scattering.

3.3. DSC

DSC thermograms of the paclitaxel and the paclitaxel-loaded PLGA NPs are shown in Fig. 2, from which we can see that an endothermic melting peak was exhibited at ~223 °C for the pristine paclitaxel. This peak, however, disappeared for the paclitaxel-loaded PLGA NPs, indicating that paclitaxel formulated in the nanoparticles existed as an amorphous state or a solid solution in the polymeric matrix.

3.4. In vitro drug release

As shown in Fig. 3, paclitaxel was released from the drug-loaded PLGA NPs in a biphasic pattern: a fast release rate in the first 3 days followed by a slow uniform release afterwards. The initial fast drug release can be ascribed to those drugs located on or near the particles surface; while the slow and uniform release could be caused by diffusion of the drugs inside the nanoparticles. In the first 3 days, about 60.0 ± 0.3% of the loaded drug was released out of the nanoparticles. The accumulative drug release in 21 days was 72.0 ± 3.2%.

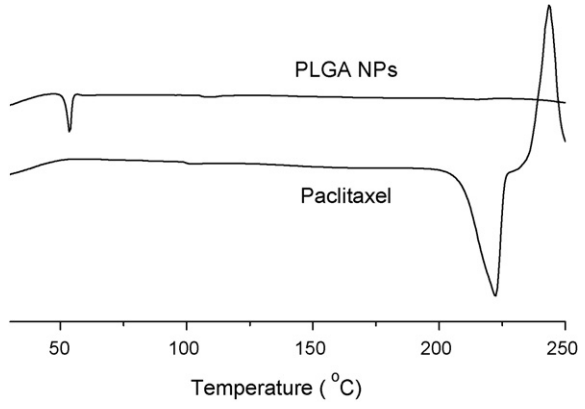


Fig. 2. DSC thermograms of paclitaxel and paclitaxel-loaded PLGA NPs.

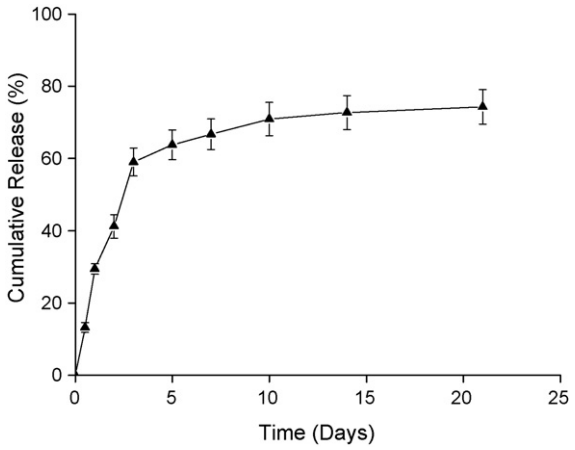
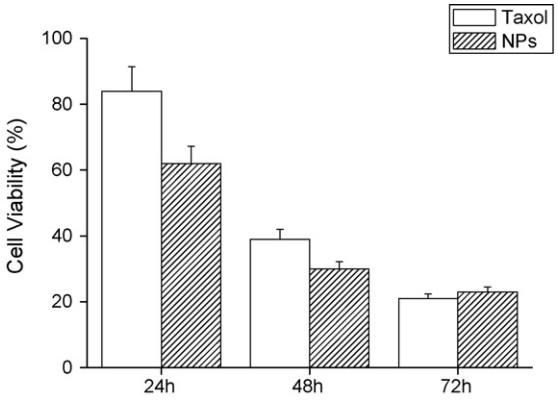


Fig. 3. *In vitro* accumulated drug release of the paclitaxel-loaded PLGA NPs.

3.5. Cell viability study

Fig. 4 shows the viability of C6 cells treated by Taxol® or the paclitaxel-loaded PLGA NPs at paclitaxel concentration of 1 μg/ml after 24, 48 and 72 h incubation. The result for the placebo PLGA NPs (without paclitaxel encapsulated) was not shown since no significant cytotoxicity was observed at the studied nanoparticle concentration levels, which implies that the polymer is biocompatible. It can be observed from Fig. 4 that the cancer cells treated by the paclitaxel-loaded NPs had lower viability (or equivalently, higher mortality) than those treated by Taxol®. The cell viability was 61.5 ± 5.2, 30.3 ± 2.1, and 22.9 ± 1.5% after 24, 48, and 72 h incubation for the paclitaxel-loaded PLGA NPs in comparison with 83.8 ± 7.4, 38.9 ± 3.0, and 21.2 ± 1.4% for Taxol®, respectively. By converting the cell viability to the cell mortality (viability + mortality = 100%) as shown in the table attached below the graph, we can see that the mortality of the C6 cells treated with paclitaxel-loaded PLGA NPs was 2.38, 1.15 and 0.97 times higher than that of the cells treated with Taxol® for 24, 48 and 72 h, respectively, which means that the paclitaxel-loaded PLGA NPs are 2.38, 1.15 and 0.97 times more effective than Taxol® for C6 cell treatment for 24, 48 and 72 h respectively. Furthermore, the NPs formulation of paclitaxel actually should have been much more effective than Taxol® if the sustainable drug release feature for the NP formulation was considered, which can be found from Fig. 3 to be 29.4, 41.2 and 59.0% of the drug encapsulated in the PLGA NPs after 24, 48 and 72 h release respectively. The measured mortality should be modified by the accumulative drug release by the following formula:

$$\text{modified mortality} = \frac{\text{measured mortality}}{\text{accumulated drug release}} \times 2$$



Mortality (%)	24 h	48h	72h
(1) Taxol®	16.2	61.1	78.8
(2) NP formulation	38.5	69.7	77.1
(2)/(1)	**2.38**	**1.15**	**0.97**
(3) Drug release	29.4	41.2	59.0
(4) Revised (2)	262	338	261
(4)/(**1**)	**16.17**	**5.54**	**3.32**

Fig. 4. C6 cell viability after 24, 48 and 72 h treatment by Taxol® or the paclitaxel-loaded PLGA NPs at the paclitaxel concentration of 1 μg/ml, respectively. The attached table shows the cell mortality both measured and modified by the accumulated drug release from the NPs.

The reason for multiplication by 2 is that (1) mortality is proportional to the area-under-the-curve of the *in vitro* PK (the drug concentration versus time curve), or simply the area under the curve (AUC) and (2) Taxol® is immediately available for the cells while drug is released from zero to certain concentration at the day observed. This means that the AUC for Taxol is a rectangular (area = concentration × time) while the AUC for the nanoparticle formulation is a triangle (area = concentration × time/2). This formula gives modified values of the cell mortality of 262, 338 and 261%, respectively, which is listed in row (4) in the attached table of Fig. 4. This might imply that with the sustainable drug release feature considered, the NP formulation could have been 16.17, 4.54 and 3.32 times more effective than Taxol® *in vitro* at paclitaxel concentration of 1 μg/ml after 24, 48 and 72 h treatment respectively. However, this kind of modification is unrealistic although it makes us believe that the NP formulation should be more effective than Taxol® as demonstrated by the viability measurement. Moreover, *in vivo* and *in vitro* drug release from the nanoparticles could be very much different (Feng, 2006).

We also did the viability experiment for C6 cells treated by Taxol® or the paclitaxel-loaded PLGA NPs at paclitaxel concentration of 0.1 and 10 μg/ml after 24, 48 and 72 h incubation, respectively. For 0.1 μg/ml paclitaxel concentration treatment, the viability of the cancer cells treated by the paclitaxel-loaded NPs was 86.6 ± 7.2, 41.2 ± 2.7, and 27.5 ± 2.1% after 24, 48, and 72 h incubation for the paclitaxel-loaded PLGA NPs in comparison with 88.2 ± 7.4, 43.9 ± 3.1, and 25.8 ± 1.8% for the cells treated with Taxol®, respectively. The corresponding mortality is thus 13.4, 58.8, 72.5% for the paclitaxel-loaded PLGA NPs treatment and 11.8, 56.1, 74.2% for the Taxol® treatment after 24, 48 and 72 h incubation, respectively, which implies that the paclitaxel-loaded PLGA NPs are 1.136, 1.045, 0.977 times more effective than Taxol® treated cells at 24, 48 and 72 h, respectively. The advantage of the nanoparticle formulation vs Taxol® seems not significant at such a low drug concentration. However, after corrected by the drug release, the nanoparticle formulation could have been 7.73, 5.09, 3.31 times more effective than Taxol® treated cells at 24, 48 and 72 h, respectively. For the treatment of higher paclitaxel concentration 10 μg/ml, the viability of the cancer cells treated by the paclitaxel-loaded NPs was 61.1 ± 5.1, 31.1 ± 2.1, and 23.5 ± 1.5% after 24, 48, and 72 h incubation for the paclitaxel-loaded PLGA NPs in comparison with 87.8 ± 7.5, 46.1 ± 3.4, and 21.3 ± 1.4% for Taxol®, respectively. The corresponding mortality data thus show that the paclitaxel-loaded PLGA NPs are 3.19, 1.278, 0.977 times more effective than Taxol® treated cells at 24, 48 and 72 h, respectively. It seems that the advantage of the nanoparticle formulation vs Taxol® is significant in short period of 1 day and becomes insignificant in long period of 3 days. However, after corrected by the drug release, the nanoparticle formulation could have been 21.7, 6.21, 3.38 times more effective than Taxol® at 24, 48 and 72 h, respectively.

3.6. CLSM

Fig. 5 shows a confocal laser scanning microscopy (CLSM) image of the C6 cells after 2 h incubation with the coumarin

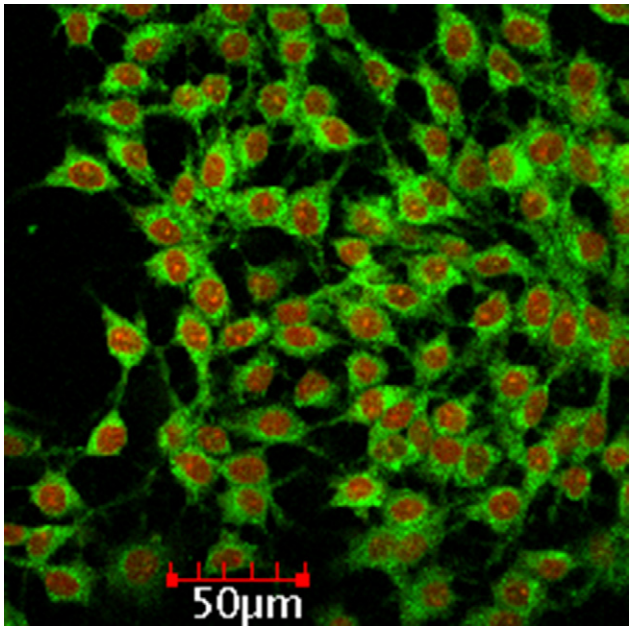


Fig. 5. Confocal laser scanning microscopy (CLSM) of C6 cells after 2 h incubation with the coumarin 6-loaded PLGA NPs at NP concentration of 0.25 mg/ml. The cell nuclei are shown in red color (PI stained) and the coumarin 6-loaded PLGA NPs are shown in green, which demonstrated the cellular uptake of the nanoparticles and nanoparticles can also be found in the nuclei. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

6-loaded PLGA NPs at the nanoparticle concentration of 0.25 mg/ml. The cell nuclei are shown in red color (PI stained) and the coumarin 6-loaded PLGA NPs are shown in green, which demonstrated the cellular uptake of the nanoparticles as well as the penetration of the nanoparticles into the nuclei.

4. Conclusion

Paclitaxel-loaded PLGA NPs were prepared by the high pressure homogenization technology. The drug-loaded nanoparticles were found of spherical shape with a size of 200–300 nm. Within the range of the instrument setting, it was found that the particles size was not significantly influenced by the homogenization pressure and the number of homogenization cycles, while increasing the number of homogenization cycle led to a decrease of drug encapsulation efficiency. The drug release profile of paclitaxel was biphasic with a fast release rate in the first 3 days followed by a slow release. A higher or comparable cytotoxicity was found for paclitaxel-loaded PLGA NPs in comparison with Taxol®, which could be ascribed to the internalization of the nanoparticles by the cells as shown in the CLSM image as well as the sustainable drug release feature of the nanoparticle formulation. The high pressure homogenization is useful for large scale industrial production of drug-loaded nanoparticles of biodegradable polymers. Moreover, nanoparticles produced by this method can have more uniform size and better redispersability after restoration than those produced by other methods such as the solvent extraction/evaporation method and the nanoprecipitation method, which will thus facilitate their biomedical applications.

Acknowledgments

This research was supported by SCS Grant U0028 (NUS R397-000-606-305, 2004, R279-000-187-305, 2005, and R279-001-187-305, 2006), Singapore Cancer Syndicate, BMRC, A*STAR (S.S. Feng: PI) and NUS FRC Grant R279-000-226-112, 2007–2008, National University of Singapore (S.S. Feng: PI).

References

Avgoustakis, K., Beletsi, A., Panagi, Z., Klepetsanis, P., Karydas, A.G., Ithakissios, D.S., 2002. PLGA-mPEG nanoparticles of cisplatin: in vitro nanoparticle degradation, in vitro drug release and in vivo drug residence in blood properties. J. Control. Rel. 79, 123–135.

Brigger, I., Dubernet, C., Couvreur, P., 2002. Nanoparticles in cancer therapy and diagnosis. Adv. Drug Deliv. Rev. 54, 631–651.

Cavallaro, G., Licciardi, M., Giammona, G., Caliceti, P., Semenzato, A., Salmaso, S., 2003. Poly(hydroxyethylaspartamide) derivatives as colloidal drug carrier systems. J. Control. Rel. 89, 285–295.

Chorny, M., Fishbein, I., Danenberg, H.D., Golomb, G., 2002. Lipophilic drug loaded Nanospheres by nanoprecipitation: effect of formulation variables on size, drug recovery and release kinetics. J. Control. Rel. 83, 389–400.

Dong, Y.C., Feng, S.S., 2004. Paclitaxel-loaded methoxy poly(ethylene glycol)-poly(lactide) (MPEG-PLA) nanoparticles by nanoprecipitation method. Biomaterials 25, 2843–2849.

Feng, S.S., Huang, G., 2001. Effects of emulsifiers on the controlled release of paclitaxel from nanoparticles of biodegradable polymers. J. Control. Rel. 71, 53–69.

Feng, S.S., Chien, S., 2003. Chemotherapeutic engineering: application and further development of chemical engineering principles for chemotherapy of cancer and other drugs. Chem. Eng. Sci. 58, 4087–4114.

Feng, S.S., Mu, L., Win, K.Y., Huang, G., 2004. Nanoparticles of biodegradable polymers for clinical administration of paclitaxel. Curr. Med. Chem. 11, 413–424.

Feng, S.S., 2004. Nanoparticles of biodegradable polymers for new-concept chemotherapy. Expert Rev. Med. Dev. 1, 115–125.

Feng, S.S., 2006. Nanoparticles of biodegradable polymers for new-concept chemotherapy—where are we now? Nanomedicine 1, 297–309.

Fernández-Urrusuno, R., Calvo, P., Remuñan-López, C., Vila-Jato, J.L., Alonso, M.J., 1999. Enhancement of nasal absorption of insulin using chitosan nanoparticles. Pharm. Res. 16, 1576–1591.

Fonseca, C., Simoes, S., Gaspar, R., 2002. Paclitaxel-loaded PLGA nanoparticles: preparation, physicochemical characterization and in vitro anti-tumoral activity. J. Control. Rel. 83, 273–286.

Govender, T., Stolnik, S., Garnett, M.C., Illum, L., Davis, S.S., 1999. PLGA nanoparticles prepared by nanoprecipitation: drug loading and release studies of a water soluble drug. J. Control. Rel. 99, 171–185.

Gref, R., Minamitake, Y., Peracchia, M.T., Trubetskoy, V., Torchilin, V., Langer, R., 1994. Biodegradable long-circulating polymeric nanospheres. Science 263, 1600–1603.

Jain, R.A., 2000. The manufacturing techniques of various drug loaded biodegradable poly(lactide-*co*-glycolide) (PLGA) devices. Biomaterials 21, 2475–2490.

Jenning, V., Lippacher, A., Gohla, S.H., 2002. Medium scale production of solid lipid nanoparticles (SLN) by high pressure homogenization. J. Microencapsul. 19, 1–10.

Jeong, Y.I., Cho, C.S., Kim, S.H., Ko, K.S., Kim, S.I., Shim, Y.H., Nah, J.W., 2001. Preparation of poly(D,L-lactide-*co*-glycolide) nanoparticles without surfactant. J. Appl. Polym. Sci. 80, 2228–2236.

Keck, C.M., Muller, R.H., 2006. Drug nanocrystals of poorly soluble drugs produced by high pressure homogenization. Int. J. Pharm. Biopharm. 62, 3–16.

Kim, S.Y., Lee, Y.M., Shin, H.J., Kang, J.S., 2001. Indomethacin-loaded methoxy poly(ethylene glycol)/poly(ε-caprolactone) diblock copolymeric nanosphere: pharmacokinetic characteristics of indomethacin in the normal Sprague-Dawley rats. Biomaterials 22, 2049–2056.

Kohler, D.R., Goldspiel, B.R., 1994. Paclitaxel (Taxol). Pharmacotherapy 14, 3–34.

Krause, K.P., Muller, R.H., 2001. Production and characterization of highly concentrated nanosuspensions by high pressure homogenisation. Int. J. Pharm. 214, 21–24.

Meerum Terwogt, J.M., Nuijen, B., Ten Bokkel Huinink, W.W., Beijnen, J.H., 1997. Alternative formulations of paclitaxel. Cancer Treat. Rev. 23, 87–95.

Miura, H., Onishi, H., Sasatsu, M., Machida, Y., 2004. Antitumor characteristics of methoxypolyethylene glycol-poly(DL-lactic acid) nanoparticles containing camptothecin. J. Control. Rel. 97, 101–113.

Mu, L., Feng, S.S., 2002. Vitamin E TPGS used as emulsifiers in the solvent evaporation/extraction technique for fabrication of polymeric nanoparticles for controlled release of paclitaxol. J. Control. Rel. 80, 129–144.

Mu, L., Feng, S.S., 2003. PLGA/TPGS nanoparticles for controlled release of paclitaxel: effects of the emulsifier and drug loading ratio. Pharm. Res. 20, 1864–1872.

Muller, R.H., Peters, K., 1998. Nanosuspensions for the formulation of poorly soluble drugs I. Preparation by a size-reduction technique. Int. J. Pharm. 160, 229–237.

Panyam, J., Labhasetwar, V., 2003. Biodegradable nanoparticles for drug and gene delivery to cells and tissue. Adv. Drug Deliv. Rev. 55, 329–347.

Redhead, H.M., Davis, S.S., Illum, L., 2001. Drug delivery in poly(lactide-*co*-glycolide) nanoparticles surface modified with poloxamer 407 and poloxamine 908: in vitro characterization and in vivo evaluation. J. Control. Rel. 70, 353–363.

Schiff, P.B., Fant, J., Horwitz, S.B., 1979. Promotion of microtubule assembly in vitro by taxol. Nature 277, 665–667.

Schultz, S., Wagner, G., Urban, K., Ulrich, J., 2004. High-pressure homogenization as a process for emulsion formation. Chem. Eng. Tech. 27, 361–368.

Singla, A.K., Garg, A., Aggarwal, D., 2002. Paclitaxel and its formulations. Int. J. Pharm. 235, 179–192.

Suh, H., Jeong, B., Rathi, R., Kim, S.W., 1998. Regulation of smooth muscle cell proliferation using paclitaxel-loaded poly (ethylene oxide)-poly (lactide/glycolide) nanospheres. J. Biomed. Mater. Res. 42, 331–338.

Wani, M.C., Taylor, H.L., Wall, M.E., Coggon, P., McPhail, A.T., 1971. Plant tumor agents. VI. The isolation and structure of Taxol, a novel antieukemic and antitumor agent from taxus brevifolia. J. Am. Chem. Soc. 93, 2325–2327.

Xie, J., Wang, C.H., 2005. Self-assembled biodegradable nanoparticles developed by direct dialysis for the delivery of paclitaxel. Pharm. Res. 22, 2079–2090.

Xu, Z., Gu, W., Huang, J., Sui, H., Zhou, Z., Yang, Y., Yan, Z., Li, Y., 2005. In vitro and in vivo evaluation of actively targetable nanoparticles for paclitaxel delivery. Int. J. Pharm. 288, 361–368.

Zhang, Z.P., Feng, S.S., 2006. In vitro investigation on poly(lactide)-Tween 80 copolymer nanoparticles fabricated by dialysis method for chemotherapy. Biomacromolecules 7, 1139–1146.