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METHOD FOR EXTRACTING EXOSOMES FROM ANIMAL UMBILICAL CORD MESENCHYMAL STEM CELLS

ABSTRACT
The present application specifically discloses a method for extracting exosomes from animal umbilical cord mesenchymal stem cells (UCMSCs). The method includes: obtaining animal umbilical cord tissue and culturing UCMSCs; extracting exosomes by culturing passage P3–P20 UCMSCs in complete medium, collecting supernatant, centrifuging to remove dead cells/debris, filtering microparticles, and centrifuging to precipitate exosomes; resuspending exosome pellets in PBS and storing at -70 to -80 °C. The extraction method of the present application offers simple operation and high exosome yield.

CLAIMS

1. A method for extracting exosomes from animal umbilical cord mesenchymal stem cells, characterized in that it comprises the following steps:
obtaining animal umbilical cord tissue and culturing umbilical cord mesenchymal stem cells;
extracting exosomes: culturing passage P3–P20 umbilical cord mesenchymal stem cells in complete culture medium, collecting supernatant, centrifuging the supernatant to remove dead cells and cell debris, filtering to remove microparticles, and then precipitating exosomes from the supernatant by centrifugation;
resuspending the exosome pellet in PBS and storing at -70 to -80 °C;
wherein said obtaining animal umbilical cord tissue and culturing umbilical cord mesenchymal stem cells specifically comprises the following steps:
S1. processing animal umbilical cord tissue by removing membrane and vascular tissues;
S2. transferring processed umbilical cord tissue to a centrifuge tube and adding collagenase for digestion at 35–37 °C for 3–6 hours;
S3. filtering the digested tissue suspension from S2 and centrifuging the filtrate to obtain a cell pellet;
S4. resuspending the cell pellet in complete medium and seeding into cell culture dishes for 24 hours;
S5. washing cultured cells with PBS buffer and replacing with fresh culture medium;
S6. repeating step S5 every 36–48 hours;
S7. subculturing cells when cell confluence reaches 70%–80%;
wherein said complete culture medium comprises, by weight percentage: basal culture medium 80%–90%, exosome-free fetal bovine serum 8%–12%, double-antibiotic solution 0.5%–1.5%, vitamin C 0.1%–0.4%, L-glutamine 1.5%–3%, transferrin 0.01%–0.05%, and ferric nitrate 0.1%–0.18%;
and the weight ratio of transferrin to ferric nitrate is 1:(2–6).
2. The method according to claim 1, characterized in that by weight percentage, said complete culture medium comprises: basal culture medium 80%–86.65%, exosome-free fetal bovine serum 10%, double-antibiotic solution 1%, vitamin C 0.2%, L-glutamine 2%, transferrin 0.03%, and ferric nitrate 0.12%.

3. The method according to claim 1, characterized in that said basal culture medium is DMEM/F12 medium.
4. The method according to claim 1, characterized in that culturing passage P3–P20 umbilical cord mesenchymal stem cells in complete culture medium further comprises electrical stimulation culture, subjecting cells to alternating electrical stimulation with an electric field strength of 100–500 mV/cm and a frequency of 5–20 Hz.
5. The method according to claim 4, characterized in that said electric field strength is 200–400 mV/cm, and said frequency is 10–20 Hz.
6. The method according to claim 4, characterized in that the waveform of the alternating current during stimulation is a bidirectional square wave.
7. The method according to claim 1, characterized in that said centrifugation method comprises:
   centrifuging at 300–600 g for 10 min at 4–6 °C, collecting supernatant;
   then centrifuging at 2,000–3,000 g for 10 min at 4–6 °C, collecting supernatant;
   subsequently centrifuging at 10,000–12,000 g for 10 min at 4–6 °C, collecting supernatant;
   finally centrifuging at 100,000–150,000 g for 1.5–2.5 hours at 4–6 °C to obtain the exosome pellet.

DESCRIPTION

METHOD FOR EXTRACTING EXOSOMES FROM ANIMAL UMBILICAL CORD MESENCHYMAL STEM CELLS

TECHNICAL FIELD

[0001] The present application relates to the field of cell technology, specifically concerning a method for extracting exosomes from animal umbilical cord mesenchymal stem cells (UCMSCs).

BACKGROUND ART

[0002]-[0004] Exosomes are small membrane-bound vesicles (30–150 nm) containing complex RNA and proteins. MSC-derived exosomes harbor various functional proteins and RNAs involved in tissue repair and cell communication. However, current exosome yields from UCMSCs remain low, hindering large-scale commercial and clinical applications.

SUMMARY OF THE INVENTION

[0005]-[0062] To significantly increase exosome yield and purity, the present application provides an extraction method utilizing a specially optimized complete culture medium (enriched with **transferrin and ferric nitrate in a 1:2 to 1:6 ratio**) combined with **bipolar alternating electrical stimulation** (100–500 mV/cm, 5–20 Hz, bidirectional square wave).

DETAILED DESCRIPTION & EXAMPLES

FORMULATION AND ELECTRICAL STIMULATION PARAMETERS

Table 1: Complete Medium Component Ratios (wt%) in Examples 1–8 & Comparative Examples 1–4

Group	Exo-Free FBS	Antibiotics	Vit C	L-Glutamine	Transferrin	Ferric Nitrate	Basal Medium
Example 1	10%	1%	0.2%	2%	0.02%	0.10%	Balance
Example 2	10%	1%	0.2%	2%	0.03%	0.10%	Balance
Example 3	10%	1%	0.2%	2%	0.04%	0.10%	Balance
Example 4	10%	1%	0.2%	2%	0.05%	0.10%	Balance
Example 5	10%	1%	0.2%	2%	0.03%	0.12%	Balance
Example 6	10%	1%	0.2%	2%	0.03%	0.15%	Balance
Example 7	10%	1%	0.2%	2%	0.03%	0.18%	Balance
Example 8	10%	1%	0.2%	2%	0.04%	0.16%	Balance
Comp. Ex. 1	10%	1%	0.2%	2%	0%	0.12%	Balance
Comp. Ex. 2	10%	1%	0.2%	2%	0.03%	0%	Balance
Comp. Ex. 3	10%	1%	0.2%	2%	0.05%	0.20%	Balance
Comp. Ex. 4	10%	1%	0.2%	2%	0.02%	0.08%	Balance

Table 2: Electrical Stimulation Parameters for Examples 5, 9–12 & Comparative Examples 5–6

Group	Electric Field Strength (mV/cm)	Frequency (Hz)
Example 5	300	15
Example 9	200	15
Example 10	400	5
Example 11	300	10
Example 12	300	20
Example 13	No Electrical Stimulation Control	
Comp. Ex. 5	500	15
Comp. Ex. 6	300	30

EXPERIMENTAL TESTING & RESULTS

[00162]-[00168] Western blot confirmed strong positive expression for CD9 and CD63 without Calnexin expression. Nanoparticle Tracking Analysis (ZetaView PMX 110) confirmed particle sizes between 80–140 nm.

Table 3: Exosome Yield, Size, and Purity Results

Group	Particle Concentration (particles/mL)	Main Peak Size (nm)	Main Peak Percentage (%)
Example 1	7.4×10^{10}	113.4	95.4%
Example 2	9.4×10^{10}	119.3	95.9%
Example 3	8.8×10^{10}	111.6	96.9%
Example 4	8.1×10^{10}	111.4	95.3%
Example 5	**11.4×10^{10}**	**120.6**	**98.2%**
Example 6	9.9×10^{10}	117.3	96.1%
Example 7	7.3×10^{10}	116.9	96.2%
Example 8	10.8×10^{10}	119.4	97.5%
Example 9	7.3×10^{10}	119.3	95.3%
Example 10	9.3×10^{10}	121.2	95.8%
Example 11	11.0×10^{10}	120.2	96.2%
Example 12	10.3×10^{10}	119.3	96.7%
Example 13	5.3×10^{10}	118.6	95.5%
Comp. Ex. 1	0.6×10^{10}	102.4	84.1%
Comp. Ex. 2	1.1×10^{10}	109.9	91.5%
Comp. Ex. 3	3.2×10^{10}	113.2	92.4%
Comp. Ex. 4	1.8×10^{10}	114.8	91.7%
Comp. Ex. 5	3.7×10^{10}	105.2	87.7%
Comp. Ex. 6	5.0×10^{10}	104.3	91.1%