Solution to Problem

[0020] The present inventors have obtained a surprising finding where when a lipid (in particular, a phospholipid) is added to a liquid culture medium and cells are cultured in suspension in the medium, a large amount of population of cell aggregates with an appropriate size can be produced. Based on this finding, the present inventors have completed the present invention. The present invention does not rely on mechanical/physical means, i.e., modifying culturing conditions, such as the viscosity of a culture medium, shear stress due to stirring, or the shape of cultureware such as a microwell plate. The present invention uses biochemical/chemical means, i.e., modifying the composition of a culture medium by using a substance present *in vivo*. In one aspect, the present invention refers to a method for producing cell aggregates, comprising a step of culturing pluripotent stem cells while suspended in a liquid culture medium comprising a lysophospholipid in a purified form, wherein the lysophospholipid has a phosphate group as a polar head group. In another aspect, the present invention refers to a method for producing cell aggregates, comprising a step of culturing pluripotent stem cells while suspended in a liquid culture medium comprising a lysophospholipid, wherein the lysophospholipid is at least one of lysophosphatidic acid and sphingosine-1-phosphoric acid. In yet another aspect, the present invention refers to a pluripotent stem cell aggregation inhibitor comprising a lysophospholipid having a phosphate group as a polar head group. Also described herein is a method for producing cell aggregates, comprising a step of culturing cells while suspended in a liquid culture medium comprising a lysophospholipid. Further described herein is the use of a lysophospholipid for inhibition of cell aggregation. Also described herein is a composition comprising a lysophospholipid, wherein the composition is a liquid culture medium or a composition added to a liquid culture medium. Also disclosed herein are the following examples.

(1) A method for producing cell aggregates, comprising a step of culturing cells while suspended in a liquid culture medium comprising a lysophospholipid. The step involves culturing cells while suspended in a liquid culture medium containing a lysophospholipid, so that an increase in the size of the cell aggregates in the culture is controlled. (2) The method according to item (1), wherein the liquid culture medium comprises the lysophospholipid in an amount of more than 0.0064 μg/mL and 100

μg/mL or less. (3) The method according to item (1) or (2), further comprising at least one of a step of adding the lysophospholipid to prepare the liquid culture medium and a step of generating the lysophospholipid through an enzymatic reaction to prepare the liquid culture medium. (4) The method according to any one of items (1) to (3), wherein the cells are cells isolated after undergoing adherent or suspension culture. (5) The method according to any one of items (1) to (4), wherein the lysophospholipid is at least one of lysophosphatidic acid and sphingosine-1-phosphoric acid.

(6) The method according to any one of items (1) to (5), wherein the liquid culture medium comprises at least one selected from the group consisting of L-ascorbic acid, insulin, transferrin, selenium, and sodium bicarbonate.

CLAIMS

[Claim 1]

A method for producing cell aggregates, comprising a step of culturing pluripotent stem cells while suspended in a liquid culture medium comprising a lysophospholipid in a purified form, wherein the lysophospholipid has a phosphate group as a polar head group.

[Claim 2]

The method according to claim 1, wherein the liquid culture medium comprises the lysophospholipid in an amount of more than 0.0064 μg/mL and 100 μg/mL or less.

[Claim 3]

The method according to claim 1 or 2, further comprising at least one of a step of adding the lysophospholipid to prepare the liquid culture medium and a step of generating the lysophospholipid through an enzymatic reaction to prepare the liquid culture medium.

[Claim 4]

The method according to any one of claims 1 or 3, wherein the pluripotent stem cells are pluripotent stem cells isolated after undergoing adherent or suspension culture.

[Claim 5]

The method according to any one of claims 1 to 4, wherein the lysophospholipid is at least one of lysophosphatidic acid and sphingosine-1-phosphoric acid.

[Claim 6]

The method according to any one of claims 1 to 5, wherein the liquid culture medium comprises at least one selected from the group consisting of L-ascorbic acid, insulin, transferrin, selenium, and sodium bicarbonate.

[Claim 7]

The method according to any one of claims 1 to 6, wherein the liquid culture medium comprises a growth factor.

[Claim 8]

The method according to claim 7, wherein the growth factor is at least one of FGF2 and TGF-β1.

[Claim 9]

The method according to any one of claims 1 to 8, wherein the liquid culture medium comprises a ROCK inhibitor.

[Claim 10]

The method according to claim 9, wherein the ROCK inhibitor is Y-27632.

[Claim 11]

A method for producing cell aggregates, comprising a step of culturing pluripotent stem cells while suspended in a liquid culture medium comprising a lysophospholipid, wherein the lysophospholipid is at least one of lysophosphatidic acid and sphingosine-1-phosphoric acid.

[Claim 12]

A pluripotent stem cell aggregation inhibitor comprising a lysophospholipid having a phosphate group as a polar head group.

[Claim 13]

The pluripotent stem cell aggregation inhibitor according to claim 12, wherein the pluripotent stem cell aggregation inhibitor comprises the lysophospholipid in an amount of more than 0.0064 μg/mL and 100 μg/mL or less.

[Claim 14]

The pluripotent stem cell aggregation inhibitor according to claim 12 or 13, further comprising a growth factor.

[Claim 15]

The pluripotent stem cell aggregation inhibitor according to claim 14, wherein the growth factor is at least one of FGF2 and TGF-β1.

[Claim 16]

The pluripotent stem cell aggregation inhibitor according to any one of claims 12 to 15, further comprising a ROCK inhibitor.

[Claim 17]

The pluripotent stem cell aggregation inhibitor according to claim 16, wherein the ROCK inhibitor is Y-27632.

[Claim 18]

The pluripotent stem cell aggregation inhibitor according to any one of claims 12 to 17, wherein the lysophospholipid is at least one of lysophosphatidic acid and sphingosine-1-phosphoric acid.