Adhesion Patterning of Mesenchymal Stem Cells on Polymeric Surfaces by Carbon Negative-Ion Implantation through a Mask and Their Differentiation into Neurons with Keeping Cell Alignment

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Abstract: We have been developing a formation method for artificially designed neuron network on polymeric surface by using a negative-ion implantation technique. The negative-ion implantation has a feature of "charge-up free" property even for insulators and isolated electrode, so that it allow us to be able to precisely control the implantation conditions of ion energy and fluence amount. In the pattering treatment, carbon negative ions were implanted into a spin-coated polystyrene film (SCPS) on glass and a silicone rubber (SR) sheet through a pattern mask, which has many narrow slits at aperture width of 50 μm and spacing distance of 150 μm (or 70 μm).

Fig. 1 shows a phase-contrast optical micrograph of PC12h (rat adrenal pheochromocytoma) cells cultured for 4 days on the C-implanted SCPS. The cells selectively adhered and self-aligned on the implantation lines as a square-mesh pattern. This pattern was done by using twice implantation with changing the direction of the pattern mask at the same conditions of 10 keV and 3 x 10^{15} ions/cm^2. The surface roughness was only 0.14 nm in Ra and 1.5 nm in peak-to-valley. It was too shallow for cell falling down there. Contact angle of SCPS surface was decreased by the ion implantation. Therefore, the self-alignment of cells was induced by the change of the hydrophilicity of the surface with intermediation of protein adsorption.

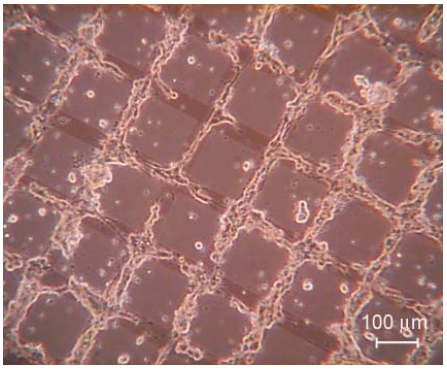

Fig. 1 Self-aligned PC12h cells on polystyrene surface and showed a square-mesh pattern.

For adhesion pattering of mesenchymal stem cells (MSCs) derived from rat bone-mallow, the SCPS surface was implanted at 10 keV and 1 x 10^{15} ions/cm^2 through the mask. The appearance of cultured MSCs on the C-implanted SCPS is shown in Fig. 2, where the adhesion of MSCs also aligned by themselves along the implantation line during cell culture.

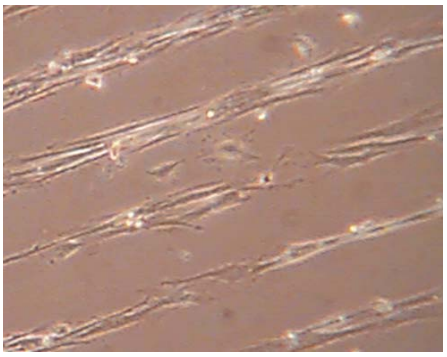

Fig. 2. Self-aligned MSCs along the implanted line on polystyrene surface.

After culturing for 3 days, we tried to induce differentiation into neuron by a method using β-melcapto-ethanol, reported by Woodbury et al. Just after changing culture medium to "induction medium" from "pre-induction medium", MSCs started to change their cell shape: cell body was shrinking around their nucleus to have round shape and was extending fine neurite-like filaments. After 180 min, the differentiation was almost completed and neuron-like shapes appeared as shown in Fig. 3. Then the cells were cultured for 10 days with a medium for neuron of Neurobasal with B27 supplement. The neuron specific enolase was detected in the differentiated cells. This means that the cells were differentiated into one kind of nerve cells.

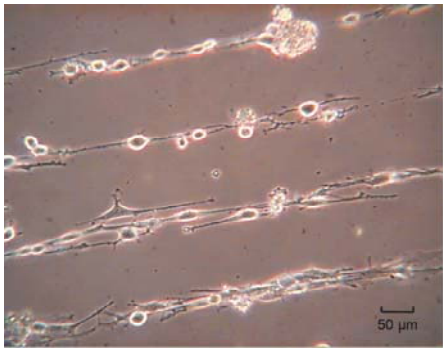

Fig. 3. Differentiated cells with keeping their adhesion pattern on polystyrene surface as they were.

For silicone rubber, the same results were obtained, but the optimal implantation condition was a little bit different from those for SCPS. The detailed results will be presented at the seminar with the fundamental properties of ion- implanted polymers.