

PH ADJUSTMENT TO IMPROVE THAW RECOVERY OF CELL BANKS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This Application claims the priority benefit of U.S. provisional application serial no. 62/022,392, filed July 9, 2014, which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0002] The present invention relates to methods of freezing cells (such as mammalian cells) for banking and freezing media for use in freezing cells.

BACKGROUND OF THE INVENTION

[0003] Cell banks are produced by first accumulating cells in a batch/perfusion cell culture process and then harvesting cells for banking. The process involves three stages: cell accumulation, harvest and cell concentration, and cell banking. A harvest process step serves to concentrate the final cell culture fluid or to extract the cells from the cell culture fluid using centrifugation. A subsequent pooling and filling process serves to prepare cell bank ampoules for long-term storage.

[0004] These traditional methods may result in inconsistent or poor viability post-thaw for select cell lines. Imperative to the cell banking process is the need for high cell viability after thawing of the frozen cells. Thus, improved methods of freezing cells for cell banking are desirable. Cell culture medium for freezing cells that allows for greater cell viability when thawed after banking would be beneficial.

[0005] All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

SUMMARY

[0006] Provided herein is a method of improving thaw recovery of cell banks comprising freezing eukaryotic cells (e.g., mammalian cells, insect cells, etc.) for banking in a freezing medium, wherein the freezing medium comprises a buffered solution and a cryoprotective agent, and wherein the freezing medium has a pH of about 6.7 to about 8.5 prior to freezing or has been adjusted to a pH of about 6.7 to about 8.5 prior to freezing.

[0006a] In one aspect, provided herein is a method of improving thaw recovery of cell banks comprising freezing mammalian cells for banking in a freezing medium, wherein the freezing medium comprises a buffered solution and a cryoprotective agent, and wherein the freezing medium has a pH of about 6.7 to about 8.5 prior to freezing.

[0007] Also provided herein is a method of freezing eukaryotic cells (e.g., mammalian cells, insect cells, etc.) for storage comprising freezing the cells in a freezing medium, wherein the freezing medium comprises a buffered solution and a cryoprotective agent, and wherein the freezing medium has a pH of about 6.7 to about 8.5 prior to freezing or has been adjusted to a pH of about 6.7 to about 8.5 prior to freezing.

[0007a] In one aspect, provided herein is a method of freezing mammalian cells for storage comprising freezing the mammalian cells in a freezing medium, wherein the freezing medium comprises a buffered solution and a cryoprotective agent, and wherein the freezing medium has a pH of about 6.7 to about 8.5 prior to freezing.

[0008] In some embodiments of the methods described above or herein, the freezing medium has a pH of about 6.7 to about 8.3, about 6.8 to about 8.3, about 6.9 to about 8.3, about 7.0 to about 8.3, about 7.1 to about 8.3, about 7.2 to about 8.3, about 7.3 to about 8.3, about 7.4 to about 8.3, about 7.5 to about 8.3, about 7.2 to about 8.0, about 7.2 to about 7.8, or about 7.5 prior to freezing.

[0009] In some embodiments of the methods described above or herein, the pH of the freezing medium has been adjusted to a pH of about 6.7 to about 8.5, about 6.7 to about 8.3, about 6.8 to about 8.3, about 6.9 to about 8.3, about 7.0 to about 8.3, about 7.1 to about 8.3, about 7.2 to about 8.3, about 7.3 to about 8.3, about 7.4 to about 8.3, about 7.5 to about 8.3, about 7.2 to about 8.0, about 7.2 to about 7.8, or about 7.5. In some embodiments of the methods described above or herein, the cells (e.g., mammalian cells or insect cells) are combined with a freezing medium before and/or after pH adjustment. In some embodiments, the adjusted pH is a target pH or a measured pH. In some embodiments, the target pH is about 6.7 to about 8.5, or any of the pH or in any pH ranges described herein. In some embodiments, the measured pH is about 6.7 to about 8.5, or any of the pH or in any pH ranges described herein. In some embodiments of the methods described above or herein, the method further comprises a step of measuring an initial pH of the freezing medium containing the cells (e.g., mammalian cells or insect cells) prior to adjusting pH of the freezing medium. In some embodiments of the methods described above or herein, the method further comprises a step of measuring the adjusted pH of the freezing medium. In some embodiments of the methods described above or herein, if the measured pH of the freezing medium is below a target pH, the method comprises repeating the adjusting step and measuring step until the adjusted pH of the freezing medium is about 6.7 to about 8.5, or any of the pH or in any pH ranges described herein.

[0010] In some embodiments of the methods described herein or above, the pH is adjusted by adding a base. In some embodiments, the base is selected from the group consisting of sodium carbonate, sodium bicarbonate, HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) sodium salt, sodium hydroxide, and potassium hydroxide. In some embodiments, the pH of the

freezing medium is adjusted by adding a base to the freezing medium according to the following formula $V_{\text{base}} = C_{\text{base}} * V_p (pH_t - pH_i)$, wherein C_{base} is a base-specific coefficient, V_{base} is a volume of the base to add to the freezing medium, V_p is the volume of the freezing medium, pH_t is the target pH, and pH_i is the initial pH. In some embodiments, the pH of the freezing medium is adjusted by adding sodium carbonate to the freezing medium according to the following formula $V_{\text{Na}_2\text{CO}_3} = 0.0085V_p (pH_t - pH_i)$, wherein $V_{\text{Na}_2\text{CO}_3}$ is a volume of 1M sodium carbonate to add to the freezing medium, V_p is the volume of the freezing medium, pH_t is the target pH, and pH_i is the initial pH. In some embodiments, the target pH is a pH between about 6.7 to about 8.5, about 7.2 to about 8.3, or any of the pH or in any pH ranges described herein. In some embodiments, the target pH is 7.5.

[0011] In some embodiments of the methods described above or herein, the cells (e.g., mammalian cells or insect cells) are in a medium having a pH of about 6.2 to about 6.6 before the cells are combined with a freezing medium.

[0012] In some embodiments of the methods described above or herein, the cryoprotective agent is DMSO (dimethyl sulfoxide), glycerol, propanediol, ethylene glycol, a macromolecule, a sugar, or a combination thereof. In some embodiments, the DMSO or glycerol in the freezing medium prior to freezing is at a concentration of about 5% to about 12.5% by volume. In some embodiments, the DMSO or glycerol in the freezing medium prior to freezing the cells (e.g., mammalian cells or insect cells) is at a concentration of about 5% to about 10% by volume.

[0013] In some embodiments of the methods described above or herein, the freezing medium containing the cells (e.g., mammalian cells or insect cells) has a cell density of about 8% to about 28% packed cell volume (PCV) prior to freezing.

[0014] In some embodiments of the method described above or herein, the method further comprises a step of cooling cell culture fluid during cell harvest and concentration process before the cells (e.g., mammalian cells or insect cells) are combined with a freezing medium. In some embodiments, the cell culture fluid is cooled to a temperature at or below about 20°C. In some embodiments, the cell culture fluid is cooled to a temperature at or below about 10°C.

[0015] Provided here is a method of freezing eukaryotic cells (e.g., mammalian cells, insect cells, etc.) for storage or improving thaw recovery of cell banks comprising (a) adjusting pH of a freezing medium containing cells to a pH of about 6.7 to about 8.5, wherein the freezing medium comprises a buffered solution and a cryoprotective agent; and (b) freezing the cells.

[0015a] In one aspect, provided herein is a method of freezing mammalian cells for storage comprising (a) adjusting pH of a freezing medium containing mammalian cells to a pH of about 6.7 to about 8.5, wherein the freezing medium comprises a buffered solution and a cryoprotective agent; and (b) freezing the mammalian cells.

[0016] In some embodiments, the pH is adjusted to a pH of about 6.7 to about 8.3, about 6.8 to about 8.3, about 6.9 to about 8.3, about 7.0 to about 8.3, about 7.1 to about 8.3, about 7.2 to about 8.3, about 7.3 to about 8.3, about 7.4 to about 8.3, about 7.5 to about 8.3, about 7.2 to about 8.0, about 7.2 to about 7.8, or about 7.5. In some embodiments, the adjusted pH is a target pH or a measured pH. In some embodiments, the target pH is about 6.7 to about 8.5, or any of the pH or in any pH ranges described herein. In some embodiments, the target pH is about 7.5. In some embodiments, the measured pH is about 6.7 to about 8.5, or any of the pH or in any pH ranges described herein.

[0017] In some embodiments of the methods described above or herein, the method further comprises a step of measuring an initial pH of the freezing medium containing the cells (e.g., mammalian cells or insect cells) prior to adjusting pH of the freezing medium. In some embodiments, the method further comprises a step of measuring the adjusted pH of the freezing medium. In some embodiments, if the measured pH of the freezing medium is below a target pH, the method comprises repeating the adjusting step and measuring step until the adjusted pH of the freezing medium is about 6.7 to about 8.5, about 7.2 to about 8.3, or any of the pH or in any pH ranges described herein.

[0018] In some embodiments of the methods described above or herein, the pH is adjusted by adding a base. In some embodiments, the base is selected from the group consisting of sodium carbonate, sodium bicarbonate, HEPES sodium salt, sodium hydroxide, and potassium hydroxide. In some embodiments, the pH of the freezing medium is adjusted by adding a base to the freezing medium according to the following formula $V_{\text{base}} = C_{\text{base}} * V_p (pH_t - pH_i)$, wherein C_{base} is a base-specific coefficient, V_{base} is a volume of the base to add to the freezing medium, V_p is the volume of the freezing medium, pH_t is the target pH, and pH_i is the initial pH. In some embodiments, the pH of the freezing medium is adjusted by adding sodium carbonate to the freezing medium according to the following formula $V_{\text{Na}_2\text{CO}_3} = 0.0085V_p (pH_t - pH_i)$, wherein $V_{\text{Na}_2\text{CO}_3}$ is a volume of 1M sodium carbonate to add to the freezing medium, V_p is the volume of the freezing medium, pH_t is the target pH, and pH_i is the initial pH. In some embodiments, the target pH is about 6.7 to about 8.5, or any of the pH or in any pH ranges described herein. In some embodiments, the target pH is about 7.5.

[0019] In some embodiments, the cells (e.g., mammalian cells or insect cells) are in a medium having a pH of about 6.2 to about 6.6 before the cells are combined with a freezing medium.

[0020] In some embodiments, the cryoprotective agent in the freezing medium is DMSO, glycerol, propanediol, ethylene glycol, a macromolecule, a sugar, or a combination thereof. In some embodiments, the DMSO or glycerol in the freezing medium containing the cells (e.g., mammalian cells or insect cells) prior to freezing is at a concentration of about 5% to about 12.5% by volume. In some embodiments, the DMSO or glycerol in the freezing medium containing the cells (e.g., mammalian cells or insect cells) prior to freezing the cells is at a concentration of about 5% to about 10% by volume.

[0021] In some embodiments, the freezing medium containing the cells (e.g., mammalian cells or insect cells) has a cell density of about 8% to about 28% packed cell volume (PCV) prior to freezing.

[0022] In some embodiments, the method further comprises a step of cooling cell culture fluid during cell harvest and concentration process before the cells (e.g., mammalian cells or insect cells) are combined with a freezing medium. In some embodiments, the cell culture fluid is cooled to a temperature at or below about 20°C, or at or below about 10°C.

[0023] Provided herein is a method of freezing eukaryotic cells (e.g., mammalian cells, insect cells, etc.) for storage or improving thaw recovery of cell banks comprising (a) adjusting pH of a freezing medium to a pH of about 6.7 to about 8.5, wherein the freezing medium comprises a buffered solution and a cryoprotective agent; (b) combining the cells with the freezing medium to form a cell pool; and (c) freezing the cells in the cell pool.

[0023a] In one aspect, provided herein is a method of freezing mammalian cells for storage comprising (a) adjusting pH of a freezing medium to a pH of about 6.7 to about 8.5, wherein the freezing medium comprises a buffered solution and a cryoprotective agent; (b) combining the mammalian cells with the freezing medium to form a cell pool; and (c) freezing the mammalian cells in the cell pool.

[0024] In some embodiments, the pH is adjusted to a pH of about 6.7 to about 8.3, about 6.8 to about 8.3, about 6.9 to about 8.3, about 7.0 to about 8.3, about 7.1 to about 8.3, about 7.2 to about 8.3, about 7.3 to about 8.3, about 7.4 to about 8.3, about 7.5 to about 8.3, about 7.2 to about 8.0, about 7.2 to about 7.8, or about 7.5. In some embodiments, the adjusted pH is a target pH or a measured pH. In some embodiments, the target pH is about 6.7 to about 8.5, or any of the pH or in any pH ranges described herein. In some embodiments, the measured pH is about 6.7 to about 8.5, or any of the pH or in any pH ranges described herein.

[0025] In some embodiments, the method further comprises a step of measuring the adjusted pH of the freezing medium. In some embodiments, if the measured pH of the freezing medium is below a target pH, the method further comprises repeating the adjusting step and measuring step until the adjusted pH of the freezing medium is about 6.7 to about 8.5, or any of the pH or in any pH ranges described herein.

[0026] In some embodiments of the methods described above or herein, the pH is adjusted by adding a base. In some embodiments, the base is selected from the group consisting of sodium carbonate, sodium bicarbonate, HEPES sodium salt, sodium hydroxide, and potassium hydroxide.

[0027] In some embodiments of the methods described above or herein, the cells (e.g., mammalian cells or insect cells) are in a medium having a pH of about 6.2 to about 6.6 before the cells are combined with a freezing medium.

[0028] In some embodiment of the methods described above or herein, the cryoprotective agent in the freezing medium is DMSO, glycerol, propanediol, ethylene glycol, a macromolecule, a sugar, or a combination thereof. In some embodiment, the DMSO or glycerol in the cell pool is at a concentration of about 5% to about 12.5% by volume, or about 5% to about 10% by volume.

[0029] In some embodiments of the methods described above or herein, the cell pool contains the cells (e.g., mammalian cells or insect cells) at a cell density of about 8% to about 28% packed cell volume (PCV).

[0030] In some embodiments of the methods described above or herein, the method further comprises a step of cooling cell culture fluid during cell harvest and concentration process before the cells (e.g., mammalian cells or insect cells) are combined with a freezing medium. In some embodiments, the cell culture fluid is cooled to a temperature at or below about 20°C. In some embodiments, the cell culture fluid is cooled to a temperature at or below about 10°C.

[0031] In some embodiments of the methods described above or herein, the cells are mammalian cells, such as Chinese hamster ovary (CHO) cells, NS0 murine myeloma cells, PER.C6® human cells, or hybridomas. In some embodiments, the cells are insect cells, such as High Five™, S2 (Schneider 2), Sf9, and Sf21. In some embodiments of the methods described above or herein, the cells (e.g., mammalian cells or insect cells) comprise a nucleic acid encoding a polypeptide. In some embodiments, the polypeptide is a therapeutic protein. In some embodiments, the therapeutic protein is selected from the group consisting of an antibody, an antibody fragment, an enzyme, and a receptor fusion protein.

[0032] Provided herein is an eukaryotic cell pool (e.g., a mammalian cell pool, or an insect cell pool) for freezing cells comprising a buffered solution, a cryoprotective agent, and eukaryotic cells comprising a nucleic acid encoding a polypeptide, wherein the medium has a pH of about 6.7 to about 8.5 or about 7.2 to about 8.3 (or any of the pH or in any

pH ranges described herein) prior to freezing the cells.

[0032a] In one aspect, provided herein is a mammalian cell pool for freezing mammalian cells comprising a buffered solution, a cryoprotective agent, and mammalian cells comprising a nucleic acid encoding a polypeptide, wherein the medium has a pH of about 6.7 to about 8.5 prior to freezing the cells.

[0032b] In some embodiments, the cells are mammalian cells, such as Chinese hamster ovary (CHO) cells, NS0 murine myeloma cells, PER.C6® human cells, or hybridomas. In some embodiments, the cells are insect cells, such as High Five™, S2 (Schneider 2), Sf9, and Sf21. In some embodiments, the cells (e.g., mammalian cells or insect cells) comprise a nucleic acid encoding a polypeptide. In some embodiments, the polypeptide is a therapeutic protein. In some embodiments, the therapeutic protein is selected from the group consisting of an antibody, an antibody fragment, an enzyme, and a receptor fusion protein.

[0033] Provided herein is a cell bank comprising a plurality of containers and each container contains (a) a freezing medium comprising a buffer and a cryoprotective agent, and (b) eukaryotic cells (e.g., mammalian cells or insect cells) comprising a nucleic acid encoding a polypeptide, wherein the freezing medium has a pH of about 6.7 to about 8.5 or about 7.2 to about 8.3 (or any of the pH or in any pH ranges described herein) prior to freezing the cell. In some embodiments, the containers are ampoules.

[0033a] In one aspect, provided herein is a cell bank comprising a plurality of containers and each container contains (a) a freezing medium comprising a buffered solution and a cryoprotective agent, and (b) mammalian cells comprising a nucleic acid encoding a polypeptide, wherein the freezing medium has a pH of about 6.7 to about 8.5 prior to freezing the cell.

[0033b] In some embodiments, the cells are mammalian cells, such as Chinese hamster ovary (CHO) cells, NS0 murine myeloma cells, PER.C6® human cells, or hybridomas. In some embodiments, the cells are insect cells, such as High Five™, S2 (Schneider 2), Sf9, and Sf21. In some embodiments, the cells (e.g., mammalian cells or insect cells) comprise a nucleic acid encoding a polypeptide. In some embodiments, the polypeptide is a therapeutic protein. In some embodiments, the therapeutic protein is selected from the group consisting of an antibody, an antibody fragment, an enzyme, and a receptor fusion protein.

[0034] It is to be understood that one, some, or all of the properties of the various embodiments described herein may be combined to form other embodiments of the present invention. These and other aspects of the invention will become apparent to one of skill in the art.

BRIEF DESCRIPTION OF THE DRAWINGS

[0035] FIG. 1 shows an example of a cell banking process flow.

[0036] FIGs. 2A-2B show thaw passage day 1 viability (FIG. 2A) and day 4 growth (PCV) (FIG. 2B) results for cell banks generated from pH adjusted pools at initial pH of 6.6 or 6.3.

[0037] FIGs. 3A-3F show thaw passage day 1 viability (FIGs. 3A, 3C, and 3E) and overall growth rate by PCV (FIGs. 3B, 3D, and 3F) vs. banking pH and the effect of adjusting pH to a