Claims

1. Method for producing an exosome containing therapeutic agent, comprising the steps:
   - Separating mononuclear cells from a bone marrow sample of a healthy donor;
   - Treating the separated mononuclear cells in a culture media at 37 °C and at 5% CO_2 for 48-72 h;
   - Separating an exosome composition from the culture media;
   - Dissolving the separated exosome composition in a phosphate buffer to obtain the exosome containing therapeutic agent;

   ~~characterized in that~~ wherein the culture media comprises a 1:1 mixture of DMEM/F12 and HEPES, 2 mmol/mL or 3.65 mg/10mL L-glutamine, 100 units/mL penicillin, 100 µg/mL streptomycin, 10% fetale bovine serum;

   wherein separating the mononuclear cells comprises:

   i. applying the mononuclear cells in the culture media to Ficoll-Urografin (F-U) solution with a density of 1.077 g/cm^3, wherein the ratio of separated mononuclear cells in the culture media to the Ficoll-Urografin (F-U) is 1:3-1:4, followed by centrifugation for 20 min at 1500 rpm;

   ii. Collecting an interphase ring with mononuclear cells after centrifugation;

   iii. Mixing the interphase ring with a Hanks salt solution in a 1:1 ratio and centrifuging the mixture for 3 min at 1500 rpm;

   iv. Optionally repeating step iii., wherein after each centrifugation mononuclear cells are separated.

2. The method of claim 1, wherein dissolving the separated exosome composition in a phosphate buffer is performed such that a ratio of 1:5 to 1:20 of separated exosome composition to phosphate buffer is obtained.

3. The method of any of the preceding claims, wherein step iii) is repeated, and wherein after each centrifugation mononuclear cells are separated. ~~separating the mononuclear cells comprises:~~

~~i. Applying the treated separated mononuclear cells in the culture media to Ficoll-Urografin (F-U) solution with a density of 1.077 g/cm³, wherein the ratio of separated mononuclear cells in the culture media to the Ficoll-Urografin (F-U) is 1:3-1:4, followed by centrifugation for 20 min at 1500 rpm;~~

~~ii. Collecting an interphase ring with mononuclear cells after centrifugation;~~

~~iii. Mixing the interphase ring with a Hanks salt solution in a 1:1 ratio and centrifuging the mixture for 3 min at 1500 rpm;~~

~~iv. Optionally repeating step iii., particularly 2 or 3 times, wherein after each centrifugation mononuclear cells are separated.~~

~~8.~~4. The method of claims 1 or 2, wherein the step of separating the exosome composition from the culture media further comprises a differential centrifugation.

~~9.~~5. The method of claim 4, wherein the differential centrifugation is performed for 10 min at 3000 rpm, followed by collecting a generated first supernatant and centrifuging the first supernatant for 30 min at 10 000 rpm, followed by collecting a generated second supernatant and ultra-centrifuging the second supernatant for 2 h at 100 000 rpm, wherein after each centrifuging step, an exosome composition is separated.

~~10. A method of treating leukaemia in a subject, comprising:~~

~~- Obtaining a bone marrow sample from a healthy donor;~~

~~- Separating mononuclear cells from the bone marrow sample;~~

~~- Treating the separated mononuclear cells in a culture media at 37 °C and at 5% CO_2 for 48-72 h;~~

~~- Separating an exosome composition from the culture media;~~

- Dissolving the separated exosome composition in a phosphate buffer to obtain the exosome containing therapeutic agent;

- Administering the obtained therapeutic agent intravenously to the subject;

characterized in that the culture media comprises a 1:1 mixture of DMEM/F12 and HEPES, 2 mmol/mL or 3.65 mg/10mL L glutamine, 100 units/mL penicillin, 100 µg/mL streptomycin, 10% fetale bovine serum.

18. The method of claim 6, wherein dissolving the separated exosome composition in a phosphate buffer is performed such that a ratio of 1:5 to 1:20 of separated exosome composition to phosphate buffer is obtained.

19. The method of claims 6 or 7, wherein separating mononuclear cells further comprises:

i. Applying the treated separated mononuclear cells in the culture media to Ficoll-Urografin (F-U) solution with a density of 1.077 g/cm³, wherein the ratio of separated mononuclear cells in the culture media to the Ficoll-Urografin (F-U) is 1:3-1:4, followed by centrifugation for 20 min at 1500 rpm;

ii. Collecting an interphase ring with mononuclear cells after centrifugation;

iii. Mixing the interphase ring with a Hanks salt solution in a 1:1 ratio and centrifuging the mixture for 3 min at 1500 rpm;

iv. Optionally repeating step iii., particularly 2 or 3 times, wherein after each centrifugation mononuclear cells are separated.

24. The method of claims 6 or 8, wherein the step of separating the exosome composition from the culture media further comprises a differential centrifugation.

25. The method of claim 9, wherein the differential centrifugation is performed for 10 min at 3000 rpm, followed by collecting a generated first supernatant and centrifuging the first supernatant for 30 min at 10 000 rpm, followed by collecting a generated second

~~supernatant and ultra-centrifuging the second supernatant for 2 h at 100 000 rpm, wherein after each centrifuging step, an exosome composition is separated.~~

~~26.~~6. ~~A culture media for producing an exosome composition, comprising a 1:1 mixture of DMEM/F12 and HEPES, 2 mmol/mL or 3.65 mg/10mL L glutamine, 100 units/mL penicillin, 100 µg/mL streptomycin, 10% fetale bovine serum.~~The method of claim 3, wherein step iii) is repeated two or three times.