disease, Parkinson's disease, and Huntington's disease.

In order to treat such mitochondria-related diseases, researches on materials that can be used for protecting mitochondria or restoring mitochondrial dysfunction, methods that allow efficient targeted delivery of such materials, or the like have been continued. However, from the viewpoint that there are problems of a rather limited therapeutic range, occurrence of side effects, or the like, there has not yet been developed an epoch-making treatment for mitochondria-related diseases.

In addition, as a method for fundamentally blocking mitochondria-related diseases, a technique for extracting a nuclear genetic material from an oocyte having mitochondrial mutations and transplanting the nuclear genetic material into an oocyte having normal mitochondria is emerging. However, biological controversy and ethical controversy have prevented relevant laws from being passed.

Accordingly, in the course of making an effort to find a method that effectively enhances intracellular mitochondrial activity while not conflicting with bioethics, the present inventors have devised a technique capable of injecting healthy mitochondria without causing cell damage.

Disclosure of Invention

Aspects of the invention are defined in the independent claims. Some optional features of the invention are defined in the dependent claims.

Thus it may be possible to provide a method for injecting mitochondria isolated from cells into specific cells. In addition, it may also be possible to provide cells having improved mitochondrial function, the cells being obtained by delivering normal mitochondria into cells at a high delivery rate without damaging the cells to which the mitochondria are to be administered.

Accordingly, there is provided a method for delivering exogenous mitochondria into recipient cells, comprising a) a step of mixing the recipient cells with mitochondria isolated from donor cells, and b) a step of centrifuging the resulting mixture.

In addition, the method may further comprise a step of adding a surfactant or a step of

performing incubation, before the step b).

Also provided is a cell into which an exogenous mitochondrion has been introduced according to the above method.

Advantageous Effects

Through the method of the present disclosure, mitochondria isolated from normal cells can be effectively injected into recipient cells. In particular, the method of the present disclosure has advantages of exhibiting a very high injection yield and causing no cell damage, since it is possible to regulate an amount of mitochondria to be delivered into cells.

Brief Description of Drawings

FIG. 1 illustrates results obtained by observing intracellular mitochondria with photofluorography.

FIG. 2 illustrates results obtained by observing isolated mitochondria with photofluorography.

FIG. 3 illustrates a schematic diagram for a mitochondrial isolation process.

FIG. 4 illustrates a graph obtained by comparing mitochondrial activity depending on a donor cell type.

FIG. 5 illustrates a graph obtained by identifying mitochondrial function by measuring an ATP content in isolated mitochondria.

FIG. 6 illustrates a result obtained by identifying high purity of isolated mitochondria by analyzing expression of mitochondrial markers and nuclear markers.

FIGS. 7A to 7C illustrate results obtained by identifying mitochondrial delivery into UC-MSCs using a mitochondrial staining pattern.

FIGS. 7D to 7F illustrate results obtained by identifying mitochondrial delivery with 3D Z-stack.

FIGS 7G to 7I illustrate results obtained by identifying a survival rate of isolated mitochondria using mitochondria-specific MitoTracker probes.

FIG. 8 illustrates results obtained by identifying a delivery rate of mitochondria, which have been delivered into UC-MSCs, through FACS analysis.

FIG. 9 illustrates a result obtained by identifying, using qPCR, delivery of UC-MSC-

times, and then mitochondria are delivered into the recipient cells through centrifugation.

Detailed Description

The present disclosure provides a method for delivering exogenous mitochondria into recipient cells, comprising a) a step of mixing the recipient cells with mitochondria isolated from donor cells, and b) a step of centrifuging the resulting mixture.

First, a step of mixing recipient cells with mitochondria isolated from donor cells is provided.

Here, "donor cells" refer to cells that provide mitochondria. In addition, the donor cells may be normal cells which contain normal mitochondria. Specifically, the donor cells may be cells isolated from a mammal including an animal or a human. In addition, the donor cells may be animal cells, and may be, each independently, any one selected from the group consisting of somatic cells, stem cells, cancer cells and a combination thereof.

In addition, the somatic cells may be any one selected from the group consisting of muscle cells, hepatocytes, fibroblasts, epithelial cells, neurons, adipocytes, osteocytes, leukocytes, lymphocytes, mucosal cells, and combinations thereof; may be any one selected from the group consisting of muscle cells, hepatocytes, fibroblasts, and combinations thereof; or may be muscle cells or hepatocytes. Preferably, the donor cells may be muscle cells with excellent mitochondrial activity.

In addition, the stem cells refer to undifferentiated cells having an ability to differentiate into various types of tissue cells. The stem cells may be any type of stem cells such as adult stem cells, mesenchymal stem cells, induced pluripotent stem cells, and tissue-derived stem cells.

In addition, the cancer cells may be any cells selected from the group consisting of squamous cell carcinoma, small cell lung cancer, non-small cell lung cancer, lung cancer, adenocarcinoma, peritoneal cancer, colon cancer, biliary tumor, nasopharyngeal cancer, laryngeal cancer, bronchial cancer, oral cancer, osteosarcoma, gallbladder cancer, kidney cancer, leukemia, bladder cancer, melanoma, brain cancer, glioma, brain tumor, skin cancer, pancreatic cancer, breast cancer, liver cancer, bone marrow tumor, esophageal cancer, colorectal cancer, stomach cancer, cervical cancer, prostate cancer, ovarian cancer, head and

neck cancer, and rectal cancer.

On the other hand, "recipient cells" refer to cells into which mitochondria are introduced. In addition, the recipient cells may be normal cells, and may be any one of white blood cells and lymphocytes which are immune-related cells. In addition, the recipient cells may be abnormal cells that contain abnormal mitochondria due to a congenital or acquired factor.

In another example of the present disclosure, the donor cells and the recipient cells may be respectively any one selected from the group consisting of somatic cells, stem cells, cancer cells, and combinations thereof.

In addition, the donor cells and the recipient cells may be allogeneic or xenogeneic to each other. The donor cells and the recipient cells may be obtained from the same subject or from different subjects.

In the step a), a method of isolating mitochondria from the donor cells can be selected from various known methods, for example, a method of isolating mitochondria using a specific buffer solution or a method of extracting mitochondria using potential difference and magnetic field.

As an embodiment for the method of isolating mitochondria, in order to maintain mitochondrial activity, mitochondria may be obtained by disrupting a cell and performing centrifugation. In an embodiment, isolation of mitochondria may be done by a step of culturing donor cells and performing a primary centrifugation of a composition that contains such cells, to produce a pellet, a step of resuspending the pellet in a buffer solution and performing homogenization, a step of performing a secondary centrifugation of the homogenized solution, to prepare supernatant, and a step of performing a tertiary centrifugation of the supernatant, to purify mitochondria. Here, from the viewpoint of maintaining cell activity, it is preferable that the time for performing the secondary centrifugation is regulated to be shorter than the time for performing the primary and tertiary centrifugations. It is possible to increase the speed as the step proceeds from the primary centrifugation to the tertiary centrifugation.

Specifically, the primary to tertiary centrifugations may be performed at a temperature of 0°C to 40°C, and preferably at a temperature of 3°C to 5°C. In addition, the centrifugations may be performed for 0.5 minutes to 50 minutes, 1 minute to 20 minutes, or 2 minutes to 30

minutes, and preferably 5 minutes. In addition, the time for performing the centrifugation can be appropriately adjusted depending on the number of centrifugations, a content of a sample, and the like. The centrifugations may be performed with a speed of 1 × g to 2,400 × g, 350 × g to 2,000 × g, or 600 × g to 1,600 × g, and preferably 1,500 × g.

In addition, the primary centrifugation may be performed with a speed of 100 to 1,000 × g, 200 to 700 × g, or 300 to 450 × g. In addition, the secondary centrifugation may be performed with a speed of 1 to 2,000 × g, 25 to 1,800 × g, or 500 to 1,600 × g. In addition, the tertiary centrifugation may be performed with a speed of 100 to 20,000 × g, 500 to 18,000 × g, or 800 to 15,000 × g.

Next, a step of delivering exogenous mitochondria into the recipient cells is provided, the step comprising a step of centrifuging the resulting mixture.

Here, the centrifugation may be performed at 100 × g, 300 × g, 500 × g, 800 × g, 1,000 × g, 1,200 × g, 1,500 × g, 1,800 × g, 2,000 × g, 2,400 × g, 3,000 × g, 5,000 × g, or 10,000 × g. In addition, the time for performing the centrifugation may be 0.1 minutes to 60 minutes, but is not limited thereto. Specifically, the time may be 1 minute, 2 minutes, 3 minutes, 5 minutes, 10 minutes, 20 minutes, or 30 minutes.

The centrifugation may be performed at a temperature of 0°C to 40°C, 20°C to 38°C, or 30°C to 37°C.

In an example of the present disclosure, in the step b), the centrifugation may be performed at a temperature of 0°C to 40°C for 0.5 to 20 minutes with 1 to 2,400 × g.

As such, by applying a centrifugal force to both the recipient cells and the mitochondria isolated from the donor cells, the mitochondria can be delivered into the recipient cells with high efficiency while causing less damage to the recipient cells. In addition, in a case where the recipient cells have mitochondria with defects, mitochondria isolated from donor cells that contain normal mitochondria can be injected into the recipient cells, so that functions of the recipient cells due to mitochondrial defects can be restored.

In addition, in the step b), it may be more effective to perform the centrifugation of the mixture in a test tube whose diameter gradually narrows toward the bottom, in terms of a centrifugal force to be applied to the recipient cells and mitochondria, and contact efficiency therebetween. The centrifugation may be performed one to three additional times under the

same condition.

In an embodiment of the present disclosure, in a case where the centrifugation is performed with a speed of 1,500 × g for 5 minutes, it was found that mitochondria are best delivered into the recipient cells.

In addition, in the step a), the mitochondria may be mixed, at varying amounts, with the recipient cells. Specifically, the mitochondria may be contained in an amount of 0.005 to 25 μg, 0.01 to 23 μg, or 0.1 to 20 μg, per 1×10^5 recipient cells. Specifically, the mitochondria may be contained in an amount of 0.1 μg, 1 μg, 3 μg, 5 μg, 10 μg, 15 μg, or 18 μg, per 1×10^5 recipient cells. In a case where the mitochondria are contained in an amount of fewer than 0.005 μg per 1×10^5 recipient cells, a somewhat decreased delivery yield may be obtained.

In addition, before performing the step b), the method may further comprise a step of adding a surfactant to the mixture obtained in the step a). The surfactant is used to enhance cell membrane permeability of the recipient cells. A time point at which the surfactant is added may be before, during, or after mixing of the recipient cells with the mitochondria. In addition, after the surfactant is added to the recipient cells, the recipient cells may be incubated for a certain period of time in order to increase the cell membrane permeability of the recipient cells. The time for which the recipient cells are left to stand may be 0.1 to 60 minutes. Specifically, the time may be 1 minute, 5 minutes, 10 minutes, 20 minutes, or 30 minutes, but is not limited thereto.

Specifically, the surfactant is preferably a nonionic surfactant, and may be a poloxamer. Here, the poloxamer is a triblock copolymer composed of a central hydrophobic chain of polyoxypropylene flanked by two hydrophilic chains of polyoxyethylene. In addition, the surfactant in the mixture may have a concentration of 1 to 100 mg/ml, 3 to 80 mg/ml, or 5 to 40 mg/ml, and preferably 10 to 30 mg/ml.

In addition, before performing the step b), the method may further comprise a step of incubating the mixture obtained in the step a) under a predetermined time and temperature condition. The incubation may be performed at a temperature of 0°C to 40°C, 20°C to 38°C, or 30°C to 37°C. In addition, the incubation may be performed for 0.1 to 4 hours, 0.5 to 3.8 hours, or 0.8 to 3.5 hours. In addition, the incubation may be performed for a predetermined time, after step b) is performed so as to deliver the mitochondria to the recipient cells. In addition, the incubation time can be appropriately selected depending on a cell type and an amount of mitochondria.

In addition, before performing the step b), the method may further comprise a step of adding a surfactant to the mixture obtained in the step a) and performing incubation. Here, the surfactant and a condition for the incubation are the same as those described above.

Also disclosed herein is a cell into which an exogenous mitochondrion has been delivered according to the method as described above.

The cell may be contained in a composition for treating a mitochondrial dysfunction disease. Specifically, a composition which contains, as an active ingredient, a cell into which an exogenous mitochondrion has been delivered according to the method as described above can be administered to treat a disease. Here, the cell of the present disclosure can be provided for administration in an effective amount to a mammal having a health condition which is directly relevant or irrelevant to the target disease.

As such, the delivery method according to the present disclosure can improve mitochondrial function of a cell into which an exogenous mitochondrion is to be introduced, and the cell obtained according to the present disclosure can be used to improve mitochondrial function and to treat or prevent a mitochondrial dysfunction disease.

Hereinafter, preferred examples of the present disclosure will be described in order to facilitate understanding of the present disclosure. However, the following examples are provided only for the purpose of easier understanding of the present disclosure, and the present disclosure is not limited by the following examples.

Preparation Example 1. Preparation of cells

Human adipocyte-derived mesenchymal stem cells (AD-MSCs; PCS-500-011, passage #7), human breast cancer cell line (MCF-7; HTB-22, passage #20), human adenocarcinoma alveolar epithelial cells (A549; CCL-185, passage #20), human leukemia cell line (K562; CCL-243, passage #20), human liver cell line (WRL-68; CL-48, passage #9), human dermal fibroblasts (HDFs; PCS-201-012, passage #9), and human natural killer cell line (NK-92; CRL-2407, passage #10) were purchased from the Animal Type Culture Collection (Manassas, VA). Human bone marrow-derived mesenchymal stem cells (BM-MSCs; MSC-001F, passage #7) were purchased from STEMCELL Technologies Inc. (Vancouver, Canada). In order to compare delivery efficiency (%) depending on a cell type

CLAIMS

1. A method for delivering exogenous mitochondria into recipient cells, comprising:

a) a step of mixing the recipient cells with mitochondria isolated from donor cells; and

b) a step of centrifuging the resulting mixture.

2. The method of claim 1, wherein, in the step a), the mitochondria and the recipient cells are present in the mixture such that the mitochondria are contained in a weight of 0.005 to 25 μg per 1×10^5 cells of the recipient cells.

3. The method of claim 1, wherein in the step b), the centrifugation is performed at a temperature of 0°C to 40°C for 0.5 to 20 minutes with 1 to 2,400 × g.

4. The method of any preceding claim, further comprising: a step of adding a surfactant before the step b).

5. The method of claim 4, wherein the surfactant in the mixture has a concentration of 1 and 100 mg/ml.

6. The method of any preceding claim, further comprising: a step of performing incubation before the step b).

7. The method of claim 6, wherein the incubation is performed at a temperature of 0°C to 40°C for 0.1 to 4 hours.

8. The method of claim 1, wherein the donor cells and the recipient cells are respectively any one selected from the group consisting of somatic cells, stem cells, cancer cells, and combinations thereof.

9. The method of claim 8, wherein the somatic cells are any one selected from the group consisting of muscle cells, hepatocytes, fibroblasts, epithelial cells, neurons, adipocytes, osteocytes, leukocytes, lymphocytes, mucosal cells, and combinations thereof.

10. The method of any preceding claim, wherein the donor cells are allogeneic or xenogeneic to the recipient cells.

11. The method of any of claims 1 to 9, wherein the donor cells and the recipient cells are obtained from the same subject or from different subjects.

12. A cell into which exogenous mitochondria have been delivered by the method according to any one of claims 1 to 11.