CELL CRYOPRESERVATIVE FORMULATIONS AND METHODS OF USE THEREOF

RELATED APPLICATIONS

[0001] This application claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application Serial No. 63/559,174, filed February 28, 2024, entitled "CELL CRYOPRESERVATIVE FORMULATIONS AND METHODS OF USE", the entire contents of which are incorporated herein by reference.

REFERENCE TO AN ELECTRONIC SEQUENCE LISTING

[0002] The contents of the electronic sequence listing (A102570066WO00-SEQ-CBD.xml; Size: 59,322 bytes; and Date of Creation: February 24, 2025) are herein incorporated by reference in its entirety.

BACKGROUND

[0003] Effective cell cryopreservation is an important aspect of many cell therapy strategies. The ability to freeze, store for various periods of time, and optionally transport cell-based therapies in a frozen state, expands their clinical use. Dimethyl sulfoxide (DMSO) is a conventional permeable type of cryoprotectant for cell products for maintaining cell quality during cryopreservation process and thaw. However, certain cryoprotectants, such as DMSO, can cause cytotoxicity when used in high concentrations in vitro and can also be harmful for patients when administered at high concentrations.

[0004] Existing methods for minimizing negative effects of certain cryoprotectants, such as DMSO, include diluting cells with physiological buffers and/or washing cells to remove the cryoprotectant and then reconstituting with a physiological buffer prior to administration into patients. However, there are several challenges in these approaches, including the need for additional processing steps, which can be costly, and short shelf life of the cell preparations. There is a need for a cryopreservative formulation that overcomes these difficulties and limitations.

SUMMARY

[0005] The ability to cryopreserve cell-based therapeutics decouples the manufacture and clinical use of cellular therapies in time and location. The design and selection of suitable cryoprotectant formulations is important to ensure cell survival and maintenance of function. This disclosure provides novel cryopreservative formulations that can be used to

cryopreserve, optionally transport, and ultimately administer cell populations to a subject in need thereof. Importantly, this disclosure contemplates using such formulations to cryopreserve cell populations, so that subsequent thawing and administration to a subject can occur without the need to wash or dilute the cells. In this way, the methods allow for freezing and thawing of cell-based therapeutics with minimal to no processing steps and manipulation. This reduces the likelihood of cell loss that can result from manipulation of cells in the post-thaw period.

[0006] This disclosure therefore provides particular cryopreservative formulations, and making of making such formulations, and methods of use thereof. The cryopreservative formulations may be used with a variety of cell types. The cryopreservative formulations provided herein result in robust post-thaw viability and cell growth. Further, such formulations are also able to preserve cell populations for extended periods of time at room temperature and at 4°C either pre-cryopreservation (e.g., as may occur in the process of cryopreserving the cell population) or post-thaw. And it has also been shown, as described in the Examples, that cell populations cryopreserved in such formulations are resistant to temperature variations of fluctuations during the cryopreserved period (e.g., as may occur in the process of transporting the cell population).

[0007] The formulations are useful for cryopreserving a variety of cell types including but not limited to retinal pigmented epithelial (RPE) cells. Thus, while many exemplifications in this disclosure involve RPE cells, it is to be understood that other cell types may be used instead. For example, the formulations provided herein may be used for photoreceptor rescue cells (PRC) (as described in WO2024044134, herein incorporated by reference in its entirety), a mesenchymal cell population such as hemangioblast-derived mesenchymal cells (HMC), vascular progenitor cells (VPC), a photoreceptor cell population, a photoreceptor progenitor cell population, a corneal epithelial cell (CEC) population, a macrophage cell population, a natural killer cell population, a T-cell population, a vascular cell population, a liver cell population, a neural cell population, and a pancreatic cell population.

[0008] One aspect of this disclosure provides a formulation comprising albumin, glucose, buffered saline, and one or two, or optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, hydroxyethyl starch (HES) having a degree of hydroxyethyl substitution ranging up to 100%, and ethylene glycol. The formulation may be, in some instances, free of dimethylsulfoxide (DMSO-free). In other instances, it may comprise less than 10%, less than 5%, less than 1%, less than 0.1%,

or less than 0.01% DMSO. In some embodiments, the formulation lacks albumin and may be referred to as albumin-free. In some embodiments, the formulation lacks glucose, and may be referred to as glucose-free. In some embodiments, the formulation lacks glucose and albumin and may be referred to as glucose-free and albumin-free. In some embodiments, the formulation comprises at least (or only) hydroxyethyl starch (HES) as the cryoprotectant, such HES having a degree of hydroxyethyl substitution ranging up to 100%.

[0009] One aspect of this disclosure provides a formulation comprising albumin, glucose, buffered saline, and one or two, or optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, pentastarch, and ethylene glycol. The formulation may be, in some instances, free of dimethylsulfoxide (DMSO-free). In other instances, it may comprise less than 10%, less than 5%, less than 1%, less than 0.1%, or less than 0.01% DMSO. In some embodiments, the formulation lacks albumin and may be referred to as albumin-free. In some embodiments, the formulation lacks glucose and may be referred to as glucose-free. In some embodiments, the formulation lacks glucose and albumin and may be referred to as glucose-free and albumin-free. In some embodiments, pentastarch may be replaced with one or more other HES having a degree of hydroxyethyl substitution ranging up to 100% including but not limited to 2-hydroxyethyl starch (2-HES).

[0010] Another aspect of this disclosure provides a cryopreservative formulation consisting essentially of albumin, glucose, buffered saline, and one or more (e.g., one or two) cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, hydroxyethyl starch (HES) having a degree of hydroxyethyl substitution ranging up to 100%, and ethylene glycol. In some embodiments, the formulation lacks albumin and may be referred to as albumin-free. In some embodiments, the formulation lacks glucose and may be referred to as glucose-free. In some embodiments, the formulation lacks glucose and albumin and may be referred to as glucose-free and albumin-free. In some embodiments, the formulation comprises at least (or only) hydroxyethyl starch as a cryoprotectant, such hydroxyethyl starch having a degree of hydroxyethyl substitution ranging up to 100%.

[0011] Another aspect of this disclosure provides a cryopreservative formulation consisting essentially of albumin, glucose, buffered saline, and one or more (e.g., one or two) cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, pentastarch, and ethylene glycol. In some embodiments, the formulation lacks albumin and may be referred to as albumin-free. In some embodiments, the

formulation lacks glucose and may be referred to as glucose-free. In some embodiments, the formulation lacks glucose and albumin and may be referred to as glucose-free and albumin-free. In some embodiments, pentastarch may be replaced with one or more other HES having a degree of hydroxyethyl substitution ranging up to 100% including but not limited to 2-hydroxyethyl starch (2-HES).

[0012] This disclosure provides various embodiments of the foregoing formulations, and these are recited below.

[0013] In some embodiments, the albumin is human albumin. In some embodiments, the albumin is recombinant human albumin.

[0014] In some embodiments, albumin is present in a range of about 2% to about 8% (w/v). In some embodiments, albumin is present in a range of about 1% to about 4% (w/v). In some embodiments, albumin is present at about 2.5% (w/v).

[0015] In some embodiments, albumin is absent from the formulation.

[0016] In some embodiments, glucose is present in a range of about 0.01% to about 1.5% (w/v). In some embodiments, glucose is present in a range of about 0.01% to about 0.5% (w/v). In some embodiments, glucose is present at about 0.09% (w/v).

[0017] In some embodiments, glucose is absent from the formulation.

[0018] In some embodiments, albumin and glucose are absent from the formulation.

[0019] In some embodiments, the buffered saline is phosphate buffered saline (PBS).

[0020] In some embodiments, the one or two, or optionally more, cryoprotectants comprise propanediol.

[0021] In some embodiments, propanediol is present in a range of about 0.1% to about 20% (v/v). In some embodiments, propanediol is present in a range of about 0.2% to about 15% (v/v). In some embodiments, propanediol is present at about 0.2%, about 2%, about 6%, about 10%, or about 15% (v/v).

[0022] In some embodiments, the one or two, or optionally more, cryoprotectants comprise dextran-40.

[0023] In some embodiments, dextran-40 is present in a range of about 1% to about 20% (w/v). In some embodiments, dextran-40 is present in a range of about 2% to about 20% (w/v). In some embodiments, dextran-40 is present in a range of about 4% to about 20% (w/v). In some embodiments, dextran-40 is present in a range of about 5% to about 15% (w/v). In some embodiments, dextran-40 is present at about 5%, about 6%, about 10%, or about 15% (w/v).

[0024] In some embodiments, the one or two, or optionally more, cryoprotectants comprise proline.

[0025] In some embodiments, the one or two, or optionally more, cryoprotectants comprise trehalose.

[0026] In some embodiments, the one or two, or optionally more, cryoprotectants comprise glycerin.

[0027] In some embodiments, the one or two, or optionally more, cryoprotectants comprise hydroxyethyl starch (HES) having hydroxyethyl substitution ranging up to 100%, including for example substitution at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 of the 11 hydroxyls in HES. Certain of these may be referred to as 2-HES, tetrastarch, pentastarch and heptastarch. The cryoprotectants may comprise two or more hydroxyethyl starches having differing degrees of hydroxyethyl substitution.

[0028] In some embodiments, hydroxyethyl starch (HES), having hydroxyethyl substitution ranging up to 100%, is present in a range of about 4% to about 20% (w/v). In some embodiments, hydroxyethyl starch (HES), having hydroxyethyl substitution ranging up to 100%, is present in a range of about 5% to about 15% (w/v). In some embodiments, hydroxyethyl starch (HES), having hydroxyethyl substitution ranging up to 100%, is present at about 5%, about 10%, or about 15% (w/v). Such hydroxyethyl starch includes but is not limited to 2-HES.

[0029] In some embodiments, the one or two, or optionally more, cryoprotectants comprise pentastarch.

[0030] In some embodiments, pentastarch is present in a range of about 4% to about 20% (w/v). In some embodiments, pentastarch is present in a range of about 5% to about 15% (w/v). In some embodiments, pentastarch is present at about 5%, about 10%, or about 15% (w/v).

[0031] In some embodiments, the one or two, or optionally more, cryoprotectants comprise ethylene glycol.

[0032] In some embodiments, ethylene glycol is present in a range of about 4% to about 6% (v/v). In some embodiments, ethylene glycol is present at about 5% (v/v).

[0033] In some embodiments, the one or two, or optionally more, cryoprotectants are propanediol and dextran-40.

[0034] In some embodiments, propanediol is present in a range of about 0.1% to about 20% (v/v) and dextran-40 is present in a range of about 0.2% to about 20% (w/v). In some embodiments, propanediol is present in a range of about 0.15% to about 20% (v/v) and

dextran-40 is present in a range of about 4% to about 20% (w/v). In some embodiments, propanediol is present in a range of about 0.2% to about 15% (v/v) and dextran-40 is present in a range of about 5% to about 15% (w/v). In some embodiments, propanediol is present at about 0.2%, about 2%, about 6%, about 10%, or about 15% (v/v) and dextran-40 is present at about 5%, about 6%, about 10%, or about 15% (w/v). In some embodiments, propanediol is present in a range of about 0.2% to about 15% (v/v) and dextran-40 is present at about 6%, about 10%, or about 15% (w/v). In some embodiments, propanediol is present at about 0.2%, about 2%, about 6%, about 10%, or about 15% (v/v) and dextran-40 is present at about 10% (w/v). In some embodiments, propanediol is present at about 10% (v/v) and dextran-40 is present at about 15% (w/v). In some embodiments, propanediol is present at about 10% (v/v) and dextran-40 is present at about 10% (w/v). In some embodiments, propanediol is present at about 6% (v/v) and dextran-40 is present at about 20% (w/v). In some embodiments, propanediol is present at about 6% (v/v) and dextran-40 is present at about 15% (w/v). In some embodiments, propanediol is present at about 6% (v/v) and dextran-40 is present at about 10% (w/v). In some embodiments, propanediol is present at about 6% (v/v) and dextran-40 is present at about 6% (w/v). In some embodiments, propanediol is present at about 6% (v/v) and dextran-40 is present at about 2% (w/v).

[0035] In some embodiments, the one or two, or optionally more, cryoprotectants are propanediol and proline.

[0036] In some embodiments, propanediol is present in a range of about 0.1% to about 20% (v/v) and proline is present in a range of about 2% to about 15% (w/v). In some embodiments, propanediol is present in a range of about 0.2% to about 15% (v/v) and proline is present in a range of about 5% to about 15% (w/v). In some embodiments, propanediol is present in a range of about 0.2% to about 15% (v/v) and proline is present in a range of about 5% to about 10% (w/v). In some embodiments, propanediol is present at about 6% (v/v) and proline is present at about 5% or about 10% (w/v).

[0037] In some embodiments, the one or two, or optionally more, cryoprotectants are propanediol and trehalose.

[0038] In some embodiments, propanediol is present in a range of about 0.1% to about 20% (v/v) and trehalose is present in a range of about 0.1% to about 20% (w/v). In some embodiments, propanediol is present in a range of about 0.2% to about 15% (v/v) and trehalose is present in a range of about 0.2% to about 15% (w/v). In some embodiments, propanediol is present at about 10% (v/v) and trehalose is present in a range of about 0.2% to about 15% (w/v). In some embodiments, propanediol is present at about 10% (v/v) and

trehalose is present at about 7% (w/v). In some embodiments, propanediol is present at about 10% (v/v) and trehalose is present at about 2% (w/v).

[0039] In some embodiments, the one or two, or optionally more, cryoprotectants are glycerin and trehalose.

[0040] In some embodiments, glycerin is present in a range of about 4% to about 16% (v/v) and trehalose is present in a range of about 2% to about 12% (w/v). In some embodiments, glycerin is present in a range of about 8% to about 12% (v/v) and trehalose is present in a range of about 4% to about 10% (w/v). In some embodiments, glycerin is present at about 10% (v/v) and trehalose is present at about 7% (w/v).

[0041] In some embodiments, the formulation comprises about 2.5% (w/v) albumin, optionally recombinant human albumin, about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 5% (v/v) propanediol and about 7.5% (w/v) dextran-40.

[0042] In some embodiments, the formulation comprises about 2.5% (w/v) albumin, optionally recombinant human albumin, about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 3% (v/v) propanediol and about 5% (w/v) dextran-40.

[0043] In some embodiments, the formulation comprises about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 3% (v/v) propanediol and about 5% (w/v) dextran-40, and is albumin-free.

[0044] In some embodiments, the formulation comprises about 2.5% (w/v) albumin, optionally recombinant human albumin, about 0.09% (w/v) glucose, buffered saline (e.g., PBS), and about 5% (w/v) dextran-40.

[0045] In some embodiments, the formulation comprises about 2.5% (w/v) albumin, optionally recombinant human albumin, about 0.09% (w/v) glucose, buffered saline (e.g., PBS), and about 7.5% (w/v) pentastarch.

[0046] Another aspect of this disclosure provides a cell preparation comprising a population of cells and any one of the foregoing formulations.

[0047] Another aspect of this disclosure provides a cell preparation comprising a population of cells and any one of the foregoing formulations, wherein the concentration of each of the one or two, or optionally more, cryoprotectants in the preparation is halved compared to its concentration in the formulation.

[0048] Another aspect of this disclosure provides a cell preparation comprising a population of cells in a 1:1 (v/v) mixture of (i) any one of the foregoing formulations and (ii) a suspension solution comprising albumin, glucose and buffered saline, each at the same

concentration as in the formulation, wherein the suspension solution does not contain a (non-albumin) cryoprotectant.

[0049] Another aspect of this disclosure provides a cell preparation comprising a population of cells in a formulation comprising albumin, glucose, buffered saline, and one or two, or optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, hydroxyethyl starch such as but not limited to 2-hydroxyethyl starch and/or pentastarch, and ethylene glycol, optionally wherein the formulation is free of dimethylsulfoxide (DMSO-free).

[0050] Other aspects of this disclosure provide a cell preparation comprising a population of cells in a formulation comprising

(a) albumin, glucose, buffered saline, and one or two, or optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, pentastarch, and ethylene glycol, optionally wherein the formulation is free of dimethylsulfoxide (DMSO-free),

(b) glucose, buffered saline, and one or two, or optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, pentastarch, and ethylene glycol, optionally wherein the formulation is free of dimethylsulfoxide (DMSO-free),

(c) albumin, buffered saline, and one or two, or optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, pentastarch, and ethylene glycol, optionally wherein the formulation is free of dimethylsulfoxide (DMSO-free),

(d) buffered saline, and one or two, or optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, pentastarch, and ethylene glycol, optionally wherein the formulation is free of dimethylsulfoxide (DMSO-free),

(e) albumin, glucose, buffered saline, and one or two, or optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, hydroxyethyl starch, and ethylene glycol, optionally wherein the formulation is free of dimethylsulfoxide (DMSO-free),

(f) glucose, buffered saline, and one or two, or optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, hydroxyethyl starch, and ethylene glycol, optionally wherein the formulation is free of dimethylsulfoxide (DMSO-free),

(g) albumin, buffered saline, and one or two, or optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, hydroxyethyl starch, and ethylene glycol, optionally wherein the formulation is free of dimethylsulfoxide (DMSO-free), or

(h) buffered saline, and one or two, or optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, hydroxyethyl starch, and ethylene glycol, optionally wherein the formulation is free of dimethylsulfoxide (DMSO-free).

[0051] Another aspect of this disclosure provides a cell preparation comprising a population of cells in a cryopreservative formulation consisting essentially of albumin, glucose, buffered saline, and one or two, or optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, hydroxyethyl starch such as but not limited to 2-hydroxyethyl starch and/or pentastarch, and ethylene glycol.

[0052] Other aspects of this disclosure provide a cell preparation comprising a population of cells in a cryopreservative formulation consisting essentially of

(a) albumin, glucose, buffered saline, and one or two, or optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, pentastarch, and ethylene glycol,

(b) glucose, buffered saline, and one or two, or optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, pentastarch, and ethylene glycol,

(c) albumin, buffered saline, and one or two, or optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, pentastarch, and ethylene glycol,

(d) buffered saline, and one or two, or optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, pentastarch, and ethylene glycol,

(e) albumin, glucose, buffered saline, and one or two, or optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, hydroxyethyl starch, and ethylene glycol,

(f) glucose, buffered saline, and one or two, or optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, hydroxyethyl starch, and ethylene glycol,

(g) albumin, buffered saline, and one or two, or optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, hydroxyethyl starch, and ethylene glycol, or

(h) buffered saline, and one or two, or optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, hydroxyethyl starch, and ethylene glycol.

[0053] This disclosure provides various embodiments of the foregoing cell preparations, and these are recited below and may also be shared with the foregoing formulations (and thus reference may be made to the prior recited embodiments as well).

[0054] In some embodiments, the albumin is human albumin, optionally recombinant human albumin. In some embodiments, albumin is present in a range of about 2% to about 8% (w/v), optionally in a range of about 1% to about 4% (w/v), further optionally at about 2.5% (w/v). In some embodiments, albumin is absent.

[0055] In some embodiments, glucose is present in a range of about 0.01% to about 1.5% (w/v), optionally in a range of about 0.01% to about 0.5% (w/v), further optionally at about 0.09% (w/v). In some embodiments, glucose is absent.

[0056] In some embodiments, the buffered saline is phosphate buffered saline.

[0057] In some embodiments, the one or two, or optionally more, cryoprotectants comprise propanediol.

[0058] In some embodiments, propanediol is present in a range of about 0.05% to about 10%, optionally in a range of about 0.1% to about 7.5%, further optionally at about 0.1%, about 1%, about 3%, about 5%, or about 7.5% (v/v).

[0059] In some embodiments, the one or two, or optionally more, cryoprotectants comprise dextran-40.

[0060] In some embodiments, dextran-40 is present in a range of about 0.5% to about 10%, optionally in a range of about 1% to about 10%, optionally in a range of about 2% to about 10%, optionally in a range of about 2.5% to about 7.5%, and further optionally at about 2.5%, about 3%, about 5%, or about 7.5% (w/v).

[0061] In some embodiments, the one or two, or optionally more, cryoprotectants comprise proline, trehalose, and/or glycerin.

[0062] In some embodiments, the one or two, or optionally more, cryoprotectants comprise hydroxyethyl starch (HES) having hydroxyethyl substitution ranging up to 100%, including but not limited to 2-HES.

[0063] In some embodiments, hydroxyethyl starch, such as but not limited to 2-HES, is present in a range of about 2% to about 10%, optionally in a range of about 2.5% to about 7.5%, and further optionally at about 2.5%, about 5%, or about 7.5% (w/v).

[0064] In some embodiments, the one or two, or optionally more, cryoprotectants comprise pentastarch. In some embodiments, the one or two, or optionally more, cryoprotectants comprise tetrastarch. In some embodiments, the one or two, or optionally more, cryoprotectants comprise heptastarch. In some embodiments, the one or two, or optionally more, cryoprotectants including two or more hydroxyethyl starches having differing degrees of hydroxyethyl substitution. As an example, the one or two, or optionally more, cryoprotectants may comprise 2-HES and pentastarch.

[0065] In some embodiments, an individual hydroxyethyl starch such as but not limited to tetrastarch, pentastarch, or heptastarch is present in a range of about 2% to about 10%, optionally in a range of about 2.5% to about 7.5%, and further optionally at about 2.5%, about 5%, or about 7.5% (w/v). In some embodiments, total hydroxyethyl starch is present in a range of about 2% to about 10%, optionally in a range of about 2.5% to about 7.5%, and further optionally at about 2.5%, about 5%, or about 7.5% (w/v).

[0066] In some embodiments, the one or two, or optionally more, cryoprotectants comprise ethylene glycol.

[0067] In some embodiments, ethylene glycol is present in a range of about 2% to about 3%, optionally at about 2.5% (v/v).

[0068] In some embodiments, the one or two, or optionally more, cryoprotectants are propanediol and dextran-40.

[0069] In some embodiments, propanediol is present in a range of about 0.05% to about 10% (v/v) and dextran-40 is present in a range of about 0.1% to about 10% (w/v), optionally wherein propanediol is present in a range of about 0.05% to about 10% (v/v) and dextran-40 is present in a range of about 2% to about 10% (w/v), optionally wherein propanediol is present in a range of about 0.1% to about 7.5% (v/v) and dextran-40 is present in a range of about 2.5% to about 7.5% (w/v), optionally wherein propanediol is present at about 0.1%, about 1%, about 3%, about 5%, or about 7.5% (v/v) and dextran-40 is present at about 2.5%, about 3%, about 5%, or about 7.5% (w/v), optionally wherein propanediol is present in a range of about 0.1% to about 7.5% (v/v) and dextran-40 is present at about 3%, about 5%, or about 7.5% (w/v), further optionally wherein propanediol is present at about 0.1%, about 1%, about 3%, about 5%, or about 7.5% and dextran-40 is present at about 5% (w/v).

[0070] In some embodiments, propanediol is present at about 5% (v/v) and dextran-40 is present at about 7.5% (w/v), or propanediol is present at about 5% (v/v) and dextran-40 is present at about 5% (w/v), or propanediol is present at about 3% (v/v) and dextran-40 is present at about 10% (w/v), or propanediol is present at about 3% (v/v) and dextran-40 is present at about 7.5% (w/v), or propanediol is present at about 3% (v/v) and dextran-40 is present at about 5% (w/v), or propanediol is present at about 3% (v/v) and dextran-40 is present at about 3% (w/v), or propanediol is present at about 3% (v/v) and dextran-40 is present at about 1% (w/v).

[0071] In some embodiments, the one or two, or optionally more, cryoprotectants are propanediol and proline.

[0072] In some embodiments, propanediol is present in a range of about 0.05% to about 10% (v/v) and proline is present in a range of about 1% to about 7.5% (w/v), optionally wherein propanediol is present in a range of about 0.1% to about 7.5% (v/v) and proline is present in a range of about 2.5% to about 7.5% (w/v), optionally wherein propanediol is present in a range of about 0.1% to about 7.5% (v/v) and proline is present in a range of about 2.5% to about 5% (w/v), and further optionally wherein propanediol is present at about 3% (v/v) and proline is present at about 2.5% or about 5% (w/v).

[0073] In some embodiments, the one or two, or optionally more, cryoprotectants are propanediol and trehalose.

[0074] In some embodiments, propanediol is present in a range of about 0.05% to about 10% (v/v) and trehalose is present in a range of about 0.05% to about 10% (w/v), optionally wherein propanediol is present in a range of about 0.1% to about 7.5% (v/v) and trehalose is present in a range of about 0.1% to about 7.5% (w/v), optionally wherein propanediol is present in a range of about 5% (v/v) and trehalose is present in a range of about 0.1% to about 7.5% (w/v), optionally wherein propanediol is present at about 5% (v/v) and trehalose is present at about 3.5% (w/v), and further optionally wherein propanediol is present at about 5% (v/v) and trehalose is present at about 1% (w/v).

[0075] In some embodiments, the one or two, or optionally more, cryoprotectants are glycerin and trehalose.

[0076] In some embodiments, glycerin is present in a range of about 2% to about 8% (v/v) and trehalose is present in a range of about 1% to about 6% (w/v), optionally wherein glycerin is present in a range of about 4% to about 6% (v/v) and trehalose is present in a range of about 2% to about 5% (w/v), and further optionally wherein glycerin is present at about 5% (v/v) and trehalose is present at about 3.5% (w/v).

[0077] In some embodiments, the cell population is a retinal pigmented epithelium (RPE) cell population, a photoreceptor cell population, a photoreceptor progenitor cell population, a photoreceptor rescue cell (PRC) population, a corneal epithelial cell (CEC) population, a mesenchymal cell population such as a hemangioblast-derived mesenchymal cell (HMC) population, a macrophage cell population, a natural killer cell population, a T-cell population, a vascular cell population, a vascular progenitor cell population, a liver cell population, a neural cell population and a pancreatic cell population.

[0078] In some embodiments, the cell population is a retinal pigmented epithelium (RPE) cell population. In some embodiments, the cell population is a hemangioblast-derived mesenchymal cell (HMC) population. In some embodiments, the cell population is a vascular progenitor cell population.

[0079] In some embodiments, the cell population is a population of cells, wherein the cells express at least one polypeptide selected from the group consisting of human leukocyte antigen E (HLA-E), human leukocyte antigen F (HLA-F), and human leukocyte antigen G (HLA-G); wherein the cells comprise a genetically engineered disruption of one or more copies of endogenous β-2 microglobulin (B2M); and wherein the cells comprise a genetically engineered disruption of one or more copies of a human leukocyte antigen (HLA) class II-related gene selected from the group consisting of regulatory factor X associated ankyrin containing protein (RFXANK), regulatory factor 5 (RFX5), regulatory factor X associated protein (RFXAP), and class II transactivator (CIITA).

[0080] In some embodiments, the cells further comprise a nucleic acid molecule encoding a suicide gene product.

[0081] In some embodiments, the cells comprise a herpes simplex virus thymidine kinase (TK) suicide gene.

[0082] In some embodiments, the cells are derived from pluripotent stem cells.

[0083] In some embodiments, the pluripotent stem cells are embryonic stem cells.

[0084] In some embodiments, the pluripotent stem cells are induced pluripotent stem cells.

[0085] In some embodiments, the cells comprise a genetically engineered disruption of all copies of endogenous B2M.

[0086] In some embodiments, the cells comprise a genetically engineered disruption of all copies of an HLA class II-related gene selected from the group consisting of RFXANK, RFX5, RFXAP, and CIITA.

[0087] In some embodiments, the cell population is a dissociated cell population, a cell sheet, a cell monolayer, or a cell aggregate.

[0088] In some embodiments, the cell population is a human cell population.

[0089] In some embodiments, the cell population is generated by in vitro differentiation of pluripotent stem cells, optionally embryonic stem cells or induced pluripotent stem cells.

[0090] In some embodiments, the cell preparation has a cell density of about 300 cells to about 10,000 cells per microliter. In some embodiments, the cell preparation has a cell density of about 300 cells to about 7,000 cells per microliter. In some embodiments, the cell preparation has a cell density of about 350 cells to about 7,000 cells per microliter. In some embodiments, the cell preparation has a cell density of about 350 cells per microliter, or about 3,000 cells per microliter, or about 3,500 cells per microliter, or about 6,500 cells per microliter, or about 7,000 cells per microliter. In some embodiments, the cell preparation has a cell density of about 3,333 cells per microliter.

[0091] In some embodiments, the cell preparation is in a volume of about 400-500 microliters, further optionally in a volume of about 450 microliters.

[0092] In some embodiments, the cell preparation is in a cryopreserved form.

[0093] In some embodiments, the cell preparation has been cryopreserved and thawed.

[0094] In some embodiments, the cell preparation is provided at room temperature (about 20 °C to about 25 °C), at about 4 °C, at about -20 °C, at about -70 °C, or at about -180 °C pre-cryopreservation or post-thaw.

[0095] In some embodiments, the cell preparation has been kept at room temperature (about 20 °C to about 25 °C) for about 1 to about 2 hours, and at about 4 °C for about 4 to about 7 hours, prior to cryopreservation, optionally at -180 °C.

[0096] In some embodiments, the cell preparation is a DMSO-free cell preparation.

[0097] Another aspect of this disclosure provides a cell preparation comprising a population of cells in a formulation comprising albumin, glucose, buffered saline, and one or two, or optionally more, cryoprotectants, and having a viability of equal to or greater than about 80% and/or a cell growth index:3 of equal to or less than 110 hours, optionally having a viability of equal to or greater than about 85% and/or a cell growth index:3 of equal to or less than 100 hours, further optionally having a viability of equal to or greater than about 90% and/or a cell growth index:3 of equal to or less than 90 hours, after

a. storage at room temperature (about 20 °C to about 25 °C) for about 1 to about 2 hours and then at about 4 °C for about 4 to about 7 hours, optionally at room temperature for about 1.5 hours and then at 4 °C for about 4.5 hours or 6.5 hours,
b. cryopreservation at -180 °C for about 1 to about 2 years, optionally for about 1 month to about 1 year,
c. cryopreservation at -180 °C and then post-thaw at about 4 °C for about 3 to about 6 hours, optionally for about 4 hours,
d. cryopreservation at -180 °C and then post-thaw at room temperature (about 20 °C to about 25 °C) for about 3 to about 6 hours, optionally for about 4 hours,
e. cryopreservation at -180 °C, then at about - 20 °C for about 0.5 to about 1 hour, then cryopreservation at -180 °C, optionally then at about - 20 °C for about 0.5 to about 1 hour, then cryopreservation at -180 °C, or
f. cryopreservation at -180 °C, then at about - 70 °C for about 2 to about 4 weeks, then cryopreservation at -180 °C.

[0098] In some embodiments, the cell preparation is a DMSO-free cell preparation. In some embodiments, the formulation or cell preparation is an albumin-free formulation or cell preparation (intending that albumin is absent). In some embodiments, the formulation or cell preparation is a glucose-free cell formulation or cell preparation (intending that glucose is absent). In some embodiments, the formulation or cell preparation is an albumin-free and a glucose-free formulation or cell preparation (intending that albumin is absent and glucose is absent). In some embodiments, the cell preparation has viability of equal to or greater than about 85% and/or a cell growth index:3 of equal to or less than 100 hours after any one of events (a) through (f). In some embodiments, the cell preparation has a viability of equal to or greater than about 90% and/or a cell growth index:3 of equal to or less than 90 hours after any one of events (a) through (f).

[0099] Another aspect of this disclosure provides a method of preparing a cell preparation, comprising contacting a population of cells with (i) any of the foregoing formulations and (ii) a suspension solution comprising albumin, glucose, and buffered saline, each at the same concentration as in the formulation, wherein the suspension solution does not contain a (non-albumin) cryoprotectant.

[00100] In some embodiments, the method further comprises cryopreserving the cell preparation. In some embodiments, the method further comprises thawing the cell preparation. In some embodiments, the method further comprises administering the cell

preparation to a subject in need thereof. In some embodiments, the cell preparation is kept at about 4 °C for about 4 hours before administration to the subject. In some embodiments, the cell preparation is kept at room temperature (about 20 °C to about 25 °C) for about 4 hours before administration to the subject. In some embodiments, the cell preparation is administered to the subject without washing or dilution post-thaw.

[00101] Another aspect of this disclosure provides a method of treating a subject having a disorder or condition comprising administering to a subject in need thereof an effective amount of any of the foregoing cell preparations after cryopreservation and thawing, without washing or dilution post-thaw.

[00102] Another aspect of this disclosure provides treating a subject having a disorder or condition comprising administering to a subject in need thereof an effective amount of the cell preparation prepared according to any of the foregoing methods for cryopreserving and thawing a cell preparation without washing or dilution post-thaw.

[00103] In some embodiments, the cell preparation is kept at about 4 °C for about 4 hours before administration to the subject. In some embodiments, the cell preparation is kept at room temperature (about 20 °C to about 25 °C) for about 4 hours before administration to the subject. In some embodiments, the disorder or condition is selected from the group consisting of retinal detachment, retinal tears, retinal dysplasia, Angioid streaks, Myopic Macular Degeneration, or retinal atrophy or associated with a number of vision-altering ailments that result in photoreceptor damage and blindness, such as, for example, choroideremia, diabetic retinopathy, macular degeneration (e.g., age-related macular degeneration, AMD), retinitis pigmentosa, Stargardt's Disease (fundus flavimaculatus), cancer, liver disease, Crohn's disease, arthritis, pulmonary artery hypertension, critical limb ischemia, Fuch's Dystropy, Corneal injury, retinal tear, and geographic atrophy such as geographic atrophy secondary to age-related macular degeneration (AMD). In some embodiments, the cell preparation is within a vial. In some embodiments, the cell preparation is within a syringe.

[00104] The summary above is meant to illustrate, in a non-limiting manner, some of the embodiments, advantages, features, and uses of the technology disclosed herein. Other embodiments, advantages, features, and uses of the technology disclosed herein will be apparent from the Detailed Description, the Drawings, the Examples, and the Claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[00105] **FIGs. 1A-1B** show results of cell viability (**FIG. 1A**) and cell growth (**FIG. 1B**) in first formulation screening using RPE cells. 5% (v/v) DMSO was used as a control. Target cell density: 3,333 cells/µL.

[00106] **FIGs. 2A-2B** show results of cell viability (**FIG. 2A**) and cell growth (**FIG. 2B**) in dextran-40 screening using RPE cells. 5% (v/v) DMSO was used as a control. Target cell density: 3,333 cells/µL.

[00107] **FIGs. 3A-3B** show results of cell viability (**FIG. 3A**) and cell growth (**FIG. 3B**) in propanediol screening using UC-RPE cells. 5% (v/v) DMSO was used as a control. Target cell density: 3,333 cells/µL.

[00108] **FIGs. 4A-4B** show results of cell viability (**FIG. 4A**) and cell growth (**FIG. 4B**) in trehalose screening using RPE cells. Target cell density: 3,333 cells/µL.

[00109] **FIGs. 5A-5B** show results of cell viability (**FIG. 5A**) and cell growth (**FIG. 5B**) in a process time evaluation using RPE cells. Process time indicates the duration from adding cryoprotectant to initiating controlled-rate freezing. Process times used correspond to 6h (1.5h RT + 4.5h 4ºC) and 8h (1.5h RT + 6.5h 4ºC). 5% (v/v) DMSO was used as a control. Target cell density: 3,333 cells/µL.

[00110] **FIGs. 6A-6B** show post-thaw stability post 4 hours at 4ºC. RPE cell viability (**FIG. 6A**) and cell growth (**FIG. 6B**) were measured after frozen vials were thawed and preserved at 4ºC for 4 hours. 5% (v/v) DMSO was used as a control. Target cell density: 3,333 cells/µL.

[00111] **FIGs. 7A-7B** show post-thaw stability post 4 hours at room temperature. RPE cell viability (**FIG. 7A**) and cell growth (**FIG. 7B**) were measured after frozen vials were thawed and preserved at room temperature for 4 hours. 5% (v/v) DMSO was used as a control. Target cell density: 3,333 cells/µL.

[00112] **FIGs. 8A-8B** show stability at -20ºC. RPE cell viability (**FIG. 8A**) and cell growth (**FIG. 8B**) were measured after temperature excursions at -20ºC. Frozen vials were transferred to -20ºC chamber and kept for 30 or 60 minutes, and then transferred back to liquid nitrogen tank; process was performed twice before evaluation. Target cell density: 3,333 cells/µL.

[00113] **FIGs. 9A-9B** show stability at -70ºC. RPE cell viability (**FIG. 9A**) and cell growth (**FIG. 9B**) were measured after temperature excursion at -70ºC. Frozen vials were transferred to -70ºC chamber and kept for 4 weeks, and then transferred back to liquid

nitrogen tank before evaluation. 5% (v/v) DMSO was used as a control. Target cell density: 3,333 cells/µL.

[00114] **FIG. 10** shows results of a multinucleation assay. Cells post thaw were seeded and cultured for 4 hours, and then cells were fixed. Stained cells were counted, and the ratio of multinucleated cells was evaluated.

[00115] **FIGs. 11A-11B** show results of cell viability (**FIG. 11A**) and cell growth (**FIG. 11B**) in a process time evaluation using UC-RPE cells. Process time indicates the duration from adding cryoprotectant to initiating controlled-rate freezing. Process times used correspond to 6h and 8h. 5% (v/v) DMSO was used as a control. Target cell density: 3,333 cells/µL.

DETAILED DESCRIPTION

[00116] Cell-based therapies including those involving transplantation of cells, *e.g.*, in the context of a regenerative medicine approach, often require formulating, storing, transporting, and/or injecting delicate or fragile cells that can be damaged or lose repopulation capacity with inappropriate handling, extensive manipulation and/or processing steps or exposure to non-physiological conditions.

[00117] Dimethyl sulfoxide (DMSO) is a conventional cryoprotectant used to protect cells from damage during cryopreservation and thaw of cell products. However, DMSO can be harmful to patients if high concentrations of DMSO are administered. DMSO can also cause cytotoxicity when used in high concentrations in cell preparations. Existing methods for minimizing such negative effects of DMSO include diluting cells with physiological buffers and/or washing cells to remove DMSO and reconstituting with a physiological buffer prior to administration into patients.

[00118] However, these approaches pose several challenges. One such challenge is that even if the cells are diluted with another solution, there may still be some cryoprotectant remaining that may be administered to a subject. This can increase the risk of side effects and thereby limit the use of such cell populations. Further, the development of an appropriate physiological buffer may be costly and labor-intensive, ultimately increasing the cost of the therapeutic. Another challenge is that processes that require dilution and/or washing of cells prior to administration are complex for clinical pharmacists and may increase contamination risk, cause loss of sterility, and have a potentially fatal effect on patients.

[00119] When the washing step is used to remove DMSO, the shelf-life of a cellular product is usually extremely short (e.g., typically 48 to 96 hours), requiring robust and

challenging scheduling for administration of the product and limiting the geographic location in which the product may be distributed and used. As with the dilution strategy, an appropriate reconstitution buffer and additional processes may be necessary, thereby increasing cost and contamination risk. Further, many clinical sites are also not set up for additional processing steps prior to administration.

[00120] Lastly, there is a degree of variability introduced as the reconstitution process will be performed by a variety of clinicians and pharmacists. This variability could lead to inconsistent dosing and resultant inconsistent therapeutic efficacy.

[00121] The present disclosure provides formulations and methods for cell cryopreservation, cell storage, cell transport, and ultimately cell administration to a subject with minimal to no processing and manipulation of the cells, and increased viability post-thaw. The formulations provided herein have several properties that make them suitable for cryopreserving cell-based therapeutics. For example, the formulations provided herein enable cryopreservation of cell-based therapeutics without using DMSO as a cryoprotectant, thereby avoiding a significant decrease in cell quality post-freezing and thaw, and increasing cell stability. Also, the formulations can be used directly post-freezing and thaw, thereby eliminating the need for dilution and reconstitution with another solution prior to administration (or transplantation) into a subject. The formulations are also capable of preserving frozen cell populations for extended periods of time (e.g., a year or longer). Further cells stored in these formulations appear resistant to changes in temperature either in a frozen state, or at room temperature or at 4°C pre-cryopreservation or post-thaw. In contrast, various currently available cryopreservative formulations require extensive cell processing procedures that may be costly, time-consuming, prone to human error, and ultimately may diminish cell survival, re-plating efficiency, and repopulation capacity of the cells contained therein. Also, notably, increased viability has been observed for cell populations that are cryopreserved using the formulations provided herein, compared to prior art cryopreservative formulations and methods. Improved cell growth post-thaw has also been observed when using the formulations provided herein. The formulations provided herein may provide for improved functionality, survival and stability of cell populations.

[00122] In addition to the aforementioned advantages, the cryopreservative formulations of this disclosure are also surprisingly and unexpectedly associated with reduced frequency (or prevalence) of multinucleated cells in culture, particularly in RPE cell cultures. Multinucleated cells, such as multinucleated RPE cells, have been observed using other formulations.

Cryopreservative Formulations

[00123] This disclosure provides novel cryopreservative formulations. Certain of these formulations are free of DMSO (DMSO-free). If DMSO is present in a formulation provided herein, it is present at less than 10% (v/v), less than 5% (v/v), less than 1% (v/v), less than 0.1% (v/v), or less than 0.01% (v/v). Certain cryopreservative formulations provided herein comprise albumin, glucose, buffered saline, and one or two, or optionally more, cryoprotectants selected from the group consisting of propanediol (1,3-propanediol), dextran-40, proline, trehalose, glycerin, hydroxyethyl starch such as but not limited to 2-HES and/or pentastarch, and ethylene glycol. In some embodiments, the cryopreservative formulations consist essentially of albumin, glucose, buffered saline, and one or two, or optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, pentastarch, ethylene glycol. And in still other embodiments, the cryopreservative formulations consist of albumin, glucose, buffered saline, and one or two, or optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, pentastarch, ethylene glycol.

[00124] Still other formulations lack albumin and/or glucose.

[00125] These formulations may be referred to as being cell-free, intending that they do not contain or comprise cells. However, as discussed in greater detail herein, such formulations may be combined with a cell population, and optionally a suspension solution, to form a cell preparation.

Albumin

[00126] Certain cryopreservative formulations provided herein comprise albumin. In some embodiments, the albumin is human albumin. In some embodiments, the albumin is recombinant human albumin. The term "recombinant human albumin" is interchangeably used with the term "rHA", "rHSA", "rhAlbumin". In some embodiments, the albumin is present in a range of about 1% to about 10% (w/v). In some embodiments, the albumin is present in a range of about 2% to about 8% (w/v). In some embodiments, the albumin is present in a range of about 2% to about 7.5% (w/v). In some embodiments, the albumin is present in a range of about 1% to about 4% (w/v). In some embodiments, the albumin is present in a range of about 2% to about 3% (w/v). In some embodiments, the albumin is present at about 1%, about 1.5%, about 2%, about 2.5%, about 3%, about 3.5%, about 4%, about 4.5%, about 5%, about 5.5%, about 6%, about 6.5%, about 7%, about 7.5%, about 8%,

about 8.5%, about 9%, about 9.5%, or about 10% (w/v)). In some embodiments, the albumin is present at about 2%, about 2.1%, about 2.2%, about 2.3%, about 2.4%, about 2.5%, about 2.6%, about 2.7%, about 2.8%, about 2.9%, or about 3% (w/v)). In some embodiments, the albumin is present at about 2.5% (w/v). The albumin may have cryoprotectant properties but it is to be understood that cryoprotectants as discussed herein do not refer to albumin. Certain of the formulations provided herein lack albumin and may be referred to as albumin-free.

Glucose

[00127] Certain cryopreservative formulations provided herein comprise glucose. In some embodiments, the glucose is present in a range of about 0.01% to about 3% (w/v). In some embodiments, the glucose is present in a range of about 0.01% to about 1.5% (w/v). In some embodiments, the glucose is present in a range of about 0.01% to about 0.5% (w/v). In some embodiments, the glucose is present in a range of about 0.02% to about 1.5% (w/v). In some embodiments, the glucose is present in a range of about 0.05% to about 1.2% (w/v). In some embodiments, the glucose is present at about 0.01%, about 0.02%, about 0.03%, about 0.04%, about 0.05%, about 0.06%, about 0.07%, about 0.08%, about 0.09%, about 0.1%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 1%, about 1.1%, or about 1.2% (w/v). In some embodiments, the glucose is present at about 0.09% (w/v). Certain of the formulations provided herein lack glucose and may be referred to as glucose-free.

Buffered Saline

[00128] The cryopreservative formulations provided herein comprise buffered saline. In some embodiments, the buffered saline is phosphate-buffered saline (PBS). In some embodiments, the buffer saline is Dulbecco's phosphate-buffered saline (DPBS).

[00129] In some embodiments, the buffered saline comprises divalent cations. Suitable divalent cations include, without limitation, *e.g.*, Ca^{2+}, Mg^{2+}, Zn^{2+}, Fe^{2+}, Mn^{2+}, Cr^{2+}, Cu^{2+}, Ba^{2+}, and Sr^{2+}. In some embodiments, the divalent cations comprise calcium. In some embodiments, the divalent cations comprise magnesium. In some embodiments, the divalent cations comprise two or more different divalent cations (e.g., calcium and magnesium). In some embodiments, the buffered saline does not comprise calcium and/or magnesium (Ca^{2+} and Mg^{2+}). In some embodiments, the buffered saline comprises one or more

pharmaceutically acceptable salts, such as but not limited to a pharmaceutically acceptable magnesium salt and/or a pharmaceutically acceptable calcium salt.

[00130] In some embodiments, the buffered saline may be excluded from any of the formulations provided herein. In some embodiments, any of the formulations provided herein may comprise a buffering agent and/or a saline solution (or electrolytes, or an electrolyte solution) in place of the buffered saline. In some instances, the formulation may lack buffered saline, but may comprise a buffering agent, and may be later combined with a saline solution (or electrolytes, or an electrolyte solution). In some instances, the formulation may lack buffered saline, but may comprise a saline solution (or electrolytes, or an electrolyte solution), and may be later combined with a buffering agent. Examples of buffering agents which may be used in the various formulations disclosed here include alkali (sodium and potassium) or alkaline earth (calcium and magnesium) carbonates, phosphates, bicarbonates, citrates, borates, acetates, phthalates, tartrates, succinates and the like, such as sodium or potassium phosphate, citrate, borate, acetate, bicarbonate and carbonate; and salts of a Group IA metal including, for example, a bicarbonate salt of a Group IA metal and a carbonate salt of a Group IA metal; an alkaline earth metal buffering agent, an amino acid, an alkaline salt of an amino acid, an aluminum buffering agent, a calcium buffering agent, a sodium buffering agent, or a magnesium buffering agent. Still other buffering agents are provided in published PCT Application No. 2008/057802, and the examples of buffering agents provided in such application are incorporated by reference herein. Examples of electrolytes that may be used in the various formulations disclosed herein include calcium chloride; sodium chloride; magnesium chloride; sodium acetate; potassium chloride; potassium acetate; sodium or potassium monobasic, dibasic or tribasic phosphate; sodium or potassium citrate; sodium or potassium tartrate; sodium benzoate; sodium or potassium sorbate; sodium or potassium phthalate; sodium or potassium metabisulphite; or other similar salts usually used in pharmaceutical products.

One or Two, or Optionally More, Cryoprotectants

[00131] The cryopreservative formulations provided herein comprise one or two, or optionally more, (non-albumin) cryoprotectants. In some embodiments, the one or two, or optionally more, cryoprotectants, in some instances, do not include DMSO. Some of the formulations provided herein comprise one (non-albumin) cryoprotectant. Some of the formulations provided herein comprise two (non-albumin) cryoprotectants. Some of the formulations provided herein comprise more than two (non-albumin) cryoprotectants. The

one or two, or optionally more, cryoprotectants are selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, hydroxyethyl starch such as but not limited to pentastarch, and ethylene glycol.

A. Propanediol (1,3-propanediol)

[00132] In some embodiments, the one or two, or optionally more, cryoprotectants comprise propanediol. In some embodiments, the propanediol is present in a range of 0.1% to about 20% (v/v). In some embodiments, the propanediol is present in a range of 0.2% to about 15% (v/v). In some embodiments, the propanediol is present in a range of 0.2% to about 6% (v/v). In some embodiments, the propanediol is present in a range of 2% to about 15% (v/v). In some embodiments, the propanediol is present in a range of 6% to about 15% (v/v). In some embodiments, the propanediol is present at about 0.2%, about 1%, about 2%, about 4% , about 6%, about 8% about 10%, about 12%, about 14%, or about 15% (v/v). In some embodiments, the propanediol is present at about 0.2%, about 2%, about 6%, about 10%, or about 15% (v/v). In some embodiments, the propanediol is present at about 0.2% (v/v). In some embodiments, the propanediol is present at about 2% (v/v). In some embodiments, the propanediol is present at about 6% (v/v). In some embodiments, the propanediol is present at about 10% (v/v). In some embodiments, the propanediol is present at about 15% (v/v). The concentration of a propanediol stock solution is 99%+ propanediol.

B. Dextran-40

[00133] In some embodiments, the one or two, or optionally more, cryoprotectants comprise dextran-40. In some embodiments, the dextran-40 is present in a range of about 1% to about 20% (w/v). In some embodiments, the dextran-40 is present in a range of about 2% to about 20% (w/v). In some embodiments, the dextran-40 is present in a range of about 4% to about 20% (w/v). In some embodiments, the dextran-40 is present in a range of about 5% to about 15% (w/v). In some embodiments, the dextran-40 is present at about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 7.5%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, or about 20% (w/v). In some embodiments, the dextran-40 is present at about 5%, about 6%, about 10%, or about 15% (w/v). In some embodiments, the dextran-40 is present at about 2% (w/v). In some embodiments, the dextran-40 is present at about 5% (w/v). In some embodiments, the dextran-40 is present at about 6% (w/v). In some embodiments, the dextran-40 is present at about 10% (w/v). In some embodiments, the

dextran-40 is present at about 15% (w/v). In some embodiments, the dextran-40 is present at about 20% (w/v).

C. Proline

[00134] In some embodiments, the one or two, or optionally more, cryoprotectants comprise proline. In some embodiments, the proline is present in a range of about 2% to about 15% (w/v). In some embodiments, the proline is present in a range of about 5% to about 15% (w/v). In some embodiments, the proline is present in a range of about 5% to about 10% (w/v). In some embodiments, the proline is present at about 5%, about 6%, about 7%, about 8%, about 9%, or about 10% (w/v). In some embodiments, the proline is present at about 5% or 10% (w/v). In some embodiments, the proline is present at about 5% (w/v). In some embodiments, the proline is present at about 10% (w/v).

D. Trehalose

[00135] In some embodiments, the one or two, or optionally more, cryoprotectants comprise trehalose. In some embodiments, trehalose is present in a range of about 0.1% to about 20% (w/v). In some embodiments, trehalose is present in a range of about 0.2% to about 15% (w/v). In some embodiments, trehalose is present in a range of about 0.2% to about 7% (w/v). In some embodiments, trehalose is present in a range of about 2% to about 12% (w/v). In some embodiments, trehalose is present in a range of about 2% to about 7% (w/v). In some embodiments, trehalose is present in a range of about 4% to about 10% (w/v). In some embodiments, trehalose is present in a range of about 6% to about 15% (w/v). In some embodiments, trehalose is present at about 2%, about 3%, about 4%, about 5%, about 6%, or about 7% (w/v). In some embodiments, trehalose is present at about 2% or about 7% (w/v). In some embodiments, trehalose is present at about 2% (w/v). In some embodiments, trehalose is present at about 7% (w/v).

E. Glycerin

[00136] In some embodiments, the one or two, or optionally more, cryoprotectants comprise glycerin. In some embodiments, glycerin is present in a range of about 4% to about 16% (v/v). In some embodiments, glycerin is present in a range of about 8% to about 12% (v/v). In some embodiments, glycerin is present at about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%,

or about 16% (v/v). In some embodiments, glycerin is present at about 10% (v/v). The concentration of a glycerin original stock solution is 99% glycerin.

[00137] The disclosure further contemplates that in any of the embodiments provided herein, "glycerin" could also encompass "glycerol".

F. Hydroxyethyl starch

[00138] In some embodiments, the one or two, or optionally more, cryoprotectants comprise hydroxyethyl starch (HES) (such as but not limited to 2-hydroxyethyl starch). Examples of such hydroxyethyl starches include tetrastarch (approximately 40% hydroxyethylated HES), pentastarch (approximately 50% hydroxyethyl substitutions), and heptastarch (approximately 70% hydroxyethyl substitutions). The degree of hydroxyethyl substitution may refer to a single molecule of HES or it may refer to a population of HES. In some embodiments, the one or two, or optionally more, cryoprotectants comprise pentastarch. Pentastarch is a subgroup of hydroxyethyl starch, having five hydroxyethyl groups out of every 11 hydroxyls, which renders it approximately 50% hydroxyethyl substituted. Pentastarch is commercially available as Pentaspan (Bristol-Myers Squibb). The Examples use a pentastarch stock solution composed of the following components: 20% (w/v) pentastarch (200 g/L), 0.18% (w/v) dextrose (1.8 g/L) and an electrolyte solution composed of: 526mg NaCl, 502mg Sodium Gluconate, 368mg Sodium Acetate·$3H_2O$, 37mg KCl, 30mg $MgCl_2 \cdot 6H_2O$ (per 100 mL volume). In some embodiments, pentastarch is present in a range of about 4% to about 20% (w/v). In some embodiments, pentastarch is present in a range of about 5% to about 15% (w/v). In some embodiments, pentastarch is present at about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, or about 20% (w/v). In some embodiments, pentastarch is present at about 5%, about 10%, or about 15% (w/v). In some embodiments, pentastarch is present at about 5% (w/v). In some embodiments, pentastarch is present at about 10% (w/v). In some embodiments, pentastarch is present at about 15% (w/v). Similar ranges apply when other HES are used in place of pentastarch.

G. Ethylene Glycol

[00139] In some embodiments, the one or two, or optionally more, cryoprotectants comprise ethylene glycol. In some embodiments, ethylene glycol is present in a range of about 2% to about 8% (v/v). In some embodiments, ethylene glycol is present in a range of about 4% to about 6% (v/v). In some embodiments, ethylene glycol is present at about 4%,

about 4.2%, about 4.4%, about 4.6%, about 4.8%, about 5%, about 5.2%, about 5.4%, about 5.6%, about 5.8%, or about 6% (v/v). In some embodiments, ethylene glycol is present at about 5% (v/v). The concentration of ethylene glycol original stock solutions is 99% ethylene glycol.

Combinations of Cryoprotectants

[00140] Cryopreservative formulations provided herein may comprise two (non-albumin) cryoprotectants. Various combinations of cryoprotectants are provided below.

Propanediol and Dextran-40

[00141] In some embodiments, the formulation comprises propanediol and dextran-40. In some embodiments, propanediol is present in a range of about 0.1% to about 20% (v/v) and dextran-40 is present in a range of about 0.2% to about 20% (w/v). In some embodiments, propanediol is present in a range of about 0.1% to about 20% (v/v) and dextran-40 is present in a range of about 4% to about 20% (w/v). In some embodiments, propanediol is present in a range of about 0.2% to about 15% (v/v) and dextran-40 is present in a range of about 5% to about 15% (w/v). In some embodiments, propanediol is present at about 0.2%, about 2%, about 6%, about 10%, or about 15% (v/v) and dextran-40 is present at about 5%, about 6%, about 10%, or about 15% (w/v). In some embodiments, propanediol is present in a range of about 0.2% to about 15% (v/v) and dextran-40 is present at about 6%, about 10%, or about 15% (w/v). In some embodiments, propanediol is present at about 0.2%, about 2%, about 6%, about 10%, or about 15% (v/v) and dextran-40 is present at about 10% (w/v). In some embodiments, propanediol is present at about 10% (v/v) and dextran-40 is present at about 15% (w/v). In some embodiments, propanediol is present at about 10% (v/v) and dextran-40 is present at about 10% (w/v). In some embodiments, propanediol is present at about 6% (v/v) and dextran-40 is present at about 20% (w/v). In some embodiments, propanediol is present at about 6% (v/v) and dextran-40 is present at about 15% (w/v). In some embodiments, propanediol is present at about 6% (v/v) and dextran-40 is present at about 10% (w/v). In some embodiments, propanediol is present at about 6% and dextran-40 is present at about 6% (w/v). In some embodiments, propanediol is present at about 6% (v/v) and dextran-40 is present at about 2% (w/v).

Propanediol and Trehalose

[00142] In some embodiments, the formulation comprises propanediol and trehalose. In some embodiments, propanediol is present in a range of about 0.1% to about 20% (v/v) and trehalose is present in a range of about 0.1% to about 20% (w/v). In some embodiments, propanediol is present in a range of about 0.2% to about 15% (v/v) and trehalose is present in a range of about 0.2% to about 15% (w/v). In some embodiments, propanediol is present at about 10% (v/v) and trehalose is present in a range of about 0.2% to about 15% (w/v). In some embodiments, propanediol is present at about 10% (v/v) and trehalose is present at about 7% (w/v). In some embodiments, propanediol is present at about 10% (v/v) and trehalose is present at about 2% (w/v).

Propanediol and Proline

[00143] In some embodiments, the formulation comprises propanediol and proline. In some embodiments, propanediol is present in a range of about 0.1% to about 20% (v/v) and proline is present in a range of about 2% to about 15% (w/v). In some embodiments, propanediol is present in a range of about 0.2% to about 15% (v/v) and proline is present in a range of about 5% to about 15% (w/v). In some embodiments, propanediol is present in a range of about 0.2% to about 15% (v/v) and proline is present in a range of about 5% to about 10% (w/v). In some embodiments, propanediol is present at about 6% (v/v) and proline is present at about 5% or about 10% (w/v).

Glycerin and Trehalose

[00144] In some embodiments, the formulation comprises glycerin and trehalose. In some embodiments, glycerin is present in a range of about 4% to about 16% (v/v) and trehalose is present in a range of about 2% to about 12% (w/v). In some embodiments glycerin is present in a range of about 8% to about 12% (v/v) and trehalose is present in a range of about 4% to about 10% (w/v). In some embodiments, glycerin is present at about 10% (v/v) and trehalose is present at about 7% (w/v).

Examples of Cryopreservative Formulations

[00145] In some embodiments, a cryopreservative formulation comprises about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 0.2%, about 2%, about 6%, about 10%, or about 15% (v/v) propanediol and about 5%, about 6%, about 10%, or about 15% (w/v) dextran-40.

[00146] In some embodiments, a cryopreservative formulation comprises about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 0.2% to about 15% (v/v) propanediol and about 6%, about 10%, or about 15% (w/v) dextran-40.

[00147] In some embodiments, a cryopreservative formulation comprises about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 0.2%, about 2%, about 6%, about 10%, or about 15% (v/v) propanediol and about 10% (w/v) dextran-40.

[00148] In some embodiments, a cryopreservative formulation comprises about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 10% (v/v) propanediol and about 15% (w/v) dextran-40.

[00149] In some embodiments, a cryopreservative formulation comprises about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 10% (v/v) propanediol and about 10% (w/v) dextran-40.

[00150] In some embodiments, a cryopreservative formulation comprises about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 6% (v/v) propanediol and about 20% (w/v) dextran-40.

[00151] In some embodiments, a cryopreservative formulation comprises about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 6% (v/v) propanediol and about 15% (w/v) dextran-40.

[00152] In some embodiments, a cryopreservative formulation comprises about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 6% (v/v) propanediol and about 10% (w/v) dextran-40.

[00153] In some embodiments, a cryopreservative formulation comprises about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 6% (v/v) propanediol and about 6% (w/v) dextran-40.

[00154] In some embodiments, a cryopreservative formulation comprises about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v)

glucose, buffered saline (e.g., PBS), about 6% (v/v) propanediol and about 2% (w/v) dextran-40.

[00155] In some embodiments, a cryopreservative formulation comprises about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 5% (v/v) propanediol and about 7.5% (w/v) dextran-40, and optionally comprises a cell population such as but not limited to an RPE cell population, an HMC population, and a VPC population.

[00156] In some embodiments, a cryopreservative formulation comprises about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 3% (v/v) propanediol and about 5% (w/v) dextran-40, and optionally comprises a cell population such as but not limited to an RPE cell population, an HMC population, and a VPC population.

[00157] In some embodiments, a cryopreservative formulation comprises about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 3% (v/v) propanediol and about 5% (w/v) dextran-40, and optionally comprises a cell population such as but not limited to an RPE cell population, an HMC population, and a VPC population.

[00158] In some embodiments, a cryopreservative formulation comprises about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), and about 5% (w/v) dextran-40, and optionally comprises a cell population such as but not limited to an RPE cell population, an HMC population, and a VPC population.

[00159] In some embodiments, a cryopreservative formulation comprises about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), and about 7.5% (w/v) pentastarch, and optionally comprises a cell population such as but not limited to an RPE cell population, an HMC population, and a VPC population.

[00160] In some embodiments, a cryopreservative formulation comprises about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 6% (v/v) propanediol and about 5% (w/v) proline.

[00161] In some embodiments, a cryopreservative formulation comprises about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 6% (v/v) propanediol and about 10% (w/v) proline.

[00162] In some embodiments, a cryopreservative formulation comprises about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 0.02% to about 1% (w/v) glucose, buffered saline (e.g., PBS), about 10% (v/v) propanediol and about 0.2% to about 15% (w/v) trehalose.

[00163] In some embodiments, a cryopreservative formulation comprises about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 10% (v/v) propanediol and about 7% (w/v) trehalose.

[00164] In some embodiments, a cryopreservative formulation comprises about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 10% (v/v) propanediol and about 2% (w/v) trehalose.

[00165] In some embodiments, a cryopreservative formulation comprises about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 10% glycerin and about 7% (w/v) trehalose.

[00166] In some embodiments, a cryopreservative formulation comprises about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), and about 5% (w/v) hydroxyethyl starch.

[00167] In some embodiments, a cryopreservative formulation comprises about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), and about 10% (w/v) hydroxyethyl starch.

[00168] In some embodiments, a cryopreservative formulation comprises about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), and about 15% (w/v) hydroxyethyl starch.

[00169] In some embodiments, a cryopreservative formulation comprises about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), and about 5% (w/v) pentastarch.

[00170] In some embodiments, a cryopreservative formulation comprises about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), and about 10% (w/v) pentastarch.

[00171] In some embodiments, a cryopreservative formulation comprises about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), and about 15% (w/v) pentastarch.

[00172] In some embodiments, a cryopreservative formulation comprises about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), and about 15% (w/v) dextran-40.

[00173] In some embodiments, a cryopreservative formulation comprises about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), and about 10% (w/v) dextran-40.

[00174] In some embodiments, a cryopreservative formulation comprises about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), and about 5% (w/v) dextran-40.

[00175] In some embodiments, a cryopreservative formulation comprises about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), and about 5% (v/v) ethylene glycol.

[00176] In some embodiments, the cryopreservative formulation may be an albumin-free version of any of the foregoing formulations (i.e., it does not contain albumin). In some embodiments, the cryopreservative formulation may be an glucose-free version of any of the foregoing formulations (i.e., it does not contain glucose). In some embodiments, the cryopreservative formulation may be an albumin-free and glucose-free version of any of the foregoing formulations (i.e., it does not contain albumin and it does not contain glucose).

[00177] In some embodiments, a cryopreservative formulation comprises glucose, buffered saline (e.g., PBS) (or optionally buffering agent and/or saline or electrolytes), and hydroxyethyl starch, and are albumin-free.

[00178] In some embodiments, a cryopreservative formulation comprises albumin (e.g., human albumin, e.g., recombinant human albumin), buffered saline (e.g., PBS) (or optionally buffering agent and/or saline or electrolytes), and hydroxyethyl starch, and are glucose-free.

[00179] In some embodiments, a cryopreservative formulation comprises buffered saline (e.g., PBS) (or optionally buffering agent and/or saline or electrolytes) and hydroxyethyl starch, and are albumin-free and glucose-free.

[00180] The disclosure contemplates and provides formulations that "comprise", "consist essentially of", or "consist of" the components provided herein. For brevity, most formulations are described herein as "comprising" their various stated components; however, it is to be understood that the disclosure provides formulations that "consist essentially of", or "consist of" the same components.

Cell Preparations

[00181] Some aspects of this disclosure provide cell preparations comprising a population of cells and the cryopreservative formulation as provided herein.

[00182] In some embodiments, the cell preparations exhibit cell viability of at least 80% (e.g., at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92% , at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% and at least 99%). In some embodiments, the cell preparations exhibit robust cell growth index. In some embodiments, the cell preparations exhibit a cell growth index:3 of at least 110 hours, at least 109 hours, at least 108 hours, at least 107 hours, at least 106 hours, at least 105 hours, at least 104 hours, at least 103 hours, at least 102 hours, at least 101 hours, at least 100 hours, at least 99 hours, at least 98 hours, at least 97 hours, at least 96 hours, at least 95 hours, at least 94 hours, at least 93 hours, at least 92 hours, at least 91 hours, at least 90 hours, at least 89 hours, at least 88 hours, at least 87 hours, at least 86 hours, at least 85 hours, at least 84 hours, at least 83 hours, at least 82 hours, at least 81 hours, or at least 80 hours. In some embodiments, the cell preparations are resistant to variations in storage temperature while maintaining robust viability and cell growth index.

[00183] Provided herein is a cell preparation comprising a population of cells in a 1:1 (v/v) mixture of (i) the cryopreservative formulation provided herein and (ii) a suspension solution comprising albumin, glucose and buffered saline, each at the same concentration as in the formulation, wherein the suspension solution does not contain a (non-albumin) cryoprotectant. In some embodiments, the cell preparation is free of DMSO (DMSO-free). If DMSO is present in a cell preparation provided herein, it is present at less than 10% (v/v), less than 5% (v/v), less than 1% (v/v), less than 0.1% (v/v), or less than 0.01% (v/v).

[00184] Provided herein is a cell preparation comprising a population of cells in a formulation provided herein comprising glucose, albumin, buffered saline, and one or two, or optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, hydroxyethyl starch such as but not limited to pentastarch, ethylene glycol, and poloxamer-188. In some embodiments, the cell preparation is free of DMSO (DMSO-free). If DMSO is present in a cell preparation provided herein, it is present at less than 10% (v/v), less than 5% (v/v), less than 1% (v/v), less than 0.1% (v/v), or less than 0.01% (v/v).

[00185] Provided herein is a cell preparation comprising a population of cells in a cryopreservative formulation consisting essentially of albumin, glucose, buffered saline, and

one or more cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, hydroxyethyl starch such as but not limited to pentastarch, and ethylene glycol.

[00186] In some embodiments, a cell preparation comprises a population of cells in a formulation provided herein comprising glucose, albumin, buffered saline, and one or two, or optionally more, cryoprotectants. In some embodiments, the components in the cell preparation are the same as those in the cryopreservative formulations provided herein except that the concentrations of the one or two, or optionally more, cryoprotectants are halved.

[00187] In some embodiments, the albumin is present in a range of about 1% to about 10% (w/v). In some embodiments, the albumin is present in a range of about 2% to about 8% (w/v). In some embodiments, the albumin is present in a range of about 2% to about 7.5% (w/v). In some embodiments, the albumin is present in a range of about 1% to about 4% (w/v). In some embodiments, the albumin is present in a range of about 2% to about 3% (w/v). In some embodiments, the albumin is present at about 1%, about 1.5%, about 2%, about 2.5%, about 3%, about 3.5%, about 4%, about 4.5%, about 5%, about 5.5%, about 6%, about 6.5%, about 7%, about 7.5%, about 8%, about 8.5%, about 9%, about 9.5%, or about 10% (w/v)). In some embodiments, the albumin is present at about 2%, about 2.1%, about 2.2%, about 2.3%, about 2.4%, about 2.5%, about 2.6%, about 2.7%, about 2.8%, about 2.9%, or about 3% (w/v)). In some embodiments, the albumin is present at about 2.5% (w/v). In some embodiments, albumin is absent (i.e., the formulation is albumin-free).

[00188] In some embodiments, the glucose is present in a range of about 0.01% to about 3% (w/v). In some embodiments, the glucose is present in a range of about 0.01% to about 1.5% (w/v). In some embodiments, the glucose is present in a range of about 0.01% to about 0.5% (w/v). In some embodiments, the glucose is present in a range of about 0.02% to about 1.5% (w/v). In some embodiments, the glucose is present in a range of about 0.05% to about 1.2% (w/v). In some embodiments, the glucose is present at about 0.01%, about 0.02%, about 0.03%, about 0.04%, about 0.05%, about 0.06%, about 0.07%, about 0.08%, about 0.09%, about 1%, about 1.1%, or about 1.2% (w/v). In some embodiments, the glucose is present at about 0.09% (w/v). In some embodiments, glucose is absent (i.e., the formulation is glucose-free).

[00189] In some embodiments, a cell preparation provided herein comprises a population of cells in a formulation comprising one or two, or optionally more, (non-albumin) cryoprotectants. In some embodiments, the one or two, or optionally more, cryoprotectants, in some instances, do not include DMSO. Some of the formulations

provided herein comprise one (non-albumin) cryoprotectant. Some of the formulations provided herein comprise two (non-albumin) cryoprotectants. Some of the formulations provided herein comprise more than two (non-albumin) cryoprotectants. The one or two, or optionally more, cryoprotectants are selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, hydroxyethyl starch such as but not limited to pentastarch, and ethylene glycol.

[00190] In some embodiments, the one or two, or optionally more, cryoprotectants comprise propanediol. In some embodiments, the propanediol is present in a range of 0.05% to about 10% (v/v). In some embodiments, the propanediol is present in a range of 0.1% to about 7.5% (v/v). In some embodiments, the propanediol is present in a range of 0.1% to about 3% (v/v). In some embodiments, the propanediol is present in a range of 1% to about 7.5% (v/v). In some embodiments, the propanediol is present in a range of 3% to about 7.5% (v/v). In some embodiments, the propanediol is present at about 0.1%, about 0.5%, about 1%, about 2% , about 3%, about 4% about 5%, about 6%, about 7%, or about 7.5% (v/v). In some embodiments, the propanediol is present at about 0.1%, about 1%, about 3%, about 5%, or about 7.5% (v/v). In some embodiments, the propanediol is present at about 0.1% (v/v). In some embodiments, the propanediol is present at about 1% (v/v). In some embodiments, the propanediol is present at about 3% (v/v). In some embodiments, the propanediol is present at about 5% (v/v). In some embodiments, the propanediol is present at about 7.5% (v/v).

[00191] In some embodiments, the one or two, or optionally more, cryoprotectants comprise dextran-40. In some embodiments, the dextran-40 is present in a range of about 0.5% to about 10% (w/v). In some embodiments, the dextran-40 is present in a range of about 1% to about 10% (w/v). In some embodiments, the dextran-40 is present in a range of about 2% to about 10% (w/v). In some embodiments, the dextran-40 is present in a range of about 2.5% to about 7.5% (w/v). In some embodiments, the dextran-40 is present at about 1%, about 1.5%, about 2%, about 2.5%, about 3%, about 3.5%, about 4%, about 4.5%, about 5%, about 5.5%, about 6%, about 6.5%, about 7%, about 7.5%, about 8%, about 8.5%, about 9%, about 9.5%, or about 10% (w/v). In some embodiments, the dextran-40 is present at about 2.5%, about 3%, about 5%, or about 7.5% (w/v). In some embodiments, the dextran-40 is present at about 1% (w/v). In some embodiments, the dextran-40 is present at about 2.5% (w/v). In some embodiments, the dextran-40 is present at about 3% (w/v). In some embodiments, the dextran-40 is present at about 5% (w/v). In some embodiments, the dextran-40 is present at about 7.5% (w/v). In some embodiments, the dextran-40 is present at about 10% (w/v).

[00192] In some embodiments, the one or two, or optionally more, cryoprotectants comprise proline. In some embodiments, the proline is present in a range of about 1% to about 7.5% (w/v). In some embodiments, the proline is present in a range of about 2.5% to about 7.5% (w/v). In some embodiments, the proline is present in a range of about 2.5% to about 5% (w/v). In some embodiments, the proline is present at about 2.5%, about 3%, about 3.5%, about 4%, about 4.5%, or about 5% (w/v). In some embodiments, the proline is present at about 2.5% or 5% (w/v). In some embodiments, the proline is present at about 2.5% (w/v). In some embodiments, the proline is present at about 5% (w/v).

[00193] In some embodiments, the one or two, or optionally more, cryoprotectants comprise trehalose. In some embodiments, trehalose is present in a range of about 0.05% to about 10% (w/v). In some embodiments, trehalose is present in a range of about 0.1% to about 7.5% (w/v). In some embodiments, trehalose is present in a range of about 0.1% to about 3.5% (w/v). In some embodiments, trehalose is present in a range of about 1% to about 6% (w/v). In some embodiments, trehalose is present in a range of about 1% to about 3.5% (w/v). In some embodiments, trehalose is present in a range of about 2% to about 5% (w/v). In some embodiments, trehalose is present in a range of about 3% to about 7.5% (w/v). In some embodiments, trehalose is present at about 1%, about 1.5%, about 2%, about 2.5%, about 3%, or about 3.5% (w/v). In some embodiments, trehalose is present at about 1% or about 3.5% (w/v). In some embodiments, trehalose is present at about 1% (w/v). In some embodiments, trehalose is present at about 3.5% (w/v).

[00194] In some embodiments, the one or two, or optionally more, cryoprotectants comprise glycerin. In some embodiments, glycerin is present in a range of about 2% to about 8% (v/v). In some embodiments, glycerin is present in a range of about 4% to about 6% (v/v). In some embodiments, glycerin is present at about 2%, about 2.5%, about 3%, about 3.5%, about 4%, about 4.5%, about 5%, about 5.5%, about 6%, about 6.5%, about 7%, about 7.5%, or about 8% (v/v). In some embodiments, glycerin is present at about 5% (v/v).

[00195] In some embodiments, the one or two, or optionally more, cryoprotectants comprise a hydroxyethyl starch such as but not limited to tetrastarch, pentastarch or heptastarch. In some embodiments, the one or two, or optionally more, cryoprotectants comprise pentastarch. Pentastarch is s a subgroup of hydroxyethyl starch, having five hydroxyethyl groups out of every 11 hydroxyls, which renders it approximately 50% substituted with hydroxyethyl. Pentastarch is commercially available as Pentaspan (Bristol-Myers Squibb).

[00196] In some embodiments, hydroxyethyl starch such as pentastarch is present in a range of about 2% to about 10% (w/v). In some embodiments, hydroxyethyl starch such as pentastarch is present in a range of about 2.5% to about 7.5% (w/v). In some embodiments, hydroxyethyl starch such as pentastarch is present at about 2.5%, about 3%, about 3.5%, about 4%, about 4.5%, about 5%, about 5.5%, about 6%, about 6.5%, about 7%, about 7.5%, about 8%, about 8.5%, about 9%, or about 10% (w/v). In some embodiments, hydroxyethyl starch such as pentastarch is present at about 2.5%, about 5%, or about 7.5% (w/v). In some embodiments, hydroxyethyl starch such as pentastarch is present at about 2.5% (w/v). In some embodiments, hydroxyethyl starch such as pentastarch is present at about 5% (w/v). In some embodiments, hydroxyethyl starch such as pentastarch is present at about 7.5% (w/v).

[00197] In some embodiments, the one or two, or optionally more, cryoprotectants comprise ethylene glycol. In some embodiments, ethylene glycol is present in a range of about 1% to about 4% (v/v). In some embodiments, ethylene glycol is present in a range of about 2% to about 3% (v/v). In some embodiments, ethylene glycol is present at about 2%, about 2.1%, about 2.2%, about 2.3%, about 2.4%, about 2.5%, about 2.6%, about 2.7%, about 2.8%, about 2.9%, or about 3% (v/v). In some embodiments, ethylene glycol is present at about 2.5% (v/v).

Combinations of Cryoprotectants

[00198] In some embodiments, a cell preparation provided herein comprises a population of cells in a formulation comprising two (non-albumin) cryoprotectants. Various combinations of cryoprotectants are provided below.

Propanediol and Dextran-40

[00199] In some embodiments, the cell preparation comprises propanediol and dextran-40. In some embodiments, propanediol is present in a range of about 0.05% to about 10% (v/v) and dextran-40 is present in a range of about 0.1% to about 10% (w/v). In some embodiments, propanediol is present in a range of about 0.05% to about 10% (v/v) and dextran-40 is present in a range of about 2% to about 10% (w/v). In some embodiments, propanediol is present in a range of about 0.1% to about 7.5% (v/v) and dextran-40 is present in a range of about 2.5% to about 7.5% (w/v). In some embodiments, propanediol is present at about 0.1% (v/v), about 1%, about 3%, about 5%, or about 7.5% and dextran-40 is present at about 2.5%, about 3%, about 5%, or about 7.5%. In some embodiments, propanediol is present in a range of about 0.1% to about 7.5% (v/v) and dextran-40 is present at about 3%,

about 5%, or about 7.5%. In some embodiments, propanediol is present at about 0.1%, about 1%, about 3%, about 5%, or about 7.5% and dextran-40 is present at about 5%. In some embodiments, propanediol is present at about 5% and dextran-40 is present at about 7.5%. In some embodiments, propanediol is present at about 5% (v/v) and dextran-40 is present at about 5%. In some embodiments, propanediol is present at about 3% (v/v) and dextran-40 is present at about 10%. In some embodiments, propanediol is present at about 3% (v/v) and dextran-40 is present at about 7.5% (w/v). In some embodiments, propanediol is present at about 3% (v/v) and dextran-40 is present at about 5% (w/v). In some embodiments, propanediol is present at about 3% and dextran-40 is present at about 3% (w/v). In some embodiments, propanediol is present at about 3% (v/v) and dextran-40 is present at about 1% (w/v).

Propanediol and Trehalose

[00200] In some embodiments, the cell preparation comprises propanediol (1,3-propanediol) and trehalose. In some embodiments, propanediol is present in a range of about 0.05% to about 10% (v/v) and trehalose is present in a range of about 0.05% to about 10% (w/v). In some embodiments, propanediol is present in a range of about 0.1% to about 7.5% and trehalose is present in a range of about 0.1% to about 7.5% (w/v). In some embodiments, propanediol is present in a range of about 5% and trehalose is present in a range of about 0.1% to about 7.5% (w/v). In some embodiments, propanediol is present at about 5% (v/v) and trehalose is present at about 3.5% (w/v). In some embodiments, propanediol is present at about 5% (v/v) and trehalose is present at about 1% (w/v).

Propanediol and Proline

[00201] In some embodiments, the cell preparation comprises propanediol (1,3-propanediol) and proline. In some embodiments, propanediol is present in a range of about 0.05% to about 10% (v/v) and proline is present in a range of about 1% to about 7.5% (w/v). In some embodiments, propanediol is present in a range of about 0.1% to about 7.5% (v/v) and proline is present in a range of about 2.5% to about 7.5% (w/v). In some embodiments, propanediol is present in a range of about 0.1% to about 7.5% and proline is present in a range of about 2.5% to about 5% (w/v). In some embodiments, propanediol is present at about 3% (v/v) and proline is present at about 2.5% or about 5% (w/v).

Glycerin and Trehalose

[00202] In some embodiments, the cell preparation comprises glycerin and trehalose. In some embodiments, glycerin is present in a range of about 2% to about 8% (v/v) and trehalose is present in a range of about 1% to about 6% (w/v). In some embodiments glycerin is present in a range of about 4% to about 6% (v/v) and trehalose is present in a range of about 2% to about 5% (w/v). In some embodiments, glycerin is present at about 5% (v/v) and trehalose is present at about 3.5% (w/v).

Examples of Cell Preparations

[00203] In some embodiments, a cell preparation provided herein comprises a population of cells in a formulation comprising about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 0.1%, about 1%, about 3%, about 5%, or about 7.5% (v/v) propanediol and about 2.5%, about 3%, about 5%, or about 7.5% (w/v) dextran-40.

[00204] In some embodiments, a cell preparation provided herein comprises a population of cells in a formulation comprising about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 0.1% to about 7.5% (v/v) propanediol and about 3%, about 5%, or about 7.5% (w/v) dextran-40.

[00205] In some embodiments, a cell preparation provided herein comprises a population of cells in a formulation comprising about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 0.1%, about 1%, about 3%, about 5%, or about 7.5% (v/v) propanediol and about 5% (w/v) dextran-40.

[00206] In some embodiments, a cell preparation provided herein comprises a population of cells in a formulation comprising about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 5% (v/v) propanediol and about 7.5% (w/v) dextran-40.

[00207] In some embodiments, a cell preparation provided herein comprises a population of cells in a formulation comprising about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 5% (v/v) propanediol and about 5% (w/v) dextran-40.

[00208] In some embodiments, a cell preparation provided herein comprises a population of cells in a formulation comprising about 2.5% (w/v) albumin (e.g., human

albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 3% (v/v) propanediol and about 10% (w/v) dextran-40.

[00209] In some embodiments, a cell preparation provided herein comprises a population of cells in a formulation comprising about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 3% (v/v) propanediol and about 7.5% (w/v) dextran-40.

[00210] In some embodiments, a cell preparation provided herein comprises a population of cells in a formulation comprising about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 3% (v/v) propanediol and about 5% (w/v) dextran-40.

[00211] In some embodiments, a cell preparation provided herein comprises a population of cells in a formulation comprising about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 3% (v/v) propanediol and about 3% (w/v) dextran-40.

[00212] In some embodiments, a cell preparation provided herein comprises a population of cells in a formulation comprising about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 3% (v/v) propanediol and about 1% (w/v) dextran-40.

[00213] In some embodiments, a cell preparation provided herein comprises a population of cells in a formulation comprising about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 3% (v/v) propanediol and about 2.5% (w/v) proline.

[00214] In some embodiments, a cell preparation provided herein comprises a population of cells in a formulation comprising about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 3% (v/v) propanediol and about 5% (w/v) proline.

[00215] In some embodiments, a cell preparation provided herein comprises a population of cells in a formulation comprising about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 0.01% to about 0.5% (w/v) glucose, buffered saline (e.g., PBS), about 5% (v/v) propanediol and about 0.1% to about 7.5% (w/v) trehalose.

[00216] In some embodiments, a cell preparation provided herein comprises a population of cells in a formulation comprising about 2.5% (w/v) albumin (e.g., human

albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 5% (v/v) propanediol and about 3.5% (w/v) trehalose.

[00217] In some embodiments, a cell preparation provided herein comprises a population of cells in a formulation comprising about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 5% (v/v) propanediol and about 1% (w/v) trehalose.

[00218] In some embodiments, a cell preparation provided herein comprises a population of cells in a formulation comprising about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 5% glycerin and about 3.5% (w/v) trehalose.

[00219] In some embodiments, a cell preparation provided herein comprises a population of cells in a formulation comprising about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), and about 2.5% (w/v) hydroxyethyl starch having hydroxyethyl substitutions ranging up to 100%.

[00220] In some embodiments, a cell preparation provided herein comprises a population of cells in a formulation comprising about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), and about 5% (w/v) hydroxyethyl starch having hydroxyethyl substitutions ranging up to 100%.

[00221] In some embodiments, a cell preparation provided herein comprises a population of cells in a formulation comprising about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), and about 7.5% (w/v) hydroxyethyl starch having hydroxyethyl substitutions ranging up to 100%.

[00222] In some embodiments, a cell preparation provided herein comprises a population of cells in a formulation comprising about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), and about 2.5% (w/v) pentastarch.

[00223] In some embodiments, a cell preparation provided herein comprises a population of cells in a formulation comprising about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), and about 5% (w/v) pentastarch.

[00224] In some embodiments, a cell preparation provided herein comprises a population of cells in a formulation comprising about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), and about 7.5% (w/v) pentastarch.

[00225] In some embodiments, a cell preparation provided herein comprises a population of cells in a formulation comprising about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), and about 7.5% (w/v) dextran-40.

[00226] In some embodiments, a cell preparation provided herein comprises a population of cells in a formulation comprising about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), and about 5% (w/v) dextran-40.

[00227] In some embodiments, a cell preparation provided herein comprises a population of cells in a formulation comprising about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), and about 2.5% (w/v) dextran-40.

[00228] In some embodiments, a cell preparation provided herein comprises a population of cells in a formulation comprising about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), and about 2.5% (v/v) ethylene glycol.

[00229] This disclosure also contemplates albumin-free versions of any of the foregoing cell preparations. This disclosure also contemplates glucose-free versions of any of the foregoing cell preparations. This disclosure also contemplates albumin-free and glucose-free versions of any of the foregoing cell preparations.

[00230] The disclosure contemplates and provides cell preparations that "comprise", "consist essentially of", or "consist of" the components provided herein. For brevity, most cell preparations are described herein as "comprising" their various stated components; however, it is to be understood that the disclosure provides cell preparations that "consist essentially of", or "consist of" the same components.

[00231] In some embodiments, the cell preparation has a cell density of about 100 cells to about 10,000 cells per microliter. In some embodiments, the cell preparation has a cell density of about 300 cells to about 7,000 cells per microliter. In some embodiments, the cell preparation has a cell density of about 350 cells to about 7,000 cells per microliter. In some embodiments, the cell preparation has a cell density of about 350 cells per microliter, or

about 3,000 cells per microliter, or about 3,500 cells per microliter, or about 6,500 cells per microliter, or about 7,000 cells per microliter. In some embodiments, the cell preparation has a cell density of about 2000 cells to about 4,000 cells per microliter, or of about 3000 cells to about 3,500 cells per microliter. In some embodiments, the cell preparation has a cell density of about 3,333 cells per microliter. In some embodiments, the cell preparation is in a volume of about 400-500 microliters. In some embodiments, the cell preparation is in a volume of about 450 microliters.

Cell populations

Types of cells

[00232] The cryopreservative formulations and cell preparations provided herein can be used in connection with different cell types and with different administration sites and routes. While ophthalmologic applications are preferred, other applications are also contemplated and embraced by the present disclosure. In some embodiments, the population of cells is selected from retinal pigmented epithelium (RPE) cells, photoreceptor cells, photoreceptor progenitor cells thereof, photoreceptor rescue cells (PRCs), corneal endothelial cells, corneal endothelial progenitor cells, corneal epithelial cells, or mesenchymal cells such as hemangioblast-derived mesenchymal cells. In some embodiments, the cell population is a retinal pigmented epithelium (RPE) cell population. In some embodiments, the cell population is a hemangioblast-derived mesenchymal cell (HMC) population. In other embodiments the population of cells is selected from macrophage, natural killer cells, T-cells, vascular cells, vascular progenitor cells, liver cells, neural cells and pancreatic cells. In some embodiments, the cell population is a vascular progenitor cell population.

Source of cells

[00233] In some embodiments, a cell population in a cell preparation provided herein may be derived from primary cells. In some embodiments, a cell population in a cell preparation provided herein may be derived from freshly harvested cells.

[00234] In some embodiments, a cell population in a cell preparation provided herein may be generated by differentiation of pluripotent stem cells, including human induced pluripotent stem cells (hiPSC), human embryonic stem cells (hESC) and somatic cells (including transdifferentiated cells and stem cells). The pluripotent stem cells may be derived from universal donor cells. The differentiation protocol may be directed or spontaneous. In some embodiments, the cell population is generated by in vitro differentiation of pluripotent

stem cells. In some embodiments, the cell population is generated by in vitro differentiation of embryonic stem (ES) cells. The embryonic stem cells may be derived from single blastomeres without embryo destruction. In some embodiments, the cell population is generated by in vitro differentiation of induced pluripotent stem cells (iPS) cells.

[00235] In some embodiments, the cell preparation comprising a population of cells suitable for transplantation into the eye of a subject is suitable for injection into the eye of the subject. In some embodiments, such a cell preparation may be used for treating retinal degeneration diseases or disorders, including, but not limited to, retinal detachment, retinal tears, retinal dysplasia, Angioid streaks, Myopic Macular Degeneration, or retinal atrophy or associated with a number of vision-altering ailments that result in photoreceptor damage and blindness, such as, for example, choroideremia, diabetic retinopathy, macular degeneration (*e.g.*, age-related macular degeneration (AMD)), retinitis pigmentosa, Stargardt's Disease (fundus flavimaculatus), and geographic atrophy including geographic atrophy secondary to AMD.

[00236] In some embodiments, the cell preparations comprise a population of RPE cells and a cryopreservative formulation provided herein. The RPE cells may be stable, terminally differentiated RPE cells that do not de-differentiate to a non-RPE cell type. The RPE cells described herein may be functional RPE cells, characterized by the ability to integrate into the retina upon corneal, sub-retinal, or other administration into a human or a non-human animal.

[00237] The RPE cells may express RPE cell markers. For example, the level of expression of markers such as RPE65, PAX2, PAX6, tyrosinase, bestrophin, PEDF, PMEL17, CRALBP, Otx2, and/or MITF may be equivalent to that in naturally occurring RPE cells. The level of maturity of the RPE cells may be assessed by measuring expression of at least one of PAX2, PAX6, PMEL17 and tyrosinase, or their respective expression levels.

[00238] The cells may be of human origin or of animal origin.

Engineered Non-Immunogenic Cells

[00239] In some embodiments, a cell population provided herein is a population of engineered cells (Universal Donor Cells or hypoimmunogenic cells or cells derived therefrom) wherein the cells express at least one polypeptide selected from the group consisting of human leukocyte antigen E (HLA-E), human leukocyte antigen F (HLA-F), and human leukocyte antigen G (HLA-G). The population of cells also comprise a genetically engineered disruption of one or more copies (e.g., all copies) of endogenous β-2

microglobulin (B2M) and/or a genetically engineered disruption of one or more copies (e.g., all copies) of a human leukocyte antigen (HLA) class II-related gene selected from the group consisting of regulatory factor X associated ankyrin containing protein (RFXANK), regulatory factor 5 (RFX5), regulatory factor X associated protein (RFXAP), and class II transactivator (CIITA) (UC-RPE). For example, the cells comprise a genetically engineered disruption of endogenous B2M and RFXANK, and express HLA-E. In another example, the cells comprise a genetically engineered disruption of all copies of endogenous B2M and all copies of RFXANK, and express HLA-E. In various aspects, the cells further comprise a nucleic acid molecule encoding a suicide gene product (e.g., a herpes simplex virus thymidine kinase (TK) suicide gene). The disruptions allow the cells to, e.g., reduce or evade immune detection, and allow production of a cell population which does not require HLA matching for therapeutic applications and provides for a homogenous population of cells with reduced occurrence of undesired cells, improved safety profiles, strong persistence in vivo, enhanced ability to engraft in a subject, and/or enhanced ability to reduce tumor burden (see, for example, WO2012145384 and WO2013158292, which are herein incorporated by reference in their entireties).

[00240] The cells, for example RPE cells (or a PSC from which the cell, for example RPE cell, is derived or an intermediate thereof) are genetically engineered to disrupt one or more copies (e.g., all copies) of the cells' endogenous B2M gene. B2M is a critical component of the HLA class I (HLA-I) complex. HLA is a cell surface complex that mediates leukocyte interactions or interactions of leukocytes with other cells. HLA-I molecules are cell surface complexes which present antigens to CD8+ cytotoxic T cells, thereby mediating cellular immunity. HLA-I molecules comprise an HLA-I heavy chain and B2M. Genetic disruption of one or more (e.g., all) copies of the B2M gene reduces or abrogates production of the B2M protein (SEQ ID NO: 5), thereby reducing or abrogating display of HLA-I complexes on the cell. A representative nucleic acid sequence encoding B2M is provided in SEQ ID NO: 1.

[00241] Also contemplated are cells, for example an RPE cell (or a PSC from which the cell is derived or an intermediate thereof) genetically engineered to disrupt one or more copies (e.g., all copies) of a subset of HLA-I. Six HLA class I alpha (α) chains have been identified, including three classical (HLA-A, HLA-B and HLA-C) and three non-classical (HLA-E, HLA-F, and HLA-G), which are responsible for specificity of peptide binding on the HLA-I binding cleft. In this regard, the disclosure contemplates cells genetically engineered to disrupt one or more copies (e.g., all copies) of an HLA-I gene selected from

HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, and HLA-G, as well as combinations thereof. For example, the cells (or a PSC from which the cell is derived or an intermediate thereof) may be genetically engineered to disrupt one or more copies (e.g., all copies) of HLA-A, HLA-B, and/or HLA-C. HLA molecules are further described in, e.g., Choo, Yonsei Med J., 48(1): 11–23 (2007); www.ebi.ac.uk/ipd/imgt/hla/; and GenBank Accession Nos. NG_029217 (HLA-A), XP_041536701 (HLA-A), 9 NG_023187 (HLA-B), NP_005505 (HLA-B), NG_029422 (HLA-C), NP_001229971 (HLA-C), NM_005516 (HLA-E), NP_005507 (HLA-E), NG_012009 (HLA-F), NP_001091949 (HLA-F), NG_029039 (HLA-G), and NP_001371219 (HLA-G).

[00242] The cells, for example RPE cells, of the disclosure (or a PSC from which the cell is derived or an intermediate thereof) are genetically engineered to disrupt one or more (e.g., all) copies of an HLA class II (HLA-II)-related gene selected from RFXANK, RFX5, RFXAP, and CIITA. HLA-II molecules are transmembrane proteins found on antigen-presenting cells (APCs) and, at times, solid organs. HLA-II molecules comprise two homologous subunits, the alpha (α) subunit and the beta (β) subunit. HLA-II related genes encode HLA-II regulatory proteins that regulate the expression of HLA class II molecules. Genetic disruption of one or more copies (e.g., all copies) of one or more of the HLA-II related genes RFXANK, RFX5, RFXAP, and/or CIITA reduces or abrogates production of the encoded proteins, thereby reducing or abrogating display of HLA-II complexes on the cell. The cell (or a PSC from which the cell is derived or an intermediate thereof) may comprise disruptions of one or more copies (e.g., all copies) of the genes encoding one of RFXANK, RFX5, RFXAP, and/or CIITA (e.g., the cell is engineered to disrupt one or more copies (e.g., all copies) of the RFXANK gene); one or more copies (e.g., all copies) of the genes encoding any combination of two of RFXANK, RFX5, RFXAP, and/or CIITA; one or more copies (e.g., all copies) of the genes encoding any combination of three of RFXANK, RFX5, RFXAP, and/or CIITA; or one or more copies (e.g., all copies) of the genes encoding all of RFXANK, RFX5, RFXAP, and CIITA). RFXANK is encoded by, e.g., the sequence of SEQ ID NOs: 3 and 5; RFX5 is encoded by, e.g., the sequence of SEQ ID NOs: 7 and 9; RFXAP is encoded by, e.g., SEQ ID NO: 11; and CIITA is encoded by, e.g., SEQ ID NO: 13. In various aspects of the disclosure, the cells are engineered to disrupt all copies of B2M and RFXANK.

[00243] Any suitable technique for introducing a disruption in a target gene (e.g., an HLA-II related gene, a B2M gene, or any other gene of interest) may be used. Many techniques for disrupting endogenous coding sequences are known in the art, including use of

gene editing systems such as CRISPR/Cas (clustered regularly interspaced short palindromic repeats and CRISPR-associated protein) systems, transcription activator-like effector nucleases (TALENs), and zinc finger nucleases, as well as targeting vectors that cleave and/or insert nucleic acid sequences into a target site in the cellular genome. An exemplary technique employs adeno- associated virus (AAV) vector-based editing, which optionally involves targeted insertion of an edited sequence (potentially a transgene) into the cellular genome so as to interrupt an endogenous coding sequence, thereby resulting in reduction or avoidance of production of the endogenous protein. In various aspects, serotype 3B AAV vectors are employed. Use of AAV vectors to engineer stem cells is further described in, e.g., Riolobos et al., Molecular Therapy, 21(6), 1232-1241 (2013), hereby incorporated by reference in its entirety and particularly with respect to the disclosure of use of AAV vectors and disruption of HLA complex production. Methods of confirming disruption of a target gene are known in the art and include, but are not limited to, PCR detection methods (e.g., real time polymerase chain reaction (qRT-PCR)), RNA sequencing, next generation sequencing methods, fluorescence-activated cell sorting (FACS), and immunostaining.

[00244] In various aspects, the engineered cells, for example RPE cells, express at least one polypeptide selected from the group consisting of HLA-E, HLA-F, and HLA-G. In the context of the disclosure, the cell is engineered to express HLA-E, HLA-F, and/or HLA-G, i.e., a nucleic acid encoding HLA-E, HLA-F, and/or HLA-G is introduced into the cell (or a PSC from which the cell is derived or an intermediate thereof) and the encoded protein is produced and displayed on the cell surface. Optionally, the engineered cells express HLA-E. Optionally, the engineered cell expresses a single chain fusion HLA-E comprising at least of portion of B2M linked, either directly or via a linker sequence, to at least of portion of the HLA-I α-chain, e.g., HLA-E. Optionally, the engineered cell expresses a single chain fusion HLA-F or HLA-G comprising at least a portion of B2M linked, either directly or via a linker sequence, to at least of portion of the HLA-I alpha-chain, e.g., HLA-F or HLA-G. The engineered cell may overexpress a protein which is naturally produced by the cell, may express a protein encoded by an exogenous nucleic acid following knock-out or disruption of the corresponding endogenous coding sequence for the protein, or may express a protein which is not naturally encoded by the wild-type cell genome. Representative DNA and protein sequences for HLA-E are provided in SEQ ID NOs: 15 and 16. Representative DNA and protein sequences for HLA-F are provided in SEQ ID NOs: 17 and 18. Representative DNA and protein sequences for HLA-G are provided in SEQ ID NOs: 19 and 20. It will be appreciated that variants of the sequences provided herein may be used and, as such, the

disclosure contemplates use of a nucleic acid comprising at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the nucleic acid sequences disclosed herein. The disclosure further contemplates use of a nucleic acid which encodes a peptide comprising an amino acid sequence having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to any of the amino acid sequences described herein. Preferably, a variant retains HLA-I functions such as, e.g., forming a functional peptide binding cleft to display peptides and/or engaging inhibitory receptors on NK cells. Cells which express HLA-E, HLA-F, and/or HLA-G minimize the risk of rejection when used as an adoptive cell therapy. In various aspects of the disclosure, the cells are engineered to disrupt all copies of B2M and RFXANK and engineered to express HLA-E, e.g., single chain fusion HLA-E.

[00245] Optionally, the cells, for example RPE cells, further comprise a nucleic acid molecule encoding a suicide gene product. Combinations of nucleic acid molecules encoding different suicide gene products also are contemplated. Suicide genes are useful in selectively killing cells by inducing apoptosis or converting a nontoxic compound to a toxic compound, resulting in death of unwanted cells. Examples of suicide gene products include, but are not limited to, caspases (e.g., caspase 9), thymidine kinases, cytosine deaminases, cytochrome P450, telomerases, and DNases. See, e.g., U.S. Patent Publication No. 2022/0025001, hereby incorporated by reference. Optionally, the nucleic acid encoding the suicide gene product is operably linked to an inducible promoter. See, e.g., Straathof et al. Blood, 105(11), 4247-4254 (2005), hereby incorporated by reference. Optionally, the engineered cells comprise a herpes simplex virus thymidine kinase (TK) suicide gene. See, e.g., Bonini, et al., Science, 276, 1719-1724 (1997), hereby incorporated by reference. In various aspects, the cell comprises a nucleic acid encoding a hygromycin-thymidine kinase fusion protein. For example, cells may comprise a nucleic acid encoding HSV TK and a nucleic acid encoding a hygromycin-thymidine kinase fusion protein.

[00246] Methods of genetically engineering a host cell to stably produce one or more gene products of interest are known in the art. Exemplary methods involve the use of viral vectors. Viral vectors may include any suitable viral vectors including, for example, retrovirus, adenovirus, parvovirus (for example, adeno-associated viruses), coronavirus, ortho-myxovirus (for example, influenza virus), rhabdovirus (for example, rabies and vesicular stomatitis virus), paramyxovirus (for example, measles and Sendai), picornavirus, alphavirus, herpesvirus (for example, Herpes Simplex virus types 1 and 2, Epstein-Barr virus, cytomegalovirus), and poxvirus (for example, vaccinia, fowlpox, and canarypox). Exogenous

nucleic acids also may be introduced into a host cell via gene editing techniques using, e.g., the systems described herein. Preferably, the nucleic acid encoding HLA-E, HLA-F, or HLA-G are integrated into cellular genome, such as into the B2M or the HLA-II loci. Thus, in various aspects, the B2M loci are disrupted by inserting in the B2M loci a nucleic acid encoding a different gene product to replace the expression of the endogenous wild type B2M protein. Alternatively or in addition, certain HLA-II loci are optionally disrupted by inserting in the HLA-II loci a nucleic acid encoding a different gene product to replace the expression of the endogenous wild type HLA-II protein.

[00247] In various aspects of the disclosure, cells of a cell population, for example, RPE cells, are derived from pluripotent stem cells. The term "pluripotent stem cells" includes embryonic stem cells, embryo-derived stem cells, and induced pluripotent stem cells, regardless of the method by which the pluripotent stem cells are derived. Pluripotent stem cells are typically defined functionally as stem cells that are: (a) capable of inducing teratomas when transplanted in immunodeficient (SCID) mice; (b) capable of differentiating to cell types of all three germ layers (e.g., can differentiate to ectodermal, mesodermal, and endodermal cell types); and (c) express one or more markers of embryonic stem cells (e.g., express Oct 4, alkaline phosphatase, SSEA-3 surface antigen, SSEA-4 surface antigen, nanog, TRA-1-60, TRA-1-81, SOX2, REX1, etc.). Pluripotent stem cells can be generated using, for example, methods known in the art. Exemplary pluripotent stem cells include embryonic stem cells. "Embryonic stem cells" (ESC) include, e.g., cells derived from the inner cell mass of human blastocysts or morulae, including those that have been serially passaged as cell lines. Embryonic stem cells, regardless of their source or the particular method used to produce them, can be identified based on, for instance, (i) the ability to differentiate into cells of all three germ layers, (ii) expression of at least Oct-4 and alkaline phosphatase, and (iii) ability to produce teratomas when transplanted into immunodeficient animals.

[00248] Another exemplary pluripotent stem cell is an induced pluripotent stem cell (iPSC). iPSCs are generated by, e.g., reprogramming a somatic cell by expressing a combination of factors (“reprogramming factors”) such as, but not limited to, Oct4, Sox2, c-Myc, Nanog, Lin28, and/or Klf4. Induced pluripotent stem cells can be generated using, as a starting point, virtually any somatic cell of any developmental stage. For example, the cell can be from an embryo, fetus, neonate, juvenile, or adult donor. Exemplary somatic cells that can be used include fibroblasts, such as dermal fibroblasts obtained by a skin sample or

biopsy, synoviocytes from synovial tissue, foreskin cells, cheek cells, or lung fibroblasts. In certain embodiments, the somatic cell is not a fibroblast.

[00249] The pluripotent stem cells can be from any species. Embryonic stem cells have been successfully derived from, for example, mice, multiple species of non-human primates, and humans. Thus, one of skill in the art can generate embryonic stem cells from any species, including but not limited to, human, non-human primates, rodents (mice and rats), ungulates (cows, sheep, etc.), dogs (domestic and wild dogs), cats (domestic and wild cats such as lions, tigers, and cheetahs), rabbits, hamsters, gerbils, squirrel, guinea pig, goats, elephants, panda (including giant panda), pigs, raccoon, horse, zebra, marine mammals (dolphin, whales, etc.) and the like. Similarly, iPSCs can be from any species. iPSCs have been successfully generated using, for instance, mouse and human cells. Accordingly, one can readily generate an iPSC using a donor cell from any species, such as any species described herein.

SEQ ID NO:	Name	Genbank
1	B2M NA seq	GenBank Accession Number NM_ 004048)
2	B2M AA seq	
3	RFXANK	GenBank Accession Numbers NM_ 134440.1
4	RFXANK	GenBank Accession Numbers NM_ 134440.1
5	RFXANK	GenBank Accession Numbers NM_ 003721.2
6	RFXANK	GenBank Accession Numbers NM_003721.2
7	RFX5	GenBank Accession Number NM_ 000449.3
8	RFX5	GenBank Accession Number NM_ 000449.3
9	RFX5	GenBank Accession Number NM_ 001025603.1
10	RFX5	GenBank Accession Number NM_001025603.1
11	RFXAP	GenBank Accession Number NM_000538.3
12	RFXAP	GenBank Accession Number NM_000538.3
13	CIITA	GenBank Accession Number NM_000246.3
14	CIITA	GenBank Accession Number NM_000246.3
15	HLA-E	GenBank Accession Number NM_005516
16	HLA-E	GenBank Accession Number NM_ 005507
17	HLA-F	GenBank Accession Number NM_018950
18	HLA-F	GenBank Accession Number NP_061823
19	HLA-G	GenBank Accession Number NM_002127
20	HLA-G	GenBank Accession Number NP_ 002118

State of cells

[00250] Cell populations may be formulated in a cell preparation as provided herein for delivery in a pharmaceutically acceptable ophthalmic vehicle, such that the preparation is

maintained in contact with the ocular surface for a sufficient time period to allow the cells to penetrate the affected regions of the eye, as for example, the anterior chamber, posterior chamber, vitreous body, aqueous humor, vitreous humor, cornea, iris/ciliary, lens, choroid, retina, sclera, suprachoridal space, conjunctiva, subconjunctival space, episcleral space, intracorneal space, epicorneal space, pars plana, surgically-induced avascular regions, the macula, or an area of geographic atrophy.

[00251] Cell populations may have several possible arrangements such as individual cells, clusters, spheres, aggregates, sheets, or any combination thereof, which may be contained in an aqueous vehicle, gel, matrix, polymer or the like. In some embodiments, the cell population is in combination with a biomaterial. In some embodiments, the cell population is part of a 3D organoid. In some embodiments, the cell population in a cell preparation provided herein is a dissociated cell population, a cell sheet, a cell monolayer, or a cell aggregate. In some embodiments, the cell population is a human cell population.

[00252] In some embodiments, a cell preparation is provided herein, in which cell populations are contained in a sheet of cells. For example, RPE cells or corneal endothelial cells or photoreceptor cells or photoreceptor rescue cells may be contained in a sheet. For example, a sheet of cells comprising RPE cells may be prepared by culturing RPE cells on a substrate from which an intact sheet of cells can be released, *e.g.*, a thermoresponsive polymer such as a thermoresponsive poly(N-isopropylacrylamide) (PNIPAAm)-grafted surface, upon which cells adhere and proliferate at the culture temperature, and then upon a temperature shift, the surface characteristics are altered causing release of the cultured sheet of cells (*e.g.*, by cooling to below the lower critical solution temperature (LCST) (see, da Silva *et al.*, Trends Biotechnol. 2007 Dec;25(12):577-83; Hsiue *et al.*, Transplantation. 2006 Feb 15;81(3):473-6; Ide, T. *et al.* (2006); Biomaterials 27, 607–614, Sumide, T. *et al.* (2005), FASEB J. 20, 392–394; Nishida, K. *et al.* (2004), Transplantation 77, 379–385; and Nishida, K. *et al.* (2004), N. Engl. J. Med. 351, 1187–1196 each of which is incorporated by reference herein in its entirety). The sheet of cells may be adherent to a substrate suitable for transplantation, such as a substrate that may dissolve *in vivo* when the sheet is transplanted into a host organism, *e.g.*, prepared by culturing the cells on a substrate suitable for transplantation, or releasing the cells from another substrate (such as a thermoresponsive polymer) onto a substrate suitable for transplantation. An exemplary substrate potentially suitable for transplantation may comprise gelatin (see, Hsiue *et al.*, *supra*). Alternative substrates that may be suitable for transplantation include fibrin-based matrixes and others.

The sheet of cells may be used in the manufacture of a medicament for the prevention or treatment of a disease of retinal degeneration.

[00253] In some embodiments, a preparation is provided herein, in which cells are in clusters, spheres, or aggregates. In some embodiments, cells provided herein are administered in combination with a biomaterial, for example a biomaterial that can be polymerized. In some embodiments, cells are administered as an organoid.

Form of Cell Preparation

[00254] In some embodiments, the cell preparation is in a cryopreserved form. The cell preparation provided herein may be cryopreserved by being cryogenically frozen at any appropriate temperature known in the art. For example, in some embodiments, the cell preparations provided herein are cryopreserved at temperatures between -100°C and -200°C. In some embodiments the cell preparations provided herein are cryopreserved at temperatures below -125°C. In some embodiments the cell preparations provided herein are cryopreserved at temperatures at or below -135°C. In some embodiments the cell preparations provided herein are cryopreserved at temperatures below -150°C. In some embodiments, the cryopreserved cell preparations provided herein are stored in liquid nitrogen or in the vapor phase of liquid nitrogen.

[00255] The cryopreserved cell preparations are produced by combining a cell population, with the cryopreservative formulation provided herein, optionally dispersing the cell population in the formulation, and then slow-freezing (e.g., controlled-rate freezing) the mixture. Slow-freezing can be accomplished manually or by device (e.g., a controlled-rate freezer, as described in the Examples). In one embodiment, the preparation is frozen at a rate of -2°C per minute until a desired, optionally intermediate, temperature is reached, such as for example -80°C. Alternatively, the rate of controlled-rate freezing may be -1°C per minute or -1.5°C per minute, etc. The cryopreserved cell preparation may be kept at the intermediate temperature for hours, days, or weeks, or longer. It may then be kept at a lower temperature. As an example, the preparation may be brought to -80°C using a slow-freeze process, after which it may be stored at -80°C overnight (or for up to about 12 or about 24 hours), after which it may be stored in the vapor phase of a liquid nitrogen tank (e.g., at about -125°C). Once at the lower temperature, it may be stored there for days, weeks, months, or even years. It may even be transported, for example to a clinical site, while in the cryopreserved form at the lower temperature, if so desired. The temperature of the cryopreservative formulation can be, for example, between 0 °C and 25 °C, for example, about 1 °C, about 2 °C, about 3 °C,

about 4 ºC, about 10 ºC, about 15 ºC, about 20 ºC, about 25 ºC. In some embodiments, the cell preparation has been cryopreserved and thawed. Once a cell preparation has been cryopreserved and thawed, it is considered a "post-thaw" cell preparation. The cell preparations provided herein may be thawed without the need for diluent, wash, or other manipulation.

[00256] The following is a non-limiting process for preparing cells for cryopreservation using any one of the cryopreservation formulas provided herein.

[00257] RPE cells are harvested from in vitro cultures, for example on day 11 or 12. They may be washed one or more times. A suitable washing buffer comprises albumin (e.g., an about 5% (w/v) albumin buffered solution). After washing, the cell pellet may be resuspended in a suitable resuspension buffer that minimally comprises albumin and glucose, and may also comprise PBS such as DPBS. Typically, the resuspension buffer will comprise the same concentration of albumin and glucose as the cryopreservative formulation. As an example, the resuspension buffer and the cryopreservative formulation may both comprise about 2.5% (w/v) albumin, about 0.09% (w/v) glucose in a buffered saline. The resuspended cells may have a cell density in the range of for example about 200 cells per microliter to about 20,000 cells per microliter, including about 1000 cells per microliter to about 10,000 cells per microliter, and about 3000 cells per microliter to about 10,000 cells per microliter. In some specific examples, the cell density may be about 6000 cells per microliter to about 7000 cells per microliter, including about 6500 cells per microliter, about 6600 cells per microliter, or about 6700 cells per microliter.

[00258] The resuspended cell mixture is then mixed 1:1 with one of the cryopreservation buffers provided herein. Such 1:1 dilution serves to half the cell density, and half the concentration of each of the cryoprotectants in the cryopreservative formulation. Accordingly, the cell density for example may be in the range of about 100 cells per microliter to about 10,000 cells per microliter, including about 500 cells per microliter to about 5,000 cells per microliter, and about 1500 cells per microliter to about 5,000 cells per microliter. In some specific examples, the cell density may be about 2000 cells per microliter to about 4000 cells per microliter, including about 3000 cells per microliter. This cell preparation may then be placed into freezing vials such as for example 2 mL CZ vials. Each vial may be filled with for example about 450 microliters of the cell preparation comprising about 1.5 million cells.

[00259] Those vials may then be subjected to cryopreservation, for example using a controlled-rate freezer and program. One non-limiting program may comprise the following

steps: (a) introduce the vial to a 4 °C environment, (b) hold the vial at 4 °C for 10 minutes, (c) ramp the temperature downwards at a rate of 2 °C per minute until -40 °C is reached, (d) ramp the temperature downwards at a rate of 10 °C per minute until -80 °C is reached, and optionally placement in liquid nitrogen (LN2) (-180 °C) thereafter.

[00260] It is to be understood that the cell pellet may be directly resuspended in a cryopreservative formulation that has been diluted 1:1 with another solution provided the formulation and such solution comprise the same concentrations of albumin, glucose and buffering agent. Thus, in some instances, the resuspension buffer or the dilution solution are identical to the cryopreservative formulation except that the cryopreservative formulation comprises one or two, or optionally more, cryopreservatives and the resuspension buffer and dilution solution do not.

[00261] When ready to be thawed, a cryopreserved vial may be placed in a 37 °C water bath for about 2-3 minutes. Thereafter, the thawed cell preparation may be gently pipetted several times (e.g., 7-10 times) in the vial. The cell preparation may be used directly and immediately, or it may be kept at 4 °C or at room temperature for 0-4 hours.

[00262] The cell preparation may also be evaluated for cell viability and cell growth potential (referred to herein as cell growth index). Viability may be measured from several aliquots of the thawed cell preparation using any standard technique or instrumentation known in the art. Typically, viability may be measured within about 5 to about 30 minutes after thawing.

[00263] Cell growth potential may be measured in a number of ways. In one approach, the cell density of the thawed cell preparation may be adjusted to about 100-200 cells per microliter (e.g., about 160 cells per microliter), and about 80 cells per microliter may be seeded per well in a 96 well plate (e.g., an e-plate). These plates may then be incubated for 7 days at 37 °C and evaluation of cell growth may be monitored during that time. One metric that may be used is the time it takes the cells to triple in number. Cells are typically seeded within about 2 hours after thawing.

[00264] In some instances, suitable cell preparations are those demonstrating equal to or greater than about 75%, or equal to or greater than about 80%, or equal to or greater than about 85%, or equal to or greater than about 90%, or equal to or greater than about 95% viability. In some instances, suitable cell preparations are those demonstrating a cell growth index:3 of less than or equal to about 100 hours, less than or equal to about 90 hours, less than or equal to about 80 hours, or less than or equal to about 70 hours. In some instances, suitable cell preparations are those demonstrating equal to or greater than about 80% viability

and a cell growth index:3 of less than or equal to about 100 hours. Other suitable cell preparations include those demonstrating equal to or greater than about 90% viability and a cell growth index:3 of less than or equal to about 80 hours.

[00265] In some embodiments, the cell preparation is kept at room temperature (about 20 °C to about 25 °C). In some embodiments, the cell preparation is kept at about 4 °C. In some embodiments, the cell preparation is kept at about -20 °C. In some embodiments, the cell preparation is kept at about -70 °C. In some embodiments, the cell preparation is kept at about -180 °C.

[00266] In some embodiments, the cell preparation has been cryopreserved, thawed, and is kept at room temperature (about 20 °C to about 25 °C).

[00267] In some embodiments, the cell preparation has been cryopreserved, thawed, and is kept at about 4 °C.

[00268] In some embodiments, cell preparation has been kept at room temperature (about 20 °C to about 25 °C) for about 1 to about 2 hours prior to cryopreservation at -180 °C. In some embodiments, cell preparation has been kept at about 4 °C for about 4 to about 7 hours, prior to cryopreservation at -180 °C. In some embodiments, cell preparation has been kept at room temperature (about 20 °C to about 25 °C) for about 1 to about 2 hours, and at about 4 °C for about 4 to about 7 hours, prior to cryopreservation at -180 °C.

[00269] Also provided herein is a cell preparation comprising a population of cells in a formulation comprising albumin, glucose, buffered saline, and one or two, or optionally more, cryoprotectants, and having equal to or greater than 80% cell viability and/or equal to or less than 110 hours cell growth index:3 after

a. storage at room temperature (about 20 °C to about 25 °C) for about 1 to about 2 hours and then at about 4 °C for about 4 to about 7 hours, optionally at room temperature for about 1.5 hours and then at 4 °C for about 4.5 hours or 6.5 hours,
b. cryopreservation at -180 °C for about 1 to about 2 years, optionally for about 1 month to about 1 year,
c. cryopreservation at -180 °C and then post-thaw at about 4 °C for about 3 to about 6 hours, optionally for about 4 hours,
d. cryopreservation at -180 °C and then post-thaw at room temperature (about 20 °C to about 25 °C) for about 3 to about 6 hours, optionally for about 4 hours,

e. cryopreservation at -180 °C, then at about - 20 °C for about 0.5 to about 1 hour, then cryopreservation at -180 °C, optionally then at about - 20 °C for about 0.5 to about 1 hour, then cryopreservation at -180 °C, or

f. cryopreservation at -180 °C, then at about - 70 °C for about 2 to about 4 weeks, then cryopreservation at -180 °C.

[00270] In some embodiments, the cell preparation is dimethylsulfoxide-free (DMSO-free). In some embodiments, the cell preparation has an equal to or greater than 85% cell viability after any of events (a) through (f). In some embodiments, the cell preparation has an equal to or greater than 90% cell viability after any of events (a) through (f). In some embodiments, the cell preparation has an equal to or greater than 95% cell viability after any of events (a) through (f).

[00271] In some embodiments, cell growth index is evaluated by cell index 3 (as shown in the Examples). In some embodiments, the cell preparations provided herein have a cell growth index:3 of equal to or less than 100 hours, equal to or less than 90 hours, or equal to or less than 80 hours following any one of (a)-(f).

[00272] In some embodiments, the cell preparations provided herein maintain robust cell growth index following any one of a-f. In some embodiments, the cell preparations provided herein maintain robust viability and cell growth index following any one of a-f. In some embodiments, the cell preparations provided herein maintain robust viability (e.g., equal to or greater than 80% or greater than 85%, and cell growth index:3 of at least 110 hours (e.g., equal to or less than 110 hours, equal to or less than 100 hour, equal to or less than 90 hours, or equal to or less than 80 hours) following any one of (a)-(f).

Methods of Making

[00273] Some aspects of this disclosure provide methods of preparing a cell preparation comprising contacting a population of cells with a cryopreservative formulation provided herein and a suspension solution comprising albumin, glucose, and buffered saline, each at the same concentration as in the formulation (e.g., about 2.5% (w/v) albumin (e.g., recombinant human albumin), about 0.09% (w/v) glucose, and buffered saline (e.g., PBS), wherein the suspension solution does not contain a (non-albumin) cryoprotectant. In some embodiments, the cell preparation comprises the cryopreservative formulation and the suspension solution in a 1:1 (v/v) ratio. In some embodiments, the method further comprises cryopreserving the cell preparation. In some embodiments, the method further comprises thawing the cell preparation. In some embodiments, the method further comprises

administering the cell preparation to a subject in need thereof. In some embodiments, the cell preparation is administered to the subject without washing or dilution post-thaw. In some embodiments, the cell preparation is kept at about 4 °C for about 4 hours before administration to the subject. In some embodiments, the cell preparation is kept at room temperature (about 20 °C to about 25 °C) for about 4 hours before administration to the subject. The population of cells may be a population of RPE cells, a population of mesenchymal stem cells such as hemangioblast-derived mesenchymal stem cells, a population of mesenchymal cells such as hemangioblast-derived mesenchymal cells, a population of photoreceptor cells, a population of photoreceptor progenitor cells, a population of photoreceptor rescue cells, a population of retinal ganglion cells, a population of retinal ganglion progenitor cells, a population of corneal endothelial cells, a population of corneal endothelial progenitor cells, or a population of vascular progenitor cells, or a population of any cell type, for example as disclosed herein. The population of cells may be generated by in vitro differentiation of pluripotent stem cells such as embryonic stem cells or induced pluripotent stem cells. The population of cells may be human cells or non-human cells.

[00274] Some aspects of this disclosure provide methods of preparing a cell preparation comprising suspending a population of cells in a cryopreservative formulation comprising:

(i) about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 0.1%, about 1%, about 3%, about 5%, or about 7.5% (v/v) propanediol and about 5% (w/v) dextran-40,

(ii) about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 5% (v/v) propanediol and about 7.5% (w/v) dextran-40,

(iii) about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 5% (v/v) propanediol and about 5% (w/v) dextran-40,

(iv) about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 3% (v/v) propanediol and about 10% (w/v) dextran-40,

(v) about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 3% (v/v) propanediol and about 7.5% (w/v) dextran-40,

(vi) about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 3% (v/v) propanediol and about 5% (w/v) dextran-40,

(vii) about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 3% (v/v) propanediol and about 3% (w/v) dextran-40,

(viii) about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 3% (v/v) propanediol and about 1% (w/v) dextran-40,

(ix) about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 3% (v/v) propanediol and about 2.5% (w/v) proline,

(x) about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 3% (v/v) propanediol and about 5% (w/v) proline,

(xi) about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 5% (v/v) propanediol and about 3.5% (w/v) trehalose,

(xii) about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 5% (v/v) propanediol and about 1% (w/v) trehalose,

(xiii) about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 5% glycerin and about 3.5% (w/v) trehalose,

(xiv) about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), and about 2.5% (w/v) pentastarch (or other hydroxyethyl starch having up to 100% substitution with hydroxyethyl),

(xv) about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), and about 5% (w/v) pentastarch (or other hydroxyethyl starch having up to 100% substitution with hydroxyethyl),

(xvi) about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), and about 7.5% (w/v) pentastarch (or other hydroxyethyl starch having up to 100% substitution with hydroxyethyl),

(xviii) about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), and about 7.5% (w/v) dextran-40,

(xix) about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), and about 5% (w/v) dextran-40,

(xx) about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), and about 2.5% (w/v) dextran-40,

(xxi) about 2.5% (w/v) albumin (e.g., human albumin, e.g., recombinant human albumin), about 0.09% (w/v) glucose, buffered saline (e.g., PBS), and about 2.5% (v/v) ethylene glycol,

(xxii) buffered saline (e.g., PBS), and about 2.5% (w/v) hydroxyethyl starch such as but not limited to tetrastarch, pentastarch or heptastarch,

(xxiii) buffered saline (e.g., PBS), and about 5% (w/v) hydroxyethyl starch such as but not limited to tetrastarch, pentastarch or heptastarch, or

(xxiv) buffered saline (e.g., PBS), and about 7.5% (w/v) hydroxyethyl starch such as but not limited to tetrastarch, pentastarch or heptastarch.

[00275] In some embodiments, the formulations may be non-albumin versions of the foregoing formulations (i.e., the formulations may be as recited above, and herein, except that such formulations may lack albumin). As demonstrated in the Examples, the cryoprotectant formulations provided herein lead to higher post-thaw cell viability when compared to DMSO containing formulations, even in the absence of albumin.

[00276] In some embodiments, the population of cells may be a population of RPE cells, a population of mesenchymal stem cells such as hemangioblast-derived mesenchymal stem cells (HMCs), a population of mesenchymal cells such as hemangioblast-derived mesenchymal cells (HMCs), a population of photoreceptor cells, a population of photoreceptor progenitor cells, a population of photoreceptor rescue cells, a population of retinal ganglion cells, a population of retinal ganglion progenitor cells, a population of corneal endothelial cells, a population of corneal endothelial progenitor cells, or a population of vascular progenitor cells. The population of cells may be generated by in vitro differentiation of pluripotent stem cells such as embryonic stem cells or induced pluripotent stem cells. The population of cells may be human cells or non-human cells.

Methods of Using

[00277] The cell preparations disclosed herein, including the cryopreserved formulations once thawed, can be used in various clinical applications. As noted herein, the cryopreserved formulations may be thawed without the need for diluent, wash, or other manipulation. In some embodiments, the cryopreserved formulations may be thawed without addition of a diluent, following which they may be administered to a subject without the need for wash or other manipulation.

[00278] In some embodiments, the cell population so formulated is intended for clinical use in a regenerative medicine approach. For example, the cell population may be a cell population that can replace cells lost or degenerated in a subject, or repair or replace a tissue that has been damaged or is dysfunctional in a subject. For example, in some embodiments, the cell population may comprise a pluripotent stem cell, for example an iPS cell or an ES cell, a multipotent stem or progenitor cell, or a functional differentiated cell, or a population or tissue comprising such cells or a combination of such cells.

[00279] In some embodiments, the cryopreserved cell preparation can be stored indefinitely or it can be stored for defined periods of time, and optionally it can be transported to a clinic or other end user location.

[00280] The cryopreserved formulations may be stored in a frozen state, such as in the vapor phase of a liquid nitrogen tank, for an extended period of time, such as those recited herein and including but not limited to at least 1 week, at least 2 weeks, at least 3 weeks, at least 4 weeks, at least 5 weeks, at least 6 weeks, at least 7 weeks, at least 8 weeks, at least 9 weeks, at least 10 weeks, at least 11 weeks, at least 12 weeks, at least 16 weeks, at least 20 weeks, at least 24 weeks, at least 28 weeks, at least 32 weeks, at least 36 weeks, at least 40 weeks, at least 44 weeks, at least 48 weeks, at least 1 year, at least 2 years, at least 3 years, at least 4 years at least 5 years or more with minimal impact on cell viability or cellular function, as measured for example by dye exclusion, re-plating efficiency, or repopulating capacity. Cells or cell populations cryopreserved and thawed according to the present disclosure may have a cell viability, re-plating efficiency, and/or repopulation capacity of at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% of the viability, re-plating efficiency, and/or repopulation capacity prior to cryopreservation.

[00281] In some embodiments, the cell preparations provided herein are formulated for administration to a subject, for example, for administration via injection, once thawed, without being diluted. In some embodiments, the cell preparation is administered with a syringe. In some embodiments, the cell preparation is administered with a cannula. Exemplary cell preparations, post-thaw may be formulated to be suitable for use in treating a human patient, *e.g.*, pyrogen-free or essentially pyrogen-free, pathogen-free, sterile, and at physiological pH and osmolarity. In some embodiments, the preparations provided herein are formulated for administration by injection into a specific site, *e.g.*, in the case of ophthalmologic preparations for treating retinal diseases or disorders, into the vitreous humor for delivery to the site of retinal or choroidal damage, including for example, to a bleb area, or to an area of geographic atrophy. In some embodiments, the cell preparation is within a storage container. In some embodiments, the storage container is a vial, ampule, bottle, syringe, a cooler package, or other suitable storage container. In some embodiments, the cell preparation is within a vial. In some embodiments, the cell preparation is within a syringe.

[00282] Cell preparations provided by the present disclosure may include additionally therapeutic agents, for example, an immunosuppressant, a pro-angiogenic agent, or nutrients or growth factors supporting survival and/or implantation of the cells in the preparation.

[00283] The volume and the number of cells in the cell preparations to be administered will depend on the specific application. Typically, for cell transplantation applications, it is desirable to reduce the volume administered as much as possible. Accordingly, the cell preparations may be formulated so that minimized volumes may be delivered. Cell concentrations for injection may be at any concentration that is effective and non-toxic. In some embodiments, the volume of the cell preparation to be administered is between 1 to 1000 µL. In some embodiments, the volume of the cell preparation to be administered is between 1 to 50 µl, or between 10 to 50 µl or between 25 to 50 µl or between 50 to 100 µl or between 50 to 200 µl or between 50 to 300 µl or between 50 to 400 µl or between 50 to 500 µl, or between 100 to 500 µl, or between 100 to 400 µl or between 100 to 300 µl, or between 100 to 300 µl or about 200 µl or about 150 µl. In some embodiments the volume of the cell preparation to be administered is about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 900, 950 or 1000 µl.

[00284] In some embodiments, the number of cells and/or the concentration of cells in a cell preparation provided herein may be determined by counting viable cells and excluding

non-viable cells. For example, non-viable cells may be detected by failure to exclude a vital dye (such as Trypan Blue), or using a functional assay (such as the ability to adhere to a culture substrate, phagocytosis, etc.). Additionally, the number of cells or the concentration of cells of a desired cell type may be determined by counting cells that express one or more cell markers characteristic of that cell type and/or excluding cells that express one or more markers indicative of a cell type other than the desired cell type.

[00285] In some embodiments, a cell preparation to be administered comprises about 100 cells per microliter to about 10,000 cells per microliter.

[00286] In some embodiments, the number of cells per vial is about 45,000 to about 4,500,000 cells per vial. In some embodiments, the number of cells per vial is about 1,500,000 cells per vial.

[00287] The aforementioned numbers of cells may be present in the cryopreserved cell preparation in a single cryovial. The cells may be present in about 10 to about 500 μL of cryopreservative formulation, including in about 100 to about 500 μL of cryopreservative formulation, or in about 200 to about 500 μL of cryoprotective formulation, or in about 300 to about 500 μL of cryopreservative formulation, or in about 400 to about 500 μL of cryopreservative formulation. In some embodiments, the cells are present in about 450 μL of cryopreservative formulation.

[00288] Some aspects of this disclosure provide methods for treating a subject having a disorder or condition. In some embodiments, the subject has a disorder or condition that would benefit from a cell transplant. In some embodiments, the method comprises administering to a subject in need thereof an effective amount of the cell preparation provided herein without washing or dilution post-thaw. In some embodiments, the method comprises administering to a subject in need thereof an effective amount of the cell preparation prepared according to a method provided herein without washing or dilution post-thaw. In some embodiments, the cell preparation is kept at about 4 °C for about 4 hours before administration to the subject. In some embodiments, the cell preparation is kept at room temperature (about 20 °C to about 25 °C) for about 4 hours before administration to the subject. In some embodiments, the cell preparation is administered in a cell dose effective to treat the disorder or condition in the subject. In some embodiments, the disorder or condition includes, but is not limited to, retinal detachment, retinal dysplasia, Angioid streaks, Myopic Macular Degeneration, or retinal atrophy or associated with a number of vision-altering ailments that result in photoreceptor damage and blindness, such as, for example, choroideremia, diabetic retinopathy, macular degeneration (*e.g.*, age-related

macular degeneration (AMD)), dry or wet age-related macular degeneration, retinitis pigmentosa, Fuch's Dystrophy, Stargardt's Disease (fundus flavimaculatus), and geographic atrophy including geographic atrophy secondary to AMD. In some embodiments, the method further comprises monitoring at least one symptom of the disorder or condition in the subject, optionally in order to determine if such symptom is lessened in severity or frequency post-administration. In other embodiments, the disorder or condition includes but is not limited to cancer, liver disease including end-stage liver disease, Crohn's disease, arthritis including osteoarthritis, pulmonary artery hypertension, and critical limb ischemia.

[00289] The terms "treatment," "treat," and "treating" refer to a clinical intervention aimed to reverse, alleviate, delay the onset of, or inhibit the progress of a disorder or condition. In some instances, the effect of the intervention may be assessed by monitoring symptoms associated with the disorder or condition. In some embodiments, the cell preparation may be administered after one or more symptoms have developed and/or after a disorder or condition has been diagnosed. In other embodiments, the cell preparation may be administered in the absence of symptoms, *e.g.*, to prevent or delay onset of a symptom or inhibit onset or progression of a disorder or condition. For example, the cell preparation may be administered to a susceptible individual prior to the onset of symptoms (*e.g.*, in light of a history of symptoms and/or in light of genetic or other susceptibility factors). The cell preparation may be administered once or more than once, including at regular and defined time points or on an as-needed basis.

[00290] The subject may be a human or a non-human mammal. In some embodiments, the subject is a non-human primate. In some embodiments, the subject is a rodent. In some embodiments, the subject is a sheep, a goat, a cattle, a cat, or a dog. In some embodiments, the subject is a research animal. In some embodiments, the subject is genetically engineered, *e.g.*, a genetically engineered non-human subject. The subject may be of either sex and at any stage of development.

[00291] The term "effective amount," as used herein, refers to an amount of a biologically active agent that is sufficient to elicit a desired biological response. For example, in some embodiments, an effective amount of a cell preparation may refer to the amount of the preparation that comprises a number of cells or amount of tissue that is sufficient to provide a therapeutic benefit to a subject having a disorder or condition, *e.g.*, sufficient to improve vision in a subject with a retinal disease or disorder. As will be appreciated by the skilled artisan, the effective amount may vary depending on various factors as, for example, on the desired biological response, *e.g.*, on the specific disorder being treated, a specific

symptom to be alleviated, or the cell or tissue being targeted, and on the subject's age, gender, disease stage, and general health status.

[00292] Some aspects of this disclosure provide methods for treating a retinal disease, wherein the methods comprise administering an effective amount of a cell preparation provided herein to the eye of a subject having a retinal disease. In some embodiments, the subject has or is diagnosed with the retinal disease. In some embodiments, the retinal disease is rod or cone dystrophy, retinal degeneration, retinitis pigmentosa, diabetic retinopathy, macular degeneration, for example wet or dry age-related macular degeneration (AMD), Leber congenital amaurosis, Stargardt disease, or geographic atrophy such as geographic atrophy secondary to AMD. In some embodiments, the cells being administered comprise RPE cells, photoreceptor cells, photoreceptor progenitor cells, photoreceptor rescue cells, corneal endothelial cells, corneal endothelial progenitor cells, or retinal ganglion cells or retinal ganglion progenitor cells, any of which may be generated by in vitro differentiation of pluripotent stem cells such as embryonic stem cells or induced pluripotent stem cells.

Kits

[00293] Some aspects of this disclosure provide kits comprising (a) a cryopreserved pharmaceutical composition comprising cells of interest as provided herein; and (b) instructions for thawing the composition, and rendering it suitable for administration to a subject. Those instructions may provide that no diluting, washing, or other manipulation of the cryopreserved composition is necessary. The kit may further comprise a device for administering the composition such as a syringe, canula, and needle.

EXAMPLES

[00294] The invention now being generally described, it will be more readily understood by reference to the following examples, which are included merely for purposes of illustration of certain aspects and embodiments of the present invention, and are not intended to limit the invention.

Example 1 – First cell preparation screening

[00295] RPEs derived from human embryonic stem cells (as described, for example, in WO2021086911, incorporated herein by reference) were pooled in a 250 mL bottle and washed with 200 mL of 0.5% rHA/DPBS by centrifugation at 500x g for 5 minutes.

[00296] RPE cells were resuspended with 2.5% rHA/DPBS/0.09% (w/v) glucose, followed by mixing with cryoprotectant buffer. As used herein, the cryopreservative formulation is sometimes referred to as the "cryoprotectant buffer". Final compositions of each cell preparation are shown in Table 1.

Table 1

Excipients	1A	1B	1C	1D	1E	1F	1G	1H	1I	1J	1K	1L	1M	1N
Propanediol (v/v)	3%	3%	3%	3%	5%	5%	-	-	-	-	-	-	-	-
Proline (w/v)	2.5%	5%	-	-	-	-	-	-	-	-	-	2.5 %	-	-
Dextran-40 (w/v)	-	-	10%	5%	5%	-	-	-	-	-	-	-	-	-
Trehalose (w/v)	-	-	-	-	-	3.5%	3.5%	-	-	-	-	-	-	-
Glycerin (v/v)	-	-	-	-	-	-	5%	-	-	-	-	-	-	-
Pentastarch (w/v)	-	-	-	-	-	-	-	2.5 %	5 %	7.5 %	-	-	-	-
Poloxamer 188 (w/v)	-	-	-	-	-	-	-	-	-	-	1 %	-	-	-
Ethylene Glycol (v/v)	-	-	-	-	-	-	-	-	-	-	-	-	2.5 %	-
DMSO (v/v)	-	-	-	-	-	-	-	-	-	-	-	-	-	5%
Glucose (w/v)	0.09%													
Recombinant Albumin (w/v)	2.5%													
DPBS w/Ca, Mg	q.s.													

[00297] Four hundred fifty (450) µL of formulated RPE cells were filled in CRYSTAL ZENITH® (CZ) vials (West Pharmaceutical Services, Inc. Exton, PA) and cryopreserved using a controlled-rate freezer at -2°C/min (THERMO SCIENTIFIC, Waltham, MA). Controlled-rate freezing was started within 3 hours of adding cryoprotectant buffer to the cells. The vials were then transferred and stored in the vapor phase of liquid nitrogen.

[00298] Post-thaw cell viability was evaluated using Vi-CELL BLU cell analyzers (Beckman Coulter Inc., Brea, CA). Cell Index (CI), is a dimensionless parameter. It is derived as a relative change in measured electrical impedance to represent cell status. The more cells are attached on the electrodes, the CI values are larger. In this experiment, cell proliferation is evaluated by the time it takes for the CI index to reach 3, meaning lower time showed higher proliferative ability. The cell growth evaluation was conducted with following condition. E-Plates 96 (ACEA Bioscience, catalog number: 111902) was coated with 0.1% Gelatin (Stemcell Tech, catalog number:7903). RPE cells were seeded to wells of the E-Plates 96 at 9,600 cells/well with 120 µL of DMEM based cell culture media containing

45%v/v DMEM with high glucose (Gibco, catalog number: 10313-021), 42.6%v/v Knockout DMEM (Gibco, catalog number: 10829-018), 1%v/v GlutaMAX-1 (100X) (Gibco, catalog number: 35050-061), 0.5%v/v MEM Non-Essential Amino Acids (Gibco, catalog number: 11140-050), 5%v/v Fetal Bovine Serum (irradiated) (Hyclone, catalog number: SH30406.02IR) and 6.4%v/v Knockout Serum Replacement (Gibco, catalog number: 10828-028). The plate with cells was incubated in CO_2 Incubator (Panasonic, Newark, NJ) at 37°C under 5%CO_2.

[00299] All cell preparations except for 3% (v/v) propanediol/2.5% (w/v) proline and 3% (v/v) propanediol/10% (w/v) dextran-40 showed over 90% cell viability and the value was comparable or superior to control 5% (v/v) DMSO cell preparation. (See FIG. 1A, Table 2.)

[00300] Post-thaw cell growth was evaluated using the xCelligence system (Agilent Technology, Santa Clara, CA). Cryopreserved RPE vials were thawed, seeded and cultured in growth medium in 96 well plates, and the cell growth was monitored in real time. Cell preparations 1A 1B, 1D, 1E, 1F, 1G, 1H, 1I, 1J, 1M and 1L showed below 80 hours at cell index 3, indicating cell growth rate of these cell preparations was in the normal range and cell preparations 1B, 1D, 1E, 1F, 1G, 1I, and 1J performed at a comparable cell growth rate to 5% (v/v) DMSO cell preparation. Cell preparations 1C and 1K showed above 80 hours at cell index 3, indicating cell growth rate of these cell preparations was relatively low. (See FIG. 1B, Table 2.)

Table 2

#	Cell preparation	Ave. of Viability (%)	SD of Viability	Cell growth (hrs) at cell index 3	SD of cell growth
1A	3% (v/v) propanediol /2.5% (w/v) proline	88.3	0.4	76.5	0.7
1B	3% (v/v) propanediol / 5% (w/v) proline	97.0	0.4	58.0	0.0
1C	3% (v/v) propanediol / 10% (w/v) dextran-40	84.4	1.0	87.5	0.7
1D	3% (v/v) propanediol / 5% (w/v) dextran-40	94.1	0.5	62.5	0.7
1E	5% (v/v) propanediol / 5% (w/v) dextran-40	96.2	0.6	53.0	1.0
1F	5% (v/v) propanediol / 3.5% (w/v) trehalose	92.6	1.4	57.7	0.6
1G	5% Glycerin / 3.5% (w/v) trehalose	91.3	0.8	55.7	1.2
1H	2.5% (w/v) pentastarch	92.4	0.9	75.1	1.1
1I	5% (w/v) pentastarch	95.5	0.6	62.8	2.8
1J	7.5% (w/v) pentastarch	95.3	0.3	56.8	0.2
1K	1% (w/v) Poloxamer188	92.0	0.9	137.7	13.4
1L	2.5% (w/v) dextran-40	93.0	0.7	77.0	5.3
1M	2.5% (v/v) ethylene glycol	90.5	2.0	66.0	2.5
1N	5% (v/v) DMSO	89.1	1.6	54.2	1.3

Example 2 – Screening of Dextran-40 density with Propanediol

[00301] RPEs derived from human embryonic stem cells (as described, for example, in WO2021086911, incorporated by reference herein) were pooled in a 250 mL bottle and washed with 200 mL of 0.5% rHA/DPBS by centrifugation at 500x g for 5 minutes.

[00302] RPE cells were resuspended with 2.5% rHA/DPBS/0.09% (w/v) glucose, followed by mixing with cryoprotectant buffer. Final compositions of each cell preparation are shown in Table 3.

Table 3

Excipients	2A	2B	2C	2D	2E
Propanediol (v/v)	3%				
Dextran-40 (w/v)	7.5%	5%	3%	1%	0.1%
Glucose (w/v)	0.09%				
Recombinant Albumin (w/v)	2.5%				
DPBS w/Ca, Mg	q.s.				

[00303] Four hundred fifty (450) µL of formulated RPE cells were filled in CRYSTAL ZENITH® (CZ) vials (West Pharmaceutical Services, Inc. Exton, PA) and cryopreserved using a controlled-rate freezer at -2ºC/min (THERMO SCIENTIFIC, Waltham, MA). Controlled-rate freezing was started within 3 hours of adding cryoprotectant buffer to the cells. The vials were then transferred and stored in the vapor phase of liquid nitrogen.

[00304] Post-thaw cell viability was evaluated using Vi-CELL BLU cell analyzers (Beckman Coulter Inc., Brea, CA) as described in Example 1. Cell preparations 2A to 2C, which are 3 to 7.5% (w/v) dextran-40 with 3% (v/v) propanediol, showed over 90% cell viability. (See FIG. 2A, Table 4.)

[00305] Post-thaw cell growth was evaluated using the xCelligence system (Agilent Technology, Santa Clara, CA). Cryopreserved RPE vials were thawed, seeded and cultured in growth medium in 96 well plates, and the cell growth was monitored in real time. Cell preparations 2A to 2C, which are 3 to 7.5% (w/v) dextran-40 with 3% (v/v) propanediol, showed below 70 hours at cell index 3, indicating cell growth rate of these cell preparations was in normal range. Cell preparations 2D to 2E, which are 0.1 to 1% (w/v) dextran-40 with 3% (v/v) propanediol, showed above 80 hours at cell index 3, indicating cell growth rate of these cell preparations was relatively low. (See FIG. 2B, Table 4.)

[00306] These results show significant cryoprotective properties of dextran-40 are observed for concentrations of 3% and higher.

Table 4

#	Cell preparation	**Ave. of Viability (%)**	**SD of Viability**	**Ave. of Cell growth (hrs) at cell index 3**	**SD of cell growth**
2A	3% (v/v) propanediol / 7.5% (w/v) dextran-40	96.3	0.3	58.0	0.0
2B	3% (v/v) propanediol / 5% (w/v) dextran-40	95.6	0.5	63.0	2.8
2C	3% (v/v) propanediol / 3% (w/v) dextran-40	93.6	0.4	68.5	2.1
2D	3% (v/v) propanediol / 1% (w/v) dextran-40	89.2	0.9	84.5	2.1
2E	3% (v/v) propanediol / 0.1% (w/v) dextran-40	86.8	1.3	83.0	0.0

Example 3 – Screening of Trehalose density with Propanediol

[00307] RPEs derived from human embryonic stem cells (as described, for example, in WO2021086911, incorporated herein by reference) were pooled in a 250 mL bottle and washed with 200 mL of 0.5% rHA/DPBS by centrifugation at 500x g for 5 minutes.

[00308] RPE cells were resuspended with 2.5% rHA/DPBS/0.09%, followed by mixing with cryoprotectant buffer. Final compositions of each cell preparation are shown in Table 5.

Table 5

Excipients	3A	3B	3C	3D	3E
Propanediol (v/v)	5%				
Trehalose (w/v)	7.5%	5%	3%	1%	0.1%
Glucose (w/v)	0.09%				
Recombinant Albumin (w/v)	2.5%				
DPBS w/Ca, Mg	q.s.				

[00309] Four hundred fifty (450) µL of formulated RPE cells were filled in CRYSTAL ZENITH® (CZ) vials (West Pharmaceutical Services, Inc. Exton, PA) and cryopreserved using a controlled-rate freezer at -2°C/min (THERMO SCIENTIFIC, Waltham, MA). Controlled-rate freezing was started within 3 hours of adding cryoprotectant buffer to the cells. The vials were then transferred and stored in the vapor phase of liquid nitrogen.

[00310] Post-thaw cell viability was evaluated using Vi-CELL BLU cell analyzers (Beckman Coulter Inc., Brea, CA) as described in Example 1. Cell preparation 3A to 3E, showed over 90% cell viability. (See FIG. 4A, Table 6.)

[00311] Post-thaw cell growth was evaluated using the xCelligence system (Agilent Technology, Santa Clara, CA). Cryopreserved RPE vials were thawed, seeded and cultured in growth medium in 96 well plates, and the cell growth was monitored in real time. All cell preparations were in the acceptable range and there were no significant differences between cell preparations. (See FIG. 4B, Table 6.)

Table 6

#	Cell preparation	Ave. of Viability (%)	SD of Viability	Ave. of Cell growth (hrs) at cell index 3	SD of cell growth
3A	5% (v/v) propanediol/ 7.5% (w/v) trehalose	92.6	0.8	62.5	2.1
3B	5% (v/v) propanediol/ 5% (w/v) trehalose	92.1	0.9	59.0	2.8
3C	5% (v/v) propanediol/ 3% (w/v) trehalose	91.0	0.7	56.5	0.7
3D	5% (v/v) propanediol/ 1% (w/v) trehalose	93.6	0.5	57.5	0.7
3E	5% (v/v) propanediol/ 0.1% (w/v) trehalose	91.9	0.4	57.0	1.4

Example 4 – Cell preparation robustness in terms of process time

[00312] RPEs derived from human embryonic stem cells (as described, for example, in WO2021086911, incorporated herein by reference) were pooled in a 250 mL bottle and washed with 200 mL of 0.5% rHA/DPBS by centrifugation at 500x g for 5 minutes.

[00313] RPE cells were resuspended with 2.5% rHA/DPBS/0.09% (w/v) glucose, followed by mixing with cryoprotectant buffer. Final compositions of each cell preparation are shown in Table 7.

Table 7

Excipients	4A	4B	4C	4D	4E	4F	4G	4H	4I	4J	4K	4L	4M
Propanediol (v/v)	5%		3%		5%		-		-		-	-	
Dextran-40 (w/v)	7.5%		5%		-		5%						
Trehalose (w/v)	-		-		1%		-						
Pentastarch (w/v)					-				5%		7.5 %		
DMSO (v/v)									-		-	5%	
Glucose (w/v)	0.09%												
Recombinant Albumin (w/v)	2.5%												
DPBS w/Ca, Mg	q.s.												

[00314] Four hundred fifty (450) µL of formulated RPE cells were filled in CRYSTAL ZENITH® (CZ) vials (West Pharmaceutical Services, Inc. Exton, PA) and cryopreserved using a controlled-rate freezer at -2ºC/min (THERMO SCIENTIFIC, Waltham, MA).

Controlled-rate freezing was started at approximately 6 hours (1.5 hours at room temperature plus 4.5 hours at 4ºC) of adding cryoprotectant buffer to the cells for preparations 4A, 4C, 4E, 4G, 4I, 4K, 4M), and at approximately 8 hours (1.5 hours at room temperature plus 6.5 hours at 4ºC) of adding cryoprotectant buffer to the cells for preparations 4B, 4D, 4F, 4H, 4J, 4L). The vials were then transferred and stored in the vapor phase of liquid nitrogen.

[00315] Post-thaw cell viability was evaluated using Vi-CELL BLU cell analyzers (Beckman Coulter Inc., Brea, CA) as described in Example 1. All cell preparations showed over 90% cell viability. Cell preparations 4A, 4B, 4C, 4D, 4G, 4H and 4K performed higher cell viability than 5% (v/v) DMSO cell preparations (4L and 4M). Cell preparation 4D showed better viability than 4B. (See FIG. 5A, Table 8.)

[00316] Post-thaw cell growth was evaluated using the xCelligence system (Agilent Technology, Santa Clara, CA). Cryopreserved RPE vials were thawed, seeded and cultured in growth medium in 96 well plates, and the cell growth was monitored in real time. All cell preparations showed below 70 hours, indicating that well-growth was observed in all cell preparations. (See FIG. 5B, Table 8.)

Table 8

#	Cell preparation	Process time post adding cryoprotectants to cryopreservation	Ave. of Viability (%)	SD of Viability	Ave. of Cell growth (hrs) at cell index 3	SD of cell growth
4A	5% (v/v) propanediol/ 7.5% (w/v) dextran-40	6h	97.9	0.2	51.0	0.0
4B		8h	94.0	2.1	51.0	0.7
4C	3% (v/v) propanediol/ 5% (w/v) dextran-40	6h	96.1	0.3	49.5	0.0
4D		8h	96.2	2.0	52.5	0.7
4E	5% (v/v) propanediol/ 1% (w/v) trehalose	6h	91.6	2.2	52.5	0.0
4F		8h	90.7	2.7	53.5	0.7
4G	5% (w/v) dextran-40	6h	95.6	0.8	53.5	0.7
4H		8h	98.1	0.4	55.5	0.0
4I	5% (w/v) pentastarch	6h	92.2	0.3	64.8	0.3
4J		8h	91.7	0.6	54.0	0.4
4K	7.5% (w/v) pentastarch	6h	94.8	0.7	60.3	0.5
4L	5% (v/v) DMSO	6h	92.5	0.8	52.5	0.0
4M		8h	91.7	0.9	53.0	0.0

Example 5 – Post thaw stability at 4°C and room temperature

[00317] RPEs derived from human embryonic stem cells (as described, for example, in WO2021086911, incorporated herein by reference) were pooled in a 250 mL bottle and washed with 200 mL of 0.5% rHA/DPBS by centrifugation at 500x g for 5 minutes.

[00318] RPE cells were resuspended with 2.5% rHA/DPBS/0.09% (w/v) glucose, followed by mixing with cryoprotectant buffer. Final compositions of each cell preparation are shown in Table 9.

Table 9

Excipients	5A	5B	5C	5D	5E	5F	5G	5H	5I	5J	5K	5L	5M	5N
Propanediol (v/v)	5%	3%	5%	-	-	-	-	5%	3%	5%	-	-	-	-
Dextran-40 (w/v)	7.5 %	5%	-	5%				7.5 %	5%	-	5 %			
Trehalose (w/v)	-	-	1%	-				-	-	1%	-			
Pentastarch (w/v)			-		5%	7.5 %				-		5%	7.5 %	
DMSO (v/v)					-	-	5%					-	-	5%
Glucose (w/v)	0.09 %													
Recombinant Albumin (w/v)	2.5%													
DPBS w/Ca, Mg	q.s.													

[00319] Four hundred fifty (450) µL of formulated RPE cells were filled in CRYSTAL ZENITH® (CZ) vials (West Pharmaceutical Services, Inc. Exton, PA) and cryopreserved using a controlled-rate freezer at -2°C/min (THERMO SCIENTIFIC, Waltham, MA). Controlled-rate freezing was started within approximately 8 hours after adding cryoprotectant buffer to cells (as described in Example 4). The vials were then transferred and stored in the vapor phase of liquid nitrogen.

[00320] Frozen vials were thawed and preserved for 4 hours at 4°C (cell preparations 5A to 5G) or room temperature (cell preparations 5H to 5N) prior to evaluation of cell viability using Vi-CELL BLU cell analyzers (Beckman Coulter Inc., Brea, CA). All cell preparations (5A to 5F) showed higher viability compared with 5% (v/v) DMSO cell preparation (5G). In particular, Cell preparation 5B had significant cell viability of 95.1% cell viability. (See FIG. 6A, Table 10.) Cell preparations 5H, 5I, 5K, 5L and 5M showed higher viability compared with 5% (v/v) DMSO cell preparation (5N). (See FIG. 7A, Table 11.)

Cell preparation 5J had 80.6% cell viability, which was slightly lower cell viability than the 5% (v/v) DMSO cell preparation (5N).

[00321] Frozen vials were thawed and preserved for 4 hours at 4ºC (cell preparations 5A to 5G) or room temperature (5H to 5N) prior to evaluating cell growth using the xCelligence system (Agilent Technology, Santa Clara, CA). Cryopreserved RPE vials were thawed, seeded and cultured in growth medium in 96 well plates, and the cell growth was monitored in real time. Cell preparations 5A, 5B, 5C, 5E, 5F showed higher cell growth rate when compared with 5% (v/v) DMSO cell preparation (5G). (See FIG. 6B, Table 10.) Cell preparation 5D performed at a comparable cell growth rate to 5% (v/v) DMSO cell preparation (5G). All cell preparations (5H to 5M) showed below 70 hours at cell index 3, indicating that well-growth was detected in all cell preparations. (See FIG. 7B, Table 11).

Table 10 (4ºC)

#	Cell preparation	Ave. of Viability (%)	SD of Viability	Ave. of Cell growth (hrs) at cell index 3	SD of cell growth
5A	5% (v/v) propanediol/ 7.5% (w/v) dextran-40	91.7	0.8	60.0	2.8
5B	3% (v/v) propanediol/ 5% (w/v) dextran-40	95.1	0.4	66.0	0.0
5C	5% (v/v) propanediol/ 1% (w/v) trehalose	86.1	1.7	66.0	0.0
5D	5% (w/v) dextran-40	88.8	0.4	74.5	2.1
5E	5% (w/v) pentastarch	91.9	0.1	58.2	0.3
5F	7.5% (w/v) pentastarch	90.4	0.7	60.2	0.5
5G	5% (v/v) DMSO	85.6	1.6	75.5	3.5

Table 11 (RT)

#	Cell preparation	Ave. of Viability (%)	SD of Viability	Ave. of Cell growth (hrs) at cell index 3	SD of cell growth
5H	5% (v/v) propanediol/ 7.5% (w/v) dextran-40	93.3	1.1	61.5	0.7
5I	3% (v/v) propanediol/ 5% (w/v) dextran-40	92.7	0.6	67.5	0.7
5J	5% (v/v) propanediol/ 1% (w/v) trehalose	80.6	5.5	69.5	3.5
5K	5% (w/v) dextran-40	90.2	0.8	67.0	0.0
5L	5% (w/v) pentastarch	88.6	0.7	56.2	0.4
5M	7.5% (w/v) pentastarch	86.6	0.5	67.7	0.4
5N	5% (v/v) DMSO	83.8	1.5	64.0	1.4

Example 6 – Cell preparation robustness in terms of temperature excursion at -70ºC

[00322] RPEs derived from human embryonic stem cells (as described, for example, in WO2021086911, incorporated herein by reference) were pooled in a 250 mL bottle and washed with 200 mL of 0.5% rHA/DPBS by centrifugation at 500x g for 5 minutes.

[00323] RPE cells were resuspended with 2.5% rHA/DPBS/0.09% (w/v) glucose, followed by mixing with cryoprotectant buffer. Final compositions of each cell preparation are shown in Table 12.

Table 12

Excipients	6A	6B	6C	6D	6E	6F	6G
Propanediol (v/v)	5%	3%	5%	-	-	-	-
Dextran-40 (w/v)	7.5%	5%	-	5%			
Trehalose (w/v)	-	-	1%	-			
Pentastarch (w/v)			-		5%	7.5%	
DMSO (v/v)					-	-	5%
Glucose (w/v)	0.09%						
Recombinant Albumin (w/v)	2.5%						
DPBS w/Ca, Mg	q.s.						

[00324] Four hundred fifty (450) µL of formulated RPE cells were filled in CRYSTAL ZENITH® (CZ) vials (West Pharmaceutical Services, Inc. Exton, PA) and cryopreserved using a controlled-rate freezer at -2ºC/min (THERMO SCIENTIFIC, Waltham, MA). Controlled-rate freezing was started within approximately 8 hours after adding cryoprotectant buffer to cells (as described in Example 4). The vials were then transferred and stored in the vapor phase of liquid nitrogen.

[00325] Frozen vials were transferred to -70ºC chamber and kept for 2 or 4 weeks, and then these vials were transferred to the tank filled vapor phase of liquid nitrogen.

[00326] Post-thaw cell viability was evaluated using Vi-CELL BLU cell analyzers (Beckman Coulter Inc., Brea, CA) as described in Example 1. After 4 weeks, Cell preparations 6A, 6B, 6D, 6E, 6F showed cell viabilities over 94% and higher viability when compared with the 5% (v/v) DMSO cell preparation (6G). (See FIG. 9A, Table 13.) Cell preparation 7C showed 84.4% cell viability, which was comparable with the 5% (v/v) DMSO cell preparation (6G).

[00327] Post-thaw cell growth was evaluated using the xCelligence system (Agilent Technology, Santa Clara, CA). Cryopreserved RPE vials were thawed, seeded and cultured in

growth medium in 96 well plates, and the cell growth was monitored in real time. All cell preparations (6A to 6G) showed below 80 hours at cell index 3, indicating that well-growth was detected in all cell preparations. (See FIG. 9B, Table 13.)

Table 13

#	Cell preparation	Ave. of Viability (%)	SD of Viability	Ave. of Cell growth (hrs) at cell index 3	SD of cell growth
6A	5% (v/v) propanediol/ 7.5% (w/v) dextran-40	97.7	0.4	55.5	2.1
6B	3% (v/v) propanediol/ 5% (w/v) dextran-40	95.7	1.2	57.0	0.0
6C	5% (v/v) propanediol/ 1% (w/v) trehalose	84.4	1.6	68.5	3.5
6D	5% (w/v) dextran-40	94.1	0.6	58.0	4.2
6E	5% (w/v) pentastarch	94.3	1.1	54.1	0.4
6F	7.5% (w/v) pentastarch	96.8	1.6	76.6	10.3
6G	5% (v/v) DMSO	87.4	1.0	57.5	0.7

Example 7 – Cell preparation robustness in terms of temperature excursion at -20ºC

[00328] RPEs derived from human embryonic stem cells (as described, for example, in WO2021086911, incorporated herein by reference) were pooled in a 250 mL bottle and washed with 200 mL of 0.5% rHA/DPBS by centrifugation at 500x g for 5 minutes.

[00329] RPE cells were resuspended with 2.5% rHA/DPBS/0.09% (w/v) glucose, followed by mixing with cryoprotectant buffer. Final compositions of each cell preparation are shown in Table 14.

Table 14

Excipients	7A	7B	7C	7D	7E	7F	7G	7H	7I	7J	7K	7L	7M	7N
Propanediol (v/v)	5%		3%		5%		-		-		-		-	
Dextran-40 (w/v)	7.5%		5%		-		5%							
Trehalose (w/v)	-		-		1%		-							
Pentastarch (w/v)					-				5%		7.5%			
DMSO (v/v)									-		-		5%	
Glucose (w/v)	0.09 %													
Recombinant Albumin (w/v)	2.5%													
DPBS w/Ca, Mg	q.s.													

[00330] Four hundred fifty (450) µL of formulated RPE cells were filled in CRYSTAL ZENITH® (CZ) vials (West Pharmaceutical Services, Inc. Exton, PA) and cryopreserved using a controlled-rate freezer at -2°C/min (THERMO SCIENTIFIC, Waltham, MA). Controlled-rate freezing was started within approximately 8 hours after adding cryoprotectant buffer to cells (as described in Example 4). The vials were then transferred and stored in the vapor phase of liquid nitrogen.

[00331] Frozen vials were transferred to -20°C chamber and kept for 30 minutes (7A, 7C, 7E, 7G, 7I, 7K, 7M) or 60 minutes (7B, 7D, 7F, 7H, 7J, 7L, 7N), and then these vials were transferred to the tank filled vapor phase of liquid nitrogen. This procedure was repeated twice in total before evaluation.

[00332] Post-thaw cell viability was evaluated using Vi-CELL BLU cell analyzers (Beckman Coulter Inc., Brea, CA) as described in Example 1. Cell preparations 7A, 7C, 7G, 7I and 7K showed viability of over 94% and higher viability when compared with the 5% (v/v) DMSO cell preparation (7M). (See FIG. 8A, Table 15.) Cell preparation 7E showed 86.1% cell viability, which was lower viability than 5% (v/v) DMSO cell preparation (7M). Cell preparations 7B, 7D, 7H, 7J and 7L showed significantly higher viability compared with 5% (v/v) DMSO cell preparation (7N). In particular, cell preparations 7B, 7D and 7L showed above 90% viability even post two exposures to -20°C for 60 minutes.

[00333] Post-thaw cell growth was evaluated using the xCelligence system (Agilent Technology, Santa Clara, CA). Cryopreserved RPE vials were thawed, seeded and cultured in growth medium in 96 well plates, and the cell growth was monitored in real time. Formulations 7A, 7C, 7E, 7G, 7I and 7K showed below 80 hours at cell index 3, indicating that well-growth was detected in all cell preparations. (See FIG. 8B, Table 15.) Cell preparations 7B and 7D showed below 80 hours at cell index 3, and cell preparation 8L showed 80.5 hours at cell index 3. These values were comparable with 5% (v/v) DMSO cell preparation (7N).

Table 15

#	Cell preparation	Temperature excursion condition	Ave. of Viability (%)	SD of Viability	Ave. of Cell growth (hrs) at cell index 3	SD of cell growth
7A	5% (v/v) propanediol 7.5% (w/v) dextran-40	30min x2	97.5	0.2	58.5	2.1
7B		60min x2	93.6	1.1	78.0	1.4
7C	3% (v/v) propanediol 5% (w/v) dextran-40	30min x2	95.0	1.7	60.5	0.7
7D		60min x2	95.4	0.6	79.0	1.4
7E	5% (v/v) propanediol 1% (w/v) trehalose	30min x2	86.1	2.1	75.0	2.8
7F		60min x2	57.9	5.7	99.0	2.8
7G	5% dextran-40	30min x2	96.5	1.7	58.0	1.4
7H		60min x2	89.4	0.9	92.5	0.7
7I	5% (w/v) pentastarch	30minx2	94.3	1.1	54.9	1.4
7J		60minx2	82.6	3.6	102.5	2.1
7K	7.5% (w/v) pentastarch	30minx2	96.8	1.6	76.9	15.6
7L		60minx2	93.9	0.5	80.5	5.7
7M	5% (v/v) DMSO	30min x2	90.4	1.5	55.5	0.7
7N		60min x2	69.8	1.4	78.5	4.9

Example 8 – Reduction in generation of multinucleated cells

[00334] RPEs derived from human embryonic stem cells (as described, for example, in WO2021086911, incorporated herein by reference) were pooled in a 250 mL bottle and washed with 200 mL of 0.5% rHA/DPBS by centrifugation at 500x g for 5 minutes.

[00335] RPE cells were resuspended with 2.5% rHA/DPBS/0.09% (w/v) glucose, followed by mixing with cryoprotectant buffer. Final compositions of each cell preparation are shown in Table 16.

Table 16

Excipients	8A	8B	8C	8D	8E	8F	8G	8H	8I
Propanediol (v/v)	5%	3%	5%	-	5%	-	-	-	-
Dextran-40 (w/v)	7.5%	5%	-	5%	-	1%			
Trehalose (w/v)	-	-	1%	-		-			
Pentastarch (w/v)			-				5%	7.5%	
DMSO (v/v)							-	-	5%
Glucose (w/v)	0.09								
Recombinant Albumin (w/v)	2.5								
DPBS w/Ca, Mg	q.s.								

[00336] Four hundred fifty (450) µL of formulated RPE cells were filled in CRYSTAL ZENITH® (CZ) vials (West Pharmaceutical Services, Inc. Exton, PA) and cryopreserved using a controlled-rate freezer at -2ºC/min (THERMO SCIENTIFIC, Waltham, MA). Controlled-rate freezing was started within approximately 6 hours after adding cryoprotectant buffer to cells (as described in Example 4). The vials were then transferred and stored in the vapor phase of liquid nitrogen.

[00337] Cryopreserved RPE cells were thawed, seeded and cultured in growth medium for 4 hours, and evaluated for the presence of multinucleated cells under a microscope. The control 5% (v/v) DMSO cell preparation (8I) resulted in 4.3% multinucleated cells and passed threshold (below 10%). (See FIG. 10, Table 17.) Cell preparations 8E, 8F and 8G showed similar level of multinucleated cells with cell preparation 8I. On the other hand, cell preparations 8A, 8B, 8C, 8D and 8H resulted in a lower percentage of multinucleated cells. In particular, the combination of Propanediol and Dextran-40 cell preparations strongly suppressed generation of multinucleated cells.

Table 17

#	Cell preparation	Average of Percent Multinucleated	SD of Percent Multinucleated
8A	5% (v/v) propanediol/ 7.5% (w/v) dextran-40	1.3	0.3
8B	3% (v/v) propanediol/ 5% (w/v) dextran-40	2.3	0.1
8C	5% (v/v) propanediol/ 1% (w/v) trehalose	3.0	0.0
8D	5% (w/v) dextran-40	2.7	0.9
8E	5% (v/v) propanediol	4.2	0.2
8F	1% (w/v) dextran-40	5.4	1.1
8G	5% (w/v) pentastarch	4.2	0.3
8H	7.5% (w/v) pentastarch	3.1	0.2
8I	5% (v/v) DMSO	4.3	0.0

Example 9 – Cell preparation robustness in terms of process time (UC-RPE)

[00338] RPEs derived from human induced pluripotent stem cells (obtained from Universal donor cells) (UC-RPE) were pooled in a 250 mL bottle and washed with 200 mL of 0.5% rHA/DPBS by centrifugation at 500x g for 5 minutes.

[00339] UC-RPE cells were resuspended with 2.5% rHA/DPBS/0.09% (w/v) glucose, followed by mixing with cryoprotectant buffer. Final compositions of each cell preparation are shown in Table 18.

Table 18

Excipients	**9A**	**9B**	**9C**	**9D**	**9E**	**9F**	**9G**	**9H**	**9I**	**9J**	**9K**	**9L**	**9M**	**9N**
Propanediol (v/v)	5%		3%		5%		-		-		-		-	
Dextran-40 (w/v)	7.5%		5%		-		5%							
Trehalose (w/v)	-		-		1%		-							
Pentastarch (w/v)					-				5%		7.5%			
DMSO (v/v)									-		-		5%	
Glucose (w/v)	0.09%													
Recombinant Albumin (w/v)	2.5%													
DPBS w/Ca, Mg	q.s.													

[00340] Four hundred fifty (450) µL of formulated UC-RPE cells were filled in CRYSTAL ZENITH® (CZ) vials (West Pharmaceutical Services, Inc. Exton, PA) and cryopreserved using a controlled-rate freezer at -2°C/min (THERMO SCIENTIFIC, Waltham, MA). Controlled-rate freezing was started at approximately 6 hours after adding cryoprotectant buffer to the cells for preparations 9A, 9C, 9E, 9G, 9I, 9K and 9M and at approximately 8 hours after adding cryoprotectant buffer to the cells for preparations 9B, 9D, 9F, 9H, 9J, 9L and 9N. The vials were then transferred and stored in the vapor phase of liquid nitrogen.

[00341] Post-thaw cell viability was evaluated using NucleoCounter® NC-200™ (ChemoMetec Inc. Bohemia, NY) device as described in Example 1. All cell preparations with 6-hour process time showed over 89% cell viability. (See Table 19.) Cell preparations 9A, 9C, 9E and 9G resulted in higher cell viability than 5% (v/v) DMSO cell preparation (9M). All cell preparations with 8-hour process time showed over 87% cell viability. (See FIG 11A.) Cell preparations 9B, 9D, 9H and 9L resulted in higher cell viability than 5% (v/v) DMSO cell preparation (9N).

[00342] Post-thaw cell growth was evaluated using the xCelligence system (Agilent Technology, Santa Clara, CA). Cryopreserved UC-RPE vials were thawed, seeded and

cultured in DMEM based growth medium in 96 well plates, and the cell growth was monitored in real time. All cell preparations showed below 70 hours, indicating that well-growth was observed in all cell preparations. (See Table 19 and FIG. 11B.)

Table 19

#	Cell preparation	Process time post adding cryoprotectants to cryopreservation	Ave. of Viability (%)	SD of Viability	Ave. of Cell growth (hrs) at cell index 3	SD of cell growth
9A	5% (v/v) propanediol/ 7.5% (w/v) dextran-40	6h	96.6	0.8	53.5	0.7
9B		8h	95.7	0.9	55.0	0.7
9C	3% (v/v) propanediol/ 5% (w/v) dextran-40	6h	95.0	0.6	55.5	0.0
9D		8h	94.6	0.8	57.0	0.7
9E	5% (v/v) propanediol/ 1% (w/v) trehalose	6h	91.8	0.9	59.0	0.0
9F		8h	87.0	1.2	62.0	0.0
9G	5% (w/v) dextran-40	6h	94.4	0.8	61.0	0.0
9H		8h	96.3	0.7	62.0	0.7
9I	5% (w/v) pentastarch	6h	89.7	2.6	53.0	4.9
9J		8h	91.7	0.6	54.0	0.4
9K	7.5% (w/v) pentastarch	6h	90.5	2.2	49.7	3.2
9L		8h	92.7	0.7	49.0	1.4
9M	5% (v/v) DMSO	6h	92.9	0.7	54.5	0.7
9N		8h	91.3	0.4	56.0	0.0

Example 10 – Screening of Propanediol density with Dextran-40 (UC-RPE)

[00343] RPEs derived from human induced pluripotent stem cells (obtained from Universal donor cells) (UC-RPE) were pooled in a 250 mL bottle and washed with 200 mL of 0.5% rHA/DPBS by centrifugation at 500x g for 5 minutes.

[00344] UC-RPE cells were resuspended with 2.5% rHA/DPBS/0.09% glucose, followed by mixing with cryoprotectant buffer. Final compositions of each cell preparation are shown in Table 20.

Table 20

Excipients	10A	10B	10C
Dextran-40 (w/v)	7.5%	5%	5%
Propanediol (v/v)	5%	3%	1%
Glucose (w/v)	0.09%		
Recombinant Albumin (w/v)	2.5%		
DPBS w/Ca, Mg	q.s.		

[00345] Four hundred fifty (450) µL of formulated UC-RPE cells were filled in CRYSTAL ZENITH® (CZ) vials (West Pharmaceutical Services, Inc. Exton, PA) and cryopreserved using a controlled-rate freezer at -2ºC/min (THERMO SCIENTIFIC, Waltham, MA). Controlled-rate freezing was started within approximately 6 hours after adding cryoprotectant buffer to cells for preparations 10A, 10B and 10C (as described in Example 4). The vials were then transferred and stored in the vapor phase of liquid nitrogen.

[00346] Post-thaw cell viability was evaluated using NucleoCounter® NC-200™ (ChemoMetec Inc. Bohemia, NY) device as described in Example 1. All cell preparations with 6-hour process time showed over 89% cell viability (see Table 21). Cell preparation 10A to 10C, showed over 90% cell viability. (See FIG. 3A, Table 21.)

[00347] Post-thaw cell growth was evaluated using the xCelligence system (Agilent Technology, Santa Clara, CA). Cryopreserved RPE vials were thawed, seeded and cultured in DMEM based growth medium in 96 well plates, and the cell growth was monitored in real time. All cell preparations were in the acceptable range and there were no significant differences between cell preparations. (See FIG. 3B, Table 21.)

Table 21

#	Cell preparation	Ave. of Viability (%)	SD of Viability	Ave. of Cell growth (hrs) at cell index 3	SD of cell growth
10A	5% (v/v) propanediol/ 7.5% (w/v) dextran-40	94.8	0.9	58.0	2.8
10B	3% (v/v) propanediol/ 5% (w/v) dextran-40	94.7	0.8	59.0	1.4
10C	1% (v/v) propanediol/ 5% (w/v) dextran-40	94.4	1.3	58.5	0.7

Example 11 – Cell preparation without Albumin

[00348] RPEs derived from human embryonic stem cells (as described, for example, in WO2021086911, incorporated herein by reference) were pooled in a 250 mL bottle and washed with 200 mL of DPBS by centrifugation at 500x g for 5 minutes.

[00349] RPE cells were resuspended with DPBS/0.09% (w/v) glucose, followed by mixing with cryoprotectant buffer. Final compositions of each cell preparation are shown in Table 22. In this Example, albumin was replaced with DPBS in both the resuspension buffer and the cryoprotectant buffer.

Table 22

Excipients	11A	11B	11C	11D	11E	11F	11G	11H	11I	11J
Propanediol (v/v)	3%	-	-	-	-	-	-	-	-	-
Dextran-40 (w/v)	5%	5%								
Pentastarch (w/v)	-	-	5%	10%						
2-Hydroxyethyl starch (HES)			-	-	5%	7.5%	10%			
DMSO (v/v)					-	-	-	5%	7.5%	10%
Glucose (w/v)	0.09%									
DPBS w/Ca, Mg	q.s.									

[00350] Four hundred fifty (450) µL of formulated RPE cells were placed in CZ vials and cryopreserved using a controlled-rate freezer at a freezing rate of -2ºC/min. Controlled-rate freezing was started within 3 hours of adding cryoprotectant buffer to the cells. The vials were then transferred and stored in the vapor phase of liquid nitrogen.

[00351] Post-thaw cell viability was evaluated using Vi-CELL BLU cell analyzers. Cell preparations with non-DMSO cryoprotectants (11A, 11B, 11C, 11D, 11E, 11F, 11G) showed higher viability than cell preparations with DMSO (11H, 11I, 11J). Cell preparations with 2-Hydroxyethyl starch (11E, 11F, 11G) showed comparable or higher viability than cell preparations with Pentastarch (HES with about 50% hydroxyethyl substitutions). These data are shown in Table 23. The findings of improved efficacy using 2-HES or Pentastarch, as cryoprotectant, can be extrapolated to other HES including but not limited to tetrastarch (approximately 40% hydroxyethylated HES) and heptastarch (approximately 70% hydroxyethylated HES).

Table 23

#	Cell preparation	Average Viability (%)	SD of Viability
11A	3% (v/v) propanediol/ 5% (w/v) dextran-40	85.5	3.3
11B	5% (w/v) dextran-40	87.2	0.5
11C	5% (w/v) pentastarch	83.3	2.3
11D	10% (w/v) pentastarch	90.2	0.8
11E	5% (w/v) 2-Hydroxyethyl starch	88.9	0.8
11F	7.5% (w/v) 2-Hydroxyethyl starch	89.5	1.9
11G	10% (w/v) 2-Hydroxyethyl starch	90.2	0.5
11H	5% (v/v) DMSO	84.1	1.1
11I	7.5% (v/v) DMSO	83.2	1.5
11J	10% (v/v) DMSO	83.4	0.8

Example 12 – Cell preparation using Vascular Progenitor Cells

[00352] A Vascular Progenitor cell is an immune-cloaked, allogeneic therapeutic cell product originating from a Class I and Class II HLA double knock-out iPSC line (Universal Donor Cell). VPCs are obtained through an expansion of iPSC seedstock via two passages followed by differentiation to VPCs over a 10-day cell culture period (hold on Day 6 at -80°C/LN_2 conditions) and culture for 4 additional days (total 10-day differentiation protocol). The cells are washed and resuspended in a suitable cryopreservation buffer and cryopreserved. Vascular Progenitor Cells and their manufacture are described in WO2021041592, herein incorporated by reference in its entirety.

[00353] Cryopreserved Vascular Progenitor Cells (VPCs) derived from human induced pluripotent stem cells (obtained from Universal donor cells) were thawed and pooled in a 50 mL tube and washed with 9 mL of 0.5% rHA/DPBS by centrifugation at 300x g for 5 minutes at 4ºC.

[00354] VPCs were resuspended with 2.5% rHA/DPBS/0.09% glucose, followed by mixing with cryoprotectant buffer. Final compositions of each cell preparation are shown in Table 24.

Table 24

Excipients	12A	12B	12C	12D
Propanediol (v/v)	3%	-	-	-
Dextran-40 (w/v)	5%	5%		
Pentastarch (w/v)	-	-	7.5%	
DMSO (v/v)				5%
Glucose (w/v)	0.09%			
Recombinant Albumin (w/v)	2.5%			
DPBS w/Ca, Mg	q.s.			

[00355] Four hundred fifty (450) µL of formulated VPCs were placed in CZ vials and cryopreserved using a controlled-rate freezer at freezing rate of -2ºC/min. Controlled-rate freezing started within 3 hours post (after) adding cryoprotectant buffer to the cells. The vials were then transferred and stored in the vapor phase of liquid nitrogen.

[00356] Post-thaw cell viability was evaluated using Vi-CELL BLU cell analyzers. Cell preparations with non-DMSO cryoprotectants (12A, 12B, 12C) showed higher viability than cell preparations with DMSO (12D). These data are shown in Table 25.

Table 25

#	Cell preparation	Average Viability (%)	SD of Viability
12A	3% (v/v) propanediol/ 5% (w/v) dextran-40	83.9	1.4
12B	5% (w/v) dextran-40	79.0	0.9
12C	7.5% (w/v) pentastarch	87.2	0.8
12D	5% (v/v) DMSO	76.2	0.6

[00357] These data show that cryopreservative formulations comprising propanediol/dextran-40, or dextran-40, or pentastarch as cryoprotectants, examples of which include 12A, 12B, and 12C, are comparable or better than cryopreservative formulations comprising DMSO when freezing VPC.

Example 13 – Cell preparation using hemangioblast-derived mesenchymal cells (HMC)

[00358] Differentiation of Hemangioblast-derived Mesenchymal stem cells (HMC) is a 33-day process starting from Human Embryonic Stem Cells (hESC). Frozen vial of 1 million of hESC is expanded to form embryoid bodies. The first step of differentiation is formation of embryoid bodies (EB) from hESC, and then EB cells are differentiated to Hemangioblast (HB). HMC are selected by plating HB onto fibronectin-coated tissue culture plates (P0). The selected HMC are expanded for one passage and cryopreserved as an intermediate substance (P1CS) for repeatable high-scale expansions. Expansion of Hemangioblast-derived Mesenchymal stem cells (HMC) is a 14-day process starting from HMC P1CS. Frozen vials of HMC P1CS are expanded in three passages. The first passage is expanded in T225 flasks. The second and third passages are expanded in XPN50 and XPN200 bioreactor disposables respectively. The HMCs harvested from the XPN200 are concentrated and washed in the UF Mini, a continuous-flow centrifuge. The HMCs are resuspended in a suitable cryopreservation buffer and cryopreserved. Hemangioblast-derived mesenchymal cells and their manufacture are described in WO2021041592 and WO2007120811 incorporated by reference herein in its entirety.

[00359] Cryopreserved hemangioblast-derived mesenchymal cells (HMCs) were thawed and pooled in a 50 mL tube and washed with 8 mL of 1% rHA/DPBS by centrifugation at 500x g for 5 minutes at 4ºC.

[00360] HMCs were resuspended with 2.5% rHA/DPBS/0.09% glucose, followed by mixing with cryoprotectant buffer. Final compositions of each cell preparation are shown in Table 26.

Table 26

Excipients	13A	13B	13C	13D
Propanediol (v/v)	3%	-	-	-
Dextran-40 (w/v)	5%	5%		
Pentastarch (w/v)	-	-	7.5%	
DMSO (v/v)				5%
Glucose (w/v)	0.09%			
Recombinant Albumin (w/v)	2.5%			
DPBS w/Ca, Mg	q.s.			

[00361] Four hundred fifty (450) µL of formulated HMCs were placed in CZ vials and cryopreserved using a controlled-rate freezer at a freezing rate of -2ºC/min. Controlled-rate freezing started within 3 hours post (after) adding cryoprotectant buffer to the cells. The vials were then transferred and stored in the vapor phase of liquid nitrogen.

[00362] Post-thaw cell viability was evaluated using Vi-CELL BLU cell analyzers. Cell preparation with non-DMSO cryoprotectants (13A, 13B, 13C) showed comparable viability to the cell preparation with DMSO (13D), as shown in Table 27.

Table 27

#	Cell preparation	Average Viability (%)	SD of Viability
13A	3% (v/v) propanediol/ 5% (w/v) dextran-40	82.4	2.4
13B	5% (w/v) dextran-40	80.4	1.7
13C	7.5% (w/v) pentastarch	83.1	2.3
13D	5% (v/v) DMSO	82.4	1.2

[00363] These data show that cryopreservative formulations comprising propanediol/dextran-40, or dextran-40, or pentastarch as cryoprotectants, examples of which include 13A, 13B, and 13C, are comparable or better than cryopreservative formulations comprising DMSO when freezing HMC.

EQUIVALENTS AND SCOPE, INCORPORATION BY REFERENCE

[00364] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents of the embodiments described herein. The scope of the present disclosure is not intended to be limited to the above description, but rather is as set forth in the appended claims.

[00365] Articles such as "a," "an," and "the" include the singular and the plural reference unless the context clearly indicates otherwise. Thus, for example, a reference to "an agent" includes a single agent and a plurality of such agents.

[00366] Claims or descriptions that include "or" between two or more members of a group are considered satisfied if one, more than one, or all of the group members are present, unless indicated to the contrary or otherwise evident from the context. The disclosure of a group that includes "or" between two or more group members provides embodiments in which exactly one member of the group is present, embodiments in which more than one members of the group are present, and embodiments in which all of the group members are present. For purposes of brevity those embodiments have not been individually spelled out herein, but it will be understood that each of these embodiments is provided herein and may be specifically claimed or disclaimed.

[00367] It is to be understood that the invention encompasses all variations, combinations, and permutations in which one or more limitation, element, clause, or descriptive term, from one or more of the claims or from one or more relevant portion of the description, is introduced into another claim. For example, a claim that is dependent on another claim can be modified to include one or more of the limitations found in any other claim that is dependent on the same base claim. Furthermore, where the claims recite a composition, it is to be understood that methods of making or using the composition according to any of the methods of making or using disclosed herein or according to methods known in the art, if any, are included, unless otherwise indicated or unless it would be evident to one of ordinary skill in the art that a contradiction or inconsistency would arise.

[00368] Where elements are presented as lists, *e.g.*, in Markush group format, it is to be understood that every possible subgroup of the elements is also disclosed, and that any element or subgroup of elements can be removed from the group. It is also noted that the term "comprising" is intended to be open and permits the inclusion of additional elements or steps. It should be understood that, in general, where an embodiment, product, or method is referred to as comprising particular elements, features, or steps, embodiments, products, or methods that consist, or consist essentially of, such elements, features, or steps, are provided

as well. For purposes of brevity those embodiments have not been individually spelled out herein, but it will be understood that each of these embodiments is provided herein and may be specifically claimed or disclaimed.

[00369] Where ranges are given, endpoints are included. Furthermore, it is to be understood that unless otherwise indicated or otherwise evident from the context and/or the understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific value within the stated ranges in some embodiments, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise. For purposes of brevity, the values in each range have not been individually spelled out herein, but it will be understood that each of these values is provided herein and may be specifically claimed or disclaimed. It is also to be understood that unless otherwise indicated or otherwise evident from the context and/or the understanding of one of ordinary skill in the art, values expressed as ranges can assume any subrange within the given range, wherein the endpoints of the subrange are expressed to the same degree of accuracy as the tenth of the unit of the lower limit of the range.

[00370] In addition, it is to be understood that any particular embodiment of the present invention may be explicitly excluded from any one or more of the claims. Where ranges are given, any value within the range may explicitly be excluded from any one or more of the claims. Any embodiment, element, feature, application, or aspect of the compositions and/or methods of the invention, can be excluded from any one or more claims. For purposes of brevity, all of the embodiments in which one or more elements, features, purposes, or aspects is excluded are not set forth explicitly herein.

[00371] All publications, patents, patent applications, publication, and database entries (e.g., sequence database entries) mentioned herein, e.g., in the Background, Summary, Detailed Description, Examples, and/or References sections, are hereby incorporated by reference in their entirety as if each individual publication, patent, patent application, publication, and database entry was specifically and individually incorporated herein by reference. In case of conflict, the present application, including any definitions herein, will control.

What is claimed is:

CLAIMS

1. A formulation comprising

(a) albumin, glucose, buffered saline, and one or two, optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, pentastarch, and ethylene glycol, optionally wherein the formulation is free of dimethylsulfoxide (DMSO-free),

(b) glucose, buffered saline, and one or two, optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, pentastarch, and ethylene glycol, optionally wherein the formulation is free of dimethylsulfoxide (DMSO-free),

(c) albumin, buffered saline, and one or two, optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, pentastarch, and ethylene glycol, optionally wherein the formulation is free of dimethylsulfoxide (DMSO-free),

(d) buffered saline, and one or two, optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, pentastarch, and ethylene glycol, optionally wherein the formulation is free of dimethylsulfoxide (DMSO-free),

(e) albumin, glucose, buffered saline, and one or two, optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, hydroxyethyl starch, and ethylene glycol, optionally wherein the formulation is free of dimethylsulfoxide (DMSO-free),

(f) glucose, buffered saline, and one or two, optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, hydroxyethyl starch, and ethylene glycol, optionally wherein the formulation is free of dimethylsulfoxide (DMSO-free),

(g) albumin, buffered saline, and one or two, optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, hydroxyethyl starch, and ethylene glycol, optionally wherein the formulation is free of dimethylsulfoxide (DMSO-free), or

(h) buffered saline, and one or two cryoprotectants, optionally more, selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, hydroxyethyl starch, and ethylene glycol, optionally wherein the formulation is free of dimethylsulfoxide (DMSO-free).

2. A cryopreservative formulation consisting essentially of

(a) albumin, glucose, buffered saline, and one or more cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, pentastarch, and ethylene glycol,

(b) glucose, buffered saline, and one or more cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, pentastarch, and ethylene glycol,

(c) albumin, buffered saline, and one or more cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, pentastarch, and ethylene glycol,

(d) buffered saline, and one or more cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, pentastarch, and ethylene glycol,

(e) albumin, glucose, buffered saline, and one or more cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, hydroxyethyl starch, and ethylene glycol,

(f) glucose, buffered saline, and one or more cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, hydroxyethyl starch, and ethylene glycol,

(g) albumin, buffered saline, and one or more cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, hydroxyethyl starch, and ethylene glycol, or

(h) buffered saline, and one or more cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, hydroxyethyl starch, and ethylene glycol.

3. The formulation of claim 1 or 2, wherein the albumin is human albumin.

4. The formulation of claim 1 or 2, wherein the albumin is recombinant human albumin.

5. The formulation of any one of the foregoing claims, wherein, if present, albumin is present in a range of about 2% to about 8% (w/v).
6. The formulation of any one of the foregoing claims, wherein, if present, albumin is present in a range of about 1% to about 4% (w/v).
7. The formulation of any one of the foregoing claims, wherein, if present, albumin is present at about 2.5% (w/v).
8. The formulation of any one of the foregoing claims, wherein, if present, glucose is present in a range of about 0.01% to about 1.5% (w/v).
9. The formulation of any one of the foregoing claims, wherein, if present, glucose is present in a range of about 0.01% to about 0.5% (w/v).
10. The formulation of any one of the foregoing claims, wherein, if present, glucose is present at about 0.09% (w/v).
11. The formulation of any one of the foregoing claims, wherein the buffered saline is phosphate buffered saline (PBS).
12. The formulation of any one of claims 1-11, wherein the one or two, optionally more, cryoprotectants comprise propanediol.
13. The formulation of claim 12, wherein propanediol is present in a range of about 0.1% to about 20% (v/v).
14. The formulation of claim 12, wherein propanediol is present in a range of about 0.2% to about 15% (v/v).
15. The formulation of claim 12, wherein propanediol is present at about 0.2%, about 2%, about 6%, about 10%, or about 15% (v/v).
16. The formulation of any one of claims 1-11, wherein the one or two, or optionally more, cryoprotectants comprise dextran-40.
17. The formulation of claim 16, wherein dextran-40 is present in a range of about 1% to about 20% (w/v).
18. The formulation of claim 16, wherein dextran-40 is present in a range of about 2% to about 20% (w/v).

19. The formulation of claim 16, wherein dextran-40 is present in a range of about 4% to about 20% (w/v).

20. The formulation of claim 16, wherein dextran-40 is present in a range of about 5% to about 15% (w/v).

21. The formulation of claim 16, wherein dextran-40 is present at about 5%, about 6%, about 10%, or about 15% (w/v).

22. The formulation of any one of claims 1-11, wherein the one or two, or optionally more, cryoprotectants comprise proline.

23. The formulation of any one of claims 1-11, wherein the one or two, or optionally more, cryoprotectants comprise trehalose.

24. The formulation of any one of claims 1-11, wherein the one or two, or optionally more, cryoprotectants comprise glycerin.

25. The formulation of any one of claims 1-11, wherein the one or two, or optionally more, cryoprotectants comprise hydroxyethyl starch having hydroxyethyl substitutions ranging up to 100%, optionally pentastarch.

26. The formulation of claim 25, wherein hydroxyethyl starch, optionally pentastarch, is present in a range of about 4% to about 20% (w/v).

27. The formulation of claim 25, wherein hydroxyethyl starch, optionally pentastarch, is present in a range of about 5% to about 15% (w/v).

28. The formulation of claim 25, wherein hydroxyethyl starch, optionally pentastarch, is present at about 5%, about 10%, or about 15% (w/v).

29. The formulation of any one of claims 1-11, wherein the one or two, or optionally more, cryoprotectants comprise ethylene glycol.

30. The formulation of claim 29, wherein ethylene glycol is present in a range of about 4% to about 6% (v/v).

31. The formulation of claim 29, wherein ethylene glycol is present at about 5% (v/v).

32. The formulation of any one of claims 1-11, wherein the one or two, or optionally more, cryoprotectants are propanediol and dextran-40.

33. The formulation of claim 32, wherein propanediol is present in a range of about 0.1% to about 20% (v/v) and dextran-40 is present in a range of about 0.2% to about 20% (w/v).

34. The formulation of claim 32, wherein propanediol is present in a range of about 0.15% to about 20% (v/v) and dextran-40 is present in a range of about 4% to about 20% (w/v).

35. The formulation of claim 32, wherein propanediol is present in a range of about 0.2% to about 15% (v/v) and dextran-40 is present in a range of about 5% to about 15% (w/v).

36. The formulation of claim 32, wherein propanediol is present at about 0.2%, about 2%, about 6%, about 10%, or about 15% (v/v) and dextran-40 is present at about 5%, about 6%, about 10%, or about 15% (w/v).

37. The formulation of claim 32, wherein propanediol is present in a range of about 0.2% to about 15% (v/v) and dextran-40 is present at about 6%, about 10%, or about 15% (w/v).

38. The formulation of claim 32, wherein propanediol is present at about 0.2%, about 2%, about 6%, about 10%, or about 15% (v/v) and dextran-40 is present at about 10% (w/v).

39. The formulation of claim 32, wherein propanediol is present at about 10% (v/v) and dextran-40 is present at about 15% (w/v).

40. The formulation of claim 32, wherein propanediol is present at about 10% (v/v) and dextran-40 is present at about 10% (w/v).

41. The formulation of claim 32, wherein propanediol is present at about 6% (v/v) and dextran-40 is present at about 20% (w/v).

42. The formulation of claim 32, wherein propanediol is present at about 6% (v/v) and dextran-40 is present at about 15% (w/v).

43. The formulation of claim 32, wherein propanediol is present at about 6% (v/v) and dextran-40 is present at about 10% (w/v).

44. The formulation of claim 32, wherein propanediol is present at about 6% (v/v) and dextran-40 is present at about 6% (w/v).

45. The formulation of claim 32, wherein propanediol is present at about 6% (v/v) and dextran-40 is present at about 2% (w/v).

46. The formulation of any one of claims 1-11, wherein the one or two, or optionally more, cryoprotectants are propanediol and proline.

47. The formulation of claim 46, wherein propanediol is present in a range of about 0.1% to about 20% (v/v) and proline is present in a range of about 2% to about 15% (w/v).

48. The formulation of claim 46, wherein propanediol is present in a range of about 0.2% to about 15% (v/v) and proline is present in a range of about 5% to about 15% (w/v).

49. The formulation of claim 46, wherein propanediol is present in a range of about 0.2% to about 15% (v/v) and proline is present in a range of about 5% to about 10% (w/v).

50. The formulation of claim 46, wherein propanediol is present at about 6% (v/v) and proline is present at about 5% or about 10% (w/v).

51. The formulation of any one of claims 1-11, wherein the one or two, or optionally more, cryoprotectants are propanediol and trehalose.

52. The formulation of claim 51, wherein propanediol is present in a range of about 0.1% to about 20% (v/v) and trehalose is present in a range of about 0.1% to about 20% (w/v).

53. The formulation of claim 51, wherein propanediol is present in a range of about 0.2% to about 15% (v/v) and trehalose is present in a range of about 0.2% to about 15% (w/v).

54. The formulation of claim 51, wherein propanediol is present at about 10% (v/v) and trehalose is present in a range of about 0.2% to about 15% (w/v).

55. The formulation of claim 51, wherein propanediol is present at about 10% (v/v) and trehalose is present at about 7% (w/v).

56. The formulation of claim 51, wherein propanediol is present at about 10% (v/v) and trehalose is present at about 2% (w/v).

57. The formulation of any one of claims 1-11, wherein the one or two, or optionally more, cryoprotectants are glycerin and trehalose.

58. The formulation of claim 57, wherein glycerin is present in a range of about 4% to about 16% (v/v) and trehalose is present in a range of about 2% to about 12% (w/v).

59. The formulation of claim 57, wherein glycerin is present in a range of about 8% to about 12% (v/v) and trehalose is present in a range of about 4% to about 10% (w/v).

60. The formulation of claim 57, wherein glycerin is present at about 10% (v/v) and trehalose is present at about 7% (w/v).

61. The formulation of claim 1, wherein the formulation comprises about 2.5% (w/v) albumin, optionally recombinant human albumin, about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 5% (v/v) propanediol and about 7.5% (w/v) dextran-40.

62. The formulation of claim 1, wherein the formulation comprises about 2.5% (w/v) albumin, optionally recombinant human albumin, about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 3% (v/v) propanediol and about 5% (w/v) dextran-40.

63. The formulation of claim 1, wherein the formulation comprises about 0.09% (w/v) glucose, buffered saline (e.g., PBS), about 3% (v/v) propanediol and about 5% (w/v) dextran-40, and is albumin-free.

64. The formulation of claim 1, wherein the formulation comprises about 2.5% (w/v) albumin, optionally recombinant human albumin, about 0.09% (w/v) glucose, buffered saline (e.g., PBS), and about 5% (w/v) dextran-40.

65. The formulation of claim 1, wherein the formulation comprises about 2.5% (w/v) albumin, optionally recombinant human albumin, about 0.09% (w/v) glucose, buffered saline (e.g., PBS), and about 7.5% (w/v) pentastarch.

66. A cell preparation comprising a population of cells and the formulation of any one of claims 1-11, 12, 16, 22-25, 29, 32, 46, 51, and 57.

67. A cell preparation comprising a population of cells and the formulation of any one of claims 13-15, 17-21, 26-28, 30-31, 33-45, 47-50, 52-56, and 58-65, wherein the concentration of each of the one or two, or optionally more, cryoprotectants is halved.

68. A cell preparation comprising a population of cells in a 1:1 (v/v) mixture of (i) the formulation of any one of claims 1-65 and (ii) a suspension solution comprising albumin, glucose and buffered saline, each at the same concentration as in the formulation, wherein the suspension solution does not contain a (non-albumin) cryoprotectant.

69. A cell preparation comprising a population of cells in a formulation comprising

(a) albumin, glucose, buffered saline, and one or two, or optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, pentastarch, and ethylene glycol, optionally wherein the formulation is free of dimethylsulfoxide (DMSO-free),

(b) glucose, buffered saline, and one or two, or optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, pentastarch, and ethylene glycol, optionally wherein the formulation is free of dimethylsulfoxide (DMSO-free),

(c) albumin, buffered saline, and one or two, or optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, pentastarch, and ethylene glycol, optionally wherein the formulation is free of dimethylsulfoxide (DMSO-free),

(d) buffered saline, and one or two, or optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, pentastarch, and ethylene glycol, optionally wherein the formulation is free of dimethylsulfoxide (DMSO-free),

(e) albumin, glucose, buffered saline, and one or two, or optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, hydroxyethyl starch, and ethylene glycol, optionally wherein the formulation is free of dimethylsulfoxide (DMSO-free),

(f) glucose, buffered saline, and one or two, or optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, hydroxyethyl starch, and ethylene glycol, optionally wherein the formulation is free of dimethylsulfoxide (DMSO-free),

(g) albumin, buffered saline, and one or two, or optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, hydroxyethyl starch, and ethylene glycol, optionally wherein the formulation is free of dimethylsulfoxide (DMSO-free), or

(h) buffered saline, and one or two, or optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, hydroxyethyl starch, and ethylene glycol, optionally wherein the formulation is free of dimethylsulfoxide (DMSO-free).

70. A cell preparation comprising a population of cells in a cryopreservative formulation consisting essentially of

(a) albumin, glucose, buffered saline, and one or two, or optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, pentastarch, and ethylene glycol,

(b) glucose, buffered saline, and one or two, or optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, pentastarch, and ethylene glycol,

(c) albumin, buffered saline, and one or two, or optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, pentastarch, and ethylene glycol,

(d) buffered saline, and one or two, or optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, pentastarch, and ethylene glycol,

(e) albumin, glucose, buffered saline, and one or two, or optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, hydroxyethyl starch, and ethylene glycol,

(f) glucose, buffered saline, and one or two, or optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, hydroxyethyl starch, and ethylene glycol,

(g) albumin, buffered saline, and one or two, or optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, hydroxyethyl starch, and ethylene glycol, or

(h) buffered saline, and one or two, or optionally more, cryoprotectants selected from the group consisting of propanediol, dextran-40, proline, trehalose, glycerin, hydroxyethyl starch, and ethylene glycol.

71. The cell preparation of any one of claims 66-70, wherein, if present, albumin is human albumin, optionally recombinant human albumin.

72. The cell preparation of any one of claims 66-71, wherein, if present, albumin is present in a range of about 2% to about 8% (w/v), optionally in a range of about 1% to about 4% (w/v), further optionally at about 2.5% (w/v).

73. The cell preparation of any one of claims 66-72, wherein, if present, glucose is present in a range of about 0.01% to about 1.5% (w/v), optionally in a range of about 0.01% to about 0.5% (w/v), further optionally at about 0.09% (w/v).

74. The cell preparation of any one of claims 66-72, wherein the buffered saline is phosphate buffered saline.

75. The cell preparation of any one of claims 66-74, wherein the one or two, or optionally more, cryoprotectants comprise propanediol.

76. The cell preparation of claim 75, wherein propanediol is present in a range of about 0.05% to about 10%, optionally in a range of about 0.1% to about 7.5%, further optionally at about 0.1%, about 1%, about 3%, about 5%, or about 7.5% (v/v).

77. The cell preparation of any one of claims 66-74, wherein the one or two, or optionally more, cryoprotectants comprise dextran-40.

78. The cell preparation of claim 77, wherein dextran-40 is present in a range of about 0.5% to about 10%, optionally in a range of about 1% to about 10%, optionally in a range of about 2% to about 10%, optionally in a range of about 2.5% to about 7.5%, and further optionally at about 2.5%, about 3%, about 5%, or about 7.5% (w/v).

79. The cell preparation of any one of claims 66-74, wherein the one or two, or optionally more, cryoprotectants comprise proline, trehalose, and/or glycerin.

80. The cell preparation of any one of claims 66-74, wherein the one or two, or optionally more, cryoprotectants comprise hydroxyethyl starch, optionally pentastarch.

81. The cell preparation of claim 80, wherein hydroxyethyl starch, optionally pentastarch, is present in a range of about 2% to about 10%, optionally in a range of about 2.5% to about 7.5%, and further optionally at about 2.5%, about 5%, or about 7.5% (w/v).

82. The cell preparation of any one of claims 66-74, wherein the one or two, or optionally more, cryoprotectants comprise ethylene glycol.

83. The cell preparation of claim 82, wherein ethylene glycol is present in a range of about 2% to about 3%, optionally at about 2.5% (v/v).

84. The cell preparation of any one of claims 66-74, wherein the one or two, or optionally more, cryoprotectants are propanediol and dextran-40.

85. The cell preparation of claim 84, wherein propanediol is present in a range of about 0.05% to about 10% (v/v) and dextran-40 is present in a range of about 0.1% to about 10% (w/v), optionally wherein propanediol is present in a range of about 0.05% to about 10% (v/v) and dextran-40 is present in a range of about 2% to about 10% (w/v), optionally wherein propanediol is present in a range of about 0.1% to about 7.5% (v/v) and dextran-40 is present in a range of about 2.5% to about 7.5% (w/v), optionally wherein propanediol is present at about 0.1%, about 1%, about 3%, about 5%, or about 7.5% (v/v) and dextran-40 is present at about 2.5%, about 3%, about 5%, or about 7.5% (w/v), optionally wherein propanediol is present in a range of about 0.1% to about 7.5% (v/v) and dextran-40 is present at about 3%, about 5%, or about 7.5% (w/v), further optionally wherein propanediol is present at about 0.1%, about 1%, about 3%, about 5%, or about 7.5% and dextran-40 is present at about 5% (w/v).

86. The cell preparation of claim 84, wherein
    a. propanediol is present at about 5% (v/v) and dextran-40 is present at about 7.5% (w/v),
    b. propanediol is present at about 5% (v/v) and dextran-40 is present at about 5% (w/v),
    c. propanediol is present at about 3% (v/v) and dextran-40 is present at about 10% (w/v),
    d. propanediol is present at about 3% (v/v) and dextran-40 is present at about 7.5% (w/v),
    e. propanediol is present at about 3% (v/v) and dextran-40 is present at about 5% (w/v),
    f. propanediol is present at about 3% (v/v) and dextran-40 is present at about 3% (w/v), or
    g. propanediol is present at about 3% (v/v) and dextran-40 is present at about 1% (w/v).

87. The cell preparation of any one of claims 66-74, wherein the one or two, or optionally more, cryoprotectants are propanediol and proline.

88. The cell preparation of claim 87, wherein propanediol is present in a range of about 0.05% to about 10% (v/v) and proline is present in a range of about 1% to about 7.5% (w/v), optionally wherein propanediol is present in a range of about 0.1% to about

7.5% (v/v) and proline is present in a range of about 2.5% to about 7.5% (w/v), optionally wherein propanediol is present in a range of about 0.1% to about 7.5% (v/v) and proline is present in a range of about 2.5% to about 5% (w/v), and further optionally wherein propanediol is present at about 3% (v/v) and proline is present at about 2.5% or about 5% (w/v).

89. The cell preparation of any one of claims 66-74, wherein the one or two, or optionally more, cryoprotectants are propanediol and trehalose.

90. The cell preparation of claim 89, wherein propanediol is present in a range of about 0.05% to about 10% (v/v) and trehalose is present in a range of about 0.05% to about 10% (w/v), optionally wherein propanediol is present in a range of about 0.1% to about 7.5% (v/v) and trehalose is present in a range of about 0.1% to about 7.5% (w/v), optionally wherein propanediol is present in a range of about 5% (v/v) and trehalose is present in a range of about 0.1% to about 7.5% (w/v), optionally wherein propanediol is present at about 5% (v/v) and trehalose is present at about 3.5% (w/v), and further optionally wherein propanediol is present at about 5% (v/v) and trehalose is present at about 1% (w/v).

91. The cell preparation of any one of claims 66-74, wherein the one or two, or optionally more, cryoprotectants are glycerin and trehalose.

92. The cell preparation of claim 91, wherein glycerin is present in a range of about 2% to about 8% (v/v) and trehalose is present in a range of about 1% to about 6% (w/v), optionally wherein glycerin is present in a range of about 4% to about 6% (v/v) and trehalose is present in a range of about 2% to about 5% (w/v), and further optionally wherein glycerin is present at about 5% (v/v) and trehalose is present at about 3.5% (w/v).

93. The cell preparation of any one of claims 66-92, wherein the cell population is a retinal pigmented epithelium (RPE) cell population, a photoreceptor cell population, a photoreceptor progenitor cell population, a photoreceptor rescue cell (PRC) population, a corneal epithelial cell (CEC) population, a mesenchymal cell population, a hemangioblast-derived mesenchymal cell (HMC) population, a macrophage cell population, a natural killer cell population, a T-cell population, a vascular cell population, a vascular progenitor cell (VPC) population, a liver cell population, a neural cell population and a pancreatic cell population.

94. The cell preparation of any one of claims 66-92, wherein the cell population is a retinal pigmented epithelium (RPE) cell population.

95. The cell preparation of any one of claims 66-92, wherein the cell population is a population of cells, wherein the cells express at least one polypeptide selected from the group consisting of human leukocyte antigen E (HLA-E), human leukocyte antigen F (HLA-F), and human leukocyte antigen G (HLA-G); wherein the cells comprise a genetically engineered disruption of one or more copies of endogenous β-2 microglobulin (B2M); and wherein the cells comprise a genetically engineered disruption of one or more copies of a human leukocyte antigen (HLA) class II-related gene selected from the group consisting of regulatory factor X associated ankyrin containing protein (RFXANK), regulatory factor 5 (RFX5), regulatory factor X associated protein (RFXAP), and class II transactivator (CIITA).

96. The cell preparation of claim 95, wherein the cells further comprise a nucleic acid molecule encoding a suicide gene product.

97. The cell preparation of claim 96, wherein the cells comprise a herpes simplex virus thymidine kinase (TK) suicide gene.

98. The cell preparation of any one of claims 95-97, wherein the cells are derived from pluripotent stem cells.

99. The cell preparation of claim 98, wherein the pluripotent stem cells are embryonic stem cells.

100. The cell preparation of claim 98, wherein the pluripotent stem cells are induced pluripotent stem cells.

101. The cell preparation of any one of claims 95-100, wherein the cells comprise a genetically engineered disruption of all copies of endogenous B2M.

102. The cell preparation of any one of claims 95-101, wherein the cells comprise a genetically engineered disruption of all copies of an HLA class II-related gene selected from the group consisting of RFXANK, RFX5, RFXAP, and CIITA.

103. The cell preparation of any one of claims 66-102, wherein the cell population is a dissociated cell population, a cell sheet, a cell monolayer, or a cell aggregate.

104. The cell preparation of any one of claims 66-103, wherein the cell population is a human cell population.

105. The cell preparation of any one of claims 66-104, wherein the cell population is generated by in vitro differentiation of pluripotent stem cells, optionally embryonic stem cells or induced pluripotent stem cells.

106. The cell preparation of any one of claims 66-105, having a cell density of about 300 cells to about 10,000 cells per microliter.

107. The cell preparation of any one of claims 66-105, having a cell density of about 300 cells to about 7,000 cells per microliter.

108. The cell preparation of any one of claims 66-105, having a cell density of about 350 cells to about 7,000 cells per microliter.

109. The cell preparation of any one of claims 66-105, having a cell density of about 350 cells per microliter, or about 3,000 cells per microliter, or about 3,500 cells per microliter, or about 6,500 cells per microliter, or about 7,000 cells per microliter.

110. The cell preparation of any one of claims 66-105, having a cell density of about 3,333 cells per microliter.

111. The cell preparation of any one of claims 66-110, in a volume of about 400-500 microliters, further optionally in a volume of about 450 microliters.

112. The cell preparation of any one of claims 66-111, in a cryopreserved form.

113. The cell preparation of any one of claims 66-111, wherein the preparation has been cryopreserved and thawed.

114. The cell preparation of any one of claims 66-111, provided at room temperature (about 20 °C to about 25 °C), at about 4 °C, at about -20 °C, at about -70 °C, or at about -180 °C pre-cryopreservation or post-thaw.

115. The cell preparation of any one of claims 66-111, wherein the cell preparation has been kept at room temperature (about 20 °C to about 25 °C) for about 1 to about 2 hours, and at about 4 °C for about 4 to about 7 hours, prior to cryopreservation, optionally at -180 °C.

116. A cell preparation comprising a population of cells in a formulation comprising albumin, glucose, buffered saline, and one or two, or optionally more, cryoprotectants, and having a viability of equal to or greater than about 80% and/or a cell growth index:3 of equal to or less than 110 hours, optionally having a viability of

equal to or greater than about 85% and/or a cell growth index:3 of equal to or less than 100 hours, further optionally having a viability of equal to or greater than about 90% and/or a cell growth index:3 of equal to or less than 90 hours, after

a. storage at room temperature (about 20 °C to about 25 °C) for about 1 to about 2 hours and then at about 4 °C for about 4 to about 7 hours, optionally at room temperature for about 1.5 hours and then at 4 °C for about 4.5 hours or 6.5 hours,
b. cryopreservation at -180 °C for about 1 to about 2 years, optionally for about 1 month to about 1 year,
c. cryopreservation at -180 °C and then post-thaw at about 4 °C for about 3 to about 6 hours, optionally for about 4 hours,
d. cryopreservation at -180 °C and then post-thaw at room temperature (about 20 °C to about 25 °C) for about 3 to about 6 hours, optionally for about 4 hours,
e. cryopreservation at -180 °C, then at about - 20 °C for about 0.5 to about 1 hour, then cryopreservation at -180 °C, optionally then at about - 20 °C for about 0.5 to about 1 hour, then cryopreservation at -180 °C, or
f. cryopreservation at -180 °C, then at about - 70 °C for about 2 to about 4 weeks, then cryopreservation at -180 °C.

117. The cell preparation of claim 116, wherein the cell preparation is a DMSO-free cell preparation.

118. The cell preparation of claim 116 or 117, wherein the cell preparation has viability of equal to or greater than about 85% and/or a cell growth index:3 of equal to or less than 100 hours after any one of events (a) through (f).

119. The cell preparation of any one of claims 116-118, wherein the cell preparation has a viability of equal to or greater than about 90% and/or a cell growth index:3 of equal to or less than 90 hours after any one of events (a) through (f).

120. A method of preparing a cell preparation, comprising contacting a population of cells with (i) the formulation of any one of claims 1-65 and (ii) a suspension solution comprising albumin, glucose, and buffered saline, each at the same concentration as in the formulation, wherein the suspension solution does not contain a (non-albumin) cryoprotectant.

121. The method of claim 120, further comprising cryopreserving the cell preparation.

122. The method of claim 121, further comprising thawing the cell preparation.

123. The method of claim 122, further comprising administering the cell preparation to a subject in need thereof.

124. The method of claim 123, wherein the cell preparation is kept at about 4 °C for about 4 hours before administration to the subject.

125. The method of claim 123, wherein the cell preparation is kept at room temperature (about 20 °C to about 25 °C) for about 4 hours before administration to the subject.

126. The method of any one of claims 123-125, wherein the cell preparation is administered to the subject without washing or dilution post-thaw.

127. A method of treating a subject having a disorder or condition comprising administering to a subject in need thereof an effective amount of the cell preparation of claim 113 without washing or dilution post-thaw.

128. A method of treating a subject having a disorder or condition comprising administering to a subject in need thereof an effective amount of the cell preparation prepared according to the method of claim 122 without washing or dilution post-thaw.

129. The method of claim 127 or 128, wherein the cell preparation is kept at about 4 °C for about 4 hours before administration to the subject.

130. The method of claim 127 or 128, wherein the cell preparation is kept at room temperature (about 20 °C to about 25 °C) for about 4 hours before administration to the subject.

131. The method of any one of claims 127-130, wherein the disorder or condition is selected from the group consisting of retinal detachment, retinal tears, retinal dysplasia, Angioid streaks, Myopic Macular Degeneration, or retinal atrophy or associated with a number of vision-altering ailments that result in photoreceptor damage and blindness, such as, for example, choroideremia, diabetic retinopathy, macular degeneration (e.g., age-related macular degeneration), retinitis pigmentosa, Stargardt's Disease (fundus flavimaculatus), cancer, liver disease, Crohn's disease, arthritis, pulmonary artery hypertension, critical limb ischemia, Fuch's Dystropy,

Corneal injury, retinal tear, and geographic atrophy (e.g., geographic atrophy secondary to age-related macular degeneration.

132. The cell preparation of any one of claims 66-119, wherein the cell preparation is within a vial.

133. The cell preparation of any one of claims 66-119, wherein the cell preparation is within a syringe.