the present inventors found that desirable results can be obtained by cell culturing using a porous polyimide film, and have thereupon devised this invention. Specifically, in cell culturing using porous polyimide films, large-diameter communicating pores that allow passage of cells in the films are utilized to allow stable growth of the cells in the spaces so that, so long as the films are adhered to each other, the cells can migrate with some degree of freedom not only within the films but also across films. By utilizing this feature, and closely contacting a porous polyimide film in which the cells are not growing to medium in which the cells are growing (a plate, dish, culture plate, microcarrier, silica porous bodies, cellulose sponge, porous polyimide film or other cell culturing medium), migration of the cells is promoted, and cell culturing and subculturing can be continued in a continuous and convenient manner.

[0019]

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

[1]

A cell culturing method including:

(1) a step of supporting cells on one or more sheet-like porous supports,

(2) a step of applying a medium to the sheet-like porous support supporting the cells and wetting the sheet-like porous support with the medium, with the medium included in some or all of the pores of the sheet-like porous support,

(3) a step of disposing the sheet-like porous support in a culturing vessel that houses medium, in such a manner that all or a portion of the surface of the sheet-like porous support wetted with the medium is exposed to a gas phase, and

(4) a step of setting the culturing vessel in an incubator and culturing the cells,

wherein the wetted state of the sheet-like porous support

surface and interior is maintained throughout the culturing.

[1a]

A cell culturing method including:

(1) a step of supporting cells on one or more sheet-like porous supports,

(2) a step of applying a medium to the sheet-like porous support supporting the cells and wetting the sheet-like porous support with the medium, with the medium included in some or all of the pores of the sheet-like porous support,

(3) a step of disposing the sheet-like porous support in a culturing vessel that houses the medium, in such a manner that all or a portion of a surface of the sheet-like porous support wetted with the medium is exposed to a gas phase, and

(4) a step of setting the culturing vessel in an incubator and culturing the cells,

wherein the wetted state of the sheet-like porous support surface and interior is maintained throughout the culturing,

wherein the sheet-like porous support is a porous polyimide film,

wherein the porous polyimide film has 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]

A culturing method according to [1], wherein in step (4), the medium is supplied into the culturing vessel in a continuous or intermittent manner, and all or a portion

of the sheet-like porous support wetted with the medium is exposed to a gas phase throughout the culturing.

[3]

A method according to [1] or [2], wherein in step (4), the step of culturing the cells is a step of culturing while supplying oxygen by oxygen-supplying means.

[4]

A method according to any one of [1] to [3], wherein in step (3), the sheet-like porous support is mounted on a rigid body.

[5]

A method according to [4], wherein the rigid body is a metal mesh.

[6]

A method according to any one of [1] to [5], wherein in step (3), a porous sheet with a larger mean pore size than the sheet-like porous supports is mounted in such a manner as to cover all or a portion of the top surface of the one or more sheet-like porous support.

[7]

A method according to [6], wherein the porous sheet with a larger mean pore size than the sheet-like porous support is selected from the group consisting of nonwoven fabrics, gauzes and sponges.

[8]

A method according to any one of [1] to [7], wherein the culturing vessel is an open vessel.

[9]

A method according to any one of [1] to [7], wherein the culturing vessel is a closed vessel.

[10]

A method according to any one of [1] to [9], wherein in step (3), two or more sheet-like porous supports are disposed in a manner layered one over the other.

[11]

A method according to any one of [1] to [10],

wherein in step (3), one or more sheet-like porous supports are disposed in a folded manner.

[12]

A method according to any one of [1] to [11], wherein the one or more sheet-like porous supports are porous polyimide films.

[13]

A method according to [12], wherein the porous polyimide film is a porous polyimide film including a polyimide obtained from a tetracarboxylic dianhydride and a diamine.

[14]

A method according to [13], 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.

[15]

A method according to [13] or [14], wherein the porous polyimide film is a porous polyimide film with a multilayer structure, having two different surface layers and a macro-void layer.

[16]

A method according to any one of [1] to [15], wherein the cells are transformed by genetic engineering technology so as to express a substance.

[17]

A method according to any one of [1] to [16], wherein the cells are selected from the group consisting of animal cells, insect cells, plant cells, yeast cells and bacteria.

[18]

A method according to [17], wherein the animal cells are cells derived from an animal belonging to the subphylum Vertebrata.

[19]

A method according to [18], wherein the cells are selected from the group consisting of CHO cells, CHO-K1 cells, Vero cells and MDCK cells.

[20]

A kit for use in the method according to any one of [1] to [19], including a porous polyimide film.

[20a]

A kit for use in the method according to any one of [1] to [19], including a porous polyimide film, wherein the porous polyimide film has 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 use of a porous polyimide film for the method according to any one of [1] to [19].

[21a]

Use of a porous polyimide film for the method according to any one of [1] to [19], wherein the porous polyimide film has 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).

[22]

A method of culturing cells by allowing them to

migrate onto a porous polyimide film, the method including contacting a porous polyimide film with a cell culture substrate in which cells are being cultured to allow the cells to migrate from the cell culture substrate onto the porous polyimide film, and culturing the cells that have been allowed to migrate onto the porous polyimide film.

[22a]

A method of culturing cells by allowing them to migrate onto a porous polyimide film, the method including contacting a porous polyimide film with a cell culture substrate in which cells are being cultured to allow the cells to migrate from the cell culture substrate onto the porous polyimide film, and culturing the cells that have been allowed to migrate onto the porous polyimide film,
wherein the porous polyimide film has 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]

A method according to [22], wherein the cell culture substrate is selected from the group consisting of plates, dishes, culture plates, culture flasks, microwell plates and glass bottom dishes, and the porous polyimide film is contacted with the top surface of the cell culture substrate.

[24]

A method according to [22], wherein the cell culture substrate is selected from the group consisting of microcarriers, silica porous bodies, cellulose sponges,

nonwoven fabrics and hollow fibers, and one or more porous polyimide films are contacted with the cell culture substrate from the top, bottom or both.

[25]

A method of culturing cells by allowing them to migrate onto a porous polyimide film, the method including contacting the top surface or bottom surface, or both, of a first porous polyimide film on which cells are being cultured, with a second porous polyimide film on which cells are not being cultured, to allow the cells to migrate from the first porous polyimide film to the second porous film, and culturing the cells that have been allowed to migrate to the second porous polyimide film.

[25a]

A method of culturing cells by allowing them to migrate onto a porous polyimide film, the method including contacting the top surface or bottom surface, or both, of a first porous polyimide film on which cells are being cultured, with a second porous polyimide film on which cells are not being cultured, to allow the cells to migrate from the first porous polyimide film to the second porous film, and culturing the cells that have been allowed to migrate to the second porous polyimide film

wherein the porous polyimide film has 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).

[26]

A method according to [25] wherein, while the first

porous polyimide film and the second porous polyimide film are in a contacted state, the film aggregate is lifted up into a gas phase.

[27]

A method according to [25] or [26], further including a step in which cells are applied to the empty first porous polyimide film wherein cells are not being cultured, and the cells are cultured on the first porous polyimide film.

[28]

A method according to [27], wherein the empty first porous polyimide film is contacted with the cell culture substrate in which cells are being cultured, and the cells are allowed to migrate from the cell culture substrate to the porous polyimide film to apply the cells to the first porous polyimide film.

[29]

A method of culturing cells by allowing them to migrate onto a porous polyimide film, the method including contacting a cell-containing biological sample with the top surface or bottom surface, or both, of a porous polyimide film, to allow the cells to migrate from the biological sample to the porous polyimide film, and culturing the cells that have been allowed to migrate to the porous polyimide film.

[29a]

A method of culturing cells by allowing them to migrate onto a porous polyimide film, the method including contacting a cell-containing biological sample with the top surface or bottom surface, or both, of a porous polyimide film, to allow the cells to migrate from the biological sample to the porous polyimide film, and culturing the cells that have been allowed to migrate to the porous polyimide film,

wherein the porous polyimide film has 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).

[30]

A method according to any one of [22] to [29], wherein the porous polyimide film is a porous polyimide film including a polyimide obtained from a tetracarboxylic dianhydride and a diamine.

[31]

A method according to [30], 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.

[32]

A method according to [30] or [31], wherein the porous polyimide film is a porous polyimide film with a multilayer structure, having two different surface layers and a macro-void layer.

[33]

A method according to [32], wherein the film thickness of the porous polyimide film is no greater than 75 μm.

[34]

A method according to any one of [22] to [33], wherein two or more porous polyimide films to which the cells have migrated are layered either above and below or left and right in the cell culture medium, and the cells are cultured.

[35]

A method according to any one of [22] to [34], which includes repeating two or more times the procedure of

allowing the cells to migrate from the cell culture substrate in which cells are being cultured, the cell-containing biological sample or the porous polyimide film in which cells are being cultured, to the porous polyimide film in which cells are not being cultured.

[36]

A kit for use in a method according to any one of [22] to [35], including a porous polyimide film.

[36a]

A kit for use in the method according to any one of [22] to [35], including a porous polyimide film, wherein the porous polyimide film has 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).

[37]

The use of a porous polyimide film for the method according to any one of [22] to [35].

[37a]

Use of a porous polyimide film for the method according to any one of [22] to [35], wherein the porous polyimide film has 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).

[Effects of the Invention]

[0020]

The present invention allows air to be efficiently supplied to cells, by culturing the cells using a sheet-like porous support such as a porous polyimide film, during which time a wet environment is maintained on the sheet-like porous support interior and surface while all or a portion of the sheet-like porous support surface is exposed to a gas phase. According to the invention there is no need for a special apparatus for oxygen supply, even in a large-scale cell culture system. Moreover, according to the invention, the sheet-like porous support is not settled deep inside the medium and hence there is not necessarily any need to move the sheet-like porous support for medium exchange. For example, medium exchange can be accomplished by creating a continuous or intermittent flow of the medium on the top or the bottom of the sheet-like porous support. In addition, since there is no need for movable parts for stirring or the like in the cell culture system employing the method of the invention, it is possible to provide a robust, highly stable cell culture system.

[0021]

Moreover, by using a porous polyimide film by the method of the invention, it has become possible to conveniently and efficiently subculture cells in a manner without a cell suspension.

[0022]

In addition, even when cells have reached confluency which is necessary for subculturing of conventional adherent cells, according to the invention a porous

What is claimed is:

1. A cell culturing method including:

(1) a step of supporting cells on one or more sheet-like porous supports,

(2) a step of applying a medium to the sheet-like porous support supporting the cells and wetting the sheet-like porous support with the medium, with the medium included in some or all of the pores of the sheet-like porous support,

(3) a step of disposing the sheet-like porous support in a culturing vessel that houses the medium, in such a manner that all or a portion of a surface of the sheet-like porous support wetted with the medium is exposed to a gas phase, and

(4) a step of setting the culturing vessel in an incubator and culturing the cells,

wherein the wetted state of the sheet-like porous support surface and interior is maintained throughout the culturing,

wherein the sheet-like porous support is a porous polyimide film,

wherein the porous polyimide film has 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 in step (4), the medium is supplied into the culturing vessel in a continuous or intermittent manner, and all or a portion of the sheet-like porous support wetted with the medium is exposed to the gas phase throughout the culturing.

3. The method according to claim 1 or 2, wherein in step (4), the step of culturing the cells is a step of

culturing while supplying oxygen by an oxygen-supplying means.

4. The method according to any one of claims 1 to 3, wherein in step (3), the sheet-like porous support is mounted on a rigid body.

5. The method according to claim 4, wherein the rigid body is a metal mesh.

6. The method according to any one of claims 1 to 5, wherein in step (3), a porous sheet with a larger mean pore size than the sheet-like porous support is mounted in such a manner as to cover all or a portion of the top surface of the one or more sheet-like porous supports.

7. The method according to claim 6, wherein the porous sheet with a larger mean pore size than the sheet-like porous support is selected from the group consisting of nonwoven fabrics, gauzes and sponges.

8. The method according to any one of claims 1 to 7, wherein the culturing vessel is an open vessel.

9. The method according to any one of claims 1 to 7, wherein the culturing vessel is a closed vessel.

10. The method according to any one of claims 1 to 9, wherein in step (3), two or more sheet-like porous supports are disposed in a manner layered one over the other.

11. The method according to any one of claims 1 to 10, wherein in step (3), one or more sheet-like porous supports are disposed in a folded manner.

12. The method according to any one of claims 1 to 11, wherein the one or more sheet-like porous supports are porous polyimide films.

13. The method according to claim 12, wherein the porous polyimide film is a porous polyimide film including a polyimide obtained from a tetracarboxylic dianhydride and a diamine.

14. The method according to claim 13, 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.

15. The method according to any one of claims 1 to 14, wherein the cells are transformed by genetic engineering technology so as to express a substance.

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

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

18. The method according to claim 17, wherein the cells are selected from the group consisting of CHO cells, CHO-K1 cells, Vero cells and MDCK cells.

19. A kit for use in the method according to any one of claims 1 to 18, including a porous polyimide film,

wherein the porous polyimide film has 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).

20. Use of a porous polyimide film for the method according to any one of claims 1 to 18,

wherein the porous polyimide film has 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. A method of culturing cells by allowing them to migrate onto a porous polyimide film, the method including contacting a porous polyimide film with a cell culture substrate in which cells are being cultured to allow the cells to migrate from the cell culture substrate onto the porous polyimide film, and culturing the cells that have been allowed to migrate onto the porous polyimide film,

wherein the porous polyimide film has 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).

22. The method according to claim 21, wherein the cell culture substrate is selected from the group consisting of plates, dishes, culture plates, culture flasks, microwell

plates and glass bottom dishes, and the porous polyimide film is contacted with the top surface of the cell culture substrate.

23. The method according to claim 21, wherein the cell culture substrate is selected from the group consisting of microcarriers, silica porous bodies, cellulose sponges, nonwoven fabrics and hollow fibers, and one or more porous polyimide films are contacted with the cell culture substrate from the top, bottom or both.

24. A method of culturing cells by allowing them to migrate onto a porous polyimide film, the method including contacting the top surface or bottom surface, or both, of a first porous polyimide film on which cells are being cultured, with a second porous polyimide film on which cells are not being cultured, to allow the cells to migrate from the first porous polyimide film to the second porous film, and culturing the cells that have been allowed to migrate to the second porous polyimide film

wherein the porous polyimide film has 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, while the first porous polyimide film and the second porous polyimide film are in a contacted state, the film aggregate is lifted up into a gas phase.

26. The method according to claim 24 or 25, further including a step in which cells are applied to the empty first

porous polyimide film wherein cells are not being cultured, and the cells are cultured on the first porous polyimide film.

27. The method according to claim 26, wherein the empty first porous polyimide film is contacted with the cell culture substrate in which cells are being cultured, and the cells are allowed to migrate from the cell culture substrate to the porous polyimide film to apply the cells to the first porous polyimide film.

28. A method of culturing cells by allowing them to migrate onto a porous polyimide film, the method including contacting a cell-containing biological sample with the top surface or bottom surface, or both, of a porous polyimide film, to allow the cells to migrate from the biological sample to the porous polyimide film, and culturing the cells that have been allowed to migrate to the porous polyimide film,

wherein the porous polyimide film has 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).

29. The method according to any one of claims 21 to 28, wherein the porous polyimide film is a porous polyimide film including a polyimide obtained from a tetracarboxylic dianhydride and a diamine.

30. The method according to claim 29, 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.

31. The method according to claim 30, wherein the film thickness of the porous polyimide film is no greater than 75 μm.

32. The method according to any one of claims 21 to 31, wherein two or more porous polyimide films to which the cells have migrated are layered either above and below or left and right in the cell culture medium, and the cells are cultured.

33. The method according to any one of claims 21 to 32, which includes repeating two or more times the procedure of allowing the cells to migrate from the cell culture substrate in which cells are being cultured, the cell-containing biological sample or the porous polyimide film in which cells are being cultured, to the porous polyimide film in which cells are not being cultured.

34. A kit for use in the method according to any one of claims 21 to 33, including a porous polyimide film,

wherein the porous polyimide film has 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).

35. Use of a porous polyimide film for the method according to any one of claims 21 to 33,

wherein the porous polyimide film has 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).