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Change in the Particle Size Distribution of poly (L-lactide) Wear Debris by γ-Ray Irradiation

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It has been known that the wear debris causes failure of implant prostheses. In this study, the convenient wear test of poly(L-lactide) (PLLA) was established and the particle size of PLLA wear debris was analyzed using the Coulter counter. Then, the changes in the particle size distribution of PLLA wear debris by γ-ray irradiation were observed dose-dependently at the dose of 10, 25 and 50 kGy. With the increasing irradiation dose, the particle size distribution of PLLA wear debris shifted toward the smaller diameter size, and the mean diameter of PLLA wear debris significantly decreased. In addition, the tensile strength and the molecular weight of irradiated PLLA were also decreased by increasing the irradiation dose. The lowering of the molecular weight by γ-irradiation resultingly caused the decreases in tensile strength of irradiated PLLA and the particle size of the wear debris derived from irradiated PLLA.

Keywords: poly(L-lactide), wear debris, particle size distribution, γ-ray irradiation

Introduction

Most total hip and knee replacements are composed of ultra high molecular weight polyethylene (UHMWPE) articulating against metal or ceramic. However, it has been well known that UHMWPE wear debris induce osteolysis and cause long term failure of hip and knee prostheses. The number of particles and the total surface area increase dramatically for particles of small diameter, thus particle number and available surface could be of great significance in promoting debris disease[1]. Vermes *et al.* reported that particulate wear debris directly activated osteoclasts and dramatically altered osteoblast function by both inducing interleukin-6 secretion and suppressing collagen synthesis[2]. Recently, it was confirmed that there was the wear resistance in the UHMWPE highly crosslinked by γ-ray irradiation[1,3].

Poly(L-lactid) (PLLA) has been used as biodegradable screws, pins and plates for internal bone fixation in the orthopedics. Ethylene oxide sterilization causes a carcinogenic risk because of the mutagenic properties of ethylene oxide[4]. In addition, autoclave sterilization causes plastic deformation and extensive hydrolytic degradation, and dry heat sterilization leads thermo-oxidative degradation of PLLA[5]. These sterilizations should not be applied to PLLA. On the other hand, in our recent study, the γ-ray irradiation of PLLA stimulated the differentiation of mouse osteoblastic cells cultured on PLLA. Then, it was suggested that, if the satisfied mechanical property was maintained, the γ-ray sterilization was suitable for PLLA devices[6-8].

In the present study, we established the convenient wear test for estimation of mechanical properties of γ-irradiated PLLA. The change in the particle size distribution of PLLA wear debris was caused by γ-irradiation, and also the molecular weights of PLLA were dose-dependently decreased by γ-irradiation.

Materials and methods

γ-Ray irradiation of PLLA

The PLLA sheets made of high molecular weight PLLA with thickness of 0.3 mm were obtained from Shimadzu Co. (Kyoto, Japan). The PLLA sheets were γ-ray irradiated at the dose of 10, 25 or 50 kGy using ^{60}Co as the radiation source. The irradiated PLLA sheets were preserved in the silica gel desiccator until next measurement.

According to the information from the manufacturer, the tensile strength of unirradiated PLLA, 25 kGy irradiated PLLA and 50 kGy irradiated PLLA were respectively 6.69 ± 0.047, 6.43 ± 0.103 and 5.80 ± 0.228 kgf/mm^2 (mean ± S.D., n = 5) in the standard tensile test, JIS K 7113 Testing Method for Tensile Properties of Plastics (Shimadzu Co., unpublished data). The tensile strength of irradiated PLLA significantly decreased ($P < 0.0001$ by one-way analysis of variance) with the increasing irradiation dose.

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Wear test

The schemes of wear test that modified the method of Miura and Takeda[9] were shown in Figure 1. The PLLA specimen was prepared; The PLLA sheets were cut out in the disk with the 14.0 mm diameter, and glass column of 11.0 mm diameter and 2.5 g weight was bonded on each PLLA disk using a cyanoacrylate adhesive (Fig. 1A). Then, the PLLA specimen was put in the cylindrical vessel of the 30.0 mm inside diameter, in which bottom plane was #400 waterproof abrasive paper (Sankyo Rikagaku Co.,Ltd., Tokyo, Japan). Five milliliter of balanced electrolyte solution, ISOTON II (Coulter Electronics Ltd., Luton, Bedfordshire, England), was added in the cylindrical vessel, and the whole vessel was gyrated of 15 mm radius at 200 rpm for 1 hour using a rotatory shaker (SRR-3, IUCHI SEIEIDO Co.,Ltd., Osaka, Japan) (Fig. 1B).

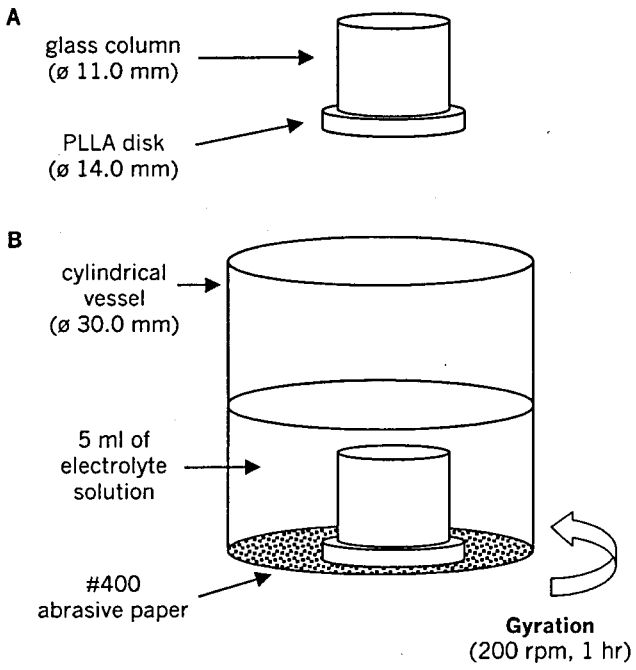

Fig. 1. Schemes of wear test

A: Glass column of 11.0 mm diameter and 2.5 g weight was bonded on the PLLA disk of 14.0 mm diameter using a cyanoacrylate adhesive.

B: The PLLA specimen was put in the cylindrical vessel of the 30.0 mm inside diameter, in which bottom plane was #400 waterproof abrasive paper, and the cylindrical vessel was gyrated at 200 rpm for 1 hour with 5 ml of electrolyte solution.

Coulter counter analysis of wear debris

The particle size of PLLA wear debris in balanced electrolyte solution obtained by wear test was measured using the Coulter counter, MULTISIZER II (Coulter Electronics Ltd.). The orifice tube with nominal aperture diameter of 100 μm was used and the particle diameter was measured in the range of 2-60 μm. The data were analyzed using MULTISIZER AccuComp Color Software Version 1.19 (Coulter Corp., Miami, Florida). The particle size distribution was obtained from mean number of each particle diameter. The mean particle diameter of PLLA wear debris was calculated from 9 times experiment.

Gel permeation chromatography

The molecular weight of PLLA was determined by gel permeation chromatography (GPC), using LC10AT (Shimadzu Co., Kyoto, Japan) equipped with refractive index detector (RID-10A, Shimadzu Co.) as a GPC apparatus. PLLA were dissolved in chloroform at a concentration of 5 mg/ml. Fifty microliters of sample solution were eluted through two GPC columns (TSKgel G5000H_{XL} + TSKgel G4000H_{XL}, each 7.8 mm i.d. × 300 mm, Tosoh, Tokyo, Japan) at a mobile phase of 1.0 ml/min chloroform. The weight average molecular weight (Mw) and the number average molecular weight (Mn) of PLLA were analyzed from the comparison with the calibration curve that was made with polystyrene standards (Showa Denko, Tokyo, Japan) using LC workstation, CLASS-LC10 (Shimadzu Co.). The polydispersity index was calculated as the ratio of Mw to Mn.

Statistical analysis

Differences of particle diameter among the groups were evaluated with one-way analysis of variance (ANOVA). When significant differences among the groups were found, Turky-Kramer test was applied for multiple comparisons. A difference was considered to be significant if $P < 0.01$.

Results and discussion

Abrasive effect for wear test on particle size distribution

Miura and Takeda made metallic alloy to be a sample and used glass tube for preparation of the specimen[9]. However, we used glass column in order to apply the load, because the specific gravity of PLLA is smaller than that of their metallic alloys (Fig. 1A). They also used alumina or zirconia balls of the 3 mm diameter that were covered in the bottom of vessel as an abrasive[9]. Though the wear test of PLLA was performed using alumina balls as the abrasive, large numbers of alumina debris were also derived as well as PLLA debris. There was no problem in their study, even if the alumina wear debris existed, because the alumina debris were almost eliminated by filtration, and they performed the cytotoxicity test of the extract of metallic alloy[9]. The cause of the derivation of alumina debris was seemed for the abrasive alumina balls to move with the PLLA specimen in the vessel. Therefore, in the present study, the waterproof abrasive paper was fixed in the bottom plane of the vessel, and only the PLLA specimen was moved in the vessel (Fig. 1B).

The effect of the roughness of the abrasive paper was investi-

gated by the measurement of the particle size distribution of PLLA wear debris. Figure 2 showed the particle size distribution obtained by use of #400, #600 or #800 abrasive paper. Each line showed the mean diameter that was obtained with 3 times experiments. When the rough abrasive paper was used, the particle size distribution shifted toward the larger diameter size. There was the reasonable relation between the roughness of the abrasive paper and particle size distribution of PLLA wear debris. The abrasive paper of various material and roughness had been marketed, therefore it was possible to apply our present method using the abrasive paper to the wear test of various samples. The roughest #400 abrasive paper, which derived the larger debris among them, was used in the present study, because it was possible that smaller debris were derived from γ-ray irradiated PLLA.

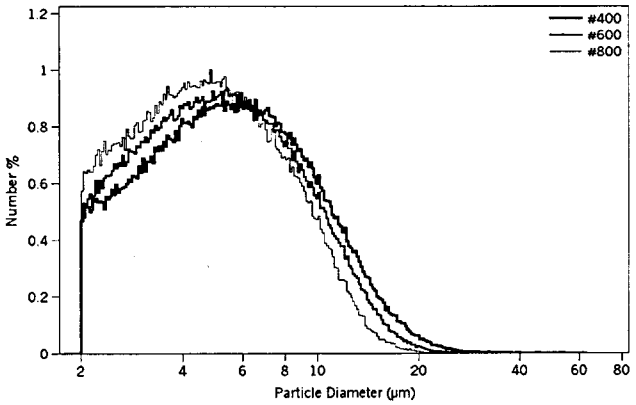

Fig. 2. The particle size distribution of PLLA wear debris obtained by the use of #400, #600 or #800 abrasive paper
Each line showed the mean diameter in 3 times experiments.

Particle size distribution of wear debris derived from γ -ray irradiated PLLA

The wear test of γ-ray irradiated PLLA was performed, and the particle size distribution of wear debris was measured by Coulter counter analysis. Figure 3 showed the particle size distributions of wear debris derived from irradiated PLLA. The center line showed the mean and the vertical width showed the mean ± 2 S.D. (n = 9). With the increasing irradiation dose, the particle size distribution of wear debris derived from irradiated PLLA shifted toward the smaller diameter size. The relationship between the irradiation dose of PLLA and the mean diameter of PLLA wear debris was shown in Figure 4. The mean diameter of PLLA wear debris was decreased 9.3% by irradiation at 50 kGy. The mean diameter of PLLA wear debris significantly decreased ($P < 0.0001$ by ANOVA) with the increasing irradiation dose. The tensile strength of irradiated PLLA also decreased with the increasing irradiation dose (Shimadzu Co., unpublished data). When the abrasive wore the PLLA specimen, the surface of PLLA would be easily cracked, because the tensile strength was lower. In fact, the minute crack had been observed on the surface of the 50 kGy irradiated PLLA disk, microscopically. Therefore, the decrease of tensile strength of PLLA by the γ-irradiation caused the decrease in particle diameter of PLLA wear debris.

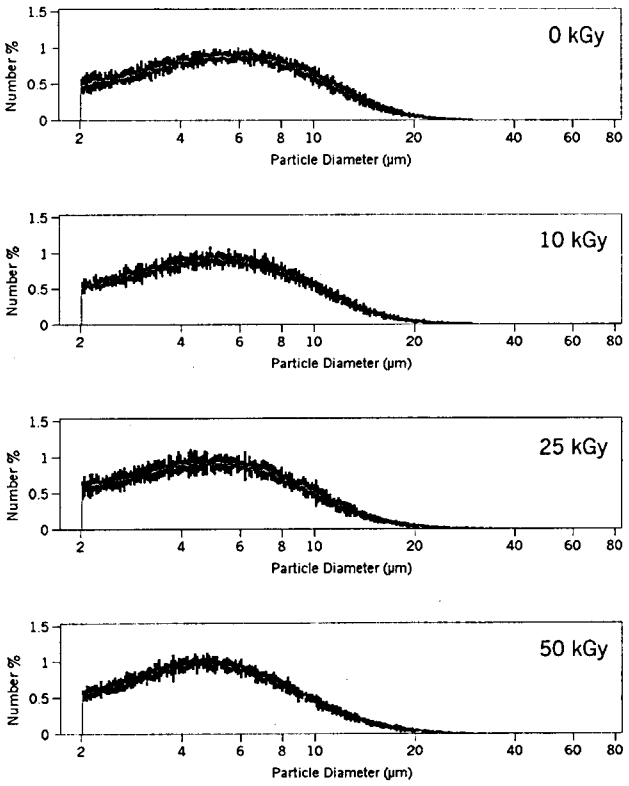

Fig. 3. The particle size distribution of wear debris derived from γ-irradiated PLLA
The center line showed the mean, and the vertical width showed the mean ± 2 S.D. (n = 9).

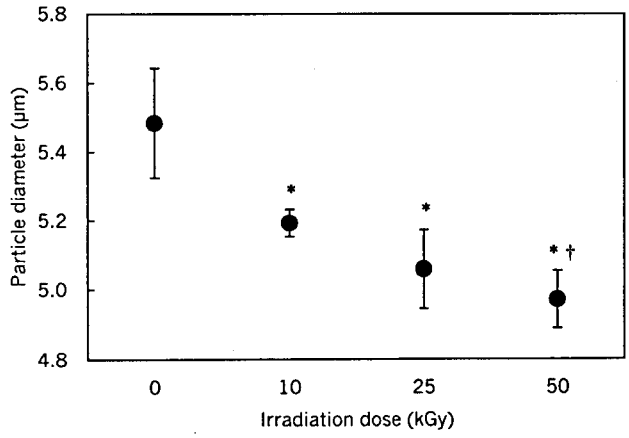

Fig. 4. The effect of γ -ray irradiation on particle size distribution of PLLA wear debris
* Significant difference compared with unirradiated PLLA at $P < 0.01$.
† Significant difference compared with 10 kGy irradiated PLLA at $P < 0.01$.

Molecular weight of γ-ray irradiated PLLA

The molecular weight of γ-ray irradiated PLLA was determined by GPC. Figure 5 showed the Mw and polydispersity index of irradiated PLLA at the dose of 10, 25 or 50 kGy. The Mw of irradiated PLLA extremely decreased with the increasing irradiation dose. The Mw of 271,000 of unirradiated PLLA was decreased to 95,000 by irradiation at 50 kGy. In contrast, the polydispersity index of irradiated PLLA was confined to the slight increase with the increasing irradiation dose, compatible with a random cleavage in the degradation mechanism [10,11]. Yoshioka *et al.* reported γ-irradiation of PLLA caused random cleavage of molecular chain with hydrolysis of ester bonds [12,13]. In addition, they detected decomposition products having a molecular weight higher than lactic acid in alkali hydrolysis products of irradiated PLLA, and they suggested crosslinkage of molecular chain also occurred [12,13]. We also analyzed of irradiated PLLA by high performance liquid chromatography after alkali hydrolysis. However, the quantity of decomposition products having a molecular weight higher than lactic acid was extremely slight (data not shown). Otto *et al.* also observed that the molecular weight of PLLA was decreased from 160,000 to 35,200 by γ-irradiation at 25 kGy[14]. Thus, γ-ray irradiation caused cleavage for molecular chain and decreased the molecular weight of PLLA. The tensile strength would have decreased as a result of the molecular weight lowering of irradiated PLLA.

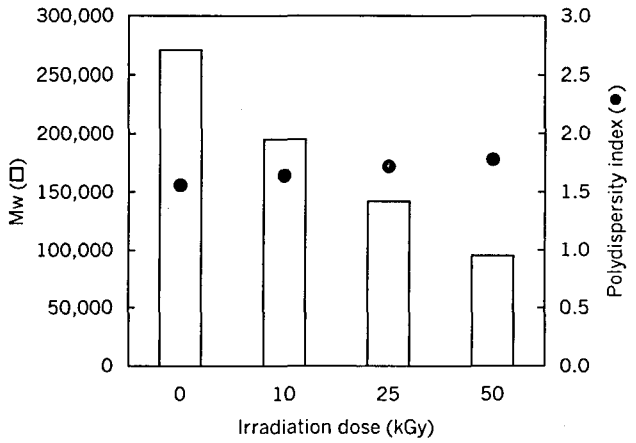

Fig. 5. The effect of γ-ray irradiation on the molecular weight and polydispersity index of PLLA
The bar showed the weight average molecular weight (Mw) and the circle showed the polydispersity index of PLLA.

Conclusions

The change in the particle size distribution of PLLA wear debris by γ-ray irradiation could be evaluated using the present method. As the results, with the increasing irradiation dose, the particle size distribution of PLLA wear debris shifted toward the smaller diameter size, and the mean diameter of PLLA wear debris significantly decreased. In addition, the molecular weight of PLLA also decreased with the increasing irradiation dose. It was indicated that the lowering of the molecular weight by γ-irradiation caused the decrease in tensile strength of irradiated PLLA and the particle size of PLLA wear debris derived from irradiated PLLA.

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