

treatment is enabled by a series of administration, migration, and differentiation of Muse cells at a state where “principle of the site” remains effective, specifically, in a state where damaged cells or their fragments still remain *in situ* such as in the site where inflammatory reaction associated with the damage occurs, for example, in the case of acute disease including myocardial infarction producing damage signal from the damaged site. However, in the case of a chronic disease in which the “principle of the site” has been already lost and the low amount of damaged signal, in the case of diseases where vascular flow is interrupted, or in the case of diseases which requires rare cell replacement, intravascular administration of Muse cells as described above may not be sufficient enough to repair tissues. As such, if it is possible to obtain *in vitro*, both stably and easily at low cost, a large amount of the desired cells from pluripotent stem cells like Muse cells by inducing differentiation, further application to various uses such as regenerative medicine, development of a new pharmaceutical agent, or safety test for pharmaceuticals can be achieved.

[CITATION LIST]

[PATENT LITERATURE]

[0009]

[PTL 1] JP 2013-252081 A

[PTL 2] JP 2004-298087 A

[PTL 3] WO 2011/007900 A

[PTL 4] WO 2014/027684 A

[PTL 5] WO 2014/133170 A

[NON PATENT LITERATURE]

[0010]

[NPL 1] Yang L, et al., Nature, Vol.453, p.524-528 (2008)

[NPL 2] Wakao, S, et al., Proc.Natl.Acad.Sci.USA, Vol.108, p.9875-9880 (2011)

SUMMARY

[TECHNICAL PROBLEM]

[0011]

A conventionally known method for differentiating pluripotent stem cells to various kinds of cells requires multi-step treatments so that it is difficult to prepare a great amount of homogeneous desired cells with a short period of time. Furthermore, it is difficult to exclude the possibility of generating tumorigenicity in cells differentiated from iPS cells which have been established by exogenous gene introduction. As such, an object of the present disclosure is to provide a method for producing, easily and with high efficiency, various differentiated cells that

are homogeneous and highly functional.

[SOLUTION TO PROBLEM]

[0012]

The present inventors have found that, in vitro, Muse cells have a phagocytic property, and, according to phagocytosis of damaged and/or dead cells (including fragments of damaged and/or dead cells), Muse cells differentiate into those cells, and thus completed the present disclosure accordingly.

[0012a] In one aspect, provided herein is a method for inducing differentiation of pluripotent stem cells in vitro into cells having the same phenotype and function as Information Presenting Cells for Differentiation (IPCD), the method comprising co-culturing pluripotent stem cells or a cell fraction having the pluripotent stem cells concentrated therein, either with damaged and/or dead cells as IPCD, and/or fragments of said damaged and/or dead cells, wherein said pluripotent stem cells phagocytize the damaged and/or dead cells as IPCD, and/or fragments of said damaged and/or dead cells, and said pluripotent stem cells are SSEA-3-positive and CD105-positive.

[0012b] In another aspect, provided herein is a method for confirming in vitro that cells contained in a cell preparation are live pluripotent stem cells positive for SSEA-3 and CD-105, including:

- (a) co-culturing cells in the cell preparation with damaged and/or dead cells, and/or fragments of said damaged and/or dead cells; and
- (b) if the cells in the cell preparation are differentiated into cells having the same phenotype and function as exodermal-lineage cells, mesodermal-lineage cells, and/or endodermal-lineage cells derived from the damaged and/or dead cells, and/or fragments of said damaged and/or dead cells, determining that the pluripotent stem cells are alive in the cell preparation.

[0012c] In another aspect, provided herein is a method for in vitro production of cells having the same phenotype and function as IPCD from pluripotent stem cells positive for SSEA-3 and CD-105, the method comprising co-culturing pluripotent stem cells or cell population with high content of the pluripotent stem cells either with damaged and/or dead cells as IPCD, and/or fragments of the damaged and/or dead cells.

[0013]

Specifically, the present disclosure provides as follows.

[1] A method for inducing differentiation of pluripotent stem cells in vitro into cells having the same phenotype and function as Information Presenting Cells for Differentiation (IPCD), the method comprising co-culturing pluripotent stem cells or cell population with high content of the pluripotent stem cells either with damaged and/or dead cells as IPCD, and/or fragments of said damaged and/or dead cells.

[2] The method according to [1] above, wherein the pluripotent stem cells phagocytize damaged and/dead cells as IPCD, and/or fragments of said damaged and/or dead cells.

[3] The method according to [1] or [2] above, wherein the pluripotent stem cells are SSEA-3-positive cells isolated from mesenchymal tissues of a body or cultured mesenchymal cells.

[4] The method according to any one of [1] to [3] above, wherein the pluripotent stem cells are CD105-positive.

[5] The method according to any one of [1] to [4] above, wherein the pluripotent stem cells are CD117-negative and CD146-negative.

[6] The method according to any one of [1] to [5] above, wherein the pluripotent stem cells are CD117-negative, CD146-negative, NG2-negative, CD34-negative, vWF-negative, and CD271-negative.

[7] The method according to any one of [1] to [6] above, wherein the pluripotent stem cells are CD34-negative, CD117-negative, CD146-negative, CD271-negative, NG2-negative, vWF-negative, Sox10-negative, Snai1-negative, Slug-negative, Tyrp1-negative, and Dct-negative.

[8] The method according to any one of [1] to [7] above, wherein the pluripotent stem cells have all of the following properties:

- (i) telomerase activity at low or under detection level;
- (ii) having the ability to differentiate into any of three germ layers;
- (iii) exhibiting no tumorigenic proliferation; and

(iv) having self-renewal ability.

[9] The method according to any one of [1] to [8] above, wherein the ratio between the number of the pluripotent stem cells and the number of the damaged and/or dead cells as IPCD is 1 : 10,000 to 10,000 : 1.

[10] The method according to any one of [1] to [9] above, wherein the damaged and/or dead cells as IPCD, and/or fragments of said damaged and/or dead cells are obtained by adding a pharmaceutical agent selected from the group consisting of apoptosis inducing agent, metabolic antagonist, alkylating agent, anthracycline, antibiotics, antimetabolic agent, topoisomerase inhibitor, proteasome inhibitor, anti-cancer agent, heat, low temperature, acid, alkali, ultrasonic wave, and physical disruption by vortex or the like.

[11] The method according to any one of [1] to [10] above, wherein the IPCD are cells derived from ectoderm.

[12] The method according to [11] above, wherein the cells derived from ectoderm are selected from the group consisting of neural cells, glial cells, pigment cells, skin cells, inner ear cells, retinal cells, corneal cells, and hair follicle cells.

[13] The method according to any one of [1] to [10] above, wherein the IPCD are cells derived from mesoderm.

[14] The method according to [13] above, wherein the cells derived from mesoderm are selected from the group consisting of cardiac muscle cells, skeletal muscle cells, smooth muscle cells, osteocytes, chondrocytes, germ line cells, and hematopoietic cells.

[15] The method according to any one of [1] to [10] above, wherein the IPCD are cells derived from endoderm.

[16] The method according to [15] above, wherein the cells derived from endoderm are selected from the group consisting of pancreatic β cells, liver cells, bile duct cells, respiratory epithelial cells, esophageal epithelial cell, vascular endothelial cells, kidney-constituting cells, bladder epithelial cells, and pancreatic exocrine cells.

[17] The method according to any one of [1] to [10] above, wherein the IPCD are rare cells.

[18] The method according to [17] above, wherein the rare cells are selected from the group consisting of motor neuron, dopamine neuron, intermediate neuron, glutamine-activated neuron, pituitary gland cells, thyroid gland cells, adrenal cortex, adrenal medulla, pressure-sensor cells of carotid artery, heart conduction system, choroid plexus epithelial cells, pancreatic-alpha cells, pancreatic-delta cells, and lung Clara cells.

[19] A method for confirming in vitro that cells contained in a cell preparation are live pluripotent stem cells, including:

(a) co-culturing cells in the cell preparation with damaged and/or dead cells, and/or

fragments of said damaged and/or dead cells; and

(b) if the cells in the cell preparation are differentiated into cells having the same phenotype and function as exodermal-lineage cells, mesodermal-lineage cells, and/or endodermal-lineage cells derived from the damaged and/dead cells, and/or fragments of said damaged and/or dead cells, determining that the pluripotent stem cells are alive in the cell preparation.

[20] A method for in vitro production of cells having the same phenotype and function as IPCD from pluripotent stem cells, the method comprising co-culturing pluripotent stem cells or cell population with high content of the pluripotent stem cells either together with damaged and/or dead cells as IPCE, and/or fragments of the damaged and/or dead cells.

[ADVANTAGEOUS EFFECTS OF DISCLOSURE]

[0014]

According to the present disclosure, by co-culturing pluripotent stem cells together with damaged and/or dead cells as IPCD, and/or together with fragments of the damaged and/or dead cells, the IPCD cell type is obtained from pluripotent stem cells. The differentiated cells that are obtained accordingly can be applied to various uses including regenerative medicine, study relating to development of a new pharmaceutical agent, and safety test for pharmaceuticals.

BRIEF DESCRIPTION OF DRAWINGS

[0015]

FIG. 1 is a view illustrating the result of determining the induction of differentiation using intact mouse cardiac muscle cells. FIGs. 1A and 1B illustrate the result of detecting mRNA expression of various markers when Muse cells are added for a case in which either culture supernatant or exosome obtained from intact mouse cardiac muscle cells is added to a culture system. FIG. 1C illustrates the result of determining the mRNA expression when Muse cells are directly contacted with intact mouse cardiac muscle cells. FIG. 1D illustrates, 3 days and 7 days after the culture based on direct contact, presence or absence of the expression of GATA-4 as determined by immunostaining and observed under a fluorescence microscope.

FIG. 2 illustrates the result of determining the induction of differentiation using damaged mouse cardiac muscle cells. It is the result of detecting mRNA expression of various markers when Muse cells are added for a case in which either culture supernatant or exosome obtained from damaged mouse cardiac muscle cells is added to a culture system (FIGs. 2A and 2B, respectively).

FIG. 3 illustrates the result of determining the phagocytic property of Muse cells. To determine the phagocytic capacity, PKH26PCL (red) was used.

CLAIMS

1. A method for inducing differentiation of pluripotent stem cells in vitro into cells having the same phenotype and function as Information Presenting Cells for Differentiation (IPCD), the method comprising co-culturing pluripotent stem cells or a cell fraction having the pluripotent stem cells concentrated therein, either with damaged and/or dead cells as IPCD, and/or fragments of said damaged and/or dead cells, wherein said pluripotent stem cells phagocytize the damaged and/or dead cells as IPCD, and/or fragments of said damaged and/or dead cells, and said pluripotent stem cells are SSEA-3-positive and CD105-positive.

2. The method according to claim 1, wherein the pluripotent stem cells have been isolated from mesenchymal tissues of a body or cultured mesenchymal cells.

3. The method according to claim 1 or 2, wherein the pluripotent stem cells are CD117-negative and CD146-negative.

4. The method according to any one of claims 1 to 3, wherein the pluripotent stem cells are CD117-negative, CD146-negative, NG2-negative, CD34-negative, vWF-negative, and CD271-negative.

5. The method according to any one of claims 1 to 4, wherein the pluripotent stem cells are CD34-negative, CD117-negative, CD146-negative, CD271-negative, NG2-negative, vWF-negative, Sox10-negative, Snail-negative, Slug-negative, Tyrp1-negative, and Dct-negative.

6. The method according to any one of claims 1 to 5, wherein the pluripotent stem cells have all of the following properties:

- (i) telomerase activity at low or under detection level;
- (ii) having the ability to differentiate into cells of three germ layers;
- (iii) exhibiting no tumorigenic proliferation; and
- (iv) having self-renewal ability.

7. The method according to any one of claims 1 to 6, wherein the ratio between the number of the pluripotent stem cells and the number of the damaged and/or dead cells as IPCD is 1 : 10,000 to 10,000 : 1.

8. The method according to any one of claims 1 to 7, wherein the damaged and/or dead cells as IPCD, and/or fragments of said damaged and/or dead cells are obtained by adding a pharmaceutical agent selected from the group consisting of apoptosis inducing agent, metabolic antagonist, alkylating agent, anthracycline, antibiotics, antimetabolic agent, topoisomerase inhibitor, proteasome inhibitor, anti-cancer agent, heat, low temperature, acid, alkali, ultrasonic wave, and physical disruption by vortex or the like.

9. The method according to any one of claims 1 to 8, wherein the IPCD are cells derived from ectoderm.

10. The method according to claim 9, wherein the cells derived from ectoderm are selected from the group consisting of neural cells, glial cells, pigment cells, skin cells, inner ear cells, retinal cells, corneal cells, and hair follicle cells.

11. The method according to any one of claims 1 to 8, wherein the IPCD are cells derived from mesoderm.

12. The method according to claim 11, wherein the cells derived from mesoderm are selected from the group consisting of cardiac muscle cells, skeletal muscle cells, smooth muscle cells, osteocytes, chondrocytes, germ line cells, and hematopoietic cells.

13. The method according to any one of claims 1 to 8, wherein the IPCD are cells derived from endoderm.

14. The method according to claim 13, wherein the cells derived from endoderm are selected from the group consisting of pancreatic β cells, liver cells, bile duct cells, respiratory epithelial cells, esophageal epithelial cell, vascular endothelial cells, kidney-constituting cells, bladder epithelial cells, and pancreatic exocrine cells.

15. The method according to any one of claims 1 to 8, wherein the IPCD are selected from the group consisting of motor neuron, dopamine neuron, intermediate neuron, glutamine-activated neuron, pituitary gland cells, thyroid gland cells, adrenal cortex, adrenal medulla, pressure-sensor cells of carotid artery, heart conduction system, choroid plexus epithelial cells, pancreatic-alpha cells, pancreatic-delta cells, and lung Clara cells.

16. A method for confirming in vitro that cells contained in a cell preparation are live pluripotent stem cells positive for SSEA-3 and CD-105, including:

(a) co-culturing cells in the cell preparation with damaged and/or dead cells, and/or fragments of said damaged and/or dead cells; and

(b) if the cells in the cell preparation are differentiated into cells having the same phenotype and function as exodermal-lineage cells, mesodermal-lineage cells, and/or endodermal-lineage cells derived from the damaged and/or dead cells, and/or fragments of said damaged and/or dead cells, determining that the pluripotent stem cells are alive in the cell preparation.

17. A method for in vitro production of cells having the same phenotype and function as IPCD from pluripotent stem cells positive for SSEA-3 and CD-105, the method comprising co-culturing pluripotent stem cells or cell population with high content of the pluripotent stem cells either with damaged and/or dead cells as IPCD, and/or fragments of the damaged and/or dead cells.