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Sunitinib release from biodegradable films of poly(L-lactide-co-caprolactone)

Seung Hee Ha [a,1], Jong-Ho Hwang [b,1], Do Hyung Kim [a,1], Min-Dae Kim [b], Cheol-Woong Choi [b], Young-Il Jeong [a,*], Chung Wook Chung [a], Cy Hyun Kim [a], Dae Hwan Kang [a,**]

[a] National Research & Development Center for Hepatobiliary Cancer, Research Institute for Convergence of Biomedical Science and Technology, Pusan National University Yangsan Hospital, Gyeongnam 626-770, Republic of Korea
[b] Department of Internal Medicine, Medical Research Institute, Pusan National University School of Medicine and Medical Research Institute, Yangsan, Republic of Korea

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ABSTRACT

The aim of this study was to prepare sunitinib-loaded biodegradable films using poly(L-lactide-co-ε-caprolactone) (PLCL) for anti-tumor drug delivery. Sunitinib-loaded PLCL film has a rough surface, while empty film has a smooth surface. PLCL film loaded with 5% (w/w) sunitinib showed an absence of a crystalline peak of sunitinib, while sharp peaks were observed at 10% (w/w) loading, indicating that sunitinib was molecularly distributed in the polymer matrix at 5% (w/w). A drug release study revealed an initial burst during the first 2 h, followed by continuous release until 24 h. Since weight loss of film was <10% for 1 week, drug release mechanism was dominantly dependent on the diffusion-mediated release of drugs to the medium. Sunitinib has a dose-dependent anti-proliferation effect against HuCC-T1 human cholangiocarcinoma cells *in vitro*. These results indicate that sunitinib-loaded PLCL film is a appropriate candidate as a vehicle for anti-tumor drug delivery.

1. Introduction

Biodegradable polymers such as poly(lactic acid), poly(glycolic acid), poly(ε-caprolactone) and their copolymers have been extensively used in drug delivery systems [1–4]. Even though various kinds of biomaterials have been used for sustained drug delivery [5–7], these biodegradable polymers have been used as a majority of coating materials for drug-eluting stents [3,4]. Most of these applications of biodegradable films have involved the development of cardiovascular stents [8–10]. Biodegradable films encapsulating anticancer agents are primarily considered for drug-eluting stents.

Sunitinib inhibits cellular signaling by targeting multiple receptor tyrosine kinases (RTKs) including platelet-derived growth factor receptors (PDGF-Rs) and vascular endothelial growth factor receptors (VEGFRs), which play a role in both tumor angiogenesis and tumor cell proliferation [11]. Inhibition of these receptors leads to reduced tumor vascularization, cancer cell death, and tumor shrinkage [11,12]. Furthermore, sunitinib displays anti-proliferative and/or apoptotic effects against tumor cells expressing target kinases [11,12]. Sunitinib directly inhibits the survival and proliferation of a variety of cancer cells [12].

The aim of this study was to prepare sunitinib-loaded PLCL films for application in anti-tumor drug delivery systems against human cholangiocarcinoma cells. Prior to their use as metallic stent coatings, we characterized and studied them against HuCC-T1 cholangiocarcinoma cell *in vitro*. Cholangiocarcinoma (CCA), which is an epithelial cancer originating from the bile ducts, is difficult to diagnose and only a small percentage of cases can be resected surgically [13–15]. Since these tumors are highly refractory to conventional chemotherapeutic agents [16], a metal stent is primarily considered to be a unique treatment option for prolongation of patient survivability [9]. Drug-eluting stents (DES) that incorporate biodegradable polymer coatings with anticancer drugs are an effective treatment option because the polymer coatings act as a drug reservoir and continuously release anti-cancer drugs to inhibit the growth of tumor cells [17]. Anti-cancer drug eluting polymer films are considered as a unique candidate for cholangiocarcinoma.

2. Experimental details

2.1. Materials

Sunitinib was purchased from LC laboratories (Woburn, MA, USA). PLCL (RESOMER® LC703S) was purchased from Evonik Ind.

* Corresponding author.
** Corresponding author at: National Research & Development Center for Hepatobiliary Cancer, Pusan National University Yangsan Hospital, Beomeo-ri, Mulgeum-eup, Yangsan, Gyeongnam 626-770, Republic of Korea.
Tel.: +82 55 360 3870; fax: +82 55 360 3879.
E-mail addresses: nanomed@naver.com (Y.-Il. Jeong), sulsulpul@yahoo.co.kr (D.H. Kang).
[1] These authors contributed equally to this work.

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Table 1
Characterization of sunitinib-loaded PLCL films.

	Weight ratio of PLCL/sunitinib (mg/mg)	Drug contents (%, w/w)	Status of film surfaces
PLCL	200/0	0	Smooth
S-PLCL1	200/10	5	Rough
S-PLCL2	200/20	10	Rough

(Evonik Röhm GmbH, Darmstadt, Germany). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), chloroform, dimethylsulfoxide (DMSO), and N,N-dimethylformamide (DMF) was purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Preparation and characterization of sunitinib-loaded PLCL films

PLCL (200 mg) and sunitinib were dissolved in 5 ml of chloroform and the mixture was cast onto a glass plate. The solvent was removed at reduced pressure. After 4 h, the sunitinib-containing PLCL films were obtained, as listed in Table 1. The crystalline properties of sunitinib-loaded PLCL films were examined with X-ray diffraction (XRD) using a *D*/max-1200 apparatus (Rigaku Co., Tokyo, Japan) using Ni-filtered Cu Kα radiation (40 kV, 20 mA). Thermal properties of sunitinib-loaded PLCL films were studied using differential scanning calorimetry (DSC) with a DSC2920 apparatus (TA Instruments, New Castle, DE, USA) between 20 °C and 350 °C with temperature increase of 10 °C/min. The morphology of films was observed by scanning electron microscopy (SEM) using a S4700 microscope (Hitachi, Tokyo, Japan).

2.3. Drug release from sunitinib-loaded PLCL films

The films incorporating sunitinib were cut into 21 mm-diameter discs, each weighing about 10 mg. Each sample was immersed into15 ml of phosphate-buffered saline (PBS, 0.1 M, pH 7.4, 1% (v/v) Tween 80) and incubated at 37 °C with shaker incubator (100 rpm). The discs were taken and freeze-dried for 2 days. Dried discs dissolved in DMF were used to measure the remaining quantity of sunitinib using a model 1201 UV spectrophotometer (Shimadzu, Tokyo, Japan) at 450 nm. All experiments were run in triplicate and data was expressed as mean ± S.D.

2.4. Degradation of PLCL films

The discs of empty PLCL films (21 mm in diameter, 10 mg) were individually immersed in 10 ml of 0.01 N NaOH solution or deionized water in 20 ml glass vials and then kept in incubator at 37 °C and 100 rpm. At predetermined time intervals, discs were recovered, washed, and subsequently dried for a week in a vacuum desiccator. Degradation of PLCL films was evaluated as a weight loss. The percentage of weight loss was calculated by following formula:

$$\text{weight loss}(\%) = \left(\frac{M_d}{M_i}\right) \times 100$$

where M_i is the initial weight of the film and M_d is the weight of the film after degradation.

2.5. Antitumor activity of sunitinib-loaded PLCL films

Human cholangiocarcinoma cells (HuCC-T1) were plated in 96-well plates (1×10^4 cells per well) with RPMI 1640 medium supplemented with 10% fetal bovine serum and incubated overnight in 5% CO_2 incubator at 37 °C. After that, sunitinib in DMSO was diluted with medium and added to 96 well plates. For cytotoxicity of empty films, empty PLCL films in DMF were diluted with a 100× volume of medium and added to 96 wells (2×10^4 cell/well). Cell viability was assessed by MTT proliferation assay.

3. Results and discussion

3.1. Characterization of sunitinib-loaded PLCL films

Sunitinib-loaded PLCL films were prepared by casting onto glass plates using organic solvent. Empty films were prepared similar way except for the absence of drug. Table 1 summarizes the characteristics of sunitinib-loaded PLCL films. Morphology of PLCL films was observed by SEM (Fig. 1). As shown in Fig. 1A, empty PLCL films had a smooth surface, whereas sunitinib-loaded PLCL films had a rough surface. PLCL films containing 10% (w/w) sunitinib (Fig. 1C) showed crumpled surface morphology, indicating that drug crystals were aggregated onto the surface of the polymer films. XRD analysis supported these results (Fig. 2). The empty PLCL film (Fig. 2A) and PLCL film containing 5% sunitinib displayed broad peak properties, while sunitinib itself had sharp crystalline peaks. Interestingly, PLCL films with 10% (w/w) sunitinib showed sharp crystalline peaks. Hydrophobic drugs normally form aggregates in the polymer matrix at higher drug loading and form drug crystals [17]. As shown in Fig. 2B, PLCL films loaded with sunitinib did not show melting point of drug while melting point of suntinib itself was observed at about 240 °C. Glass transition temperature (T_g) of empty PLCL films and sunitibin-loaded PLCL films appeared at about 40 °C and, melting and decomposition of empty PLCL films might have occurred at about 200 °C.

3.2. Sunitinib release and degradation of sunitinib-loaded PLCL films

The initial burst effect was observed until 2 h and then sunitinib was released continuously from the PLCL films (Fig. 3). The release rate of sunitinib was slightly faster at lower drug loading (S-PLCL1) than higher drug loading. As shown in Fig. 2A, hydrophobic drugs

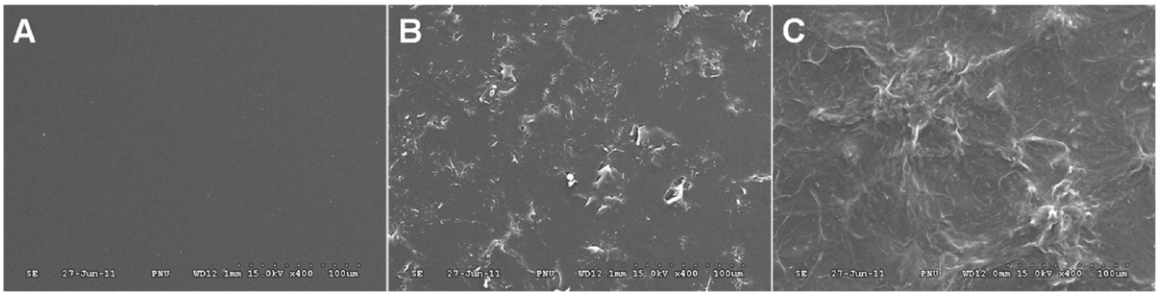

Fig. 1. Morphology of empty PLCL film and sunitinib-loaded PLCL films observed by SEM (400×). (A) PLCL; (B) S-PLCL1; (C) S-PLCL2 in Table 1.