

COMPOSITIONS AND METHODS OF CRYOPRESERVING CELLS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application Serial No. 62/964,074, filed January 21, 2020, the disclosure of which is hereby incorporated by reference.

BACKGROUND

[0002] Freezing of cells has long been used to preserve living cells after the cells have been removed or separated from a donating organism. However, the cryopreservation and recovery of living cells remains challenging. This is at least because cells subjected to freezing and thawing conditions are exposed to harsh conditions which in turn result in a generally low survivability rate.

[0003] Freezing is destructive to most living cells. Generally, as the extracellular medium freezes, cells attempt to maintain osmotic equilibrium across the membrane leading to intracellular water loss, which in turn increases intracellular solute concentration until intracellular freezing occurs. It is believed that both intracellular freezing and solution effects are responsible for cell injuries. Such cell injuries include, for example damage to cells plasma membrane which results from osmotic dehydration of the cells.

SUMMARY

[0004] The inventors have surprisingly discovered cryopreservation media that allows for freezing and subsequent thawing of viable cells. The frozen and thawed cells using the media and methods described herein retain high survivability, allowing the cells to be used for various applications, including for example, adoptive cell transfer methods.

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[0004a] In an aspect of the present disclosure, there is provided a composition of cryopreserved mammalian immune cells comprising mammalian immune cells that are primary cells or derived from induced pluripotent stem cells; and a cryopreservation medium comprising: between 1 w/v % and 10 w/v % dimethyl sulfoxide (DMSO), between 0.25 w/v % and 5 w/v % disaccharide, and human serum albumin (HSA).

[0004b] In a further aspect of the present disclosure, there is provided a kit comprising the composition disclosed herein.

[0004c] In another aspect of the present disclosure, there is provided a method of cryopreserving mammalian immune cells that are primary cells or derived from induced pluripotent stem cells, the method comprising:

(a) contacting the cells with a cryopreservation medium comprising between 1 w/v % and 10 w/v % dimethyl sulfoxide (DMSO), between 0.25 w/v % and 5 w/v % disaccharide, and human serum albumin (HSA); and

(b) cooling the cells by 1°C/minute to a temperature of -80°C.

[0004d] In a further aspect of the present disclosure, there is provided a method of cryopreserving and recovering viable mammalian immune cells that are primary cells or derived from induced pluripotent stem cells, the method comprising:

(a) contacting the cells with a cryopreservation medium comprising between 1 w/v % and 10 w/v % dimethyl sulfoxide (DMSO), between 0.25 w/v % and 5 w/v % disaccharide, and human serum albumin (HSA);

(b) cooling the cells by 1°C/minute to a temperature of -80°C thereby cryopreserving the cells; and

(c) thawing the cryopreserved cells.

[0005] In some examples, a cryopreservation medium for cryopreserving mammalian cells is provided, the medium comprising: dimethyl sulfoxide (DMSO), disaccharide, human serum, and IL-7 or IL-15.

[0006] In some examples, a cryopreservation medium for cryopreserving mammalian cells is provided, the medium comprising: dimethyl sulfoxide (DMSO), disaccharide, human serum albumin, and IL-7 or IL-15.

[0007] In some examples, the cryopreservation medium includes IL-7 and IL-15.

We claim:

1. A composition of cryopreserved mammalian immune cells comprising mammalian immune cells that are primary cells or derived from induced pluripotent stem cells; and a cryopreservation medium comprising: between 1 w/v % and 10 w/v % dimethyl sulfoxide (DMSO), between 0.25 w/v % and 5 w/v % disaccharide, and human serum albumin (HSA).
2. The composition of claim 1, wherein the disaccharide is sucrose.
3. The composition of claim 3, further comprising 1 ng/mL to 50 ng/mL cytokines.
4. The composition of claim 3, wherein the cytokines are selected from IL-7 and IL-15.
5. The composition of claim 4, wherein IL-7 is present at a final concentration between 1 ng/mL and 50 ng/mL.
6. The composition of claim 5, wherein IL-7 is present at a final concentration of 5 ng/mL.
7. The composition of claim 6, wherein IL-15 is present at a final concentration between 1 ng/mL and 50 ng/mL.
8. The composition of claim 7, wherein the IL-15 is present at a final concentration of 5 ng/mL.
9. The composition of any one of claims 1-8, further comprising a mammalian cell culture medium at between 10 w/v% and 90 w/v%.
10. The composition of claim 1, further comprising one or more amino acids.

11. The composition of claim 1, further comprising one or more inorganic salts.
12. The composition of claim 1, wherein the pH is between 7 and 8.
13. The composition of claim 1, wherein the mammalian immune cells are lymphocytes.
14. The composition of claim 13, wherein the mammalian immune cells are T cells.
15. The composition of claim 13, wherein the mammalian immune cells are natural killer (NK) cells.
16. The composition of claim 1, wherein the mammalian immune cells are induced pluripotent cell (iPSC)-derived cells.
17. The composition of claim 16, wherein the iPSC-derived cells are iCART cells.
18. The composition of claim 1, comprising the mammalian immune cells at a concentration of 1×10^6 cells/mL to 3×10^8 cells/mL.
19. The composition of claim 1, comprising between 0.5 w/v % and 30 w/v % human serum albumin (HSA).
20. The composition of claim 19, wherein the HSA is present at a final concentration of 2.5 w/v%.
21. The composition of claim 1, comprising 5 w/v % DMSO.
22. A kit comprising the composition of claim 1.
23. A method of cryopreserving mammalian immune cells that are primary cells or derived from induced pluripotent stem cells, the method comprising:

(a) contacting the cells with a cryopreservation medium comprising between 1 w/v % and 10 w/v % dimethyl sulfoxide (DMSO), between 0.25 w/v % and 5 w/v % disaccharide, and human serum albumin (HSA); and

(b) cooling the cells by 1°C/minute to a temperature of -80°C.

24. A method of cryopreserving and recovering viable mammalian immune cells that are primary cells or derived from induced pluripotent stem cells, the method comprising:

(a) contacting the cells with a cryopreservation medium comprising between 1 w/v % and 10 w/v % dimethyl sulfoxide (DMSO), between 0.25 w/v % and 5 w/v % disaccharide, and human serum albumin (HSA);

(b) cooling the cells by 1°C/minute to a temperature of -80°C thereby cryopreserving the cells; and

(c) thawing the cryopreserved cells.

25. The method of claim 24, wherein the mammalian immune cells are suitable for adoptive cell therapy.