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ARTICLE



Characterization of biodegradable poly(L-lactide) tube over accelerated degradation

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Abstract

The next-generation bioresorbable polymeric stents are undergoing continual research and development, especially how to control and optimize their degradation behaviors. The aim of this paper is to study the properties of a poly(L-lactide) (PLLA) tube over accelerated degradation in vitro. Spherical nanoindentation was particularly adopted to measure the mechanical properties of PLLA tube; while differential scanning calorimetry and gel permeation chromatography were used for crystallinity and molecular weight measurements. Overall, the material degradation was captured by the local stress-strain response obtained with spherical nanoindentation, and further confirmed with chemical analyses. The results are particularly useful for development of bioresorbable polymeric stents with improved performance.

KEYWORDS

biodegradable polymeric stents, differential scanning calorimetry, gel permeation chromatography, mechanical properties, spherical nanoindentation

1 | INTRODUCTION

Biodegradable polymers are an important class of materials desirable for biomedical applications. At present, there is a growing interest in using biodegradable polymers for manufacturing stents due to their resorption with time. This technology offers a potential reduction of the long-term risks, which are associated with permanent stenting, including stent fracture, impaired vessel homeostasis and neoatheroscleorsis. Mechanical and chemical characterizations of a bioresorbable poly(L-lactide) (PLLA) stent were previously conducted over two-year in vitro degradation using pyramidal nanoindentation, atomic force microscopy and chemical analytical techniques. No obvious degradation was observed over the two-year period, in terms of decline of mechanical properties of the scaffold. This was recognized as being usual for PLLA

degradation in vitro, due to a lack of environmental and physiological stimuli. Additionally, this behavior is indicative of the mechanism of bulk degradation in PLLA, where depletion of mechanical properties comes at the later stages of degradation.

In literature, various methods have been adopted to investigate the degradation of PLLA, [4-6] including the attempts to change, or even tune, its degradation behavior for improved performance. [7-9] For instance, Diaz et al. [7] demonstrated that the degradation behavior of porous PLLA scaffolds could be tuned through the addition of nanohydroxyapatite (nHA) particles. Also, the plasticizers such as D- and L-lactide were found effective in altering the thermal and mechanical properties of PLLA over degradation. [9] By mixing with titanium dioxide (TiO₂), a transformation of less ordered to better organized crystalline structure was discovered for PLLA. [10]

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In addition, Naseem et al. [6] confirmed the role of natural ageing in altering the mechanical properties of PLLA stents using atomic force microscopy nanoindentation. The work of Oosterbeek et al.[8] indicated that blending PLLA with novel polyethylene glycol functionalized poly(L-lactide-co-ε-caprolactone) provided an effective method of tuning the timescale and mechanical properties of PLLA during degradation. Furthermore, Dreher et al.[11] demonstrated that fatigue loading imposed during degradation could increase the stiffness of a stent sub-unit significantly and lower the load at fracture, highlighting an interplay between deformation and degradation for PLLA. These results are potentially important in defining appropriate in vitro conditions for preclinical evaluations of bioresorbable polymeric stents.

Accelerated degradation provides a faster means of assessing the degradation mechanism in specimens that take a long time to degrade. Numerous strategies have been employed to accelerate in vitro degradation including the application of stress/strain, increase in temperature of the degradation medium and controlled variation of pH level. [12-15] Miller and Williams [12] studied both invitro and in-vivo stabilities of a biodegradable suture material, focusing on the effect of mechanical strain which may be imposed during handling prior to surgery. It was clear from the experiments that the rate of hydrolysis was affected by the magnitude of the strain imposed on the suture, where higher strain correlated with a faster rate of hydrolysis. Cam et al. [15] investigated the degradation behavior of PLLA with high molecular weight in an alkaline medium. By using 0.01 N NaOH at 37°C, it was possible to shorten the degradation time without disruption to the mechanism of bulk degradation of PLLA. Weir et al. [16] assessed the degradation of PLLA at elevated temperatures, and confirmed accelerated degradation at 50°C and 70°C. The accelerated process followed the same two-stage bulk degradation mechanism as occurs at human-body temperature (37°C), which is typical for semicrystalline aliphatic polyesters.^[16] Agrawal et al.^[14] investigated degradation of a PLA co-polymer at a range of temperatures, from 25°C to 80°C, aiming to assess the characteristics of degradation at temperatures both below and above the glass transition temperature (T_g) . They concluded that the activation energies calculated from the Arrhenius equations, which was used to calculate the effect of temperature change on reaction rate, varied greatly for temperatures above and below T_{o} . It was therefore recommended that any tests which were performed at a temperature above T_g should not be applied to predict the material behavior at a temperature below $T_{\rm g}$. [14]

Indentation tests can be performed using indenter tips of different geometries, yielding different mechanical responses, especially for polymers. Pyramidal indentation is commonly used to investigate material properties by penetrating the sample surface with a sharp tip such as Berkovich. The use of sharp tip in indentation is associated with multiple advantages such as well-defined plastic deformation and easy penetration. However, pyramidal indentation can be problematic and causes discrepancies in the data for complex biomaterials. According to previous work, the property variation measured for polymers is down to several contributing factors including the local variation of properties, indentation size effect and the intrinsic bimodal behavior of the materials.[17] The indentation size effect in epoxy was investigated by Alisafaei et al. [18] using different indenter tips, with penetration depth ranging between 30 and 3000 nm. For a three-sided Berkovich tip, elastic modulus showed an increase with decreasing penetration depth; while no size effect was seen for indentation with a spherical tip. This is due to the higher order of displacement gradient associated with sharper tips, and a variation of indentation depth causes a change in strain gradient for the Berkovich tip. However, the strain gradient remains almost constant for indentation with a spherical tip, as the gradient is solely dependent on the tip radius instead of penetration depth.^[18] The use of spherical or blunter tips extends the elastic-plastic deformation during indentation. Furthermore, the load-displacement data can be transformed into stress-strain curves to determine the vield strength of the materials.^[19] During spherical indentation, the material behavior is dependent on the actual-yield strain ratio. Specifically, a high ratio is associated with plastic behavior and a low ratio represents elastic behavior. Actual strain intends to increase with progressive penetration depth, and thus, a transition from purely elastic to plastic response can be observed from the stress-strain curves obtained during spherical indentation.[20,21]

Spherical indention has been used to study the mechanical properties of polymers, including modulus and stress-strain curves, but little work has been done to quantify the property change of bioresorbable polymers over the process of degradation. In this paper, accelerated degradation was carried out for PLLA tube at 50°C to study the property change with degradation time. The PLLA tubes, used for stent manufacturing via laser cutting, were supplied by Abbott Vascular. The tube was segmented using a sterile blade, and sections were placed in glass vials filled with PBS (pH 7.4) and maintained at elevated temperature (50°C). Spherical indentation was carried out on dried samples using a nanoindenter (Micromaterials) to assess the change in mechanical properties. Given the bulk degradation nature of PLLA and limited mass loss at initial stages, gel permeation chromatography was also used to assess the degradation of the polymer by measuring the change of molecular weight with time.

2 | SAMPLE PREPARATION AND DEGRADATION TESTS

PLLA tubes, used to manufacture ABSORB stents through laser cutting, were supplied by the manufacturer (Abbott Vascular). The dimensions of the material were confirmed using optical imaging (SmartScope Flash 200), where the wall thickness was found to be $\sim 150 \ \mu m$ (Figure 1).

A sharp blade was used to section the tubes in a sterile environment (to reduce potential surface contamination to the sample). Each section was weighed prior to incubation in glass vials filled with PBS, which was changed fortnightly to maintain a pH level of 7.4. A total of 14 samples were prepared from the original tube. Vials were maintained at a temperature of 50°C using an incubating oven, with daily motioning. This temperature was chosen based on previously defined DSC data, [3] preliminary tests and literature review. [16] Samples to be assessed were extracted from the incubating oven and dried at 37°C for 48 hours to ensure removal of all moisture. In this study, the in-vitro tests were conducted to evaluate the degradation behavior of the PLLA tube at a benchtop, and do not represent the behavior of the manufactured stents which undergo additional crimping and sterilization processes.

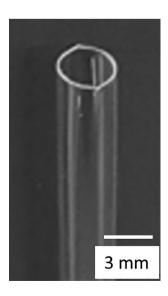


FIGURE 1 PLLA tube

3 | PROPERTY CHARACTERIZATION OVER DEGRADATION

3.1 | Mass loss

Mass loss of samples was analyzed over their incubation time in PBS. An Ultra-Micro balance (Sartorius SE2) was used for measurements, prior to and after incubation in PBS. Incubated samples underwent drying for 48 hours in an oven (37°C; below the glass transition temperature) before measurements. This was to remove retained moisture during their incubation period. The mass loss was then calculated as:

Mass loss (%) =
$$\frac{\text{Change in mass}}{\text{Original mass}} \times 100\%$$
 (1)

3.2 | Differential scanning calorimetry

Here, differential scanning calorimetry (DSC) was used to assess the thermal behavior of the polymer by measuring the heat flux of the sample due to temperature change. By heating the samples, the thermodynamic property of the polymer can be visualized over the whole process. Small pieces were taken from the polymer samples at a weight of 3 to 5 mg and underwent DSC testing (Universal V4, TA instruments). The difference in weights of the specimens does not affect the results outcome. The analyses were carried out for tube sections incubated over 30, 90, and 120 days to determine the crystallinity. DSC testing was also carried out for virgin sample (day 0) for comparison purpose.

It should be noted that PLLA is a semicrystalline material. Particularly, the medical grade PLLA normally has a high proportion of crystallinity, aiming to increase the mechanical strength of the material. Specifically, the degree of crystallinity was calculated from the following equation:

Crystallinity (%) =
$$\frac{\Delta H_f - \Delta H_c}{\Delta H_{f100\%}}$$
 (2)

where ΔH_f is the enthalpy of melting obtained from the sample DSC data, ΔH_c stands for the enthalpy of crystallization in the exothermal process, and $\Delta H_{f100\%}$ is the enthalpy of melting for 100% crystalline PLLA. There is a wide variation for the enthalpy of melting for 100% crystalline polymer PLLA in literature, we used the most reported one which is about 93 J/g. [22] As the

crystallization behavior was not found for PLLA in the exothermal process, ΔH_c was taken as 0 in our calculations. [22,23]

3.3 | Nanoindentation

A Platform 3 indenter (Micromaterials, Wrexham) was used for nanoindentation in this study. The tests were carried out using a spherical indenter tip with a radius of 10 μ m. The loading and unloading rates were set as 0.5 mN/s and 5 mN/s, respectively. Distances between indents were kept at 40 μ m to eliminate their mutual interactions. Based on the Oliver-Pharr theory, the top portion (~20%) of unloading curve was used to calculate the elastic modulus of the sample. The Young's modulus and the Poisson's ratio of the diamond indenter tip were taken as 1140 GPa and 0.07, respectively, in the calculations.

The Hertz's model is primarily used for theoretical analyses in indentation, where frictionless contact is assumed between two isotropic elastic bodies. The model can be modified to give a relationship between stress and strain for spherical indentation^[20,21]:

$$\sigma_{ind} = \frac{4E^*}{3\pi} \varepsilon_{ind}, \sigma_{ind} = \frac{P}{\pi a^2}, \varepsilon_{ind} = \frac{a}{R_{eff}}$$
 (3)

where σ_{ind} , ε_{ind} , E^* , P, a, and R_{eff} denote the indentation stress, indentation strain, reduced elastic modulus, load, radius of the contact boundary and effective radius of the indenter.

3.4 | Gel permeation chromatography

The gel permeation chromatography (GPC) analysis is based on separation of molecules of different sizes in solution. This method was used here to obtain the change in molecular weight for the samples with incubation time. The eluent, with a concentration of 1 mg/mL, was used to dissolve the polymer sections (~0.5 g). The injection volume used in this study was 100 µL for each sample. The polymer sections were fully dissolved in the eluent before being injected onto the columns (two PLgel 5 µm Mixed C columns and a guard column). The measurements were taken in CHCl₃ + 2% triethylamine, with a 1 mL/min flow rate, using a refractive index (RI) detector. Both the columns and the RI detector were maintained at 40°C. The GPC data were processed using the Cirrus SEC software according to the polystyrene (PS-M) standard.

4 | RESULTS AND DISCUSSION

All 14 samples incubated in the study had rigid cylindrical integrity. Within 120 days of incubation, the material became extremely brittle and unsuitable for testing. Therefore, a 120-day limit was defined as the upper experimental threshold.

4.1 | Mass loss

After drying, samples were weighed to assess the mass loss. Figure 2A shows the results of percentage mass loss with time over 120 days of incubation. It can be ascertained that there was minimal mass loss of the samples over this period. As previously discovered with this material, mass loss is an occurrence which transpires in the later stages of degradation. Although the samples are brittle and succumb easily to breakage after 120 days, this does not deem the sample "fully degraded" as there is an obvious presence of solid material. Polymer chain scission occurs from the moment the sample is incubated into the PBS medium, which manifests as a drop in molecular weight. It can be presumed that the cleaved chains are still too bulky at the 120-day period to diffuse

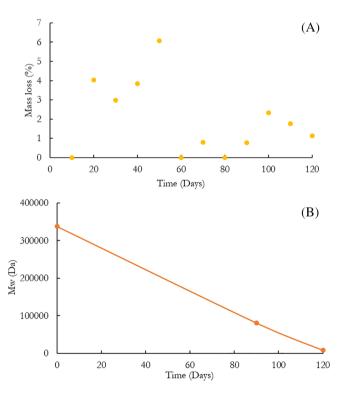


FIGURE 2 A, Mass loss and B, molecular weight (Da) of tube samples with degradation over 120 days [Color figure can be viewed at wileyonlinelibrary.com]

out of the tube sample, giving the reason for the insignificant mass loss.

4.2 | GPC

Figure 2B indicates the occurrence of degradation for the PLLA material due to a great decline of the molecular weight, from an initial molecular weight (Mw) of 338 kDa at day 0 to 8 kDa at day 120. This illustrates the occurrence of chain scission in the polymer, with the presence of smaller fragments. In this instance, the chain of lower molecular weight is still too large to dissipate from the polymer material, and therefore remains in the core of the material. This is the reason that no great weight loss is observed. After 120 days, the samples become extremely brittle and crumble, suggesting that the samples are still not in the final stages of degradation. The breakage of the sample could be attributed to the shorter-length chains and reconfiguration of the internal structure of the material.

4.3 | DSC

DSC results show an interesting trend (Figure 3), that is, a rapid increase in crystallinity of the material from 0 to 30 days and a gradual decline from 30 days to 120 days. Crystallinity peaks at 30 days and continuously declines after this point, but it does not decline to the initial crystallinity value. At 120 days, the percentage crystallinity of the sample remains 15% higher than that of the initial state. This means that the amorphous regions are first targeted by degradation and with their removal from the polymer core, the remaining sample consists of a high percentage of polymer in a crystalline state. Between day 30 and 120, crystallinity decreases as the crystalline

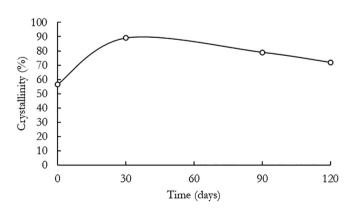


FIGURE 3 Crystallinity of in vitro degraded samples with time

regions succumb to degradation and cleavage of these long chains occurs, causing the drop of crystallinity. The melt enthalpy obtained from the DSC analyses is given in Table 1, from which the crystallinity was calculated using Equation (3).

4.4 | Spherical indentation of virgin samples

With spherical indentation, the modulus is calculated with the slope of each unloading segment, as illustrated by Field and Swain. [20,21] Figure 4 displays the modulus vs the indentation depth (n=4), with each test containing 100 load-unloading cycles. There is a rapid increase of modulus to a certain depth, with the value peaking at around 500 nm of penetration depth. Then, a continual decline is observed until the end of indentation test at which the penetration reaches a depth of approximately 3000 nm.

To assess whether this is a natural response of the material or an effect of the multiple loading-unloading cycles, singular indentation tests were carried out on the virgin tube at a load range of 1 to 23 mN. This enabled the assessment of Young's modulus without continuous loading-unlading cycles. Figure 5 gives the plot of the Young's modulus calculated from the

TABLE 1 Melt enthalpy and crystallinity for samples over degradation times

Times (day)	Enthalpy of melt (J/g)	Crystallinity (%)
0	52.68	56.66
30	70.69	76.01
90	77.07	82.87
120	76.78	82.56

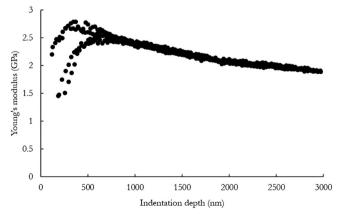


FIGURE 4 Young's modulus obtained with continual and cyclic indents (n = 4)

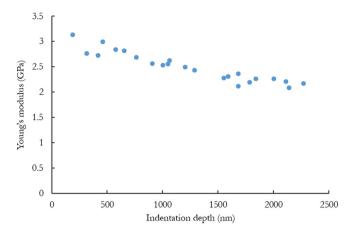


FIGURE 5 Young's modulus of the virgin state PLLA tube obtained with singular indent [Color figure can be viewed at wileyonlinelibrary.com]

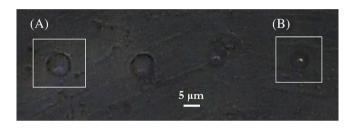


FIGURE 6 Residual impressions left by, A, continual cyclic loading and unloading (100 cycles) up to 23 mN and B, a singular load of 23 mN [Color figure can be viewed at wileyonlinelibrary.com]

singular indentation tests, and there is no initial peak or incremental increase in modulus. Instead, there is a slight decline of modulus with the increase of indentation depth and load level, representing an indentation size effect or a variation of properties across the initial layers of the stent. Figure 6 illustrates the residual impressions which are left from (a) 100 load-cycles (with the last cycle being at 23 mN) and (b) singular indentation at 23 mN. It is obvious that the area of contact for the former is significantly larger than that for the latter. This will affect the modulus value due to the difference in area of contact. It can be concluded that continuous indentation cycles, used in spherical indentation, can present inaccurate mechanical properties of polymer materials, for which material hardening could be a cause.

4.5 | Spherical indentation of degraded samples

There is an increase in the modulus, at the same indentation depth, from day 0 to 30 (Figure 7); followed by a

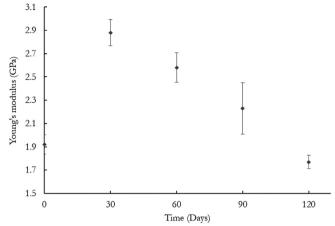


FIGURE 7 Young's modulus obtained with the first indent at 23 mN on degraded tube surface

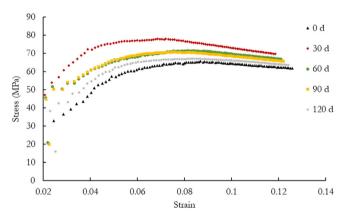


FIGURE 8 Stress-strain response of the material over degradation [Color figure can be viewed at wileyonlinelibrary.com]

decline until 120 days. The 120-day sample, which is the sample prior to brittle facture upon manual handling, shows a lower modulus than that of the virgin tube. Although the samples are brittle after the 120-day incubation, it cannot be said that the sample is fully degraded. Instead, the polymer chains within the structure have come to a state which cannot maintain integrity, therefore giving a weaker response.

Here, 100 cycles of indentation were conducted for one indent on the degraded specimens. The test was performed at a 30-day interval (Figure 8). Degradation of PLLA is confirmed from the change of the stress-strain response. Compared with the tube sample in a virgin state (day 0), there is an observed increase in stress at the same strain levels. The change is also seen for 60-and 90-day samples, where the stress levels drop consistently with degradation. At 120 days, a lower stress response is observed when compared to the virgin sample at the same levels of strain, indicating a significant

weakening of the material. The results appear concurrent with the phenomena typically occurring in PLLA, where material stiffness increases with internal shift of the polymeric chains and increasing crystallinity, followed by a decline due to continuous degradation of the sample.

5 | CONCLUSIONS

Spherical indentation can demonstrate degradation of this material type, through the stress-strain response at a local level. The effect of material hardening on PLLA properties should be considered, as a high number of cycles can affect the material behavior during indentation testing. Given this, the value for the first load cycle on this type of material should be taken as the true value as opposed to the values obtained with subsequent loading cycles. For accelerated degradation at higher temperature (50°C), material degradation can be observed on a shorter time scale when compared to samples incubated at human body temperature (37°C). Mass loss presented here is minimal over the degradation period studied, consistent with previous work. This also confirms that the mechanism of accelerated degradation remains unchanged for an elevated temperature which is below the glass transition temperature.

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