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Surface characterization and assessment of cell attachment capabilities of thin films fabricated by ion-beam irradiation of poly(L-lactic acid) substrates

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ABSTRACT

The ion-beam irradiation of substrates of poly(L-lactic acid) (PLLA), a biodegradable polymer, gave rise to exfoliatable thin films when the substrate was immersed in an aqueous solution. The thin films exhibited excellent cell affinity, and hence, can be useful in bioengineering applications. In this study, we characterized both surfaces of the thin films and evaluated their cell attachment capabilities. Each surface was analyzed by X-ray photoelectron spectroscopy (XPS) and dynamic force microscopy (DFM). These analyses showed that carbonization took place at both surfaces. In addition, no significant changes were noticed in the topographies of the two surfaces. Finally, the cell attachment capabilities of the surfaces were determined by culturing mouse fibroblasts on them. The cells attached firmly to the bottom as well as the top surface of the film and were well spread out. These results could be attributed to the carbonization of the surfaces of the thin-film. Such thin films, fabricated by the irradiation of a biodegradable polymer, are expected to find wide application in areas such as tissue regeneration and cell transplantation.

1. Introduction

In order to construct three-dimensional tissue and regulate its cellular functions, it is important to be able to control the arrangement, stacking, and orientation of cells. Recently, a number of techniques have been developed for the fabrication of single-cell sheets without the need for enzymatic digestion by using thermoresponsive polymers [1], particle membranes [2], and by employing electrochemical detachment [3].

These cellular sheets maintain their structure through cell-to-cell interactions and can feasibly be attached to other surfaces through extracellular matrix proteins [4,5]. It has also been reported that electrically communicative myocardial tissue can be obtained by stacking monolayered sheets of cells [6]. Thus, the use of cellular sheets is a powerful method of constructing three-dimensional tissues and for studying cell transportation. However, such cellular sheets, which consist of only cells, are easily foldable and wrinkle owing to cytoskeletal reorganization [4].

Our previous study demonstrated that thin films can be fabricated by the irradiation of poly(L-lactic acid) (PLLA) with a beam of He^+ [7]. This procedure for the formation of the thin film is schematically illustrated in Fig. 1. The irradiated surface, which could be exfoliated from the substrate in the form of a thin film when immersed in a cell culture medium, exhibited excellent cell attachment properties. The thickness of the film was approximately 1.2 μm. Using this technique, cellular films can be obtained without requiring enzymatic digestion. The thin film attaches cells together and can be transferred to other surfaces without cell dissipation occurring. It was surmised that the thin film would be very promising as scaffolds for cell support for the purposes of tissue regeneration and cell transplantation.

Numerous studies have been performed to date on the surface properties and cell attachment of the top surface of the thin film [8,9]. No study has investigated the bottom surfaces of the thin film. The cell-attachment capabilities of the bottom surface are also important for the stacking and/or transporting of the cellular films. In this study, we evaluated the surface properties and cell attachment capabilities of both surfaces of the thin film formed by ion-beam irradiation. In addition, we also investigated the mechanism of formation of the thin film.

2. Materials and methods

2.1. Substrates and ion-beam irradiation

PLLA sheets (Ecoloju, Mitsubishi Jyushi, Japan) were cut into squares with sides of 3 cm and used as the substrates. A square-sized area on the substrate, with a side of 2 cm, was irradiated with He^+ ions with an acceleration energy of 150 keV and a fluence

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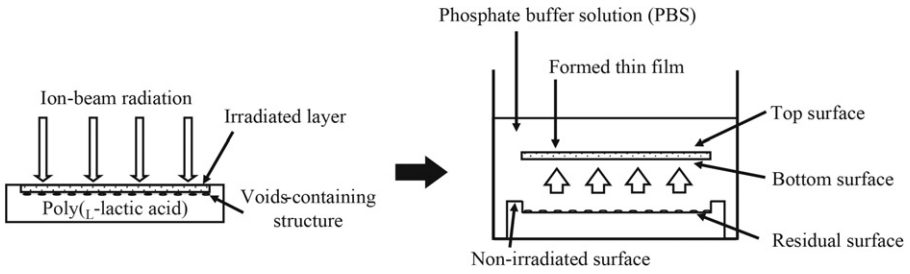

Fig. 1. Schematic diagram of the process for the formation of the thin film by ion-beam irradiation of the PLLA substrate.

of 1×10^{15} ions/cm^2. The beam current density was maintained at 0.05 μA/cm^2 to prevent the specimens from heating.

2.2. Surface characterization

The irradiated specimens were placed at the bottom of culture dishes with a diameter of 60 mm, soaked in a phosphate buffered saline (PBS) solution, and incubated at 37 °C in a humidified atmosphere with 5% CO_2. After being exfoliated from the PLLA substrate, the formed thin films were washed several times with deionized water. Then, their surfaces were characterized. The two surfaces of the exfoliated thin film were classified as top and bottom. The surface of the substrates was labeled as non-irradiated surface and as residual surface after the formed thin film had been exfoliated (shown in Fig. 1).

All the surfaces were characterized by X-ray photoelectron spectroscopy (XPS) (JEOL, JPS-9010MC) using Mg Kα radiation (1253.6 eV). Detailed C1s spectra of the surfaces were recorded with an energy step of 0.1 eV. All the binding energies were referenced to the C1s peak of neutral carbon at 285.0 eV. Through curve fitting, the overlapping peaks were resolved into their various components using the combinations of Gaussian and Lorentzian functions after Shirley-type background subtraction. For comparison, we also characterized the non-irradiated surface of the substrate and the top surface of the thin film before the exfoliation process.

The topography of each surface was analyzed using dynamic force microscope (DFM) (SII NanoTechnology Inc., Japan). The measurements were performed with a Si cantilever at a resonant frequency of 136 kHz. The scan size was 5-μm-square. The average surface roughness (Ra) was measured at three separate random areas and the values averaged.

2.3. Assessment of cell-attachment capabilities

In order to evaluate the cell attachment capabilities, mouse fibroblasts (L929, Riken Cell Bank, Japan) were cultured on each of their surfaces. The thin film specimens were sterilized under ultraviolet light for 10 min prior to cell cultivation. The L929 cells were suspended in a culture medium (RPMI 1640, Nissui Pharm. Co., Japan) supplemented with 10% fetal bovine serum (Sanko Jyunyaku Co., Japan) and antibiotics. The cells were seeded on each surface at a density of 5×10^4 cells/ml and incubated at 37 °C in a humidified atmosphere with 5% CO_2. The attachment behavior of the cells was observed with an optical microscope equipped with phase-contrast objectives (IX-70, Olympus Co., Tokyo, Japan). Five random photographs were taken ranging from the center to the periphery of the specimen. The attached cells were categorized either as "spread" cells or "rounded" cells. The cell-spreading ratio was calculated by the following formula: cell spreading (%) = (spreading cell count/total cell count) × 100. Data were expressed as mean ± standard deviation and analyzed using Dunnett's multiple comparison test. Significant differences were determined at $p < 0.05$.

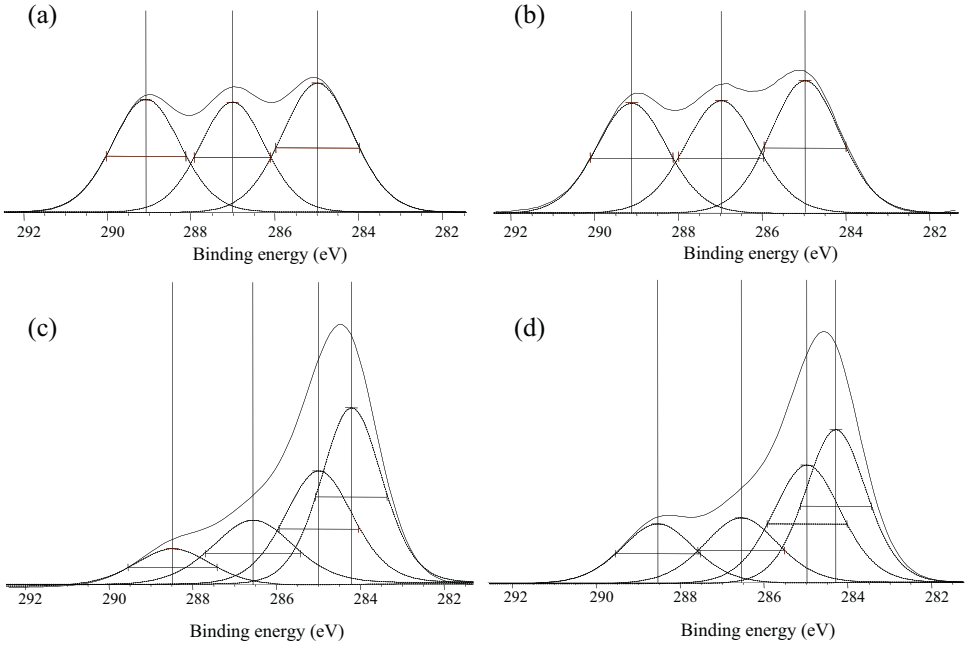

Fig. 2. XPS C1s spectra of the (a) non-irradiated surface and (b) residual surface of the substrate and (c) the top and (d) bottom surfaces of the thin film.