Gradient Cell Density Formation and Adipogenic Differentiation of Mesenchymal Stem Cells on a Micropattened Surface

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Introduction: Manipulation of stem cell functions such as adhesion, proliferation, and differentiation remains a great challenge in tissue engineering and regenerative medicine. Many factors including soluble growth factors, mechanical stimuli, surface morphology and chemical groups have been reported to affect the differentiation of mesenchymal stem cells (MSCs). Cell density has also been reported as a factor affecting cell functions such as proliferation and differentiation [1]. However, until now the effect of cell density on cell functions has been studied by culturing cells separately at different cell densities. It is desirable to compare the effect more directly if cells at different cell densities can be cultured simultaneously on a single surface. The present study used UV-photolithographic process to prepare non-adhesive poly(vinyl alcohol) (PVA)-micropatterned surfaces that allowed the formation of a cell density gradient. The effect of density of human MSCs on their adipogenic differentiation was investigated by culturing MSCs on the PVA-micropatterend surfaces.

Methods: Photoreactive poly(vinyl alcohol) derivative bearing azidophenyl groups (AzPhPVA) was synthesized according to the previous report [2]. An aqueous solution of AzPhPVA (100 μL of 200 μg/mL) was dropped onto a cell culture polystyrene plate (2 × 2 cm) and air-dried in the dark. The AzPhPVA-coated plate was covered with a photomask with transparent stripe with widths varying from 20 to 1000 μm separated by 200 μm nontransparent stripes and was then exposed to UV light. After UV exposure, the plate was washed with ultrapure water to remove unreacted polymers. The PVA-micropattern formed on the cell culture polystyrene plate was observed with an optical microscope. The micropatterned plate was then seeded with a suspension solution of human bone marrow-derived MSCs at an initial cell density of 5.00×10^3 cells/cm^2. The MSCs were precultured in DMEM serum medium for 3 days. During cell culture, cells on the micropatterned surface were observed with a phase contrast microscope to confirm the formation of cell gradient. After 3 days of preculture, the culture medium was changed to adipogenic differentiation medium consisting of DMEM supplemented with 10% fatal bovine albumin, 1 μM dexamethasone, 0.5 mM methyl-isobutylxanthine, 10 μg/mL insulin, and 100 μM indomethacin. After MSCs were cultured in the adipogenic differentiation medium for 1, 2, and 3 weeks, the cells were rinsed with PBS three times, fixed with 4% paraformaldehyde solution for 1 h at 4 ºC, and then stained with Oil Red O for 2 h. To analyze gene expression during adipogenic differentiation, MSCs were cultured at different cell density separately and quantitative real time PCR (qRT-PCR) was performed. TaqMan® probes and primer pairs for fatty acid binding protein-4 (FABP4), lipoprotein lipase (LPL), and peroxisome proliferator activated receptor γ2 (PPAR γ 2) were used. The expression level of 18S rRNA was used as an endogenous control and gene expression levels relative to *GAPDH* were calculated using comparative Ct method.

Results : Observation by optical microscopy demonstrated that grafted PVA formed the same striped micropattern as that of the photomask. MSCs were seeded on the PVA-micropatterned surfaces and cultured in serum medium. The cells distributed evenly on the PVA-micropatterned surfaces immediately after cell seeding. However, after 1 day of culture cells adhered only on the polystyrene stripes, not on the PVA stripes. T he cells on the PVA stripes moved to the polystyrene stripes during cell culture because PVA did not support cell adhesion. Therefore, a gradient cell pattern was formed on the PVA-micropatterned surfaces. The cell number on each polystyrene stripe was counted and cell density was calculated. The cell density ranged from 5×10^3 o 3×10^4 cells/cm^2 from the narrowly separated polystyrene stripe to the widely separated polystyrene stripe The measured cell density was in a good agreement with the designed width ratio of the PVA stripe to the polystyrene stripe. The result indicates that cell density could be controlled by changing the ratio of the cell adhesive and non-adhesive areas on the PVA-micropatterned surfaces. After 1 to 3 week culture in the adipogenic differentiation medium, lipid vacuoles were observed at all cell densities. The cells at the lowest density did not become confluent and some cells existed individually. The cells at the highest density became confluent and contacted with each other. The number of lipid vacuoles increased with culture period. The number of lipid vacuoles was small at the lowest density cells and abundant at the highest density cells. The cells at different cell densities showed almost the same degrees of Oil Red O staining. qRT-PCR analysis showed that PPARγ2, LPL, and FABP4 genes were expressed at all the cell densities examined in this study. The expression level of the genes increased with culture time. There was no significant difference in the expression level of these genes among these cell densities. The results suggest that there was no apparent effect of cell density on the adipogenic differentiation of human MSCs at the cell densities.

Conclusions: A gradient cell density pattern of human MSCs was formed on a PVA-micropatterned surface The MSCs at cell densities ranging from $5 .00 \times 10^3$ to 3.00×10^4 cells/cm^2 showed similar adipogenic differentiation. The micropatterning technique will be a useful tool to generate a gradient of cell density to compare the effect of cell density on stem cell functions.

References :

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