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Vitrification of Human Amnion-derived Mesenchymal Stem Cells

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Background & Objectives: Human amnion-derived mesenchymal stem cells (AMSCs) are multipotent stem cells capable of differentiating into mesenchymal lineages. Stem cell-like characteristics of human AMSCs suggest that these cells are a promising new clinical tool for cell therapy. For effective manipulation and clinical application of human AMSCs lines, it is necessary to establish reliable methods for long-term cryopreservation. Slow cooling method using dimethylsulfoxide is effective for cryopreservation of wide variety of cell lines. A rapid cooling method, vitrification, has been used for cryopreservation of zygotes or embryos which can reduce damage resulted from ice crystal formation within the cytoplasm during freezing process compared to slow freezing. The objective of this study was to investigate whether a vitrification can be a useful method to cryopreserve human AMSCs.

Method: Human amnions were obtained during cesarean delivery with patients' consent. Amnions were subjected to a series of enzymatic digestion and mesenchymal cell-like cells were cultured in vitro. At passage 2, human AMSCs were cryopreserved by vitrification method using 20% ethylene glycol (EG20) and 40% ethylene glycol containing 9% ficoll and 0.3 M sucrose (EFS40). Two weeks later, the vitrified human AMSCs were thawed and then cultured for one week. The expressions of established stem cell surface markers were examined by FACS analysis in the thawed human AMSCs at passage 3. Non-vitrified human AMSCs at passage 3 were served as control.

Results: Human AMSCs were successfully vitrified. The survival rate after thawing was 82% as judged by tryphan blue staining. Their morphology was indistinguishable from non-vitrified control cells. Vitrified-thawed human AMSCs continued to express surface markers of mesenchymal stem cells such as CD44, CD49d, CD59, CD90, CD105, HLA-ABC and HAL-G, but negative for CD31, CD34, CD45, CD106, CD117 and HLA-DR. These patterns of expression were identical to control.

Conclusions: Vitrification method can be successfully applied to cryopreservation of human AMSCs and our finding will be valuable for future utilization of human AMSCs.