geometry could contribute differentiating neuronal cells, we used mouse N2a neuroblastoma stem cells on different micropatterns of convex pillars or walls.

Methods: N2a cells were plated on poly-L-lysine-coated SiO2 surfaces at a concentration of 1×10^5/ml. After the cells were attached firmly for 18h, the cells were induced to differentiate into neurons in the presence of dibutyryl cyclic AMP (dbcAMP) and 17β-estradiol for 48~96 h. The cell were fixed in 2% paraformaldehyde after washing in phosphate buffered saline, pH 7.4, and stained with a lipohillic dye, 3,3'-dioctadecyloxacarbocyanine perchlorate (DiO) to clearly show axonal growth. Cells were also analyzed by scanning electron microcopy.

Results: Different patterns of SiO2 gave a marked guidance of axonal growth according to the formed walls of SiO2. The micropatterns also influenced orientations of axonal growth and neurite development.

Conclusion: Thus, the results suggest that physical microenvironment could also serve as a mechanical cue for the guidance of axonal growth in differentiating neuronal cells.

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P-8 Maintenance and Differentiation of Embryonic Stem Cells without Feeder Layer

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Objectives: Although certain cell lines from mouse (mESCs) and human embryonic stem cells (hESCs) can be grown under feeder-free conditions, most of the cell lines can be maintained only on various feeder cells. Feeder-free ES cell culture system is one of critical components towards generating and maintaining clinical grade of hESC lines for the application of cell replacement therapy in near future. To overcome the feeder-dependent or conditioned medium culture systems for proliferation and differentiation of ESCs, we employed a mixture of polymeric natural product gel as a substrate to hold ESCs in suspension to develop a feeder-free system.

Methods: Both mESC (J1 and D3) and hESC lines (Miz-Med-4 and 6) were cultured according to conventional ES cell culture system established in sDMEM containing LIF. These cells were encapsulated in the suspension matrix gel in either single cells or multi-cells in the matrice depending on the sizes of matrix gel.

Results: Both J1 and D3 mESCs were capable of proliferation under the identical condition used in conventional culture without feeder layer. hESCs (Miz-Med-4 and 6) also showed cell proliferation as judged by diameter of the cell colony formed within the matrix even from a single cell. The mES cell colony formed within the matrix showed pluripotent cell lineage makers, including alkaline phosphatase, SSEA-1, nanog, Oct-4 and Sox2, commonly used. Upon the removal of LIF from the medium, both mESCs and hESCs showed varying degrees of cell differentiation depending on the duration of induction of cell differentiation.

Conclusion: The developed feeder-free ESC culture system allowed us to eliminate preparation and maintenance of feeder layer for cell proliferation, and subsequent steps of embryoid body formation and replating ESCs onto substrates for cell differentiation. The newly-established ESC culture system should provide opportunities to develop clinical grade of

hESCs as well as study signaling molecules involved in stem cell niche.

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P-9 Effect of Flavonoid on in vitro Proliferation of Mouse Embryonic Stem Cells

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Objectives: Embryonic stem cells are recognized as an excellent cell culture model for studying developmental mechanisms and their therapeutic modulations. Flavonoids are a diverse group of naturally occurring polyphenolic compounds with wide-ranging biological properties (e.g., antiviral, anti-inflammatory, mutagenic, antimutagenic, proliferative, and other effects). Thus, the aim of this work was to determine if flavonoid can promote mouse ES (mES) cell proliferation.

Methods: The D3 and G4-2 mES cells were plated in 24-well plates (1×10^4 cells/well) in the presence of various concentrations (0.5~200 μM) of flavonoids (10 types), respectively. To examine the toxicity of flavonoid in the mES cell proliferation, we tried to test MTT assay. Flavonoid effect was examined during one day or three passages culture. The total cell number was measured with a hemocytometer. Using western blot analysis, Akt, Erk and START3 expression related to proliferation was checked.

Results: After one day treatment, in two types of treatment groups (3,2-dihydroxyflavone and 3,4-dihydroxyflavone), flavonoid effect for the mES cell proliferation was detected, while others were absolutely not. MTT assay showed the mES cell proliferation was increased about 1.5 folds at a concentration of 1 to 10 μM in the two flavonoid treatment groups. The results were similar in both D3 and G4-2 mES cells. However, after treatment of the two flavonoids during three passages, we confirmed the proliferation effects on mES cells were slightly different, 3,2-dihydroxyflavone effect was continued in 5 μM concentration but 3,4-dihydroxyflavone effect was in 1 μM. The alteration of cell cycle in response to 3,4'-hydroxyflavonoid was examined. 3,4-dihydroxyflavone induced the phosphorylation of Akt, Erk and STAT3 in a dose dependent manner on western blot analysis. Also, 3,4'-dihydroxyflavone increased the levels of the cell cycle regulator proteins in a dose dependent manner.

Conclusion: In the present study, we concluded special types of flavonoids bring to positive effect on the D3 and G4-2 mES cell proliferation.