Cell Function Inducible Micropattern Surface for Maintenance of Undifferentiated Embryonic Stem Cells

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Statement of Purpose: Embryonic stem (ES) cells have a unique ability to differentiate into all functional cells. It is important to maintain the undifferentiated state of ES cells during ordinary culture period. Even in the case of the undifferentiated culture condition (e.g. on the feeder cells or in the medium containing leukemia inhibitory factor, LIF), the ES cells have to be done passage when the formed colonies are large. In other words, it is considered that the undifferentiated ES cells depend on not only the stimulation by LIF but also the size of formed colonies. In this study, a photoreactive phospholipid polymer was synthesized, and the various dimension of micropattern surfaces were prepared by UV irradiation with a photomask. Then, the mouse ES cells were cultured on the micropattern surface to control the undifferentiated state of them.

Methods: A phospholipid polymer consisting of 2-methacryloyloxyethyl phosphorylcholine (MPC, 90mol%) and methacrylic acid was synthesized, and is referred to as PMAc. The carboxylic group of methacrylic acid unit in PMAc was modified to photoreactivity with 4-azidoaniline hydrochloride and water-soluble carbodiimide. From this procedure, a photoreactive phospholipid polymer (Az-PMAc, Figure 1) was obtained. An aqueous solution of Az-PMAc was cast on a polystyrene plate, and air-dried at room temperature. The plate coated by Az-PMAc was covered with a photomask and then was UV-irradiated with a UV lamp. The plate was repeatedly washed with distilled water. The micropattern surface was immersed in 0.1wt% gelatin solution for 30min at room temperature. Mouse ES cells (129/Sv) were plated on the micropattern surface. The ES cells were cultured in Knockout™ DMEM containing 15% Knockout™ serum replacement, 2mM L-glutamine, 100μM nonessential amino acid, 100μM 2-mercaptoethanol and 1000 U/mL LIF, and maintained in the cell culture incubator (37°C, 5%CO_2). After 3 days, the ES cells were fixed by 3.8% formaldehyde solution, and the cells were stained by using an alkaline phosphatase (ALP) immunostaining kit to confirm the undifferentiated state.

Results / Discussion: The micropattern surface was prepared by using Az-PMAc. Figure 2 (upper) shows the phase contrast image of typically pattern surface (Az-PMAc as aisle, and lattice (gelatin) as cell growth area). The ES cells were plated on the micropattern surface. It has been reported that the surface modified by Az-PMAc was concentrated by the phospholipid polar groups like a biomembrane, and the Az-PMAc area reduced the nonspecific protein adsorption[1]. Therefore, the ES cells adhered on the only lattice area (100x100 μm) which was adsorbed by gelatin. It was observed that the ES cells proliferated in the only lattice area and they formed colonies. After 3 days, it was estimated that the cells were reached confluence in the lattice. The

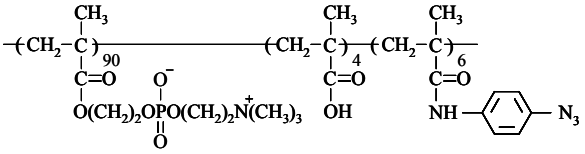

Figure 1 Chemical structure of photo-reactive phospholipid polymer, Az-PMAc.

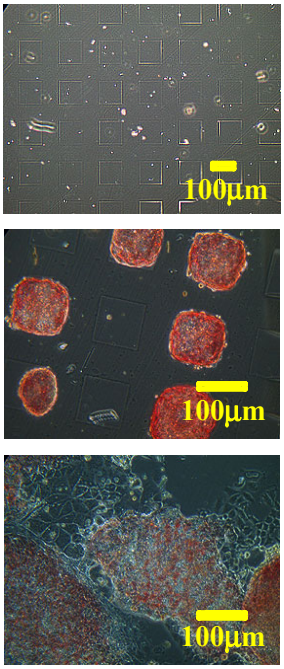

Figure 2 Phase contrast microscope images (Upper: micropattern surface, Middle: inside micropattern after ALP stain (day 3), Bottom: outside micropattern after ALP stain (day 3)

undifferentiated state of the ES cells was confirmed by ALP staining. Figure 2 (middle and bottom) shows the phase contrast images of the ES cells after ALP staining. When the ES cells maintained undifferentiated state, the colonies are stained ruby. From this result, the ES cells grown in lattice area can maintain highly undifferentiated state. On the other hand, the ES cells grown outside micropattern could not be stained. Namely, it was confirmed that the overgrowth cells outside micropattern lost the undifferentiated state. It cannot be considered that the ES cells lost the undifferentiated state because of the lack of stimulation factor such as LIF, since the medium was exchanged everyday.

Conclusion: In this study, the micropattern surface could regulate the cell function (pluripotency of ES cells) by controlling its size. This study demonstrated a new vision of micropattern surface, and it was concluded that micropattern surfaces would be able to contribute for tissue engineering and regenerative medicine.

Reference:

1) Konno T. et al., Biomaterials. 2005;**26**:1381-1388.