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Controlled Drug Delivery of Ampicillin-Poly(L-lactic acid) Films for the Treatment of Otitis Media

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중이염 치료용 암피실린-폴리락트산 필름의 약물방출조건

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A new local drug delivery device to treat otitis media (OM) has been developed. This device consists of a biodegradable poly(L-lactic acid) (PLLA) film containing antibiotic (ampicillin, AMP), which can be placed into the middle ear cavity and release the therapeutic concentration of AMP for prolonged period. Biodegradable films containing AMP (10 w/w%) were prepared by solution casting method using a suspension of the drug in a PLLA/CH_2Cl_2 solution (molecular weight of PLLA, 100,000 (100 K) and 300,000 (300 K), respectively). PLLA-AMP films were characterized by FTIR, DSC, and SEM. *In vitro* release of AMP from AMP-PLLA films were examined. The release pattern of AMP from AMP-PLLA films remained consistent from 1 day to 14 days, and the release rates of AMP from AMP-100K-PLLA film and AMP-300K-PLLA film were 0.7384 μg/ml/day, 0.4107 μg/ml/day, respectively.

Keywords—Biodegradable film, Poly(L-lactic acid), Local drug delivery system, Ampicillin

Otitis media (OM) is one of the most common inflammatory disorders in human beings[1] and one of the most common diseases in the otolayngologic field. There are numerous studies that document the high incidence and prevalence of its different forms and manifestations.[2-6] OM is classified into two large groups. Acute otitis media (AOM) generally causes ear pain and predominantly purulent middle ear effusion, but infection may be present even with mucoid effusion and chronic otitis media (COM) includes additional pathology, such as suppurative discharge through a rupture tympanic membrane, growth of granulation tissue in the middle ear, damage to the ossicles, hearing loss, cholesteatoma, and/or mastoiditis.[7] The infecting bacterial organisms generally responsible are: *S. pneumoniae* (40%), *H. influenzae* (24%), group A β-hemolitic streptococci (4%), *Staphylococcus aureus* (2%) and with the balance being aseptic or viruses (30%). In neonates, *Escherichia coli*, group B streptococci, *S. aureus*, *Listeria* and *Klebsiella pneumoniae* are the common infecting organisms.[8,9]

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In children over 6 years of age and adults, *S. pneumoniae*, group A β-hemolytic streptococci and viruses are the etiologic agents. Studies of young children document *H. influenzae* as a causative agent more frequently (up to 49.5% of causes in children).[10]

To prevent damage to the ear and a possible loss of hearing, it is important to treat bacteria OM with medications. The treatment of OM is medication and operations. Various antimicrobial drugs are commonly used to treat AOM. However, these drugs are only partially effective at best and treatment failure is not uncommon.[11] Therefore, proper management of middle ear inflammation is desirable. Although systemic administration of antibiotics is useful, high oral doses are required to achieve effective concentration in the middle ear cavity, and long-term use may lead to the development of resistant bacterial strains.[12,13] These disadvantages have led researchers to focus on localized delivery of antibiotics directly at the diseased site.

Several experimental models for studying antimicrobial drug in OM are studied[14,15] and various degradable devices are under investigation for the delivery of antibiotics into the middle ear of experimental animals.[16] Especially, advantages of polymeric controlled release drug delivery system to treat OM involve (1) removal of disadvantages according to operation such as poor results of operation by reason of lesion of middle ear and related structures, difficulty of choice of operation in cholesteatomatous OM, problems of material selection used for ossiculoplasty, and anxiety and evasion on operation, (2) localization for purposes of local therapy, (3) the maximum effect of treatment based on local application, (4) targeting to specific diseased tissues, and (5) decrease of toxic effect owing to overdose or repeated medication.

Poly(L-lactic acid) (PLLA) (Fig. 1A), due to its biocompatibility and biodegradability, has been widely used for the controlled release of drugs.[17-29] Several antibiotics and antibacterial drugs such as trimethoprim, amoxicillin, sulfamethoxazole, cefaclor and sulfisoxazole are known to be effective against OM. Since AMP (Fig. 1B) has broad spectrum of antibacterial activity and high efficacy against gram-positive and gram-negative bacteria, it has been widely used for the treatment of OM and related conditions.

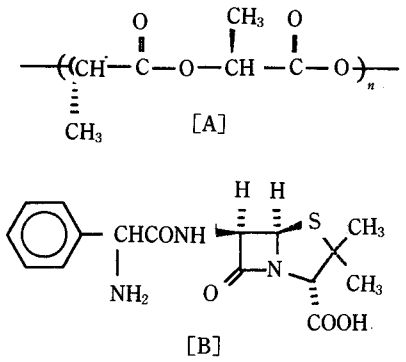


Figure 1—The chemical structure of [A] poly(L-lactic acid) and [B] 6-[(aminophenyl-acetyl)amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (ampicillin).

The objective of this research is to develop a biodegradable, PLLA, film that is capable of delivering therapeutic concentrations of AMP for prolonged periods in middle ear cavity.

EXPERIMENTAL

Materials

Poly(L-lactic acid)s-MW: 100,000, lot no. 423425 and MW: 300,000, lot no. 424478-were purchased from Polysciences, Inc., Warrington, PA. Number average (Mn) and weight average (Mw) molecular weights of initial polymers, were evaluated by gel permeation chromatography (GPC, Waters 150C, Milford, MA). Ampicillin anhydrous (lot no. 71H 0594) and ammonium dihydrogenphosphate (lot no. OB1137) were obtained from Sigma Chemical Co., St. Louis, MO and Junsei Chemical Co., Tokyo, Japan, respectively. Methylene chloride and methanol were purchased from Mallinckrodt Specialty Chemicals Co., Paris, KY, and J. T. Baker Inc., Phillipsberg, NJ.

All the chemicals were of analytical grade and used without further purification.

Preparation of AMP-PLLA Films

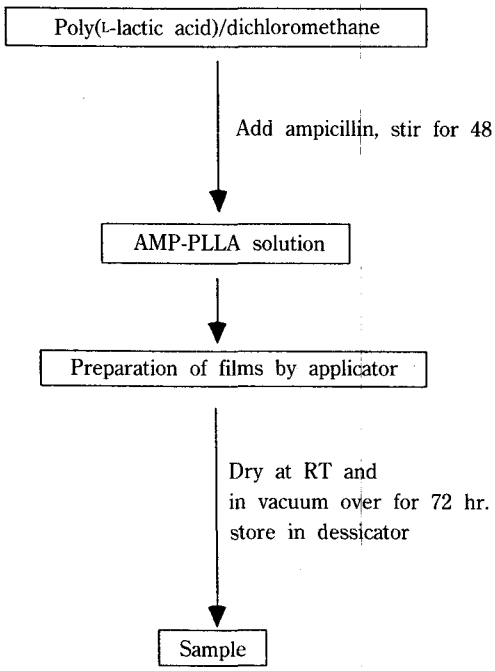


Figure 2—Preparation of AMP-100K-PLLA and AMP-300K-PLLA films by the solvent casting method in dichloromethane.

AMP-PLLA films were prepared by solvent casting method (Fig. 2). To a solution of PLLA(100 K, 0.9 g) in 6.0 m*l* methylene chloride was added AMP 0.1 g under room temperature, and the suspension was stirred for 48 hours. The AMP-PLLA films were casted by pouring this dispersion onto glass molds, and allowing them to dry initially at room temperature for 48 hours. The AMP-100 K-PLLA films were cut into strips (10 mm in length and width) and were dried at room temperature *in vacuo* for a further 3 days. The AMP-300 K-PLLA films were also prepared using PLLA (300K) and AMP with the same method as described above.

Fourier Transform Infrared Spectrophotometry

Transmission infrared spectra were obtained from AMP-PLLA films casted onto NaCl plates from a 1.0 w/v% sample in methylene chloride solution using a Mattson Alpha Centauri Fourier transform infrared (FTIR) spectrophotometer.

Differential Scanning Calorimetry

DSC thermograms were obtained using a DuPont, DSC 2910, differential scanning calorimeter fitted to a DuPont Instruments, Thermal Analyst 2100. Samples weighing between 2 to 6 mg, crimped in aluminum cells, were heated at a rate of 10℃ /min over a temperature range of 30 to 250℃ in an atmosphere of nitrogen. Glass transition temperatures (T_g) were taken from the midpoint of the transition.

Scanning Electron Microscopy

Scanning electron microscope was used to study the surface morphology of the AMP-PLLA films before and after dissolution. After being sputter-coated with gold using an Eiko IB3 ion coater, the films were examined in a Hitachi, model S-510, SEM.

Film Weight and Thickness Variation

Twelve samples (10 mm length×10 mm width) from each batch of films were weighed and the mean weights were calculated. Film thickness of each sample was measured using a micrometer gauge (Mitutoyo, no. 7301).

Determination of Drug Content in the AMP-PLLA Films

Triplicate samples of AMP-PLLA films were initially dissolved in 1 m*l* of methylene chloride in a screw-capped test tube and 2 m*l* of distilled, deionized water was added. The mixer was shaken for 30 min to extract the drug into the aqueous layer. After centrifugation at 3000 rpm for 20 min and standing for 24 hours, an aliquot of the aqueous layer was taken and reversed-phase HPLC method was used to calculate drug contents in the AMP-PLLA films.

***In Vitro* Release of AMP from AMP-PLLA Films**

A weighed amount of the films was introduced into a capped test tube with 1.0 m*l* of phosphate buffered saline (PBS, pH 7.4) and the test tube was placed in a waterbath whose temperature was maintained at 37℃. At predetermined time intervals up to 14 days, films were removed from the medium and placed into 1.0 m*l* of fresh PBS. These samples were kept in a freezer until HPLC analysis.

HPLC Analysis

The HPLC system consisted of an SP8880 auto-

sampler, an SP8810 precision isocratic pump, and a Spectra 100 variable wavelength detector set at 223 nm and absorbance attenuation of 0.01 aufs (all from Spectra-Physics, San Jose, CA). Data acquirement and peak integration were accomplished with an SP4290 integrator (Spectra-Physics, San Jose, CA). A Waters μ-Bondapak C_{18}, 3.9 mm I.D.×300 mm long, 10 μm particle size, stainless steel column with a Guard-Pak™ precolumn (Waters, Milford, MA) was used. The mobile phase was methanol:water:low UV PIC® A reagent (25:75:0.5, v/v%), at a flow rate of 1.50 ml/min. The autosampler was set at auto mode, 20 μl injection volume, and 20 min run time. Under these conditions, the retention time for AMP was about 9.70 min, and its standard curve was linear within the range of 3-100 μg/ml with $r^2>0.99$.

RESULTS AND DISCUSSION

Characterization of AMP-PLLA Films

Number-average, weight-average molecular weights and polydispersity determined by GPC were summarized in Table I. The polydispersity index of PLLA (100K) and PLLA (300K) were 1.61 and 1.65, respectiely, suggesting that the molecular weight distributions are comparatively narrow.

Table I—*Number average (M_n), weight-average (M_w) molecular weights and polydispersity index of poly(L-lactic acid)s, as determined by GPC*

Compound	$\bar{M}_w$	$\bar{M}_n$	polydispersity
PLLA(100K)	67,319	41,809	1.61
PLLA(300K)	227,938	138,386	1.65

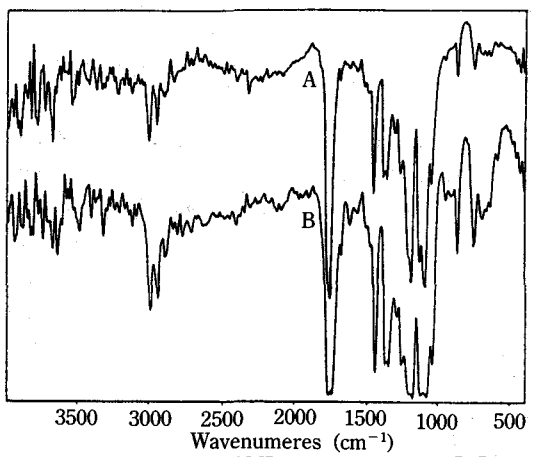


Figure 3—IR spectra of AMP-100 K-PLLA film [A] and AMP-300 K-PLLA [B] film.

Fig. 3 shows the transmission IR spectra of AMP-PLLA films measured by the NaCl cell method. The absorption peak characteristics of AMP-100K-PLLA and AMP-300K-PLLA films were observed at 1749 and 1759 cm^{-1}. The β-lactam ketone group of AMP shows strong absorption peak around 1750 cm^{-1}. The absorption peaks at 1070-1150 cm^{-1} are due to ester group of PLLA. It was found that AMP decomposition did not occur in the preparation of films.

A series of thermograms of the AMP, PLLA (100K), AMP-100K-PLLA, PLLA (300K) and AMP-300K-PLLA are presented in Fig. 4 and their thermal analysis data are listed in Table II. PLLA (100K) (Fig. 4B) and PLLA (300K) (Fig. 4D) show endothermic peak at 174.41℃ and 187.05℃, respectively, corresponding to their melting points. As shown in Fig. 4, differential scanning calorimetry confirmed the absence of any chemical interaction between AMP and PLLA (100K and 300K). AMP-100K-PLLA and AMP-300K-PLLA displayed the glass transition temperatures (T_g) at 59.92℃ and 59.51℃, respectively. The glass transition was observed as a discontinuity due to an increase in heat capacity of AMP-PLLA films on the ordinate of the DSC curve, as shown in Fig. 4. The T_g is evident as a small endothermic rise, represented by the midpoint of the rise measured from the extension of the pre- and post-transition base-

Table II—*Thermal properties of raw materials and prepared films*

Compound	M.P.	T_g
raw materials		
Poly(L-lactic acid) MW 100,000	174.41℃	
MW 300,000	187.05℃	
Ampicilin anhydrous	227.97℃	
prepared films		
AMP-100K-PLLA film	172.88℃	59.92℃
AMP-300K-PLLA film	178.24℃	59.51℃

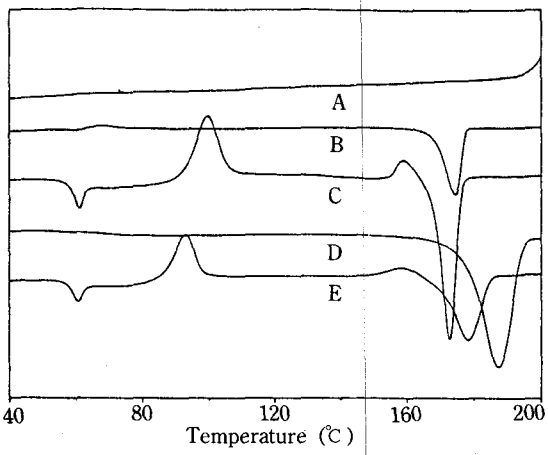


Figure 4—DSC thermograms for (A) AMP, (B) PLLA (100K), (C) AMP-100K-PLLA film, (D) PLLA (300K) and (E) AMP-300K-PLLA film.

Table III—*Average thickness and weight of AMP-100 K-PLLA and AMP-300K-PLLA films*

Compound	Thickness (mm)	Weight (mg)
AMP-100K-PLLA	0.02± 0.00*	1.05± 0.04
AMP-300K-PLLA	0.02± 0.00	1.38± 0.03

*: Values are Mean± SD (n=6).

lines, i.e., when the transition assumes half the value of this change. The area under the anomalous endothermic peak is determined by extrapolating the post-transition baseline. The thermograms of AMP-PLLA films demonstrated cool crystallinity zone around 90-100℃. On the other hand, it was found that the AMP-PLLA films have lower melting points than the raw material PLLA (100K) and PLLA (300K) by 2℃ and 9℃, respectively. This lower melting points seems to be due to the formation of small crystals by the action of the dispersed ampicilin as a crystal seed.

Table III summarizes the average thickness and weight of AMP-PLLA films. The thickness of the films was controlled constantly by using applicator. The mean weight of AMP-100K-PLLA and AMP-300K-PLLA films (10 mm length×10 mm width) were 1.05± 0.04 and 1.38± 0.03 mg, respectively.

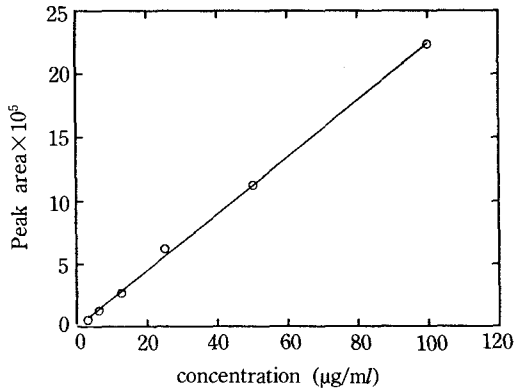


Figure 5—Calibration curve of ampicillin by HPLC assay (Y=22446X+3511.4, r=0.999).

Drug Content

Fig. 5 shows one of the calibration plots obtained from the standard solutions containing 3~100 μg of AMP. The linearity of calibration curve was determined to show that a directly proportional relationship exists between the peak area ratios and the concentrations of the AMP. Y=22446X+3511.4 (r^2>0.99). The retention time of AMP was 9.70 min.

The experimentally determined drug content of the AMP-PLLA films ranges from 98.70 to 98.90% of the theoretical drug content for all six drug loadings. Therefore, it was found that AMP was homogeneously dispersed in PLLA (100K and 300 K) films, which was confirmed by SEM (Table IV).

***In Vitro* Release**

Fig. 6 shows the *in vitro* release of AMP from AMP-PLLA films in PBS (pH 7.4) at 37℃. In the initial stage up to 5 hours, relatively constant

Table IV—*Measurement of drug content of AMP-PLLA (100 K & 300 K) films*

Compound	Theoretical drug loading (w/w%)	Experimental drug loading (w/w%)	Percent theoretical drug content (%)
AMP-100K-PLLA	10.0	9.89± 0.39*	98.90
AMP-300K-PLLA	10.0	9.87± 0.30	98.70

*: Values are Mean± SD (n=6).

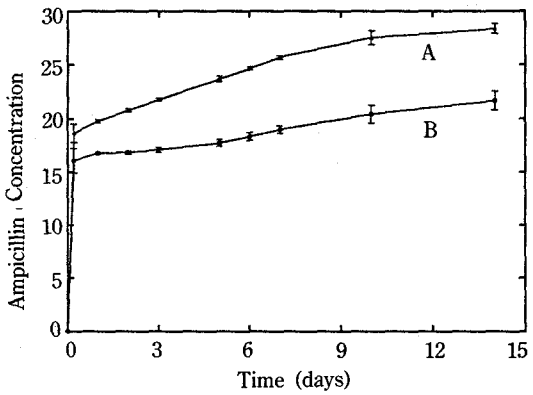
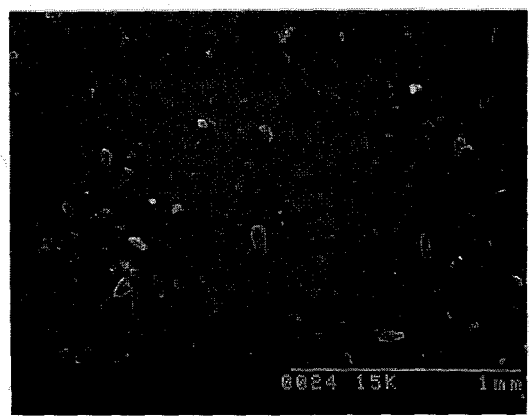
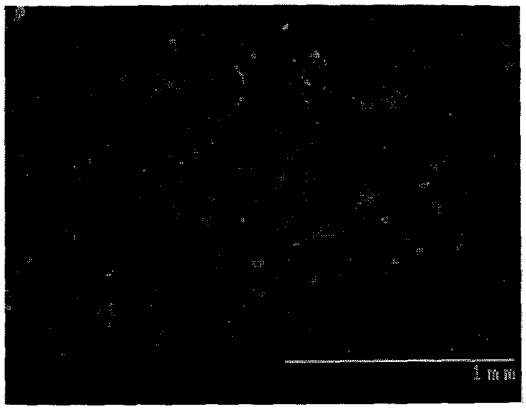


Figure 6—*In vitro* release profiles of AMP from AMP-PLLA films
A: AMP-100K-PLLA film, Release rate: Y=0.7384X+19.504 (r=0.970), B: AMP-300K-PLLA film, Release rate: Y=0.4107X+16.023 (r=0.993).

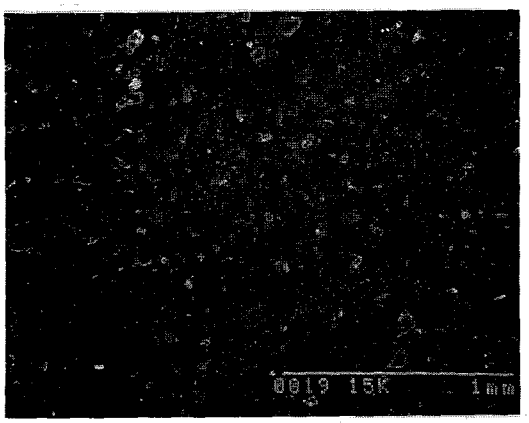


A

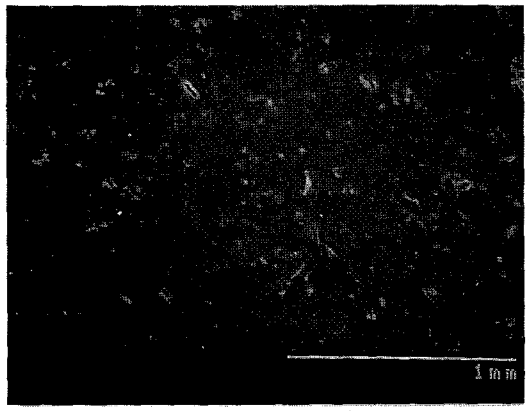


B

Figure 7—Scanning electron micrographs of AMP-100 K-PLLA film (×50).
A: before *in vitro* release test, B: after *in vitro* release test.



A



B

Figure 8—Scanning electron micrographs of AMP-300 K-PLLA film (×50).
C: before *in vitro* release test, D: after *in vitro* release test.

burst release of 3.74 μg/hr/cm^2 and 3.22 μg/hr/cm^2 were observed for AMP-100K-PLLA and AMP-300K-PLLA, respectively, due to rapid dissolution of AMP from the surface of the films. The constant percentage of drug released during the burst phase, which was independent of molecular weight of PLLA, indicates uniform dispersion of drug in the films containing varying molecular weight and the constant surface area of the films.

The rate and extent of drug release from the AMP-100K-PLLA films were compared to AMP-300K-PLLA at pH 7.4. The release rate of AMP-100K-PLLA and AMP-300K-PLLA after 5 hours were 0.74 and 0.41 μg/ml/day, respectively. This might be caused by a difference in viscosity (mol.

wt.) of PLLA.

It was found that the AMP-PLLA films have not inflection point in release curve during 14 days, suggesting that drug releases from AMP-PLLA films via mainly simple diffusion of the freely water soluble drug.

Since the thickness of the polymer envelope, the size, surface characteristics and shape of the films can influence the release rate of the active ingredient,[30] it was important to determine the kinetic order of this rate.

Scanning Electron Microscopy

The morphological and surface characteristics of AMP-100K-PLLA and AMP-300K-PLLA films were examined by SEM. The electron micrographs were presented in Figures 7 and 8. SEM views indicate that the films have a smooth surface prior to drug release (Fig. 7A and 8A), while after the release, irregular pores were evident (Fig. 7B and 8B). The formation of pores in the AMP-PLLA films suggests that the release of AMP from these AMP-PLLA films was by dissolution of the drug and by subsequent diffusion through the pores.

CONCLUSIONS

Biodegradable films for localized release of AMP into the middle ear cavity were prepared using the PLLA (100K and 300K). The release rates from AMP-100K-PLLA and AMP-300K-PLLA films are 0.74 μg/ml/day and 0.41 μg/ml/day, respectively. The maintenance of therapeutic levels for long periods of time indicates that our formulations are effective for the treatment of OM. *In vivo* release experiments using mice and rats are currently under investigation.

ACKNOWLEDGEMENT

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ABBREVIATIONS

PLLA: Poly(L-lactic acid); PLLA (100K): poly(L-lactic acid) (MW 100,000); PLLA (300K): poly(L-lactic acid) (MW 300,000); AMP: ampicillin anhydrous; AMP-100K-PLLA: poly(L-lactic acid) (MW 100,000) film loaded with 10 w/w% of ampicillin; AMP-300K-PLLA: poly(L-lactic acid) (MW 300,000) film loaded with 10 w/w% of ampicillin; AMP-PLLA: AMP-100K-PLLA and AMP-300K-PLLA.

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