optimal cryoprotectants in the cryopreservation of human embryonic stem cells (ESC).

Materials and Methods: Human ESC clumps established at Seoul National University Hospital (SNUhES 1) were cryopreserved with the vitrification method using the EM grid. EDS and EFS40 were used as vitrification solutions.

EDS step1: DMEM + 10% ethylene glycol + 10% DMSO

step2: DMEM + 20% ethylene glycol + 20% DMSO + 0.5 M sucrose

EFS40: DMEM + 40% ethylene glycol + 18% Ficoll + 0.5 M sucrose

Results: Between the EDS and EFS40 groups, there was no significant difference in the recovery rate after cryopreservation of human ESC. The formation rates of ESC colonies in the vitrified groups were significantly lower than those in the control ESC group ($p<0.05$, $p<0.05$). In addition, the formation rate of ESC colonies in the EDS group was significantly higher than that in the EFS40 group ($p<0.05$). The ESC colonies in the vitrified groups were significantly smaller after culture duration of 2 and 4 days, respectively, compared with the control ESC group ($p<0.01$, $p<0.05$). However, these effects could be reduced to nonsignificant level by the additional culture of ESC colonies. The vitrified human ESC retained the properties of pluripotent cells, including the expression of cell surface markers for the undifferentiated cells such as alkaline phosphatase and SSEA-4 (stage-specific embryonic antigen-4), and the expression of transcription factor Oct-4 (octamer-binding transcription factor-4), and the normal karyotype.

Conclusion: The vitrification method using the EM grid and EDS solution was confirmed to be very effective for the cryopreservation of human ESC.

P-11 Maintenance of Human Embryonic Stem Cells Derived from Frozen-thawed Blastocysts on Feeder-free Culture Condition

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Objective: This study was to confirm whether the established human embryonic stem (hES) cell growth can be maintained without mouse embryonic feeder (MEF) cells.

Materials and Methods: The hES cells (MB02 and MB03) derived from frozen-thawed blastocysts were subcultured until 10th passage (about 2 month, 40 population doublings) on MEF feeder. And then some of these hES colonies were cultured on feeder-free condition using Matrigel-coated plate/STO cell conditioned medium. Characterization of hES cells cultured on feeder or off feeder were taken by alkaline phosphatase staining, karyotyping, cell surface marker staining, Oct4 gene expression and telomerase activity.

Results: The hES cells cultured on feeder-free condition during subculture (about 6 month, 120 population doublings) indicated stable proliferation rate, normal karyotype. high telomerase activity. Similar to cells cultured on feeders, hES cells maintained under feeder-free conditions expressed Oct4, alkaline phosphatase, surface marker (SSEA-4, TRA-1-60, TRA-1-81). Also, embryoid bodies cultured on gelatin dish

in differentiation medium were examined by RT-PCR, some specific factors represented three embryonic germ layers were determined in vitro (NF-M, keratin, enolase, cAct and amylase).

Conclusion: The established hES cells derived from frozen-thawed blastocysts can be maintained on feeder-free condition without loss of human cell characteristics.

P-12 In vitro Differentiation of Human Embryonic Stem Cells into Cardiomyocytes

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Objective: This study was to investigate differentiation of human embryonic stem (hES) cells into cardiomyocytes according to treatment factors and culture duration.

Materials and Methods: To differentiate into cardiomyocytes, embryoid bodies induced from established hES (MB03) cells were cultured in 0.75% DMSO, 0.75% DMSO + 1 μM retinoic acid (RA), 1 μM retinoic acid (RA) and 10 ng/ml bFGF added DMEM (+10% hyclon FBS) medium for 1, 2 and 3 weeks. To demonstrate differentiation into cardiomyocytes, we did RT-PCR with primers about cardiomyocytes once a week for 3 weeks.

Results: In RT-PCR, MLC-2A and MLC-2V as cardiomyocyte markers were expressed in all groups from 1 week. Especially, cardiac actin was just expressed in 0.75% DMSO treatment from 2 weeks.

Conclusion: This study showed that DMSO has an effect on differentiation of hES cells into cardiomyocytes from 1 week to 2 weeks.

P-13 Functional Cardiomyocytes Formation Derived from Parthenogenetic Mouse Embryonic Stem Cells

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Objective: This study was to establish the use of parthenogenetic mouse ES cells as a reproducible differentiation system for mouse cardiomyocytes.

Materials and Methods: To induce differentiation, parthenogenetic mouse ES cells were dispersed by dissociation and the formation of ES cell aggregates in differentiation medium. After 7 days in differentiation culture, the embryoid bodies (EBs) were plated onto gelatin-coated dish. Cultures were observed daily using an inverted light microscope to determine the day of contraction onset and total duration of continuous contractile activity for each contracting focus. The effects of dimethyl sulfoxide (DMSO) and