Method for producing an exosome containing therapeutic agent

Field of disclosure

The present invention is directed to a method for producing an exosome containing therapeutic agent, a method for treatment with an exosome containing therapeutic agent and a culture media for producing an exosome containing therapeutic agent.

Background, prior art

RU 2593003 C2 discloses method for treatment of tumors is known in which a patient is treated with an exosome containing medicament.

A major disadvantage of this method is its insufficient specificity, which is due to the fact that the exosomes are collected from a cell line and not from a human donor. Even leukemia cell lines do not resemble phenotypical features of a particular tumor of the patient and do not include mutations which may at least partially contribute to the development of resistance against a therapeutic agent. Furthermore, this method does not allow for restoration of the immune system, which is usually suppressed by administration of chemotherapeutic agents during treatment. K562/i-S9 cell line is used as the one that produces exosomes which are then loaded with the therapeutic agent according to RU 2593003 C2. This cell line cannot reflect the genetic and surface antigen landscape of a patient`s target cells, especially if the patient has a tumor different from hemoblastosis. Exosomes which are collected from the patient`s target cells cultured *in vitro* resemble antigen and receptor specificity of the cells these exosomes are targeted for what provides them more effective transfer to the target cells. Furthermore, these exosomes are not immunogenic as far as they are collected from autologous biomaterial.

It is an object of the present invention to provide an efficient therapeutic agent for the treatment of leukemia.

It is a further object of the invention to provide a method of treating leukaemia in a subject.

Summary of disclosure

In a first aspect, the invention is directed to a 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% CO2 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.

The culture media comprises a 1:1 mixture of DMEM/F12 (Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12, according to Dulbecco, R. & Freeman, G. (1959) Virology 8, 396 and Smith et al. (1960) Virology 12, 185) and HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), 2 mmol/mL or 3.65 mg/10mL L-glutamine, 100 units/mL penicillin, 100 µg/mL streptomycin, 10% fetale bovine serum.It is understood that the method for producing an exosome containing therapeutic agent and in particular step of separating mononuclear cells from the bone marrow sample of a healthy donor, is performed in vitro.

Typically, the mononuclear cells may be collected by centrifugation.

In some embodiments, 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.

In further embodiments, 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/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., particularly 2 or 3 times, wherein after each centrifugation mononuclear cells are separated.

Typically, the Hanks salt solution consists of 185.41 mg/l calcium chloride dihydrate, 48 mg/l disodium hydrogenphosphate, 97.72 mg/l magnesium sulfate, 400 mg/l potassium chloride, 8 g/l sodium chloride, 60 mg/l potassium dihydrogenphosphate und 1 g/l glucose.

In other embodiments, the step of separating the exosome composition from the culture media further comprises a differential centrifugation.

In some embodiments, 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.

According to a second aspect, the invention is directed to 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 (Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12, according to Dulbecco, R. & Freeman, G. (1959) Virology 8, 396 and Smith et al. (1960) Virology 12, 185) and HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), 2 mmol/mL or 3.65 mg/10mL L-glutamine, 100 units/mL penicillin, 100 µg/mL streptomycin, 10% fetale bovine serum.

In some embodiments, 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.

In some embodiments, treating the 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/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., particularly 2 or 3 times, wherein after each centrifugation, an exosome composition is separated.

In other embodiments, the step of separating the exosome composition from the culture media further comprises a differential centrifugation.

In some embodiments, 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.

According to a third aspect, the invention is directed to 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.

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 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.

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 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/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., particularly 2 or 3 times, wherein after each centrifugation mononuclear cells are separated.

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.

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.

6. 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.

7. 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.

8. 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/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., particularly 2 or 3 times, wherein after each centrifugation mononuclear cells are separated.

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

10. 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.

11. 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.