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]

~~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~~

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]

~~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~~

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]

~~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~~

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 ($7.2NaHCO_3$)		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 derivative thereof~~, 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 derivative thereof~~, 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~~s~~ 3, claim 4 when dependent on claim 3, and claim 5 when dependent on claim 4 when dependent on claim 3~~ to 5~~, 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 derivative thereof~~, 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 derivative thereof~~, 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 derivative thereof~~, 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 derivative thereof~~, 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 derivative thereof~~, 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~~s~~ 20, claim 21 when dependent on claim 20, and claim 22 when dependent on claim 21 when dependent on claim 20~~ to 22~~, 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.