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STEM-CELLBANKER for Use in Mesenchymal Stem Cells (MSCs) Cryopreservation

Mesenchymal stem cells (MSCs) are multipotent stem cells that is able to differentiate into a variety of cell types. Diffentiations of MSCs into osteoblasts, chondrocytes, adipocytes have been shown in *in vitro* or *in vivo*. Recent study shows MSCs a promising approach for regenerative medicine for a wide range of applications such as application to bone and cartilage repair, regeneration of cardiomyocytes or the like.

A proven record of successful cryopreservation for MSCs using STEM-CELLBANKER has been established in our collaborative study with Graduate School of Comprehensive Human Sciences Tsukuba.

(Publication: Under construction)

Materials and Method

Human umbilical cord blood-derived MSCs was cultured and collected by centrifugation. Cell concentration was adjusted to 2 x 10^5 cells/ml with STEM-CELLBANKER solution. The mixture was transfer to a cryotube and frozen directly in –80℃ deep freezer for 4 days without programmed freezing, followed by cryopreservation in –196℃ liquid nitrogen tank for 72 days. After thawing the cells in 37℃ waterbath, cell-washing was conducted in a tube with mixture of DMEM (9ml) and FBS (1ml) for 2 times. Cells were centrifuged 1000rpm/s for 5min at 4℃ and followed by cell counting (for cell viability).Cells with concentration of 4 x 10^5 cells/ml were plated in 35mm dish for cell proliferation determination. Number of living cells was counted at 4^{th} day and 8^{th} day after culture. At the same time, cells with concentration of 5 x 10^4 were cultured in 24-well plate with 2 differentiation media as following for cell differentiation determination.

	Osteoblast Differentiation Medium	Fat cell Differentiation Medium
Composition of Medium	IMDM + 1v/v% FBS 0.1 μM dexamethasome 50 μg/ml ascorbic acid 10mM β-glycerol phosphate 10ng/ml hEGF	IMDM + 1v/v% FBS 0.1 μM dexamethasome 0.5mM 3-isobutyl-1- methylxanthine 0.1mM indomethacin

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Result:

- Cell viability

An average of more than 90% of cell viability after thawing was observed.

- Cell proliferation ability

Figure 1 shows proliferation of MSCs after thawing.

Thawed MSCs was indicated for possessing cell proliferation ability as normal.

- Cell differentiation ability

Figure 2a. shows Osteoblast differentiation of MSCs after thawing.

Figure 2b. shows Fat cell differentiation of MSCs after thawing.

Thawed MSCs was indicated for possessing cell differentiation ability as normal.

Figure 1

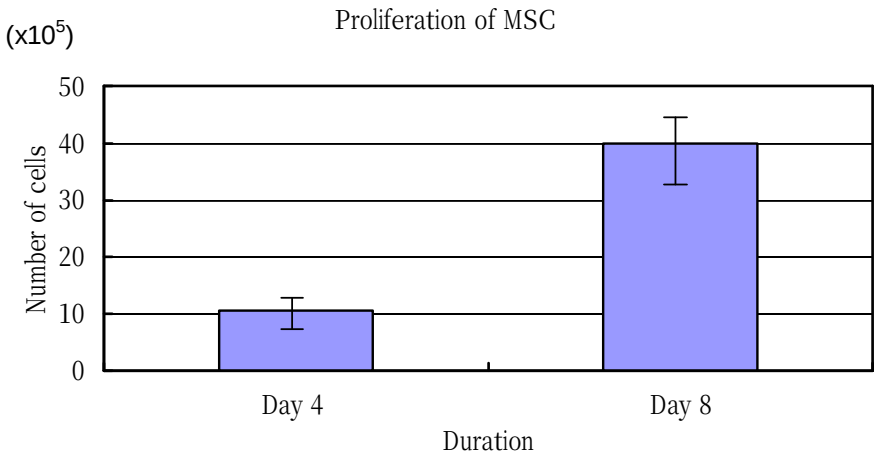

Fig. 1 shows the proliferation of MSCs after thawing. Increase of cells number was observed on Day 4 and Day 8 indicating that thawed MSCs possesses cell proliferation ability as normal.

Figure 2a (Staining with Alizalin Red)

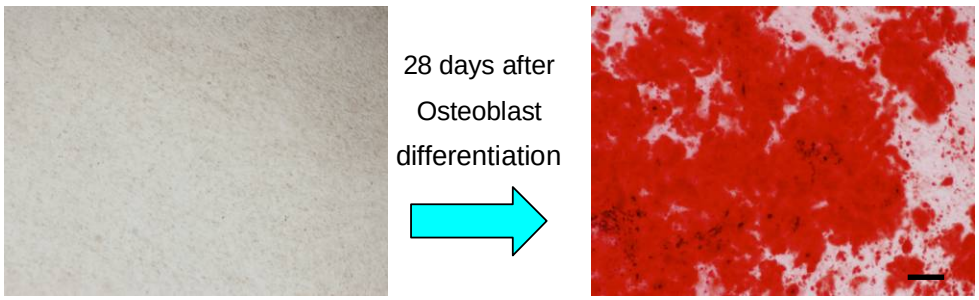

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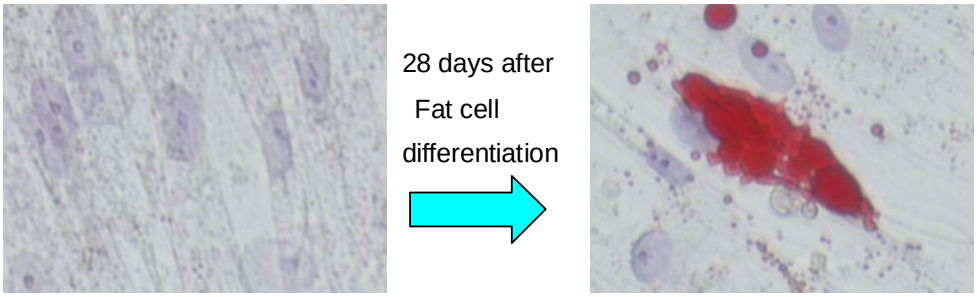
Figure 2b (Staining with Oil Red O)



Conclusion

STEM-CELLBANKER showed as high as 90% cell viability while retaining normal cell proliferation and differentiation ability of MSCs. The procedures for cell freezing and cell thawing are very simple. Cells can be frozen directly in -80 degree Celsius for long-term preservation with high cell viability.

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