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The Nanoparticles for Combating Acne Vulgairs: *In-vitro* Efficacy of Lauric Acid-Loaded PCL-PEG-PCL on *Propionbactrium acne*

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***Abstract*—Poly(ε-caprolactone)-poly(ethylene glycol)-poly(ε-caprolactone) (PCL-PEG-PCL, PCEC) copolymers possess amphiphilicity, biodegradability, and great biocompatibility. The PCEC nanoparticles are widely used as a drug delivery system. Free fatty acids showing antibacterial activity has been reported. Amount other fatty acids, lauric acid (LA) is demonstrated its strongest bactericidal activity against Propionibacterium acnes (P. acnes). But LA is poor water soluble. This study evaluated the antibacterial activity of LA-loaded PCEC nanoparticles against P. acnes. As free LA dissolved in 5% DMSO, the minimum bactericidalclicliho concentration (MBC) was 80 μg/mL to against P. acnes. In contrast, MBC of PCEC-LA nanoparticles were 40 μg/mL. It is thought that PCEC-LA nanoparticles possess potency for treating acne vulgaris.**

Keywords- Poly(ε-caprolactone)-poly(ethylene glycol)-poly(ε-caprolactone; Lauric acid; Propionibacterium acnes; antibacterial; minimum bactericidal concentration.

I. INTRODUCTION

Acne vulgaris is one of the most common of skin diseases caused by the acne bacteria Acne vulgaris usually affects the face, chest and upper back and present in adolescence and early adult life. *Propionibacterium acnes* belongs to the Gram-positive bacteria, the bacteria is not form spores, is absolutely anaerobic bacteria, commonly found in deep within the hair follicle [1, 2]. In recent years, there are many polymeric nanoparticles anticancer used as agent for acne vulgaris therapy. Poly(ethylene glycol) (PEG) and Poly(ε-caprolactone) (PCL) are widely used for biomedical applications because of their great biocompatibility. PEG is a water-soluble biocompatible, non-toxic polymer, and absence of antigenicity or immunogenicity polymer which has been widely used for drug delivery system many clinical applications [3, 4]. PCL is biodegradable and biocompatible polyester. It is rather hydrophobic and it degrades very slowly by simple hydrolysis because of its semicrystalline structure and low glass transition temperature [5, 6]. Further, PEG/PCL block copolymer can improve their hydrophilicity and biodegradability to use as controlled drug delivery system. The PCL-PEG-PCL (PCEC) copolymer is a candidate for drug delivery system because of its biodegradability, good biocompatibility, low toxicity, amphiphilic property [7, 8, 9].

A number of natural antimicrobial products such as FFAs and their esters show possess antibacterial and antiviral activity to against Gram-positive bacteria [10, 11, 12, 13]. The mechanism of antibacterial activity of FFAs is not fully understood, it may be due to they disrupted bacterial membranes and increased membrane permeability. Lauric acid is a kind of fatty acid which shows the strongest bactericidal activity against *P. acnes in vitro* [14].

Herein, we report the antimicrobial activity of LA loaded PCL-PEG-PCL nanoparticles (PCEC-LA NPs) against *P. acne*. The prepared PCEC-LA NPs and their antibacterial activity were demonstrated. So the PCEC-LA NPs might be a novel agent for acne vulgaris therapy.

II. MATERIALS

ε-caprolactone, 18-crown-6, dimethyl sulfoxide and noble agar were obtained from Sigma Aldrich (USA, St. Louis). Poly(ethylene glycol) was purchased from Showa (Japan, Tokyo). 2-Bromo-2′-acetonaphthone was obtained from Alfa Aesar (USA, New York). Reinforced clostridial medium was from OXOID (England, Hampshire). Acetonitrile was purchased from ECHO (Taiwan, Miaoli).Agar was purchased from BD (Sparks, MD).

III. PREPARATION AND CHARACTERIZATION OF LA-LOADED PCEC NPS

In this work, a series of biodegradable triblock poly(ε-caprolactone)-poly(ethylene glycol)-poly(ε-caprolactone) copolymers were synthesized by ring-opening copolymerization. Briefly, PEG and ε-CL were introduced into a dry glass flask under a nitrogen atmosphere, and amount of $Sn(Oct)_2$ was added into the reaction vessel under agitation. The reaction system was kept at 130 °C for 24 h. The resultant PCEC copolymer was first dissolved in dichloromethane, then reprecipitated from the filtrate using cold ether/n-hexane, after which the mixture was filtered and vacuum dried. The purified PCEC copolymers were kept in desiccators for using. The chemical composition and molecular weight of the copolymer were characterized by 1H nuclear magnegtic resonance (1H NMR) (Bruker 500MHz, Bruker, Massachusetts, USA), Fourier transform infared spectroscopy (FT-IR) (FT/IR 410, Jasco, Tokyo, Japan), differential scanning calorimetry (DSC) (Jade DSC, Perkinelmer, Waltham, USA) and gel permeation

chromatography (GPC) (Viscotek GPCmax, Viscotek, Texas, USA), respectively. The critical micelle concentration (CMC) were determined by fluorescent probe, 1,6-diphenyl-1,3,5-hexatriene (DPH). The micelles were prepared by thin film hydration method. The surface zeta potential (mV) of PCEC-LA NPs and PCEC NPs were measured using the BI-Zeta (Brookhaven Instruments Corporation, New York, USA). The mean diameters of PCEC-LA NPs and PCEC NPs were determined through dynamic light scattering (DLS) (90plus, Brookhaven Instruments Corporation/ New York). To quantitate lauric acid which encapsulated in micelles by derivation of naphthacyl ester, and quantitated by High-performance liquid chromatography (HPLC) (Alliance ® 2695, Waters, Massachusetts, USA).

IV. Preparation Of Bacteria

P. acnes (BCRC 10723) was cultured in Reinforced Clostridial Medium (RCM) (OXOID, England, Hampshire) under anaerobic condition using Gas-Pak with rotary shaking at 37 °C. And then the culture was diluted 1:100 and cultured at 37 °C with rotary shaking until reaching around OD_{600} = 0.7 (logarithmic growth phase) under anaerobic condition. The bacteria were harvested by centrifugation at 5,000 g for 10 min, washed with sterile PBS, and suspended to appropriate amount of sterile PBS for further experiments.

V. *In-Vitro* Antibacterial Activity Of Pcec-La Nps

The antimicrobial activities of LA or PCEC-LA NPs against *P. acnes* were investigated. *P. acnes* (1×10^6 CFU/mL) was incubated with LA, or PCEC-LA NPs (0–100 μg/mL) in 5% DMSO in PBS at 37 °C for 5 h under anaerobic condition, and 5% DMSO in PBS was used as a control. After incubation, the samples were diluted 1:10 to $1:10^6$ in PBS, and 5 μL of dilutions was spotted on agar plates. Agar plates were incubated at 37 °C under anaerobic condition for 3 days, and CFU (colony forming units) of *P. acnes* was quantified.

VI. Results And Disscutions

A. Characterization of PCEC NPs and LA-loaded PCEC NPs

A series of PCEC copolymers were synthesized by ring-opening copolymerization of ε-CL initiated by PEG using $Sn(Oct)_2$ as a catalyst. ^{1}H-NMR, FT-IR, and GPC were used to characterize the chemical structure and molecular weight of the PCEC triblock copolymer. In the chemical structure of PCEC copolymer, the molecular weight of PEG was 4 kDa while the molecular weight of PCL varied from 2k to 10kDa. Table I summarizes the characterizations of PCEC copolymers. It can be seen that the conversion rates were very close to one, except $PC_{100}E_{40}C_{100}$ where longer PCL may hinder the ring-opening polymerization. Figure 1 displayed the particle size and zeta potential of series PCEC-LA NPs. The particle size of $PC_{20}E_{40}C_{20}$-LA, $PC_{50}E_{40}C_{50}$-LA, and $PC_{100}E_{40}C_{100}$-LA NPs were 24.70 ± 3.63, 67.77 ± 5.59, and 89.33 ± 2.55 nm, respectively. Moreover, the zeta potential of $PC_{20}E_{40}C_{20}$-LA, $PC_{50}E_{40}C_{50}$-LA, and $PC_{100}E_{40}C_{100}$-LA NPs were -4.160 ±2.20, -16.57 ± 4.16, and -18.383 ± 3.03 mV, respectively. The particle size measured by DLS indicates that the encapsulation of LA decreased the particle size and zeta potential as well. Sutton et al explained that the decrease in particle size after the encapsulation of doxorubicin, hydrophobic drug, was mainly contributed from the hydrogen boding between drug and the core of PEG-b-PCL. However, the close packing of PCL segment with hydrophobic drug could not be ruled out [14]. Therefore, we expected the presence of the close packing of LA in the core of PCEC micelles led to the size reduction found in this study.

TABLE I. Characterization Of Pcec Copolymer

	M_n (g/mol)	$\overline{M}_{nm}$ (g/mol)	$\overline{M}_n$ (g/mol)	$\overline{M}_w$ (g/mol)	PDI	CMC (wt%)
$PC_{20}E_{40}C_{20}$	8000	8127	8320	9727	1.169	4.94×10^{-3}
$PC_{50}E_{40}C_{50}$	14000	13698	10831	13999	1.293	4.33×10^{-3}
$PC_{100}E_{40}C_{100}$	24000	22571	14154	18838	1.331	2.63×10^{-3}

The results showed that the PCEC NPs with and without LA were negatively charged. The encapsulated LA led to a decrease of the zeta-potential of PCEC-LA NPs. That was because of carboxyl group deprotonated, the nanoparticles encapsulated LA result in zeta potential decreased (Fig. 1). The loading yield of LA in PCEC-LA NPs was determined by HPLC. The loading yield of LA in PCEC-LA NPs was determined by HPLC. Because the functional group of LA is a carboxylic group which has negligible UV absorbance. So using the HPLC technique to separate LA from PCEC-LA NPs, LA must be derivatized with naphthacyl estere first. After being derivatized with 2-bromo-2'-acetonaphthone, derivatized LA could be detected by the UV/VIS detector at 245 nm. The standard curve was generated by measured the UV absorbance intensity of a series of derivatized LA samples ranging from 0 to 2250 μM (Fig. 2). The drug loading contents of PC20E40C20-LA, PC50E40C50-LA, and PC100E40C100-LA NPs were 3.27 ± 0.15, 9.75 ± 2.35, and 15.42 ± 4.02 %, respectively. Further, we found the PC50E40C50-LA NPs prepared by 10 mg of PCEC and 2 mg LA possess the highest LA concentration (323.75 μg/mL).

TABLE II. The Mbc Of Free La And Pcec-La Nps

	Free LA	$PC_{20}E_{40}C_{20}$-LA	$PC_{50}E_{40}C_{50}$-LA	$PC_{100}E_{40}C_{100}$-LA
MBC	80	40	40	40

*Free LA: Lauric acid dissolved in 5% DMSO Unit: μg/mL; n=3

B. In-vitro antibacterial activity of PCEC-LA NPs

In order to determine the antimicrobial activity of series PCEC-LA NPs. Samples of free LA, $PC_{20}E_{40}C_{20}$-LA, $PC_{50}E_{40}C_{50}$-LA, and $PC_{100}E_{40}C_{100}$-LA NPs (0, 20, 40, 60, 80,

and 100 μg/mL LA) were incubated with *P. acnes* (1×10^6 CFU/mL) for 5 h under anaerobic condition. In antibacterial study, the effect of PCEC-LA NPs were compared with free LA dissolved in organic solvent (5% DMSO), and evaluated by minimum bactericidal concentration (MBC) (Table. II). The experimental results showed as free LA dissolved in 5% DMSO, the MBC were 80μg/mL. In contrast, the MBC of PCEC-LA NPs was 40 μg/mL. Figure. 3 showed that free LA with 80 μg/mL LA completely killed the bacteria. As shown in Fig. 4, $PC_{20}E_{40}C_{20}$-LA, $PC_{50}E_{40}C_{50}$-LA, and $PC_{100}E_{40}C_{100}$-LA NPs completely killed *P .acne* when their concentration was 40 μg/mL.

VII. CONLUSIONS

In this study, the antibacterial activity of PCEC-LA NPs was evaluated. We successfully resolve the poor water solubility issue of LA by loading LA into PCEC NPs. The size of the PCEC-LA NPs was about 24-89 nm. We further demonstrated that after loading LA into PCEC NPs, its antimicrobial activity against *P. acnes* was well. The result showed PCEC-LA NPs complete killing of *P. acnes* at the LA concentration of 40 μg/mL. The PCEC-LA NPs might be a novel agent for acne vulgaris therapy.

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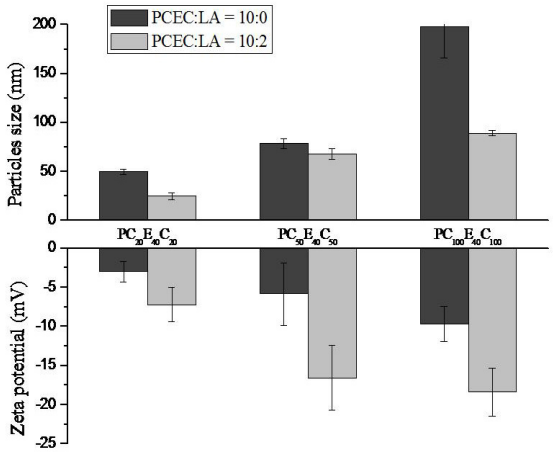

Figure 1. Characterization of PCEC-LA NPs. The size and surface zeta potential (mV) of the PCEC-LA NPs were determined by dynamic light scattering. Data represents mean ± SD of three individual experiment

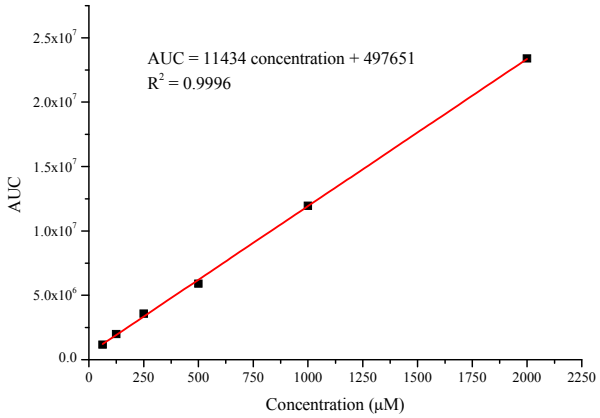

Figure 2. The linear calibration standard curve of derived LA by HPLC. Data represents mean ± SD of three individual experiments.

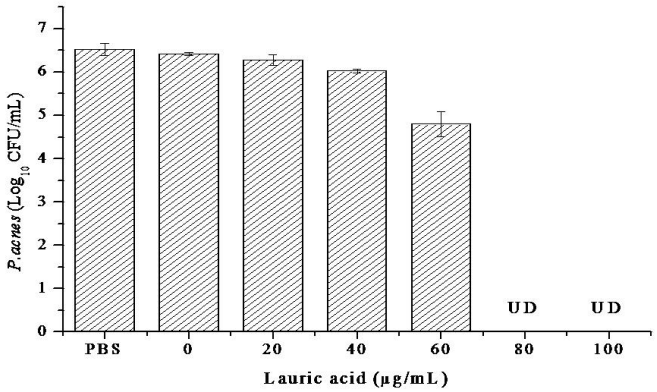

Figure 3. Antimicrobial activity of free lauric acid against P. acnes. Free lauric acid were incubated with P. acnes (1 × 106 CFU/mL), for 5 h under anaerobic condition to test their antimicrobial activity. The results showed that 80 μg/mL Free lauric acid completely killed bacteria. Data represents mean ± SD of three individual experiments. UD: undetectable.

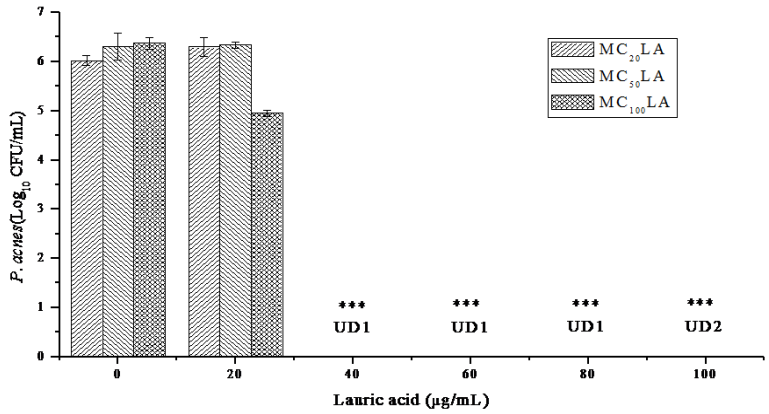

Figure 4. Antimicrobial activity of PCEC-LA NPs against P. acnes. PCEC-LA NPs were incubated with P. acnes (1 × 106 CFU/mL), for 5 h under anaerobic condition to test their antimicrobial activity. The results showed that 40 μg/mL PCEC-LA NPs completely killed bacteria. Data represents mean ± SD of three individual experiments. UD: undetectable.