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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