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.