P108

Differentiation of Mesenchymal Stem Cell on the Single Line Pattern in Nano-Size Fabricated on the Electron Beam Reactive Mask Material

Aya Tsubokura, Yoshifumi Kawagishi, Satomi Aoki and Naoya Takeda*

*Department of Life Science and Medical Bioscience, School of Advanced Science and Engineering, Waseda University, TWIns Bldg., 2-2 Wakamatsu-cho, Shinjuku-ku, Tokyo 162-8480, Japan, * Tel: +81-3-5369-7323, e-mail: ntakeda@waseda.jp, aya-tsubokura@akane.waseda.jp*

Living cells communicate actively with their microenvironments, which include neighboring cells, biosignaling factors, and surrounding matrix and materials. Conventional biology have particularly focused on neighboring cells and biosignaling factors, and examined how they affect cell behavior, *e.g.* proliferation, differentiation, and signal transduction. Recently, the surrounding matrices and materials attract growing interests, because these substrates have proved to not just provide the cells with adhesion sites but also affect cell's behaviors. Stiffness or topography of the scaffolds changes differentiating directions of the mesenchymal stem cell (MSC). However, in the previous studies, the surface was pre-coated or patterned with ECM proteins to accelerate cell adhesion, which may affect the MSC differentiation. Moreover, in some cases, cell culture was performed in a group, so effect of the neighboring cells was not excluded. In our study, linearly grooved single pattern in nano-size was fabricated and applied to the culture site for human mesenchymal stem cells (Fig. 1). The narrow line pattern was capable for single cell culture. Thus, how this nano patterned substrate affected differentiation of the MSC was exclusively examined.

Electron beam (EB) lithography and a polymer resist as the mask material, which have been originally developed for semiconductor processing, were directly applied to create the novel cell patterning system. The positive resist mainly consisting of poly[1-chloro-(methyl acrylate)-*co*-(1-methylstylene)] (ZEP520A, Zeon, Tokyo, Japan) was spin-coated on a cover glass in thickness of 100 nm and 400 nm. With EB irradiation, individual linear grooves ranging from 100 nm to 1 μm in width were lithographed on the resist (Fig. 1a). The rest of the polymer surface was modified with Pluronic F108, a copolymer containing hydrophilic PEG blocks, to prevent non-specific cell adhesion. The bone marrow human MSC (hbmMSC) was seeded and cultured on these line patterns without pre-coating any ECM protein to promote cell adhesion and without applying any biosignaling factors to induce differentiation.

The hbmMSCs were extraordinarily extended along the line, probably accompanying mechanical stress. As far as the number of the single cell on the line pattern, 500 nm width was most optimal. The adhesion property depended on depth of the pattern. The cells firmly adhered and remarkably stretched along a line pattern when the resist was 100 nm thickness (100 nm depth, Fig. 1b). Fluorescent staining of phospho-paxillin showed that the cells formed focal adhesions along the line pattern. On the other hand, for the 400 nm depth (Fig. 1c), focal adhesion existed only at both edges of the spindle shaped body, and the cells actively migrated back and forth along the line. In this case, qRT-PCR experiments showed mRNAs expression of the neural markers (Nestin and Tuj1) and osteogenic markers (RunX2 and OCN) increased in comparison with flat surface culture, while not for the 100 nm depth at all. These results strongly suggested that the simple nano line pattern could initiate differentiation of single hbmMSC when width and depth were appropriately designed.

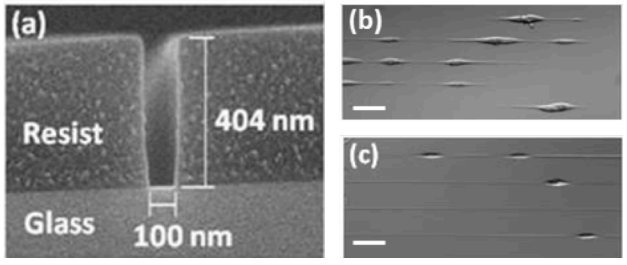

Fig. 1. (a) SEM image of cross section of the EB lithographed nano line pattern. (b,c) hbmMSC cultured on the line patterns of 500 nm width. (b) 100 nm depth and (c) 400 nm depth. Scale bar =100 μm.