In order to maintain the undifferentiated state, colonies containing deviated cells are carefully removed by pipetting operations under a microscope apparatus upon subculture. A device performing such an operation of removing colonies, for example, an observing device combined with pipetting by robot handling, has been developed as well.

[0006]

Further, Patent Document 1 discloses culture of pluripotent stem cells in the presence of activin for proliferating pluripotent stem cells such as iPS cells while maintaining undifferentiated states thereof.

[Prior Art Document]

[Patent Document]

[0007]

[Patent Document 1] JP2012-143229A

[SUMMARY OF THE INVENTION]

[0007a]

In one aspect, the present invention provides a method for culturing a stem cell having pluripotency, the method comprising: culturing the stem cell in a culture medium to form a colony of the stem cell; and removing a cell deviated from an undifferentiated state, which has emerged in the colony, from the colony (i) by adding hemagglutinin (HA) of a neurotoxin complex of Clostridium botulinum having an E-cadherin function inhibitory activity to the culture medium, or (ii) by exchanging the culture medium with a culture medium that contains hemagglutinin (HA) of a neurotoxin complex of Clostridium botulinum having an E-cadherin function inhibitory activity, wherein the stem cell is a human pluripotent stem cell.

[0007b]

In another aspect, the present invention provides a method for removing a cell deviated from an undifferentiated state, the cell being a cell that has emerged during culture of a stem cell having pluripotency, the method comprising: culturing the stem cell in a culture medium; and removing a cell deviated from an undifferentiated state from a colony (i) by adding hemagglutinin (HA) of a neurotoxin complex of Clostridium botulinum having an E-cadherin function inhibitory activity to the culture medium containing the colony in which the cell deviated from the undifferentiated state has emerged by the culture, or (ii) by exchanging the culture medium with a culture medium that contains hemagglutinin (HA) of a neurotoxin complex of

Clostridium botulinum having an E-cadherin function inhibitory activity, wherein the stem cell is a human pluripotent stem cell.

[0007c]

In yet another aspect, the present invention provides a method for culturing a stem cell having pluripotency, the method comprising: culturing the stem cell in a culture medium to form a colony of the stem cell; adding hemagglutinin (HA) of a neurotoxin complex of Clostridium botulinum having an E-cadherin function inhibitory activity to the culture medium in which the colony is formed, or exchanging the culture medium with a culture medium that contains hemagglutinin (HA) of a neurotoxin complex of Clostridium botulinum having an E-cadherin function inhibitory activity; and forming a colony of a cell in an undifferentiated state by culturing the stem cell in the culture medium containing the hemagglutinin, wherein the stem cell is a human pluripotent stem cell.

[0007d]

In yet another aspect, the present invention provides a method for culturing a stem cell having pluripotency, the method comprising: adding hemagglutinin (HA) of a neurotoxin complex of Clostridium botulinum having an E-cadherin function inhibitory activity to a culture medium; and maintaining an undifferentiated state of the stem cell by culturing the stem cell in the culture medium containing the hemagglutinin, wherein the stem cell is a human pluripotent stem cell.

[0007e]

In yet another aspect, the present invention provides a kit comprising: a medium component for a stem cell having pluripotency; and hemagglutinin (HA) of a neurotoxin complex of Clostridium botulinum having an E-cadherin function inhibitory activity, wherein the hemagglutinin is a complex composed of two or three components selected from the group consisting of three hemagglutinin subcomponents HA1, HA2, and HA3 of the neurotoxin complex of Clostridium botulinum and a substance that can inhibit cell migration.

[Problem to be Solved by the Invention]

[0008]

As described above, a deviation phenomenon tends to occur in subculture of pluripotent stem cells such as human iPS cells, and to maintaining the undifferentiated state is difficult. After several times of subculture, it is likely that the deviation phenomenon occurs in many iPS cell colonies and the colonies can become inferior colonies that contain cells deviated from the

undifferentiated state. Complicated operations such as careful culturing and careful colony sorting are therefore indispensable. From the viewpoint of promoting the stem cell industry as well, a method for maintaining undifferentiated states of pluripotent stem cells, that involves less complicated operations and can be performed by a non-expert, has been desired.

[0009]

The present disclosure, in one aspect, provides a method by which "a cell deviated from the undifferentiated state", which emerges in a colony during culture of a stem cell having pluripotency, can be removed.

[Means for Solving the Problem]

[0010]

The present disclosure, in one aspect, relates to a method for culturing a stem cell having pluripotency, the method including performing cell culture in the presence of a substance that

4. A method for culturing a stem cell having pluripotency, the method comprising:

adding hemagglutinin (HA) of a neurotoxin complex of Clostridium botulinum having an E-cadherin function inhibitory activity to a culture medium; and

maintaining an undifferentiated state of the stem cell by culturing the stem cell in the culture medium containing the hemagglutinin,

wherein the stem cell is a human pluripotent stem cell.

5. The method according to any one of claims 1 to 4, wherein

the human pluripotent stem cell is either a human iPS cell or a human ES cell.

6. The method according to any one of claims 1 to 5, wherein

the hemagglutinin is a complex composed of two or three components selected from the group consisting of three hemagglutinin subcomponents HA1, HA2, and HA3 of the neurotoxin complex of Clostridium botulinum.

7. The method according to claim 6, wherein

the subcomponent HA3 of the complex is of Clostridium botulinum type A or Clostridium botulinum type B.

8. The method according to any one of claims 1 to 7, wherein

the culture is subculture.

9. The method according to any one of claims 1 to 8, wherein a concentration of the hemagglutinin in the culture medium is 10 nM or more.

10. A kit comprising:

a medium for a stem cell having pluripotency; and

hemagglutinin (HA) of a neurotoxin complex of Clostridium botulinum having an E-cadherin function inhibitory activity, wherein the hemagglutinin is a complex composed of two or three components selected from the group consisting of three hemagglutinin subcomponents HA1, HA2, and HA3 of the neurotoxin complex of Clostridium botulinum and

a substance that can inhibit cell migration.

11. The kit according to claim 10 for the method according to any one of claims 1 to 9.

12. The kit of claim 10 or 11, wherein the substance that can inhibit cell migration is a Rac-1 inhibitor.

METHOD FOR CULTURING PLURIPOTENT STEM CELLS

ABSTRACT

Provided is a method by which cells deviated from the undifferentiated state, which emerge in a colony during culture of stem cells having pluripotency, can be removed. In one aspect, provided is a method for culturing stem cells having pluripotency, the method including performing cell culture in the presence of a substance that can inhibit cell-cell adhesion. In another aspect, provided is a method for removing cells deviated from the undifferentiated state, the cells being cells that have emerged or may possibly emerge during culture of stem cells having pluripotency, the method including performing cell culture in the presence of a substance that can inhibit cell-cell adhesion. In still another aspect, provided is a method for maintaining the undifferentiated state of stem cells having pluripotency, the method including performing cell culture in the presence of a substance that can inhibit cell-cell adhesion.

Fig. 1