의 기본 배양액 간에서는 DMEM-F12 배양액에서 배아 줄기세포군의 부분적 분화률이 높게 나타난 것을 제외하고는 미분화 특성을 유지하는데는 차이가 없는 것으로 나타났다.

P-30 A New Protocol for Effective Cryopreservation of hES Cells by Minimum Volume Cooling (MVC) Method

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Objective: Human embryonic stem (hES) cells are being very important resources for study on cell replacement therapy, other medical applications or basic scientific research, and thus their efficient cryopreservation is inevitably needed. This study was to examine whether the newly developed MVC vitrification method can be used for the freezing of hES cells.

Materials and Methods: In this study, hES cell colonies (MB03) were cryopreserved by either the slow-cooling method using StrataCooler® (Stratagene) as a control method or MVC vitrification method using modified French-mini straw (500 μl, IMV) (we designated it as MVC straw). hES cell colonies cultured on mouse embryonic fibroblast (STO cell, ATCC) feeder using ES culture medium were mechanically dissected into several small clumps following by collagenase treatment. In experiment I, with the slow-cooling method, about 20~30 clumps of hES cells were transferred into a cryo-vial containing 1 ml freezing medium (10% DMSO added ES culture medium). The vials were slowly cooled in a StrataCooler® at -80℃ deep-freezer and then plunged in liquid nitrogen (LN_2) on next day. The vials were rapidly thawed in a water bath at 37℃. At thawing, freezing medium is gradually diluted in ES culture medium for 20~30 min. hES cell clumps were recovered using micropipette and then plated onto a fresh feeder layer. In experiment II, hES cell clumps were vitrified using MVC straw which were prepared by cutting end place of one side. Ten hES cell clumps were loaded onto one MVC straw using micropipette followed by exposure in two vitrifiation solution (VS); 10% ethylene glycol (EG) and 10% FBS added D-PBS for 5 min in the first step, then 30% EG + 0.5 M sucrose (S) + 10% FBS added D-PBS for 20 sec in the second step. And then MVC straw was plunged directly into LN_2. Thawing was rapidly performed by 5-step (1 MS, 0.5 MS, 0.25 MS, 0.125 MS and 10% FBS added D-PBS). Then recovered hES cell clumps were plated onto a fresh feeder layer.

Results: Pluripotent hES cell clumps were successfully cryopreserved by slow-cooling or vitrification method, and the cells retained hES characteristics after thawing. All clumps were recovered without any cell loss. At day 2 after thawing, cell survivabilities in vitrificaion method, which we confirmed as attachment of hES cell clumps onto feeder cell layer, were higher (94.3%, 66/70) than that in slow-cooling method (82.8%, 53/64), respectively. However, in MVC vitrification method, survived hES cell clumps were growing out with stable proliferation rate and they indicated normal karyotype, positively immunostained (AP, SSEA-4, TRA-1-60) using surface marker antibodies and high telomerase activity. Also, it

was determined that the vitrified-thawed hES cells also can be differentiated into all three embryonic germ layer cells in vitro.

Conclusion: This result demonstrates that hES cells can be successfully cryopreserved by MVC vitrification method without loss of human cell characteristics.

P-31 Functional Cardiomyocytes Formation Derived from Mouse Embryonic Stem Cells

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Objective: Pluripotent ES cells differentiate spontaneously into beating cardiomyocytes via embryo-like aggregates. This study was to establish a reproducible cadiomyocyte differentiation protocol using mouse embryonic stem (mES03) cells.

Materials and Methods: mES03 cells growing in colonies were dissociated and allowed to re-aggregated in suspension (EB formation). To launch cardiomyocyte differentiation, EBs were treated with 0.75% dimethyl sulfoxide (DMSO) for 4 days in suspension (4-/4+ or 4+/4-) and then were plated onto gelatin-coated dish. During differentiation, onset of contraction, duration, and frequency of the activities were recorded for each active foci. Contraction frequencies in DMSO treated groups (4-/4+ or 4+/4-) were compared with that of non-DMSO treated control group (4-/4-). To confirm generation of cardiomyocytes, contracting cell masses were mechanically dissected, enzymatically dispersed using trypsin-EDTA, plated onto glass coverslips, and then incubated for 48 hrs. Attached cells were stained with antibodies against cardiomyocytes specific markers.

Results: In DMSO-treated cells, spontaneous and rhythmic contractions were noticed as early as 7 days after plating onto adherent surface. In 35 days, approximately 45% or 50% of the EBs exhibited spontaneous contraction in either 4+/4- or 4-/4+ group. No statistic differences, however, were obvious on treatment methods, counting of beating foci suggested that 4-/4+ treatment group exhibited slightly more beatings than that of 4+/4- treatment group. Cells within the spontaneously contracting colonies were stained positively with muscle specific anti-sarcomeric α-actinin antibody and cardiac specific anti-cardiac troponin I antibody.

Conclusion: This study indicates that mES03 cell-derived cardiomyocytes displayed biochemical properties of cardiomyocytes and DMSO enhanced development of cardiomyocytes in 4-/4+ method.