COMPOSITION FOR ANTI-AGING

FIELD OF THE INVENTION

[0001] The present invention is related to an antiaging composition. The present invention is further related to a use of a conditioned medium for the preparation of the antiaging composition.

BACKGROUND OF THE INVENTION

[0002] Mitochondrion is a very important organelle in cells which perform the function of respiration and synthesis of ATP (adenosine triphosphate) and further regulate the metabolism of cells [1, 2]. If the mitochondrion is damaged, free radicals would accumulate in the cells and prevent the mitochondria from generating ATP and cell metabolism, and finally result in cell aging. Thus, numerous anti-oxidative or mitochondria-restoring natural products, such as vitamin C, vitamin E, or β-carotene, are used as additives in foods or cosmetics. However, although the natural products were proved to be effective, adverse effect would still occurs if it is overdosed. For example, excessive intake of vitamin C causes nephrolithiasis, excessive intake of vitamin E causes anticoagulant response and higher probability of stroke, and excessive intake of β-carotene increase the susceptibility of lung cancer [5, 6, 7]. Therefore, it is in great need to develop a novel natural product or composition for antiaging.

SUMMARY OF THE INVENTION

[0003] In one aspect provided herein is an antiaging composition, comprising a conditioned medium, wherein the conditioned medium is the medium in which umbilical cord-derived mesenchymal stem cells and oxidative stress-damaged cells are co-cultured.

[0004] In another aspect provided herein is a use of a conditioned medium for the preparation of antiaging pharmaceutical composition, wherein the conditioned medium is the medium in which umbilical cord-derived mesenchymal stem cells and oxidative stress-damaged cells are co-cultured.

[0005] In a specific embodiment, the oxidative stress-damaged cells are HEK-293.

[0006] According to the present invention, the composition or the pharmaceutical composition is for the use in ameliorating the aging of damaged epithelial cells.

[0007] According to the present invention, the composition or the pharmaceutical composition is for the use in ameliorating the aging of damaged skin cells.

[0008] In a specific embodiment of the present invention, the composition is a cosmetic composition. In a preferred embodiment, the cosmetic composition is suitable for topical administration on the skin of a subject in need.

[0009] It should be understood that both the foregoing general description and the following detailed description are exemplary and illustrative only and are not restrictive of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] The foregoing summary, as well as the following detailed description of the invention, will be better understood when read in conjunction with the appended drawings. For the purpose of illustrating the invention, there are shown in the drawings embodiments which are presently preferred.

[0011] In the drawings:

[0012] Fig. 1 shows the restoration of mitochondria activity of damaged epithelial cell by the conditioned medium of umbilical cord-derived mesenchymal stem cell. Graphics showing (A) the respiratory efficiency of mitochondria of HEK-293 cells, (B) basic respiratory efficiency, (C) efficiency of ATP generation, and (D) maximum respiratory efficiency. The data is presented as the average± S.D. of at least 3 independent experiments. *$p < 0.05$, **$p < 0.01$, compare to the group of basic medium. 1: basic medium, 2: umbilical cord-derived mesenchymal stem cell conditioned medium-1 (UC-MSC-CM (1)), 3: umbilical cord-derived mesenchymal stem cell conditioned medium-2 (UC-MSC-CM (2))

[0013] Fig. 2 shows the amelioration of aging of damaged epithelial cell by the conditioned medium of umbilical cord-derived mesenchymal stem cell. Graphics showing (A) the overall aging of HEK-293 treated by basic medium or umbilical cord-derived mesenchymal stem cell conditioned medium (UC-MSC-CM), (B) the percentage of severe aging of HEK-293 treated by basic medium or umbilical cord-derived mesenchymal stem cell conditioned medium (UC-MSC-CM). The data is presented as the average± S.D. of at least 3 independent experiments. **$p < 0.01$, compare to the group of basic medium.

[0014] Fig. 3 shows the amelioration of aging of damaged skin cell by the conditioned medium of umbilical cord-derived mesenchymal stem cell. Graphics showing (A) the overall aging of HaCaT treated by basic medium or umbilical cord-derived mesenchymal stem cell

conditioned medium (UC-MSC-CM), (B) the percentage of severe aging of HaCaT treated by basic medium or umbilical cord-derived mesenchymal stem cell conditioned medium (UC-MSC-CM). **p < 0.01, compare to the group of basic medium.

DESCRIPTION OF THE INVENTION

[0015] In one aspect provided herein is an antiaging composition, comprising a conditioned medium, wherein the conditioned medium is the medium in which umbilical cord-derived mesenchymal stem cells and oxidative stress-damaged cells are co-cultured.

[0016] In another aspect provided herein is a use of a conditioned medium for the preparation of antiaging pharmaceutical composition, wherein the conditioned medium is the medium in which umbilical cord-derived mesenchymal stem cells and oxidative stress-damaged cells are co-cultured.

[0017] In a specific embodiment, the oxidative stress-damaged cells are HEK-293.

[0018] In a specific embodiment, the oxidative stress damage is caused by the treatment of H_2O_2. In one embodiment, HEK-293 cell is treated by H_2O_2 for 4 hours to cause the oxidative stress damage.

[0019] In a specific embodiment, the umbilical cord-derived mesenchymal stem cells is the cells which is positive for group of surface markers including CD73, CD90 and EphA2, and is negative for group of surface markers including CD34, CD45 and HLA-DR.

[0020] In a specific embodiment, the percentage of umbilical cord-derived mesenchymal stem cells that express EphA2 is over 90%.

[0021] According to the present invention, the composition or the pharmaceutical composition is for the use in the treatment of mitochondria damage.

[0022] According to the present invention, the composition or the pharmaceutical composition is for the use in the restoration of the respiratory efficiency and ATP generation of mitochondria of damaged cells.

[0023] According to the present invention, the composition or the pharmaceutical composition is for the use in ameliorating the aging of damaged skin cells.

[0024] In a specific embodiment, the composition is cosmetic composition. Preferably, the cosmetic composition is suitable for topical administration on skin. For example, the cosmetic composition comprises an effective amount of conditioned medium. According to a specific

embodiment, the cosmetic composition further comprises an acceptable carrier and is prepared as a topical preparation.

[0025] The present invention is further illustrated by the following examples, which are provided for the purpose of demonstration rather than limitation.

[0026] Example 1: The restoration of the activity of mitochondria of damaged epithelial cells by umbilical cord-derived mesenchymal stem cell conditioned medium

[0027] 1. Material and Method

[0028] 1.1 Preparation of umbilical cord-derived mesenchymal stem cell conditioned medium

[0029] HEK-293 cells were inoculated on 24-wells plate ($5x10^4$ cells/well) for 24 hours. Umbilical cord-derived mesenchymal stem cells were inoculated on 0.4 μm trans-well culture plate ($5x10^4$ cells/well) for 24 hours. After that, the medium was removed from the 24-wells plate, the medium comprising 200 μM H_2O_2 was added, and the cells were cultured for 4 hours. The medium was removed from trans-well plate and basic medium was added. After treating for 4 hours by H_2O_2, the trans-well plate was disposed on the 24-wells plate, and umbilical cord-derived mesenchymal stem cells and HEK-293 cells were co-cultured in a 37℃ humidified incubator for 44 hours. An then, the medium in the trans-well plate and 24-wells plate was collected and stored in -80°C refrigerator, which is the umbilical cord-derived mesenchymal stem cell conditioned medium.

[0030] 1.2 Analysis of mitochondria activity of HEK-293 cells

[0031] According to the manual of manufacturer, HEK-293 cells were inoculated on Seahorse XF24 plate ($3.5x10^4$ cells/well) for 16 hours. The medium was removed, the medium comprising 100 μM H_2O_2 was added, and the cells were further cultured for 4 hours. Then, basic medium and umbilical cord-derived mesenchymal stem cell conditioned medium was added and cells were cultured in a 37℃ humidified incubator for 44 hours. 75 μL Oligomycin (20 μM), 85 μL FCCP (40 μM), and 95 μL Antimycin A (20 μM) were respectively added according to three sequential time point. XFe 24 Analyzer was used to analyze the mitochondria activity of HEK-293 cells.

[0032] 2. Result

[0033] The activity of ATP generation of mitochondria was evaluated by adding Oligomycin, which inhibit the ATP generation of mitochondria. The respiratory efficiency of mitochondria was evaluated by adding FCCP. The intactness of mitochondria was reported by

adding Antimycin A. As the result shown in figure 1, umbilical cord-derived mesenchymal stem cell conditioned medium is able to restore the activity of mitochondria of damaged epithelial cells, including basic respiratory efficiency, maximum respiratory efficiency, and ATP generation.

[0034] Example 2: The amelioration of the aging of damaged epithelial cells by umbilical cord-derived mesenchymal stem cell conditioned medium

[0035] 1. Material and method

[0036] 1.1 Preparation of umbilical cord-derived mesenchymal stem cell conditioned medium

[0037] The medium were prepared according to the same method disclosed in Example 1.

[0038] 1.2 Analysis of the aging of HEK-293 cells

[0039] HEK-293 cells were inoculated on 24-wells plate ($4x10^4$ cells/well) for 24 hours. After that, the medium was removed from the 24-wells plate, the medium comprising 200 μM H_2O_2 was added, and the cells were cultured for 4 hours. Umbilical cord-derived mesenchymal stem cell conditioned medium was thawed in 4℃. After treating for 4 hours by H_2O_2, basic medium and the umbilical cord-derived mesenchymal stem cell conditioned medium were added, and HEK-293 cells were cultured in a 37℃ humidified incubator for 48 hours. The medium was then replaced by the medium comprising 100 nM Bafilomycin A1 for 1 hour and further was replaced by the medium comprising 33 μM C_{12}FDG for 2 hours. After that, all medium was removed and the cells were washed by PBS. The cell suspension was collected after TrypLE treatment. The suspension was centrifuged (300 g) for 5 minutes and the supernatant was removed. Cells were re-suspended by PBS. Finally, FITC intensity was analyzed by flow cytometry. Overall aging was measured according to FITC intensity of all cells and is present as n-fold according to control group (normal). When the FITC intensity of cells is 2.5 times higher than normal control group, it is determined as severe aging.

[0040] 2. Result

[0041] C_{12}FDG is a substrate of β-galactosidase, and it would be sliced by β-galactosidase after moving into cell and emit fluorescence. Cell aging can be determined by the detection of fluorescence of cells by flow cytometry. It is shown that umbilical cord-derived mesenchymal stem cell conditioned medium ameliorates the overall aging of damaged epithelial cells and reduces the percentage of severe aged cells (Fig. 2).

[0042] Example 3: The amelioration of the aging of damaged skin cells by umbilical cord-derived mesenchymal stem cell conditioned medium

[0043] 1. Material and Method

[0044] 1.1 Preparation of umbilical cord-derived mesenchymal stem cell conditioned medium

[0045] The medium were prepared according to the same method disclosed in Example 1.

[0046] 1.2 Analysis of aging of HaCaT cells

[0047] HaCaT cells were inoculated on 12-wells plate ($2x10^5$ cells/well) for 24 hours. After that, the medium was removed from the 12-wells plate, the medium comprising 200 μM H_2O_2 was added, and the cells were cultured for 4 hours. Umbilical cord-derived mesenchymal stem cell conditioned medium was thawed in 4℃. After treating for 4 hours by H_2O_2, basic medium and the umbilical cord-derived mesenchymal stem cell conditioned medium were added, and HaCaT cells were cultured in a 37℃ humidified incubator for 72 hours. The medium was then replaced by the medium comprising 100 nM Bafilomycin A1 for 1 hour and further was replaced by the medium comprising 33 μM C_{12}FDG for 2 hours. After that, all medium was removed and the cells were washed by PBS. The cell suspension was collected after TrypLE treatment. The suspension was centrifuged (300 g) for 5 minutes and the supernatant was removed. Cells were re-suspended by PBS. Finally, FITC intensity was analyzed by flow cytometry. Overall aging was measured according to FITC intensity of all cells and is present as n-fold according to control group (normal). When the FITC intensity of cells is 2.5 times higher than normal control group, it is determined as severe aging.

[0048] 2. Result

[0049] C_{12}FDG is a substrate of β-galactosidase, and it would be sliced by β-galactosidase after moving into cell and emit fluorescence. Cell aging can be determined by the detection of fluorescence of cells by flow cytometry. It is shown that umbilical cord-derived mesenchymal stem cell conditioned medium ameliorates the overall aging of damaged skin cells and reduces the percentage of severe aged cells (Fig. 3).

[0050] Reference:

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CLAIMS

What is claimed is:

1. A use of a conditioned medium for the preparation of anti-aging composition, wherein the conditioned medium is the medium in which umbilical cord-derived mesenchymal stem cells and oxidative stress-damaged cells treated with H_2O_2 are co-cultured.

2. The use as claimed in claim 1, wherein the composition is for the use in the treatment of damage of mitochondria and the restoration of the respiratory efficiency and ATP generation of mitochondria in damaged cells.

3. The use as claimed in claim 1, wherein the composition is for the use in ameliorating the aging of damaged epithelial cells or skin cells.