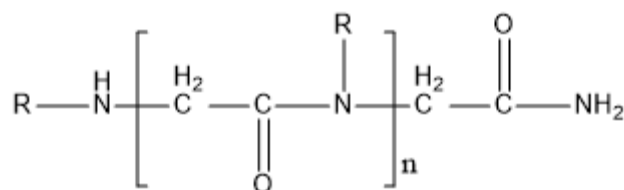


CLAIMS

1. A DMSO-free cryopreservation solution, comprising, per 100 mL, 0.01-50 g of a biomimetic ice growth inhibition material, 5.0-45 mL of a polyalcohol, a water-soluble saccharide at 0.1-1.0 mol L⁻¹, 0-30 mL of serum and the balance of a buffer, wherein the biomimetic ice growth inhibition material is selected from an atatic PVA having a syndiotacticity of 15%-60%, a molecular weight of 10-500 kDa and a degree of hydrolysis of greater than 80%, and/or a combination of said atatic PVA and an amino acid biomimetic ice growth inhibition material.

2. The cryopreservation solution according to claim 1, wherein the amino acid biomimetic ice growth inhibition material is selected from one or two or more of a polyamino acid with a degree of polymerization ≥ 2 , an amino acid and a peptide compound;

preferably, the peptide compound is a polypeptide, a glycopeptide derivative or a compound of formula (I):



formula (I),

wherein R is selected from substituted or unsubstituted alkyl, and the substituent may be selected from -OH, -NH₂, -COOH, -CONH₂ and the like; for example, R is substituted or unsubstituted C₁₋₆ alkyl, and preferably R is -CH₃, -CH₂CH₃ or -CH₂CH₂COOH; n is an integer greater than or equal to 1 and less than or equal to 1000.

3. The cryopreservation solution according to claim 1 or 2, wherein ~~the ice growth inhibition material is a PVA, and~~ the content of the atatic PVA is 0.1-6.0 g;

preferably, the polyalcohol may be C2-5 polyalcohol, such as any one of ethylene glycol, propylene glycol and glycerol;

preferably, the water-soluble saccharide may be at least one of a non-reducing disaccharide, a water-soluble polysaccharide and a glycoside, for example, selected from sucrose, water-soluble cellulose (e.g., hydroxypropyl methylcellulose), trehalose and polysucrose;

preferably, the buffer may be at least one of DPBS, hepes-buffered HTF buffer and other cell buffers;

according to the present invention, the serum can be selected from human serum albumin or a substitute thereof, such as sodium dodecyl sulfate, for a human-derived cryopreservation object, and can be fetal bovine serum or bovine serum albumin for a non-human-derived cryopreservation object.

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4. The cryopreservation solution according to any one of claims 1 to 3, wherein the biomimetic ice growth inhibition material is a polyamino acid or an amino acid, and the content of the ice growth inhibition material is 0.01-50 g;

preferably, the ice growth inhibition material is a combination of a PVA and an amino acid and/or a polyamino acid, for example consists of 0.1-5.0 g of the PVA, 8.0-35 g of the amino acid and/or 1.0-9.0 g of the polyamino acid.

5. The cryopreservation solution according to any one of claims 1 to 4, wherein ~~the PVA is selected from one of or a combination of two or more of an isotactic PVA, a syndiotactic PVA and an atactic PVA; for example, the PVA has a syndiotacticity of 15%-60%, preferably 50%-60%;~~

~~preferably, the PVA is selected from a PVA having a molecular weight of 10-500 kDa or higher;~~

~~preferably, the PVA is selected from a PVA having a degree of hydrolysis of greater than 80%;~~

preferably, the polyamino acid is a homopolymer (with a degree of polymerization ≥ 2) of at least one selected from lysine, arginine, proline, threonine, histidine, glutamic acid, aspartic acid, glycine and the like;

preferably, the polypeptide is selected from a peptide consisting of 2 to 8 different amino acids, such as a dipeptide, a tripeptide or a tetrapeptide, for example selected from one or two or more of L-Thr-L-Arg (TR), L-Thr-L-Pro (TP), L-Arg-L-Thr (RT), L-Pro-L-Thr (PT), L-Thr-L-Arg-L-Thr (TRT), L-Thr-L-Pro-L-Thr (TPT), and L-Ala-L-Ala-L-Thr (AAT);

preferably, the glycopeptide derivative is synthesized by a saccharide and an amino acid, for example is a molecule consisting of gluconolactone (GDL) and an ice-philic amino acid through chemical bonding, and for example is at least one of GDL-L-Thr, GDL-L-Gln, GDL-L-Asn, GDL-L-Phe, GDL-L-Tyr, GDL-L-Val and GDL-L-Ser.

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6. The cryopreservation solution according to any one of claims 1 to 5, wherein the content of the polyalcohol content is 6.0-28 mL;

preferably, the content of serum is 0;

preferably, the content of the water-soluble saccharide is 0.1-1.0 mol L⁻¹;

preferably, the pH of the cryopreservation solution is 6.5-7.6.

7. A preparation method of the cryopreservation solution according to any one of claims 1 to 6, comprising: dissolving an ice growth inhibition material in a buffer, cooling to room temperature before adjusting the pH, dissolving other components in the rest buffer, and mixing after cooling;
5 wherein

preferably, the preparation method comprises:

(1) dissolving ~~a~~the atatic PVA in a portion of a buffer and cooling to room temperature before adjusting the pH to give a solution 1;

(2) optionally, dissolving a polyamino acid or an amino acid in a portion of the buffer and
10 cooling to room temperature before adjusting the pH to form a solution 2;

(3) dissolving a water-soluble saccharide in a portion of the buffer and adding other components except serum after the water-soluble saccharide is completely dissolved to prepare a solution 3; and

(4) mixing the solution 1, optionally the solution 2 and the solution 3 after they are cooled to
15 room temperature, and adjusting the pH and making up to a predetermined volume with the buffer to give the cryopreservation solution;

optionally, when the cryopreservation solution comprises serum, the serum is added when the cryopreservation solution is used.

20 8. A DMSO-free freezing equilibration solution comprising, per 100 mL, 0-5.0 g of an atatic PVA, 5.0-45 mL of a polyalcohol, 0-30 mL of serum and the balance of a buffer; wherein the atatic PVA has a syndiotacticity of 15%-60%, a molecular weight of 10-500 kDa and a degree of hydrolysis of greater than 80%;

preferably, the content of the PVA is 0.1-4.0 g.

25 9. The freezing equilibration solution according to claim 8, wherein the freezing equilibration solution comprises, per 100 mL, 7.5-15 mL of the polyalcohol, 10-20 mL of the serum and the balance of the buffer;

preferably, the freezing equilibration solution comprises, per 100 mL, 0.5-3.5 g of the atatic
30 PVA, 7.5-15 mL of the polyalcohol and the balance of the buffer.

10. A DMSO-free cryopreservation reagent comprising the cryopreservation solution according to any one of claims 1 to 6 and the freezing equilibration solution according to any one of claims 8 to 9, wherein the cryopreservation solution and the freezing equilibration solution are
35 independently present;

preferably, the content of serum is 0, and the freezing equilibration solution comprises, per 100 mL, 0.5-2.5 g of an atatic PVA, 7.5-15 mL of a polyalcohol and the balance of a buffer;

wherein the atatic PVA has a syndiotacticity of 15%-60%, a molecular weight of 10-500 kDa and a degree of hydrolysis of greater than 80%.

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11. Use of the cryopreservation solution according to any one of claims 1 to 6 and/or the freezing equilibration solution according to any one of claims 8 to 9 in cryopreservation of biological tissues.

10 12. The use according to claim 11, wherein the biological tissue is selected from at least one of an oocyte, an embryo, a stem cell, an organ and tissue.