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Improvement of cell attachment capabilities of poly-L-lactic acid films by modification of surface properties with ion-beam irradiation

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ABSTRACT

In this study, the correlation between surface properties, including the carbon-bonding state for ion-beam irradiated poly(L-lactic acid) films, and cell attachment capabilities was investigated. H^+, N_2^+, or Kr^+ beams were used to irradiate the film-surface at an energy of 50 keV with fluences ranging from 1×10^{13} to 1×10^{15} ions/cm^2. Chemical compositions, carbon structure, and wettability of the irradiated surfaces were evaluated by X-ray photoelectron spectroscopy (XPS), Raman spectroscopy, and contact angle measurement of water. Carbonization proceeded with ion mass and/or irradiation fluence, and subsequently the irradiated surfaces became hydrophobic compared to the non-irradiated surface. Mouse fibroblasts (L929) were seeded onto the non-irradiated and irradiated surfaces to evaluate cell attachment capabilities. These results indicate that a moderate amount of C=C groups is suitable for cell attachment and proliferation, and that an excess is inhibitory.

1. Introduction

Tissue engineering scaffolds play a critical role in replacing or repairing damaged tissues. Biodegradable polymers have attracted a great deal of attention as ideal scaffold, since polylactic acid (PLA), polyglycolic acid (PGA), and poly(lactic-co-glycolic acid) (PLGA) have been used for tissue engineering applications owing to their biodegradability and good mechanical strength. However, for these polymers, further application progress would be difficult because of poor cell/material interaction derived from the high hydrophobicity and lack of reactive functional groups. In the field of tissue engineering research, the low cell attachment capabilities of these scaffolds are a significant problem, leading to an inflammatory response after implantation.

Surface modification is a method for improving the interaction between scaffolds and cells and/or natural tissues. Ion-beam irradiation has been known to be effective for improving cell attachment and has been applied in surface modification of various polymers [1–6]. This technique can be well controlled and has good reproducibility in terms of chemical compositions, structures, and bonding states on irradiated polymer surfaces.

Enhancement of cell attachment capabilities of polymers was suggested to be attributable to a decrease in the contact angle [1–3,5] and carbonization [3,4] induced by ion-beam irradiation. Particularly, surface carbonization markedly enhanced cell attachment. However, the effect of carbonization on cell attachment capabilities is not clearly understood. Applying irradiated polymers to biomedical applications requires unequivocal information regarding the relationship between surface properties, including carbonization, and cell attachment capabilities.

In this study, ion-beam irradiation was applied to the surface of PLLA films, using various ions and fluences. The chemical compositions and carbonization of irradiated PLLA surfaces were evaluated by XPS and Raman spectroscopy, while the effect of irradiation on surface wettability was also examined. We discuss the relationship between these surface properties and cell attachment capabilities.

2. Materials and methods

2.1. Ion-beam irradiation

Poly-L-lactic acid film (PLLA, 30×30 mm^2, Mitsubishi Jyushi, Japan) was used as a substrate. H^+, N_2^+, or Kr^+ beams were used to irradiate the films at an energy of 50 keV with fluences ranging from 1×10^{13} to 1×10^{15} ions/cm^2, respectively. The current density was kept 0.05 μA/cm^2 to prevent the substrates from heating under a chamber pressure of less than 5×10^{-4} Pa.

2.2. Surface characterization

2.2.1. XPS measurement

Chemical compositions and carbon bonding states of non-irradiated and irradiated surfaces were analyzed by X-ray photoelectron spectroscopy (XPS; JEOL, JPS-9010MC, Japan) using Mg Kα radiation (1253.6 eV). All binding energies were referenced to the C1s neutral

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carbon peak at 285.0 eV. Using curve fitting, the overlapping peaks were resolved into multiple components with the combination of Gaussian and Lorentzian functions after Shirley-type background subtraction.

2.2.2. Raman spectroscopy

Raman spectra were recorded with a Raman spectrometer (Labram300; Horiba Jobin Yvon, Ltd., Japan) with an He–Ne laser (632.814 nm) within the wavenumber region of 1000–2000 cm^{-1} under ambient atmosphere and at a spectral resolution of 4 cm^{-1}. The exposure time and accumulation for measuring the Raman spectra were 5 s and 10 times, respectively.

2.2.3. Contact angle measurement

The contact angle of water on films was measured by the sessile drop method using a contact angle meter (CA-X; Kyowa Interface Science Co., Ltd., Japan). The water used for measurement was distilled water, and the amount of water per droplet was about 1.8 μl. The data for 5 measurements on different positions were averaged.

2.3. Cell attachment capabilities

Mouse fibroblasts (L929: Riken Cell Bank, Japan) were suspended in a culture medium (RPMI 1640: Nissui Pharm. Co., Japan) supplemented with 10% fetal bovine serum (FBS: Sanko-jyunyaku Co., Japan). Non-irradiated and irradiated PLLA films were sterilized under ultraviolet (UV) irradiation for 10 min on both sides and fixed to the bottom of 60-mm culture dishes to prevent floating in culture medium. L929 cells were seeded on non-irradiated and irradiated surfaces at a density of 5×10^4 cells/ml and incubated at 37 °C in a humidified atmosphere with 5% CO_2 for 24 and 48 h. After incubation, unattached cells were removed by rinsing with phosphate buffered saline (PBS). Cell attachment and morphology were observed with a phase contrast microscope (IX-70: Olympus Co., Tokyo, Japan). The percentage of L929 coverage on non-irradiated and irradiated surfaces were calculated at 5 different positions of the optical micrographs and averaged for each specimen. This experiment was repeated 3 times.

3. Results and discussion

3.1. Surface characterization

The chemical compositions and carbon bonding states of non-irradiated and irradiated PLLA surfaces were characterized by XPS. Existential elements were identified from the survey spectra. Only C1s and O1s peaks were observed in all the spectra (data not shown).

Using the TRIM code [7], the average penetration depth of irradiated ions in PLLA films was estimated to be 780, 100, and 60 nm with H^+-, N_2^+-, and Kr^+-irradiated surfaces, respectively. We assumed

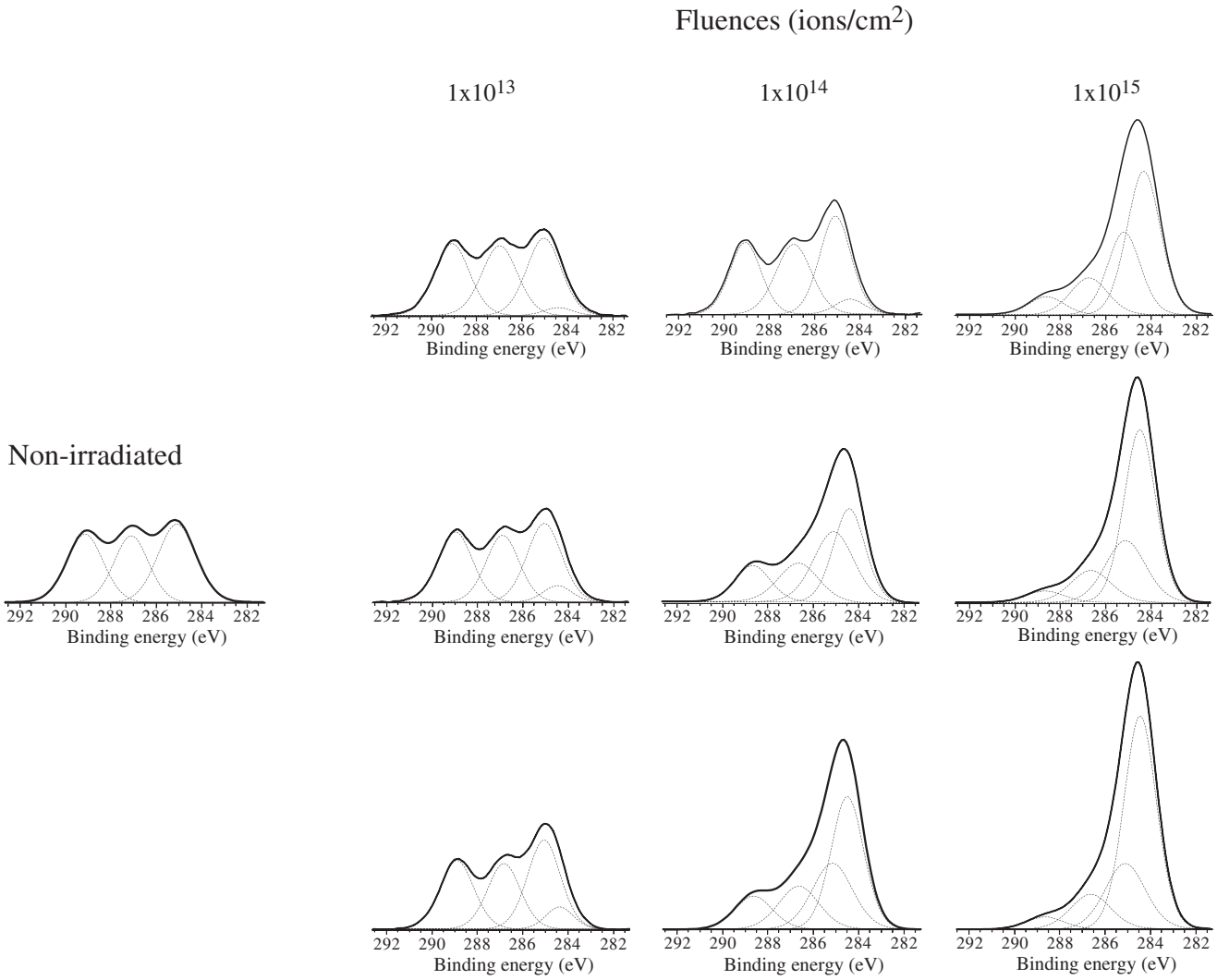

Fig. 1. XPS C1s spectra of non-irradiated and irradiated PLLA surfaces with H^+, N_2^+, or Kr^+ at fluences 1×10^{13}, 1×10^{14}, and 1×10^{15} ions/cm^2.