Patent Document 1: Japanese unexamined Patent Application Publication No. 2012-115253

Patent Document 2: International Publication No. WO 2014/208053

Non-Patent Documents

[0006]

Non-Patent Document 1: Gage, F.H. Science 287: 1433-1438 (2000)

Non-Patent Document 2: Morrison, S.J. *et al.*, Cell 96: 737-749 (1999)

Non-Patent Document 3: Batle, E. *et al.*, Cell 111: 251-263 (2002)

Non-Patent Document 4: Lane, T.A. *et al.*, Transfusion 49: 1471-1481 (2009)

Summary of the Invention

In one aspect, the present disclosure provides a mammalian cell preservation solution comprising trehalose, or a salt thereof and a hydrogen carbonate as a pH modifier, and having a pH of 6.5 to 8.5.

In another aspect, the present disclosure provides a powder formulation comprising trehalose, or a salt thereof and a hydrogen carbonate as a pH modifier, for preparing the preservation solution according to the disclosure.

In another aspect, the present disclosure provides a method of preserving a mammalian cell, comprising a step of preserving a mammalian cell in a preservation solution comprising trehalose, or a salt thereof and a hydrogen carbonate as a pH modifier, and having a pH of 6.5 to 8.5.

Object to be Solved by the Invention

[0007]

An object of the present invention is to provide, for instance, a mammalian cell preservation solution that can effectively suppress a decrease in cell viability occurring when mammalian cells are preserved in liquid or a decrease in self-renewal potential occurring when mammalian stem cells are preserved in liquid, and that is less likely to cause a harmful effect on the life of a mammal at the time of *in vivo* administration of mammalian cells to the mammal.

Means to Solve the Object

[Table 15]

	Immediately after start of preservation	Preservation period		
		6 h	24 h	48 h
Example 15	21.3±1.9	21.9±3.2	22.0±2.7**	15.2±3.6***
Comparative Example 8	21.3±1.9	20.8±1.4	14.9±2.5	1.70±1.4
Example 16	21.6±2.5	21.0±2.0	16.3±5.4	10.0±7.1*
Comparative Example 9	21.6±2.5	22.7±1.6	14.5±3.4	1.10±0.8

The numbers in the Table each represent the ratio of the number of colonies to the number of cells seeded (mean ± standard deviation [SD]; n = 4). The "**" and "***" in Example 15 indicate a statistically significant difference ($p < 0.01$ and $p < 0.001$, respectively) from Comparative Example 8 having the same preservation period. In addition, the "*" in Example 16 indicates a statistically significant difference ($p < 0.05$) from Comparative Example 9 having the same preservation period.

[0085]

II To Examine pH Modifier

1. Materials and Methods

[Mammalian Cells]

Human bone marrow-derived mesenchymal stem cells (hBM-MSC) designated in Table 16 below were used in the following Experiments.

[0086]

[Table 16]

Doner's age, sex, source	31 years old, male, bone marrow
Number of doners	1
Lot No.	0000527428
Passage number when purchased	Passage 2
Preservation method	Preserved in solution nitrogen
Manufacturer	Lonza Walkersville, Inc.

Provider	KATAYAMA CHEMICAL INDUSTRIES

[0087]

[To Prepare Test solution]

CSP-01 solution was prepared like in Example 1, and the CSP-01 solution (5.6 unadjusted) was obtained by adding, to lactated Ringer's solution (Lactec Injection; manufactured by Otsuka Pharmaceutical Factory, Inc.), α,α-trehalose (manufactured by Hayashibara Co., Ltd. or manufactured by FUJIFILM Wako Chemicals) and low-molecular-weight Dextran L Injection (10% [w/v] dextran-containing lactated Ringer's solution) (manufactured by Otsuka Pharmaceutical Factory, Inc.) so that the trehalose or the dextran had a final concentration of 3% (w/v) or 5% (w/v), respectively. CSP-01 solution (7.3 NaOH) was obtained by adding 0.5 mol/L sodium hydroxide solution to have a pH of 7.300. A sodium bicarbonate solution was added as a pH modifier to prepare CSP-01 solution (7.2 $NaHCO_3$). The CSP-01 solution (7.2 $NaHCO_3$) had a pH of 7.158. Note that the pH of each test solution was a value measured at room temperature (21.2 to 24.0°C).

[0088]

[To Prepare hBM-MSC-Containing Lactated Ringer's Solution]

The hBM-MSC-containing lactated Ringer's solution was prepared in accordance with the following procedures [1] to [8].

[1] Add 4×10^5 hBM-MSC to a 75-cm^2 flask and culture the cells in an incubator at 37°C and 5% CO_2 in the presence of a culture medium kit (manufactured by Lonza,

Cell viability (%) = (Total cell count − Dead cell count)/Total cell count × 100.

[0091]

2. Results

[Experiment]

The cell viability (see Figure 4) when hBM-MSC were preserved at 5°C for 3 days in 3 different tests designated in Table 17 below was measured in accordance with the protocol described in the above section [To Measure Cell viability]. Table 18 and Figure 4 show the results.

[0092]

[Table 17]

Group (n = 4)	pH (measured value)	Temperature(°C)
CSP-01 (5.6 unadjusted)	5.599	23.7
CSP-01 (7.3NaOH)	7.300	24.0
CSP-01 (7.2$NaHCO_3$)	7.158	21.2

[0093]

[Table 18]

Test solution	Cell viability (mean ± SD)	
	Before preservation	Day 3 after preservation
CSP-01 (5.6 unadjusted)	98.5±0.4	79.8±7.6
CSP-01 (7.3NaOH)		62.7±4.3
CSP-01 (7.2$NaHCO_3$)		87.9±1.7

[0094]

The results revealed that hBM-MSC were preserved at 5°C for 3 days; and the cell viability was significantly lower in the case of preservation in CSP-01 (7.3 NaOH)

CLAIMS

1. A mammalian cell preservation solution comprising trehalose, or a salt thereof and a hydrogen carbonate as a pH modifier, and having a pH of 6.5 to 8.5.

2. The preservation solution according to claim 1, wherein the hydrogen carbonate is sodium bicarbonate.

3. The preservation solution according to claim 1 or 2, further comprising a polysaccharide, or a salt thereof.

4. The preservation solution according to any one of claims 1 to 3, wherein the preservation solution is an isotonic solution.

5. The preservation solution according to claim 4, wherein the isotonic solution is a lactated Ringer's solution.

6. The preservation solution according to any one of claim 3, claim 4 when dependent on claim 3, and claim 5 when dependent on claim 4 when dependent on claim 3, wherein the polysaccharide is dextran.

7. The preservation solution according to any one of claims 1 to 6, wherein a concentration of the trehalose, or a salt thereof is 2.0 to 6.0% (w/v).

8. The preservation solution according to claim 6, or claim 7 when dependent on claim 6, wherein a concentration of the dextran, or a salt thereof is 4.0 to 7.0% (w/v).

9. The preservation solution according to any one of claims 1 to 8, wherein the preservation solution is for preserving a mammalian cell at 0 to 40°C.

10. The preservation solution according to any one of claims 1 to 9, wherein the preservation solution is for preserving a mammalian cell for a period of 6 h to 14 days.

11. The preservation solution according to any one of claims 1 to 10, wherein the preservation solution is used for suppressing a decrease in viability of a mammalian cell.

12. The preservation solution according to any one of claims 1 to 11, wherein the preservation solution is used for suppressing a decrease in self-renewal potential of a mammalian cell.

13. The preservation solution according to any one of claims 1 to 12, wherein the preservation solution is used for mammalian cell transplantation.

14. The preservation solution according to any one of claims 1 to 13, wherein the mammalian cell is a mammalian stem cell.

15. The preservation solution according to claim 14, wherein the mammalian stem cell is a mammalian mesenchymal stem cell.

16. A powder formulation comprising trehalose, or a salt thereof and a hydrogen carbonate as a pH modifier, for preparing the preservation solution according to any one of claims 1 to 15.

17. The powder formulation according to claim 16, wherein the hydrogen carbonate is sodium bicarbonate.

18. A method of preserving a mammalian cell, comprising a step of preserving a mammalian cell in a preservation solution comprising trehalose, or a salt thereof and a hydrogen carbonate as a pH modifier, and having a pH of 6.5 to 8.5.

19. The method of preserving a mammalian cell according to claim 18, wherein the hydrogen carbonate is sodium bicarbonate.

20. The method of preserving a mammalian cell according to claim 18 or 19, wherein the preservation solution further comprises a polysaccharide, or a salt thereof.

21. The method of preserving a mammalian cell according to any one of claims 18 to 20, wherein the preservation solution is an isotonic solution.

22. The method of preserving a mammalian cell according to claim 21, wherein the isotonic solution is a lactated Ringer's solution.

23. The method of preserving a mammalian cell according to any one of claim 20, claim 21 when dependent on claim 20, and claim 22 when dependent on claim 21 when dependent on claim 20, wherein the polysaccharide is dextran.

24. The method of preserving a mammalian cell according to any one of claims 18 to 23, wherein the mammalian cell is preserved in the preservation solution for 6 h to 14 days.