

## MICROFABRICATION OF A BIODEGRADABLE POLYMER BY ION BEAM IRRADIATION FOR A NEW CO-CULTURE SYSTEM OF CELLS

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**INTRODUCTION:** For the visualization of the advanced medical services such as an order-made medical treatment and a point-of-care system, improvement and generalization of diagnosis materials and technologies were requested. From the ecology, biodegradable materials are expected to enlarge their application to the fields of diagnosis and biosensing. Poly-(L-lactide) (PLLA) is one of the biodegradable polymers which are already used as implanting materials, and it remains in a human body for years after implantation. However, ion beam irradiation into PLLA film accelerates its degradation, and the irradiated layer detaches to be a thin sheet from the remaining film. When this film is used for cell culture, the irradiated layer can detach with cells cultured on it. This phenomenon can be utilized to produce patterned culture of cells, which are useful for tissue engineering, regenerative medicine, and diagnosis techniques. In this study, we attempt the microfabrication of biodegradable polymers by ion beam irradiation for the patterning culture of human and mammalian cells to establish a new technique of patterning culture.

**METHODS:** PLLA films (LACTY, SHIMADZU, Japan) were irradiated by He<sup>+</sup> ion beam at the acceleration energy of 50 keV, 100 keV, 150 keV and fluency of  $1 \times 10^{13} \sim 1 \times 10^{15}$  ions/cm<sup>2</sup> at the beam current of 0.1 μA/cm<sup>2</sup> over a stainless steel mask with several kinds of patterns. Then, laminin (extracted from EHS tumors of mouse, Harbor Bio-Products, USA) or laminin pentapeptide (AnaSpec Inc. USA) was coated at the concentration of 1-10 μg/mL for 2h at 37°C. Then, human hepatocellular carcinoma cells, Hep G2, were cultured on the PLLA film for 2h in a CO<sub>2</sub>-incubator. The irradiated layer was detached from the PLLA film with cells over them, and human embryonic fibroblasts HEL299 were inoculated and cultured for 1h in the CO<sub>2</sub>-incubator.

**RESULTS & DISCUSSION:** Figure 1 shows the depth of the grooves prepared by detaching the irradiated layer. By changing the acceleration

energy of He<sup>+</sup> ion beam, the depth can be controlled between 1.4-2.7 μm.

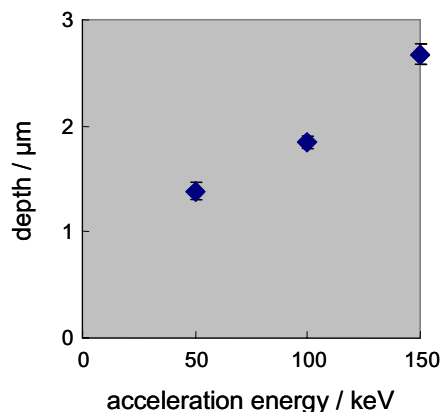


Fig. 1: Depth of the grooves fabricated on biodegradable polymer by ion beam irradiation.

An example of the images of cells after the detachment of the irradiated layer was shown in Figure 2 (a). Hep G2 was cultured in a pattern because the cells on the irradiated layer were removed. Then, HEL299 was inoculated over Hep G2 and cultured for 1d. An example of the images of co-cultured cells was shown in Figure 2 (b). HEL299 was mainly adhered to the detached area whereas Hep G2 still remained on unirradiated area.

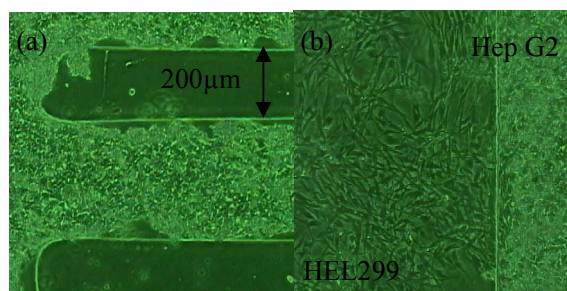


Fig. 2: Hep G2 cells cultured in a pattern(a) and patterned co-culture of Hep G2 and HEL299 cells (b).

**CONCLUSIONS:** As a conclusion, modification of PLLA surface with ion beam irradiation is effective to produce patterned co-culture system after the detachment of the irradiated area as a thin sheet.