Degradation of Poly(L-Lactide) Films Under Ultraviolet-Induced Photografting and Sterilization Conditions

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ABSTRACT: The degradation of polymers under ultraviolet (UV) irradiation has been a great concern for biomaterial and agricultural applications. The major objective of this research was to study the effect of UV irradiation on the representative bulk and surface properties of poly (L-lactide) (PLA) films. Two UV sources with different spectral outputs and intensities were chosen so that one of them could be used for surface modification and the other could be used for UV sterilization of the PLA films. The results established that the molecular weight of PLA decreased significantly during irradiation from the photografting lamp under atmospheric conditions. Irradiation through a Pyrex container was shown to minimize polymer degradation during UV exposure from the photografting lamp. The PLA films UV-irradiated under the sterilization lamp for 12 h revealed a similar reduction in the molecular weight and no change in the surface hydrophilicity. However, significantly less photodegradation was observed under the sterilization lamp when the samples were held in a Pyrex container. J Appl Polym Sci 106: 1042–1047, 2007

Key words: degradation; photochemistry; radiation

INTRODUCTION

Poly(L-lactide) (PLA) is being widely investigated as a scaffold material to provide two-dimensional and three-dimensional matrices for cell adhesion and subsequent tissue development.[1,2] The unmodified hydrophobic PLA surface generally has low cell affinity.[3] The surface modification of PLA has the potential to expand the market for PLA into various biological and other application areas in which enhanced wettability, cell selectivity, or receptor immobilization is required. Researchers have used a variety of experimental techniques to introduce a desired chemical functionality into PLA.[4–12] Photoinduced grafting and photopolymerization have been shown to be convenient ways of achieving the surface functionalization of polymer films.[8–12] These photografting studies used higher grafting temperatures (>40°C) and, more importantly, used high-power ultraviolet (UV) lamps (800–2000 W). Additionally, these studies used a reaction chamber equipped with a quartz window, which allowed lower UV wavelengths (wavelength < 300 nm) to reach the samples. These conditions are not well suited for PLA surface grafting because of the greater extent of PLA degradation at higher temperatures and under high-power UV irradiation, particularly at lower wavelengths. Therefore, we conducted grafting studies under relatively mild conditions, using a low-power UV lamp (100 W) at room temperature and a Pyrex test tube in the photopolymerization step.[13] The Pyrex test tube was expected to prevent a significant fraction of wavelengths below 300 nm from reaching the samples.[14]

Biomaterial sterility is an important concern for tissue engineering applications. Several sterilization methods for PLA-based materials have been described elsewhere.[15–17] These methods use high-pressure steam (120–135°C), dry heat (160–190°C), ethylene oxide gas, and irradiation (UV, γ-ray, or ionizing). UV irradiation has been used extensively as a method for the sterilization of PLA-based materials because of its simplicity and low cost of operation[18] and has been shown to be effective in inhibiting the germination of various fungi.[19–22] However, Ho and Pometto[23] showed that the degradation of PLA plastic films was enhanced by a factor of 55–97% by UV irradiation over a period of 8 weeks.[23] Copinet et al.[24] further studied the effect of simultaneous UV irradiation with relative humidity and temperature changes. Their study revealed that although the extent of PLA degradation was primarily governed by changes in the relative humidity and temperature, the degradation rate was enhanced

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by UV irradiation (wavelength ~ 315 nm) over a period of 30 days. Fischbach et al.[18] studied the effects of UV irradiation for shorter exposure times (up to 24 h; the light intensity was not reported) on the properties of PLA-based materials that are relevant to tissue engineering. However, the study was focused on diblock copolymer films of poly(ethylene glycol) and PLA (thickness ~ 10–30 μm) with a number-average molecular weight (M_n) of the PLA block of about 10,000 Da. Here we present results for molecular weight changes as a function of the UV sterilization time for relatively thick (~ 125 μm) unmodified PLA homopolymer films (M_n ~ 110,000 Da).

In this area of PLA modification and its potential applications, there are two issues of concern. One is the degradation during the surface-modification process in which UV irradiation is used. The second is the possible molecular weight degradation during subsequent UV sterilization of PLA. Although we did not characterize the sterilization of PLA films, the issues of polymer degradation under UV irradiation in both surface modification and sterilization processes are addressed in this communication.

EXPERIMENTAL

Materials

PLA pellets were supplied by Cargill Dow LLC (Minnetonka, MN). Chloroform (99.9% w/w), concentrated H_2SO_4, and H_2O_2 (30% w/w) were obtained from Fisher Scientific (Pittsburgh, PA). Methylene chloride (CH_2Cl_2; 99% w/w) was purchased from Aldrich Chemicals (St. Louis, MO). All chemicals were used as received.

Experimental procedures

Film preparation

The PLA films were solvent-cast into a 3.5-in.-diameter Petri dish cleaned by exposure to a piranha solution, concentrated H_2SO_4 and 30% H_2O_2 (70 : 30 v/v). PLA pellets (1.1 g) were dissolved in 90 mL of CH_2Cl_2, the solution was poured into the Petri dish, and the solvent was allowed to evaporate at a very slow rate to achieve a nominal PLA film thickness of about 125 μm.

UV treatment

Two UV sources with different spectral outputs and intensities were chosen so that one of them could be used for surface modification (photografting lamp) and the other could be used for UV sterilization of the PLA films (sterilization lamp). A UV processor (model 60000, Oriel Corp.) equipped with a 100-W mercury arc lamp (model 8261, Oriel) with a wavelength range of 232–500 nm was used as the photografting lamp. PLA film specimens (0.5 cm × 3 cm) were irradiated with the photografting lamp at an intensity of ~ 25 mW/cm^2 at 365 nm, which was measured at the substrate surface by the placement of an OAI 306 UV power meter in the plane of the substrate surface. Alternatively, the PLA films were placed under a Philips TUVG30T8 30-W mercury vapor UVC lamp (henceforth called the sterilization lamp) integrated into a laminar air-flow hood (Labconco Corp., Kansas City, MO), with a distance of 60 cm between the films and lamp.

Experiments

In the first experiment, a PLA film was exposed to the photografting lamp, and M_n of the film was measured after various exposure times. In the second experiment, a PLA film was exposed to the sterilization lamp, and M_n of the film was measured after various exposure times. Then, the first and second experiments were performed with a PLA film enclosed in a Pyrex test tube.

Analytical techniques

Gel permeation chromatography (GPC) was used to measure the molecular weights of the PLA films after the UV treatments. A Waters Associates model 2695 separation module equipped with two consecutive Polymer Labs PLgel 5-μm Mixed-D and Mixed-E columns and a Waters Associates 2410 refractive-index detector set at 35°C was used. Samples were dissolved in chloroform to achieve a nominal concentration of 2 mg/mL. Chloroform was used as an eluent at a flow rate of 1 mL/min. Polystyrene standards of narrow molecular weight distribution (Polysciences, Inc.; weight-average molecular weight ~ 580–377,400 Da) were used for the calibration.

Contact-angle measurements were performed on a Kruss G10 static contact-angle apparatus. The water contact angles were calculated with the sessile drop method and reported as averages of 10 readings with ±95% confidence intervals.

Statistical analysis

A statistical evaluation of the GPC data was performed with an analysis of variance (ANOVA) and multiple-comparison least-square difference (LSD) procedures. All results are reported as means plus or minus the 95% confidence intervals.

RESULTS AND DISCUSSION

Several researchers have reported degradation studies of PLA-based materials under prolonged UV irradiation of 4–8 weeks.[23,24] The major objective of our

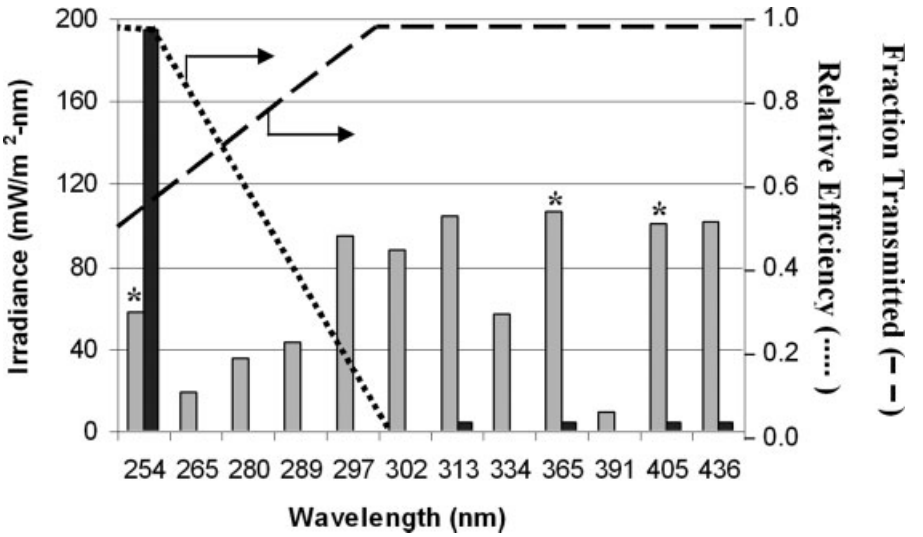

Figure 1 Spectral output of the UV sources between 200 and 450 nm. An Oriel lamp was used for photografting (gray bars), and a Labconco UV system was used for sterilization (black bars). The dotted line represents a typical variation of the relative sterilization efficiency with the wavelength.[19] The dashed line represents the fraction of the incident UV irradiation transmitted by the Pyrex test tube.[14] The excitation wavelengths for benzophenone, a commonly used photoinitiator, when irradiated with UV are shown by asterisks.[9]

study was to investigate the effect of a shorter period of UV exposure on the bulk and surface properties of PLA films. The molecular weight was chosen as the bulk property of interest because it directly affects the mechanical properties of the polymer.[25] The water contact angle, a measure of surface hydrophilicity, was selected as a representative surface property because it influences the suitability of a biomaterial for tissue engineering applications.[26] Two types of UV sources were considered in this study, namely, the photografting lamp and the sterilization lamp. The PLA films were exposed to the UV irradiation either directly under atmospheric conditions or while enclosed in a Pyrex test tube. The latter was used to investigate the effect of lower wavelength UV irradiation (wavelength $<$ 300 nm) on the polymer film properties.

Figure 1 shows the spectral output between 250 and 450 nm for the UV sources used. The sterilization lamp showed that more than 95% of the UV irradiation was present in a single peak at 254 nm, whereas the photografting lamp showed output at several other wavelengths (the data were taken from the manufacturers, Oriel and Philips). The sterilization efficacy depends on the wavelength(s) and corresponding intensities of UV irradiation used for a particular species (e.g., a specific strain of bacteria) to be sterilized. Studies reported in the literature reveal that sterilization effects are produced by wavelengths below 320 nm, with the optimum effect occurring around 254 nm.[19,21,22] As shown in Figure 1, the sterilization efficiency decreases significantly for wavelengths greater than 254 nm to a value of about 2% at 300 nm.[19] The Pyrex prevented a significant fraction of wavelengths below 300 nm from reaching the samples,[14] and benzophenone, a commonly used photoinitiator, initiated the photografting when irradiated with wavelengths of 250, 365, and 400 nm (shown by asterisks in Fig. 1).[9] Therefore, on the basis of this background information, it follows that if the film samples were enclosed in a Pyrex container, UV irradiation from the photografting lamp could

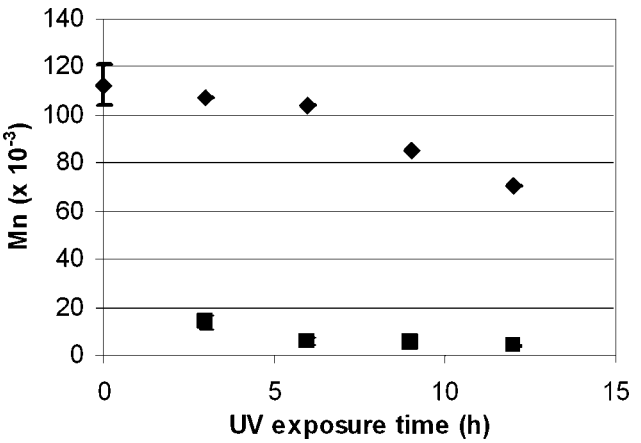

Figure 2 M_n of PLA as a function of the exposure time to the UV lamp used for photografting (■) for direct exposure and (◆) with the specimens in a Pyrex test tube. The error bar represents a 95% confidence interval. M_n of PLA irradiated with the UV lamp used for photografting for direct exposure showed a statistically significant difference from M_n of the respective PLA films irradiated in the Pyrex test tube, as determined by ANOVA and LSD.

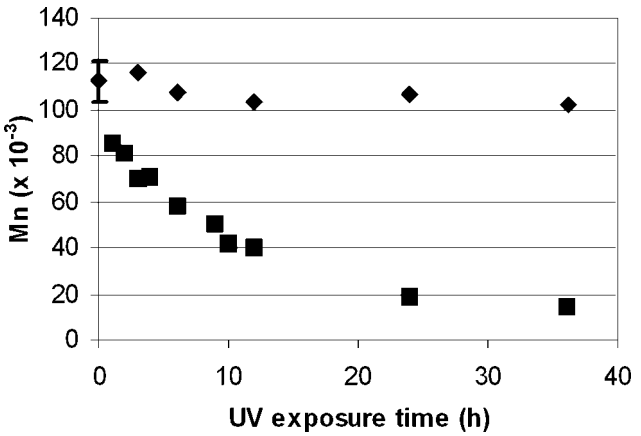

Figure 3 M_n of PLA as a function of the exposure time to the sterilization lamp (■) for direct exposure and (◆) with the specimens in a Pyrex test tube. The error bar represents a representative 95% confidence interval. M_n of PLA irradiated with the sterilization lamp for direct exposure showed a statistically significant difference from M_n of the respective PLA films irradiated in the Pyrex test tube, as determined by ANOVA and LSD.

promote photografting but with a relatively low sterilization efficiency, and the UV irradiation from the sterilization lamp could promote only sterilization and not photografting.

Effects of UV irradiation on the PLA films

First, a PLA film was irradiated with the photografting lamp for various exposure times. Figure 2 shows that the PLA molecular weight decreased significantly within a UV exposure time of 12 h. M_n of the untreated PLA film was 110,000 Da. M_n of the PLA film decreased to about 4000 Da after UV exposure for 12 h. The PLA film after the UV exposure was noticeably fragile and therefore was not UV-irradiated for longer than 12 h. The PLA film enclosed in a Pyrex test tube and irradiated with UV (see the diamonds in Fig. 2) did not show a significant reduction in the molecular weight up to a UV exposure time of 6 h. The PLA molecular weight decreased to about 70,000 Da after 12 h of UV irradiation. These experiments demonstrated that the PLA molecular weight was less affected when the irradiated films were enclosed in a Pyrex container.

Figure 3 shows GPC results for the PLA film exposed to the sterilization lamp. When the films were exposed directly under atmospheric conditions, the molecular weight decreased significantly up to a UV sterilization time of 12 h (shown by squares in Fig. 3). Specifically, M_n of the PLA film after UV exposure for 12 h was about 40,000 Da. The degradation rate decreased further for longer sterilization times, resulting in an M_n value of about 20,000 Da after 36 h of UV exposure. The films were stiff but not fragile as in the previous case. The PLA film enclosed in a Pyrex test tube and irradiated with UV for a similar time did not show a significant decrease in the molecular weight (shown by diamonds in Fig. 3). These experiments corroborated the earlier results that the PLA molecular weight was less affected when the irradiated films were enclosed in a Pyrex container.

Contact-angle measurements were performed on PLA films before and after exposure to the photografting lamp and sterilization lamp. The untreated PLA film was hydrophobic with a water contact angle of 82 ± 1°. This water contact angle changed only slightly even after 12 h of UV exposure. These results suggested that both hydrophilic and hydrophobic groups were created almost equally because of the photodegradation (the degradation mechanisms are discussed later), and the hydrophobic character of the PLA backbone was still predominant in the degradation products.

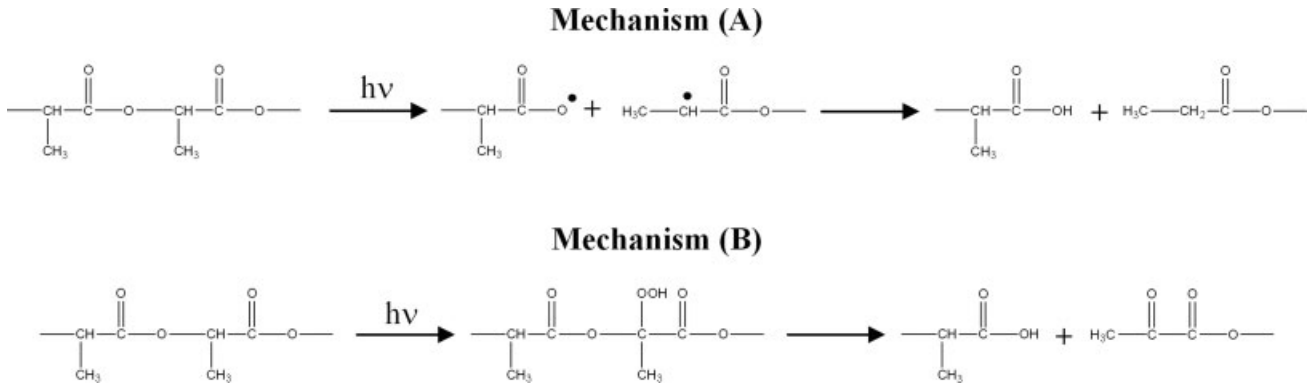

Figure 4 Basic mechanisms proposed to predict the degradation products of PLA. Mechanism A involves a photolysis reaction leading to breakage of the backbone C—O bond. Mechanism B involves photooxidation of PLA leading to the formation of a hydroperoxide derivative and its subsequent degradation to compounds containing a carboxylic acid and diketone end groups.

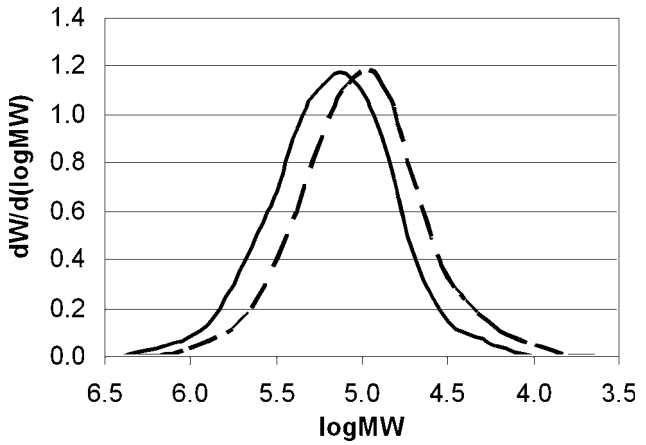

Figure 5 Representative molecular weight distribution curves for (—) neat PLA and (- - -) PLA films exposed to the sterilization lamp for 12 h. A similar unimodal molecular weight distribution was observed for all UV exposure times.

Mechanisms of PLA degradation under UV irradiation

Several mechanisms have been proposed in the literature for the degradation of polyester compounds due to UV irradiation. Scheirs and Gardette[27,28] examined the effect of UV irradiation on poly(ethylene terephthalate), poly(butylene naphthalate), and poly(ethylene naphthalate). Sakai et al.[29] studied the UV degradation of PLA in the presence of a photosensitizer. Figure 4 shows two basic mechanisms proposed to predict the degradation products of PLA. Mechanism A involves a photolysis reaction leading to breakage of the backbone C—O bond. Alternatively, mechanism B involves photooxidation of PLA leading to the formation of a hydroperoxide derivative and its subsequent degradation to compounds containing a carboxylic acid and diketone end groups. Furthermore, the photolysis of the diketone may lead to the homolytic cleavage of the C—C bond between the two carbonyl groups, resulting in two carbonyl radicals. This radical pair can undergo cage escape to form several photodecomposed products.[30]

Both mechanisms are in agreement with the acquired GPC and contact-angle results. The GPC results showed a reduction in the PLA molecular weight but a unimodal molecular weight distribution for all UV exposure times (see Fig. 5). More specifically, the UV degradation of PLA is a random process (i.e., each bond of a particular type is equally likely to break), and the degradation products are likely to have similar molecular weights as they have the same backbone structure with different end groups. On the basis of the molecular weight reduction observed after the UV irradiation (see Figs. 2 and 3), one can expect the generation of a number of end groups in comparison with neat PLA. However, the mechanisms indicate that both hydrophilic and hydrophobic end groups were created after the degradation of the PLA chains and probably suggest why the water contact angle did not change after UV irradiation under both the photografting lamp and the sterilization lamp.

CONCLUSIONS

A significant reduction in the PLA molecular weight was observed when the films were directly exposed to UV irradiation under atmospheric conditions. The molecular weight was less affected when the irradiated films were enclosed in a Pyrex container, presumably because the Pyrex blocked a significant fraction of the lower wavelength UV irradiation (wavelength < 300 nm). However, the representative surface property, namely, the water contact angle, did not change significantly during the 12-h exposure to either lamp. Two mechanisms for PLA degradation under UV irradiation have been proposed. Further studies are needed to ascertain the predominant mechanism, if any, for PLA degradation observed under the conditions of these experiments.

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