

Review

Cryopreservation of Adipose-Derived Mesenchymal Stem Cells

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Mesenchymal stem cells (MSCs) have the potential to differentiate into cells of mesodermal origin such as osteoblasts, adipocytes, myocytes, and chondrocytes. They possess an immunosuppressive effect, which makes them a viable cell population for the cell-based therapy of treatment-resistant immune diseases. Adipose-derived mesenchymal stem cells (ASCs) have been demonstrated to have the ability to acquire the properties of subcutaneous adipose tissue particularly easily, and cryopreservation is currently performed as a routine method for preserving ASCs to safely acquire large numbers of cells. However, many studies have reported that cellular activity after freezing and thawing may be affected by the solutions used for cryopreservation. Dimethyl sulfoxide (DMSO) is commonly used as a cryopreservation medium as it diffuses into the cell through the plasma membrane and protects the cells from the damage caused by freezing. As substitutes for DMSO or animal-derived serum, cell banker series, polyvinylpyrrolidone (PVP), sericin and maltose, and methyl cellulose (MC) have been investigated for their clinical applications. It is critical to develop a reliable cell cryopreservation protocol for regenerative medicine using MSCs.

Key words: Adipose-derived mesenchymal stem cells (ASCs); Cryopreservation; Dimethyl sulfoxide (DMSO); Cell banker series; Polyvinylpyrrolidone (PVP)

INTRODUCTION

Mesenchymal stem cells (MSCs) were initially separated from the bone marrow for osteogenesis and hematopoietic cell regeneration (8,21,22). MSCs maintain the potential to differentiate into cells of mesodermal origin such as osteoblasts, adipocytes, myocytes, and chondrocytes (Fig. 1); moreover, MSCs possess immunosuppressive effects, which implies that they may have possible clinical applications in regenerative medicine and in therapies for treatment-resistant immune disorders (7,15,28–31,34). In addition to bone marrow-derived MSCs (BMSCs), MSCs can be obtained from, for example, umbilical cord tissue and blood, placental, and adipose tissues (13). Adipose-derived MSCs (ASCs) are normally

isolated from the subcutaneous adipose tissue, which allows them to be acquired in large numbers. ASCs proliferate rapidly with a high cellular activity, making them an ideal source for obtaining MSCs (25). Obtained ASCs can be cryopreserved; however, a number of studies have indicated that the cellular activity of ASCs after freezing and thawing is affected by cryopreservation solutions. For example, dimethyl sulfoxide (DMSO) and glycerol are commonly used as cryopreservation solutions to protect the cells from the damage caused by freezing, but they can also decrease the survival rate or induce cell differentiation (9,24). Polyvinylpyrrolidone (PVP), sericin and maltose, and methyl cellulose (MC) have been tested as alternatives to DMSO and glycerol as well as for the substitution of

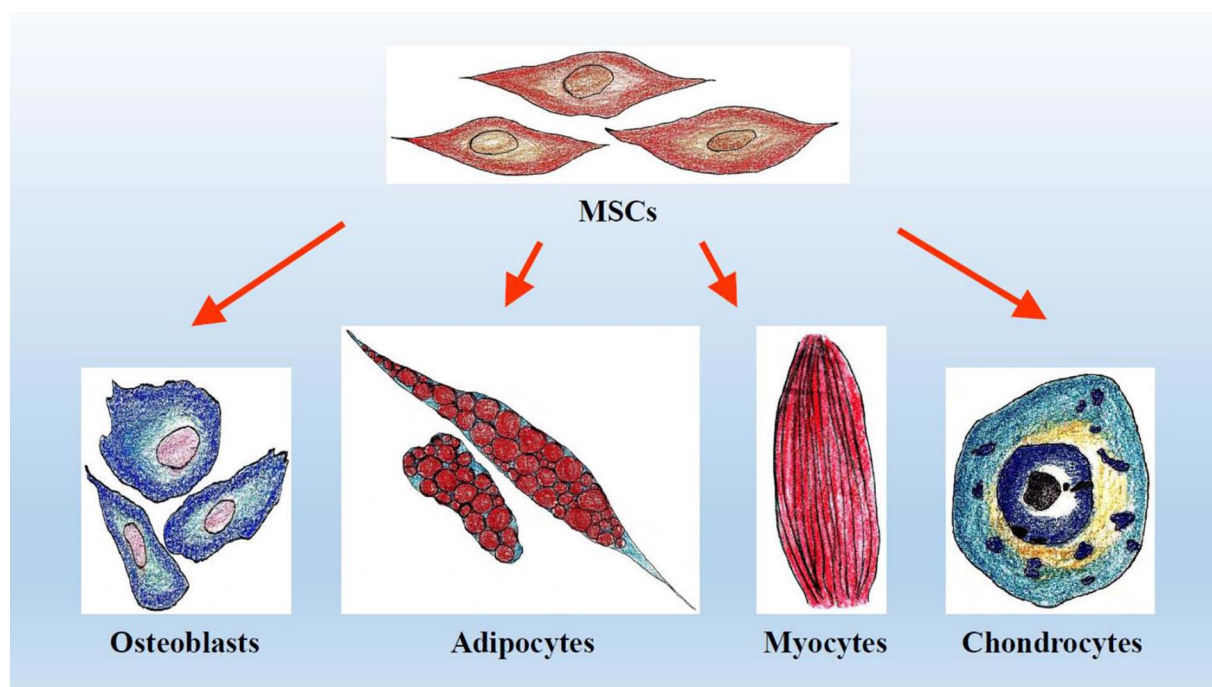


Figure 1. Scheme of mesenchymal stem cells (MSCs) and their differentiated cells.

animal-derived serum in the cryopreservation of cultured cells to be used for clinical applications (18,26,35,36). We review some of the currently available cryopreservation solutions that can be used for the cryopreservation of acquired ASCs (or MSCs).

THE EFFECT OF DMSO AS A CRYOPRESERVATION AGENT

DMSO is a solution that is commonly used for the cryopreservation of cultured mammalian cells because it is cheap and has a relatively low cell toxicity. However, when the cells will be used for clinical application, negative effects such as a decline in the survival rate following freezing, preserving, and thawing have been reported (1,3,7,14,16,37,38). Moreover, the induction of cell differentiation due to DNA methylation and modification of histones has been reported in HL-60 cells (human promyelocytic leukemia cell line from 36-year-old female) (4). However, Liu et al. reported that the survival rate and differentiation potency after freezing and thawing was not affected by slow freezing and rapid thawing in 10% DMSO (17). Thirumala et al. reported a combination of 80% serum [fetal calf serum (FCS) or human serum (HS)] and 10% DMSO had a survival rate of more than 80% in either FCS or HS. When tested with DMSO concentrations from 0 to 10% in a serum-free state, apoptotic, necrotic, and live cells were 20%, 50%, and 30%, respectively, following freezing and thawing when no DMSO was added, while apoptotic or necrotic cells were less

than 5%, and live cells were more than 80% in 2–10% DMSO. The presence of serum or the type of serum used in the presence of DMSO had no effect on the survival rate or on the differentiation potency of ASCs (35).

COMPARISON BETWEEN THE CELL BANKER SERIES AND DMSO

The cell banker series (Nippon Zenyaku Kogyo Co., Ltd., Fukushima, Japan) allows for rapid cell cryopreservation at -80°C and has been shown to be associated with a better survival rate following freezing and thawing. Serum-containing cell bankers 1 and 1+ can be used for cryopreservation of almost all mammalian cells, making them the standard cell banker series. Moreover, nonserum-type cell banker 2 is optimal for the cryopreservation of cells in serum-free culture conditions. STEM-CELLBANKER (cell banker 3) is a cell cryopreservation solution that satisfies the concept of a chemically defined known ingredient that is xeno-free and that optimizes the preservation performance of valuable cells such as somatic stem cells and induced pluripotent stem cells (27).

Oishi et al. investigated the cell survival rate and differentiation potency of adipocytes and osteoblasts when mouse ASCs were frozen and thawed in 10% DMSO and cell banker series 1, 1+, and 2. The cell survival rate of the ASCs after freezing and thawing was more than 90% in cell banker 2, approximately 90% in cell banker 1 and 1+, and less than 80% in 10% DMSO. The proliferation potential of the cells cultured for 7 days following freezing and

thawing was 8.6×10^5 cells/ml in cell banker 2, 7.0×10^5 cells/ml in cell banker 1+, 6.8×10^5 cells/ml in cell banker 1, and 5.8×10^5 cells/ml in 10% DMSO, with a relatively high proliferation potential in cell banker 2 compared to the other cryopreservation solutions. The differentiation potency of adipocytes after 7 days of culturing the frozen and thawed ASCs was maintained in all cryopreservation solutions. However, the differentiation potency of osteoblasts was maintained only using cell banker 2. Overall, cell banker 2 was the most effective cryopreservation solution for ASCs (34). Miyamoto et al. compared the survival rate, proliferation potential, and multilineage potential of cells following freezing and thawing in 10% DMSO and cell banker 2 in human ASCs. The cell survival rate following freezing and thawing was more than 95% in both cryopreservation solutions. However, the cell proliferation potential and multilineage potential was significantly better in the cell banker 2 solution (26).

Al-Saqi et al. compared the cell survival rate and differentiation potency of ASCs between 10% DMSO and cell banker 3. While there were no differences in the cell form before and after freezing and thawing observed between the two types of cryopreservation solutions, the cell survival rate was significantly higher in cell banker 3 than 10% DMSO ($90.4 \pm 4.5\%$ in cell banker 3 and $79.9 \pm 3.8\%$ in 10% DMSO). The differentiation potency with fat and osteoblasts did not differ significantly between cell banker 3 and 10% DMSO (2).

OTHER CRYOPRESERVATION SOLUTIONS AND SERUM-FREE CRYOPRESERVATION SOLUTIONS

PVP, MC, and sericin and maltose have been tested as cryopreservation solutions of ASCs as alternatives to DMSO or to animal-derived serum for the purpose of clinical application of cultured cells. PVP, which is a macromolecular polymer, lowers the freezing point and inhibits the increase of extracellular salt concentration, thereby stabilizing the cell membrane and protecting cells from impairment due to freezing. Thirumala et al. investigated the survival rate of ASCs and their differentiation potency into adipocytes and osteoblasts after freezing and thawing using PVP as a substitute for DMSO. When tested at levels of 1%, 5%, 10%, 20%, and 40% PVP, the highest survival rate of ASCs was found to be 69.7% in 10% PVP. The cell survival rate slightly increased to 72.1% when 10% PVP was supplemented with 10% HS. On the other hand, the cell survival rate in 10% DMSO + 10% HS was 82.5%, indicating DMSO to be more effective than PVP. The potential to differentiate into adipocytes and osteoblasts did not differ significantly between the two groups, showing that PVP is an effective cryopreserving reagent for ASCs (36).

MC is a macromolecular polymer that is used as a cell cryopreservation reagent to substitute animal-derived serum

(5,23,33). Thirumala et al. compared the cell survival rate when ASCs were cryopreserved with i) 1% MC, ii) 1% MC + 10% HS, iii) 1% MC + 10% FCS, and iv) 1% MC + 10% DMSO. The cell survival rate after freezing and thawing was the highest in 1% MC + 10% DMSO ($79.8\% \pm 2.8\%$), while the survival rates in all other groups were less than 50%. Although MC can be substituted for serum in the cryopreservation of ASCs, the presence of DMSO is essential for cellular activity (35).

Miyamoto et al. tested the cocoon-derived protein, sericin, as the main component to substitute animal-derived serum. Human ASCs were cryopreserved using the sericin-containing cryopreservation solution (10% DMSO + 1% sericin + 0.1 mol/L maltose) or 10% DMSO alone, and the survival rate, proliferation potential, and multilineage potential to differentiate into adipocytes and osteocytes of the thawed cells were compared. The survival rate was more than 95% in both solutions, and there was no significant difference between the solutions. The sericin-containing cryopreservation solution is more effective than 10% DMSO alone in terms of preserving proliferation potential and multilineage potential (26).

CONCLUSION

Since the acquisition of subcutaneous adipose tissue is less invasive, ASCs can be obtained more easily than BMSCs. A number of ASCs can be prepared before transplantation because the proliferative rate of ASCs is high (20,32). Moreover, the antigenicity of ASCs may be low (6), suggesting that ASCs could be used in the treatment of several diseases (10–12,19). Several studies have demonstrated the effectiveness of cryopreservation solutions, including DMSO, cell banker series, PVP, sericin and maltose, and MC for ASCs. Since several new solutions for cryopreservation such as FM-1 (Kyokuto Pharmaceutical Industrial Co., Ltd, Tokyo, Japan) have been recently released, they should be evaluated in a further study.

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