Covalent Grafting of Poly(L-lactide) to Tune the In Vitro Degradation Rate

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The in vitro rate of degradation was purposely affected by covalently grafting the surface of poly(L-lactide) (PLLA). PLLA films were surface modified by our vapor-phase nondestructive photografting technique. Films were grafted for 20 min with one of the following monomers: acryl amide (AAm), *N*-vinyl pyrrolidone (VP), or acrylic acid (AA) and thereafter incubated in vitro in a phosphate-buffered saline solution at 37 °C for 154 days. The films were studied with contact angle measurements, SEM, ATR-FTIR, SEC, and DSC. The analyses verified that the in vitro rate of degradation was enhanced and that the grafted surface layer did remain covalently attached to the surface during the initial stages of incubation.

Introduction

An increase in the rate of degradation of poly(L-lactide) (PLLA) opens up new possibilities for the material in several biomedical applications. Degradable polymers such as aliphatic polyesters, and polylactides (PLA) in particular, are extensively used in biomedical applications such as tissue engineering and drug release. PLLA is biocompatible and is used when a temporary support is needed.[1] The slow degradation of PLLA is, however, considered to be a disadvantage in many applications.[2] To improve the host−implant interactions of biomaterials, various surface modifications are used. To achieve a stable and long lasting surface structure, covalent attachment is important.

Early attempts to control the rate of degradation of PLA were made by copolymerization with monomers such as ϵ-caprolactone,[3] glycolic acid,[4] 1,5-dioxepan-2-one,[5] α-malate,[6] and glycine.[7] Copolymerizations with hydrophilic monomers such as ethylene glycol[8] or hydroxyethyl acrylate monomers[9] with the specific aim of increasing the degradation rate have also been performed. Blending of PLLA with other materials such as poly(hexamethylene succinate)[10] or thioridazine[11] is an alternative to copolymerization. The drawbacks of copolymerizing and blending are that the bulk properties of the material are changed, which is a disadvantage in many applications. Recent advances in polymer chemistry have paved the way for new strategies to tune the degradation rate by new macromolecular architectures.[12,13] Surface treatment is another potential way of increasing the degradation rate. A few surface treatment methods for controlling the degradation of PLA are described in the literature. Examples of these are plasma glow[14] and NaOH[15] treatments, which etch the outer layer of the material. Another surface treatments is, for example, photografting in monomer solution.[16] Oxygen plasma is also used for the surface treatment of polyesters.[17] Surface modifications are also used to achieve better host−implant reactions,[18,19] as the surface is the first that comes into contact with the living tissue.[20] By modifying the surface of PLA, it is possible to improve both the degradation rate and the wettability. As the surface comes into contact with the living tissue first, and since PLA and many other resorbable polymers are hydrophobic, an increased wettability is very important from a biocompatibility perspective. However, a difficulty with current surface modification techniques is that they often initialize degradation during the modification step, and this is not desired. For example, etching causes degradation and removal of the top layer of the substrate. Radiation techniques initialize degradation during the modification of PLA and several other resorbable biomaterials. We have earlier developed a new solvent-free vapor-phase photografting technique that overcomes the problem of degradation during the surface modification process of resorbable substrates[21,22] and this is also viable for the covalent surface modification of biostable polymers.[23,24] We have used our technique to covalently surface modify degradable polymers with various vinyl monomers, to increase the hydrophilicity and provide biocompatibility of the substrate surfaces.

To further control cell reactions to a substrate, covalent surface modification with reactive groups and subsequent immobilization of different bioactive moieties is a very important tool, as we have shown earlier.[25,26] In biomedical applications, hydrolysis is the most important mechanism of degradation,[27] and the degradation rate is affected by various factors, including the molecular weight, polydispersity, morphology, and surface area accessible to the surrounding media. The first step of the degradation is water uptake, and hydrolysis thereafter causes breakage of the backbone ester bonds.

Our hypothesis is that we directly influence the in vitro rate of PLLA degradation by using our technique for nondestructive solvent-free vapor-phase photografting. We also hypothesize that the vapor-phase photografted surface layer remains attached to the surface during the initial degradation.

Experimental Procedures

Materials. *N*-Vinylpyrrolidone (VP), 99+%, was purchased from Aldrich and distilled at 100 °C under reduced pressure before use and was stored cold. Acryl amide (AAm) was purchased from Aldrich and was used as received. Acrylic acid (AA) was purchased from Acros (99.5%) and distilled at 45 °C at reduced pressure before use and stored cold. Benzophenone (BPO), 99+%, was used as received from Acros. Chloroform (99.5%, Aldrich) and ethanol (99.5%, Lab-Scan) were used

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as received. PLLA (M_n = 145 600, M_w = 191 400) in pellet form was a kind gift from Tenova. Substrate films were prepared by dissolving 4 g of polymer in 100 mL of chloroform and pouring the clear solution onto a silanized glass mold. The solvent was allowed to slowly evaporate from the covered molds, and the films were dried. Circular pieces with a diameter of 15 mm were cut from the films and used as sample substrates in the subsequent grafting and in vitro degradation studies.

Vapor-Phase Grafting. Vapor-phase grafting has been described elsewhere.[21,22] The grafting procedure was performed in our specially constructed reactor in a water bath for 20 min at 50 °C. The system was degassed thoroughly before each grafting. In the case of coupling with AA, this was done through a freeze-pump thawing cycle. After grafting, the films were rinsed and soaked in deionized water and ethanol followed by drying under vacuum. The same washing procedure was used for the pristine samples.

In Vitro Degradation. The washed and dried samples of grafted and pristine PLLA films were degraded in vitro in 17 mL of a 0.1 M phosphate buffered saline solution at pH 7.4 and 37 °C in rubber-sealed serum bottles. The samples were removed from the bottles at various times from 0 to 154 days and thereafter rinsed in deionized water and dried under vacuum.

Characterization. The molecular weights were determined from filtered samples with size exclusion chromatography (SEC) on a system consisting of a Waters 717plus auto sampler, a Waters model 510 apparatus equipped with three PLgel 10 μm mixed-B columns, 300 mm × 7.5 mm (Polymer Labs), and an PL-ELS 1000 evaporative light scattering detector (Polymer Labs). Chloroform was used as an eluent at 25 °C with a flow rate of 1.0 mL/min. Polystyrene standards with narrow molecular weight distributions (M_w/M_n = 1.06) were used for calibration.

Fourier transform infrared spectrometry (FTIR) spectra were recorded on a PerkinElmer Spectrum 2000 FTIR equipped with an attenuated total reflectance (ATR) crystal accessory (Golden Gate).The surface was analyzed to a depth of approximately 1 μm. All spectra were calculated means from 32 scans at a 2 cm^{-1} resolution with correction for atmospheric water and carbon dioxide.

The static contact angles of surfaces were measured on an apparatus constructed at the department. The samples to be analyzed were placed on a flat and well-lit surface in front of a Sanyo VCC4100 Color CCD video camera, equipped with a Cosmicar 25 mm 1:1.4 television lens, connected by means of a 20 mm spacer to increase the optical magnification. The video signal was transferred to a computer using an IC−-PCI frame grabber card from Imaging Technology Inc. The live feed from the camera was captured and processed with OPTIMAS 6.2 software from the Optimas Corporation. The contact angle data for each sample are averages of four individual measurements.

Surface topographies were examined by SEM using a JEOL JSM 5400 scanning microscope. Samples were mounted on metal stubs and sputter-coated with gold−palladium (Denton Vacuum Desc II).

The melting and glass transition temperatures and ΔH for the synthesized monomer and polymers were measured by differential scanning calorimetry (DSC). The instrument used was a temperature- and energy-calibrated Mettler Toledo differential scanning calorimeter 820 purged with nitrogen gas. The samples were loaded into 40 μL aluminum caps. Thermograms were recorded from 0 to 160 °C. The heating rate was 10 °C/min, and the nitrogen flow was set to 80 mL/min.

Results and Discussion

PLLA was covalently surface modified by a single-step vapor-phase photografting technique with either AA, AAm, or VP. The grafted substrates were degraded in vitro in a phosphate-buffered saline solution for various times.

Appearance. The undegraded grafted samples were semi-flexible and opaque. The degraded samples changed gradually during the degradation process to milky white and became harder and more brittle. This process was faster for the grafted samples than for the pristine PLLA. This behavior was most clearly seen for the films grafted with AA, which became too fragile to handle within a month of degradation and broke into fragments in the degradation vials.

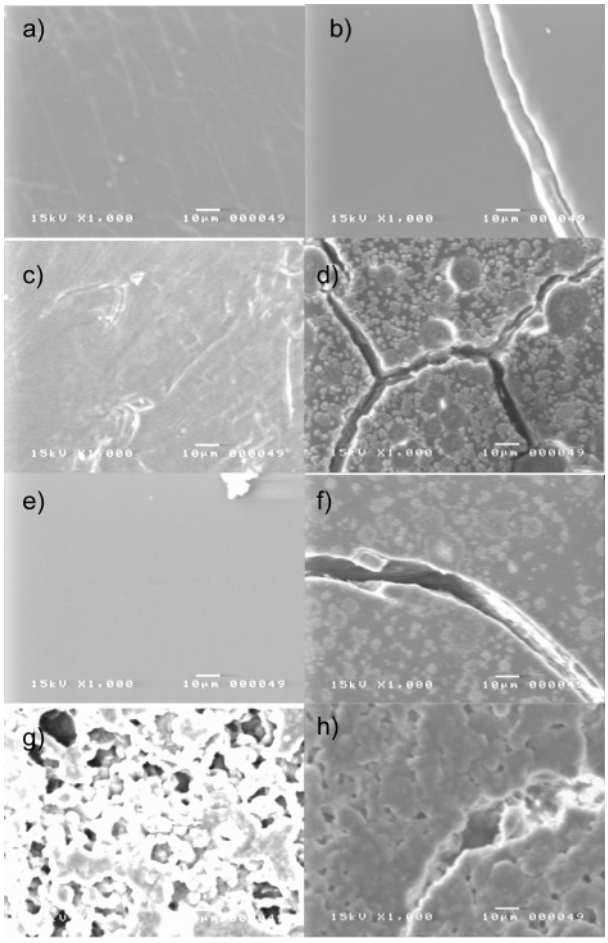

Figure 1. SEM micrographs (×1000) of PLLA degradation samples: (a) pristine at 0 days, (b) pristine at 154 days, (c) VP grafted at 0 days, (d) VP grafted at 154 days, (e) AAm grafted at 0 days, (f) AAm grafted at 154 days, (g) AA grafted at 0 days, and (h) AA grafted at 154 days.

Weight. The weights of the samples were measured before and after degradation. Many of the degraded samples were very brittle and thus difficult to handle, especially the AA-grafted samples. This made the removal from the degradation buffer without losing any fragments of the samples very difficult. Even so, it is clear that the weight of the samples decreased with time. After 154 days, 75−79% of the original weight remained. There were no larger differences between the different samples, except that the AA-grafted films had a somewhat lower weight.

Surface Topography. The surface topographies of the degraded substrates were studied with SEM. The micrographs are shown in Figure 1.

Samples degraded in buffered saline solution for 42, 84, and 154 days were compared to pristine substrates. The analyses showed that the pristine PLLA samples had quite a smooth surface, which became cracked with degradation time. The VP-grafted PLLA films were smooth before degradation and were totally cracked at 154 days of degradation. The AAm-grafted samples like the VP samples were smooth before degradation. After 84 days of degradation, the surface was full of dots. Macroscopic cracks were seen after 154 days of degradation.

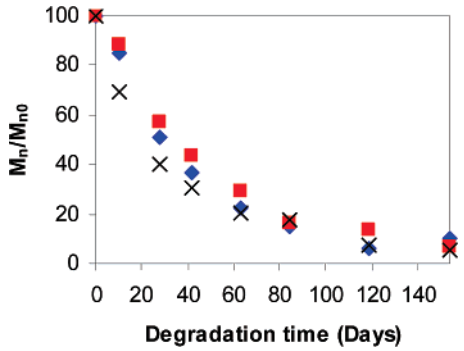

Figure 2. Time dependence of M_n/M_{n0} for (◆) pristine PLLA and PLLA grafted with (■) VP and (×) AAm.

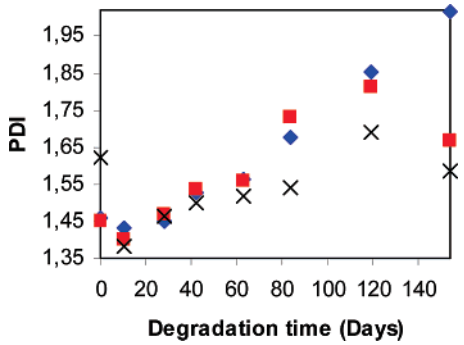

Figure 3. Time dependence of PDI for (◆) pristine PLLA and PLLA grafted with (■) VP and (×) AAm.

The surface of the films grafted with AA had a porous structure before incubation. This is explained by the higher graft yield for these films, which gives a higher concentration of grafts that are visible on the surface. This has been seen in previous work with a high graft yield.[22] The AA-grafted films became somewhat swollen with time. The samples degraded for 42 days developed macroscopic cracks that increased in number and size with time.

Molecular Weight. The molecular weights of the samples were assessed by SEC. The decrease in molecular weight, given as a percentage of the initial molecular weight before degradation, is shown in Figure 2.

The degradation rate was somewhat faster for the AAm-grafted films during the initial degradation. The VP-grafted and pristine substrates had a similar degradation rate. The AA-grafted films could not be studied by SEC because of their lack of solubility in $CHCl_3$, THF, DMF, and H_2O. The appearance of the AA-grafted films, which became very fragile with degradation time, indicated that the molecular weights of these films were even lower than those of the AAm-grafted films.

The polydispersity index (PDI) was calculated for all the samples assessed with SEC, and its time dependence is displayed in Figure 3.

The PDI of the pristine PLLA increased almost linearly with degradation time, showing that there was a larger variety of chain lengths in the most degraded samples. The VP- and AAm-grafted samples showed an increase in PDI with a peak at 119 days of degradation, and thereafter a decreasing PDI. This indicates that the longer chains have been degraded to shorter ones at the end of the degradation and/or that the shortest oligomers have been lost to a greater extent by erosion. This was expected because the greater wettability of the grafted substrates facilitates water uptake. Degradation creates carboxylic chain ends that are known to autocatalyze ester hydrolysis.[28] The acidic oligomers formed inside the material by degradation will also contribute to the autocatalytic effect.[1] Only those groups that are soluble in aqueous medium surrounding them escape from the matrix material.[27] The groups in the inner parts of the material take a longer time to diffuse out, increasing the autocatalytic effect.[28] The lactic acid formed as the final degradation product is removed from the body by normal metabolic pathways as water and carbon dioxide.[20]

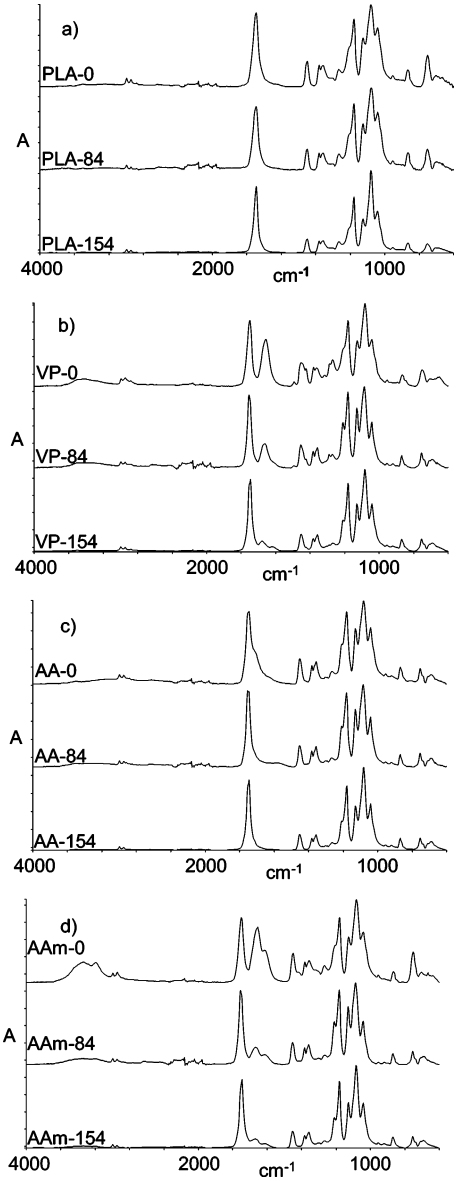

Figure 4. ATR-FTIR spectra of (a) PLLA, (b) PLLA grafted with VP, (c) PLLA grafted with AA, and (d) PLLA grafted with AAm after 0, 84, and 154 days.

Surface Composition. The surface compositions of the films were monitored with ATR-FTIR. The spectra are shown in Figure 4.

Pristine PLLA shows a characteristic FTIR spectrum with a C═O peak at 1747 cm^{-1}, and the appearance of this spectrum does not change significantly during the course of degradation. Chain scission results in hydroxyl and carboxyl terminal groups, which would show in the FTIR spectrum if they were trapped in the matrix, but the gradual weight loss of the PLLA samples indicates that the shorter chain segments produced by degradation are eroded from the samples and dissolved in the degradation medium. Films grafted with VP, AAm, or AA show, as expected, additional C═O bands originating from amide C═O groups (VP and AAm) or carboxylic acid (AA). The general

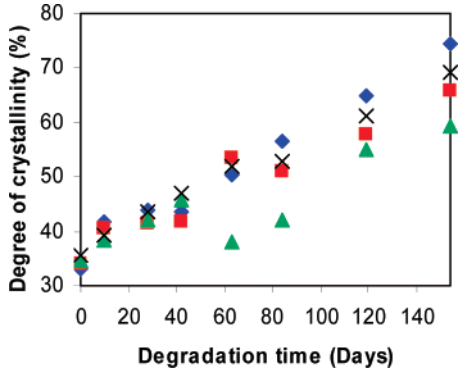

Figure 5. Change in the degree of crystallinity with degradation time for (◆) pristine PLLA and PLLA grafted with (■) VP, (▲) AA, and (×) AAm.

trend is for the intensity of the bands stemming from the graft chains to decrease with increasing degradation time. These segments of the substrate are obviously more easily eroded than the bulk chain segments since they are more soluble in water. However, even after longer degradation times, fractions of the graft chains are still attached to the sample surface. This verifies that the functionalities are not lost upon incubation and that they are indeed covalently attached and not just physically adsorbed.

Morphology. The morphologies of the degraded substrates were studied with DSC. The films are all semi-crystalline. In the first stages of degradation, an increase in matrix crystallinity with time is observed (Figure 5).

This is attributed to two separate mechanisms: the additional crystallization of partly degraded chains and tie chains connecting crystalline regions and the preferential degradation of the amorphous regions, which permits a rearrangement of the crystals and leads to recrystallization. Since water penetrates amorphous regions more easily than crystalline regions, the degradation begins in the amorphous parts, which hold the crystal blocks together.[29] The degradation of PLLA proceeds much faster in the bulk than on the surface because of the autocatalytic effect.[30] This is caused by the water penetrating the amorphous parts and creating a negative gradient of water into the inner parts of the specimen at the start of water uptake. The degree of crystallinity increased the most in the case of pristine PLLA, and this helps to explain why grafted samples lost their physical integrity to a markedly greater extent than the non-grafted PLLA. The degree of crystallinity was calculated from ΔH using a value of $\Delta H^\circ = 106$ J/g.[31] The increase in crystallinity was accompanied by a slight increase in the melting temperature (Figure 6), followed by a gradual decrease.

The glass transition temperature remained unchanged at about 55 °C for all samples throughout the time scale of observation (Figure 7).

Wettability. The wettability of the degraded substrates was studied with static contact angle measurements. Some of the degraded samples were, however, too brittle to handle without breaking, and it was then not possible to study all the samples.

Covalent surface grafting with VP, AA, and AAm resulted in PLLA surfaces with a significantly greater hydrophilicity than the pristine PLLA (Table 1). While the latter showed a static contact angle greater 80°, the grafted surfaces showed values in the region of 45−60°. The contact angle of pristine PLLA remained at 70−75° during the early stages of degradation and did not show any significant decrease until 154 days of degradation. An increase in wettability is to be expected after a long degradation period, given that chain scission produces

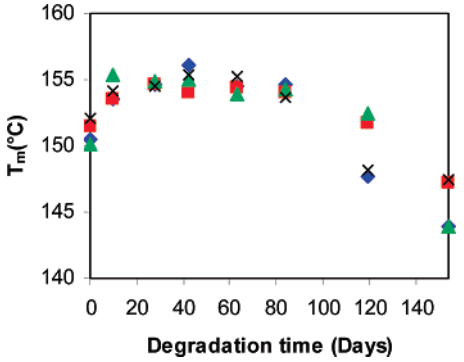

Figure 6. Change in melting temperature with degradation time for (◆) pristine PLLA and PLLA grafted with (■) VP, (▲) AA, and (×) AAm.

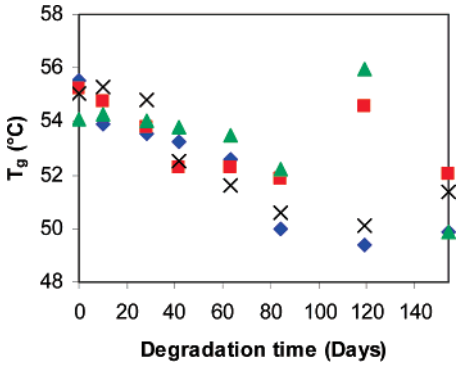

Figure 7. Change in glass transition temperature with degradation time for (◆) pristine PLLA and PLLA grafted with (■) VP, (▲) AA, and (×) AAm.

Table 1. Static Contact Angle Measurements for Pristine PLLA and PLLA Grafted with VP, AA, and AAm

degradation time (days)	static contact angle (deg)			
	PLA	VP	AA	AAm
0	83	58	53	50
10	76	52	*a*	60
28	74	45	*a*	53
42	74	44	*a*	50
63	71	43	*a*	*a*
154	64	*a*	*a*	*a*

[a] Substrates too brittle to study.

shorter chain segments with hydroxyl and carboxylic acid terminal groups. These grafted samples that could be tested consistently exhibited static contact angles of 20−30° lower than those for pristine PLLA throughout the degradation. Samples grafted with AA became too brittle to study after only a week of degradation in vitro.

Conclusion

We have successfully increased the rate of degradation of PLLA by nondestructive vapor-phase surface modification with vinyl monomers. We have also shown that the grafted surface layers remain attached to the surface upon incubation. PLLA substrates were covalently surface modified by solvent-free vapor-phase photografting where AAm, AA, or VP was coupled to the surface. The films were degraded in vitro in a phosphate-buffered saline solution at 37 °C for 154 days. The surface properties were studied with ATR-FTIR, SEC, SEM, DSC, and contact angle measurements. The grafting has increased the wettability, and this gives improved surface properties from a

biomedical point of view. Analyses verify that the grafted surface layers remain attached to the surface of the substrate surface upon incubation. The grafted films show a faster degradation rate in terms of mechanical characteristics and weight loss than the pristine substrates. The slow degradation of PLLA is a problem in many applications, and our new vapor-phase grafting technique open up new possibilities for controlling the degradation rate.

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