DEGRADATION ANALYSIS OF RADIOSENSITIZER CONTROL RELEASE SYSTEM BASED ON THE NMR SPECTROSCOPY

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Abstract

The degradation process of multilayer radiosensitizer releasing systems was conducted and examined via the nuclear magnetic resonance spectroscopy. Copolymers of glycolide and lactide with different chain microstructure were used as biocompatible drug carriers. Metronidazole was used as radiosensitizer. The changes of copolymers chain microstructure were monitored during 16 weeks of hydrolytic degradation of material in artificial Cerebro-spinal Fluid Solution. This study shows distinct differences in the rate of copolymer degradation and changes of the degree of randomness, directly connected with the type of comonomers and the comonomeric molar ratio. During 16 weeks of hydrolytic degradation, copolymers with L-lactidyl units remain resistant to hydrolysis while materials with D,L-lactidyl units are completely degraded. Examined materials can be used as carriers of agents for different types of short-, mid- and long-term therapies based on control release systems.

Keywords: biodegradable polyesters, NMR spectroscopy, hydrolytic degradation, polymeric chain microstructure

[Engineering of Biomaterials 113 (2012) 16-18]

Introduction

Among great variety of biomedical materials compatible with human tissues, aliphatic polyesters especially the poly(lactide-co-glycolide) copolymers are considered one of the most valuable and commonly used. Undergoing both hydrolytic and enzymatic degradation to glycolic acid and lactic acid they can be easily metabolized and eliminated by most human cells. It has been proved that PLGA chain microstructure determines the rate of polymer degradation and can be modified or even defined by proper conditions of material synthesis. Such factors as temperature, time, the type of polymerization (bulk, solution), type and amount of initiator used significantly influence the properties of synthesized material and its degradation time [1-3]. The high biocompatibility of PLGA combined with designable material properties make this copolymer suitable as carrier for creating drug control release systems. In our previous study the radiosensitizer metronidazole release system was tested in the *in vitro* conditions and the drug release profiles from different type of carriers were compared.

The purpose of this system is to be placed directly onto the brain tumor tissue to bypass the blood-brain barrier and obtain stable radiosensitizer release after proper amount of time without causing any toxic interactions within the brain tissue [4]. In this study, further hydrolytic degradation analysis of three different PLGA copolymers was carried out in order to detect and describe changes in copolymer chain for selecting the optimal material for brain glioma radiotherapy.

Materials and methods

Polymeric materials poly(glycolide-co-D,L-lactide) and poly(glycolide-co-L-lactide) were synthesized in Centre of Polymer and Carbon Materials PAScs, Zabrze by the ring-opening copolymerization held in bulk, using $Zr(acac)_4$ as nontoxic initiator. Molar ratio of comonomers/initiator was 1000:1. Obtained copolymers were dissolved in chloroform, precipitated in methyl alcohol and next dried at 25°C under reduced pressure to constant weight.

Artificial Cerebro-spinal Fluid Solution (aCFS) was prepared according to producer's (Alzet) instructions by stirring proper amounts of NaCl, KCl, $CaCl_2$, $MgCl_2 \times 6H_2O$, $Na_2HPO_4 \times 7H_2O$ and $NaH_2PO_4 \times H_2O$ with distilled water.

The initial copolymers and degradation products composition was confirmed by the ^{1}H-NMR analysis and the chain microstructure was examined by the ^{13}C-NMR measurement (600 MHz on AVANCE II Ultra Shield Plus, Bruker) using DMSO as a solvent.

The copolymers molecular masses M_n and M_w were investigated by gel permeation chromatography (GPC, Physics SP 8800 chromatograph) with the use of chloroform as a solvent.

The copolymers glass-transition temperature T_g was determined by differential scanning calorimetry (TA DSC 2010 apparatus, TA Instruments, New Castle, DE).

Mono and triple layered radiosensitizer release systems containing metronidazole were obtained as previously described [4] and put into glass ampoules filled with amount of aCFS proportional to their weight (1 ml of aCFS per 15 mg of polymer). Material samples were gathered every two weeks and the whole amount of buffer was changed every single week to maintain the dynamic system similar to *in vivo* conditions. Incubation with constant ampoules shaking was held at 37°C (Memmert Precision Incubator INE 400) for 16 weeks.

Results and Discussion

Three different samples referring to three PLGA copolymeric materials were examined: A1 (PLA:GA, comonomeric ratio 84:16), B1 (PDLA:GA, comonomeric ratio 84:16), C1 (PDLA:GA, comonomeric ratio 47:53). The material properties are shown in TABLE 1. All materials were obtained in presence of $Zr(acac)_4$ as initiator and show semibloc chain microstructure (FIG. 1), with the degree of randomness (R) varied from 0,37 for A1 to 0,61 for B2 (TABLE 1).

TABLE 1. Characterization of obtained materials.

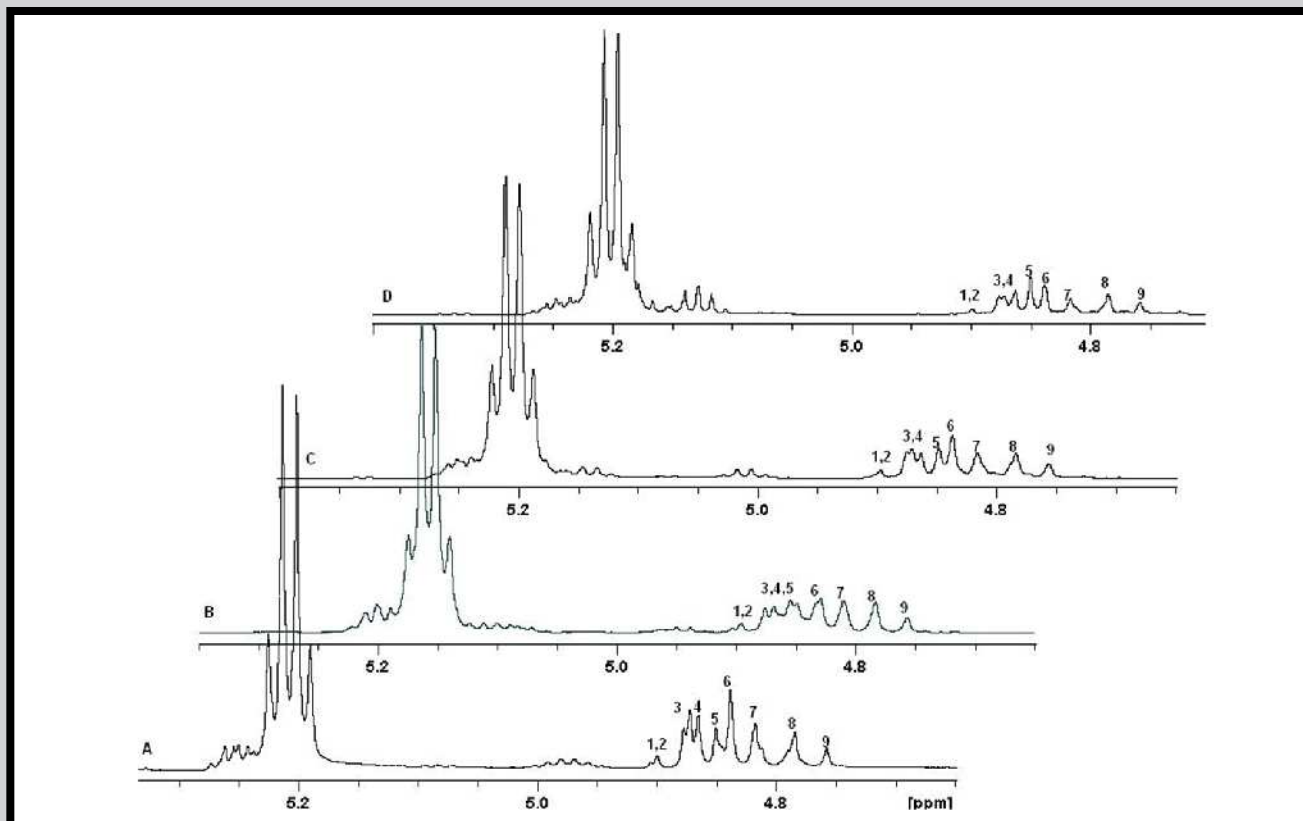| Sample	A1	B1	B2
material	PLA:GA	PDLA:GA	PDLA:GA
molar ratio	84:16	84:16	47:53
T_g [°C]	60.6	57.1	51
M_n [Da]	75 300	63 500	46 400
M_w [Da]	254 700	142 800	99 200
M_w/M_n	3.38	2.25	2.14
R	0.37	0.47	0.61

FIG. 1. NMR spectra of A1 PLA/GA,84/16 after 0 (A), 4 (B), 8 (C) and 16 (D) weeks of degradation. Methylene proton range of lactidyl units 5.1-5.3 ppm, methylene proton range of glycolidyl units 4.75-4.9 ppm. Sequences: 1- GLGGG or GGGLG, 2- LGGLG or GLGGL, 3- GGGGG, 4- LLGGL + LGGLL, 5- GGGGL + LGGGG, 6- LLGGG + GGGLL, 7- LLGLL + GLGLL + LLGLG + GLGLG, 8- GGGLG or GLGGG, 9- LGGGL + GLGGL or LGGLG.

TABLE 2. Lactidyl and glycolidyl units content changes during degradation.

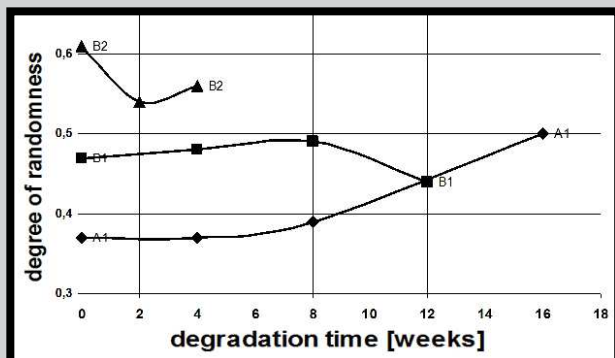| Sample	Degradation time [weeks]	Lactidyl units LL [mole %]	Glycolidyl units GG [mole %]
A1	0	84	16
	4	86	14
	8	88	12
	16	89	11
B1	0	84	16
	4	87	13
	8	94	6
	12	85	15
B2	0	47	53
	2	54	46
	4	69	31
	8	45	55

FIG. 2. Changes of degree of randomness during PLGA degradation.

The highest R value in the B2 sample (PDLA:GA, 47:53) results directly from the comonomeric ratio of this copolymer. With almost even amounts of comonomers, B2 possess the highest chance of random distribution of glycolidyl and lactidyl units in the copolymer chain during the synthesis. Similar results were described before [5] for the $Zr(acac)_4$ initiator. Sample A1 containing L-lactidyl units possess the lowest degree of randomness and also has the highest molar masses and polymeric dyspersion (M_w/M_n) coefficient. During the 16 weeks of hydrolytic degradation, the slowest degradation rate for A1 sample was observed. Sample A1 also maintain the disc shape for the whole examined period. The changes in lactidyl and glycolidyl units content N_{LL} and N_{GG} (TABLE 2) indicate faster but stable degradation of glycolidyl units in comparison to L-lactidyl units in A1 copolymer. It is confirmed by slowly decrease of average glycolidyl blocks length (L_{GG}) (FIG. 3) and insignificant changes in average length of lactidyl blocks (L_{LL}) (FIG. 4). The L_{GG} value decreases steadily for the whole experimental period and confirms stable degradation of glycolidyl units in the A1 sample. On the contrary, the L_{LL} values increase during 8th week of degradation experiment and then decrease to the initial value in 16th week. Probably, in the first period of degradation only the short, alternating blocks of GLG type (formed by transestryfication reaction during copolymerization) are degrade and in consequence both L_{LL} and N_{LL} values increase. After 8 weeks, the hydrolization of longer lactidyl blocks begins but still glycolidyl blocks degrade faster so the L_{LL} value decrease and N_{LL} increase to 89% [6-9].

Both copolymeric materials containing D,L-lactidyl units B1 and B2 degrade faster than A1 sample containing only L-lactidyl units which is proved by the split of initial disc-shape matrices into smaller various-shape pieces.
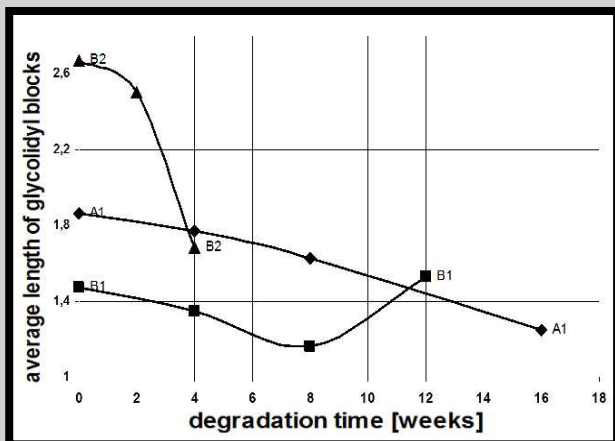
FIG. 3. Changes of average length of glycolidyl blocks during PLGA degradation.

FIG. 4. Changes of average length of lactidyl blocks during PLGA degradation.

The degradation of glycolidyl units for B1 and B2 is faster than in A1 (TABLE 2, FIG. 3) but only in the first period of experiment - during 8 weeks. After 8 weeks, the D,L-lactidyl units degrade much faster for B1 and B2 than the L-lactidyl units in A1 (TABLE 2, FIG. 4). The N_{GG} decreases rapidly from 16% to 6% for B1 and from 53% to 31% for B2 in the first period of degradation (TABLE 2) which is probably connected with easier water penetration to glycolidyl domains than lactidyl chains. However, after 4 (B2) or 8 (B1) weeks of degradation, the N_{LL} starts to decrease and the comonomeric molar ratio become similar to the initial value. It is probable that longer lactidyl chains start to split and thus the L_{LL} value decreases (FIG. 4, B1). The increase of N_{GG} value for B1 and B2 after 8 weeks can be explained by possible inhibition of further glycolic units degradation. This could be probably caused by presence of high crystalline domains within glycolidyl chains resistant to buffer influence, which is well noticeable for sample B1 (FIG. 3). The other explanation is the difference between the lactidyl and glycolidyl units degradation rates, also leading to the increase of N_{GG} though both copolymers degrade [6-11].

It's worth noting that further examination of samples B1 and B2 to the complete 16-weeks period couldn't be proceeded due to their fast degradation rate and insufficient amount of remaining material for the purpose of NMR analysis. However, it is expected that sample B2 would reveal the decrease of L_{LL} and grow of L_{GG} after 8 weeks of degradation, similar to sample B1 and confirmed by the N_{GG} and N_{LL} values (TABLE 2).

Conclusions

The nuclear magnetic resonance analysis reveals significant changes in the polymer chain microstructure for all examined materials. The poly(L-lactide-co-glycolide) (84:16) shows the longer degradation time, caused by the presence of long L-lactidyl chains resistant to hydrolytic degradation. During the 16 weeks of degradation poly(L-lactide-co-glycolide) with comonomeric ratio 84/16 (A1) remain unhydrolized in comparison to PDLA:GA, 84:16 (B1) and PDLA:GA, 47:53 (B2). It may be of good usage for stable release systems applied to harder tissues, where no mechanical damage can be done by the remaining polymer matrice. The poly(D,L-lactide-co-glycolide) copolymers B1 and B2 do not maintain the initial shape and after 16 weeks of degradation are almost completely disintegrated. They might be of great value as short- and mid-term release systems applied to soft tissues. The degree of randomness changed insignificantly for all examined copolymers which posses the semi-block chain microstructure.

Acknowledgements

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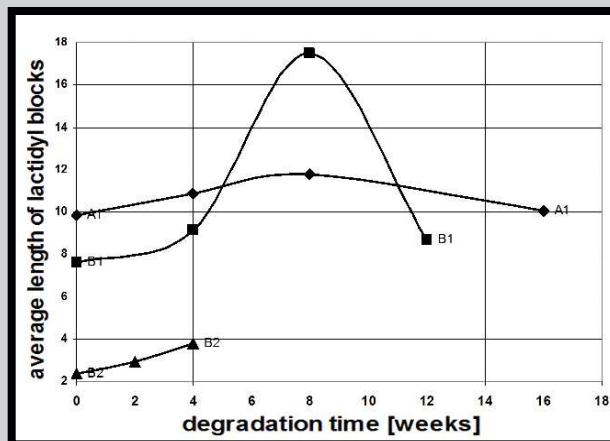
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