Applied Surface Science 278 (2013) 211–213

Contents lists available at SciVerse ScienceDirect

Applied Surface Science

journal homepage: www.elsevier.com/locate/apsusc

Antimicrobial activity of biopolymer–antibiotic thin films fabricated by advanced pulsed laser methods

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ARTICLE INFO

Article history:
Received 2 July 2012
Accepted 9 January 2013
Available online 18 January 2013

Keywords:
Poly(D,L-lactide)
Gentamicin
Matrix assisted pulsed laser evaporation
Drug delivery
Antimicrobial activity

ABSTRACT

We report on thin film deposition by matrix assisted pulsed laser evaporation (MAPLE) of two polymer–drug composite thin film systems. A pulsed KrF* excimer laser source ($\lambda = 248$ nm, $\tau = 25$ ns, $\nu = 10$ Hz) was used to deposit composite thin films of poly(D,L-lactide) (PDLLA) containing several gentamicin concentrations. FTIR spectroscopy was used to demonstrate that MAPLE-transferred materials exhibited chemical structures similar to those of drop cast materials. Scanning electron microscopy data indicated that MAPLE may be used to fabricate thin films of good morphological quality. The activity of PDLLA–gentamicin composite thin films against *Staphylococcus aureus* bacteria was demonstrated using drop testing. The influence of drug concentration on microbial viability was also assessed. Our studies indicate that polymer–drug composite thin films prepared by MAPLE may be used to impart antimicrobial activity to implants, medical devices, and other contact surfaces.

1. Introduction

The region in proximity to an implant is prone to infection because the immune system is locally compromised. Therefore, a major focus of biomedical engineering research is maximizing the efficacy of antimicrobial agents to common microorganisms [1,2]. For example, efforts are underway to enhance the effectiveness of antibacterial agents against pathogenic bacteria [3]. Efforts are also underway to use nanoscale materials (e.g., thin films) in order to impart biologically-relevant functionalities to medical implants [4]. For example, applying antimicrobial thin films to medical implants is a more effective method to prevent implant infection than systemic antibiotic delivery. Antimicrobial thin films may prevent bacteria from entering the biofilm state, which is more resistant to antimicrobial agents, and causing either local or systemic infections [5–8].

We have examined deposition of thin films containing biodegradable polymers that serve as carriers for antimicrobial agents by means of matrix assisted pulsed laser evaporation (MAPLE). MAPLE is a laser technique that is based on a cryogenic approach for transferring organic and polymeric materials onto a substrate in a "protected" manner [9,10]. In previous work, deposition of poly(D,L-lactide) (PDLLA) thin films, as well as poly(1,3-bis-(p-carboxyphenoxy propane)-co-(sebacic anhydride)) 20:80 gentamicin sulfate multilayered thin structures using MAPLE was reported [11,12]. In this work, thin films containing PDLLA and several gentamicin concentrations were prepared using MAPLE. Fourier transform infrared (FTIR) spectroscopy was used to compare the chemical structure of MAPLE-transferred materials with that of drop cast materials. Scanning electron microscopy (SEM) was used to examine the morphology of the MAPLE-deposited thin films. The antibacterial activity of PDLLA–gentamicin films against *Staphylococcus aureus* was demonstrated using drop testing. These studies indicate that MAPLE is a promising approach for deposition of biodegradable polyesters that contain antimicrobial agents.

2. Experimental procedure

2.1. Materials

Commercially obtained PDLLA (Sigma–Aldrich, Inc., St. Louis, MO, USA) was used as the polymer matrix in the PDLLA–gentamicin composite films. A gentamicin solution with a concentration of 10 mg/ml was obtained from a commercial source (MP Biomedical, Solon, Ohio, USA). Ethyl acetate was used to solvate the commercially obtained PDLLA material. Solutions consisting of PDLLA,

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http://dx.doi.org/10.1016/j.apsusc.2013.01.062

gentamicin, and ethyl acetate were prepared with two gentamicin-ethyl acetate ratios: 1:100 (PDLLA–gentamicin 1%), henceforth referred to as RC4, and 1:25 (PDLLA–gentamicin 4%), henceforth referred to as RC5; it should be noted that both solutions contained 2 wt% PDLLA. In order to obtain solid targets for MAPLE processing, the solutions were poured into a pre-cooled copper target holder at 173 K and were subsequently immersed in liquid nitrogen for 30 min.

2.2. Experimental conditions

MAPLE was used to deposit PDLLA–gentamicin composite thin films. The laser was operated at a repetition rate of 10 Hz and a laser fluence of 500 mJ/cm^2 for 30,000 laser pulses; a laser spot area of 8 mm^2 was used. The target was rotated at a rate of 0.4 Hz during film deposition to avoid heating and damage by the pulsed laser beam. During deposition, the laser beam scanned the entire target surface at an angle of 45° with respect to the target normal. All of the depositions were conducted at room temperature; the residual pressure was ~7.5 Pa. The thin films were grown at a target–substrate separation distance of 4 cm. The target temperature was maintained at a temperature of ~173 K by active liquid nitrogen cooling. PDLLA–gentamicin 1% and PDLLA–gentamicin 4% thin films were deposited onto both side polished Si ⟨1 0 0⟩ substrates for FTIR and SEM analysis. PDLLA–gentamicin 1% and PDLLA–gentamicin 4% thin films were deposited onto quartz substrates for drop testing. Prior to placement within the deposition chamber, the substrates were successively cleaned into an ultrasonic bath with acetone, ethanol and deionized water for 15 min; the substrates were subsequently dried in a jet of high purity nitrogen under UV exposure from a VL-115UV lamp (Vilber Lourmat, Marne-la-Vallee Cedex, France). In order to provide comparison data, polymer–drug composite thin films were prepared by drop casting on Si ⟨1 0 0⟩ substrates.

2.3. Characterization methods

FTIR spectra were recorded with a FTIR-8400S instrument (Shimadzu Corp. Kyoto, Japan), which was operated over 7800–350 cm^{-1} using 8 cm^{-1} resolution. Film microstructure was evaluated using a Evo 50 XVP scanning electron microscope (Zeiss, Oberkochen, Germany). The MAPLE-deposited thin films were sputtered with a thin layer of gold prior to imaging. The activity of the PDLLA–gentamicin composite thin films against *S. aureus* bacteria was demonstrated using drop testing. Two experimental sample sets on quartz substrates were examined, PDLLA–gentamicin 1% (RC4) thin films and PDLLA–gentamicin 4% (RC5) thin films.

The antibacterial activity of the MAPLE-deposited PDLLA–gentamicin composite thin films was assessed using the drop test [13–15]. Samples of unmodified glass with the same dimensions as MAPLE-modified quartz were used as a negative control. *S. aureus* ATCC 29213 (American Type Culture Collection, Manassas, VA, USA) was cultured on trypticase soy agar at 37 °C for 24 h. UV exposure in the biosafety cabinet for 15 min was used to sterilize the MAPLE-modified samples and the unmodified samples. *S. aureus* from the culture plate was added to a 10 ml sterile saline solution; a cell density of 10^8 CFU/ml was obtained using this approach. Cell density was determined by visual comparison using a 0.5 McFarland Standard. The cell suspension was then diluted with sterile saline to a concentration of 10^6 CFU/ml.

The samples were placed into sterile Petri dishes. 100 μl of the 10^6 CFU/ml cell suspension was pipetted onto the surface of each sample; all of the suspension remained on the surface of the sample. The samples were evaluated at time intervals of 0, 1, 2, and 4 h. At the desired time interval, 5 ml of sterile 1× phosphate buffered saline (PBS) was applied to the sample. 10 μl of the suspension in the Petri dish was applied to brain heart infusion agar (BHI) and spread using glass beads. The plates were then inverted and incubated for 24 h at 37 °C. After 24 h, the number of colonies on the plates was counted and the cell density in the suspensions on the samples was determined.

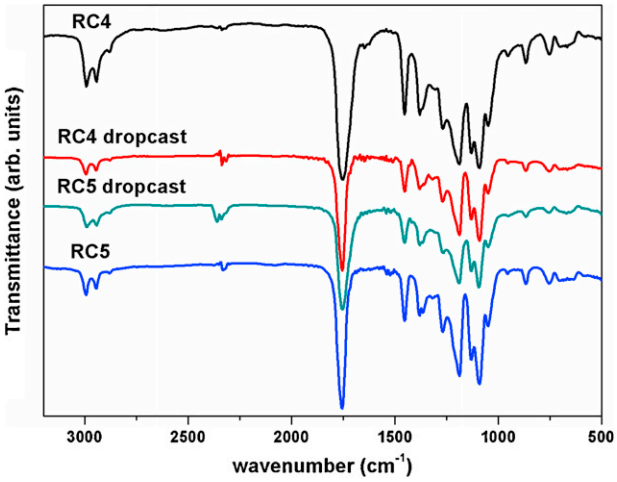

Fig. 1. Typical FTIR spectra of PDLLA–gentamicin 1% drop cast material (red curve), PDLLA-gentamicin 4% drop cast material (blue curve), PDLLA–gentamicin 1% thin film obtained by MAPLE at 500 mJ/cm^2 laser fluence (black curve), and PDLLA–gentamicin 4% thin film obtained by MAPLE at 500 mJ/cm^2 laser fluence (green curve). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

3. Results and discussion

Fig. 1 contains typical Fourier transform infrared spectra for drop cast PDLLA–gentamicin 1%, shown in the figure as RC4 drop cast (red curve), and PDLLA–gentamicin 4%, shown in the figure as RC5 drop cast (blue curve). Fig. 1 also contains typical Fourier transform infrared spectra for the PDLLA–gentamicin 1% thin film, RC4 (black curve), and the PDLLA–gentamicin 4% thin film, RC5 (green curve), which were prepared using MAPLE at a fluence of 500 mJ/cm^2. All of the functional absorption peaks found in the FTIR spectra of the MAPLE-deposited PDLLA–gentamicin composite thin films, particularly in the regions centered at 3000–2750 cm^{-1}, 2361–2342 cm^{-1}, 1754 cm^{-1}, 1269 cm^{-1}, and 1051 cm^{-1}, showed very close similarities to those of the corresponding drop cast materials [11]. FTIR analysis revealed that MAPLE is suitable technique for transfer of PDLLA–gentamicin composite materials since the chemical structures of the MAPLE-transferred materials and the drop cast materials were similar. Scanning electron micrographs (Fig. 2) show that the PDLLA–gentamicin 1% thin film (RC4) had a more uniform surface topography than the PDLLA–gentamicin 4% thin film (RC5). In particular, a larger density of particulates with sizes in the sub-micrometer and micrometer range was noted on the surface of the PDLLA–gentamicin 4% thin film.

Fig. 3

The cell density of the 100 μl suspension was calculated from the number of colonies on the agar plates; this data is provided in Table 1. The number of surviving cells was then normalized to

Table 1
Cell density in suspension on sample surface.

Time	Glass	RC4	RC5
0 h	184	N/A	N/A
1 h	134	371	461
2 h	147	328	396
4 h	21	53	1

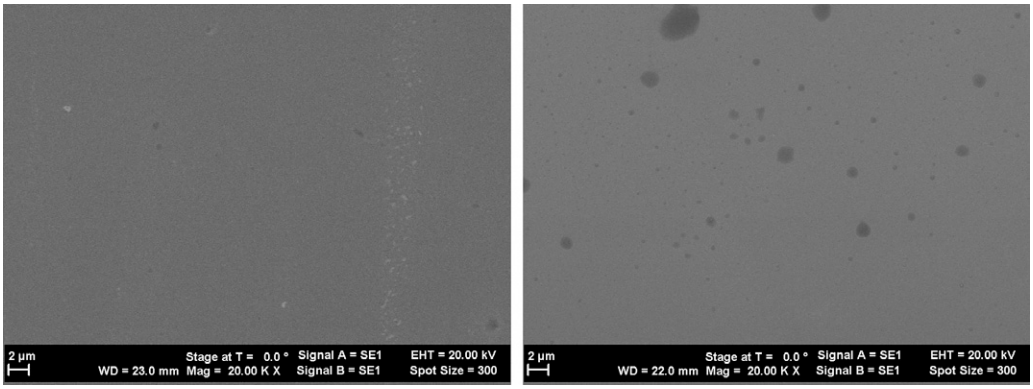

Fig. 2. Scanning electron micrographs of PDLLA–gentamicin 1% (left) and PDLLA–gentamicin 4% (right) thin films obtained by MAPLE at 500 mJ/cm^2 laser fluence.

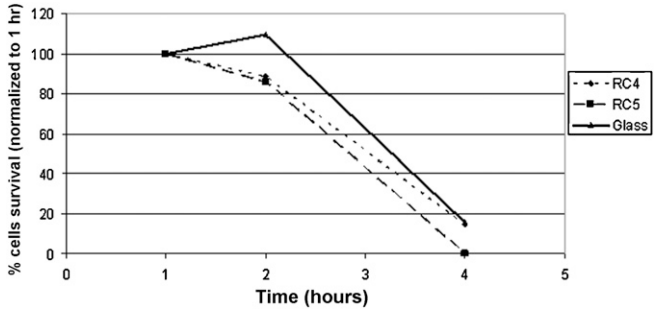

Fig. 3. Drop test data.

Table 2
Cell survival in suspension on sample surface.

Time (h)	Glass	RC4	RC5
1	100	100	100
2	109.7	88.4	85.9
4	15.7	14.3	0.2

the number of surviving cells at 1 h; this value was noted as the percentage of surviving cells. Data on the percentage of surviving cells was provided in Table 2.

The suspension cell densities for all of the samples were generally lower at longer time points. This decrease was attributed to dehydration of the bacteria as water evaporated from the surface. A negative control is used to facilitate determining the antibacterial activity of the PDLLA–gentamicin composite thin films. The results indicate that the two test materials, the PDLLA–gentamicin 1% thin film (RC4) and the PDLLA–gentamicin 4% thin film (RC5), had lower *S. aureus* survival than the glass control. The film with the higher gentamicin concentration was noted to possess greater antibacterial activity; in particular, the PDLLA–gentamicin 4% thin film (RC5) exhibited stronger antibacterial activity than PDLLA–gentamicin 1% thin film (RC4). The difference between the PDLLA–gentamicin 1% thin film (RC4) and the negative control was 19.4% at 2 h and 8.8% at 4 h; the difference between the PDLLA–gentamicin 4% thin film (RC5) and the negative control was 21.7% at 2 h and 98.6% at 4 h.

4. Conclusions

This study has demonstrated that MAPLE may be used to successfully grow PDLLA–gentamicin composite thin films that exhibit antimicrobial properties. FTIR confirmed that the MAPLE deposition process produced PDLLA–gentamicin composite thin films with chemical structures similar to those of drop cast thin films. We demonstrated that thin films containing the antibacterial agent gentamicin effectively inhibited growth of *S. aureus*. We anticipate that MAPLE may have potential application in the medical device industry, including use as a medical implant coating that imparts resistance to microbial growth and biofilm formation.

Acknowledgments

This work was supported by the Romanian National Authority for Scientific Research (CNCS-UEFISCDI) through Project Number PN-II-ID-PCE-2011-3-0888 (209/5.10.2011). Gabriel Socol gratefully acknowledges support from the European Social Fund POSDRU 2007–2013 through Contract POSDRU/89/1.5/S/60746.

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