In Vitro Degradation of Poly(lactic-*co*-glycolic) Acid Random Copolymers

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Summary: We have investigated the *in vitro* degradation of poly(lactic-*co*-glycolic) acid copolymer with a lactic to glycolic ratio of 65/35. The degradation studies were performed on solvent-cast films of controlled thickness and shape. The samples were then incubated at 37 °C in phosphate buffered saline solution. The degradation was followed using potentiometry, light microscopy, gravimetry, gel permeation chromatography and differential scanning calorimetry. Water was found to diffuse inside the film as soon as the sample was placed in the degradation media. Wrinkles formed on the upper layer while degradation took place via chain scission in the bulk of the film. After 10 days, this led to the creation of a vesicle where liquid low molecular weight oligomers were trapped inside a thin film of high molecular weight polymer. This thin film acted as a membrane allowing only low molecular weight compounds to diffuse out of the film.

Keywords: biodegradable; biomaterials; block copolymers

Introduction

Biodegradable polymers have become important biomedical materials. In particular linear aliphatic polyester polymers have been found to be very useful in biomedical applications due to their easy processability, good mechanical strength and excellent degradation properties due to their hydrolysable backbones. Polyglycolic acid (PGA), polylactic acid (PLA) and their random block copolymers poly (lactic-*co*-glycolic) acid (PLGA) are good examples of polyesters with good properties for biomedical applications and are currently used in degradable and absorbable sutures, implants, artificial skin graft and drug release systems. The main advantage of such synthetic biocompatible polymers is that degradation is due to simple hydrolysis of the ester backbone[1] in aqueous solution and that the degradation products can ultimately be metabolized or eliminated by the body.[2]

One of the key parameters that needs to be controlled is the dynamics of degradation, which needs to be tailored for the intended application. Several factors are known to influence the rate of degradation of PLGAs including chemical architecture (e.g.: molecular weight, length of lactic and glycolic blocks, ratio of lactic and glycolic acid), structure and morphology (e.g.: crystallinity, shape of the specimen) and therefore the way the materials are processed and the environment in which the polymer is placed (e.g.: body fluid, digestive fluid). The exact relationship between these parameters and the degradation of PLGA is still poorly understood and is the focus of this research work.

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Experiment Parts

Materials and Film Preparation

The PLGA random copolymer with a lactic to glycolic molar ratio of 65/35 was supplied

by AstraZeneca, Macclesfield, UK. The polymer was amorphous with a glass transition at $T_g = 38.3 \pm 0.5\,^{\circ}C$. The PLGA number-average molecular weight was $M_n = 4700 \pm 500$ g mol^{-1} and the weight-average molecular weight was $M_w = 8500 \pm 500$ g mol^{-1}. All the other chemicals were purchased from Sigma-Aldrich and used as received. The PLGA films were prepared by solvent casting from dichloromethane (DCM). A 29 % (wt.) solution of PLGA in DCM was prepared and stirred for 2 hours at room temperature to ensure full dissolution of the polymer. A fixed amount (600 μL) of solution was then collected using a pipette and spread on a glass side inside a glass ring of 1.3 cm internal diameter. The samples were then dried in an oven at 100 °C for 24 hours. This methodology ensured that all films contained the same amount of polymer (0.3 mg) and were of the same thickness (0.3 mm). Phosphate buffered saline solution (PBS) was prepared by dissolving 8 g of sodium chloride (NaCl), 1.38 g of sodium phosphate dibasic (Na_2HPO_4), 0.19 g of potassium phosphate monobasic (KH_2PO_4) and 0.2 g of sodium azide (NaN_3) in one liter of distilled water. The pH was adjusted to 7.4 using hydrochloric acid (HCl). The *in vitro* degradation studies were carried out by placing the polymer films into 20 mL of PBS solution in sealed jars, which were then placed into an incubator at $37.0 \pm 0.1\,^{\circ}C$.

Microscopy

The sample surface was photographed at regular time intervals during the degradation using a digital Zeiss AxioCamMR camera attached to an Olympus Stereo Zoom microscope. In order to avoid disruption of the sample surface, the photographs were taken while the sample was still inside the buffer solution.

pH Measurements

The pH of the degradation media was checked at regular time intervals. The pH measurements were performed using a glass bodied combination pH electrode linked to a Hydrus 300 meter (Fisher). The pH-meter was calibrated before each set of measurements with three standard buffer solutions purchased from Acros Organics: phthalate buffer solution pH = 4.01, phosphate buffer solution pH = 7.00, borate buffer solution pH = 10.01.

Water Content and Mass Loss Measurements

Samples were weighed before being placed in the degradation media in order to get the initial mass of the films, m_{ini}. At regular time intervals the films were then recovered, gently washed with distilled water, swabbed with blotting paper and immediately weighed in order to obtain the wet mass of the films, m_{wet}. Finally the samples were dried in an oven at 100 °C for a week and weighed again to obtain the dry mass left after degradation, m_{dry}. The water content and mass loss of the film were then calculated through Equation (1) and (2).

$$Water\ content = \frac{m_{wet} - m_{dry}}{m_{dry}} \quad (1)$$

$$Mass\ loss = \frac{m_{ini} - m_{dry}}{m_{ini}} \quad (2)$$

Gel Permeation Chromatography (GPC)

The number- and weight-average molecular weights (M_n and M_w) were determined at 35 °C by GPC using tetrahydrofuran (THF) as solvent. A Viscotek 301 Triple Detector Array equipped with two TSKgel GMHHR-N 300×7.8 mm columns and a differential refractometer detector was used. The GPC was calibrated using polystyrene standards (580 to 170,800 g mol^{-1}) purchased from Polymer Laboratories Ltd. Therefore all molecular weights are given in polystyrene equivalent. The films were recovered from the jars at regular time intervals, gently washed with distilled water, swabbed with blotting paper and immediately dissolved in THF (4 mg mL^{-1}). 100 μL of the solution was then injected into the GPC using a VE 5200

autosampler. Three measurements were performed for each sample.

Differential Scanning Calorimetry (DSC)

DSC experiments were performed using a DSC Q100 from TA Instruments equipped with an autosampler and a RCS cooling system. 5 to 10 mg aliquots of wet sample were extracted at different degradation times and placed in a standard aluminum pan that was not hermetically sealed. Each sample was subsequently heated from room temperature to 150 °C inside the DSC at 20 °C min^{-1} in order to evaporate the water. The glass transition was then measured during a 2nd heating cycle where the sample was heated from −50 °C to 100 °C at 10 °C min^{-1}. The data were analysed using Universal Analysis 2000 software provided with the instrument. A minimum of 3 measurements were performed for each degradation time.

Results and Discussion

We have investigated the *in vitro* degradation in 20 mL of PBS at 37 °C of a PLGA with a lactic to glycolic ratio of 65/35. Films were created by solvent casting from DCM. In Figure 1 (top), the pH evolution of the PBS media as a function of the degradation time for PLGA 65/35 film is presented. A decrease in pH from 7.4 to 7.0 is observed

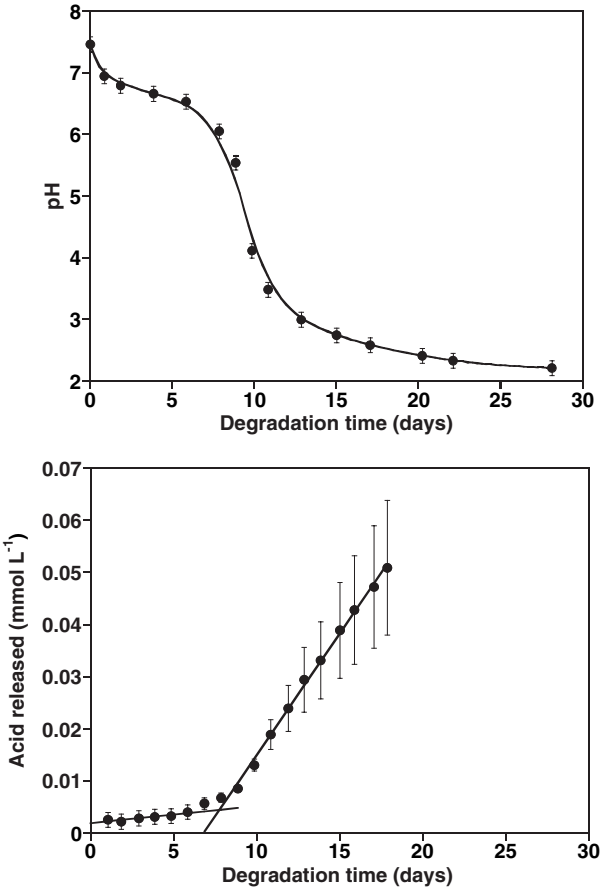

Figure 1.
pH evolution of the media (top) and acid released into the media (bottom) for PLGA 65/35 film degraded in 20 mL of PBS buffer at 37 °C.

during the first day of degradation. Following this initial drop, the pH remains roughly constant for the next four days before dropping sharply from 6.8 to 3 between 7 and 13 days of degradation. After 13 days, the pH decreases slowly and after 28 days of degradation the pH of the media is 2.2.

The decrease in pH is due to the degradation of the polymer and the release of acidic compounds into the media. In order to measure the amount of acid released, the PBS buffer was titrated with lactic and glycolic acid. The same titration curves were obtained for both acids. The titration curve obtained was used to calculate the amount of acid release in the media. In Figure 1 (bottom), the acid released in the media versus the degradation time is presented. Two distinct release regimes are observed. From 0 to 8 days of degradation, a slow release of acid into the media is observed at a rate of $(4.2 \pm 0.6) \times 10^{-4}$ mmol L^{-1} $days^{-1}$. From 8 days onwards, the rate of acid release increases significantly and becomes one order of magnitude higher at: $(4.7 \pm 0.1) \times 10^{-3}$ mmol L^{-1} $days^{-1}$.

In order to investigate the change in morphology of the films, we used optical microscopy to photograph the surface of the films. To avoid sample disruption, the films were photographed while inside the buffer solution. In Figure 2, the photographs of the film surface at different degradation times are presented. During the initial days of degradation, small wrinkles appear on the surface of the film and the sample becomes cloudy. During the first 10 days of degradation, the sample becomes white and wrinkles on the surface increase in size (Figure 2). A physical examination of the sample reveals the presence of a soft surface layer (wrinkles) while the bottom layer of the sample remains hard.

PLGAs are known to be hygroscopic polymers. In Figure 3 (left), the water content of the film is presented versus the degradation time. As can be seen as soon as the film is placed inside the buffer solution water diffuses inside. The film water content reaches 100% (wt.) after only 9 days of degradation. The mass loss was also measured as a function of degradation time and is presented in Figure 3 (right). As can be seen during the first 10 days of degradation, the film mass loss is relatively low at only 10% after 10 days. The large uptake of water by the film results in a significant increase in volume of the film. The presence of a soft surface layer and a hard bottom layer suggests that the majority of the water has diffused into the top layer. The wrinkling of the surface suggests

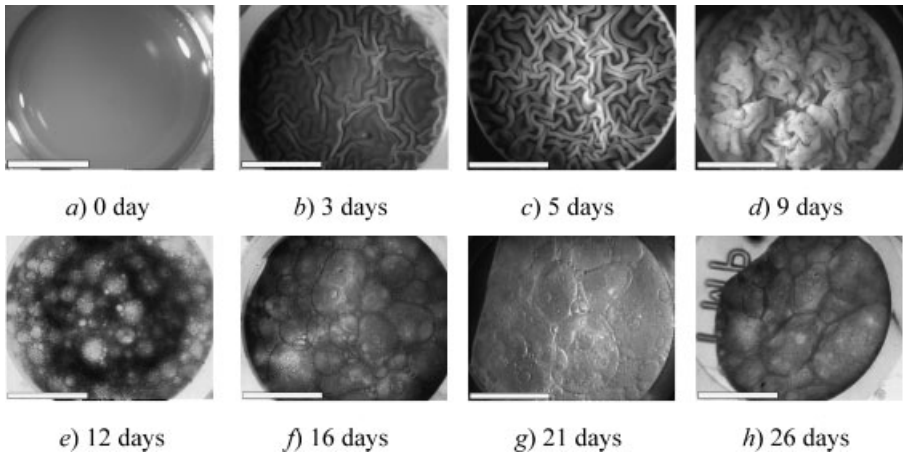

Figure 2.
Optical micrographs of PLGA 65/35 films surface degraded in 20 mL of PBS buffer solution at 37 °C at different degradation times. The scale bar represents 5 mm.

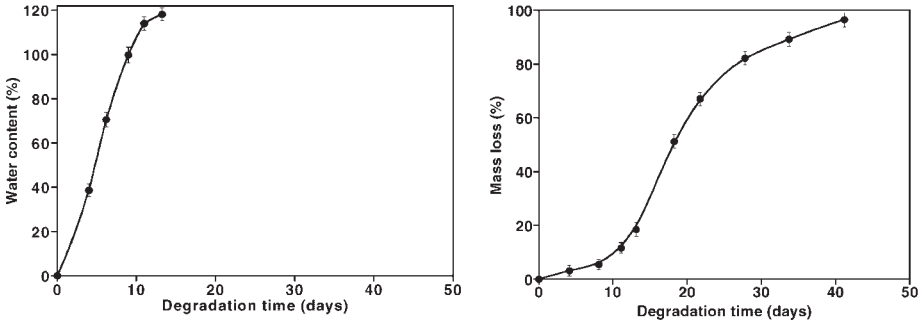

Figure 3.
Water content (left) and mass loss (right) for PLGA 65/35 films degraded in 20 mL of PBS buffer solution at 37 °C.

that the swelling of the surface layer is not uniform across the whole surface and that part of the top layer remains attached to the hard bottom layer.

At 10 days, a dramatic change in the film morphology is observed. The wrinkles disappear and the film surface becomes smooth and rounded. After 10 days of degradation, bubbles begin to appear inside the film and they increase in number and size with increasing degradation time. At long degradation times, the film shrinks and becomes smaller. After 10 days of degradation, if the sample is taken out of the buffer the film collapses and liquid escapes from the interior of the film. This observation and the apparition of bubbles indicate that the inside of the film becomes liquid. This suggest that chain scission occurs inside the films resulting in the formation of low molecular weight oligomers that will be liquid and form bubbles inside the film at low enough molecular weight.

GPC was used to measure the molecular weight of the polymer as a function of the degradation time. In Figure 4, the number-average molecular weight M_n of the polymer is presented as a function of the degradation time. M_n is found to decrease as soon as the film is placed in the buffer solution confirming that chain scission occurs in the bulk of the film. After 15 days, the M_n of the polymer is 680 g mol^{-1} and slowly becomes constant. At the same time, the rate of acid release increases significantly. This suggests that only oligomers with low enough M_n are able to diffuse outside of the film. This is confirmed by the

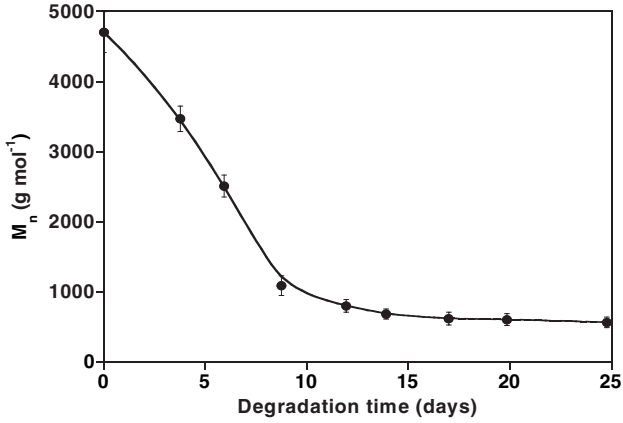

Figure 4.
Number-average molecular weight, M_n, for PLGA 65/35 film degraded in 20 mL of PBS buffer solution at 37 °C.

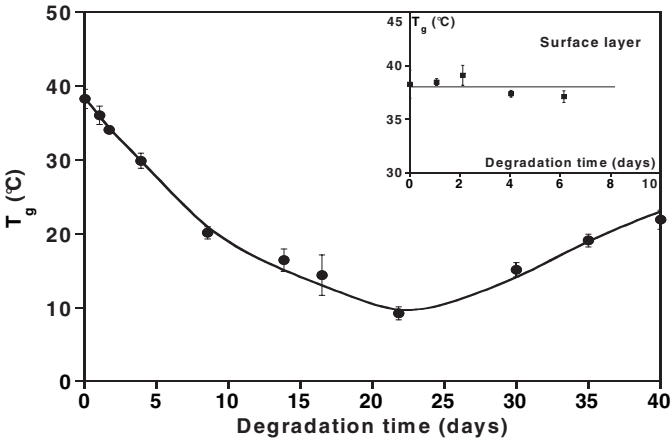

Figure 5.
Glass transition T_g (°C) of PLGA 65/35 degraded in PBS of: ● the bottom layer of the sample and ■ the top layer of the sample. Scanning rate: 10 °C min^{-1}, aluminium pans, nitrogen atmosphere.

observed increase in the rate of film mass loss after 10–15 days (Figure 3 right).

DSC was used to measure the glass transition (T_g) of the dry samples as a function of the degradation time. In Figure 5 the evolution of the T_g against the degradation time is presented. T_g is seen to decreases from 38.3 °C to 9.2 °C over the first 22 days of degradation and subsequently to increase again to 20 °C over the latter 18 days. The T_g of PLGA is a function of the molecular weight of the polymer. As said earlier as soon as the samples are placed in the liquid media the average molecular weight of the polymer decreases resulting in the observed decrease in T_g during the first 15 to 20 days of degradation.

In order to measure the glass transition of the soft surface layer, the material forming the wrinkles was scooped off the top of the sample and its T_g measured using the same procedure. This was only possible for the first 8 days of degradation during which the wrinkles are observed. In the insert of Figure 5 the T_g obtained for the material forming the surface layer of the sample is presented. As can be seen the T_g of this surface layer remains roughly constant suggesting that very little chain scission is occurring in the top layer of the film. This result suggests that a slower degradation process occurs at the surface in comparison to the bulk of the sample which results in the formation of a vesicle after 10 days of degradation where low molecular weight compounds are trapped inside a high molecular weigh polymer skin. The hydrolysis of PLGA is known to be catalysed by acids.[1,3,4] In the bulk of the sample the acid compounds formed by the hydrolysis reaction are trapped and the environment becomes highly acidic resulting in the autocatalysis of the degradation reaction. The slow degradation of the surface layer is thought to be due to the absence of any autocatalytic degradation reaction. This is because the surface layer is in direct contact with the buffer solution hence any acidic compounds formed can either diffuse into the media immediately or are neutralized by the buffer solution resulting in the absence of autocatalysis.

After 22 days of degradation the T_g of the sample increases again while the average molecular weight of the sample remains constant (Figure 4). As shown by other authors the glycolic units are usually degraded preferentially.[5,6] This implies that with time the ratio of lactic units in the remaining polymer increases. PLA has a higher glass transition than PGA.[7] The increase in the T_g after 20 days of degradation is therefore thought to result from the preferential degradation of glycolic units

resulting in the remaining polymer containing an increasing proportion of lactic units with increasing degradation time.

Conclusions

We have investigated the *in vitro* degradation of a random poly(lactic-*co*-glycolic) copolymer with a lactic to glycolic ratio of 65/35 in PBS buffer solution at pH 7.4 and at 37 °C. As soon as the polymer is placed in the buffer, water diffuses inside the film and a soft surface layer forms. As more water is accommodated by the film, its volume increases leading to a wrinkling of the surface layer. Simultaneously, degradation of the polymer inside the film starts via random hydrolysis resulting in the decrease of the M_n of the polymer. The decrease in molecular weight was confirmed by the reduction in the bulk polymer glass transition over the first 20 days of degradation. A differentiation occurs between surface and bulk degradation rates with the latter going much faster. This was confirmed by measuring the glass transition of the material forming the surface layer of the film. The T_g of this surface material was found to remain constant suggesting a slower degradation rate. This is thought to be due to the highly acidic environment inside the film resulting in the autocatalysis of the degradation reaction. Since the surface layer is in direct contact with the buffer, the acidity is neutralised and therefore the autocatalysis reaction does not occur. This results in the surface degradation being much slower.[8,9] This difference leads to the formation of a vesicle after 10 days of degradation where low molecular weight compounds are trapped inside a thin layer of high molecular weight polymer. This thin layer acts as a membrane and allows only oligomers with low enough molecular weight to diffuse out into the media. Once the diffusion process starts the film mass loss rate of the film increases significantly. At the later stages of degradation the glass transition of the residual polymer is found to increase. This is thought to be due to the preferential degradation of the glycolic units resulting in the polymer having a higher lactic unit content and therefore a higher T_g.

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