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Preparation and cellular uptake of PLGA particles loaded with lamivudine

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Poly(D,L-lactide-co-glycolide) (PLGA) nanoparticles loaded with lamivudine and coated with bovine serum albumin (BSA) were prepared via a double emulsion method. The influences of experiments parameters such as volume of inner aqueous phase, concentration of organic phase and ultrasonication time on the particle size and drug entrapment efficiency were investigated, obtaining PLGA particles with a diameter of ~260 nm and drug entrapment efficiency of ~35%. The particles were observed by scanning electron microscopy and transmittance electron microscopy, showing a core-shell structure. BCA assay found that 58 mg BSA was present on/in 1 g LPB particles. The loaded lamivudine showed a burst release at beginning and sustained release until 24 h in physiological conditions. Low pH could accelerate the release of lamivudine from PLGA particles, making the PLGA particles potential intelligent intracellular drug carriers. The PLGA particles were readily internalized into the human liver cells within a short time and increased gradually with the prolongation of incubation time regardless of the loading of lamivudine. The particles either resided within lysosomes or transferred to cytoplasm, but could not enter into the cell nucleus. The cell viability was not significantly influenced in the presence of the particles regardless of lamivudine encapsulation, suggesting that this kind of particles may be a good candidate for the intracellular anti-hepatitis B drug delivery.

PLGA, lamivudine, cell uptake, intracellular distribution, drug delivery

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Hepatitis B caused by hepatitis B virus (HBV) is an infectious inflammatory illness of the liver, and is also considered as a causative factor of the development of hepatocellular carcinoma [1]. HBV infection is one of the most common infections over the world and especially severe in China. Of the 350–400 million individuals worldwide infected with the HBV, one-third resides in China, and about 5% of them are chronically infected [2,3]. Every year, estimated 300000 people die from HBV-related diseases in China, including 180000 patients with hepatocellular carcinoma.

Currently, medications for treatment of hepatitis B infection are mainly divided into antiviral drugs (nucleoside analogues) and immune system modulators (interferon). Although none of these drugs can clear the infection, they can stop the virus from replicating, thus minimizing liver damage. Among the antiviral drugs, lamivudine (LAM, Figure 1(a)), a cytosine nucleoside analogue, has shown good results in the treatment of HBV infection in human beings [4,5]. Once phosphorylated to active metabolites, lamivudine can inhibit the HBV reverse transcriptase enzyme competitively and act as a chain terminator of DNA synthesis. However, this drug exhibits very short physiological half-life, and therefore, requires a specially designed dosage everyday, which may cause high level resistance and other side effects [6]. Thus, protection in physiological environment and increase of residence time of lamivudine inside cells would greatly improve the treatment efficiency. To achieve this aim, lamivudine has been encapsulated into various colloidal carriers such as biodegradable polymer spheres [7] and micelles [8], and conjugated to polysaccharide

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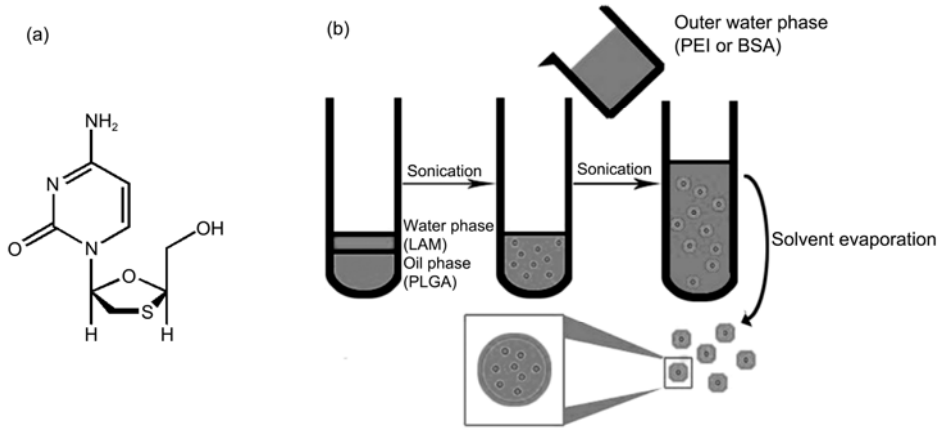

Figure 1 (a) Chemical structure of lamivudine; (b) preparation process of LPB particles.

and dendrimers [9,10].

Poly(lactide-co-glycolide) (PLGA) nanoparticles are widely used as carriers for drug, gene and vaccine delivery because of its good biocompatibility and biodegradability, low cytotoxicity and easy preparation [11,12]. The PLGA particles may protect the drug, gene and vaccine against degradation and ensure their transport and delivery [13,14], and also can control the release rate and achieve targeting [15–18]. Among the method for preparation of PLGA particles, the emulsification-solvent evaporation is the most frequently employed one [19]. The water/oil/water (W/O/W) double emulsion technique has been successfully developed and used to incorporate hydrophilic molecules such as hydrophilic drugs, proteins and nucleotide into the lumen of biodegradable particles [20–23].

In our previous study, PLGA particles are used to encapsulate hydrophobic dyes and anti-cancer drug by oil/water emulsion employing bovine serum albumin (BSA) as stabilizers, which are simultaneously loaded onto the particles during the formulation process. The surface immobilized macromolecules enable further covalent grafting of poly(ethylene glycol) (PEG) and folic acid (FA), or physical modification via layer-by-layer (LBL) assembly [24–26]. Herein we shall use W/O/W double emulsion to encapsulate water soluble lamivudine with BSA as macromolecular surfactant. The preparation parameters are optimized with respect to the particle size and drug encapsulation efficiency. The physiochemical properties of resultant particles (the so-called LAM-PLGA-BSA, LPB) are characterized in terms of chemical composition, morphology, surface charge property and drug release kinetics. Finally, cellular uptake kinetics and intracellular distribution of the LPB particles by human liver cells and their influences on the cell viability shall be explored.

1 Materials and methods

1.1 Reagents

Poly(lactide-co-glycolide) (PLGA, LA∶GA=75∶25, M_W= 130 kD), bovine serum albumin (BSA) and 4,6-diamidino-2-phenylindole (DAPI) were obtained from Sigma-Aldrich. MitoTracker® Green and LysoTracker® Green were purchased from Invitrogen Co., Ltd. All other chemicals were analytical grade and used without further treatment. Milli-Q water was used throughout the experiments.

1.2 Particles preparation

PLGA particles were prepared by means of a double emulsion-solvent evaporation method (Figure 1(b)). Briefly, 0.1–0.4 mL lamivudine (Figure 1(a)) aqueous solution (10 mg/mL, internal aqueous phase) or pure water (blank PLGA/BSA particles) was emulsified in 1 mL PLGA dichloromethane solution (oil phase) using an ultrasonicator (MISONIX Ultrasonic liquid Processors) for desired time (10–120 s). The power of ultrasonic was set as 6 W. Four different PLGA concentrations (2%, 4%, 6%, 8%; w/v) were employed in the oil phase. This primary W/O emulsion was rapidly added into 4 mL 3% (w/v) BSA aqueous solution (external aqueous phase) and emulsified again for the same time period. The resultant double emulsion was then poured into 50 mL Milli-Q water, stirred for 1 h at room temperature to allow evaporation of the organic solvent. The PLGA particles were collected by centrifugation at 15000 r/min for 15 min, washed with Milli-Q water for three times, and freeze-dried overnight under vacuum. PLGA particles containing Nile red or coumarin were similarly prepared by addition of 0.2 mg/mL Nile red or coumarin into PLGA solution before mixing with lamivudine solution.

1.3 Particles characterization

(1) Morphology. The morphology of the PLGA particles was analyzed by scanning electron microscopy (SEM, HITACHI S-520) and transmission electron microscopy (TEM, Philips TECNAL-10). A drop of the PLGA particles suspension was added onto a clean glass and copper grid with carbon membrane for SEM and TEM observation,

respectively.

(2) Size and surface charge measurements. The size and surface charge of the particles were determined using Beckman Delsa^{TM} Nano (Beckman Coulter). The data was averaged from three parallel experiments.

(3) BSA content assay. To determine the BSA amount on the particles, a certain amount PLGA particles was dissolved in a solution of 5% (w/v) sodium dodecyl sulfate (SDS) in NaOH (pH 13). The system was sonicated at 40°C for 40 min and centrifuged at 15000 r/min for 15 min. The amount of BSA was measured from the supernatant by a BCA protein assay kit.

(4) Drug loading determination. The drug content was determined by a high-pressure liquid chromatography (HPLC) method. Briefly, a certain amount of particles was dissolved in 1 mol/L NaOH solution, and the suspension was stirred at 40°C for 30 min until complete dissolution of the polymer spheres. The resultant transparent solution was neutralized with 1 mol/L HCl and diluted to a certain volume. Then the solution was filtered and injected into the HPLC equipment to determine drug content. Samples were passing through a C18 pre-column and a Jupiter C18 reverse-phase (150×3.9 mm (I.D.)) with 5 mm packing particles (Phenomenex, Torrance, CA). The mobile phase was 0.02 mol/L phosphate buffer saline (pH 7.2), acetonitrile and methanol (91∶0.1∶8.9). The mobile phase was degassed and filtered before use. Flow rate was set at 1.5 mL/min and the UV-vis detector was set at 274 nm. The 20 μL sample was injected and the measurement was repeated in triplicate and averaged.

In this study, drug loading (DL) and entrapment efficiency (EE) are expressed as [27]:

$$\mathrm{DL}(\%) = \frac{\text{Amount of drug}}{\text{Amount of (polymer} + \text{drug)}} \times 100, \quad (1)$$

$$\mathrm{EE}(\%) = \frac{\text{Drug loading amount}}{\text{Drug feeding amount}} \times 100. \quad (2)$$

(5) Release of lamivudine from the LPB particles [28,29]. The release behavior of lamivudine encapsulated inside the LPB particles was studied at 37°C. Briefly, 50 mg LPB particles were suspended in 2 mL PBS, and sealed into a dialysis bag. The dialysis bag was then immersed into 48 mL PBS, and stirred for designed time intervals with a magnetic stirrer. At each time interval, 1 mL of PBS outside the dialysis bag was taken out from the beaker and the content of lamivudine was measured with a UV-vis spectrometer. The 1 mL of fresh PBS was added to the beaker to keep the constant volume. Each data was averaged from 3 parallel experiments.

1.4 *In vitro* cell culture experiments

(1) Cell culture. HepLL cells are immortalized human liver cells [30] which kindly donated by Dr. Jun Li at First Affiliated Hospital, Zhejiang University, and cultured with regular growth medium consisting of high-glucose DMEM (Gibco) containing 100 μg/mL streptomycin, 100 U/mL penicillin, and 10% fetal bovine serum, and incubated in a 5% CO_2 incubator at 37°C with 100% humidity.

(2) Cellular uptake of PLGA particles. HepLL cells were seeded on a 24-well plate at a density of 5×10^4 cells per well and allowed to attach for 16 h. To determine the particles uptake rate and amount as a function of incubation time, the cells were incubated with 100 μg/mL of coumarin-labeled PLGA particles for different time. Then the cells were washed three times with PBS to remove free PLGA particles and detached by trypsin. Finally the uptake of coumarin-labeled particles was determined by flow cytometry (FACS Calibur, Becton Dickinson BD).

(3) Intracellular distribution. Fluorescent staining of lysosomes, mitochondria, and cell nuclei was carried out and confocal laser scanning microscopy (CLSM, LSM 510, Carl Zeiss) was used to observe the intracellular distribution of PLGA particles. Briefly, after incubation with 100 μg/mL of Nile red-labeled particles for desired time, the HepLL cells were carefully washed with PBS for three times, then incubated with LysoTracker® Green and MitoTracker® Green at 37°C for 30 min, respectively. Finally the cells were stained with DAPI and observed under CLSM.

(4) Cell viability. The influences of PLGA particles on cell viability were assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, 10^4 HepLL cells in 100 μL medium were seeded into a well of a 96-well plate and incubated overnight. To determine the cell viability as a function of particles concentration and incubation time, the cells were incubated with various concentrations of particles for 24 h and incubated with 100 μg/mL of particles for different time, respectively. After removal of the particles, the cells were cultured in fresh medium supplemented with 0.5 mg/mL MTT at 37°C for another 4 h. The blue crystals generated by the mitochondria dehydrogenase were dissolved with 150 μL dimethyl sulfoxide (DMSO) and the absorbance at 570 nm was measured by a microplate reader (Model 550, Bio-Rad). The data was normalized to the particle free control group.

2 Results and discussion

2.1 Optimization of fabrication parameters

The LPB particles were prepared by a W/O/W double emulsion-solvent evaporation method. The particle size and the drug loading and entrapment efficiency of LPB particles were influenced by parameters such as the volume of inner aqueous phase and organic phase, concentration of organic phase, ultrasonication time, and so on [31].

(1) Effect of volume of inner aqueous phase. Firstly, in-

fluence of the volume of inner aqueous phase on the physiochemical properties of the as-prepared PLGA particles in terms of particle size, surface charge, drug loading and entrapment efficiency was investigated. Table 1 shows no significant change in the particle size along with increase of the volume of inner aqueous phase when it was below 0.3 mL. However, the particle size significantly increased with further increase of the volume, which should be resulted from the less stable W/O emulsion and thereby the coalescence of suspended droplets. Meanwhile, the drug loading and entrapment efficiency increased with the volume of the inner aqueous phase, reached 1.92% and 40.35% for the batch with 0.4 mL internal aqueous phase, respectively. The surface zeta potential was around −30 mV due to the adsorption of negatively charged BSA and not obviously influenced by the polymer concentration. Considering the balance between the particle size and the drug encapsulation efficiency, 0.3 mL inner aqueous phase was used for the following experiments.

(2) Effect of the PLGA concentration of organic phase. The influence of PLGA concentration in organic phase on the particle size and drug loading and encapsulation efficiency was then evaluated. The results are summarized in Table 2. As the concentration of polymer increased from 2% to 8%, the particle diameter increased from 257 to 567 nm. It can be explained by enhancement of viscosity of the PLGA solution at higher concentration, leading to poorer dispersion. On the other hand, with the increase of the PLGA concentration in the oil phase, the drug encapsulation efficiency decreased from 34.9% to 24.4%, and the drug loading efficiency decreased as well from 1.66% to 1.16%. The surface charge property was not obviously influenced by the volume of inner aqueous phase.

(3) Effect of ultrasonication time. During preparation of particles with the double emulsion method, the second emulsification critically influences the particle size. Hence, the intensity and time of emulsification need to be finely tuned. The particle size and drug loading efficiency as a function of ultrasonication time are summarized in Table 3. With the increase of the ultrasonication time, the particle size decreased remarkably in the first 40 s, i.e. from 380 to 280 nm, and then reached a plateau. Meanwhile, both the drug loading efficiency (from 0.68% at 10 s to 1.68% at 60 s) and drug entrapment efficiency (from 13% at 10 s to ~35% at 60 s) were sharply improved at longer ultrasonication time, and then leveled off at still longer time. Therefore, 60 s would be necessary to form well dispersed emulsion, preventing droplet coalescence during the second emulsion process and subsequent drug leakage into the outer aqueous phase. Surface zeta potential of the particles was almost constant in the experiments, suggesting that the surface charge property of the particles mainly depends on the selected macromolecular surfactant instead of the preparation parameters.

Taking all the results into consideration, the optimal fabrication conditions are 0.3 mL of inner aqueous phase, 2% (w/v) PLGA in dichloromethane and double-60 s ultrasonication.

2.2 Characterization of LPB nanoparticles

The morphology of the LPB particles was characterized by SEM and TEM (Figure 2). The LPB particles show a spherical morphology with relative narrow size distribution and

Table 1 Influence of volume of inner aqueous phase on particle size, surface charge and drug loading (DL) and entrapment efficiency (EE) of the LPB particles[a)]

Volume of inner phase (mL)	Size (nm)	Zeta potential (mV)	DL (%)	EE (%)
0.1	249±35	−31.0±2.5	0.75±0.10	15.8±2.2
0.2	260±47	−32.3±0.9	1.32±0.08	27.3±1.7
0.3	257±27	−29.3±4.2	1.66±0.23	34.9±4.8
0.4	384±68	−34.2±3.4	1.92+0.28	40.4±5.9

a) All batches were emulsified using an ultrasonicator (6 W) for 60 s. The 1 mL PLGA dichloromethane solution (2% (w/v), oil phase) was employed. The volume of the BSA aqueous solution (3% (w/v), external aqueous phase) was 4 mL.

Table 2 Influence of concentration of organic phase on particle size, surface charge and drug loading and entrapment efficiency of the LPB particles[a)]

Concentration of the organic phase (%, w/v)	Size (nm)	Zeta potential (mV)	DL (%)	EE (%)
2	257±27	−29.3±4.2	1.66±0.23	34.9±4.8
4	391±21	−28.6±3.7	1.43±0.31	30.0±6.5
6	496±25	−30.5±1.8	1.30±0.16	27.3±3.4
8	567±35	−32.0±1.9	1.16±0.21	24.4±4.4

a) All batches were emulsified using an ultrasonicator (6 W) for 60 s. The 0.3 mL lamivudine aqueous solution (10 mg/mL, internal aqueous phase) was employed. The volume of the BSA aqueous solution (3% (w/v), external aqueous phase) was 4 mL.

Table 3 Influence of ultrasonication time on particle size, surface charge and drug loading and entrapment efficiency of the LPB particles[a)]

Ultrasonication time (s)	Size (nm)	Zeta potential (mV)	DL (%)	EE (%)
10	382±38	–28.6±5.2	0.62±0.38	13±8
20	341±26	–31.7±4.2	0.80±0.29	16.8±6.1
30	315±35	–26.8±3.3	0.78±0.24	16.4±5
40	280±14	–30.5±3.0	1.23±0.17	25.8±3.6
50	275±25	–32.9±3.7	1.52±0.14	31.9±2.9
60	257±27	–29.3±4.2	1.66±0.23	34.9±4.8
90	265±19	–27.9±2.8	1.61±0.26	33.8±5.5
120	248±21	–28.5±3.6	1.68±0.13	35.3±2.7

a) All batches were emulsified using an ultrasonicator (6 W) for desired time. The 0.3 mL 10 mg/mL lamivudine aqueous solution and 1 mL 2% (w/v) PLGA dichloromethane solution were employed as internal aqueous phase and oil phase, respectively. The volume of the BSA aqueous solution (3% (w/v), external aqueous phase) was 4 mL.

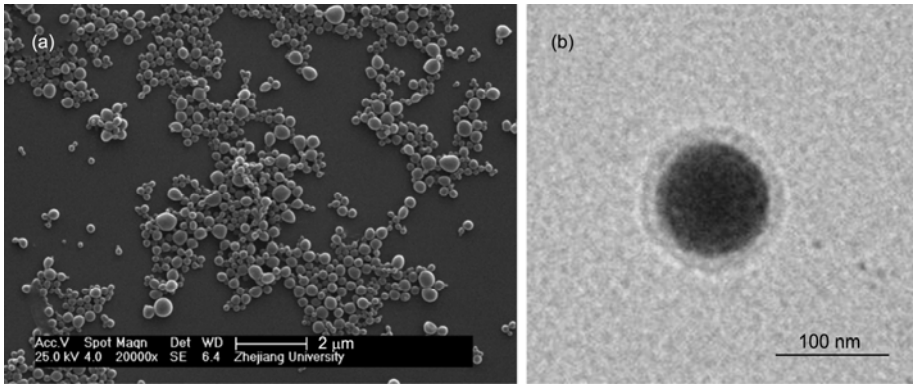

Figure 2 SEM (a) and TEM (b) images of LPB particles.

good dispersity (Figure 2(a)). As shown in Figure 2(b), a thick shell was found around the LPB particles, confirming the existence of BSA layers. According to the results of BCA assay, 58 mg BSA was present on/in 1 g LPB particles. The average diameter of the LPB particles was 257 nm in the wet state (as measured by DLS) and 212 nm in the dry state (as measured by TEM and image-analysis software). The difference of the measured size is attributed to the hydrophilic BSA molecules on the particle surface. Furthermore, the surface zeta potentials of the LPB particles in 10 mmol/L NaCl solution was –29.3±4.2 mV, which was improved to –10.9±2.4 mV in cell culture medium with 10% FCS due to adsorption of serum proteins.

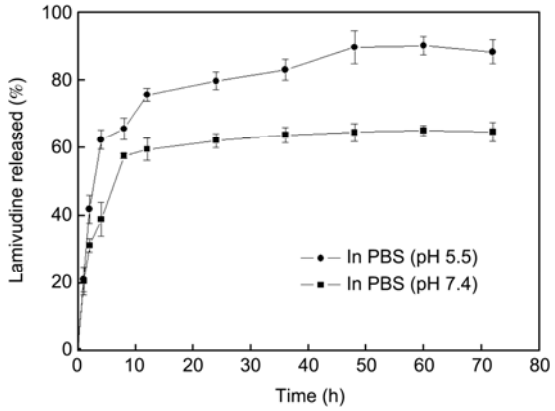

Figure 3 Release profile of lamivudine from LPB particles at 37°C in different mediums.

2.3 Drug release *in vitro*

The release behavior of lamivudine from the LPB particles was characterized in PBS with different pH values to mimic physiological and intra-lysosome environment at 37°C (Figure 3). At pH 7.4, an obvious burst release was observed during the first 2 h, and then the release was slowed down and reached to a plateau after 8 h, during which about 60% of the drug was released. The burst effect is caused by lamivudine molecules adsorbed or located near the particle surface. At pH 5.5 (similar as in lysosomes), the release became faster, and more than 60% of the drug was released at first 4 h. The release became much slower afterwards and reached a plateau after 48 h, during which 90% of the encapsulated lamivudine was released. The faster release in acidic environment might be attributed to protonation of the amine group in lamivudine, leading to the change of charge balance and a larger repulsion between the molecules. Nev-

ertheless, the LPB particles might protect the encapsulated drug in physiological condition and release quickly when they are internalized by cells, and particularly transported into lysosomes.

2.4 Cellular uptake

To make the PLGA particles detectable via fluorescence microscopy and flow cytometry, hydrophobic dyes, coumarin and Nile red were pre-loaded during the particle fabrication, respectively. Due to their very poor solubility in aqueous medium, no detectable release from the particles was found after incubation in the cell culture medium at least for 24 h at 37°C (data not shown). Loading of the trace amount of coumarin/Nile red did not bring significant influences on the particle size and morphology as well as surface charge, colloidal stability and protein adsorption property. Normalization of the fluorescence intensity of each type of the coumarin loaded particles was performed for the quantitative measurements by FCM. To investigate the uptake kinetics, the HepLL cells were incubated with 100 μg/mL coumarin-labeled LPB particles and blank PLGA (PB) particles for different time intervals, respectively, and then quantified with FCM by determining the green fluorescence-emitting cells (Figure 4). For the FCM study, the logarithmic fluorescence intensity of particle-free control cells was set below 10. Therefore, the cells possessing fluorescence intensity larger than 10 were considered as positive ones [32,33]. As shown in Figure 4, the cellular uptake amount of both particles monotonously increased along with the prolongation of the culture time. For both types of the particles, the fluorescence intensity was already quite high after incubation for 4 h, indicating that internalization of both types of PLGA particles is very fast. During the next 20 h, the fluorescence intensity increased monotonously, and then leveled off, revealing that the uptake process was completed in 24 h. There is no significant difference in the uptake kinetics between the two types of particles, suggesting

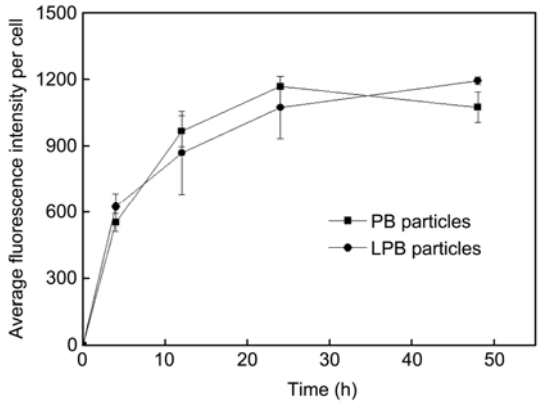

Figure 4 Uptake of LPB particles by HepLL cells as a function of culture time with a particle concentration of 100 μg/mL. Data was measured by flow cytometry and averaged to each cell.

the encapsulation of lamivudine does not influence on the cellular uptake process.

2.5 Intracellular distribution

During the process of cellular uptake, the exogenous particles are initially enclosed into endosomes, which mature into multivesicular bodies or late endosomes and eventually fuse with lysosomes [34]. Mitochondria are important intracellular energy metabolism places, and are closely related to the signals which induce cell apoptosis. The internalization process and the cellular distribution of the Nile red-labeled LPB particles were microscopically monitored by staining the lysosomal compartments with LysoTracker Green, the mitochondria with MitoTracker Green, and the cell nuclei with DAPI, respectively. Figure 5 shows that some LPB particles were internalized after 12 h incubation and further increased after 24 h. Only a few particles could be observed and colocalized with lysosomes after 12 h incubation. After 24 h incubation, more LPB particles were overlapped with lysosomes (yellow) while many LPB particles were located in the cytoplasm (red) rather than lysosomes. This result implies that many LPB particles can escape from the lysosomes. Although many particles were found around nucleus, no colocalization signals of the particles and cell nucleus could be found even after 24 h co-incubation, suggesting that the particles could not penetrate into cell nucleus.

As shown in Figure 6, very a few Nile red-labeled LPB particles overlapped with the mitochondria after 12 h incubation. When the incubation time was extended to 24 h, a few LPB particles were colocalized with the mitochondria, indicating the possible interaction of the particles with cell organelles such as mitochondria.

2.6 Cytotoxicity

MTT assay was used to assess the viability of the HepLL cells after incubation with various concentrations of PB and LPB particles for 24 h and incubated with 100 μg/mL of particles for different time, respectively. Figure 7(a) shows that in general the cell viability was largely maintained after incubation with both types of particles. The cell viability slightly decreased along with the increase of particles concentration, which decreased to 79.2% and 81.3% of particles free controls when the cells were exposed to 200 μg/mL PLGA-BSA (PB) and LPB particles, the highest concentration tested. As shown in Figure 7(b), both PB and LPB particles showed very low cytotoxicity to HepLL cells during the 48 h culture period. Thus, LPB particles are considered to be safe as potential drug carriers and encapsulation of lamivudine does not bring additional toxicity to normal cells. In next step, the cell model with hepatitis B virus infection would be created and the anti-virus effect of the LPB particles would be studied to elucidate the potential application

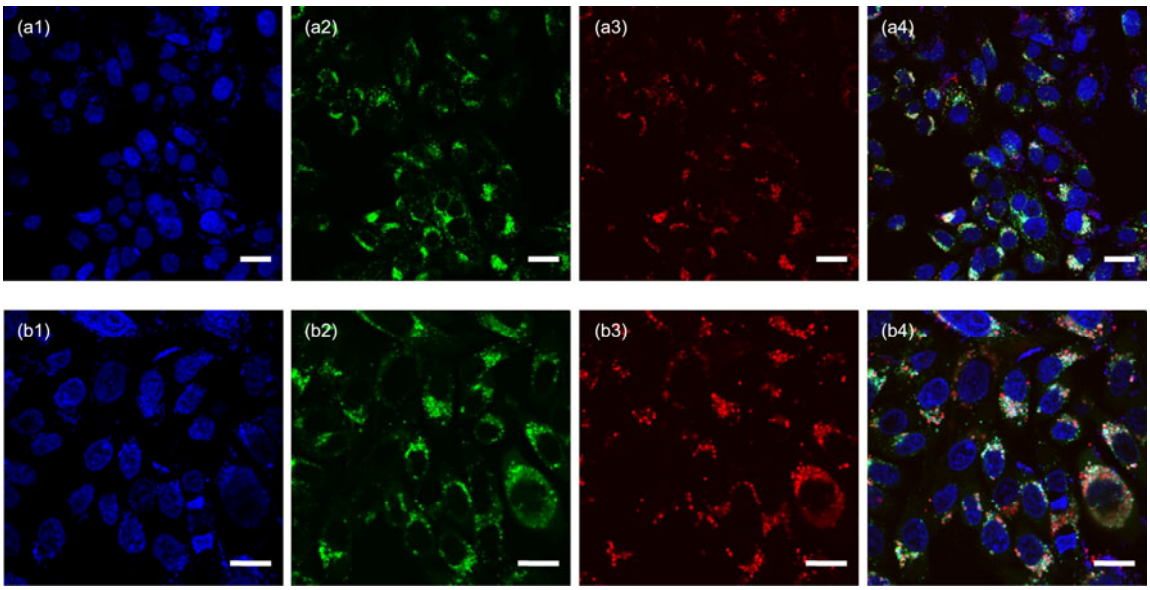

Figure 5 CLSM images of HepLL cells incubated with 100 μg/mL LPB particles for 12 h (a) or 24 h (b), respectively. The cell nuclei were stained by DAPI (a1, b1), the lysosomes were stained by LysoTracker (a2, b2) and the LPB particles were labeled by Nile red (a3, b3). (a4, b4) The merge images of nucleus (blue), lysosomes (green) and LPB particles (red). Scale bar: 10 μm.

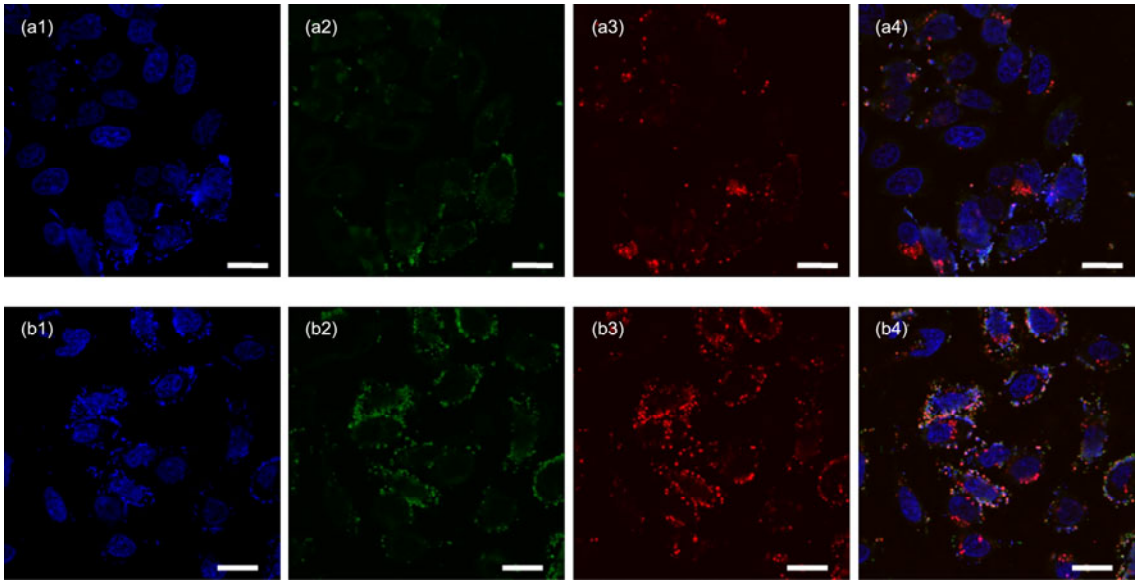

Figure 6 CLSM images of HepLL cells incubated with 100 μg/mL LPB particles for 12 h (a) or 24 h (b), respectively. The cell nuclei were stained by DAPI (a1, b1), the mitochondria were stained by MitoTracker (a2, b2) and the LPB particles were labeled by Nile red (a3, b3). (a4, b4) The merge images of nucleus (blue), mitochondria (green) and LPB particles (red). Scale bar: 10 μm.

of this kind of drug carriers.

3 Conclusion

The lamivudine-loaded PLGA particles were prepared by a W/O/W double emulsion-solvent evaporation method with BSA as macromolecular surfactant. By varying the experimental conditions such as volume of inner aqueous phase, concentration of organic phase and sonication parameters, the particle size and drug encapsulation efficiency were optimized and finally the LPB particles with ~260 nm in diameter, ~35% drug loading efficiency and negative surface charge were obtained. The lamivudine inside LPB particles showed a burst release at beginning and sustained release until 24 h in physiological conditions. The release of lamivudine from LPB particles was accelerated in acidic medium, making the LPB particles potential intelligent intracellular drug carriers. The LPB particles were readily internalized into human liver cells within a short time and

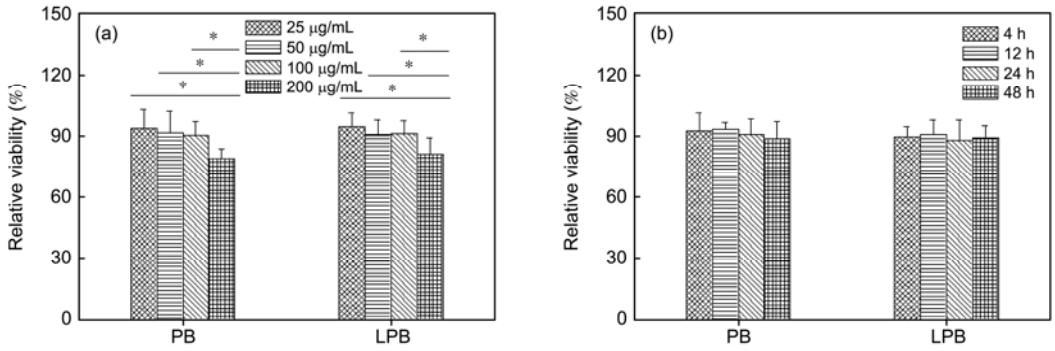

Figure 7 Relative cell viability after incubation of HepLL cells with the PB or LPB particles as a function of (a) concentration with a culture time of 24 h, and (b) culture time with a particle concentration of 100 μg/mL.

increased gradually with the prolongation of incubation. After ingested into the cells, the LPB particles would either reside within the lysosomes or transfer to cytoplasm, but could not enter into the cell nucleus. The cell viability was not significantly influenced by the ingestion of the particles regardless of lamivudine encapsulation.

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