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Controlled poly(L-lactide-co-trimethylene carbonate) delivery system of cyclosporine A and rapamycine – the effect of copolymer chain microstructure on drug release rate

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ABSTRACT

The effect of poly(L-lactide-co-TMC) chain microstructure (and its changes during degradation) on immunosuppressive drugs' release process was analyzed. Three kinds of poly(L-lactide-co-TMC) (PLATMC) – two semiblock and one random were used to prepare matrices containing cyclosporine A or rapamycine and drug free matrices. All of them degraded slowly enough to provide long term delivery of immunosuppressive agents. Moreover, copolymer chain microstructure determined the effect of drug loading on the degradation process. It was observed that matrices without drug obtained from semiblock copolymer degraded differently than matrices containing cyclosporine A or rapamycine, whereas all kinds of matrices obtained from random PLATMC degraded in similar way. This is the evidence that the only in case of semiblock copolymer factors concerning the presence of drug and the kind of drug influenced degradation process. Based on the obtained results, correlations between copolymer degradation and drug release process are proposed. According to our outcomes, regular drug release process may be obtained from highly randomized copolymers ($R \approx 1$) that remain amorphous during degradation process. Determination of this factor may help in development of biodegradable systems, in which drug release rate and profile can be tailored by synthesis of polymer with appropriate chain microstructure.

1. Introduction

Biodegradable polymers obtained from lactide and trimethylene carbonate (TMC) are interesting materials for applications in medical and pharmaceutical fields. PTMC degrades by surface erosion without acidic products, that could allow to obtain zero order drug release kinetics as well as protection of labile drug molecules (Nair and Laurecin, 2007; Hang et al., 2004). As an amorphous polymer with T_g below body temperature, PTMC is expected to exhibit high permeability to drugs, which makes it promising candidate for drug delivery applications (Pego et al., 2003). Poly(L-lactide) (PLLA) is a crystalline polymer which degrades very slowly. Polylactides undergo hydrolytic degradation via the bulk erosion mechanism by random scission of ester bonds (Nair and Laurecin, 2007). Copolymerization has been widely used to reach desired material characteristics in the final polymers (Pego et al., 2003). Introduction of carbonate linkages into a polymer chain is an effective way to attain a spectrum of properties such as degradation behavior and mechanical performance (Matsumura et al., 1999). Copolymers of TMC with L-lactide may be also interesting in developing alternative delivery systems of agents whose dosage forms cause many side effects, as cyclosporine A (CyA) and rapamycine (sirolimus). CyA is a cyclic undecapeptide, used in prophylaxis and therapy of graft rejection in all types of solid organ and bone marrow transplantation, as well as in treatment of a number of autoimmune diseases. It acts by selective inhibition of interleukin-2 release during the activation of T-cells and causes suppression of the cell-mediated immune response. However, prolonged repeated treatment with CyA may cause many side effects like nephrotoxicity, gingival hyperplasia and neurological disorders (Lallemand et al., 2003; Li et al., 2005). Several controlled delivery systems of CyA have been studied so far: microspheres, nanospheres, and emulsion based on biodegradable aliphatic polyesters including poly(lactide-co-glycolide), polylactide, and poly(ε-caprolactone) (Li et al., 2005; Sinha et al., 2004). Biodegradable matrices with immunosuppressive agent (cyclosporine A or rapamycine) could be administered locally providing sustained, prolonged release. Local

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immunosuppression may reduce the drug specific and general adverse consequences of systemic immunosuppression. Targeting to lymphatics has been suggested as the parameter to improve CyA formulations (Gref et al., 2001; Katayama et al., 1995). The possibility to obtain slow release of CyA after implant administration onto the surface of thoracic duct was reported (Katayama et al., 1995). Rapamycin possesses immunosuppressive properties and is used clinically to prevent acute renal allograft rejection. As mTOR (mammalian target of rapamycin) inhibitor, rapamycin belongs to a new class of immunosuppressants, which in contrast to cyclosporine A does not inhibit calcineurin (Nehaus et al., 2001; Koehl et al., 2005). However, it may cause toxic effects such as hyprlipidaemia and tromboleukopaenia (Johnson, 2003). Studies have been reported on the release of rapamycine from biodegradable matrices made of poly(D,L-lactide) and poly(D,L-lactide-co-glycolide) (Alexis et al., 2004). Most studies concerning this agent are related to rapamycine eluting stents, because it has been shown to inhibit vascular smooth muscle cell proliferation and migration (Wilensky and Klugherz, 2005; Marx and Marks, 2001; Pan et al., 2007).

Poly(L-lactide-co-trimethylene carbonate) (PLATMC) has not been considered as delivery system of cyclosporine A or rapamycine, so far. The aim of this study was to determine the usefulness of this kind of copolymer as carrier of immunosuppressive drugs as well as to elucidate the effect of PLATMC chain microstructure on cyclosporine A and rapamycine release behaviors. Determination of this factor may help in development of biodegradable systems, in which drug release rate and profile can be tailored by synthesis of polymer with appropriate chain microstructure. In fact, the final properties of a copolymer, including thermal and mechanical properties, biodegradability, are strongly dependent on the chain microstructure (Lee et al., 2005; Hua et al., 2009). It has been reported that copolymers with designed morphologies (semicrystalline, multiblock, and highly randomized structures) can be achieved by using zirconium (IV) acetylacetonate as non-toxic initiator at various reaction temperatures. Relatively low temperature (about 110 °C) minimizes intermolecular transesterification and allows obtaining semicrystalline copolymers with multiblock structure. Higher temperature (up to 180 °C) favors transesterification and in consequence, amorphous copolymers are obtained with highly randomized chain structures (Dobrzyński and Kasperczyk, 2006). The influence of the PLAGA and PLACL chain microstructure on release process of doxorubicine was confirmed (Kasperczyk et al., 2009). Preliminary studies on PLATMC and PLACL matrices showed the importance of copolymer chain mictrostructure in determining the cyclosporine A and rapamycine release profiles (Kasperczyk et al., 2006). However, in the aim of determination of the factors that can be used to tailor degradation process and drug release kinetics, few copolymers of the same kind that differ in chain microstructure should be taken into account.

2. Materials and methods

2.1. Synthesis of copolymers

Three kinds of poly(L-lactide-co-TMC) (PLATMC) were used to prepare matrices containing cyclosporine A or rapamycine: two semiblock (PLATMC 28:72 and PLATMC 72:28) and a random PLATMC 72:28. Copolymers were synthesized according to the method described in literature (Dobrzyński and Kasperczyk, 2006). Briefly, the copolymers were obtained by ring-opening polymerization of TMC and L-lactide in the presence of low toxic $Za(Acac)_4$ as initiator. Copolymerization was performed in bulk at 110 °C (PLATMC 28:72; semiblock PLATMC 72:28) or 120 °C (random PLATMC 72:28) in sealed glass ampoules for 72 h. The obtained copolymers were dissolved in dichloromethane, precipitated with methanol, and dried at 50 °C under vacuum up to constant weight.

2.2. Characterization of copolymers

The molecular weights and molecular weight distribution of the copolymers were determined by gel permeation chromatography with a Physics SP 8800 chromatograph equipped with Styragel columns and Shodex SE 61 detector. Tetrahydrofuran was used as the eluent, and the flow rate was 1 mL/min. The molecular weights were calibrated with polystyrene standards.

The composition of the copolymers was determined by ^{1}H NMR and ^{13}C NMR spectroscopy. ^{1}H NMR spectra were recorded at 600 MHz and ^{13}C NMR at 125 MHz with AVANCE II Ultra Shield Plus, Bruker 600 MHz spectrometer and a 5-mm sample tube. $CDCl_3$ was used as solvent. The spectra were obtained at 28 °C with 32 scans, 3.74 s acquisition time and 7 μs pulse width for ^{1}H NMR, and 30,000 scans, 1.8 s acquisition time, 9 μs pulse width and 3 s delay time between pulses for ^{13}C NMR.

The thermal properties were examined by differential scanning calorimetry (DSC) with a TA DSC 2010 apparatus (TA Instruments, New Castle, DE) calibrated with high purity indium and gallium. The samples were scanned from −20 °C to 220 °C at a heating rate of 20 °C/min and then quenched into liquid nitrogen.

2.3. In vitro release of cyclosporine A and rapamycine

Three kinds of matrices (matrices with 10% of cyclosporine A, 10% of rapamycine and without drug) were prepared by solution of each kind of copolymer in methylene chloride (Aldrich). After solvent evaporation at ambient temperature, the matrices were dried under reduced pressure to yield samples with 1.2 cm diameter and 0.5 mm thickness. The drug load in matrices (ca. 10% of copolymer content) was confirmed by means of UV–vis spectrometry.

The various samples were immersed in phosphate buffer saline (PBS) and incubated at 37 °C. At regular time periods (every third or fourth day), the solution was renewed and the drug concentration was determined. After 14, 35, 70 and 182 days of experiment, the matrices were withdrawn for the monitoring of degradation.

The concentration of released drug during the 227 days' period was determined by means of UV–VIS spectroscopy (Spectrophotometer V-570, UV-VIS–NIR–JASCO). The PBS solution was also renewed in the case of drug free matrices, and was taken as reference.

2.4. Microstructure characterization of copolymers during in vitro drug release

Copolymers microstructure was characterized at the beginning and during degradation process based on the parameters determined from ^{1}H and ^{13}C NMR spectra: percentage content of lactidyl (F_{LL}) and carbonate (F_T) units in copolymer; the average length of lactidyl (l_{LL}) and carbonate (l_T) blocks in copolymer chains and randomization ratio (R) (Dobrzyński and Kasperczyk, 2006).

3. Results and discussion

3.1. Characterization of copolymers

Three PLATMC copolymers were synthesized to evaluate the influence of comonomer molar ratio and copolymer chain microstructure on drug release profiles: two semiblock copolymers with reverse comonomer compositions, PLATMC 28:72 (R = 0.57) and PLATMC 72:28 (R = 0.5), and a third copolymer, PLATMC 72:28 (R = 0.85) which had the same composition as PLATMC 72:28

Table 1
Characterization of poly(L-lactide-co-trimethylene carbonate) matrices (R – randomization ratio; l_{LL}, l_T – the average length of lactidyl and carbonate blocks; M_n – number-average molecular mass; I_p – polydispersity index ($I_p = M_w/M_n$); T_g – glass transition temperature; T_m – melting temperature; I/M – initiator to monomer molar ratio).

Copolymer	Average length of the blocks	R	M_n (kDa)	I_p	T_g(°C)[a]	T_m(°C)[b]	Copolymerization conditions		
							I/M	Temp.(°C)	Time(h)
PLATMC (28:72) (R = 0.57)	l_{LL} = 1.6 l_T = 4.1	0.57	17.5	2.0	8.0	–	1/800	110	72
PLATMC (72:28) (R = 0.5)	l_{LL} = 6.3 l_T = 2.4	0.5	36.0	2.6	42.3	159	1/800	110	72
PLATMC (72:28) (R = 0.85)	l_{LL} = 3.9 l_T = 1.5	0.85	86.7	2.2	31.4	151	1/1000	120	72

[a] Obtained by DSC (second heating).
[b] Obtained by DSC (first heating).

(R = 0.5), but was synthesized at higher temperature. The copolymers were characterized in detail by using ^{1}H and ^{13}C NMR, DSC and GPC. Table 1 presents the characteristics of the 3 copolymers used to prepare matrices with immunosuppressive drugs. The Mn of PLATMC 72:28 (R = 0.85) is 86,700 with a polydispersity index (I_p) of 2.2, while the M_n of PLATMC 28:72 and PLATMC 72:28 (R = 0.5) are much lower. It is also noted that PLATMC 72:28 (R = 0.5) presents higher I_p value (I_p = 2.6). The average length of the lactidyl and TMC blocks was determined from ^{13}C NMR. PLATMC 72:28 (R = 0.5) exhibits a semiblock structure with l_{LL} = 6.3 and l_T = 2.4. With the same gross composition, PLATMC 72:28 (R = 0.85) presents a more random structure with l_{LL} = 3.9 and l_T = 1.5. This finding is assigned to the higher reaction temperature in the case of PLATMC 72:28 (R = 0.85). On the other hand, PLATMC 28:72 (R = 0.57) presents the lower l_{LL} and higher l_T values as compared to PLATMC 72:28. DSC shows that PLATMC 28:72 (R = 0.57) is an amorphous polymer with a glass transition temperature (T_g) at 8 °C (Table 1). In contrast, both PLATMC 72:28 (R = 0.5) and PLATMC 72:28 (R = 0.85) are semicrystalline with T_g at 42 °C and 31.4 °C and melting temperature (T_m) at 159 °C and 151 °C, respectively.

Fig. 1 shows the ^{13}C NMR spectra of the copolymers. TLT sequence (signal 2) was detected in all cases, indicating the occurrence of second mode transesterification during copolymerization.

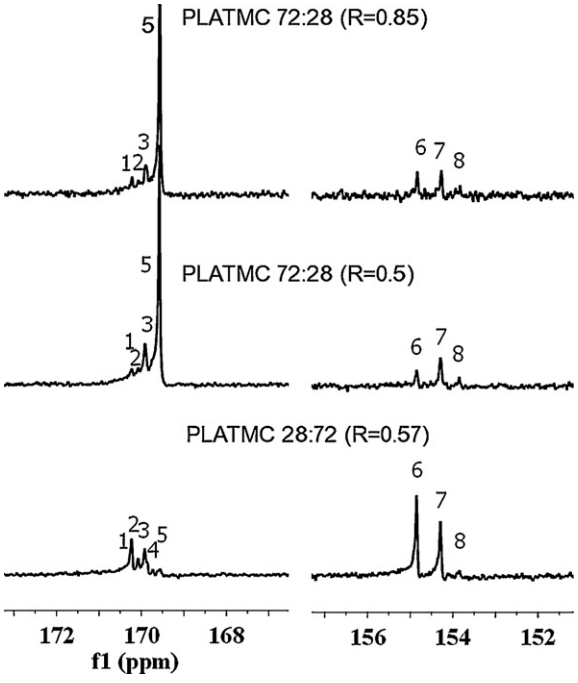

Fig. 1. ^{13}C NMR spectra of copolymers used to prepare matrices with immunosuppressive drugs. Carbonyl carbon region of lactyl unit: 1 – TLLT + LLLLT + TLLLT; 2 – TLT; 3 – TLLT + TLLLL + TLLLT + LLLLT; 4 – TLLLT + TLLLL; 5 – LLLLL; Carbonyl carbon region of carbonate unit 6 – TT = TTT + LLTT + TLTT; 7 – TLL = TTLL + LLTLL + TLTLL; 8 – TLT = TTLT + LLTLT + TLTLT.

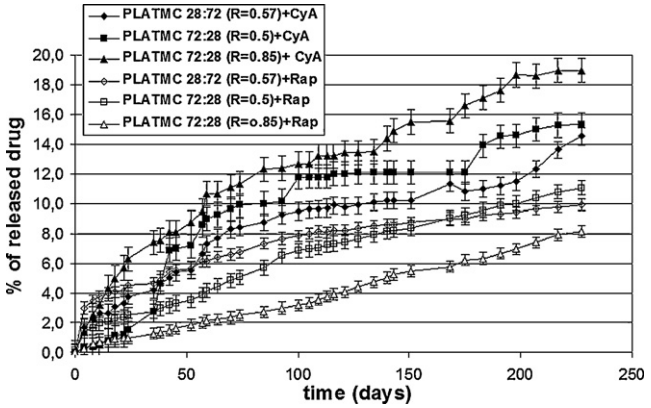

Fig. 2. Cumulative release of cyclosporine A and rapamycine from different PLATMC matrices during 227 days. Each point represents the mean ± SD of three points.

3.2. Cyclosporine A and rapamycine release profiles and changes in matrices microstructure during degradation

Detailed analysis of drug release process (Figs. 2, 3 and 5) and changes in copolymer chain microstructure during degradation (Tables 2, 3 and 4, Fig. 4) of the 3 PLATMC matrices containing CyA or rapamycine has been conducted.

3.2.1. Matrices with immunosuppressive drugs obtained from PLATMC 28:72 (R = 0.57)

After the burst effect, cyclosporine A was released steadily from PLATMC 28:72 (R = 0.57) matrices (Figs. 2 and 3). Between day 14 and 35, the drug was released in range of 67 μg/1 g of copolymer to 169.87 μg/1 g of copolymer. The amount of released CyA increased to 130.6–263.5 μg/1 g of copolymer from day 57 and 68. This was probably due to significant degradation of lactidyl units. In fact, the lactidyl content decreased from 28% to 23% in this period (Table 2). After 70 days, cyclosporine A was released very evenly, as a consequence of insignificant changes in copolymer chain microstructure.

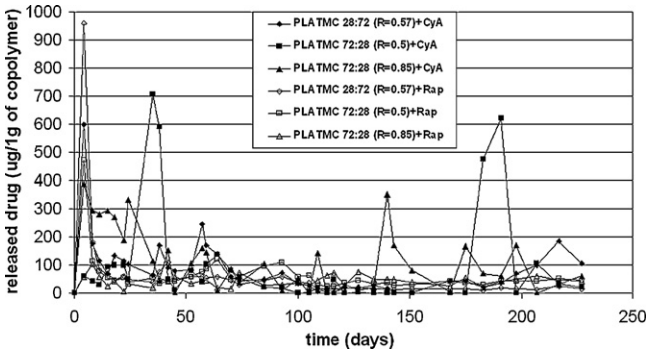

Fig. 3. Release profile of cyclosporine A and rapamycine from different PLATMC matrices during 227days. Each point represents the mean of three points.

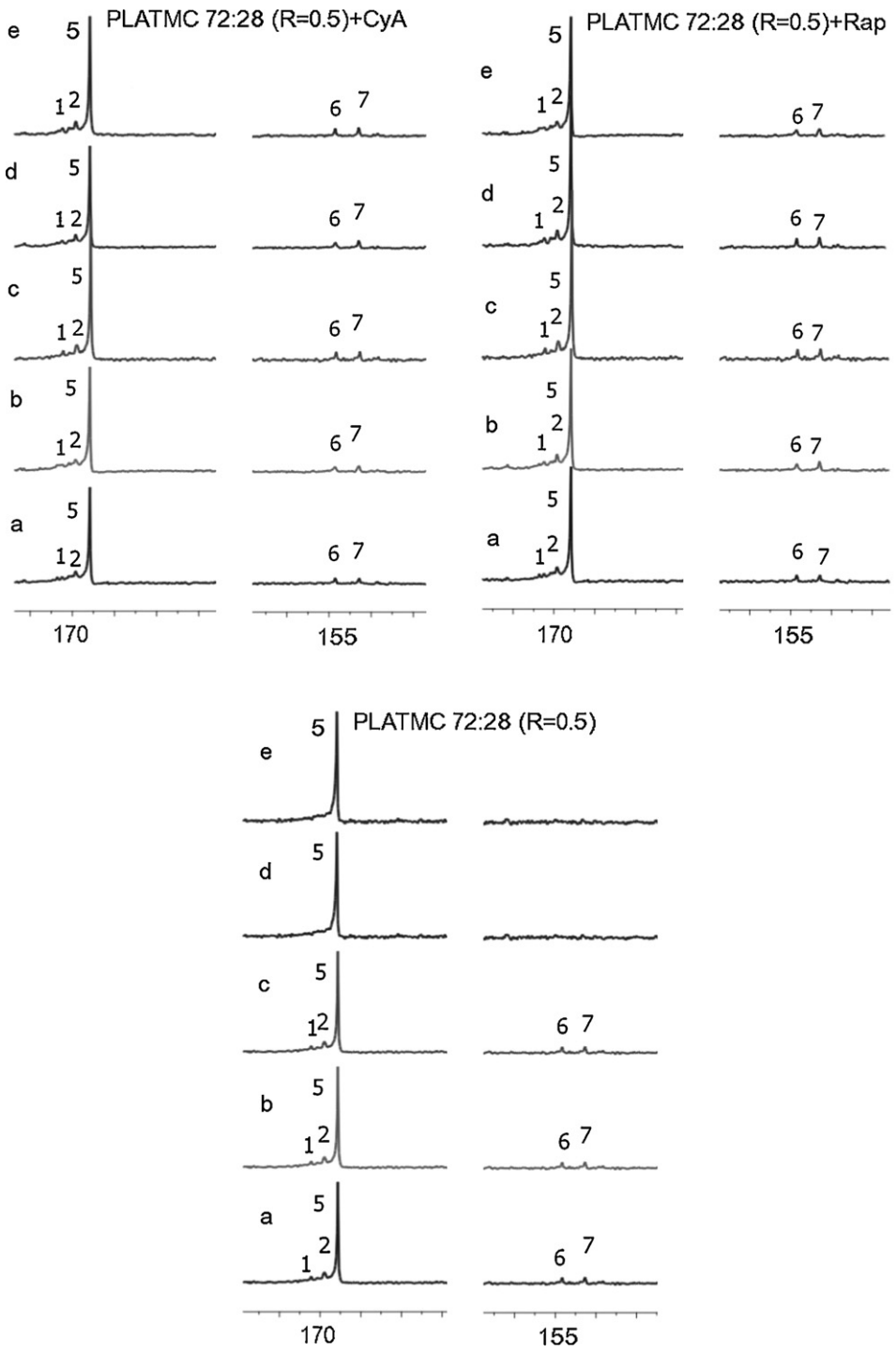

Fig. 4. ^{13}C NMR spectra of matrices made of PLATMC 72:28 ($R = 0.5$): with CyA; with rapamycine and without drug, before degradation (a) and after 14 (b), 35 (c), 70 (d) and 182 (e) days of degradation. Carbonyl carbon region of lactyl unit: 1 – TLL̲T + LLLL̲T + TLLL̲T; 2 – TL̲T; 3 – TL̲LT + TL̲LLL + TL̲LLT + LL̲LLT; 4 – TL̲LLT + TL̲LLL; 5 – LL̲LLL; Carbonyl carbon region of carbonate unit 6–TT = TTT + LLTT + TLTT; 7–TLL = TTLL + LLTLL + TLTLL; 8–TLT = TTLT + LLTLT + TLTLT.

Similarly, the burst effect was also observed in case of matrices containing rapamycine (Figs. 2 and 3), followed by stabilization of drug release after day 10. Simultaneously, slight decrease of lactidyl units was observed, without significant changes of the chain microstructure.

Degradation of drug containing and drug free matrices proceeded differently. In case of drug free matrices, the content of lactidyl units decreased by 9% between day 70 and 182 (Table 2), in agreement with literature data (Hua et al., 2009). In fact, it was reported that lactidyl component is preferentially degraded as compared to TMC one in the amorphous zones, leading to lactidyl content decrease (Hua et al., 2009). In the meantime, l_{LL} decreased and l_T increased, and the degree of randomness increased. The decrease of lactidyl content was less important in case of drug containing matrices. Little changes of chain microstructure are observed (Table 2). The regular degradation apparently favored steady drug release profiles. It should also be noted that more CyA than rapamycine was released probably due to the higher solubility of the former.

Table 2
Microstructure of PLATMC 28:72 (R = 0.57) matrices: without drug; containing cyclosporine A or rapamycine (F_{LL}, F_T – percentage content of lactidyl and carbonate unit l_{LL}, l_T – average length of lactidyl and carbonate sequences; R – randomization ratio) after 14, 35, 70, 105 and 182 days of degradation.

Matrix	Time (days)	F_{LL}	F_T	l_{LL}	l_T	R
PLATMC 28:72 (R- = 0.57)	0	28	72	1.6	4.1	0.57
	35	27	73	1.5	4.1	0.57
	70	27	73	1.5	3.9	0.59
	182	18	88	1.2	5.4	0.61
PLATMC 28:72 (R- = 0.57) + CyA	0	28	72	1.6	4.1	0.57
	14	27	73	1.5	4.2	0.58
	35	28	72	1.5	4.0	0.57
	70	23	77	1.4	4.7	0.56
	182	24	76	1.4	4.5	0.57
PLATMC 28:72 (R- = 0.57) + Rap	0	28	72	1.6	4.1	0.57
	14	27	73	1.6	4.1	0.56
	35	27	73	1.5	4.2	0.57
	70	26	74	1.5	4.3	0.56
	182	26	74	1.5	4.3	0.58

Table 3
Microstructure of PLATMC 72:28 (R = 0.5) matrices: without drug; containing cyclosporine A or rapamycine (F_{LL}, F_T – percentage content of lactidyl and carbonate unit l_{LL}, l_T – average length of lactidyl and carbonate sequences; R – randomization ratio) after 14, 35, 70, 105 and 182 days of degradation.

Matrix	Time (days)	F_{LL}	F_T	l_{LL}	l_T	R
PLATMC 72:28 (R = 0.5)	0	72	28	6.3	2.4	0.5
	35	72	28	6.3	2.4	0.49
	70	74	26	6.3	2.2	0.52
	182	87	13	11.9	1.8	0.60
PLATMC 72:28 (R = 0.5) + CyA	0	72	28	6.3	2.4	0.5
	14	72	28	5.4	2.1	0.57
	35	72	28	5.0	1.9	0.62
	70	71	29	6.4	2.6	0.47
	182	72	28	5.2	2.0	0.60
PLATMC 72:28 (R = 0.5) + Rap	0	72	28	6.3	2.4	0.5
	14	72	28	4.8	1.9	0.64
	35	72	28	5.6	2.2	0.54
	70	72	28	6.1	2.4	0.50
	182	73	27	5.8	2.1	0.54

3.2.2. *Matrices with immunosuppressive drugs obtained from more blocky PLATMC 72:28 (R = 0.5)*

Interestingly, the burst effect observed for PLATMC 28:72 (R = 0.57) was not observed in case of PLATMC 72:28 (R = 0.5) matrices with CyA (Fig. 2). Increased amount of released CyA was noticed

Table 4
Microstructure of PLATMC 72:28 (R = 0.85) matrices: without drug; containing cyclosporine A or rapamycine (F_{LL}, F_T – percentage content of lactidyl and carbonate unit l_{LL}, l_T – average length of lactidyl and carbonate sequences; R – randomization ratio) at the beginning and after 14, 35, 70, 105 and 210 days of degradation.

Matrix	Time (days)	F_{LL}	F_T	l_{LL}	l_T	R
PLATMC 72:28 (R = 0.85)	0	72	28	3.9	1.5	0.85
	35	71	29	3.7	1.5	0.80
	70	71	29	3.2	1.3	0.91
	105	71	29	3.3	1.4	0.88
	210	69	31	3.1	1.4	0.87
PLATMC 72:28 (R = 0.85) + CyA	0	72	28	3.9	1.5	0.85
	35	69	31	2.9	1.3	0.93
	70	69	31	2.9	1.3	0.95
	105	69	31	3.1	1.4	0.89
	210	71	29	3.2	1.3	0.92
PLATMC 72:28 (R = 0.85) + Rap	0	72	28	3.9	1.5	0.85
	35	71	29	3.3	1.3	0.90
	70	68	32	3.0	1.4	0.87
	105	70	30	3.3	1.4	0.85
	210	72	28	3.3	1.3	0.85

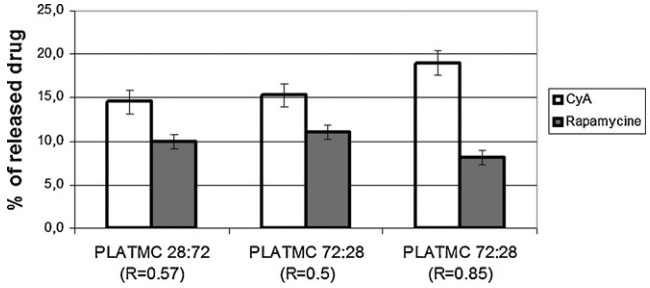

Fig. 5. Comparison of the amount of released immunosuppressive drugs from poly(L-lactide-co-TMC) matrices during 227 days. Each point represents the mean ± SD of three points.

at day 36 (706.6 μg/1 g of copolymer). Between day 35 and 70, changes in copolymer microstructure were detected with increase of l_{LL}, l_T, and decrease of randomization ratio (Table 3, Fig. 4). The increase of randomness between day 70 and 182 caused stabilization of CyA release first, but after 100 days (especially between day 134 and 174) decrease or inhibition of drug release. After day 175, the drug was released unevenly.

More regular release profile of rapamycine was observed, except from 6 days at the beginning, when more drug was released (at an average of 473.1 μg/1 g of copolymer) (Fig. 3). Increase of the average length of both lactidyl and carbonate blocks was detected between day 35 and 70 (Table 3, Fig. 4), together with some fluctuation of the randomness.

It appears that drug containing matrices degraded differently from drug free matrices (Table 3, Fig. 4). In the latter case, carbonate units were preferentially degraded. After 182 days, the LA:TMC unit ratio increased to 87:13. This phenomenon is typical of PLATMC with high contents of lactidyl units (Hua et al., 2009). As shown in ^{13}C NMR spectrum (Fig. 4), the signals arising from random sequences of carbonyl carbons of lactyl and carbonate units disappeared beyond 70 days of degradation. Only signals arising from LLLLL sequences remained. On the contrary, comonomer molar ratio in drug containing matrices did not change significantly during the degradation process, and no significant increase of the average length of lactidyl units was observed. Apparently, incorporation of drug molecules into the polymeric matrice favored more uniform degradation of both components.

3.2.3. *Matrices with immunosuppressive drug obtained from more random PLATMC 72:28 (R = 0.85)*

More random PLATMC 72:28 (R = 0.85) was used in comparison with the degradation process and drug release profile of semiblock PLATMC 72:28 (R = 0.5). Table 4 presents changes of copolymer chain microstructure of PLATMC 72:28 (R = 0.85) matrices with CyA, rapamycine and without drug.

Similarly as in case of PLATMC 72:28 (R = 0.5), the burst effect was not observed in PLATMC 72:28 (R = 0.85) matrices with CyA (Figs. 2 and 3). The process proceeded unevenly from day 70 to 105. In the meantime, increase of the average lengths of lactidyl and carbonate units was detected (Table 4). Fluctuations in cyclosporine A release (from 0 to 352 μg per 1 g of copolymer) were observed after day 105.

Much more even release profile was observed in case of matrices with rapamycine (Figs. 2 and 3). The maximum was noted at day 8 (103.86 μg per 1 g of copolymer) and the lowest amount at day 22 (5.65 μg per 1 g of copolymer). However, as shown in Table 4, there were not significant changes in chain microstructure and copolymer composition from the beginning until day 210.

Similar degradation behaviors were observed for the 3 matrices obtained from random PLATMC 72:28 (R = 0.85) (Table 4), in contrast to semiblock PLATMC 72:28 (R = 0.5). The 3 PLATMC 72:28

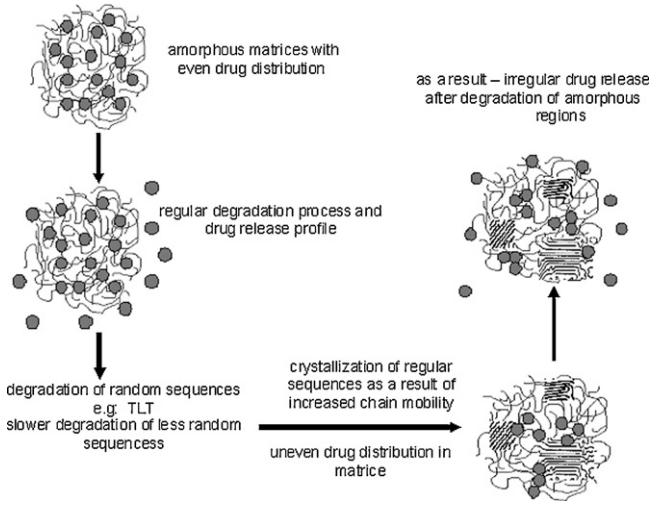

Fig. 6. Schematic presentation of the influence of copolymer chain microstructure changes on the drug release profile.

($R = 0.85$) matrices exhibited almost equal removal of carbonate and lactidyl units during degradation. In case of drug free matrices, slightly faster degradation of lactidyl units was observed (3% decrease after 210 days).

3.3. Comparison of cyclosporine A and rapamycine release profiles

The release profiles of immunosuppressive drugs from the 3 PLATMC copolymers (two semiblock and one random) have been analyzed during 227 days (Fig. 5). Slow degradation was observed in all cases, thus showing that they are promising candidates for long term delivery of immunosuppressive agents.

Similar amounts of released drugs were determined for PLATMC 28:72 ($R = 0.57$) and PLATMC 72:28 ($R = 0.5$) (respectively: 14.5% and 15.3% of CyA and 9.9% and 11.1% of rapamycine), despite the different copolymer compositions. Both copolymers exhibited a semiblock structure, which determined their degradation process and simultaneously drug release rate. The highest amount of cyclosporine A was released from more random PLATMC 72:28 ($R = 0.85$). In case of semiblock PLATMC matrices, even drug release profile was observed only in case of PLATMC 28:72 ($R = 0.57$) whose degradation proceeded in regular way with little changes of randomization ratio. In case of PLATMC 72:28 ($R = 0.5$), fluctuations of released drug appeared along with decrease of copolymer randomness. On the other hand, more random PLATMC 72:28 ($R = 0.85$) synthesized at higher temperature presents different drug release behaviors. Degradation of short sequences allowed to release more CyA and had an impact on the even rapamycine release.

Interestingly, in case of matrices obtained from semiblock copolymers (PLATMC 28:72 ($R = 0.57$) and PLATMC 72:28 ($R = 0.5$)), drug free matrices degraded differently from those containing cyclosporine A or rapamycine. In case of random PLATMC 72:28 ($R = 0.85$), all matrices degraded in similar way. No difference was observed in case of random copolymer matrices which exhibited more regular degradation process. Apparently, the presence of drug molecules in matrices made of semiblock copolymer slowed down degradation process. In fact, it was reported that interactions between polymer carboxyl endgroups and basic drugs can modify the degradation and drug release rates. Two opposing effects have been observed. Drug release may be accelerated, when basic drugs behave as a base catalyst acting on the ester bonds in polymer chains, which enhances the polymer degradation and drug release. However, drug release may also be delayed, when basic drugs neutralize carboxyl endgroups, thereby decreasing the autocatalytic effect of the acidic chain ends on polymer degradation and water penetration into the matrix (Frank et al., 2005; Miyajima et al., 1999). In case of caffeine base, the effect of drug on polymer degradation was dependent of its content. Matrices with low content of caffeine ($\leq$2%) accelerated considerably the degradation with respect to caffeine-free devices (Li et al., 1996). Thus, this work shows that the effect of drug molecule on degradation process is also dependent on copolymer microstructure.

4. Conclusions

Detailed analysis of changes in PLATMC chain microstructure during degradation process and its influence on CyA and rapamycine release has been conducted. Three kinds of PLATMC were used to prepare matrices containing cyclosporine A or rapamycine: two semiblock (PLATMC 28:72 ($R = 0.57$); PLATMC 72:28 ($R = 0.5$)) and one random (PLATMC 72:28 ($R = 0.85$)). All of them degraded slowly enough to provide long term delivery of immunosuppressive agents. Moreover, copolymer chain microstructure determined the effect of drug loading on the degradation process. In case of matrices obtained from semiblock PLATMC 28:72 ($R = 0.57$) and PLATMC 72:28 ($R = 0.5$), drug free matrices degraded differently from those containing cyclosporine A or rapamycine. In case of random PLATMC 72:28 ($R = 0.85$), similar degradation behaviors are observed for all kinds of matrices. Based on the obtained results, correlations between copolymer degradation and drug release process are proposed (Fig. 6). Amorphous matrices with even drug distribution provide regular degradation and drug release process. Decrease of randomization ratio caused uneven drug release. It was reported that initially amorphous copolymers containing larger amounts of lactidyl units are able to crystallize during degradation because of the presence of relatively long LLA blocks (Hua et al., 2009). This may cause irregular drug release or fluctuations after degradation of regions surrounding accumulated drug molecules. It is thus concluded that regular drug release process may be obtained from highly randomized copolymers ($R \approx 1$). The copolymers should undergo regular degradation without rapid changes of randomness, because significant decrease of randomization ratio can cause fluctuations in drug release. Initially amorphous copolymers can remain amorphous during degradation because of highly random units' distribution (Hua et al., 2009). These kinds of copolymers can be obtained by varying reaction conditions that stimulate transestrification of the second mode (e.g. temperature and/or time increase). It has been shown that using zirconium (IV) acetylacetonate as initiator of L-lactide and TMC copolymerization allows to obtain copolymers of practically desired composition (Dobrzyński and Kasperczyk, 2006).

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