Macromolecular Research, Vol. 11, No. 5, pp 352-356 (2003)

The Effect of Gamma Irradiation on PLGA and Release Behavior of BCNU from PLGA Wafer

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Received May 30, 2003; Revised Aug. 25, 2003

Abstract: The objectives of this study were to investigate the influence of gamma irradiation for sterilization on poly(D,L-lactide-*co*-glycolide) (PLGA) with different molecular weight and the effect of gamma irradiation on the release behavior of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU, carmustine) from PLGA wafer with various irradiation doses. The effect of gamma irradiation on PLGA was evaluated by gel permeation chromatography (GPC), differential scanning calorimetry (DSC), and electron paramagnetic resonance (EPR). The weight average molecular weight (M_w) and glass transition temperature (T_g) of PLGA decreased after gamma irradiation. The extent of M_w reduction was dependent on irradiation dose and PLGA molecular weight. Using EPR spectroscopy, we successfully detected gamma irradiation induced free radicals in PLGA. The gamma irradiation increased the release rate of BCNU from PLGA wafer at applied irradiation doses except 2.5 Mrad of irradiation dose in this study.

Keywords: PLGA wafer, gamma irradiation, molecular weight, free radical, BCNU, release profile.

Introduction

Biodegradable polymer-based drug delivery systems are widely used to control the drug release. One of the synthetic biodegradable polymers is poly(D,L-lactide-*co*-glycolide) (PLGA). PLGA is lactide and glycolide copolymer and has been approved for drug delivery use by the Food and Drug Administration. It provides many advantages such as regulating varying degradation period according to mole fraction of lactide and glycolide[1] and producing biocompatible and toxicologically safe by-products that are further eliminated by the normal metabolic pathways. It has been widely used as carriers in controlled-release delivery systems[2-8] and in tissue engineering area[9-18] due to above reasons.

One of the most important points for pharmaceutical products is that they has to be sterilized before their application in clinic to make sure the elimination of injurious microorganisms. Aseptic processing in a clean room can be used to manufacture germless terminal products but sterilization of final products is preferred due to aspect of economy and convenience. The common sterilization methods are ethylene oxide (EO) gas,[19] dry heat,[20] steam,[21] organic solvent (ethanol),[22] plasma treatment,[23] and gamma irradiation.[24] Among these, sterilization method using EO gas has the residual possibility of EO showing fatal toxicity. Dry heat and steam sterilization can cause rigorous degradation of heat and moisture sensitive polymers and drugs such as proteins. Sterilization using organic solvent requires drying process of residual solvent results in loss of drug. Plasma treatment sterilization used recently has the possibility of the structural change of polymer by heat generated during the process. In this respect, sterilization mentioned above is not proper for the sterilization of PLGA and gamma irradiation is preferred. Moreover, 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU, carmustine) used as a model drug in this study, an important chemotherapeutic agent used for brain tumors,[25-29] is a heat sensitive drug reported that the decomposition of BCNU at room temperature is 6% after 3 hrs and 8% after 6 hrs.[30]

Although gamma irradiation remains the only accepted method, recently it has been researched about the effect on biodegradable polymers.[31-33] It has been reported that gamma irradiation induces free radicals in polymer matrix by chain scission and decreased molecular weight of polymer. The extent of molecular weight reduction was gamma irradiation dose dependent and it affects polymer degradation and

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release profile of drug from polymer device. But there is no research in the published papers about the effect of gamma irradiation on molecular weight of PLGA according to PLGA molecular weight. In this study, we investigated the influence of gamma irradiation on PLGA with different molecular weight with various irradiation doses by gel permeation chromatography (GPC), differential scanning calorimetry (DSC), and electron paramagnetic resonance (EPR). PLGA (M_w : 8K g/mole) showing the least reduction of molecular weight after gamma irradiation was chosen for matrix of BCNU to limit the change of release profile. The effect of gamma irradiation on the release profile of BCNU from PLGA wafer with various irradiation doses was also investigated by high performance liquid chromatography (HPLC).

Experimental

Materials. PLGA having molecular weight of 8, 20, 33, 90, and 110 K g/mole (mole ratio of lactide to glycolide, 50/50, 75/25, 50/50, 75/25, and 50/50 Resomer RG 502H, 752, 503H, 756, and 506, respectively) were purchased from Boehringer Ingelheim (Germany) (Figure 1). BCNU was purchased from Sigma Chemical Co. (St. Louis, MO, USA) and stored at 20 °C until use. Methanol (Junsei, Japen), methylene chloride (MC, Tedia, Japan), and chloroform (Burdick & Jackson, Muskegon, MI, USA) were used as received. All other chemicals were reagent grade. Deionized water was prepared by a Milli-Q purification system from Millipore (Molsheim, France).

Preparation of BCNU-loaded PLGA Wafer. BCNU and PLGA 8K g/mole were mixed physically and compression molded into wafers using Carver Press (MH-50Y CAP 50 tons, Japan) at 20 kg_f/cm^2 for 5 sec at room temperature. Amount of BCNU against PLGA was 3.85 wt%. The wafers were 3 × 1 mm^2 in size with a flat surface and stored at 0 °C until use.

Gamma Irradiation of PLGA and BCNU-loaded PLGA Wafer. Gamma irradiation was performed with ^{60}Co irradiation at KAERI (Korea Atomic Energy Research Institute, Daejeon, Korea). The irradiation doses were 0, 2.5, 5, and 7.5 Mrad at dose rates of 0.8 Mrad/hr. The sample was surrounded by dry ice during irradiation to protect the sample from heat and moisture.

Molecular Weight Analysis. Molecular weight of PLGA before and after gamma irradiation was determined by GPC equipped with a differential refractive index detector (Shodex® RI-71) and three columns (Shodex® GPC K-802, Asahipak GF-510 HQ, and GF-7M HQ) in series maintained 35 °C. Sample molecular weight averages were determined relative to polystyrene monodisperse standards with molecular weights ranging from 1,270 to 1,950,000 g/mole (Shodex® SM-105). Chloroform was used as mobile phase at 0.6 mL/min.

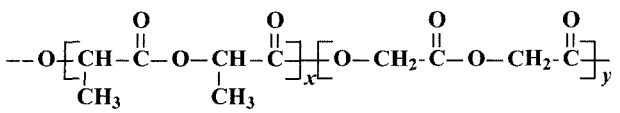

Figure 1. Chemical structure of PLGA.

EPR Measurements. The EPR measurements were performed at room temperature by using EPR spectrometer (EMX, Bruker, Germany) with the following settings: microwave frequency : 9.61 GHz; modulation amplitude : 5.0 G; center field : 3425.0 G; scan range : 120 G at KAERI (Korea Atomic Energy Research Institute, Daejeon, Korea)

Determination of Glass Transition Temperature. Glass transition temperature (T_g) of PLGA was determined before and after irradiation by DSC (Mettler TA 2910, AC, USA). The samples were heated in closed aluminum pans between 0 to 120 °C, at a heating rate of 10 °C/min under a constant flow of nitrogen.

***In Vitro* Release Test.** Release of BCNU from PLGA wafer was monitored for a period of incubation in phosphate buffered saline (PBS, pH 7.4) at 37 °C. Wafers were individually placed in 20 mL of PBS with constant shaking at 110 rpm. At specific time following incubation wafers were retrieved and freeze-dried for 48 hrs. BCNU-loaded PLGA wafers were dissolved respectively in 2 mL of MC. PLGA was precipitated by adding 18 mL of methanol and after centrifugation, 20 μL of aliquots of supernatant were analyzed by HPLC. Due to the unstable property of BCNU in the release test condition the amount of BCNU released into PBS was calculated by the amount of BCNU remained in the wafer after specific release test period. All samples were analyzed using HPLC system equipped with a Model P-2000 pump, a Model AS-3000 autosampler, and a Model UV-1000 UV detector at 248 nm(Thermo Separation Products, Fermont, CA) The column used was μ-Bondapak™ C_{18} (3.9 × 300 mm^2, Waters, Milford, MA). Mobile phase was deionized water/methanol (6:14 v/v) mixture and flow rate was adjusted to 1.0 mL/min.

Results and Discussion

Gamma Irradiation Effect on PLGA Molecular Weight. Figure 2 shows percentage of weight average molecular weight (M_w) change of PLGA for 0, 2.5, 5, and 7.5 Mrad irradiation doses. No changes in shape, color, and size of irradiated PLGA have been observed for all irradiation doses. M_w was decreased after irradiation and the extent of M_w reduction was accelerated by increasing irradiation dose. This indicates that irradiation caused a substantial degradation of PLGA. It has been explained that random chain scission and free radicals in PLGA were occurred after irradiation. Interestingly, the obtained M_w of PLGA was decreased more related to PLGA molecular weight after irradiation. PLGA 8 K g/mole demonstrated the least change of M_w among

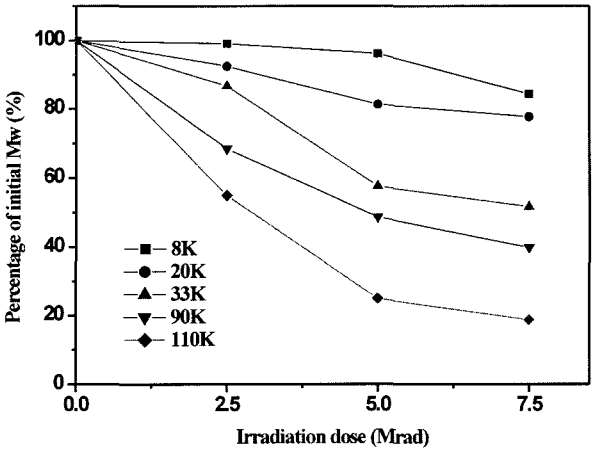

Figure 2. Changes of weight average molecular weight of PLGA before and after gamma irradiation with various irradiation doses.

irradiated PLGA in this study, a 15.7% drop at 7.5 Mrad, but only a slight 0.9% reduction at 2.5 Mrad. The M_w reduction for PLGA 110K g/mole was the largest drop among irradiated PLGA in this study, a 45% drop at 2.5 Mrad and 81% drop at 7.5 Mrad. It could be suggested that high molecular weight PLGA was higher than low molecular weight PLGA about the reduction rate of M_w by random polymer chain scission after irradiation. Generally, gamma irradiation occurs degradation or cross-linking of polymer through the formation of free radicals. The above results indicate that PLGA used in this study was more degradation type than cross-linking type after irradiation.

EPR Analysis. EPR measurements were performed at room temperature to observe the formation of free radical in PLGA 8K g/mole after irradiation under dry ice to protect from moisture and oxygen. Non-irradiated PLGA did not give any signal (not shown). Gamma irradiation leads to the appearance of the tertiary macroalkyl radical -Ċ(CH_3)- that yields a signal in EPR spectra of Figure 3. The signal intensity of EPR spectra is proportion to concentrations of the chain scission radical -Ċ(CH_3)-, (a), (b), and (c) in Figure 3, EPR spectra of PLGA 8K g/mole after irradiation at 2.5, 5, and 7.5 Mrad, respectively, are suggested that the signal intensity of EPR spectra is increased with increasing irradiation dose. This indicates that the concentration of free radicals in polymer matrix was increased with increasing irradiation dose. The results of EPR spectra suggest that molecular weight is decreased more due to acceleration of polymer degradation with increasing irradiation dose. In Figure 3, (c) is EPR spectrum of PLGA 8K g/mole measured by EPR spectrometer at room temperature under air right away after irradiation and Figure 4 is EPR spectrum of PLGA 8K g/mole measured by EPR spectrometer at same condition after 2 hrs from irradiation at same dose. The signal intensity of EPR spectrum in (c) of Figure 3 is higher than that of EPR spectrum in Figure 4. This indicates that the concentration of free radicals is

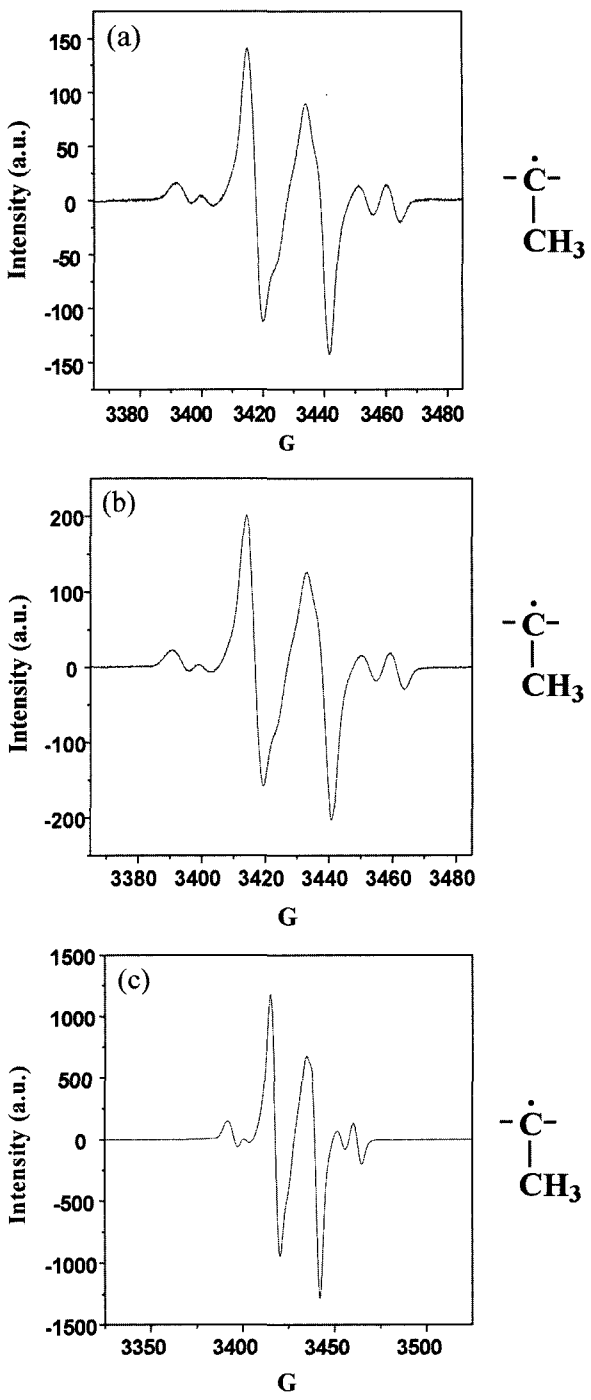

Figure 3. EPR spectra of PLGA 8K g/mole after gamma irradiation at (a) 2.5 Mrad, (b) 5 Mrad, and (c) 7.5 Mrad.

decreased under air with time. The reason of decrease of the concentrations of free radicals is admission of oxygen and it is reported that half life of free radicals is about 5 hrs.[33] EPR signal was not detected at room temperature under air after 12 hrs from irradiation (not shown).

DSC Analysis. Table I shows T_g of PLGA before and after

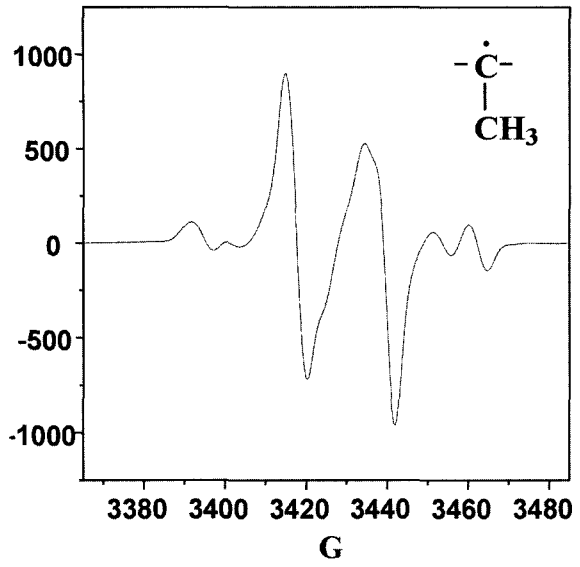

Figure 4. EPR spectrum of gamma irradiated PLGA 8K g/mole after 2 hrs in air (Irradiation dose ; 7.5 Mrad).

Table I. Glass Transition Temperature of PLGA before and after Gamma Irradiation with Various Irradiation Doses

	0 Mrad	2.5 Mrad	5 Mrad	7.5 Mrad
	T_g (°C)			
PLGA 8K	42.17	41.50	39.65	37.64
PLGA 20K	49.05	48.68	46.37	45.88
PLGA 33K	48.51	47.88	45.53	43.82
PLGA 90K	58.31	53.86	51.69	50.77
PLGA 110K	52.62	50.79	50.37	46.25

gamma irradiation. T_g of PLGA 8K g/mole was 42.17 °C before irradiation but T_g of PLGA 8K g/mole after irradiation was reduced to 41.50 °C at 2.5 Mrad, 39.65 °C at 5 Mrad, and 37.64 °C at 7.5 Mrad, respectively. T_g of PLGA with various molecular weight was found to be reduced dependent on the irradiation dose. This reduction of T_g after irradiation could be thought due to the changes in the polymer matrix and the reduction of molecular weight occurring radiolytic degradation such as random chain scissions.

***In Vitro* Release Study.** Figure 5 shows BCNU release profile in PBS, 37 °C from PLGA wafer after irradiation with various irradiation doses. The drug release profile irrespective of irradiation dose showed near first order release profile (initial burst release followed constant slow release). The release rate was increased with increasing irradiation dose. The release rate of drug from the PLGA matrix is mainly dependent on not only diffusion of drug through the matrix but also degradation of PLGA. M_w of PLGA was decreased after irradiation and the extent of M_w reduction was accelerated by increasing irradiation dose. This leads to a decrease of the extent of polymer chain entanglement and to an increased mobility of the macromolecules.[34] And also the interaction of high irradiation dose with polymer can influence the chemical and physical properties of the polymer.[35] So the release profile of BCNU from PLGA wafer after irradiation could be affected by above reasons. But the release profile of BCNU from PLGA wafer after irradiation at 2.5 Mrad was almost identical compared to those of BCNU from non-irradiated PLGA wafer. It could be explained that a slight 0.9% reduction of M_w of PLGA 8K g/mole at 2.5 Mrad was occurred in contrast to a 15.7% drop at 7.5 Mrad. Therefore the irradiation dose of 2.5 Mrad is suitable for sterilization for drug delivery PLGA device to limit polymer degradation. This dose is the minimum requirement for the sterilization of pharmaceutical products in agreement with Good Manufacturing Practice (GMP).[36]

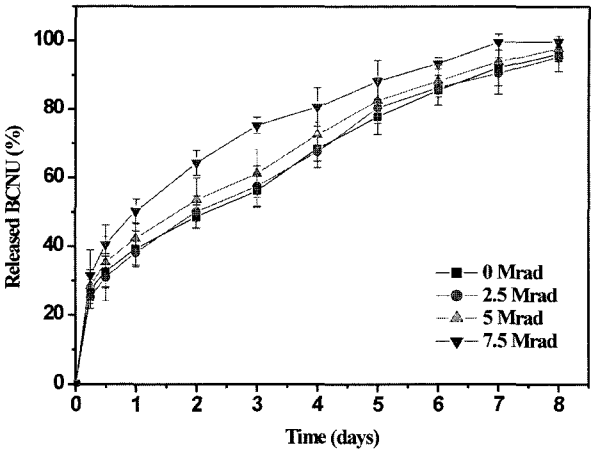

Figure 5. Release profile of BCNU from PLGA 8K g/mole wafer after gamma irradiation with various irradiation doses.

Conclusions

The influence of gamma irradiation on PLGA with different molecular weight and the effect of irradiation on the release profile of BCNU from PLGA wafer with various irradiation doses were investigated. M_w of PLGA was decreased after irradiation and the extent of M_w reduction was dependent on irradiation dose and PLGA molecular weight. Gamma irradiation leads to the appearance of the tertiary macroalkyl radical -Ċ (CH_3)-. EPR spectra suggest that M_w is decreased more due to acceleration of polymer degradation with increasing irradiation dose. The reduction of T_g after irradiation could be thought due to the changes in the polymer matrix and the reduction of molecular weight occurring radiolytic degradation such as random chain scissions. The drug release profile of BCNU from PLGA wafer irrespective of irradiation dose showed near first order release kinetics. The release rate was increased with increasing irradiation dose. However,

the release profile of BCNU from PLGA wafer after irradiation at 2.5 Mrad was almost identical compared to those of BCNU from non-irradiated PLGA wafer. Therefore the irradiation dose of 2.5 Mrad is suitable for sterilization for drug delivery PLGA device. Studies on the effect of gamma irradiation on the release behavior of BCNU from PLGA wafer with various molecular weight and the storage effect on the release behavior of BCNU from PLGA wafer after gamma irradiation are in progress.

Acknowledgements. This work was supported by KMOCIE (Grant No. B49-990-5411-05-1-3).

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