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Polymeric Micelles for Acyclovir Drug Delivery

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Abstract

Polymeric prodrug micelles for delivery of acyclovir (ACV) were synthesized. First, ACV was used directly to initiate ring-opening polymerization of ϵ -caprolactone to form ACV-polycaprolactone (ACV-PCL). Through conjugation of hydrophobic ACV-PCL with hydrophilic methoxy poly(ethylene glycol) (MPEG) or chitosan, polymeric micelles for drug delivery were formed. ^1H NMR, FTIR, and gel permeation chromatography were employed to show successful conjugation of MPEG or chitosan to hydrophobic ACV-PCL. Through dynamic light scattering, zeta potential analysis, transmission electron microscopy, and critical micelle concentration (CMC), the synthesized ACV-tagged polymeric micelles were characterized. It was found that the average size of the polymeric micelles was under 200 nm and the CMCs of ACV-PCLMPEG and ACV-PCL-chitosan were 2.0 mg L^{-1} and 6.6 mg L^{-1} , respectively. The drug release kinetics of ACV was investigated and cytotoxicity assay demonstrates that ACV-tagged polymeric micelles were non-toxic.

Keywords

acyclovir; ring-opening polymerization; micellar drug delivery; methoxypolyethylene glycol; chitosan

1. Introduction

Due to the fact that nanoparticles can be prepared using a variety of polymers, biodegradable polymers have been extensively studied for the field of polymer therapeutics [1-3]. While there have been many biodegradable polymeric nanoparticles synthesized for drug delivery, polymeric micelles have numerous advantages over other proposed colloidal delivery systems [4-7]. Many studies have shown the promise micelles have for drug delivery because they can be tailored for prolonged blood circulation time, cellular selectivity, and for their controlled release capabilities [8-10]. In addition, micelles can be specifically synthesized to increase a drug's solubility and bioavailability [11-14].

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Appendix A. Supplementary data

Acyclovir (ACV) is a guanosine-based prodrug most commonly used for the treatment of infections caused by herpes simplex virus (HSV) type 1 and 2, varicella zoster virus and, to a lesser extent, cytomegalovirus and Epstein-Barr virus [15]. Moreover, prodrug ACV can be converted to its cytotoxic phosphorylated form by herpes simplex virus thymidine kinase (HSVTK) gene for cancer therapy [16]. That is, if the HSV-TK gene is delivered to actively dividing cancer cells, and ACV is subsequently administered to the cells, the TK enzyme phosphorylates ACV, yielding toxic metabolites which cause death in prodrug treated HSV-TK expressing cells [17-19]. However, due to ACV's poor water solubility and ensuing low bioavailability, alternative delivery approaches are required to increase the therapeutic potential of ACV. Several methods reported are to couple ACV to biocompatible hydrophilic polymers [20-22] or encapsulation into drug carriers [23-25]. Although these processes increase the bioavailability of ACV as well as offer a practical treatment for patients, they are labor-intensive and cost-ineffective. Recently, we have shown that ACV can be used as an initiator to proceed ring-opening polymerization of ϵ -caprolactone (ϵ -CL) to form hydrophobic ACV-polycaprolactone (ACV-PCL) [26], which makes this an economically attractive approach compared with the aforementioned conjugation and encapsulation methods.

Polymeric micelles consist of an inner core made of a hydrophobic block copolymer and an outer corona made of the hydrophilic block of the copolymer. PCL, having been widely used as the core-forming hydrophobic segment of nanoparticles, was selected as the model polymer for this study. PCL is a semi-crystalline, linear resorbable aliphatic polyester. It has been commonly used in drug delivery systems because it is biodegradable and biocompatible. [27-29] PCL is commonly synthesized by ring-opening polymerization of ϵ -CL using an alcohol as an initiator and stannous (II) octoate ($\text{Sn}(\text{Oct})_2$) as a catalyst [30, 31]. In addition to using alcohol as the initiator, methoxy-poly(ethylene oxide) and starch have been employed as macroinitiators to form amphiphilic polymers. [32, 33]. In this study, prodrug ACV possessing hydroxyl groups was used as the initiator to obtain prodrug-PCL. Then, a hydrophilic compound (MPEG or chitosan) was grafted on the hydrophobic prodrug-PCL to form the amphiphilic block copolymer which already has the drug attached started from the ring-opening polymerization. These synthesized ACV-tagged amphiphilic polymers can self-assemble in aqueous medium to form polymeric prodrug micelles for use as nanocarriers in drug delivery.

Individual conjugation of ACV-PCL to a wide array of biocompatible hydrophilic polymers to form polymeric micelles each has their own advantages for drug delivery. With this in mind, we choose to assess the successful conjugation of two model hydrophilic polymers, MPEG and chitosan, to hydrophobic ACV-PCL. Chitosan is a natural polysaccharide derived from deacetylation of chitin. Chitosan's biocompatible and biodegradable features have attracted much attention in biomedical and pharmaceutical research [28, 34]. Similarly, MPEG is a biocompatible hydrophilic polymer commonly used in polymeric micelle formation. MPEG is inexpensive, non-toxic and is widely used to covalently modify biological macromolecules and surfaces [10, 35, 36]. Hence, ACV-PCL-MPEG and ACV-PCL-chitosan copolymers were synthesized. The chemical structure and physical properties of the copolymers were characterized and micelle formation investigated. The drug release

profiles of ACV from polymeric prodrug micelles and the biocompatibility of polymeric prodrug micelles were investigated in this study.

2. Experimental Section

2.1. Materials

ACV was purchased from TCI (Tokyo, Japan). N,N' -dicyclohexyl carbodiimide (DCC), ϵ -CL, pyrene, and succinic anhydride were purchased from Acros Organics (Geel, Belgium). $\text{Sn}(\text{Oct})_2$, CDCl_3 with 1% tetramethylsilane (TMS), deuterated dimethyl sulfoxide (DMSO-d_6), dimethyl sulfoxide (DMSO), tetrahydrofuran (THF), dichloromethane (DCM), methanol, 2-propanol, hexane, toluene, methoxypolyethylene glycol amine (MPEG- NH_2 ; MW = 5,000), and chitosan oligosaccharide lactate (MW = 5,000) were all purchased from Sigma-Aldrich (St. Louis, MO). Ethyl ether was purchased from J.T. Baker (Austin, TX). N -Hydroxysuccinimide (NHS) was purchased from Alfa Aesar (Ward Hill, MA). Acetone was purchased from Pharmco-AAPER (Shelbyville, KY). Pyridine and hydrochloric acid (HCl) were purchased from EMD (Philadelphia, PA). Sodium chloride (NaCl) and magnesium sulfate were purchased from Showa (Tokyo, Japan). All reagents were used as received without further purification.

2.2. Characterization Methods

Gel permeation chromatography (GPC) analyses were performed on a Waters 1525 binary HPLC pump equipped with a Waters 2414 refractive index detector (Milford, MA). Waters styragel HR 3 (MW = 500 – 30,000) and HR 4E (MW = 50 – 100,000) columns were equipped. Molecular weight calibration was performed with polystyrene standards that covered a MW range of 400 – 4.3×10^4 . GPC analyses were performed in THF at a flow rate of 1 mL min^{-1} with an injected volume of 50 μL . ^1H NMR spectra were obtained from a Varian Unity/Inova 400 MHz instrument (Sparta, NJ). To obtain FTIR spectra by a Jasco FTIR-4200 spectrometer (Tokyo, Japan), a small amount of polymeric sample was loaded onto a silicon wafer and THF was added dropwise to dissolve the sample and evaporated afterwards. This was repeated until the entire sample was dissolved and a film had formed.

2.3. Synthesis of ACV-tagged Amphiphilic Polymers

2.3.1. Synthesis of ACV-PCL—ACV (50 mg) was weighed and mixed with ϵ -CL (2.25 mL) under a sonication bath for 5 min at room temperature. $\text{Sn}(\text{Oct})_2$ (0.5 wt% of ϵ -CL) was then added into the mixture. The entire solution was placed into a 3-necked round-bottom flask. The system was purged with nitrogen and immersed in an oil bath at 140°C for 24 h. The crude product was cooled to room temperature, dissolved in DCM, and precipitated by cold methanol. The product was then vacuum dried by a rotary evaporator at 40°C .

2.3.2. Synthesis of ACV-PCL-COOH—ACV-PCL (0.5 mmol) and succinic anhydride (1 mmol) were weighed and dissolved in toluene in a 3-necked round-bottom flask. One mmol pyridine was added and the solution was reacted under nitrogen at 70°C for 48 h. The product was then precipitated by cold hexane, and spun down. The pellet was re-dissolved in DCM and washed twice each with 10% (v/v) HCl and saturated NaCl solution. The organic phase was isolated and dried with magnesium sulfate then filtered. The carboxylated ACV-

PCL was recovered by precipitation in cold hexane and then vacuum dried by rotary evaporation at 40°C.

2.3.3. Synthesis of ACV-PCL-NHS—ACV-PCL-COOH (0.54 mmol) and NHS (2.7 mmol) were weighed and mixed in 15 mL DCM, and then DCC (2.7 mmol) was added. The reaction was run under a nitrogen environment at room temperature for 24 h. The precipitated byproduct 1,3-dicyclohexylurea was removed by vacuum filtration. The filtrate was added into 35 mL diethyl ether and cooled to 4°C for 4 h to precipitate ACV-PCL-NHS. The precipitate was collected by centrifugation at 3,500 rpm for 5 min, washed with 2-propanol and solvent removed by rotary evaporation at 40°C.

2.3.4. Synthesis of ACV-PCL-MPEG—ACV-PCL-NHS (10 mg) and MPEG-NH₂ (10 mg) were weighed and dissolved by 20 mL DCM in a round-bottom flask. The flask was purged with nitrogen and the solution was stirred for 24 h. The solution was then dialyzed (MWCO = 6-8 kD, Spectra/Por, Rancho Dominguez, CA) against pure DCM to remove remaining MPEG-NH₂. ACV-PCL-MPEG was recovered by rotary evaporation at 40°C.

2.3.5. Synthesis of ACV-PCL-chitosan—ACV-PCL-NHS (10 mg) was dissolved in 5 mL acetone and slowly added to chitosan solution (20 mg chitosan oligosaccharide lactate dissolved in 25 mL deionized water). The mixture, purged with nitrogen, was stirred in a round-bottom flask for 24 h. The reacted solution was vacuum dried to remove acetone and then lyophilized. The amphiphilic polymer was then dissolved in DCM and dialyzed (MWCO = 6-8 kD, Spectra/Por) against pure DCM to remove unreacted chitosan. ACV-PCL-chitosan was recovered by rotary evaporation at 40°C.

2.4. Preparation of Polymeric Prodrug Micelles

ACV-PCL-MPEG and ACV-PCL-chitosan micelles were formed similarly. Briefly, 10 mg of ACV-tagged amphiphilic polymer was dissolved in 2 mL acetone. The solution was then added dropwise to 10 mL deionized water under sonication. Acetone was removed by rotary evaporation and the final solution was collected by filtering through a 0.45 µm filter.

2.5. Determination of Critical Micelle Concentration

The CMC was estimated by using pyrene as a fluorescent probe [37]. Briefly, 1 mg mL⁻¹ of polymeric prodrug micelle was formed. Various amounts of deionized water and micellar solution were added respectively to glass vials to obtain micellar concentrations ranging from 5×10^{-7} to 1 mg mL⁻¹. Pyrene in acetone was then added separately to the prepared vials to get a final concentration of pyrene in water of 6.0×10^{-7} mg mL⁻¹, slightly lower than the saturation solubility of pyrene in water [38]. The solutions were then allowed to equilibrate for 8 h. Fluorescent spectra were determined by a plate reader (Synergy MX, BioTek, Winooski, VT) with an excitation wavelength of 334 nm.

2.6. Size and Morphology of Polymeric Prodrug Micelles

The average particle size of polymeric prodrug micelles was determined by a dynamic light scattering (DLS) instrument (Zetasizer Nano ZS, Malvern Instruments, United Kingdom) equipped with a red laser at a wavelength of 633 nm and scattering angle of 90° at 25°C.

The zeta potential of the micelles dispersed in deionized water was determined with a zeta potential analyzer (Zetasizer Nano ZS). Transmission electron microscopy (TEM) image of micelles was performed on a JEM-4000FX (JEOL, Tokyo, Japan) at 80 kV. The TEM samples were prepared by adding 10 μL of micellar solution (1 mg mL^{-1}) onto a Formvar grid for 5 min and wicking away solution in excess. The samples were negatively stained with 10 μL phosphotungstic acid solution (2 wt%) for 10 sec and then 15 sec, wicking away excess staining solution each time.

2.7. Drug Release Kinetics

Polymeric prodrug micelles at a concentration of 1 mg mL^{-1} were made in phosphate buffered saline (PBS) (1 M, pH 7.4) at 25°C . Two mL of solution was placed in a dialysis tube (Float-ALyzer, Spectra/Por) with a MWCO of 3.5 – 5 kD. The dialysis bag was then immersed in 50 mL PBS at room temperature. At specified time intervals, 5 μL of sample was removed and replaced with fresh PBS. The amount of ACV released was analyzed by a plate reader (BioTek) at 254 nm. All experiments were carried out in triplicate.

2.8. Cytotoxicity Test

24-well plates were seeded with human colorectal HT29 cells (HTB-38; ATCC, Manassas, VA) suspended in 0.5 mL Dulbecco's modified Eagles' medium (DMEM, Corning Cellgro, Manassas, VA) supplemented with 10% fetal bovine serum (FBS, Atlanta biologicals, Flowery Branch, GA) and 1% penicillin-streptomycin (Sigma) and incubated at 37°C in 5% CO_2 balanced with humidified air for 24 h. In each well, 500 μL of 1 mg mL^{-1} ACV-PCL-MPEG or ACVPCL-chitosan polymeric micelles (filtered by a $0.22 \mu\text{m}$ filter) was added. After incubation for 48 h, cell viability was assessed using MTT assay. 200 μL of sterile MTT solution (4 mg mL^{-1}) was added into the culture wells and incubated for 4 h. The medium containing unreacted MTT was removed and 300 μL DMSO was added to dissolve the insoluble purple formazan crystals formed in cellular mitochondria. The absorbance at 590 nm was measured with a plate reader (BioTek) and results were recorded as viability percentage calculated against the control group without micellar challenge. All experiments were carried out in triplicate.

3. Results and Discussion

3.1. Synthesis and Characterization of Amphiphilic Prodrug Polymers

ACV-PCL was synthesized through ring-opening polymerization of $\epsilon\text{-CL}$, which was initiated exclusively by ACV (Scheme 1A). The number average molecular weight (M_n) of ACV-PCL polymer after 24 h reaction time was approximately $1.7 \times 10^4 \text{ g mol}^{-1}$ with polydispersity index (PDI) of 1.64 as measured from GPC analysis (as shown in Table S1 in the supporting information). The ^1H NMR spectra of guanosine-based prodrug ACV and ACV-PCL 24 h post-synthesis are shown in Fig. S1(i) and (ii), respectively, in the supporting information. Judging from the ACV-PCL spectrum, chemical shifts at 3.75 (e, f- CH_2), 5.44 (d- CH_2), 7.68 (b-CH) were assigned to protons in ACV, while the peaks at $\delta=1.36$ (3- CH_2), 1.64 (2- CH_2), 2.29 (1- CH_2) and 4.04 (4- CH_2) correspond with the backbone chain of PCL polymer. The characteristic resonances of PCL and ACV were both observed in the synthesized polymer. Although the characteristic peaks of ACV in Fig.

S1(ii) are much lower than those representing PCL, the evidence of coupling of ACV's protons in synthesized ACV-PCL is clear.

ACV-PCL was further conjugated with either MPEG or chitosan as shown in scheme 1 B-E. Successful conjugation of MPEG or chitosan was confirmed by GPC, ^1H NMR and FTIR analyses. The increase in M_n of ACV-PCL to 22.4 kDa from 17.5 kDa corresponds with the addition of MPEG (Table S1 in the supporting information). Fig. 1 shows the ^1H NMR of MPEG-NH₂ and ACV-PCL-MPEG. The peak at 3.63 (A-OCH₂) attributed to MPEG can be clearly seen in Fig. 1 (iv). Due to the conjugation of MPEG to ACV-PCL through amide linkage (Scheme 1D), the change of the peak at 1.79 (B-NH₂) from a singlet in Fig. 1 (iii) to a multiplet in Fig. 1 (iv) further confirms conjugation of MPEG to ACV-PCL. Further characterization of successful grafting of MPEG to hydrophobic ACV-PCL was examined through FTIR analysis. FTIR spectra of ACV-PCL (A), MPEG-NH₂ (B) and ACV-PCL-MPEG (C) are shown in Fig. 2. C-H stretching vibrations can be seen from 2945-2852 cm⁻¹ for all samples. The typical bending vibration of 1634 cm⁻¹ for the NH₂ bending amide I band can be seen in ACV-PCL and ACVPCL-MPEG spectra, and is attributed to ACV. PCL and MPEG blocks were characterized by prominent absorptions at 1726 cm⁻¹ (C=O) and 1112 cm⁻¹ (C-O-C), respectively. These peaks are found in ACV-PCL-MPEG copolymer ascertaining successful bonding of MPEG to ACV-PCL.

GPC analysis revealed that the M_n of ACV-PCL increased from 17.5 kDa to 21.7 kDa after grafting with chitosan and had a PDI of 1.42 (Table S1 in the supporting information). Successful conjugation of chitosan to ACV-PCL was characterized similar to ACV-PCL-MPEG. Fig. 3 reveals the ^1H NMR of chitosan (v) and ACV-PCL-chitosan (vi). As shown in Scheme 1E, conjugation of chitosan to ACV-PCL is made via amide linkage. The peak at 1.79 (A-NH₂) from a singlet to a multiplet in Fig. 3 (vi) confirms conjugation of chitosan to ACV-PCL. Moreover, the peaks from the aromatic protons on C₃ – C₆ can be seen from δ = 3.28 – 3.85, slightly shifted downward from the peaks shown in the original chitosan sample. Fig. 4 depicts the FTIR spectra of ACV-PCL (A), chitosan (B), and ACV-PCL-chitosan (C). OH stretching from 3429 – 3167 cm⁻¹ as well as peaks at 1634 cm⁻¹ and 1521 cm⁻¹ corresponding to the N-H bending vibrations of the amide I and II bands are attributed to ACV and chitosan, respectively. The aforementioned peaks as well as the carbonyl absorption at 1726 cm⁻¹ associated with PCL and a peak at 1065 cm⁻¹ (C-O-C) seen in each spectra are found in ACV-PCL-chitosan. The FTIR results are in line with the results from ^1H NMR and demonstrate successful synthesis of ACV-PCL-chitosan.

3.2. Formation and Characterization of ACV-tagged Polymeric Micelles

Due to the amphiphilic nature of the synthesized ACV-PCL copolymers (i.e., ACV-PCL-MPEG and ACV-PCL-chitosan), they can self-assemble into polymeric prodrug micelles in aqueous medium. Here, ACV-PCL acts as the hydrophobic segment and MPEG or chitosan serve as the hydrophilic segments. Polymeric micelles of ACV-PCL copolymer were prepared via the evaporation method [39]. The CMC using pyrene as a hydrophobic fluorescent probe was carried out to prove the formation of the micellar structures. Pyrene can preferentially partition into the interior hydrophobic microdomains and change the intensities of the first and third bands in the pyrene fluorescence spectrum [38] For ACV-

PCL-MPEG the shift found in the first and third bands was at I_{335}/I_{330} while for ACV-PCL-chitosan it was at I_{337}/I_{332} . It is believed that this change is due to different polymer compositions. Fig. 5A shows the CMC values of ACV-tagged polymeric micelles in aqueous medium. It was determined that the CMCs for ACV-PCL-MPEG and ACV-PCL-chitosan were 2.0 and 6.6 mg L⁻¹, respectively (Fig. 5A (i) and (ii)). Kim *et al.* reported that the CMC values of block copolymers depends upon the block copolymer architecture [40]. In this study, the hydrophobic block was kept constant, as ACV-PCL, while the hydrophilic block was changed to either MPEG or chitosan. The low CMC value obtained for ACV-tagged polymeric micelles could provide evidence for their stability in dilute solutions.

In order to characterize the morphology of ACV-tagged polymeric micelles, DLS and TEM analyses were carried out. Fig. 5B shows the size and morphology of ACV-tagged polymeric micelles. As can be seen from DLS results, sizes of the two types of polymeric prodrug micelles were under 200 nm. ACV-PCL-MPEG (Fig. 5B (i)) and ACV-PCL-chitosan (Figure 5B (ii)) micelles showed size distributions with mean diameters of 141.8 and 172.7 nm, respectively. The morphology of the micelles was further examined by TEM as shown in the insets of Fig. 5B. The size of the micelles in the TEM images was slightly lower than the results from DLS with an average particle size of ~100-150 nm. This size fluctuation is due to the fact that DLS records the hydrodynamic radius of particles which is often times slightly larger than the actual particle size. Zeta potential analysis of ACV-tagged polymeric micelles was also performed. An average zeta potential for ACV-PCL-MPEG micelles was 1.4 mV. Due to the fact that MPEG has a neutral charge, the results obtained for ACV-PCL-MPEG micelles are reasonable. In contrast, the detected zeta potential of ACV-PCL-chitosan micelles was 32.3 mV. This is attributed to the positive charge of the hydrophilic chitosan segment deployed on micellar nanoparticles.

To determine the drug loading percentage of acyclovir per mg of micelle formulation, the absorbance of ACV-tagged polymeric micelles at $t = 0$ and $t = 48$ h was examined and compared to a standard calibration curve of ACV ranging from 0.002 to 1.0 mg/mL. It was found that ACV comprised 8.7% and 3.2% of ACV-PCL-MPEG and ACV-PCL-chitosan micelles, respectively. The difference in ACV loading percentage is due to different conditions employed for the synthesis of ACV-PCL-MPEG and ACV-PCL-chitosan amphiphilic copolymers. While MPEGNH₂ can dissolve in organic solvent, chitosan requires an aqueous solvent for it to dissolve. Therefore, for ACV-PCL-chitosan synthesis, the ester bond between ACV and PCL is already weakened, thereby resulting in less ACV coupled with PCL than ACV-PCL-MPEG which is synthesized under organic solvent environment.

3.3. In Vitro Release of ACV from Polymeric Micelles

The *in vitro* release behaviors of ACV from both types of polymeric prodrug micelles in PBS at 25°C were studied and the results are shown in Fig. 6. Similar biphasic drug release profile of ACV-PCL-MPEG and ACV-PCL-chitosan micelles was observed, with initial burst release within the first 2 h (~ 50% accumulative release) followed by a sustained release pattern up to 48 h. The release of ACV from both polymeric prodrug micelles was caused by the hydrolysis of ester linkage between ACV and PCL. In the first phase, the rate

of drug release from ACV-PCL MPEG micelles was relatively rapid than that from ACV-PCL-chitosan ones. The reason for this phenomenon is speculated to be twofold. First, MPEG chains probably are more hydrophilic and flexible than chitosan chains on micellar nanoparticles, leading to a faster hydrolysis and concomitant ACV diffusion from hydrophobic PCL cores. Second, the diffusion of ACV molecules might be slowed down by the positively charged chitosan on ACV-PCL-chitosan micelles. Such different initial release trend continued and resulted in higher ACV accumulative release from ACV-PCL-MPEG micelles (96%) than from ACV-PCL-chitosan micelles (82%).

3.4. Cytotoxicity Study

Biocompatibility is a vital necessity of polymer materials being used for drug delivery applications. To evaluate if ACV-tagged polymeric micelles showed any cytotoxicity towards HT29 colorectal cells, MTT assay was performed. As shown in Fig. 7, the viability of HT29 cells after treatment for 48 h with either ACV-PCL-MPEG or ACV-PCL-chitosan has little change compared with the untreated control cells (i.e., cells without any micelle challenge). These results demonstrate that ACV-tagged polymeric micelles do not exhibit apparent toxicity, and are biocompatible.

4. Conclusion

In this study, ACV-PCL-MPEG and ACV-PCL-chitosan polymeric micelles were synthesized and characterized. ACV was used to directly initiate polymerization of ϵ -CL to form hydrophobic ACV-PCL. Compared to conventional methods of incorporating ACV into polymeric carriers by chemical conjugation or physical encapsulation, our approach is advantageous in terms of eliminating drug-loading steps, enhancing drug-carrying capacity, and decreasing production cost. By grafting ACV-PCL with either MPEG or chitosan, ACV-tagged amphiphilic polymers can self-assemble as micellar nanoparticles in aqueous medium. Structural analyses such as ^1H NMR and FTIR were performed to confirm their conjugation. ACV-tagged polymeric micelles were found to have a mean size under 200 nm by both DLS and TEM and have a low CMC. Moreover, both ACV-PCL-MPEG and ACV-PCL-chitosan micelles were nontoxic to HT29 colorectal cells. ACV-tagged polymeric micelles are potential carriers for therapeutic and anticancer drug delivery.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Acyclovir was used directly to initiate polymerization of ϵ -caprolactone.
- ACV-tagged micelles were self-assembled by conjugation with hydrophilic poly(ethylene glycol) or chitosan.
- ACV-tagged polymeric micelles are non-toxic.
- ACV-tagged micelles are advantageous in terms of eliminating drug-loading steps, enhancing drug-carrying capacity, and decreasing production cost.

Scheme 1.

Synthetic scheme of (A) ACV-PCL, (B) ACV-PCL-COOH, (C) ACV-PCL-NHS, (D) ACV-PCL-MPEG, and (E) ACV-PCL-chitosan.

Fig. 1.
¹H NMR spectra of (iii) MPEG and (iv) ACV-PCL-MPEG.

Fig. 2.
FTIR spectra of (A) ACV-PCL, (B) MPEG-NH₂, and (C) ACV-PCL-MPEG.

Fig. 3.
¹H NMR spectra of (v) chitosan and (vi) ACV-PCL-chitosan.

Fig. 4.
FTIR spectra of (A) ACV-PCL, (B) chitosan, and (C) ACV-PCL-chitosan.

Fig. 5. (A) Plot of the intensity ratio (I_I/I_{III}) versus concentration of ACV-tagged polymeric micelles, and (B) particle size distribution of (i) ACV-PCL-MPEG and (ii) ACV-PCL-chitosan. Insets represent TEM images.

Fig. 6.
In vitro drug release profiles of ACV-PCL-MPEG (—■—) and ACV-PCL-chitosan (—▲—)
polymeric micelles in PBS (mean $\pm$ SD, $n = 3$).

Fig. 7.

Viability of HT29 colorectal cancer cells challenged with ACV-tagged polymeric micelles at different dosages. ■ Control (without micelle challenge), ▨ ACV-PCL-MPEG micelles, and ■ ACV-PCL-chitosan micelles (mean $\pm$ SD, $n = 3$).