cryopreservation (cell arousal) to be carried out conveniently and efficiently in a consistent manner from use to re-freezing.

[Means for Solving the Problems]

[0015]

The present inventors have found that cells can be efficiently cultured for long periods by conducting the cell culturing using a porous polyimide film.

The present invention preferably includes, but is not limited to, the following modes.

[Mode 1]

A long term cell culturing method including:

(1) applying cells to a porous polyimide film, and

(2) applying the porous polyimide film to which the cells have been applied, to a cell culture medium and culturing the cells for 30 days or longer.

[Mode 1a]

A long term cell culturing method including:

(1) applying cells to a porous polyimide film, and

(2) applying the porous polyimide film to which the cells have been applied, to a cell culture medium and culturing the cells for 30 days or longer,

wherein the porous polyimide film with a multilayer structure, having at least two surface layers (an A-surface and a B-surface), and a macro-void layer sandwiched between the two surface layers,

a mean pore size of the holes in the A-surface is smaller than a mean pore size of the holes in the B-surface, and

the macro-void layer has a partition bonded to the surface layers (the A-surface and the B-surface), and a plurality of macro-voids surrounded by the partition and the surface layers (the A-surface and the B-surface).

[Mode 2]

The method according to mode 1, wherein the cells are cultured for 60 days or longer in step (2).

[Mode 3]

The method according to mode 1, wherein the cells

are cultured for 120 days or longer in step (2).

[Mode 4]

The method according to any one of modes 1 to 3, using two or more porous polyimide films layered either above and below or left and right in the cell culture medium.

[Mode 5]

The method according to any one of modes 1 to 4, wherein the porous polyimide films are:

i) folded,

ii) wound into a roll,

iii) connected as sheets or fragments by a filamentous structure, or

iv) bound into a rope,

and used by suspension or anchoring in the cell culture medium in the cell culturing vessel.

[Mode 6]

The method according to any one of modes 1 to 5, wherein in the culturing of step (2), all or some of the porous polyimide films are not in contact with the liquid phase of the cell culture medium.

[Mode 7]

The method according to any one of modes 1 to 6, wherein in the culturing of step (2), the total volume of the cell culture medium in the cell culturing vessel is 10,000 times or less of the total sum of the porous polyimide film volume including the cell survival zone.

[Mode 8]

The method according to any one of modes 1 to 7, wherein in the culturing of step (2), the total volume of the cell culture medium in the cell culturing vessel is 100 times or less of the total sum of the porous polyimide film volume including the cell survival zone.

[Mode 9]

The method according to any one of modes 1 to 8, wherein the culturing in step (2) is carried out in a system in which a cell culture medium is continuously or

intermittently supplied to a cell culturing vessel from cell culture medium supply means installed outside of the cell culturing vessel.

[Mode 10]

The method according to mode 9, wherein the cell culture medium is circulated between the cell culture medium supply means and the cell culturing vessel.

[Mode 11]

The method according to mode 9 or 10, wherein the system is a cell culturing apparatus including a culturing unit which is the cell culturing vessel, and a culture medium-supply unit which is the cell culture medium supply means, wherein

the culturing unit is a culturing unit that houses one or more porous polyimide films to support cells, and that comprises a culture medium supply port and a culture medium discharge port, and

the culture medium-supply unit is a culture medium-supply unit comprising a culture medium housing vessel, a culture medium supply line, and a liquid conveyance pump that conveys the medium continuously or intermittently through the culture medium supply line, the first end of the culture medium supply line contacting the medium in the culture medium housing vessel, and the second end of the culture medium supply line being in communication with the culturing unit interior via the culture medium supply port of the culturing unit.

[Mode 12]

The method according to mode 13, wherein the culturing unit is a culturing unit that does not comprise an air supply port, an air discharge port and an oxygen exchange membrane.

[Mode 13]

The method according to mode 11 or 12, wherein the culturing unit further comprises a culture medium discharge line, the first end of the culture medium discharge line being connected to the culture medium

housing vessel, the second end of the culture medium discharge line being in communication with the culturing unit interior via the culture medium discharge port of the culturing unit, and the medium being able to circulate through the culture medium-supply unit and the culturing unit.

[Mode 14]

The method according to any one of modes 1 to 13, wherein the cells are selected from the group consisting of animal cells, insect cells, plant cells, yeast cells and bacteria.

[Mode 15]

The method according to mode 14, wherein the animal cells are cells derived from an animal belonging to the subphylum Vertebrata.

[Mode 16]

The method according to mode 15, wherein the cells are selected from the group consisting of CHO cells, CHO-K1 cell lines, CHO DP-12 cell lines, CHO cell-related lines, Vero cells and MDCK cells.

[Mode 17]

The method according to any one of modes 1 to 16, wherein the porous polyimide film is a porous polyimide film including a polyimide obtained from a tetracarboxylic dianhydride and a diamine.

[Mode 18]

The method according to mode 17, wherein the porous polyimide film is a colored porous polyimide film obtained by forming a polyamic acid solution composition including a polyamic acid solution obtained from a tetracarboxylic dianhydride and a diamine, and a coloring precursor, and then heat treating it at 250°C or higher.

[Mode 19]

A cell culturing apparatus for use in the method according to any one of modes 1 to 18, including a porous polyimide film.

[Mode 19a]

A cell culturing apparatus for use in the method according to any one of modes 1 to 18, including a porous polyimide film,
wherein the porous polyimide film with a multilayer structure, having at least two surface layers (an A-surface and a B-surface), and a macro-void layer sandwiched between the two surface layers,
a mean pore size of the holes in the A-surface is smaller than a mean pore size of the holes in the B-surface, and the macro-void layer has a partition bonded to the surface layers (the A-surface and the B-surface), and a plurality of macro-voids surrounded by the partition and the surface layers (the A-surface and the B-surface).

[Mode 20]

A cell culturing apparatus according to mode 19, wherein two or more porous polyimide films are layered either above and below or left and right.

[Mode 21]

A kit for use in the method according to any one of modes 1 to 13, including a porous polyimide film.

[Mode 21a]

A kit for use in the method according to any one of modes 1 to 18, including a porous polyimide film and a cell culture medium,
wherein the porous polyimide film with a multilayer structure, having at least two surface layers (an A-surface and a B-surface), and a macro-void layer sandwiched between the two surface layers,
a mean pore size of the holes in the A-surface is smaller than a mean pore size of the holes in the B-surface, and the macro-void layer has a partition bonded to the surface layers (the A-surface and the B-surface), and a plurality of macro-voids surrounded by the partition and the surface layers (the A-surface and the B-surface).

[Mode 22]

The use of a porous polyimide film for a long term cell culturing method.

[Mode 22a]

Use of a porous polyimide film for a long term cell culturing method which cultures cells for 30 days or longer,

wherein the porous polyimide film with a multilayer structure, having at least two surface layers (an A-surface and a B-surface), and a macro-void layer sandwiched between the two surface layers,

a mean pore size of the holes in the A-surface is smaller than a mean pore size of the holes in the B-surface, and the macro-void layer has a partition bonded to the surface layers (the A-surface and the B-surface), and a plurality of macro-voids surrounded by the partition and the surface layers (the A-surface and the B-surface).

[Mode 23]

A cell cryopreservation method, including:

(1) a step of supporting cells on a porous polyimide film,

(2) a step of placing the cell-supporting porous polyimide film under conditions in which the cells freeze, to freeze the cells supported on the porous polyimide film, and

(3) a step of storing the cell-supporting porous polyimide film under conditions in which the frozen state is maintained.

[Mode 23a]

A cell cryopreservation method, including:

(1) a step of supporting cells on a porous polyimide film,

(2) a step of placing the cell-supporting porous polyimide film under conditions in which the cells freeze, to freeze the cells supported on the porous polyimide film, and

(3) a step of storing the cell-supporting porous polyimide film under conditions in which the frozen state is maintained,

wherein the porous polyimide film with a multilayer

structure, having at least two surface layers (an A-surface and a B-surface), and a macro-void layer sandwiched between the two surface layers,
a mean pore size of the holes in the A-surface is smaller than a mean pore size of the holes in the B-surface, and the macro-void layer has a partition bonded to the surface layers (the A-surface and the B-surface), and a plurality of macro-voids surrounded by the partition and the surface layers (the A-surface and the B-surface).

[Mode 24]

The method according to mode 23, wherein in step (1), the cells are seeded on the porous polyimide film to support the cells on the porous polyimide film.

[Mode 25]

The method according to mode 23, wherein in step (1), the cells are seeded and cultured on the porous polyimide film to support the cells on the porous polyimide film.

[Mode 26]

The method according to any one of modes 23 to 25, which further includes, following step (3),

(4) a step of placing the cell-supporting porous polyimide film under conditions in which the cells thaw, to thaw the cells supported on the porous polyimide film.

[Mode 27]

The method according to mode 26, which further includes, following step (4),

(5) a step of applying the cell-thawed porous polyimide film to a cell culture medium and culturing the cells.

[Mode 28]

The method according to mode 27, wherein in step (5), the cells are cultured until the cultured cells proliferate even outside of the porous polyimide film.

[Mode 29]

The method according to mode 27 or 28, wherein in step (5), the one or more separate non-cell-supporting porous polyimide films are applied onto the cell culture medium together with the cell-thawed porous polyimide film, and culturing is carried out to support the cells on the one or more separate porous polyimide films.

[Mode 30]

The method according to any one of modes 27 to 29, which further includes, following step (5),

(6) a step of placing all or a portion of the cell-supporting porous polyimide film under conditions in which the cells freeze, to freeze the cells supported on the porous polyimide film, and

(7) a step of storing the cell-supporting porous polyimide film under conditions in which the frozen state is maintained.

[Mode 31]

The method according to mode 30, wherein steps (1) to (7) are repeated several times.

[Mode 32]

The method according to any one of modes 23 to 31, wherein the cells are selected from the group consisting of animal cells, insect cells, plant cells, yeast cells and bacteria.

[Mode 33]

The method according to mode 32, wherein the animal cells are cells derived from an animal belonging to the subphylum Vertebrata.

[Mode 34]

The method according to mode 32 or 33, wherein the cells are adherent cells.

[Mode 35]

The method according to mode 32 or 33, wherein the cells are suspension cells.

[Mode 36]

The method according to any one of modes 23 to 35,

wherein the porous polyimide film is a porous polyimide film including a polyimide obtained from a tetracarboxylic dianhydride and a diamine.

[Mode 37]

The method according to mode 36, wherein the porous polyimide film is a colored porous polyimide film obtained by forming a polyamic acid solution composition including a polyamic acid solution obtained from a tetracarboxylic dianhydride and a diamine, and a coloring precursor, and then heat treating it at 250°C or higher.

[Mode 38]

The method according to mode 36 or 37, wherein the porous polyimide film is a porous polyimide film with a multilayer structure, having two different surface layers and a macro-void layer.

[Mode 39]

A porous polyimide film for cryopreservation of cells.

[Mode 39a]

A porous polyimide film for cryopreservation while supporting cells,
wherein the porous polyimide film with a multilayer structure, having at least two surface layers (an A-surface and a B-surface), and a macro-void layer sandwiched between the two surface layers,
a mean pore size of the holes in the A-surface is smaller than a mean pore size of the holes in the B-surface, and the macro-void layer has a partition bonded to the surface layers (the A-surface and the B-surface), and a plurality of macro-voids surrounded by the partition and the surface layers (the A-surface and the B-surface).

[Mode 40]

A porous polyimide film to be used in the method according to any one of modes 23 to 28.

[Mode 40a]

A porous polyimide film to be used in the method according to any one of modes 23 to 37,

wherein the porous polyimide film with a multilayer structure, having at least two surface layers (an A-surface and a B-surface), and a macro-void layer sandwiched between the two surface layers,
a mean pore size of the holes in the A-surface is smaller than a mean pore size of the holes in the B-surface, and the macro-void layer has a partition bonded to the surface layers (the A-surface and the B-surface), and a plurality of macro-voids surrounded by the partition and the surface layers (the A-surface and the B-surface).

[Mode 41]

A kit for use in the method according to any one of modes 23 to 38, including a porous polyimide film.

[Mode 41a]

A kit for use in the method according to any one of modes 23 to 37, including a porous polyimide film and a cell cryopreservation liquid,
wherein the porous polyimide film with a multilayer structure, having at least two surface layers (an A-surface and a B-surface), and a macro-void layer sandwiched between the two surface layers,
a mean pore size of the holes in the A-surface is smaller than a mean pore size of the holes in the B-surface, and the macro-void layer has a partition bonded to the surface layers (the A-surface and the B-surface), and a plurality of macro-voids surrounded by the partition and the surface layers (the A-surface and the B-surface).

[Mode 42]

The use of a porous polyimide film for the method according to any one of modes 23 to 38.

[Effects of the Invention]

[0016]

The present invention allows convenient and stable long term culturing of cells to be performed using a porous polyimide film. According to the invention, the porous polyimide film that is to serve as the culture support has large diameter communicating pores through

which cells can pass, and the three-dimensional space is ensured at the final stage even when very large volumes of cells are growing in the space. Hence, there is less likelihood of a limit to the culturing period such as occurs in a confluent state in classical plate culturing. Moreover, according to the invention there is no particular need for pretreatment of the porous polyimide film by collagen or the like during the cell culturing. Furthermore, since the porous polyimide film is a flexible thin-film material, bending, folding or cutting into a free shape is more convenient. Consequently, the

What is claimed is:

1. A long term cell culturing method including:

(1) applying cells to a porous polyimide film, and

(2) applying the porous polyimide film to which the cells have been applied, to a cell culture medium and culturing the cells for 30 days or longer,

wherein the porous polyimide film with a multilayer structure, having at least two surface layers (an A-surface and a B-surface), and a macro-void layer sandwiched between the two surface layers,

a mean pore size of the holes in the A-surface is smaller than a mean pore size of the holes in the B-surface, and

the macro-void layer has a partition bonded to the surface layers (the A-surface and the B-surface), and a plurality of macro-voids surrounded by the partition and the surface layers (the A-surface and the B-surface).

2. The method according to claim 1, wherein the cells are cultured without subculture operations in step (2).

3 The method according to claim 1 or 2, wherein the cells are cultured for 60 days or longer in step (2).

4. The method according to claim 1 or 2, wherein the cells are cultured for 120 days or longer in step (2).

5. The method according to any one of claims 1 to 4, using two or more porous polyimide films layered either above and below or left and right in the cell culture medium.

6. The method according to any one of claims 1 to 5, wherein the porous polyimide films are:

i) folded,

ii) wound into a roll,

iii) connected as sheets or fragments by a filamentous structure, or

iv) bound into a rope,

and used by suspension or anchoring in the cell culture medium in the cell culturing vessel.

7. The method according to any one of claims 1 to 6, wherein in the culturing of step (2), all or some of the porous

polyimide films are not in contact with the liquid phase of the cell culture medium.

8. The method according to any one of claims 1 to 7, wherein in the culturing of step (2), the total volume of the cell culture medium in the cell culturing vessel is 10,000 times or less of the total sum of the porous polyimide film volume including a cell survival zone.

9. The method according to any one of claims 1 to 8, wherein in the culturing of step (2), the total volume of the cell culture medium in the cell culturing vessel is 100 times or less of the total sum of the porous polyimide film volume including the cell survival zone.

10. The method according to any one of claims 1 to 9, wherein the culturing in step (2) is carried out in a system in which a cell culture medium is continuously or intermittently supplied to a cell culturing vessel from cell culture medium supply means installed outside of the cell culturing vessel.

11. The method according to claim 10, wherein the cell culture medium is circulated between the cell culture medium supply means and the cell culturing vessel.

12. The method according to claim 10 or 11, wherein the system is a cell culturing apparatus including a culturing unit which is the cell culturing vessel, and a culture medium-supply unit which is the cell culture medium supply means, wherein

the culturing unit is a culturing unit that houses one or more porous polyimide films to support cells, and that comprises a culture medium supply port and a culture medium discharge port, and

the culture medium-supply unit is a culture medium-supply unit comprising a culture medium housing vessel, a culture medium supply line, and a liquid conveyance pump that conveys the medium continuously or intermittently through the culture medium supply line, the first end of the culture medium supply line contacting the medium in the culture medium housing vessel, and the second end of the culture medium supply line being in communication with the culturing unit interior via the culture

medium supply port of the culturing unit.

13. The method according to claim 12, wherein the culturing unit is a culturing unit that does not comprise an air supply port, an air discharge port and an oxygen exchange membrane.

14. The method according to claim 12 or 13, wherein the culturing unit further comprises a culture medium discharge line, the first end of the culture medium discharge line being connected to the culture medium housing vessel, the second end of the culture medium discharge line being in communication with the culturing unit interior via the culture medium discharge port of the culturing unit, and the medium being able to circulate through the culture medium-supply unit and the culturing unit.

15. The method according to any one of claims 1 to 14, wherein the cells are selected from the group consisting of animal cells, insect cells, plant cells, yeast cells and bacteria.

16. The method according to claim 15, wherein the animal cells are cells derived from an animal belonging to the subphylum Vertebrata.

17. The method according to claim 16, wherein the cells are selected from the group consisting of CHO cells, CHO-K1 cell lines, CHO DP-12 cell lines, CHO cell-related lines, Vero cells and MDCK cells.

18. The method according to any one of claims 1 to 17, wherein the porous polyimide film is a porous polyimide film including a polyimide obtained from a tetracarboxylic dianhydride and a diamine.

19. The method according to claim 18, wherein the porous polyimide film is a colored porous polyimide film obtained by forming a polyamic acid solution composition including a polyamic acid solution obtained from a tetracarboxylic dianhydride and a diamine, and a coloring precursor, and then heat treating it at 250°C or higher.

20. A cell culturing apparatus for use in the method

according to any one of claims 1 to 19, including a porous polyimide film,
wherein the porous polyimide film with a multilayer structure, having at least two surface layers (an A-surface and a B-surface), and a macro-void layer sandwiched between the two surface layers,
a mean pore size of the holes in the A-surface is smaller than a mean pore size of the holes in the B-surface, and
the macro-void layer has a partition bonded to the surface layers (the A-surface and the B-surface), and a plurality of macro-voids surrounded by the partition and the surface layers (the A-surface and the B-surface).

21. The cell culturing apparatus according to claim 20, wherein two or more porous polyimide films are layered either above and below or left and right.

22. A kit for use in the method according to any one of claims 1 to 19, including a porous polyimide film and a cell culture medium,
wherein the porous polyimide film with a multilayer structure, having at least two surface layers (an A-surface and a B-surface), and a macro-void layer sandwiched between the two surface layers,
a mean pore size of the holes in the A-surface is smaller than a mean pore size of the holes in the B-surface, and
the macro-void layer has a partition bonded to the surface layers (the A-surface and the B-surface), and a plurality of macro-voids surrounded by the partition and the surface layers (the A-surface and the B-surface).

23. Use of a porous polyimide film for a long term cell culturing method which cultures cells for 30 days or longer,
wherein the porous polyimide film with a multilayer structure, having at least two surface layers (an A-surface and a B-surface), and a macro-void layer sandwiched between the two surface layers,
a mean pore size of the holes in the A-surface is smaller than a mean pore size of the holes in the B-surface, and

the macro-void layer has a partition bonded to the surface layers (the A-surface and the B-surface), and a plurality of macro-voids surrounded by the partition and the surface layers (the A-surface and the B-surface).

24. A cell cryopreservation method, including:

(1) a step of supporting cells on a porous polyimide film,

(2) a step of placing the cell-supporting porous polyimide film under conditions in which the cells freeze, to freeze the cells supported on the porous polyimide film, and

(3) a step of storing the cell-supporting porous polyimide film under conditions in which the frozen state is maintained,

wherein the porous polyimide film with a multilayer structure, having at least two surface layers (an A-surface and a B-surface), and a macro-void layer sandwiched between the two surface layers,

a mean pore size of the holes in the A-surface is smaller than a mean pore size of the holes in the B-surface, and

the macro-void layer has a partition bonded to the surface layers (the A-surface and the B-surface), and a plurality of macro-voids surrounded by the partition and the surface layers (the A-surface and the B-surface).

25. The method according to claim 24, wherein in step (1), the cells are seeded on the porous polyimide film to support the cells on the porous polyimide film.

26. The method according to claim 24, wherein in step (1), the cells are seeded and cultured on the porous polyimide film to support the cells on the porous polyimide film.

27. The method according to any one of claims 24 to 26, which further includes, following step (3),

(4) a step of placing the cell-supporting porous polyimide film under conditions in which the cells thaw, to thaw the cells supported on the porous polyimide film.

28. The method according to claim 27, which further includes, following step (4),

(5) a step of applying the cell-thawed porous polyimide film to a cell culture medium and culturing the cells.

29. The method according to claim 28, wherein in step (5), the cells are cultured until the cultured cells proliferate even outside of the porous polyimide film.

30. The method according to claim 28 or 29, wherein in step (5), the one or more separate non-cell-supporting porous polyimide films are applied onto the cell culture medium together with the cell-thawed porous polyimide film, and culturing is carried out to support the cells on the one or more separate porous polyimide films.

31. The method according to any one of claims 28 to 30, which further includes, following step (5),

(6) a step of placing all or a portion of the cell-supporting porous polyimide film under conditions in which the cells freeze, to freeze the cells supported on the porous polyimide film, and

(7) a step of storing the cell-supporting porous polyimide film under conditions in which the frozen state is maintained.

32. The method according to claim 31, wherein steps (1) to (7) are repeated several times.

33. The method according to any one of claims 24 to 32, wherein the cells are selected from the group consisting of animal cells, insect cells, plant cells, yeast cells and bacteria.

34. The method according to claim 33, wherein the animal cells are cells derived from an animal belonging to the subphylum Vertebrata.

35. The method according to claim 33 or 34, wherein the cells are adherent cells.

36. The method according to claim 33 or 34, wherein the cells are suspension cells.

37. The method according to any one of claims 24 to 36, wherein the porous polyimide film is a porous polyimide film including a polyimide obtained from a tetracarboxylic

dianhydride and a diamine.

38. The method according to claim 37, wherein the porous polyimide film is a colored porous polyimide film obtained by forming a polyamic acid solution composition including a polyamic acid solution obtained from a tetracarboxylic dianhydride and a diamine, and a coloring precursor, and then heat treating it at 250°C or higher.

39. A porous polyimide film for cryopreservation while supporting cells,
wherein the porous polyimide film with a multilayer structure, having at least two surface layers (an A-surface and a B-surface), and a macro-void layer sandwiched between the two surface layers,
a mean pore size of the holes in the A-surface is smaller than a mean pore size of the holes in the B-surface, and
the macro-void layer has a partition bonded to the surface layers (the A-surface and the B-surface), and a plurality of macro-voids surrounded by the partition and the surface layers (the A-surface and the B-surface).

40. A porous polyimide film to be used in the method according to any one of claims 24 to 38,
wherein the porous polyimide film with a multilayer structure, having at least two surface layers (an A-surface and a B-surface), and a macro-void layer sandwiched between the two surface layers,
a mean pore size of the holes in the A-surface is smaller than a mean pore size of the holes in the B-surface, and
the macro-void layer has a partition bonded to the surface layers (the A-surface and the B-surface), and a plurality of macro-voids surrounded by the partition and the surface layers (the A-surface and the B-surface).

41. A kit for use in the method according to any one of claims 24 to 38, including a porous polyimide film and a cell cryopreservation liquid,
wherein the porous polyimide film with a multilayer structure, having at least two surface layers (an A-surface and a

B-surface), and a macro-void layer sandwiched between the two surface layers,
a mean pore size of the holes in the A-surface is smaller than a mean pore size of the holes in the B-surface, and
the macro-void layer has a partition bonded to the surface layers (the A-surface and the B-surface), and a plurality of macro-voids surrounded by the partition and the surface layers (the A-surface and the B-surface).

42. Use of a porous polyimide film for the method according to any one of claims 24 to 38,
wherein the porous polyimide film with a multilayer structure, having at least two surface layers (an A-surface and a B-surface), and a macro-void layer sandwiched between the two surface layers,
a mean pore size of the holes in the A-surface is smaller than a mean pore size of the holes in the B-surface, and
the macro-void layer has a partition bonded to the surface layers (the A-surface and the B-surface), and a plurality of macro-voids surrounded by the partition and the surface layers (the A-surface and the B-surface).