This study is aimed at investigating the characters and developmental potential of HAM from post-cryopreservation.

**Methods:** HAM were isolated via enzymatic procedures and cultured, and an aliquot was cryopreserved. After thawing, cells were cultured in adipogenic, chondrogenic and osteogenic inductive media, and also induced to insulin-producing cells under suitable culture conditions. Cells were analyzed by immunocytochemistry, reverse transcriptase-polymerase chain reaction (RT-PCR) and emzyme-linked immunosorbent assay (ELISA).

**Results:** HAM from pre- and post-cryopreservation had similar cellular morphology and similar expression of protein such as collagen, fibronectin, CD44, CD54, CD106, SSEA3, SSEA4, HLA ABC, vWF and vimentin. RT-PCR analysis of the HAM from post-cryopreservation also showed expression of specific maker, Oct4, SCF, BMP-4, FGF-5, nestin, CK18, similar to HAM from pre-cryopreservation. Under appropriate culture conditions, the cells differentiated into adipocytes, chodrocytes and osteocytes. And in medium supplemented with glucagon-like peptide (GLP-1), activin A and nicotinamide, HAM efficiently differentiated into insulin producing cells and secreted insulin and C-peptide.

**Conclusion:** Post-cryopreserved HAM still have developmental potential and the cells can be induced to differentiate into insulin-producing cells. So it could be used for further experiment and treatment of type 1 diabetes.

## P-4 Directed Differentiation of Mouse Embryonic Stem Cells into Early Progenitors of Three Germ Layers within 3D Matrix

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**Objectives:** Embryonic stem cells (ESCs) can renew indefinitely and differentiate in vitro into various cell lineages depending on inducing agents used. Current protocols for cell differentiation from pluripotent ESCs involve formation of embryoid body to reinforce cell-cell interactions to generate mixed cell lineages. We hypothesized that it would be possible to achieve homogeneous cell differentiation if ESCs are cultured under a condition in which all the cells receive similar inducing signals in the presence of minimal cell interactions. To achieve this, we developed a direct differentiation culture system using micro-sized 3D matrix from the proliferated ESCs without forming EB.

**Methods:** Mouse D3 ESCs on STO feeder layer in sDMEM containing LIF and 15% FBS were dissociated completely into single cell suspension to prepare 3D matrix culture. Five to 10 cells were allocated in a 3D matrix gel using a glass micropipette. To prepare RNA, $0.5\sim1\times10^4$ cells were cultured in a milli-sized 3D matrix in a similar manner to that of micro-sized 3D matrix. Directed differentiation of the cells in matrix was achieved by adding 0 (control, vehicle alone), 1, 10 and 500 nM retinoic acid into sDMEM containing 2% FBS without LIF for 4 (4+0) days, respectively. Subsequently, the cells were cultured in sDMEM containing 15% FBS without LIF for 4 (4+4) and 8 (4+8) days to allow further differentiation and analyzed for early germ layer progenitors using various cell lineage markers.

**Results:** Different concentrations of RA showed less proliferation than that of the culture with LIF, resulting in different cell morphologies visibly. The cells in low RA concentrations at 0 and 1 nM expressed Brachyury at 4+0, whereas the cells in higher RA at 10 and 500 nM expressed nestin in immunocytochemical and RT-PCR analyses. Furthermore, GATA-4 and Foxa2 were intensively expressed in culture at 10 nM RA, suggesting endo-mesodermal cell lineages.

**Conclusion:** From these results, it was proposed that pure population of early progenitors can be obtained using 3D

matrix by both minimizing cell-cell interactions and providing a uniform milieu to differentiating ESCs with the inducing agent.

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## P-5 Isolation of Single Cell-derived Mouse Embryonic Stem Cell Clones from Mixed Cell Population

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**Objectives:** Since mouse embryonic stem cells (ESCs) have generally been cultured on the feeder layer in vitro for long time, the pluripotency of ESCs depends entirely on the quality of the feeder layer. Therefore, minor alterations in the microenvironment of feeder layer could affect status of the cells, generating slightly different cell lineages without notice. Thus, some of the ESC lines turn into preferring to differentiate into certain directions upon induction. It would be a laborious task to separate pluripotent cells from the mixed cell population with early germ layer stem cells. In particular, establishment of a single cell-derived cell clones should be a useful tool to study further. To isolate single cell-derived ESCs, we used a 3D matrix culture system in present study.

**Methods:** Mouse J1 ESCs were cultured on STO feeder layer in sDMEM containing LIF and 15% FBS for proliferation. To obtain stable GFP expressing J1 ESCs (J1-GL), cells were selected with G418 for 1 week. 3D matrix culture was performed by making single cell suspension for every 4 days. At the end of culture, high or low of GFP expressing cell clones were selected for candidate colonies. They were then used for further proliferation and establishment of single cell-derived ESC lines, designated J1-GL-H and J1-GL-L, respectively.

**Results:** The isolated J1-GL-H and J1-GL-L ESCs showed similar proliferation rates to those of wild type and the mother cell clone (J1-GL). To verify whether these cell lines have pluripotency as in wild type J1 or original J1-GL, various stem cell markers including alkaline phosphatase, SSEA-1, nanog, Oct-4 and Sox2 were examined cytochemically and at RNA level. Upon retinoic acid (RA) treatment with different concentrations, the established cell lines underwent differentiation into 3 germ layer lineages.

**Conclusion:** Taken these results including cell proliferation and differentiation together, the developed technology should be useful in establishing homogeneous cell population and isolation of single cell-derived clone safely without using feeder layer cells. Furthermore, the ESC culture system should provide opportunities toward obtaining clinical grade of human ESCs as well as studying signaling molecules in a controlled way by eliminating cell-cell interactions and growth factors secreted by feeder layer.

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