

## Properties of Adipose Tissue Derived Mesenchymal Stem Cells with Long-Term Cryopreservation

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**Abstract :** Adult mesenchymal stem cells (MSCs) have been investigated using preclinical approaches for tissue regeneration. Porcine MSCs (pMSCs) are capable of growing and attaching to plastic with a fibroblast-like morphology and then differentiating into bone, adipose, and cartilage tissues *in vitro*. This study was conducted to investigate the proliferating abilities, differentiation potentials, and multipotency of miniature pig adipose tissue-derived MSCs (mpAD-MSCs) with or without long-term cryopreservation, considering that cryostorage has the potential for use in clinical applications. After confirming the characteristics of the mpAD-MSCs, we examined the effect of long-term cryopreservation (> 2 years) on expression of cell surface markers (CD34, CD90 and CD105), proliferating abilities (cumulative population doubling level, doubling time, colony-forming unit, and MTT assay) and differentiation potentials into mesodermal cell lineages. As a result, the expression of cell surface markers is similar between thawed and fresh mpAD-MSCs. However, long-term cryopreservation significantly lowered the differentiation potentials (adipogenic, chondrogenic, and osteogenic) of mpAD-MSCs. When compared with fresh mpAD-MSCs, thawed mpAD-MSCs exhibited lower expression of mesodermal cell lineage-related genes such as peroxisome proliferator-activated receptor- $\gamma$ 2, lipoprotein lipase, collagen Type II  $\alpha$ 1, osteonectin, and osteocalcin. Interestingly, long-term cryostored mpAD-MSCs exhibited significantly higher cell viability than the fresh mpAD-MSCs. Long-term cryopreservation induced a 30% increase in the cell viability of mpAD-MSCs when compared with the fresh mpAD-MSCs at 5 days after thawing. However, long-term cryopreservation significantly lowered expression of stemness markers such as Oct3/4, Sox2, and Nanog. Furthermore, long-term cryopreservation negatively affected expression of senescence-associated genes such as telomerase reverse transcriptase and heat shock protein 90 of mpAD-MSCs when compared with the fresh mpAD-MSCs. The results from this study might be important for the successful application of MSCs in clinical trials after long-term cryopreservation.

**Keywords :** mesenchymal stem cells, cryopreservation, stemness, senescence

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