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Comparison of Dimethyl Sulfoxide and Ethylene Glycol for Cryopreservation of Porcine Mesenchymal Stem Cells

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Cryopreservation has been extensively applied to many cell and tissue types. Recently, due to the use of a variety of stem cells and the emerging higher standards for cell and tissue banking, specifically stem cell banking there has been a renewed interest in optimizing cryopreservation techniques. Hence, the present study was under taken to compare the viability, apoptosis and necrosis of porcine mesenchymal stem cells cryopreserved in dimethyl sulfoxide (DMSO) and ethylene glycol (EG) by rapid freezing-slow thawing method without the use of programmable freezer. Bone marrow aspirates obtained from the femur and tibia of a young adult porcine (~6 months of age), passed through a ficoll density gradient and were plated. After 48 h, non-adherent cells were removed and the adherent fibroblast-like cells were cultured for 10~14 days in a-DMEM supplemented with 10% FBS at 38.5℃ in a humidified atmosphere of 5% CO_2 in air with media replacement every 3rd day (passage 0). Cells were harvested when they reached 80~90% confluence (passage 8) using 0.25% (w/v) trypsin-EDTA solution and pelleted at 300 ×g for 5 min. MSCs were characterized by their growing cell-surface antigen profile CD $105^{pos.}$ MSCs were transferred into pre-cooled (4℃) cryo-vials containing 1 mL of 10% DMSO (group I) and 1.5 M EG (group II), cooled to -1℃/min up to -70℃ in a deep freezer overnight, then immediately plunged into LN_2 and stored for 7 days. Cryo-vials were thawed at 37℃ in a water bath and freezing medium was gradually diluted with a-DMEM. Fresh MSCs were kept as

control (group Ⅲ). In three replicates, cell viability, apoptosis and necrosis rates were evaluated with Vybrant Apoptosis Assay kit #3 using fluorescence-activated cell sorter (FACS). Statistical assay was performed one-way ANOVA by SPSS 10.0 ($p<0.05$). Fresh MSCs exhibited a significantly ($p<0.05$) higher viability (73.8%) than that of group Ⅰ (51.4 %) and group Ⅱ (53.1%), respectively. No significant difference was observed in the apoptosis rate among the groups. Necrosis rate in group Ⅰ (38.4%) and group Ⅱ (36.5%) was significantly ($p<0.05$) higher than that of fresh MSCs (10.4%), respectively. However, the dead cell rate in group Ⅰ (8.1%) and group Ⅱ (7.9%) was lower than that of fresh cell (12.6%), respectively. In conclusion, porcine MSCs could be effectively cryopreserved in dimethyl sulfoxide and ethylene glycol by slow freezing-rapid thawing method without the use of programmable freezer. Further, analysis of certain genes that play important roles on cell survival could increase our understanding of cryopreservation of the stem cells to develop a suitable protocol.

Key words) *MSCs, Cryopreservation, Dimethyl sulfoxide, Ethylene glycol, Cell viability, Apoptosis, Porcine*

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