(19) Patent Publication **CN 119464200 A**
(12) Patent Application Publication
(10) Pub. No.: CN 119464200 A
(43) Pub. Date: Feb. 18, 2025

(21) Application No.: 202411705611.6
(22) Filing Date: Nov. 26, 2024
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(51) Int. Cl.: C12N 5/0775 (2010.01)

A FREEZE-DRYING LYOPROTECTANT FOR SIRT6 EXTRACELLULAR VESICLES, SIRT6 EXTRACELLULAR VESICLE LYOPHILIZED POWDER, AND PREPARATION METHODS AND APPLICATIONS THEREOF

ABSTRACT
The present application relates to the field of biomedicine, specifically disclosing a lyoprotectant for SIRT6 extracellular vesicles, SIRT6 extracellular vesicle lyophilized powder, and preparation methods and applications thereof. The SIRT6 extracellular vesicle lyoprotectant provided in the present application comprises the following component contents: polyvinyl alcohol 0.8–1.5 g/100 mL, dextran 0.5–0.8 g/100 mL, trehalose 5.5–6 g/100 mL, mannitol 2.5–4 g/100 mL, vitamin C 0.35–0.4 g/100 mL, ectoin (hydroxyectoine) 0.15–0.3 g/100 mL, superoxide dismutase 0.8–1.2 g/100 mL, with phosphate-buffered saline (PBS) as the solvent. The present application also provides SIRT6 extracellular vesicle lyophilized powder and a preparation method thereof. The SIRT6 extracellular vesicle lyoprotectant of the present application can reduce structural damage to SIRT6 extracellular vesicles caused by freeze-drying, maintaining structural integrity and biological functionality.

CLAIMS

1. A SIRT6 extracellular vesicle lyoprotectant, characterized in that it comprises the following components per 100 mL: polyvinyl alcohol 0.8–1.5 g, dextran 0.5–0.8 g, trehalose 5.5–6 g, mannitol 2.5–4 g, vitamin C 0.35–0.4 g, ectoin 0.15–0.3 g, and superoxide dismutase 0.8–1.2 g, with phosphate-buffered saline (PBS) as the solvent.
2. The SIRT6 extracellular vesicle lyoprotectant according to claim 1, characterized in that it comprises the following components per 100 mL: polyvinyl alcohol 1.0–1.2 g, dextran 0.5–0.8 g, trehalose 5.5–6 g, mannitol 2.5–4 g, vitamin C 0.35–0.4 g, ectoin 0.2–0.25 g, and superoxide dismutase 0.8–1.2 g, with phosphate-buffered saline (PBS) as the solvent.
3. The SIRT6 extracellular vesicle lyoprotectant according to claim 1, characterized in that it comprises the following components per 100 mL: polyvinyl alcohol 1.0 g, dextran 0.6 g, trehalose 6 g, mannitol 3.5 g, vitamin C 0.4 g, ectoin 0.25 g, and superoxide dismutase 1.0 g, with phosphate-buffered saline (PBS) as the solvent.
4. The SIRT6 extracellular vesicle lyoprotectant according to any one of claims 1 to 3, characterized in that the concentration of said phosphate-buffered saline (PBS) is 0.01 M, and the pH is 7.4.
5. A SIRT6 extracellular vesicle lyophilized powder, characterized in that it comprises SIRT6 extracellular vesicles and the SIRT6 extracellular vesicle lyoprotectant according to any one of claims 1 to 4.

6. A method for preparing the SIRT6 extracellular vesicle lyophilized powder according to claim 5, characterized in that it comprises the following steps: adding the lyoprotectant to SIRT6 extracellular vesicles, followed by pre-freezing, primary freeze-drying, and secondary freeze-drying to obtain the SIRT6 extracellular vesicle lyophilized powder; wherein the concentration of said SIRT6 extracellular vesicles in the lyoprotectant is 5–10 g/100 mL.
7. The method for preparing the SIRT6 extracellular vesicle lyophilized powder according to claim 6, characterized in that: the temperature of said pre-freezing is -10 to -15 °C for a duration of 10–20 min; the temperature of said primary freeze-drying is -35 to -50 °C for a duration of 30–60 min; and the temperature of said secondary freeze-drying is -75 to -85 °C for a duration of 1.5–2.5 h.
8. A SIRT6 extracellular vesicle injection, characterized in that it comprises the SIRT6 extracellular vesicle lyophilized powder according to claim 5.
9. A method for preparing the SIRT6 extracellular vesicle injection according to claim 8, characterized in that it comprises the following steps: adding normal saline to the SIRT6 extracellular vesicle lyophilized powder for resuspension at a resuspension ratio of 0.05–0.1 mg/mL.

DESCRIPTION

A FREEZE-DRYING LYOPROTECTANT FOR SIRT6 EXTRACELLULAR VESICLES, SIRT6 EXTRACELLULAR VESICLE LYOPHILIZED POWDER, AND PREPARATION METHODS AND APPLICATIONS THEREOF

TECHNICAL FIELD

[0001] The present application relates to the field of biomedicine, specifically concerning a freeze-drying lyoprotectant for SIRT6 extracellular vesicles, SIRT6 extracellular vesicle lyophilized powder, and preparation methods and applications thereof.

BACKGROUND ART

[0002] In the biomedical field, extracellular vesicles serve as crucial intercellular communication tools with enormous potential in the treatment and diagnosis of various diseases. In recent years, with rapid advancements in biotechnology, the application range of extracellular vesicles has continually expanded, exhibiting significant advantages particularly in drug delivery systems, disease biomarker detection, and cellular therapies. However, extracellular vesicles face numerous challenges in practical application, especially regarding maintaining stability and bioactivity during long-term storage and transportation.

[0003] To address these challenges, researchers typically employ various methods to protect the structure and function of extracellular vesicles. Common approaches include utilizing various lyoprotectants, such as sugars (e.g., sucrose, glucose), polyols (e.g., glycerol, sorbitol), proteins (e.g., BSA), and other additives (e.g., surfactants). Through physical and chemical interactions, these lyoprotectants alleviate damage caused by water removal during freeze-drying, thereby improving vesicle stability and activity.

[0004] SIRT6 extracellular vesicles are multigene-modified human umbilical cord mesenchymal stem cell (hUCMSC) extracellular vesicles extracted from SIRT6-immortalized hUCMSCs. Possessing low immunogenicity, high stability, and the ability to naturally cross biological barriers, SIRT6 extracellular vesicles have become an attractive option for treating diseases such as osteoarthritis (OA). However, although existing lyoprotectants for SIRT6 extracellular vesicles improve stability to a certain extent, they often fail to completely prevent structural damage to the vesicles during freeze-drying, leading to impaired biological functionality.

[0005] Therefore, designing a more effective lyoprotectant to reduce structural damage during freeze-drying and ensure structural integrity and functionality is of paramount importance for the clinical application of SIRT6 extracellular vesicles.

SUMMARY OF THE INVENTION

[0006] To reduce structural damage during freeze-drying and ensure structural integrity and functionality post-lyophilization, the present application provides a SIRT6 extracellular vesicle lyoprotectant, SIRT6 extracellular vesicle lyophilized powder, and preparation methods and applications thereof.

[0007] In a first aspect, the SIRT6 extracellular vesicle lyoprotectant provided by the present application adopts the following technical scheme:

A SIRT6 extracellular vesicle lyoprotectant, comprising the following components per 100 mL: polyvinyl alcohol 0.8–1.5 g, dextran 0.5–0.8 g, trehalose 5.5–6 g, mannitol 2.5–4 g, vitamin C 0.35–0.4 g, ectoin 0.15–0.3 g, and superoxide dismutase 0.8–1.2 g, with phosphate-buffered saline (PBS) as the solvent.

[0008] The present application provides a SIRT6 extracellular vesicle lyoprotectant that significantly reduces structural damage to SIRT6 extracellular vesicles during freeze-drying, effectively maintaining structural integrity and biological activity. Specifically, polyvinyl alcohol exhibits excellent film-forming properties and stabilizing effects, forming a protective film during freeze-drying to prevent vesicle rupture. Dextran and trehalose, as non-reducing sugars, form a stable vitreous (glassy) structure that reduces ice crystal formation during water evaporation, thereby mitigating physical damage. Mannitol acts as a bulking agent, aiding in maintaining osmotic pressure balance across cell membranes and reducing loss of intra-vesicular contents. Vitamin C provides antioxidant effects to scavenge free radicals and prevent oxidative damage; ectoin stabilizes the activity of extracellular vesicles, particularly offering excellent protection during freeze-thaw cycles, thereby reducing vesicle denaturation and damage while maintaining structural integrity and activity. Superoxide dismutase further clears excessive free radicals, protecting intra-vesicular biomolecules from oxidative damage. Through the synergistic action of these components, the present application effectively protects extracellular vesicle structure, significantly reduces loss rates during lyophilization, enhances the recovery rate of SIRT6 extracellular vesicles and the stability of carried bioactive substances post-freeze-drying, extends shelf life, and establishes a solid foundation for subsequent research and therapeutic applications.

[0009] Optionally, the SIRT6 extracellular vesicle lyoprotectant comprises the following components per 100 mL: polyvinyl alcohol 1.0–1.2 g, dextran 0.5–0.8 g, trehalose 5.5–6 g, mannitol 2.5–4 g, vitamin C 0.35–0.4 g, ectoin 0.2–0.25 g, and superoxide dismutase 0.8–1.2 g, with phosphate-buffered saline as the solvent.

[0010] In some embodiments, the content of polyvinyl alcohol may be 0.8–1.0 g/100 mL, 0.8–1.2 g/100 mL, 0.8–1.5 g/100 mL, 1.0–1.2 g/100 mL, 1.0–1.5 g/100 mL, or 1.2–1.5 g/100 mL.

[0011] In a specific embodiment, the content of polyvinyl alcohol may also be 0.8 g/100 mL, 1.0 g/100 mL, 1.2 g/100 mL, or 1.5 g/100 mL.

[0012] In some embodiments, the content of ectoin may be 0.15–0.2 g/100 mL, 0.15–0.25 g/100 mL, 0.15–0.3 g/100 mL, 0.2–0.25 g/100 mL, 0.2–0.3 g/100 mL, or 0.25–0.3 g/100 mL.

[0013] In a specific embodiment, the content of ectoin may also be 0.15 g/100 mL, 0.2 g/100 mL, 0.25 g/100 mL, or 0.3 g/100 mL.

[0014] Optionally, the SIRT6 extracellular vesicle lyoprotectant comprises the following components per 100 mL: polyvinyl alcohol 1.0 g, dextran 0.6 g, trehalose 6 g, mannitol 3.5 g, vitamin C 0.4 g, ectoin 0.25 g, and superoxide dismutase 1.0 g, with phosphate-buffered saline as the solvent.

[0015] Optionally, the concentration of phosphate-buffered saline is 0.01 M, with a pH of 7.4.

[0016] In a second aspect, the present application provides a SIRT6 extracellular vesicle lyophilized powder, comprising SIRT6 extracellular vesicles and the SIRT6 extracellular vesicle lyoprotectant according to any one of claims 1 to 4.

[0017] In a third aspect, the present application provides a method for preparing a SIRT6 extracellular vesicle lyophilized powder, comprising the steps of: adding lyoprotectant to SIRT6 extracellular vesicles, subjecting the mixture to pre-freezing, primary freeze-drying, and secondary freeze-drying to obtain the SIRT6 extracellular vesicle lyophilized powder; wherein the concentration of SIRT6 extracellular vesicles in the lyoprotectant is 5–10 g/100 mL.

[0018] Optionally, the pre-freezing temperature is -10 to -15 °C for 10–20 min; the primary freeze-drying temperature is -35 to -50 °C for 30–60 min; and the secondary freeze-drying temperature is -75 to -85 °C for 1.5–2.5 h.

[0019]-[0028] Specific parameters for freezing temperatures and durations in various embodiments include pre-freezing (-10 °C or -15 °C for 10 min or 20 min), primary freezing (-35 °C, -40 °C, -45 °C, or -50 °C for 30 min, 40 min, 50 min, or 60 min), and secondary freezing (-75 °C, -80 °C, or -85 °C for 1 h, 1.5 h, or 2.5 h).

[0029] In a fourth aspect, the present application provides a reconstitution method for SIRT6 extracellular vesicle lyophilized powder, comprising: adding normal saline to the SIRT6 extracellular vesicle lyophilized powder for resuspension at a ratio of 0.05–0.1 mg/mL.

[0030] In summary, the present application offers the following beneficial effects:

1. By utilizing polyvinyl alcohol, trehalose, ectoin, etc., a lyoprotectant is prepared that significantly reduces damage to SIRT6 extracellular vesicle structures during lyophilization, effectively preserving structural integrity and bioactivity.
2. By further controlling polyvinyl alcohol (1.0–1.2 g/100 mL) and ectoin (0.2–0.25 g/100 mL), superior protective performance for SIRT6 extracellular vesicles is achieved.

DETAILED DESCRIPTION

[0031]-[0035] The raw materials used in the present application (e.g., PVA CAS: 9002-89-5, Dextran 70 CAS: 58798-70-2, Mannitol CAS: 87-78-5, Vitamin C CAS: 50-81-7, Ectoin CAS: 165542-15-4, SOD CAS: 9054-89-1) are commercially available. SIRT6 extracellular vesicles were extracted according to Example 11 of CN118726486A.

PREPARATION EXAMPLES 1–4

[0038]-[0040] Preparation Examples 1–4 provide SIRT6 extracellular vesicle lyoprotectants differing in PVA content as shown in Table 1 below. Components (PVA, Dextran 0.6g, Trehalose 6g, Mannitol 3.5g, Vitamin C 0.4g, Ectoin 0.25g, SOD 1.0g per 100 mL 0.01M PBS pH 7.4) were dissolved and mixed thoroughly.

Table 1: Polyvinyl Alcohol (PVA) Addition Amount in Preparation Examples 1–4

Preparation Example	PVA Addition Amount (g / 100 mL)
1	0.8
2	1.0
3	1.2
4	1.5

PREPARATION EXAMPLES 5–7

[0041]-[0042] Preparation Examples 5–7 provide SIRT6 extracellular vesicle lyoprotectants differing in Ectoin addition amounts as shown in Table 2.

Table 2: Ectoin Addition Amount in Preparation Examples 2 & 5–7

Preparation Example	Ectoin Addition Amount (g / 100 mL)
2	0.25
5	0.15
6	0.20
7	0.30

COMPARATIVE PREPARATION EXAMPLES 1–3

[0043]-[0047] Comparative Preparation Example 1 contains 0 g PVA; Comparative Preparation Example 2 contains 0 g Ectoin; Comparative Preparation Example 3 contains PVA 2.0g, Dextran 1.0g, Trehalose 8g, Mannitol 1.5g, Vitamin C 0.3g, Ectoin 0.35g, SOD 1.5g.

EXAMPLES 1–13 & COMPARATIVE EXAMPLES 1–5

[0048]-[0059] Lyophilized powders were prepared using 10 mg SIRT6 extracellular vesicles in 100 mL lyoprotectant with freezing parameters detailed in Table 3.

Table 3: Freezing Parameters for Examples 2 & 8–13

Example	Pre-freezing		Primary Freezing		Secondary Freezing	
	Temp (°C)	Time (min)	Temp (°C)	Time (min)	Temp (°C)	Time (h)
2	-10	20	-35	60	-75	2.5
8	-15	10	-35	60	-75	2.5
9	-10	20	-40	50	-75	2.5
10	-10	20	-45	40	-75	2.5
11	-10	20	-50	30	-75	2.5
12	-10	20	-35	60	-80	1.0
13	-10	20	-35	60	-85	1.5

PERFORMANCE TESTING

[0060] The lyoprotectant performance was evaluated by measuring relative SIRT6 protein expression levels before and after lyophilization. Lyophilized powders were resuspended in saline (0.08 mg/mL). The initial SIRT6 expression level before freeze-drying was set as 1.0. Results are shown in Table 4.

Table 4: Relative SIRT6 Protein Expression Levels After Reconstitution

Group	Relative SIRT6 Expression	Group	Relative SIRT6 Expression
Example 1	0.95	Example 10	0.98
Example 2	0.99	Example 11	0.97
Example 3	0.98	Example 12	0.99
Example 4	0.96	Example 13	0.98
Example 5	0.97	Comp. Example 1	0.77
Example 6	0.98	Comp. Example 2	0.81
Example 7	0.96	Comp. Example 3	0.88
Example 8	0.98	Comp. Example 4	0.72
Example 9	0.99	Comp. Example 5	0.64

[0061] As shown in Table 4, SIRT6 extracellular vesicle lyophilized powders provided in Examples 1–13 maintained relative SIRT6 expression levels of 0.95–0.99 after reconstitution, whereas Comparative Examples 1–5 retained only 0.64–0.88. This demonstrates that utilizing the lyoprotectant of the present application in combination with the multi-step freezing process preserves the structural integrity and bioactivity of SIRT6 extracellular vesicles, holding significant value for clinical applications.