The degradation of poly(trimethylene carbonate) implants: the role of molecular weight and enzymes

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ABSTRACT The degradation of poly(trimethylene carbonate) (PTMC) implants with different molecular weights was investigated *in vitro* and *in vivo*. The results showed that PTMC degraded *in vitro* enzymolysis case and *in vivo* via surface erosion. The effect of the molecular weight on the degradation rate of PTMC was investigated in light of hydrophilicity and form-stability. A higher degradation rate was seen in the high molecular weight PTMC and its more hydrophobic surface with better form-stability. Furthermore, PTMC had a higher degradation rate of *in vitro* enzymolysis because of the surfactants role of lipase in diffusion of the degradation products. The results indicate that the form-stabilized PTMC is a promising candidate for clinical subcutaneous implants especially due to their tunable degradation rate.

Keywords: Poly(trimethylene carbonate); *In vitro* degradation; *In vivo* implantation; Molecular weight; Lipase; Form-stability

Introduction

The implantable contraceptive Norplant is one of the most convenient and safe methods for long-acting and effective contraception [1~5]. Although the contraceptive effect is excellent, the

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1 implants must be removed at the end of use because the silastic carrier does not degrade [6, 7].
2 Significant research efforts have been devoted to biodegradable polymeric carriers as potential
3 tools to eliminate removal.

4 There are currently numerous biodegradable polymers under investigation for use in the
5 biomedical and pharmaceutical fields [8~11]. Most research interests have been focused on
6 poly(lactide) (PLA), poly(glycolide) (PGA) and their copolymers [12~25]. However, the utility of
7 PLA and PGA as drug carriers in implantable applications has been hampered by their acidic
8 degradation products. Several studies have shown that the decrease in local pH caused by the
9 acidic degradation products is detrimental to cells, drugs, or proteins and can lead to inflammatory
10 reactions at the site of PLA or PGA implantation [26~28]. Thus, PLA, PGA, and their copolymers
11 do not appear to be the most suitable carriers for long-term contraceptive implants; therefore,
12 further study into alternative materials is urgently required.

13 One promising candidate material is poly(trimethylene carbonate) (PTMC). PTMC is an
14 amorphous polymer with a relatively low glass transition temperature (T_g) of -17°C [29, 30]. In
15 contrast to PLA/PGA, acidic compounds are not produced upon the degradation of PTMC [31, 32].
16 Since reported by Zhu [29], the degradation behavior of PTMC has been extensively studied
17 [29~32]. The previous studies indicate that PTMC is more susceptible to enzymolysis versus
18 hydrolysis as indicated by its much faster degradation. This is most likely due to the enzymatic
19 activity [31]; however, the exact role of enzymes in PTMC degradation remains unclear.

20 The *in vivo* degradation rate of PTMC is still a matter of debate because it is unpredictable.
21 The initial PTMC molecular weight has a large influence on its *in vivo* erosion rate—higher
22 molecular weight polymers degrade significantly faster versus smaller ones [31]. However, few
23 reports thoroughly explain this phenomenon, and further research is required. Importantly, the
24 initial molecular weight also plays a remarkable role in the mechanical properties of PTMC. The
25 low molecular weight PTMC exhibits poor mechanical properties and tends to creep at room
26 temperature [31] due to its amorphous structure and lower T_g . High molecular weight PTMC has
27 outstanding mechanical performance and combines high flexibility with high tensile strength [33]
28 with excellent creep resistance to sustain dimensional stability.

29 For long-term biodegradable contraceptive implants, good form-stability with a low
30 degradation rate is required. Although the form-stability is enhanced, high molecular weight

1 PTMC has an undesired faster degradation rate *in vivo* [31]. The low molecular weight PTMC has
2 slow degradation but poor form-stability, which may compromise its efficacy or even risk patient
3 safety [34]. It is not suitable for long-term implants. The exact combination of good form-stability
4 and low degradation rate in PTMC remains elusive. Thus, the influence of molecular weight on
5 the degradation rate and form-stability of PTMC is a critical issue to be addressed.

6 In this paper, PTMC rods with different molecular weights were prepared as long-term
7 implants. The *in vitro* enzymatic and hydrolytic degradation were performed to elucidate the role
8 of enzymes in the degradation of PTMC. The rods were also implanted subcutaneously in the back
9 of rats. The dimension and mass loss were monitored to clarify the effect of molecular weight on
10 form-stability and degradation rate of PTMC.

11 **Experimental**

12 **Instruments**

13 ¹H NMR spectra were recorded with an ARX 300 (Bruker, Switzerland) using chloroform
14 solution with tetramethylsilane as an internal reference. The molecular weight and its distribution
15 (Mn and PDI) were determined by GPC (Waters, U.S.) with a Waters Model 1515 isocratic
16 high-performance liquid chromatography pump, a Waters Model 2414 differential refractive index
17 detector, and a Waters Styragel HT4 chromatographic column using THF as the solvent and a flow
18 rate of 1 mL/min at 35°C. The Mn and PDI were calculated using polystyrene as standard. The
19 phase transition of the copolymers was determined with a Netzsch DSC 200 F3 (Netzsch,
20 Germany) equipped with a liquid nitrogen cooling system. The temperature range was between
21 -100°C and 100°C, and the heating rate was 10°C/min under nitrogen. The thermal stability of the
22 polymers under nitrogen atmosphere used a Netzsch TGA 209 F3 (Netzsch, Germany) from room
23 temperature to 550°C and a heating rate of 10°C/min. The mechanical properties of the networks
24 were measured using an Instron 1121 universal testing machine (Instron, U.S.) with a crosshead
25 speed of 50 mm/min in accordance with GB/T 1040.1-2006. Tests were done on triplicate samples,
26 and the results were presented as an average of the tested samples. The water contact angles of the
27 different PTMC polymers were determined using thin films spin-coated from chloroform onto
28 glass slides. A contact angle-measuring instrument OCA 15 pro (Dataphysics, Germany) equipped
29 with a video measuring system determined the contact angles of sessile drops at room temperature.

The pH of the media before and after degradation was monitored with a combination microelectrode (InLabMicro™, Toledo-Mettler) and a digital pH meter (FiveEasy™, Toledo-Mettler). The scanning electron microscopy (SEM) images were obtained with an XL30ESEM-FEG microscope (FEI-Philips, Netherlands) or FEI inspect F50 microscope (FEI, UAS). The sample surfaces were sputter coated with Au to avoid charging.

Materials

TMC was purchased from Daigang Biomaterial Co., Ltd, recrystallized twice from acetic ether and dried for 24 h at 37°C under reduced pressure before polymerization. Stannous octoate [Sn(Oct)₂] (95%) and lipase solutions (from *Thermomyces lanuginosus*, ≥100,000 U/g) were used as received from Sigma-Aldrich. All other solvents and reagents used were of analytical grades and purified by standard methods.

Preparation of PTMC implants

The PTMC implants were directly synthesized via a bulk ring-opening polymerization reaction of TMC in the presence of Sn(Oct)₂ as the catalyst. Under nitrogen, dried TMC and an anhydrous toluene solution of Sn(Oct)₂ were added to glass ampoules containing clean and dry Teflon tubes with an inner diameter of 3 mm. The toluene was later removed. The ampoules were purged with dry nitrogen, heat-sealed under vacuum and vigorously shaken to obtain a homogeneous mixture of the monomers and the catalyst. The ampoules were then conditioned in an oil bath pre-heated to 130±2°C for 24 h. After the reaction, the ampoules were quenched at room temperature, and the polymer implants were easily removed from the Teflon tubes and used without any further purification.

In vitro degradation

The PTMC implants with a length of 10 mm were weighed and conditioned in lipase solutions (from *Thermomyces lanuginosus*, ≥100,000 U/g), which were refreshed twice a week. The degradation experiments were performed in triplicate at 37°C and gentle shaking. At regular time intervals, the specimens were removed and washed with distilled water. After blotting with a tissue, the specimens were weighted and then vacuum dried at 37°C to a constant weight for further analysis. The diameter of the dried specimens was also recorded as was the pH of the

media containing the degradation products. The mass loss, water uptake and loss in diameter were calculated via the following equations:

$$\text{Mass loss (\%)} = \frac{w_i - w_d}{w_i} \times 100 \quad \text{Equation (1)}$$

$$\text{Water uptake (\%)} = \frac{w_w - w_d}{w_d} \times 100 \quad \text{Equation (2)}$$

$$\text{Loss in diameter} = \frac{d_i - d_d}{d_i} \times 100 \quad \text{Equation (3)}$$

Here, w_i , w_w and w_d represent the initial weight, wet and dry weight of the samples, respectively.

Terms d_i and d_d represent the initial diameter and the diameter of the dry samples, respectively.

The hydrolytic degradation of the PTMC implants was studied as a control. The implants were cut to 40 mm in length and immersed in vials containing 20 mL of pH 7.4 phosphate buffered saline (PBS) that was replaced once a week. The vials were placed in a 37°C incubator. At 10, 20, 30, 40 and 50 weeks, the samples were removed from buffer and washed with distilled water. The specimens were dried at 37°C in vacuum to constant weight. The mass loss was then determined according to Equation 1.

In vivo degradation

Animal experiments were performed according to the Guidelines of the Liaoning Administrative Committee on Animal Research. The *in vivo* implantation was performed as previously described [35]. Briefly, 20 mm long implants were sterilized by soaking in 75% ethanol for 3 h, rinsed with distilled water and air-dried before subcutaneous implantation into adult male Wistar rats (200 g). For one polymer at each study time point, six specimens were implanted bilaterally into the subcutaneous tissue of the back of one rat.

After implantation, the rats were sacrificed and shaved at predetermined time points. The cylinders were then gently removed from the surrounding fibrous tissue capsules with tweezers. The samples were washed with distilled water and dried at 37°C in vacuum to a constant weight, and the mass loss was then determined according to Equation 1.

Results and discussion

Synthesis and properties of the PTMC implants

To evaluate the effect of molecular weight on PTMC degradation, a series of PTMC implants

with varying molecular weights was prepared via the bulk ring-opening polymerization of TMC in the presence of catalytic $\text{Sn}(\text{Oct})_2$. In all cases, the monomer conversion was quite high (>96%) as determined by ^1H NMR. An overview of the properties of the resulting PTMC implants is given in Table 1.

Table 1 Properties of the PTMC implants prepared via the bulk ring-opening polymerization of TMC.

No.	M/C	M_n ^{a)}	PDI ^{a)}	$[\eta]$ ^{b)}	T_g	T_d	E ^{c)}	σm ^{d)}	Advancing contact angle ^{e)}
		kDa		$\text{dl}\cdot\text{g}^{-1}$	$^{\circ}\text{C}$	$^{\circ}\text{C}$	MPa	MPa	$^{\circ}$
PTMC ₇₂	500	72	1.37	1.15	-15.9	248.0	3.12±0.08	0.94±0.12	58.7±0.38
PTMC ₁₃₅	1000	135	1.25	1.86	-15.0	252.6	4.99±0.23	1.02±0.10	67.5±0.52
PTMC ₁₉₄	5000	194	1.14	2.49	-14.5	254.8	5.15±0.30	2.43±0.44	70.28±0.43
PTMC ₂₅₆	10000	256	1.11	3.88	-14.3	258.9	6.61±0.60	3.19±0.50	78.7±0.76
PTMC ₃₂₉	20000	329	1.07	6.54	-13.9	267.0	10.9±0.47	4.70±0.49	100.97±0.30

a): calculated from GPC analysis.

b): derived from ubbelohde viscometer with tetrachloroethane at room temperature.

c): E = Young's modulus.

d): σm = tensile strength.

e): PTMC films were spin-coated on glass and then conditioned in water for 1 week at 37°C

The molecular weight of the resulting implants was controlled by adjusting the molar ratio of monomer to catalyst (M/C). As shown in Table 1, the molecular weight was carefully tuned in a range of 72~329 kDa as the M/C increased from 500 to 20,000. This indicates that the M/C is an efficient and facile variable to control the polymer molecular weight. As the M/C increased, the active centers for polymerization decreased. In that case, the polymer would form a long chain with a rapid increase in the average molecular weight [36].

The PTMC implants of different molecular weights were amorphous as determined by DSC. The T_g was dependent on polymer molecular weight and varied from -15.9 to -13.9°C. The T_g increased with increasing molecular weight. The results indicate that all polymers are in the rubbery state at physiologic temperatures and are suitable for subcutaneous implant. The thermal stability of the PTMC implants also increased with increasing molecular weight, which may be due to the large molecular chain that retards the thermal degradation.

The mechanical properties of the prepared PTMC implants are also listed in Table 1. The

molecular weight plays an important role on the mechanical properties. As the molecular weight increased, the modulus and tensile strength of the PTMC rods increased from 3.12 ± 0.08 to 10.9 ± 0.47 MPa and from 0.94 ± 0.12 to 4.70 ± 0.49 MPa, respectively. These high mechanical properties eliminate the viscous flow as observed for low molecular weight PTMC. This enhances the form-stability of the biomedical devices based on PTMC.

The hydrophilicity of a polymer has important effects on its degradation behavior [37]. To evaluate the hydrophilicity of the PTMC implants, the water contact angle on the surface of each implant was measured and listed in Table 1. Obviously, the advancing contact angle increased across the series and reached a maximum of $100.97 \pm 0.30^\circ$ for PTMC₃₂₉. The values of the water contact angles indicate relatively more hydrophobic polymer surfaces as the molecular weight increased.

In vitro degradation

The PTMC implants with different molecular weights were immersed into the 37°C lipase solutions for different time periods, and Figure 1A shows the mass loss of the PTMC implants as a function of the degradation time.

Figure 1 The mass loss (A) and Mn (B) of PTMC *in vitro* enzymatic degradation as function of degradation time.

As shown in Figure 1A, all the PTMC implants showed significant mass loss with a linear relationship between the mass loss and the degradation time. The initial molecular weight had a significant effect on the mass loss of the PTMC implants for *in vitro* enzymatic degradation. High molecular weight PTMC implants showed a rapid mass loss over time. For example, after 12 weeks, $50.93 \pm 0.05\%$ of the initial mass of PTMC₃₂₉ had been lost, but only $11.64 \pm 0.04\%$ for PTMC₇₂ at the same time point.

During the *in vitro* enzymatic degradation, the molecular weight of all PTMC implants was measured as a function of the degradation time—no obvious molecular weight loss was found across all cases (Figure 1B). Even when weight loss reached 40~50%, the molecular weight of the residual PTMC remained close to the initial value. This indicates that the degradation mechanism of PTMC implants in the presence of lipase was surface erosion.

In our previous study [35], the *in vitro* degradation rate constant of the TMC and ϵ -caprolactone (CL) copolymers were calculated via the mass loss based on a first-order kinetic model. The following equation was used:

$$R_{t_2} = R_{t_1} - k(t_2 - t_1) \quad \text{Equation (4)}$$

Term k is the rate constant, and R_t is mass loss of the tested samples at degradation time of t .

We calculated the *in vitro* enzymatic degradation rate constant of the PTMC implants via the similar strategy. The linear fitting data from mass loss (Figure 1A) versus the degradation time are listed in Table 2. As seen in Table 2, the *in vitro* enzymatic degradation rate of different molecular weight PTMC determined from the changes in mass loss significantly increased from 0.94 to 4.25 %/w as the molecular weight increased from 72 to 329 kDa..

Table 2 Enzymatic degradation rates of PTMC rods in lipase at 37°C determined from the mass loss.

No.	<i>Mn</i>	Rate constant <i>k</i>	Correlation coefficient
	<i>kDa</i>	%/w	<i>R</i>
PTMC ₇₂	72	0.94	0.979
PTMC ₁₃₅	135	1.46	0.994
PTMC ₁₉₄	194	2.02	0.990
PTMC ₂₅₆	256	3.81	0.997
PTMC ₃₂₉	329	4.25	1.000

Versus the TMC-CL copolymers [35], the *in vitro* enzymatic degradation rate of the PTMC was much lower. This was because of the higher catalytic activity of the enzymes in cleaving ester bonds than in cleaving carbonate bonds [35]. In addition, the mobility of the polymer chain also significantly influences the polymer degradation behavior [38]. Because the poly(ϵ -CL) has a Tg of -65°C, the Tg of the TMC-CL copolymers is much lower than that of PTMC. The lower Tg

enhances the chain mobility of TMC-CL copolymers. This makes the polymer chains more accessible to enzymes and results in higher degradation.

The increase in enzymatic degradation rate with increasing molecular weight may be related to the hydrophilicity of PTMC because the hydrophilicity has important effects on the polymer degradation behavior [37]. As shown in Table 1, the water contact angle increases with increases in molecular weight. It illustrates that PTMC samples became more hydrophobic with increasing molecular weight. It has been reported that the lipase activity is higher on the hydrophobic surface [39, 40]. Hence, we concluded that the lipase activity is high for degrading high molecular weight PTMC.

The degradation behavior of PTMC was not only influenced by the molecular weight, but also by the physical form. The *in vitro* enzymatic degradation of the PTMC₂₅₆ films (id=10 mm, thk=0.5 mm) prepared by solvent casting was compared to PTMC₂₅₆ rods (id=3 mm, length=10 mm). Figure 2 presents the mass loss of the films and rods made of the same materials as a function of degradation time. Obviously, the films degraded faster than the rods. After 10 weeks, the mass loss of the PTMC₂₅₆ films was more than 92%. This is 2-fold higher than that of the PTMC₂₅₆ rods after 12 weeks. The effective surface area of the PTMC films was about $50 \pi \text{ mm}^2$, which was much larger than the $30 \pi \text{ mm}^2$ of the rods. The PTMC samples with a higher surface area had a faster degradation rate. This also indicates that the PTMC was degraded in lipase via a surface erosion process.

Figure 2 The mass loss of PTMC with different molecular weights and physical shapes during *in vitro* enzymatic degradation as a function of time.

Figure 2 also shows that the PTMC films with relatively lower molecular weights degraded

1 faster than the high molecular weight rods. This indicates that the effect of morphology was more
 2 significant than molecular weight effects on PTMC enzymatic degradation. These data concur
 3 with *in vitro* enzymatic degradation of the TMC-CL copolymers [35]. Hence, the important role of
 4 morphology on the biodegradable polymers should be given more keen attention and taken into
 5 account during the design of biodegradable devices.

6 The form-stability of biodegradable implants is an important property that should be given
 7 much attention because it not only greatly influences the final utility of the biomedical devices,
 8 but also plays a significant role in the degradation behavior of the implants. Figure 3 shows the
 9 form-stability of the PTMC implants during *in vitro* enzymatic degradation. The low molecular
 10 weight PTMC implants had poor form-stability; high molecular weight samples were better. The
 11 PTMC₇₂ implants changed from cylindrical to spherical at week 1, while the PTMC₃₂₉ retained its
 12 initial appearance with no deformation throughout the degradation process. Therefore, a high
 13 molecular weight is critical for the PTMC implants to sustain form-stability.

15 Figure 3 The macroscopic observation of the PTMC at different times in 37°C lipase.

16 The effective surface area markedly influenced the degradation rate. The effective surface
 17 area of the low molecular weight PTMC implants decreased as became spherical. Consequently,
 18 the degradation rate decreased with decreasing surface area. The results once again confirmed that
 19 the degradation rate increased with increasing molecular weight of the PTMC implants.

Figure 4 The loss in diameter as function of degradation times (A) and the relationship between the mass loss and loss in diameter (B) of PTMC₃₂₉ in lipase solutions at 37°C.

Although the shape of the PTMC₃₂₉ implants was still rod-like (Figure 3), the diameter decreased significantly upon enzymatic degradation. Figure 4A clearly shows the loss in diameter of the PTMC₃₂₉ implants as a function of degradation time. The linear relationship between mass loss and loss in diameter shown in Figure 4B implies that the decrease in mass occurred simultaneously with a decrease in diameter for the polymeric rods. This indicates a surface erosion process during the *in vitro* enzymatic degradation of the PTMC implants.

Figure 5 The degradation behavior of the PTMC₂₅₆ implants with and without the lipase.

As a control, the *in vitro* hydrolytic degradation of the PTMC implants was carried out in pH 7.4 PBS. The degradation results of the PTMC₂₅₆ implants with and without lipase are presented together in Figure 5 to clarify the role of lipase in the degradation behavior of PTMC.

Figure 5A shows the mass loss of the PTMC₂₅₆ implants as a function of degradation time. The PTMC₂₅₆ implants degraded with lipase show a greater mass loss than those degraded without lipase. The mass loss was more than 46% after 12 weeks in the enzymatic degradation; however, the mass loss was only 3.6% after 50 weeks if the lipase was absent.

With increasing mass loss, the molecular weight of the PTMC₂₅₆ rods slightly decreased with the degradation time. Importantly, no significant molecular weight differences could be observed between samples with and without lipase.

The PDI of the PTMC₂₅₆ implants was measured as a function of degradation time. A significant distinction in PDI changes was seen for the rods with hydrolytic and enzymatic degradation. In the presence of the lipase, the PDI changed within the range of 1.11-1.12 with degradation. However, the PDI became broader (1.11-1.16) in the absence of lipase. This clearly demonstrates that the residual PTMC implants after enzymatic degradation had a narrower PDI than the hydrolyzed samples.

To thoroughly and quantitatively elucidate the affect of the lipase on the degradation behavior, the *in vitro* degradation rate constant of the PTMC₂₅₆ implants with and without lipase were calculated based on Equation 4. The linear fitting of the data from the plots of mass loss (Figure 5A) versus degradation time are listed in Table 3.

Table 3 The degradation rates of PTMC₂₅₆ implants with or without lipase determined from the mass loss.

Samples	Media	Rate constant <i>k</i>	Correlation coefficient
		%/w	<i>R</i>
PTMC ₂₅₆ implants	Lipase	3.81	0.997
	PBS	0.07	0.961

Obviously, the degradation rate constant of the PTMC₂₅₆ implants was much higher in the presence of lipase (a 50-fold increase; Table 3). We concluded that the lipase could accelerate the degradation of the PTMC implants by enhancing the loss of the degradation products. It acts as a surfactant to interact with the degradation products and accelerate their dispersal into the surrounding media [41]. A similar observation was seen with the *in vitro* degradation of the TMC-CL copolymers in our previous studies.

The difference in the PDI changes between enzymatic and hydrolytic degradation of the PTMC₂₅₆ implants (Figure 5C) might be due to the difference in degradation rate. In the enzymatic degradation, the degradation products are separated from the residual samples and dispersed into the surrounding media via the surfactant role of the lipase. Due to the absence of lipase in hydrolysis, more degradation products accumulate in the degradation areas and then results in a wider PDI. The SEM measurements observed the surfactant role of the lipase. Figure 6 shows the morphology changes in the surface of the PTMC₂₅₆ implants degraded with and without lipase as a function of the degradation time.

Figure 6 SEM of the surface of the PTMC₂₅₆ implants before and after degradation without (A) and with (B) lipase.

Obviously, the surface of the PTMC implants degraded without lipase did not change with degradation (Figure 6A). In contrast, small holes appeared on the surface of samples after 1 week of degradation in the presence of lipase (Figure 6B). The appearance of the holes indicated loss of degradation products. As the degradation progressed, the erosion became remarkable, and the size and deepness of the holes increased. This indicated that more degradation products were separated from the bulk with the aid of lipase versus hydrolysis. The SEM results validated this hypothesis and visually demonstrate the surfactant role of the lipase. Figure 7 presents schematics of the hydrolytic and enzymatic degradation of the PTMC implants.

Figure 7 Hydrolytic and enzymatic degradation of the PTMC.

It has been demonstrated that PTMC can be degraded without the formation of the acidic degradation products. There is fair evidence to support this view from the changes in pH of the degradation media. In Figure 8, the pH of the lipase solution was monitored during degradation. As expected, acidification did not occur during degradation. The pH of lipase remained constant near 6.02 unequivocally indicating that no acidic products were formed. Importantly, the release of acidic products was independent of PTMC molecular weight.

Figure 8 The pH profile of lipase solutions after enzymatic degradation of PTMC rods with different molecular weights.

Degradation may cause changes in the thermal response of the polymers [42, 43]. Thus, we measured the implants' thermal properties by DSC and TGA during degradation. The changes in the thermal properties of the PTMC implants as a function of the degradation time and sample molecular weight are listed in Figure 9. As shown in Figure 9A, the Tg of the PTMC₇₂ and PTMC₁₃₅ first increased from the initial values of -15.9 and -15.0 °C to -15.4 and -14.8 °C at 2

weeks and 1 week, respectively. They then decreased during degradation. The increase in T_g was attributed to the anti-plasticization effect of the hydrogen-bonded bridges established by water molecules penetrating the inter-chain spaces of the polymers after hydration. Similar results were reported by other groups [44~48].

Figure 9 The T_g (A) and T_d (B) of PTMC implants as a function of degradation time in 37°C lipase solutions.

As shown in Table 1, the low molecular weight PTMC had higher hydrophilicity, which enhanced the anti-plasticization effect of the hydrogen-bonded bridges and resulted in higher T_g during the early stage of degradation. In contrast to PTMC₇₂ and PTMC₁₃₅, the T_g of the high-molecular weight rods (PTMC₃₂₉) continuously decreased with degradation due to the lack of hydrogen-bonded bridges for their more hydrophobic characteristics..

The thermal stability of the PTMC implants subjected to degradation was monitored with degradation. The changes in T_d of the different molecular weight PTMC implants as a function of degradation time are shown in Figure 9B. The results were normalized with respect to their initial values (T_{d0}). It is clear that the T_d of the samples decreased with degradation, especially the low molecular weight PTMC. This was attributed to the accumulation of the low molecular weight degradation products. For example, the PTMC₇₂ had lost the thermal stability to 83.6% of its initial values after 12 weeks; PTMC₃₂₉ was more stable.

The thermal properties of the PTMC₂₅₆ implants degraded with and without lipase were monitored during degradation (Figure 10). The T_g and T_d of the PTMC₂₅₆ decreased with degradation for both cases. This decrease in the thermal properties was also due to the accumulation of the low molecular weight degradation products. Although there were less degradation products (narrower PDI; Figure 5C), the decrease in T_g and T_d of the enzymolysis samples was much faster than that in hydrolysis samples. This may be due to integration of the

enzymes into the residual samples.

Figure 10 The changes in Tg (A) and Td (B) of the PTMC₂₅₆ after degradation with and without lipase.

The mechanical properties of the PTMC implants also deteriorated with degradation. The mechanical properties (Young's modulus, E ; and tensile strength, σ) of the PTMC₂₅₆ *in vitro* hydrolytic degradation are given in Figure 11. The results were normalized with respect to their initial values (E and σ_0).

Figure 11 The modulus and tensile strength of PTMC₂₅₆ as a function of degradation time in 37°C PBS.

As seen in Figure 11, the Young's modulus and tensile strength of the PTMC₂₅₆ implants decreased during degradation. The modulus and tensile strength decreased to 67.2% and 65.1% of the initial values after 50 weeks. The loss in the mechanical properties of the implants was attributed to the formation of defects induced by the mass loss for hydrolysis samples. A small defect has a large effect on the strength because failure occurs at cracks or holes in the samples. The defects increased with increasing mass loss indicating that the loss in mechanical properties is proportional to the mass loss.

The enzymatic degradation samples were too short to be clamped for tensile testing, and their

mechanical properties were not measured after degradation. However, we did conclude that the mechanical properties of the PTMC rods after lipase treatment decreased much faster than the hydrolysis samples as seen in the degradation rate data (Table 3).

In vivo degradation

The PTMC implants with different molecular weights loaded inside trocars were subcutaneously implanted in the back of rats for pre-clinical studies. The mass loss and molecular weight of the post-degradation samples were monitored and evaluated during degradation (Figure 12).

Figure 12 The mass loss (A) and Mn (B) of PTMC implanted in the back of rats as function of the degradation time

Figure 12A shows that the mass loss was dependent on the molecular weight of the PTMC implants *in vivo*. Higher mass loss was detected for the high molecular weight PTMC. For example, the mass loss of PTMC₃₂₉ was above 94% after 12 weeks. After 32 weeks, PTMC₂₅₆ had lost more than 96% of its mass, but at the same time only 2.18% and 3.67% mass loss was seen for PTMC₇₂ and PTMC₁₃₅, respectively. These results are similar to the enzymolysis data.

Similarly, the molecular weight of the residual samples slight decreased with degradation (Figure 12B) due to the cleavage of the carbonate bonds. The positive mass loss and slight decrease in molecular weight strongly demonstrated that surface erosion occurred during the *in vivo* degradation process.

The *in vivo* degradation rate constants of the PTMC implants with different molecular weights were also calculated based on Equation 4. The linear fitting data for mass loss (Figure 12A) versus degradation time is listed in Table 4.

1

Table 4 *In vivo* degradation rates of PTMC rods determined from the mass loss.

No.	<i>M_n</i>	Rate constant <i>k</i>	Correlation coefficient
	<i>kDa</i>	%/w	<i>R</i>
PTMC ₇₂	72	0.07	0.942
PTMC ₁₃₅	135	0.11	0.959
PTMC ₁₉₄	194	0.92	0.951
PTMC ₂₅₆	256	3.06	0.983
PTMC ₃₂₉	329	8.51	0.990

2 Table 4 presents the *in vivo* degradation rates of the PTMC implants determined from the
3 mass loss as a function of molecular weight. The *in vivo* degradation rate of the high molecular
4 weight PTMC was much higher. For example, the degradation rate of PTMC₃₂₉ was 121 and 77
5 times higher than that of PTMC₇₂ and PTMC₁₃₅, respectively. Similar results have been seen by
6 others [31]. The higher *in vivo* degradation rate of the high molecular weight PTMC was because
7 it was more hydrophobic (Table 1). As stated above, the enzyme activity is high on the
8 hydrophobic surface; enzymes will degrade the high molecular weight PTMC preferentially.

9

10 Figure 13 The water uptake of PTMC implants during the *in vivo* degradation as a function of degradation time.

11 Besides, water uptake occurred during the degradation seems to be an important factor in the
12 degradation of the PTMC implants. The absorbed water molecules could lead to plasticization of
13 the polymer and thus facilitate enzymatic attack. Thus, the degradation rate of PTMC implants
14 that had higher water uptake was faster than that of polymers with lower ones. Figure 13 displays
15 the water uptake profiles of the PTMC implants during the *in vivo* degradation. The water uptake
16 of PTMC₇₂ was about 3.06% after 32 weeks. The PTMC₂₅₆ and PTMC₃₂₉ presented higher water

uptakes of 5.70% and 7.55% after 32 weeks and 10 weeks *in vivo* implantation, respectively. Hence, increased degradation was observed due to the higher water uptake of the PTMC implants with higher molecular weight.

The increase of the water uptake with molecular weight could be attributed to the changes in surface morphology of the PTMC implants during the *in vivo* degradation. The SEM results showed that the changes in surface morphology depend very strongly on the PTMC molecular weight (Figure 14). No significant difference in the surface morphology was seen for low molecular weight PTMC₇₂ before and after *in vivo* implantation (Figure 14A), but the surface of the high molecular weight PTMC₃₂₉ became rough and pitted (Figure 14B). The roughness of the surface increased with the PTMC molecular weight at the same time point. The rough surface could store more water than the smooth ones and then the higher water uptake was observed. Furthermore, the increase in surface roughness with degradation time also indicates that a surface erosion mechanism is occurring during *in vivo* degradation. Similar observation also could be found during the *in vitro* enzymatic degradation.,

Figure 14 SEM of the surface of the low-molecular weight PTMC₇₂ (A) and high-molecular weight PTMC₃₂₉ (B) before and after degradation *in vivo*.

Figure 15 shows the deformation of the PTMC implants during *in vivo* degradation. Similarly, the form-stability of the low molecular weight PTMC *in vivo* was poor. The implants of PTMC₇₂ and PTMC₁₃₅ changed from cylindrical to an oblate sphere at week 4, and the form-stability deteriorated seriously with degradation. Finally, obvious oblates were formed at week 32. The deformation of the amorphous PTMC implants is attributed to the viscous flow caused by lower T_g, and the lower molecular weight that promotes polymers creep at physiological temperatures. Simultaneously, the effective surface area of the implants was at a minimum because of the oblate

1 shape.

Figure 15 The macroscopic observation of the PTMC at different implantation times in the back of rats.

In contrast, the form-stability of the high molecular weight PTMC was better with no formation seen. Hence, the form-stability should be responsible for the significant difference in degradation rate between the low molecular weight and high molecular weight PTMC because of the variation in the surface area. The results indicate that the better the form-stability, the higher the degradation rate. The data confirmed once again that degradation rate of the high molecular weight PTMC was faster due to its better form-stability. Furthermore, these results confirm the *in vitro* enzymolysis case. The good *in vitro/in vivo* correlation of the PTMC degradation indicates that the *in vitro* data offer a clear and concise experimental rationale to better guide *in vivo* studies in future experiments.

Figure 16 The loss in diameter (A) and the relationship between the mass loss and loss in diameter (B) of PTMC₃₂₉ as function of degradation times *in vivo*.

Similar to what was seen for *in vitro* enzymatic degradation, the diameter of the high molecular weight PTMC₃₂₉ implants implanted *in vivo* decreased with degradation time (Figure

16A). The linear relationship between mass loss and loss in diameter shown in Figure 16B also implies that the PTMC implants degraded *in vivo* via surface erosion.

Figure 17 The T_g (A) and T_d (B) of PTMC as a function of degradation time *in vivo*.

Similar to the *in vitro* cases, the *in vivo* degradation also influenced the thermal properties of the PTMC implants. As seen in Figure 17A, the T_g of the PTMC implants increased first in the early stage due to the establishment of hydrogen-bonded bridges. The T_g then decreased with *in vivo* degradation due to the accumulation of low molecular weight degradation products. The thermal stability of the PTMC implants also decreased with accumulation of the degradation products (Figure 17B).

Conclusions

The degradation behavior of PTMC implants was quantitatively studied *in vitro* and *in vivo*. The results indicate that the *in vitro* enzymatic degradation and *in vivo* degradation of PTMC took place via surface erosion. In comparison of the hydrolysis case, the enzymatic degradation rate of PTMC determined from the mass loss data was much higher. We concluded that the lipase acted as a surfactant to promote the dissolution of the degradation products into the surrounding media and thus accelerate the degradation.

The degradation rate of PTMC was dependent on its molecular weight. Higher mass loss and subsequent higher degradation rates were detected for the high molecular weight PTMC. This is because of its higher lipase activity on the more hydrophobic surface of PTMC as the molecular weight increased. The form-stability also influenced the degradation behavior of PTMC. The lower the molecular weight, the worse the form-stability. The decrease in the surface area of the low molecular weight PTMC *in vitro* or *in vivo* causes a lower degradation rate. These results

show that the form-stability and degradation rate of PTMC could readily be tuned by adjusting the molecular weight. Future studies will focus on enhancing the form-stability of the low molecular weight PTMC via chemical cross-linking as well as the degradation behaviors of the resulting cross-linked networks.

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