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Formation of micro-patterned cellular chips by ion-beam irradiation into Poly-$_L$-lactic acid

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We performed H^+, H_2^+ and He^+ ion-beam irradiation into biodegradable polymer sheets of Poly-$_L$-lactic acid (PLLA) at an energy of 150 keV with a fluence of 1×10^{15} ions/cm^2. The irradiated surface was investigated by means of FT-IR-ATR, Raman spectroscopy, and a contact angle meter. Ion-beam irradiated layer was exfoliated from the substrate in a water solution, and thin films were obtained. Next, H^+, H_2^+ and He^+ ion-beam irradiation was performed at an energy of 150 keV with a fluence of 1×10^{15} ions/cm^2 with a stainless-steel mask on the sample surface. We fixed the sheets to the bottom of a cell culture dish and seeded mouse fibroblast (L929) cells on the sample surface. These cells attached and spread on the micro-patterned domains, and the domains spontaneously exfoliated from the substrate. Consequently, micro-patterned cellular chips were obtained. These techniques and production methods are expected to apply to scaffolds, artificial organs and cell diagnosis tools.

Keywords: PLLA, ion-beam irradiation, micro-patterning, cellular chips

1. INTRODUCTION

Poly-$_L$-lactic acid (PLLA) is a biodegradable polymer that hydrolyzes into low molecules when implanted in a living body. These products are ultimately resolved into carbon and water which are non-toxic to the body. Figure 1 shows a structural formula for Poly-$_L$-lactic acid. Therefore, PLLA is used as scaffolds for tissue engineering such as sutures, wound healing materials, and drug delivery systems. It has been reported that He^+ ion-beam irradiated specimens spontaneously form thin films in a water solution [1, 2]. In this report, we investigated the formation of thin films and micro-patterned cellular chips by H^+, H_2^+ and He^+ ion-beam irradiation into PLLA sheets.

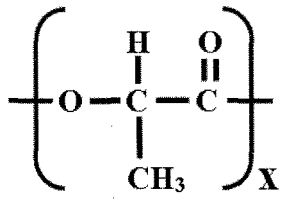


Fig.1 Structural formula of PLLA.

2. EXPERIMENTAL

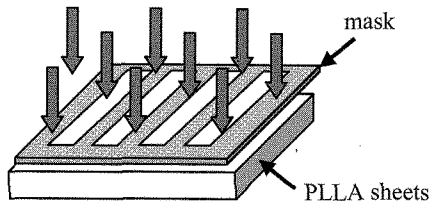
PLLA sheets (Ecoloju: Mitsubishi Jyushi Co. Ltd., Japan) were cut into 3 cm squares and used as substrates. We performed H^+, H_2^+ and He^+ ion-beam irradiation into 2 cm square of substrates at an energy of 150 keV with a fluence of 1×10^{15} ions/cm^2 at room temperature using the RIKEN 200 kV Low-Current Implanter. The beam current density was kept 0.05 μA/cm^2 to prevent heating of the specimen. The irradiated surface was investigated by means of Fourier transform infrared spectroscopy (Nexus 470: Thermo Nicolet, USA), Raman spectroscopy (Labram: Jovin Yvon), and a sessile water drop method using a contact angle meter (CA-X: Kyowa Interface Science Co., Ltd Japan). Functional group analyses were carried out with the FT-IR-ATR spectroscopy. Each spectrum was obtained after 64 scans from 1000 to 4000 cm^{-1}. Raman scattering measurements were performed at room temperature with a 632.817 nm He-Ne laser. The exposure time was five seconds, with ten scans accumulated in order to improve the S/N ratio. In the contact angle measurement, distilled water (Otsuka Seiyaku) was used for the measurement, and the volume of each water droplet was about 1.8 μl. Each determination was obtained by averaging the results of at least five measurements.

To investigate the development of thin films, we fixed the sheets to the bottom of a cell-culture dish to prevent floating on a water solution, filled the dish with phosphate-buffered saline (PBS) solution (pH=7.4) and incubated them at 37 ℃ in a humidified atmosphere with 5 % CO_2. We performed the cross-sectional SEM (JSM-6330F: JEOL, Japan) observation of irradiated specimens to measure the film thickness.

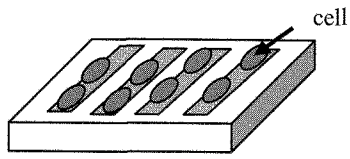
We performed the formation of micro-patterned cellular chips by combining the formation of a self-assembled thin film with the improvement in cell affinity induced by ion-beam irradiation. Figure 2 show schematic diagrams. The H^+, H_2^+ and He^+ ion-beam irradiation was performed

at an energy of 150 keV with a fluence of 1×10^{15} ions/cm^2 with a stainless-steel mask on the sample surface. H^+ ion-beam irradiated linear domain with a width of 80 μm, H_2^+ ion-beam irradiated circular domain with a width of 200 μm and He^+ ion-beam irradiated circular domain with a width of 120 μm. Mouse fibroblast (L929) cells were suspended in a culture medium (RPMI 1640: Nissui Pharm. Co., Japan) supplemented with 10.0 % fetal bovine serum (FBS: Sanko-Jyunyaku Co., Japan). Micro-patterned sheets were fixed to the bottom of a dish to prevent floating on a culture medium, L929 cells were seeded 6 ml on the ion-beam irradiated surface at a density of 5×10^4 cells/ml and then incubated at 37 ℃ in a humidified atmosphere with 5 % CO_2. The extent of cell attachment and spreading were observed with an optical microscope equipped with phase contrast objectives, using a CCD camera (IX-70: Olympus Co., Tokyo Japan).

1. Micro-patterned ion-beam irradiation



2. Seeding Cell



3. Forming the patterned cellular chips

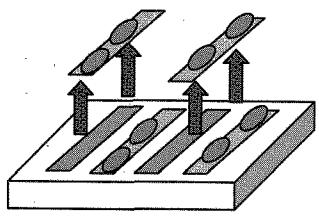


Fig.2 Schematic diagram of the formation of micro-patterned cellular chips by ion-beam irradiation.

3. RESULTS AND DISCUSSION

Figure 3 depicts FT-IR-ATR spectra for (a) non-irradiated, (b) H^+, (c) H_2^+ and (d) He^+ ion irradiation at an energy of 150 keV with a fluence of 1×10^{15} ions/cm^2. The spectrum for the non-irradiated PLLA surface had three main peaks at 1080 cm^{-1}, 1180 cm^{-1} (assigned to C-O-C and C-O stretching vibration), and 1740 cm^{-1} (assigned to C=O stretching vibration) [3]. The three peaks decreased dramatically for the ion-beam irradiated surfaces, and C=O stretching vibration shifted from 1740 cm^{-1} to 1720cm^{-1} owing to conformation transformed to carboxyl from carbonyl group [4, 5]. Moreover, new peak appeared at 3200 to 3400 cm^{-1} due to hydroxyl (O-H) groups. These results indicate that chemical bonds were destroyed and new functional groups were induced by the ion-beam irradiation.

Figure 4 illustrates Raman spectra for (a) non-irradiated, (b) H^+, (c) H_2^+ and (d) He^+ ion irradiation at an energy of 150 keV with a fluence of 1×10^{15} ions/cm^2 in the region of 1000 to 2000 cm^{-1}. The Raman spectra for H^+ and H_2^+ showed almost no difference from the non-irradiated PLLA. However, new broad peaks were formed under He^+ ion irradiation. The higher-frequency band is characteristic of polycrystalline graphite (G band) and the lower frequency band is associated with a disordered graphite structure (D band) [6,7]. Thus, the He^+ ion-beam irradiated surface structure was destroyed and amorphous carbon was formed. These results indicate that heavy ions contribute to the formation of amorphous carbon. The appearance of new functional groups and carbon structures was controlled by ion species.

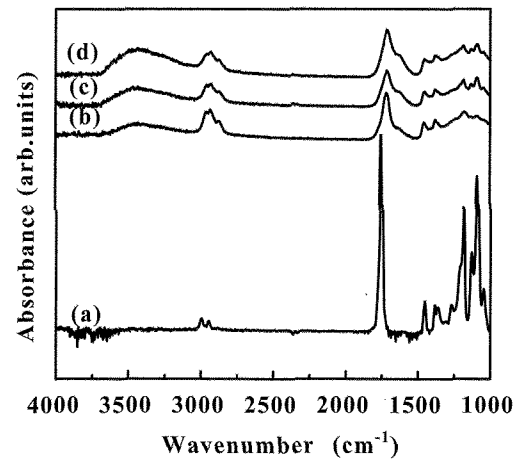


Fig.3 FT-IR-ATR spectra of (a) non-irradiated, (b) H^+, (c) H_2^+ and (d) He^+ ion-beam irradiated PLLA at an energy of 150 keV with a fluence of 1×10^{15} ions/cm^2 in the region of 1000 to 4000 cm^{-1}.

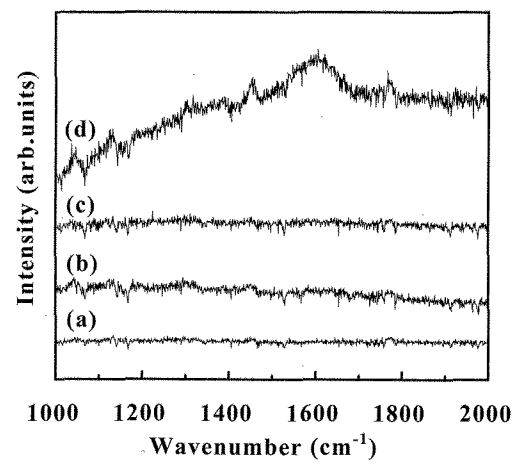


Fig.4 Raman spectra of (a) non-irradiated, (b) H^+, (c) H_2^+ and (d) He^+ ion-beam irradiated PLLA at an energy of 150 keV with a fluence of 1×10^{15} ions/cm^2 in the region of 1000 to 2000 cm^{-1}.

Figure 5 presents the contact angle of water for non-irradiated, H^+, H_2^+ and He^+ ion-beam irradiated PLLA at an energy of 150 keV with a fluence of 1×10^{15} ions/cm^2. The contact angle of the non-irradiated sample was 62°, but this increased dramatically under ion-beam irradiation.

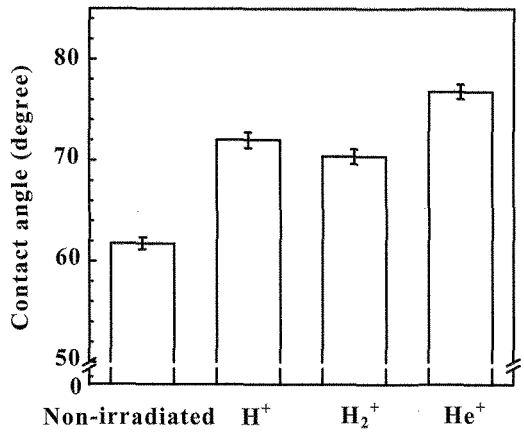


Fig.5 Contact angle of water for non-irradiated, H^+, H_2^+, and He^+ ion-beam irradiated PLLA at an energy of 150 keV with a fluence of 1×10^{15} ions/cm^2.

Figure 6 shows micrographs of the self-assembled thin films. We obtained thin films by H^+, H_2^+ and He^+ ion-beam irradiation. Figure 7 shows cross-sectional SEM photographs of the ion-beam irradiated specimen. It is clear that void space existed between the substrate and the ion-beam irradiated layer. The film thickness was about 1.5 μm in the H^+ ion beam irradiated specimens, 0.8 μm in the H_2^+ ion-beam irradiated, and 1.2 μm in the He^+ ion irradiated. The lighter irradiated ions, the longer projected range is. However, the film thickness produced by He^+ ion-beam irradiation was thicker compared with those formed by H_2^+ ion-beam irradiation. This reason is 150 keV H_2^+ ions with a fluence of 1×10^{15} ions/cm^2 corresponds to single H^+ ions with an average energy of 75 keV with a fluence of 2×10^{15} ions/cm^2 [8]. Ion-beam irradiation caused structural change near the position of energy deposition peak. The energy deposition peak of H_2^+ and He^+ ion-beam irradiation at an energy of 150 keV with a fluence of 1×10^{15} ions/cm^2 was estimated from TRIM calculation [9] to be 1.0 μm and 1.3 μm respectively, using a density of 1.24 g/cm^3 for PLLA. The experimental values also agreed with the theoretical value. Thus, the film thickness was controlled by ion species. It has been reported that Kr^+ ion-beam irradiation at an energy of 150 keV with a fluence of 1×10^{15} ions/cm^2 does not form thin films because mechanical strength was decreased by amorphous carbonization after ion-beam irradiation [1]. In fact light ion irradiation, such as H^+, H_2^+ and He^+, is suitable for developing thin films.

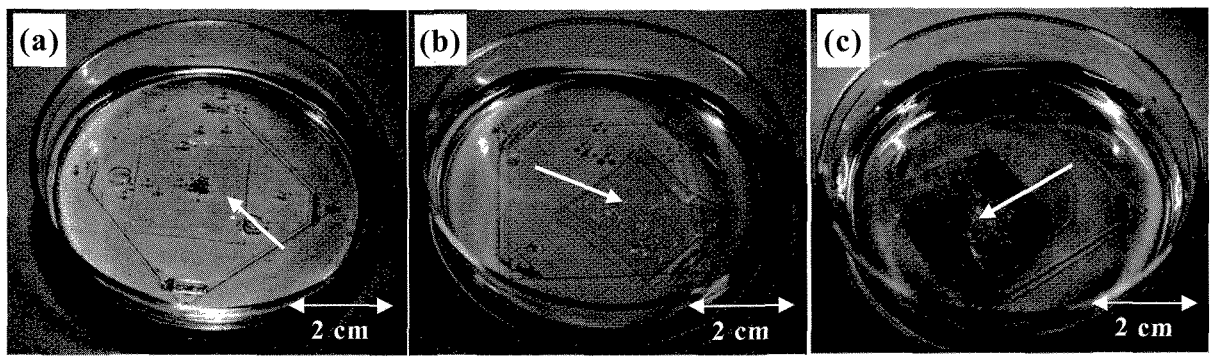


Fig.6 Micrographs of the self-assembled thin film produced by (a) H^+, (b) H_2^+ and (c) He^+ ion-beam irradiated PLLA at an energy of 150 keV with a fluence of 1×10^{15} ions/cm^2.

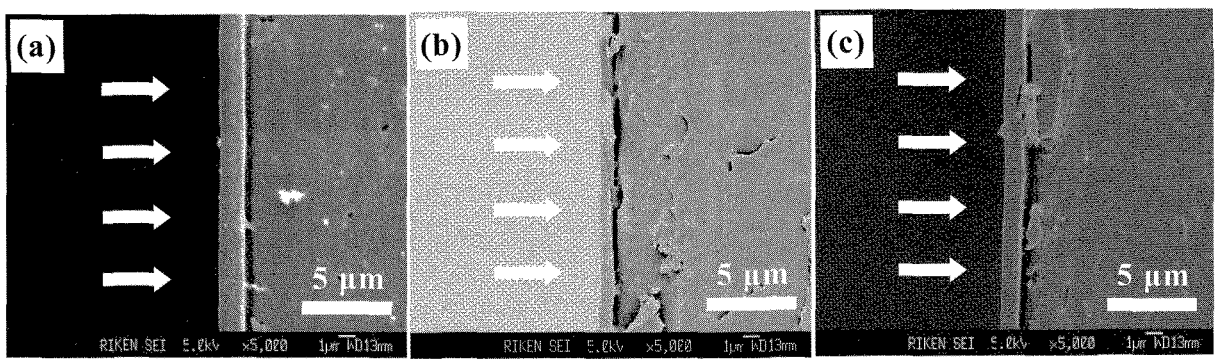


Fig.7 Cross-sectional SEM photograph of (a) H^+, (b) H_2^+ and (c) He^+ ion-beam irradiated specimen at an energy of 150 keV with a fluence of 1×10^{15} ions/cm^2.

Figure 8 shows phase-contrast photographs of cellular chips from (a) H^+, (b) H_2^+ and (c) He^+ ion-beam irradiation after an incubation period of 48 h. The seeded cells attached and spread on the micro-patterned domains compared with non-irradiated area, and the domains exfoliated spontaneously from the substrate. It is considered that cell adhesive proteins adsorption increased due to the formation of new functional groups and new carbon structure induced by ion-beam irradiation [10]. Accordingly, micro-patterned cellular chips were obtained. The size and shape of these chips were controlled by changing the mask pattern.

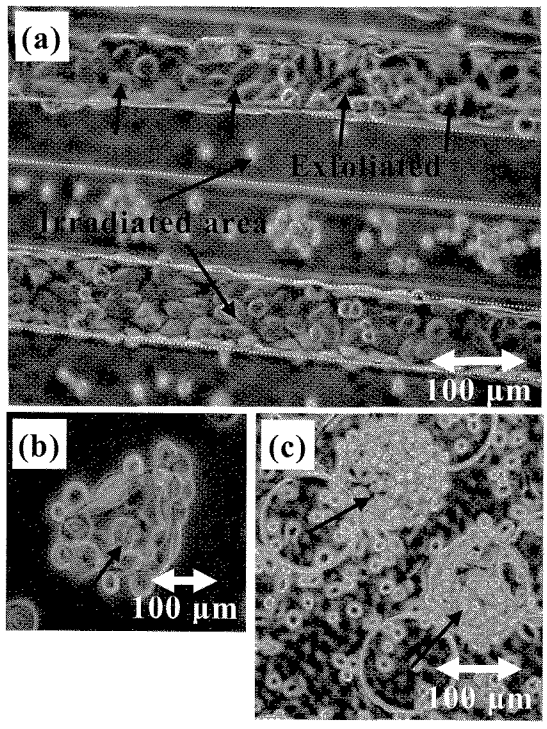


Fig.8 Phase contrast photographs of patterned cellular chips by (a) H^+, (b) H_2^+ and (c) He^+ ion-beam irradiation at an energy of 150 keV with a fluence of 1×10^{15} ions/cm^2 after an incubation period of 48 h.

4. CONCLUSION

We obtained thin films by H^+, H_2^+ and He^+ ion-beam irradiation at an energy of 150 keV with a fluence of 1×10^{15} ions/cm^2. The film thickness was controlled by the ion species. Ion-beam irradiation improved cell attachment properties. It is considered that the formation of new carbon structures and new functional groups were induced, and the contact angle increased dramatically by ion-beam irradiation. We performed the formation of micro-patterned cellular chips by combining the formation of a self-assembled thin film with the improvement in cell affinity induced by H^+, H_2^+ and He^+ ion-beam irradiation. The size and shape of the cellular chips were controlled by changing the mask pattern. Moreover, these chips have good cell affinity and biodegradable properties. Thus, they are expected to be useful in repairing defects in tissues such as skin, cardiac muscle, and the liver.

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