CELL CULTURE DEVICE, METHOD FOR CULTURING CELLS, AND METHOD FOR PRODUCING PRODUCT

BACKGROUND

Technical Field

[0001] The present disclosure relates to a method for culturing cells, a method for producing a product, and a cell culture device.

Related Art

[0002] In a case of culturing cells, a culture solution may be extracted for various purposes from the culture vessel using a pump. For example, in JP2010-29109A, a culture solution is extracted from the culture vessel for the intended purpose of capturing microorganisms that contaminate the culture solution.

[0003] US6544424B describes that a culture solution extracted from the culture vessel is filtered by a tangential flow type method using a diaphragm pump.

SUMMARY

[0004] Embodiments of the present invention provide a cell culture device and a method for culturing cells, with which both good cell proliferation properties and the suppression of diaphragm damage can be achieved in a case where a cell suspension is extracted with a diaphragm pump having a small capacity, and a method for producing a product using the method for culturing cells.

[0005] The means for solving the above problems includes the following aspects.

[0006] According to a first aspect of the present disclosure, there is provided a cell culture device including:

a culture vessel containing a cell suspension; and

a diaphragm pump for extracting the cell suspension from the culture vessel,

in which a volume of a space defined by a diaphragm of the diaphragm pump and a plane F including a surface of an outer edge part of the diaphragm on a side far from an apex of the diaphragm is 1 cm^3 or more and 20 cm^3 or less,

in a case where an angle formed by a straight line connecting a foot of a perpendicular line which is drawn from the apex of the diaphragm to the plane F to each point in the diaphragm and by the perpendicular line is denoted by an angle A at each point, a

thickness H of the diaphragm is within a range of 0.5 mm or more and 1.5 mm or less at any point in a region where the angle A is from 0° to 75°, and

in a case where a thickness of the diaphragm at the apex of the diaphragm is denoted by H_T, the thickness H of the diaphragm satisfies Expression 1 at any point in the region where the angle A is from 0° to 75°,

$1 \leq H_T/H \leq 1.75$ (Expression 1),

wherein the volume of the space and the thickness are measured in a state in which external pressure is not applied to the diaphragm.

In an embodiment of the cell culture device, in a case where a thickness of the diaphragm at a thickest point among points in a region where the angle A of the diaphragm is from 75° to 88° is H_Y and a thickness of the diaphragm at a point where the angle A is 90° is denoted by H_Z, H_Z and H_Y satisfy $H_z < H_Y$, and

the thickness H of the diaphragm satisfies $H \leq H_z$ at any point in the region where the angle A is from 0° to 75°.

In an embodiment of the cell culture device, in a case where a thickness of the diaphragm at a thickest point among points in a region where the angle A of the diaphragm is from 75° to 88° is denoted by H_Y, the thickness H of the diaphragm satisfies Expression 2 at any point in the region where the angle A is from 0° to 75°,

$1 < H_Y/H \leq 3$ (Expression 2).

In an embodiment of the cell culture device, a material of the diaphragm is silicone rubber.

In an embodiment of the cell culture device, a tensile stress σ of the diaphragm at an elongation rate of 50% satisfies Expression 3,

$0.4\ \text{MPa} \leq \sigma \leq 1.5\ \text{MPa}$ (Expression 3).

According to a second aspect of the present disclosure, there is provided a method for culturing cells, including:

culturing cells in a cell suspension contained in a culture vessel; and

extracting the cell suspension from the culture vessel with a diaphragm pump,

in which a volume of a space defined by a diaphragm of the diaphragm pump and a plane F including a surface of an outer edge part of the diaphragm on a side far from an apex of the diaphragm is 1 cm^3 or more and 20 cm^3 or less,

in a case where an angle formed by a straight line connecting a foot of a perpendicular line which is drawn from the apex of the diaphragm to the plane F to each point

in the diaphragm and by the perpendicular line is denoted by an angle A at each point, a thickness H of the diaphragm is within a range of 0.5 mm or more and 1.5 mm or less at any point in a region where the angle A is from 0° to 75°, and

in a case where a thickness of the diaphragm at the apex of the diaphragm is denoted by H_T, the thickness H of the diaphragm satisfies Expression 1 at any point in the region where the angle A is from 0° to 75°,

$1 \leq H_T/H \leq 1.75$ (Expression 1),

wherein the volume of the space and the thickness are measured in a state in which external pressure is not applied to the diaphragm.

In an embodiment of the method for culturing cells, in a case where a thickness of the diaphragm at a thickest point among points in a region where the angle A of the diaphragm is from 75° to 88° is H_Y and a thickness of the diaphragm at a point where the angle A is 90° is denoted by H_Z, H_Z and H_Y satisfy $Hz < H_Y$, and the thickness H of the diaphragm satisfies $H \leq H_Z$ at any point in the region where the angle A is from 0° to 75°.

In an embodiment of the method for culturing cells, in a case where a thickness of the diaphragm at a thickest point among points in a region where the angle A of the diaphragm is from 75° to 88° is denoted by H_Y, the thickness H of the diaphragm satisfies Expression 2 at any point in the region where the angle A is from 0° to 75°,

$1 < H_Y/H \leq 3$ (Expression 2).

In an embodiment of the method for culturing cells, a material of the diaphragm is silicone rubber.

In an embodiment of the method for culturing cells, a tensile stress σ of the diaphragm at an elongation rate of 50% satisfies Expression 3,

$0.4 \text{ MPa} \leq \sigma \leq 1.5 \text{ MPa}$ (Expression 3).

In an embodiment of the method for culturing cells, at a time at which the diaphragm pump is in operation, a pressure P in an inside of the diaphragm pump that drives the diaphragm satisfies Expression 4,

atmospheric pressure - 0.1 MPa ≤ P ≤ atmospheric pressure + 0.1 MPa (Expression 4).

In an embodiment of the method for culturing cells, the cells are CHO cells.

In an embodiment, the method for culturing cells further comprising:

subjecting the cell suspension extracted from the culture vessel to a separation treatment by which the extracted cell suspension is separated, by using a separation

membrane, into a first solution having a cell concentration higher than a cell concentration in the cell suspension and a second solution having a cell concentration lower than the cell concentration in the cell suspension by a tangential flow filtration method,

returning the first solution to the culture vessel; and

adding a medium anew to the culture vessel.

According to a third aspect of the present disclosure, there is provided a method for producing a product, including:

culturing cells that produce a product, using the method for culturing cells according to the second aspect; and

recovering the product from a cell suspension of the cells.

In an embodiment of the method for producing a product, the product is an antibody.

[0007] According to the embodiments of the present invention, there are provided a cell culture device and a method for culturing cells, with which both good cell proliferation properties and the suppression of diaphragm damage can be achieved in a case where a cell suspension is extracted with a diaphragm pump having a small capacity, and a method for producing a product using the method for culturing cells.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008] Fig. 1 is a view illustrating an example of a configuration of a cell culture device according to the present disclosure.

Fig. 2 is a view illustrating an example of a configuration of a diaphragm pump in the cell culture device according to the present disclosure.

Fig. 3A is a view for explaining an angle A at each point on a diaphragm.

Fig. 3B is a view for explaining a volume defined by the diaphragm.

In Figs. 4A to 4D are views illustrating determination standards for a state of a diaphragm in Examples and Comparative Examples.

Fig. 5 is a graph showing determination standards for proliferation properties and viability in Examples and Comparative Examples.

Fig. 6 is a graph showing changes in cell concentration and viability in Example 1.

DETAILED DESCRIPTION

[0009] Hereinafter, a cell culture device, a method for culturing cells, and a method for

CLAIMS

1. A cell culture device comprising:

a culture vessel containing a cell suspension; and

a diaphragm pump for extracting the cell suspension from the culture vessel,

wherein a volume of a space defined by a diaphragm of the diaphragm pump and a plane F including a surface of an outer edge part of the diaphragm on a side far from an apex of the diaphragm is 1 cm^3 or more and 20 cm^3 or less,

in a case where an angle formed by a straight line connecting a foot of a perpendicular line which is drawn from the apex of the diaphragm to the plane F to each point in the diaphragm and by the perpendicular line is denoted by an angle A at each point, a thickness H of the diaphragm is within a range of 0.5 mm or more and 1.5 mm or less at any point in a region where the angle A is from 0° to 75°, and

in a case where a thickness of the diaphragm at the apex of the diaphragm is denoted by H_T, the thickness H of the diaphragm satisfies Expression 1 at any point in the region where the angle A is from 0° to 75°,

$1 \leq H_T/H \leq 1.75$ (Expression 1),

wherein the volume of the space and the thickness are measured in a state in which external pressure is not applied to the diaphragm.

2. The cell culture device according to claim 1,

wherein in a case where a thickness of the diaphragm at a thickest point among points in a region where the angle A of the diaphragm is from 75° to 88° is H_Y and a thickness of the diaphragm at a point where the angle A is 90° is H_Z, H_Z and H_Y satisfy $Hz < H_Y$, and

the thickness H of the diaphragm satisfies $H \leq H_Z$ at any point in the region where the angle A is from 0° to 75°.

3. The cell culture device according to claim 1 or 2,

wherein in a case where a thickness of the diaphragm at a thickest point among points in a region where the angle A of the diaphragm is from 75° to 88° is denoted by H_Y, the thickness H of the diaphragm satisfies Expression 2 at any point in the region where the angle A is from 0° to 75°,

$1 < H_Y/H \leq 3$ (Expression 2).

4. The cell culture device according to any one of claims 1 to 3,

wherein a material of the diaphragm is silicone rubber.

5. The cell culture device according to any one of claims 1 to 4,

wherein a tensile stress σ of the diaphragm at an elongation rate of 50% satisfies Expression 3,

$0.4\ \text{MPa} \leq \sigma \leq 1.5\ \text{MPa}$ (Expression 3).

6. A method for culturing cells, comprising:

culturing cells in a cell suspension contained in a culture vessel; and

extracting the cell suspension from the culture vessel with a diaphragm pump,

wherein a volume of a space defined by a diaphragm of the diaphragm pump and a plane F including a surface of an outer edge part of the diaphragm on a side far from an apex of the diaphragm is 1 cm^3 or more and 20 cm^3 or less,

in a case where an angle formed by a straight line connecting a foot of a perpendicular line which is drawn from the apex of the diaphragm to the plane F to each point in the diaphragm and by the perpendicular line is denoted by an angle A at each point, a thickness H of the diaphragm is within a range of 0.5 mm or more and 1.5 mm or less at any point in a region where the angle A is from 0° to 75°, and

in a case where a thickness of the diaphragm at the apex of the diaphragm is denoted by H_T, the thickness H of the diaphragm satisfies Expression 1 at any point in the region where the angle A is from 0° to 75°,

$1 \leq H_T/H \leq 1.75$ (Expression 1),

wherein the volume of the space and the thickness are measured in a state in which external pressure is not applied to the diaphragm.

7. The method for culturing cells according to claim 6,

wherein in a case where a thickness of the diaphragm at a thickest point among points in a region where the angle A of the diaphragm is from 75° to 88° is denoted by H_Y and a thickness of the diaphragm at a point where the angle A is 90° is denoted by H_Z, H_Z and H_Y

satisfy $Hz < H_Y$, and

the thickness H of the diaphragm satisfies $H \leq H_Z$ at any point in the region where the angle A is from 0° to 75°.

8. The method for culturing cells according to claim 6 or 7,

wherein in a case where a thickness of the diaphragm at a thickest point among points in a region where the angle A of the diaphragm is from 75° to 88° is denoted by H_Y, the thickness H of the diaphragm satisfies Expression 2 at any point in the region where the angle A is from 0° to 75°,

$1 < H_Y/H \leq 3$ (Expression 2).

9. The method for culturing cells according to any one of claims 6 to 8,

wherein a material of the diaphragm is silicone rubber.

10. The method for culturing cells according to any one of claims 6 to 9,

wherein a tensile stress σ of the diaphragm at an elongation rate of 50% satisfies Expression 3,

$0.4 \text{ MPa} \leq \sigma \leq 1.5 \text{ MPa}$ (Expression 3).

11. The method for culturing cells according to any one of claims 6 to 10,

wherein at a time at which the diaphragm pump is in operation, a pressure P in an inside of the diaphragm pump that drives the diaphragm satisfies Expression 4,

atmospheric pressure - 0.1 MPa ≤ P ≤ atmospheric pressure + 0.1 MPa (Expression 4).

12. The method for culturing cells according to any one of claims 6 to 11,

wherein the cells are CHO cells.

13. The method for culturing cells according to any one of claims 6 to 12, further comprising:

subjecting the cell suspension extracted from the culture vessel to a separation treatment by which the extracted cell suspension is separated, by using a separation

membrane, into a first solution having a cell concentration higher than a cell concentration in the cell suspension and a second solution having a cell concentration lower than the cell concentration in the cell suspension by a tangential flow filtration method,

returning the first solution to the culture vessel; and

adding a medium anew to the culture vessel.

14. A method for producing a product, comprising:

culturing cells that produce a product, using the method for culturing cells according to any one of claims 6 to 13; and

recovering the product from a cell suspension of the cells.

15. The method for producing a product according to claim 14,

wherein the product is an antibody.