(19) Patent Publication **CN 109957542 A**
(12) Patent Application Publication
(10) Pub. No.: CN 109957542 A
(43) Pub. Date: Jul. 02, 2019

(21) Application No.: 201910177644.0
(22) Filing Date: Mar. 09, 2019
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(51) Int. Cl.: C12N 5/0775 (2010.01); A01N 1/02 (2006.01); A61K 35/28 (2015.01); A61P 39/06 (2006.01)

PREPARATION METHOD OF HUMAN UMBILICAL CORD MESENCHYMAL STEM CELL FORMULATION FOR ROUTINE ANTI-AGING TREATMENT

ABSTRACT

The present invention relates to a preparation method of a human umbilical cord mesenchymal stem cell (hUCMSC) formulation for routine anti-aging treatment. The method comprises: obtaining umbilical cord tissue pieces, primary stem cell culture, stem cell passage, cryopreservation, resuscitation & culture, and final hUCMSC formulation preparation. Resuspension and concentration adjustment (4×10^4–6×10^4 cells/mL) are performed using 5% glucose injection for passages P_e (where e = 6, 7, 8, 9, 10). The invention improves stem cell reserves while guaranteeing high viability, proliferation capacity, and phenotypic specificity.

CLAIMS

1. A method for preparing a human umbilical cord mesenchymal stem cell formulation for routine anti-aging treatment, characterized in that it comprises steps of obtaining umbilical cord tissue pieces, primary stem cell culture, stem cell passage, stem cell cryopreservation, stem cell resuscitation & culture, and preparation of the stem cell formulation;
wherein said stem cell cryopreservation step comprises: when P_n generation human umbilical cord mesenchymal stem cells reach 80%–90% confluence, discarding supernatant, adding 0.5 wt% TrypLE Express solution, stopping digestion with complete culture medium upon completion, centrifuging and discarding supernatant, washing cells with physiological saline, resuspending in serum-free MSC freezing medium, aliquoting into cryovials and sealing, placing into a controlled-rate freezing container at -80 °C for 12–24 hours, and immediately transferring to liquid nitrogen;
wherein said formulation preparation step comprises: resuspending passage P_e human umbilical cord mesenchymal stem cells in 5% glucose injection, gently pipetting until uniform, supplementing 5% glucose injection, and adjusting cell concentration to 4×10^4–6×10^4 cells/mL to obtain the final formulation; wherein e = 6, 7, 8, 9, 10.
2. The method according to claim 1, characterized in that said serum-free MSC cryopreservation formulation comprises, by weight parts: serum-free medium 80 parts, dimethyl sulfoxide (DMSO) 10 parts, L-menthyl glucoside 5 parts, glycerol 4 parts, and N-acetylglucosamine 1 part.
3. The method according to claim 1, characterized in that obtaining umbilical cord tissue pieces comprises: disinfecting umbilical cord tied at both ends, placing in physiological saline containing 1 wt% double-antibiotic; removing tied ends, cutting the middle segment into 0.8–1.2 cm pieces, rinsing blood with antibiotic-saline; dissecting tissue, removing blood vessels and epidermis to collect Wharton's jelly, rinsing Wharton's jelly, and mincing into 1 mm × 1 mm × 1 mm tissue pieces.
4. The method according to claim 1, characterized in that primary culture comprises: seeding tissue pieces adherently into culture dishes, standing at 37 °C / 5% CO_2 for 1–2 hours, adding complete medium, changing half medium every 3 days, standing for 14 days, discarding tissue and medium, washing with saline, digesting with 0.5 wt% TrypLE Express at 37 °C

for 2–3 minutes until cells round up, adding complete medium to stop digestion, and pipetting to yield P_1 stem cell suspension.

5. The method according to claim 1, characterized in that stem cell passage comprises: at 80%–90% confluence of P_n cells, digesting with TrypLE Express, stopping with complete medium, centrifuging, washing with saline, resuspending in serum-free medium, seeding into T-175 flasks at 37 °C / 5% CO_2 to obtain P_{n+1} cells.
6. The method according to claim 1, characterized in that resuscitation comprises: thawing P_n cryovials in a 37 °C water bath, transferring to saline, centrifuging, resuspending in serum-free medium, seeding into flasks at 37 °C / 5% CO_2 to obtain P_{n+1} cells.
7. The method according to claim 2, 5, or 6, characterized in that said serum-free medium is HY STEMCELL Human MSC Serum-Free Medium.
8. The method according to claim 1, 2, 5, or 6, characterized in that the target stem cell suspension concentration is 1×10^7 cells/mL.

DESCRIPTION

PREPARATION METHOD OF HUMAN UMBILICAL CORD MESENCHYMAL STEM CELL FORMULATION FOR ROUTINE ANTI-AGING TREATMENT

TECHNICAL FIELD

[0001] The present invention relates to the field of biomedicine, specifically concerning a preparation method of a human umbilical cord mesenchymal stem cell (hUCMSC) formulation for routine anti-aging treatment.

BACKGROUND ART

[0002]-[0003] Aging is a complex physiological process accompanied by organ function decline and age-related diseases. Stem cell therapy offers huge promise in regenerative medicine and anti-aging applications. However, challenges remain in perfecting stem cell banking and cryopreservation technologies to maintain viability and potency.

SUMMARY OF THE INVENTION & FORMULATION

[0004]-[0014] The present invention provides a complete SOP for hUCMSC anti-aging formulations, featuring a high-efficiency **serum-free cryopreservation solution**:

> **Proprietary Serum-Free Cryopreservation Formula (by weight parts):**
> • Serum-Free Culture Medium: 80 parts
> • Dimethyl Sulfoxide (DMSO): 10 parts
> • L-Menthyl Glucoside: 5 parts
> • Glycerol: 4 parts
> • N-Acetylglucosamine: 1 part

[0023] The final clinical formulation is suspended in **5% Glucose Injection** at a concentration of **4×10^4–6×10^4 cells/mL** (passages P_6 to P_{10}), ensuring immediate clinical applicability, safety, and osmotic stability.

EXPERIMENTAL VALIDATION & PHENOTYPIC STABILITY

[0029] **Flow Cytometry Surface Marker Phenotyping:**

Specific surface antigens (CD34-, CD45-, CD44+, CD90+) were evaluated post-thaw after 3 days of culture:

- **Fresh Unfrozen Controls (Comparative Ex. 1 & 2):**
  P_6: CD34-/CD44+ = 99.9%, CD45-/CD90+ = 99.9%
  P_{10}: CD34-/CD44+ = 99.9%, CD45-/CD90+ = 99.8%
- **Post-Thaw Resuspended Cells (Examples 1 & 2):**
  P_6: CD34-/CD44+ = 99.6%, CD45-/CD90+ = 98.9%
  P_{10}: CD34-/CD44+ = 99.5%, CD45-/CD90+ = 98.6%

[0030] **Cell Proliferation & Growth Curve Analysis:**

Growth curves over 7 days confirmed that post-thaw hUCMSCs restored rapid exponential proliferation, maintaining near-identical growth kinetics to unfrozen cells while significantly outperforming standard conventional freezing methods.