

Polymer Nanoparticles Prepared by Supercritical Carbon Dioxide for in Vivo Anti-cancer Drug Delivery

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Abstract: A new approach for producing polymer nanoparticles made of bovine serum albumin-poly(methyl methacrylate) conjugate by precipitating in supercritical CO₂ is reported. The nanoparticles were loaded with the anti-tumor drug camptothecin. With albumin serving as a nutrient to cells, the drug-encapsulated nanoparticle shows an enhanced ability to kill cancer cells compared to that of the free drug in solution both in vitro and in vivo.

Keywords: Protein; Polymer; Nanoparticle; Drug delivery

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Introduction

Polymeric nanoparticles have much medical promise with a large number of therapeutic nanoparticles presently in clinical trials or approved for clinical use [1-4]. Ge *et al.* [5] developed a new type of protein-polymer hybrid nanoparticles made of hydrophilic denatured bovine serum albumin (BSA) covalently bonded to hydrophobic poly(methyl methacrylate) (PMMA).

Based on their study, here we report a new approach for producing such nanoparticles made of bovine serum albumin-poly(methyl methacrylate) conjugate by precipitating in supercritical CO₂. BSA-PMMA nanoparticles can be prepared with hydrophobic drugs being encapsulated by a simple precipitation using supercritical carbon dioxide (SC-CO₂) as an anti-solvent. SC-CO₂ is also non-toxic, non-flammable, and FDA approved. Such a process can readily be scaled to kilogram quantities, and represents a new approach with great potential to produce dry polymeric nanoparticles for sus-

tained drug delivery [6]. In this work we use the solution enhanced dispersion by supercritical fluids (SEDS) method to produce camptothecin (CPT)-encapsulated BSA-PMMA nanoparticles. The nanoparticle shows an enhanced ability to kill cancer cells compared to that of the free drug in solution both in vitro and in vivo.

The BSA-PMMA conjugate was synthesized by the method described by Ge *et al.* previously [5]. In a typical experiment, BSA (lyophilized powder, from Sigma-Aldrich) was dissolved in dimethyl sulfoxide (DMSO) at 50°C at a concentration of 2 mg/mL, followed by addition of acrylic acid N-hydroxysuccinimide ester (NAS) (in DMSO at a concentration of 20 mg/mL) with a molar ratio of NAS to BSA of 130:1. After reaction at 25°C for 5 hours, methyl methacrylate (MMA) was added, followed by addition of 2,2'-azobis(2-methylpropionitrile) (AIBN) (8 mM) to initiate the polymerization at 70°C. The conjugate was collected by precipitation in a methanol/ethyl ether (1:8, v/v) mixture. The BSA:PMMA weight ratio in the conjugate was determined as ~4:1 by ¹H-NMR.

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Then, 2 mg/mL of BSA-PMMA conjugate and 0.25 mg/mL of CPT were dissolved in chloroform, followed by subjecting to the SEDS process. Briefly, the solution was injected (1 mL/min) through a nozzle with 250 μm internal diameter into a SC-CO₂ (150 g/min) at 40°C and 100 bar. CPT is used for treating a wide range of tumors [7]. However, it is poorly soluble in water [8]. After preparing the CPT-encapsulated BSA-PMMA nanoparticles by precipitating in SC-CO₂ (Fig. 1(a)), we estimated the yield of our SEDS process to be $\sim 88\%$. The scanning electron microscopy (SEM) image (Fig. 1(b)) shows the nanoparticles have an average size around 200~300 nm. The size of CPT-loaded BSA-PMMA nanoparticles dispersed in PBS (pH 7.4) at a concentration of 7 mg/mL was measured to be 310 nm (PDI = 0.121) by dynamic light scattering (mean size: 310 nm, size distribution: ± 27 nm).

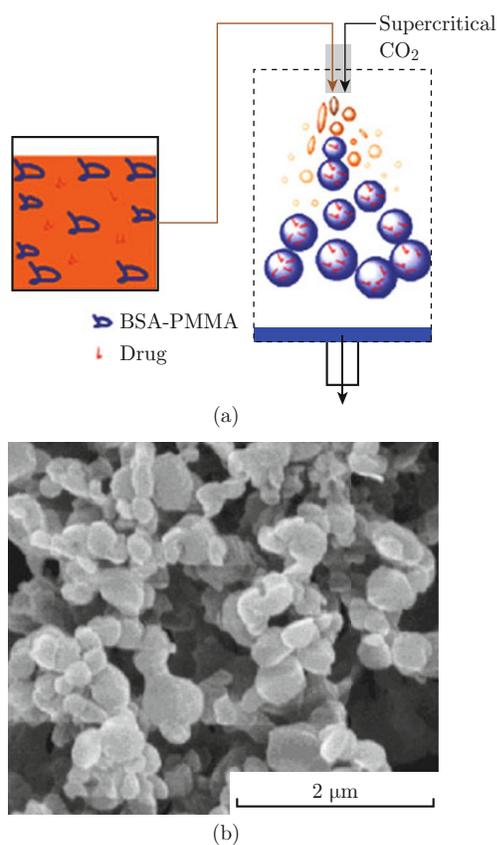


Fig. 1 (a) Preparation of drug-loaded nanoparticles by precipitation in SC-CO₂; (b) SEM image of BSA-PMMA nanoparticles.

The drug loading was determined as 12.5 wt% in the BSA-PMMA nanoparticles. The drug release profile was studied by dispersing the CPT-loaded nanoparticles in a release medium (PBS, pH 7.4 containing 2% (w/v) Tween 80) [9]. As shown in Fig. 2(a), at the drug loading ratio of 12.5 wt%, the encapsulated CPT was released from the nanoparticles over a period of 48

hours with a low initial burst. And the encapsulation ratio was determined to be ~ 10 wt%. At the early stage of drug release, the diffusion of CPT from nanoparticles probably played an important role and resulted in a burst release. At the late stage, the degradation of BSA-PMMA nanoparticles caused more drug molecules to gradually be released.

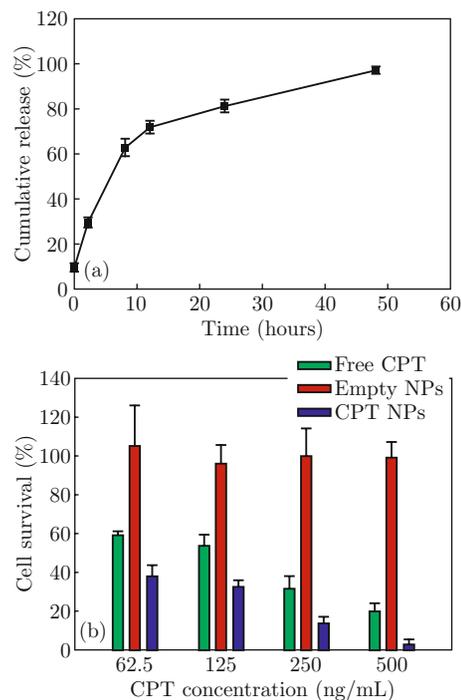


Fig. 2 (a) Sustained release of CPT from nanoparticles; (b) In vitro anti-cancer activities of free CPT and CPT nanoparticles.

The CPT-encapsulated BSA-PMMA nanoparticles were analyzed for their ability to retard the proliferation of tumor cells. Human colorectal cancer cells HCT116 were plated in 96-well microplates at 5.0×10^3 cells per well in 190.0 μL of complete medium and allowed to adhere under incubation at 37°C and 5% CO₂. Twenty-four hours later, HCT116 cells were exposed to CPT in a 10% v/v DMSO solution containing CPT or CPT-encapsulated nanoparticles with the same drug concentration. The final concentration of DMSO in the cell culture medium was 0.5% (v/v), which had no measurable effect on cell viability. Empty nanoparticles were used as a control. The viability of cell populations was then assessed by the MTT method [10] at 72 h. As shown in Fig. 2(b), the dose-dependent cytotoxic effect of the CPT solution was evident cumulating in more than 50% HCT116 survival at 62.5 ng/mL after 72 h. The encapsulation of CPT into the nanoparticles resulted in marked improvements of the anti-tumor activities. After 72 h, less than 10% survival was observed at the CPT concentration above 250 ng/mL. Calculated from the experiment, the IC₅₀ for free CPT was around

87.5 ng/mL, while the IC₅₀ for CPT in nanoparticles was only around 23.5 ng/mL. The empty nanoparticles showed excellent biocompatibility. Around 100% cell survival was observed for cells treated with different concentrations of empty nanoparticles.

We tested the *in vivo* anti-tumor efficiency of CPT-encapsulated BSA-PMMA nanoparticles by *i.v.* injection into mice with subcutaneous colon cancer tumors. NOG (NOD/LtSz-scid IL2R γ null) mice (15-16 weeks old) were injected *s.c.* with 2×10^7 HCT116 colon cancer cells approximately 10-18 days before dosing. Tumor sizes were measured for the duration of the experiment. Treatment was initialized when the mean tumor size reached approximately 300-500 mm³ (day 1). The animals were sorted into 2 groups, each group having six mice. For group one, as the positive control, free CPT was administered by intraperitoneal injection (*i.p.* injection). The dose of CPT (9 mg/kg) was based on the literature [11]. CPT is very insoluble in aqueous solution and is acutely lethal when given to mice by *i.v.* injection at 9 mg/kg (0.18 mg/20 g mouse) due to the particulate matter in the drug suspension. Thus, we followed the previous protocol [11] for the administration of free CPT. In the experiment, the CPT was suspended in a vehicle of 0.5% methylcellulose and 0.1% Tween 80 and administered by *i.p.* injection as an attempt to maximize its efficiency. Mice were given CPT at 9 mg/kg once daily on day 1 and 7 with a dosing volume of 200 μ L. For group two, mice were treated with CPT nanoparticles. The dry powder of CPT nanoparticles was suspended in PBS (pH 7.4) at a concentration of 7 mg/mL with the assistance of probe sonication. The nanoparticles showed excellent dispersability in aqueous solution because of the hydrophilic shell of BSA on the nanoparticles. The treatment was administered intravenously by tail vein injection (*i.v.* injection) once daily on day 1 and 7 at 9 mg of CPT/kg. The small size and narrow size distribution of the nanoparticles in PBS (pH 7.4) made this formulation very suitable for *i.v.* injection. As shown in Fig. 3, the tumor volume in CPT nanoparticle-treated mice was significantly smaller than that of mice treated with free CPT. At day 30, the median tumor volume of the CPT nanoparticle-treated mice is 448 mm³, which is almost the same compared with the tumor volume at day 1 (437 mm³). However, for free CPT-treated group, at day 30, the median tumor volume 3515 mm³, which is 7 times increased compared with the tumor volume at day 1. Thus, a significant prohibition of tumor growth was observed when using CPT encapsulated nanoparticles compared with free CPT.

PMMA is biocompatible but not biodegradable *in vivo*. In our study, we choose the PMMA as the hydrophobic core of the BSA-PMMA nanoparticle is because of its good biocompatibility and the hydrophobicity which could be used to encapsulate hydrophobic

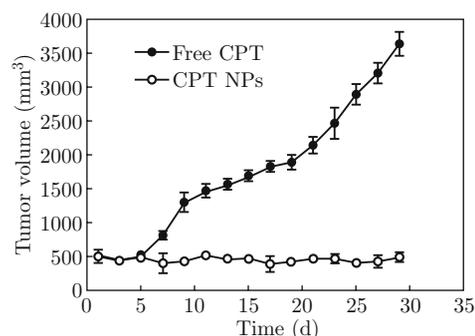


Fig. 3 *In vivo* anti-tumor efficiency of free CPT and CPT-encapsulated BSA-PMMA nanoparticles.

drugs. A number of studies have tried to develop PMMA-based nanoparticles for drug delivery [12]. No obvious toxicity was observed by *i.v.* injection of PMMA nanoparticles *in vivo* [13]. In our study we utilized the BSA which is biodegradable to copolymerize with PMMA. One BSA molecule was covalently bound with about 49 PMMA chains. Each single PMMA chain attached only has 5-7 of repeating units (MMA) in the BSA-PMMA conjugate we used for *in vivo* study. After the degradation of BSA *in vivo*, only oligomers of MMA (5-7 of repeating units with 500-700 Da in molecular weight) would exist. In the *in vitro* and *in vivo* study, we observed that empty BSA-PMMA nanoparticles have good biocompatibility and no toxicity to cells or mice.

In summary, we have developed a new method to produce protein-polymer hybrid nanoparticles for anti-cancer drug delivery. We have demonstrated that the hybrid nanoparticles prepared by precipitating in SC-CO₂ have excellent biocompatibility and efficient cell uptake. The preparation process is simple and easy to scale up. As an example of this type of drug delivery vehicles, compared with free drug formulation, the camptothecin-encapsulated BSA-PMMA nanoparticles shows enhanced anti-tumor activity both *in vitro* and *in animals*.

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