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Tunable Degradation of Piperazine-Based Polyurethane Ureas

Changshun Ruan,[1,2] Yang Hu,[1,2] Lixin Jiang,[1,2] Qingqing Cai,[1,2] Haobo Pan,[1,2] Huaiyu Wang[1,2]

[1]Center for Human Tissue and Organ Degeneration, Institute of Biomedicine and Biotechnology, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen 518055, China

[2]Shenzhen Key Laboratory of Marine Biomedical Materials, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen 518055, China

Correspondence to: H. Wang (E-mail: hy.wang1@siat.ac.cn)

ABSTRACT: To manipulate the degradation of polymeric biomaterial for potential applications in tissue regeneration, a series of piperazine-based polyurethane ureas (P-PUUs) were designed and prepared with poly(D,L-lactic acid) diol (PDLLA diol), 1,6-hexamethylene diisocyanate (HDI), and piperazine (PP). The number of piperazine units [Num(pp)] in the P-PUUs could be precisely calculated by a specified equation and could be controlled by the regulation of the PDLLA diol/HDI/PP ratio. Then, the *in vitro* degradation of the P-PUUs was investigated by the detection of the variation of the pH value, the weight loss ratio, the surface morphologies, and the molecular weight loss over 12 weeks. The results reveal that the degradation stability and the degradation rate of the P-PUUs could be manipulated by Num(pp), and a linear correlation between the degradation rate of the P-PUUs and Num(pp) in the polymer was demonstrated; this implied the tunable degradation of the P-PUUs. Such a linear correlation is expected to benefit to tissue regeneration as the degradation rate of P-PUUs for specific tissue defects can be well tuned once the tissue regenerative period is known. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40527.

KEYWORDS: biodegradable; biopolymers & renewable polymers; functionalization of polymers; ring-opening polymerization

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INTRODUCTION

The stimulation of tissue healing depends on the physiochemical properties of biomedical scaffold materials,[1–3] in particular, a suitable architecture, excellent biocompatibility, and satisfactory biodegradability. Furthermore, biomaterials for tissue regeneration, temporary templates or substrates, should play a key role in supporting tissue construction. Thus, the degradation rate of an ideal regenerative biomedical material should be comparable to the tissue growth rate, as the implant should have enough mechanical properties initially to bear loads, and over time, the load bearing could be transferred onto the newly formed tissues. In this respect, the degradation of regenerative biomedical materials must be tunable by precise design, to match the tissue growth rate.[4,5]

Much is known about the factors affecting the degradation and degradation rate, but less attention has been paid to the low-molecular-weight products during hydrolysis. Aliphatic polyesters, derived from cyclic monomers, for example, ε-caprolactone, lactide (LA), and glycolide, have been extensively documented with great potential in tissue regeneration. However, their release of acidic degradation products *in vivo* leads to a local acidic microenvironment; this is the main reason for the uncontrollable degradation of these polymers due to acidity-caused autoaccelerating degradation behavior in the degradation process.[6] The attempt to control acidic component release has been extensively studied. Albertsson and coworkers[7,8] demonstrated that the degradation rate and release rate of acidic degradation products from biomedical polyesters could be controlled through the macromolecular design of caprolactone (CL)/1,5-dioxepan-2-one (DXO) copolymers with the same compositions but different macromolecular structures, that is, DXO/CL/DXO triblock, CL/DXO multiblock, and random crosslinked CL/DXO copolymers. The major drawback of this process was that both the feedstocks we used were acidic monomers, and the degradation products were still acidic. To prevent these negative effects, the neutralization of acidic degradation products through the addition of alkalinity salts[9] or blending with polymers that degrade alkalinity products[10] has been proposed. Luo, Niu, and coworkers[11,12] prepared a novel polymer 1,4-Butanediamine grafted poly(D,L-lactic acid) (BDPLA) by grafting alkaline 1,4-butanediamine (BDA) onto poly(D,L-lactic acid) (PDLLA). The grafted BDA weakened or neutralized the acidity of PDLLA degradation products. Hence, the resulting BDPLA was better than PDLLA in terms of stability and compatibility. However, the proposed graft chemical process was tedious and uncontrolled, and the grafting ratio of BDA was

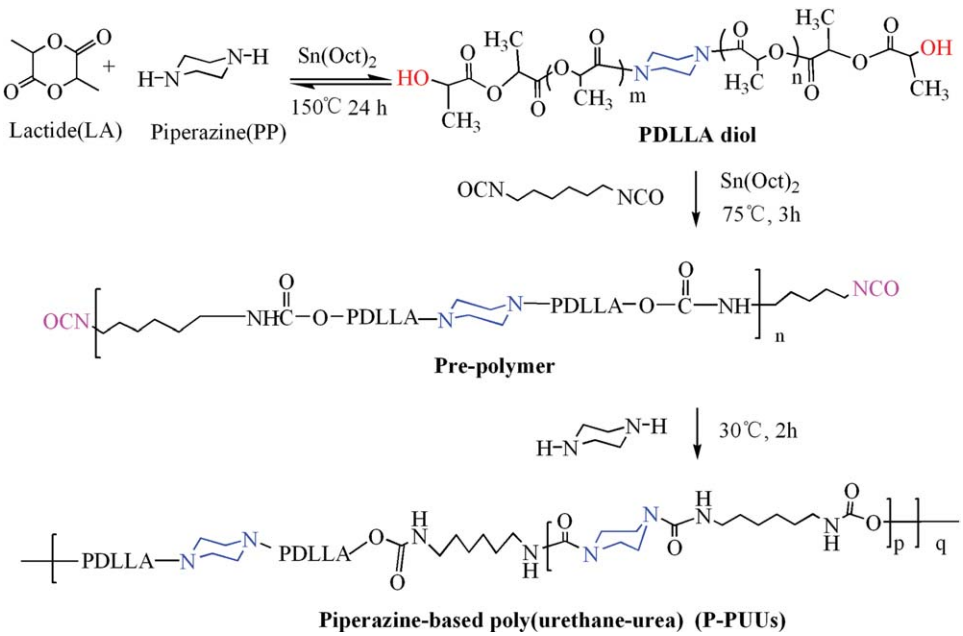

Figure 1. Synthesis route for the P-PUUs. Stannous octoate ($Sn(Oct)_2$) [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

very low. Our research team[13] has been dedicated to improving the degradability stability of PDLLA-based biomaterials by comparing the effects of different chain extenders on the degradation properties of PDLLA-based polyurethanes. Three types of segmented polyurethanes based on poly(D,L-lactic acid) diol (PDLLA diol) and hexamethylene diisocyanate (HDI) were synthesized with three chain extenders: piperazine (PP), 1,4-butanediol (BDO), and BDA, respectively. It was a surprise that the alkaline chain extender PP and BDA could mitigate the acidity-caused autoaccelerating degradation, and the degradation rate decreased. Moreover, a series of novel piperiazine-based polyurethane urea (P-PUUs) with different numbers of piperazine units [Num(pp)] were designed and synthesized in our previous research.[14,15] Unlike diamine and diol, PP had only one active point in each side for linking to —NCO; this could make its corresponding polyurethane have a low crosslinking degree. Moreover, as a chain extender, PP not only made the synthesis process of the P-PUUs easy to control but also improved the physical properties of the P-PUUs, including the mechanical properties and shape-memory behavior. On the basis of previous research,[13–15] we aimed to tune the degradation rate of the P-PUUs in this study to make them fulfill the requirement of scaffold materials in tissue regeneration. In particular, we investigated the *in vitro* degradation of the P-PUUs by determining the weight loss ratios, surface morphologies, molecular weights, and pH values of the incubation media over 12 weeks.

EXPERIMENTAL

Materials

P-PUUs were synthesized and characterized, as described in our previous article.[14,15] The synthesis route and chemical structures are illustrated in Figure 1. The Num(pp) in P-PUUs were controlled by the regulation of the ratio of the P-PUU feedstocks, and it could be precisely calculated according to eq. (1):

$$\mathrm{Num(pp)} = \frac{M_n}{M_n(\mathrm{pre})} + \frac{M_n}{M_n(\mathrm{pre})} \times \frac{M_n(\mathrm{pre})}{M_n(\mathrm{diol})} \quad (1)$$

where M_n is the number-average molecular weight of the P-PUUs, M_n(pre) is the number-average molecular weight of prepolymer, and M_n(diol) is the number-average molecular weight of the PDLLA diol. On the right side of eq. (1), the latter part represents the number of PP molecule used to initiate chain-opening polymerization of PDLLA diol and the first part calculates the number of PP molecule as the chain extender.

Preparation and Degradation of Polymer Films

The polymers films for *in vitro* degradation experiments were prepared as follows: 3.0 wt % P-PUUs/PDLLA chloroform solution were placed in a polytetrafluoroethylene mold to form uniform films by evaporating solvent over 48 h.[16] Then these films were vacuum-dried to constant weight. All experiments were performed at room temperature. The obtained glabrous transparent films were cut into 14 mm × 4 mm × 1 mm pieces, then UV-sterilized for 30 min before the degradation study.[12] The samples were assessed *in vitro* for pH variation (ΔpH) and weight loss ratio using double-distilled water (ddH_2O) and phosphate-buffered solution (PBS; 0.1 M, pH 7.4) as degradation medium, respectively. PBS solution consisted of KCl, KH_2PO_4, NaCl and Na_2HPO_4. All experiments were performed in a shaking incubator (37 ± 0.5°C, 50 rpm).

Variation of the pH value during Degradation

To test the pH value of degradation medium, 5 mL of ddH_2O was added to each sample in the vial and the pH value was measured once a week for 12 weeks using a pHS-25bpH meter (China).[13] Three repeats were prepared for each polymer for statistical accountability. The ΔpH of each week was calculated according to eq. (2):

$$\Delta pH_t = pH_t - pH_{t(-1)} \quad (2)$$

where pH_t and $pH_{(t-1)}$ are the pH values at the t and $t-1$ weeks during degradation.

Table I. Basic Data for the Polymers Used in the Degradation Experiments

Polymer[a]	Diol/HDI/PP	$M_{n(pre)} \times 10^4$ (g/mol)	$M_n \times 10^4$ (g/mol)	PDI	Num(pp)[b]
PDLLA	—	—	6.053	1.08	—
P-PUU4K	1/1/0	—	5.017	1.11	14.0
P-PUU4K1.1	1/1.1/0.1	2.212	5.585	1.15	18.1
P-PUU4K1.2	1/1.2/0.2	1.994	6.232	1.23	20.5
P-PUU4K1.3	1/1.3/0.3	1.838	6.854	1.29	22.8
P-PUU4K1.4	1/1.4/0.4	1.776	7.532	1.36	25.2

The M_n value of the PDLLA diol was 3592 g/mol (as measured by ^{1}H-NMR).
[a] For the sample designations, see the main text.
[b] Calculated with eq. (1).

Weight Loss of P-PUUs during Degradation

In the examination of weight loss, 5 mL of sterile PBS solution was added to the vials with P-PUU samples, and 36 samples were prepared for each polymer.[13] At the end of each week, three vials with each P-PUU were taken out, rinsed with distilled water, and vacuum-dried at room temperature to constant weight. The weight loss ratio was calculated as follows:

$$\text{Weight loss}\,(\%) = 1 - \frac{W_t}{W_o} \times 100\% \quad (3)$$

where W_0 is the original weight of every P-PUU sample and W_t is the weight of the sample after t weeks of degradation. The data are expressed as the averages of triplicates.

Molecular Weight Change and Degradation Rate

Gel permeation chromatography with multiangle laser light scattering (laser photometer Dawn EOSTM, Wyatt Technology Corp.) was used to determine the molecular weight of the original and degraded samples. Three Agilent 1100 high performance liquid chromatography (HPLC) columns (300 × 8.0 mm^2) were used in series with tetrahydrofuran as the eluent at a flow rate of 1 mL/min. According to the hypothesis for polymer hydrolytic degradation,[17,18] the constant (k) values of the degradation rate for the P-PUUs films were estimated by the assumption of their exponential decreases in M_n and with eq. (4):

$$\ln[M_n(t_2)] = \ln[M_n(t_1)] - k\Delta t$$
$$\Delta t = t_2 - t_1 \quad (4)$$

where $M_n(t_1)$ and $M_n(t_2)$ are the number-average molecular weight values of the samples at time 1 (t_1) and time 2 (t_2) during degradation.

Morphology Observation

Scanning electron microscopy (SEM) was used to observe the surface morphologies of the samples after they were immersed in media for 0 and 8 weeks. The samples were dried and gold-coated before SEM determination (TESCAN VEGA II LMU, Czechoslovakia).

RESULTS AND DISCUSSION

Tunable Functionalization of the P-PUUs with Num(pp)

In this study, the M_n of PDLLA diol, which was used as the starting material for the P-PUUs, was 3592 g/mol, as determined by ^{1}H-NMR. According to the diol/HDI/PP molar ratios in the reaction, P-PUUs were divided into five groups, that is,

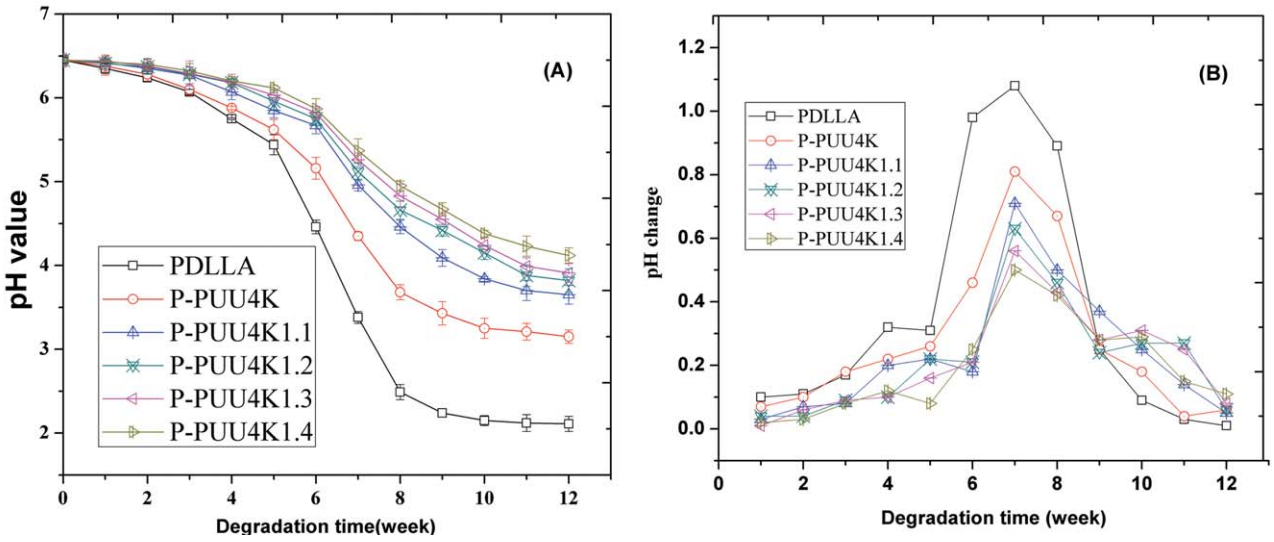

Figure 2. (A) pH values and (B) ΔpH values of the incubating media as a function of the time during degradation. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

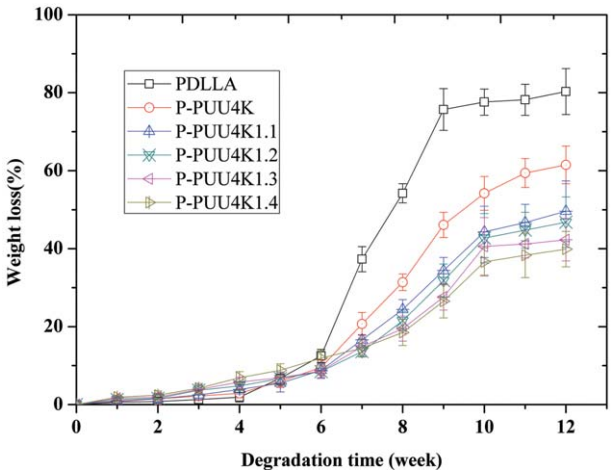

Figure 3. Weights of various polymers as a function of the time during degradation. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

4K, 4K1.1, 4K1.2, 4K1.3, and 4K1.4, with diol/HDI/chain extender ratios of 1/1/0, 1/1.1/0.1, 1/1.2/0.2, 1/1.3/0.3, and 1/1.4/0.4, respectively. As shown in Figure 1, dual introductions of PP into the polyurethane backbone brought a number of PP rings into the P-PUUs, and the Num(pp) in each sample could be precisely calculated according to eq. (1). The basic data for these polymers are listed in Table I. During polymer synthesis, the ratio increase of PDLLA diol to HDI to PP from 1:1:0 to 1:1.4:0.4 led to a significant increase in the Num(pp) in the P-PUUs from 14.0 to 25.2. It was evident that the Num(pp) in the P-PUUs could be readily tuned by the variation of the ratio of the monomers. Although the molecular weight of the P-PUUs varied from 5.017×10^4 to 7.532×10^4 g/mol as the Num(pp) increased, the polydispersity indices (PDI = Weight-average molecular weight/M_n) remained around 1.2. This indicated that the molecular structure of the obtained P-PUUs were mostly linear with low crosslinking during polymer synthesis.

Variation of the pH Values during Degradation

The degradation properties of the P-PUUs, such as the variation of the pH value, the weight loss, and the surface morphologies, were characterized by an *in vitro* degradation model according to our previous study.[13,15,17] In this study, more attention was paid to the exploration of the relationship between the Num(pp) in the P-PUUs and the degradation behaviors.

Figure 2 reveals the ΔpH of ddH_2O during the degradation process of the P-PUUs. The tendency of the sample ΔpH was the same, and it was in accordance with our previous results,[13] in which ΔpH could be divided into three stages on the basis of the variation degree [Figure 2(B)]: 0–5, 5–10, and 10–12 weeks. During the initial 5 weeks, the pH values of all of the samples were above 5.5, and the ΔpH value was less than 0.2. The polymer was primarily swollen and hydrated rather than degraded. In the second time frame of 5–10 weeks, the pH value varied obviously, and the highest ΔpH value was detected in the seventh week as 1.08 for PDLLA, 0.81 for P-PUU4K, 0.71 for P-PUU4K1.1, 0.63 for P-PUU4K1.2, 0.56 for P-PUU4K1.3, and 0.50 for P-PUU4K1.4; this was attributed to the release of acidic or alkaline groups from the breakage of ester/urethane/urea bonds. After 10 weeks, all of the samples were in the stable phase with tiny pH changes of less than 0.30. At last, the pH values were 2.11 for PDLLA, 3.15 for P-PUU4K, 3.65 for P-PUU4K1.1, 3.82 for P-PUU4K1.2, 3.91 for P-PUU4K1.3, and 4.12 for P-PUU4K1.4. At all time points, the pH value of PDLLA was clearly the lowest, and its variation was more intense than that of the others. The pH values of the degradation medium increased with Num(pp) in the P-PUUs. This suggested that the acidic environment during degradation was mitigated.

Weight Loss of the P-PUUs during Degradation

The weight loss of the P-PUUs during degradation is shown in Figure 3. The degradation process of these samples comprised two stages. During the initial 5 weeks, the degradation degrees of all of the polymer samples were slight, and the films were

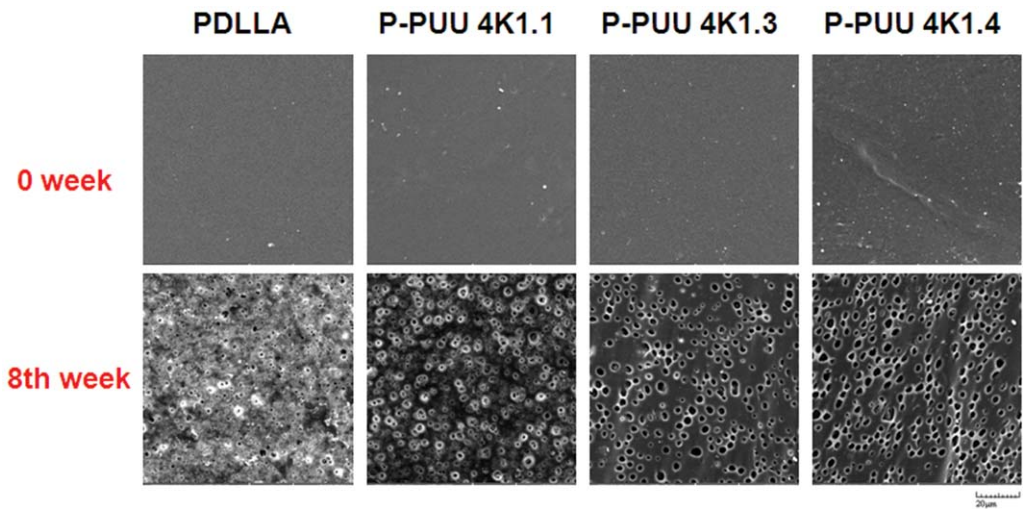

Figure 4. SEM micrographs showing the surface morphologies of the various polymers before and after 8 weeks of degradation (1500×). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

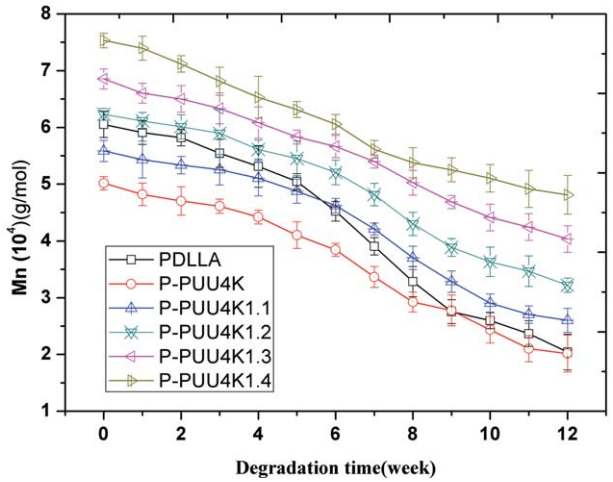

Figure 5. Variation of M_n as a function of the time during degradation. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

still transparent in appearance. After the 5th week, an obvious weight loss of the polymers was observed, and this was in line with our previous study.[13] The involvement of PP reduced the weight loss ratio of the P-PUUs, and the weight loss ratio of the P-PUUs decreased as a function of increasing Num(pp). The weight loss of PDLLA was 76.3% after it was soaked in PBS for 12 weeks, whereas those of P-PUU4K, P-PUU4K1.1, P-PUU4K1.2, P-PUU4K1.3, and P-PUU4K1.4 were 61.5, 49.6, 46.8, 42.3, and 39.9%, respectively.

SEM Observation of the P-PUUs after Degradation

The results of the weight loss experiments were verified by the SEM observation. The surface morphologies of four kinds of polymers (PDLLA, P-PUU4K1.1, P-PUU4K1.2, and P-PUU4K1.4) before and after 8 weeks of degradation are revealed together in Figure 4. All of the pristine polymer films exhibited a smooth and nonporous surface. After 8 weeks of soaking in medium, the PDLLA film was dramatically destroyed, and its surface was rougher than that of the P-PUUs. There were thickly dotted pores on the surface of the P-PUUs, and P-PUU4K1.4 had fewer pores in comparison with the other two kinds of P-PUUs. It was evident that the PP units could retard the degradation of the P-PUUs.

Generally speaking, the degraded products from the biodegradable polyurethanes were lactic acid residues from the PDLLA diol segments and diamine units from the urethane and urea fragments. The former acidic residues could be partially neutralized by the latter alkaline products in a local microenvironment.[5] For the P-PUUs, the introduced PP components not only relieved ΔpH but also obviously decreased the weight loss of the polymers. Actually, there were two reasons for this phenomenon. First, the PP components presented alkalinity during the degradation process of the P-PUUs, which neutralized the acidic degradation products from the PDLLA diol then mitigated the acidity-caused autoaccelerating degradation behavior and decreased the degradation rate. Second, Num(pp) could accommodate the hydrophilicity of the P-PUUs. It has been well documented that the hydrophilicity order was Urea > Urethane > Ester, but the hydrolysis sensitivity order was Urea < Urethane < Ester.[13,17,19,20] As the chain extender, PP brought a number of urea/urethanes in the P-PUUs. With increasing Num(pp) in the P-PUUs, the hydrophilicity of the P-PUUs were improved by the compromise of the hydrolysis rate. Therefore, the introduction of PP could improve the stability of the P-PUUs during degradation.

Molecular Weight Change and Degradation Rate

The M_n values of the PDLLA and P-PUUs as a function of time during degradation are plotted in Figure 5. As shown, the polymers with different Num(pp) hydrolyzed differently. The process of M_n change could be divided into two stages. The former phase comprised the initial 5 weeks. At this period, the degradation rates were very slow, as all polymers were mainly in the hydration stage, and the change of chemical structure was tiny with the only evidence as M_n decrease. This is in line with the previous report that the degradable polymer broke down in a

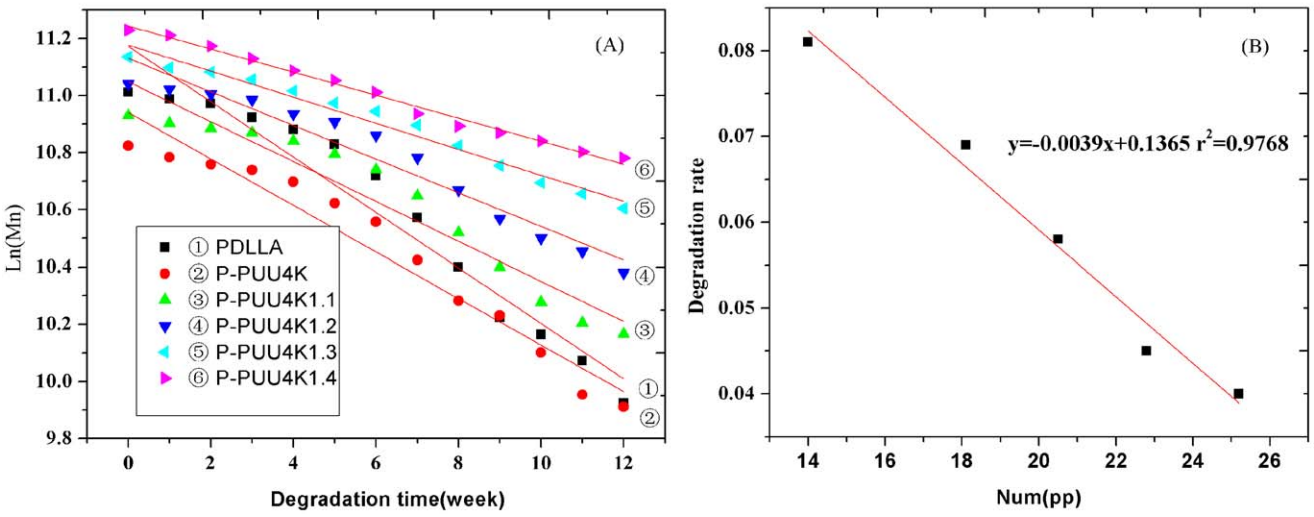

Figure 6. (A) Natural logarithms of the M_n values as a function of the degradation time and (B) line fitting of the polymer degradation rates to Num(pp). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table II. *k* Values of the Hydrolytic Degradation Rate

	Sample					
	PDLLA	P-PUU4K	P-PUU4K1.1	P-PUU4K1.2	P-PUU4K1.3	P-PUU4K1.4
Slope (*k*)	0.0969	0.0814	0.0698	0.0589	0.0456	0.0404
Standard error	0.0072	0.0056	0.0059	0.0043	0.0022	0.0017
R^2	0.9379	0.9462	0.9202	0.9427	0.9736	0.9901

controlled manner, first by decrease in molecular weight, then weight loss.[21] The later phase started from the 5th week when the polymers degradation accelerates. However, the phase duration and degradation rate of various polymers differed from one another. It was obvious that the degradation rate of PDLLA was the quickest among all the polymers in the second phase.

To further analyze the degradation rate of the P-PUUs, the natural logarithm of their M_n as a function of the degradation time is depicted in Figure 6(A). In addition, the degradation rates of all of the copolymers were quantitatively evaluated by eq. (4) on the basis of the general hypothesis of hydrolytic degradation, and the *k* values of the hydrolytic degradation rate are summarized in Table II.

It was clear from Figure 6(A) and Table II that PDLLA degraded faster than the P-PUUs and the *k* values decreased with Num(pp) in the P-PUUs. The P-PUUs with more Num(pp) were endowed with better stability. More interestingly, the degradation rate of the P-PUUs was in linear correlation with the Num(pp) in the polymers, as shown in Figure 6(B). Therefore, the P-PUUs were capable of tunably degradation by the regulation of Num(pp). According to this linear correlation and the known Num(pp), the degradation rate of the P-PUUs could be accurately estimated. Such a linear correlation could be widely applied to tissue regeneration, as the degradation rate of the targeted P-PUUs could be well tuned to meet the specific requirements of various tissue defects.

CONCLUSIONS

A series of P-PUUs with a tunable degradation rate was obtained with PP functionalization. The Num(pp) in the P-PUUs could be controlled by the regulation of the PDLLA diol/HDI/PP monomer ratio. The relationship between Num(pp) in the P-PUUs and the degradation properties was studied by the determination of the ΔpH, weight loss ratio, surface morphology, and molecular weight variation. The results indicated that the degradation stability of the P-PUUs could be well tuned by their Num(pp) values. Furthermore, the degradation rate of the P-PUUs decreased linearly with Num(pp) in the P-PUUs. Such a linear correlation could be widely applied to tissue regeneration as the degradation rate of the targeted P-PUUs could be precisely controlled to meet the specific requirements of various tissue defects. In conclusion, the P-PUUs polymers prepared in this study were tunable in degradation rate and, hence, are desirable for serving as biomedical scaffold materials.

ACKNOWLEDGMENTS

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