

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.

[0004a] In one aspect of the present disclosure, there is provided a cryopreservation medium for cryopreserving mammalian cells, the medium comprising: dimethyl sulfoxide (DMSO), disaccharide, human serum, and IL-7 or IL-15.

[0004b] In another aspect of the present disclosure, there is provided a cryopreservation medium for cryopreserving mammalian cells, the medium comprising: dimethyl sulfoxide (DMSO), disaccharide, human serum albumin, and IL-7 or IL-15.

[0004c] In a further aspect of the present disclosure, there is provided a cryopreservation medium for cryopreserving mammalian cells, the medium comprising: between about 1 w/v % and 10 w/v % dimethyl sulfoxide (DMSO), between about 0.25 w/v % and 5 w/v % disaccharide, and between about 10 w/v % and 90 w/v % human serum.

[0004d] In another aspect of the present disclosure, there is provided a cryopreservation medium for cryopreserving mammalian cells, the medium comprising: between about 1 w/v % and 10 w/v % dimethyl sulfoxide (DMSO), between about 0.25 w/v % and 5 w/v % disaccharide, and between about 0.5w/v % and 30 w/v % human serum albumin.

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

[0004f] In another aspect of the present disclosure, there is provided a method of cryopreserving mammalian cells, the method comprising:

- (a) contacting the cells with a cryopreservation medium disclosed herein; and
- (b) cooling the cells by about 1°C/minute to a temperature of -80°C or below.

[0004g] In a further aspect of the present disclosure, there is provided a method of cryopreserving and recovering viable cells, the method comprising:

- (a) contacting the cells with a cryopreservation medium disclosed herein;
- (b) cooling the cells by about 1°C/minute to a temperature of -80°C or below 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.

[0008] In some examples, a cryopreservation medium for cryopreserving mammalian cells is provided, the medium comprising: between about 1 w/v % and 10 w/v % dimethyl sulfoxide (DMSO), between about 0.25 w/v % and 5 w/v % disaccharide, and between about 10 w/v % and 90 w/v % human serum.

[0009] In some examples, a cryopreservation medium for cryopreserving mammalian cells is provided, the medium comprising: between about 1 w/v % and 10 w/v % dimethyl sulfoxide (DMSO), between about 0.25 w/v % and 5 w/v % disaccharide, and between about 0.5w/v % and 30 w/v % human serum albumin.

[0010] In some examples, the disaccharide is sucrose.

[0011] In some examples, the medium further comprises D-glucose.

[0012] In some examples, the cryopreservation medium includes one or more cytokines. In some examples, the cytokines are selected from IL-7 and IL-15.

[0013] In some examples, the cryopreservation medium includes both IL-7 and IL-15.

[0014] In some examples, IL-7 is present at a final concentration of between about 1 ng/mL and 50 ng/mL.

[0015] In some examples, IL-7 is present at a final concentration of about 5 ng/mL.

[0016] In some examples, IL-15 is present at a final concentration of between about 1 ng/mL and 50 ng/mL.

[0017] In some examples, the IL-15 is present at a final concentration of about 5 ng/mL.

[0018] In some examples, the cryopreservation medium further includes a mammalian cell culture medium. In some examples, the mammalian cell culture medium is present at about between 10 w/v% and 90 w/v%. In some examples, the mammalian cell culture medium does not comprise non-human animal components.

[0019] In some examples, the cryopreservation medium further includes one or more amino acids.

[0020] In some examples, the cryopreservation medium further includes one or more inorganic salts.

[0021] In some examples, the pH between about 7 and 8. For example, the pH is about 7.2, 7.3, 7.4, 7.5, 7.6 7.7 and 7.8. In some examples, the pH is about 7.4.

[0022] In some examples, a kit is provided that includes the cryopreservation medium described herein.

[0023] In some examples, a method of cryopreserving mammalian cells is provided the method includes: (a) contacting the cells with a cryopreservation medium described herein; and (b) cooling the cells by about 1°C/minute to a temperature of -80°C or below.

[0024] In some examples, a method of cryopreserving and recovering viable cells is provided, the method comprising: (a) contacting the cells with a cryopreservation medium described herein; (b) cooling the cells by about 1°C/minute to a temperature of -80°C or below thereby cryopreserving the cells; and (c) thawing the cryopreserved cells.

[0025] In some examples, the thawed cryopreserved cells are not washed prior to subsequent culture or transplantation into a subject.

[0026] In some examples, the thawed cryopreserved cells have enhanced cell survival in comparison to cells frozen and thawed with a cryopreservation medium not disclosed herein. For example, the thawed cryopreserved cells have enhanced cell survival in comparison to cells frozen and thawed with a generally commercially available cryopreservation medium.

[0027] In some examples, the thawed cryopreserved cells have enhanced cell survival in vitro.

[0028] In some examples, the thawed cryopreserved cells have enhanced cell survival following transplantation into a subject.

[0029] In some examples, the mammalian cells are lymphocytes or progenitor cells.

[0030] In some examples, the mammalian cells are genetically modified lymphocytes or progenitor cells.

[0031] In some examples, the mammalian cells are induced pluripotent cell (iPSC)-derived lymphocytes or progenitor cells.

[0032] In some examples, the lymphocytes are T cells or natural killer (NK) cells.

[0033] In some examples, the progenitor cells are iPSC, hematopoietic progenitor cells (HPC), or embryonic stem cells (ESC).

[0034] In some examples, the mammalian cells are suitable for adoptive cell therapy.

[0035] Various aspects of the invention are described in detail in the following sections. The use of sections is not meant to limit the invention. Each section can apply to any aspect of the invention. In this application, the use of “or” means “and/or” unless stated otherwise. As used herein, the singular forms “a”, “an”, and “the” include both singular and plural referents unless the context clearly dictates otherwise.

BRIEF DESCRIPTION OF THE DRAWINGS

[0036] **FIG. 1** illustrates an experimental design that includes different cryo-formulation numbers and their compositions, number of mice each formulation was tested on, and iCART cell activation and transduction days of cells.

[0037] **FIG. 2** is a schematic illustration of preparation of iCART cells from frozen iPSC-derived T cells (“iT cells”).

[0038] **FIG. 3A** illustrates an experimental design to test the *in vivo* efficacy of iCART cells that were cryopreserved using cryo-formulation#2, cryo-formulation#6, and cryo-formulation#10. The design includes formulation compositions, number of mice each formulations were tested on, dose/s of cells administered to mice, and activation/collection days of cells. **FIG. 3B** illustrates the mean *in vivo* efficacy of iCART cells that were cryopreserved using cryo-formulation#2, cryo-formulation#6, and cryo-formulation#10. **FIG. 3C** illustrates the *in vivo* efficacy of iCART cells in each mice that were cryopreserved using cryo-formulation#2, cryo-formulation#6, or cryo-formulation#10. The *in vivo* efficacy of iCART cells was expressed in terms of total radiance flux.

[0039] **FIG. 4** illustrates an experimental design to test the effect of low- and high-washing of cryo-formulations on the *in vivo* efficacy of wild-type iCART cells. The design include three different formulations, doses of iCART cells, number of mice each formulations were tested on, and routes of administrations of target cells (i.e., Nalm6 cells) and iCART cells.

[0040] **FIG. 5** illustrates the *in vivo* efficacy of iCART cells that were cryopreserved using formulations illustrated in **FIG. 4**. The *in vivo* efficacy of iCART cells expressed in terms of luciferase expression was measured one week post-administration of iCART cells.