

The present inventors still further found that a porous polyimide film with a spatial structure morphologically approximating bone marrow structure can be used for healing of bone injury sites.

5 [0016]

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

[Mode 1]

10 A method of culturing bone marrow cells that includes applying bone marrow cells to a porous polyimide film and culturing them.

[Mode 1a]

15 A method of culturing bone marrow cells that includes applying bone marrow cells to a porous polyimide film and culturing them, wherein the porous polyimide film has a three-layer structure consisting of an A-surface layer having a plurality of pores, a B-surface layer having a plurality of pores, and a macro-void layer sandwiched between the two surface layers,
20 a mean pore size in the A-surface layer is smaller than a mean pore size in the B-surface layer, and the macro-void layer has a partition bonded to the A-surface layer and the B-surface layer, and a plurality of macro-voids surrounded by the partition, the A-surface
25 layer, and the B-surface layer.

[Mode 2]

The method according to mode 1, wherein the bone marrow cells are marrow stromal cells.

[Mode 3]

30 The method according to mode 1, wherein the bone marrow cells are bone marrow-derived blood cells.

[Mode 4]

35 The method according to any one of modes 1 to 3, wherein the bone marrow cells are cells harvested from mammalian bone marrow.

[Mode 5]

The method according to any one of modes 1 to 3,

wherein the bone marrow cells are primary cultured cells from cells harvested from mammalian bone marrow.

[Mode 6]

5 The method according to any one of modes 1 to 5, further including differentiation of bone marrow cells to hematocytes by culturing.

[Mode 7]

10 The method according to any one of modes 1 to 6, further including applying bone marrow cells to a porous polyimide film and then adding a differentiation-inducing accelerating substance and culturing, to accelerate differentiation from the bone marrow cells to hematocytes in a manner specific to the spatial structure of the porous polyimide film.

15 [Mode 8]

 The method of preparing hematocytes, including recovering hematocytes obtained using a method according to mode 6 or 7.

[Mode 9]

20 The method according to any one of modes 6 to 8, wherein the hematocytes are erythroid progenitor cells or erythrocytes.

[Mode 10]

 The method of culturing bone marrow cells including:

25 (1) a step of applying a first cell group to a porous polyimide film and culturing it, and

 (2) a step of applying a second cell group to the porous polyimide film after the culturing in step (1), and culturing it,

30 wherein the second cell group consists of bone marrow cells.

[Mode 10a]

 A method of culturing bone marrow cells including:

35 (1) a step of applying a first cell group to a porous polyimide film and culturing it, and

 (2) a step of applying a second cell group to the porous polyimide film after the culturing in step

(1), and culturing it,

wherein the second cell group consists of bone marrow cells, and

wherein the porous polyimide film has a three-layer

5 structure consisting of an A-surface layer having a plurality of pores, a B-surface layer having a plurality of pores, and a macro-void layer sandwiched between the two surface layers,

10 a mean pore size in the A-surface layer is smaller than a mean pore size in the B-surface layer, and

the macro-void layer has a partition bonded to the A-surface layer and the B-surface layer, and a plurality of macro-voids surrounded by the partition, the A-surface layer, and the B-surface layer. [Mode 11]

15 The method according to mode 10, wherein the first cell group is selected from the group consisting of animal cells, insect cells, plant cells, yeast cells and bacteria.

[Mode 12]

20 The method according to mode 11, wherein the first cell group consists of bone marrow cells derived from a mammal.

[Mode 13]

25 The method according to any one of modes 10 to 12, further including differentiation of bone marrow cells to hematocytes by the culturing in step (2).

[Mode 14]

30 The method according to any one of modes 10 to 13, further including applying the second cell group in step (2) to a porous polyimide film and then adding a differentiation-inducing accelerating substance and culturing, to accelerate differentiation from the bone marrow cells, as the second cell group, to hematocytes in a manner specific to the spatial structure of the porous

35 polyimide film.

[Mode 15]

A method of preparing hematocytes, including

recovering hematocytes obtained using a method according to mode 13 or 14.

[Mode 16]

5 The method according to any one of modes 13 to 15, wherein the hematocytes are erythroid progenitor cells or erythrocytes.

[Mode 17]

10 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]

15 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]

20 The method according to mode 17 or 18, 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 20]

25 The method according to any one of modes 1 to 19, using two or more porous polyimide films layered either above and below or left and right in the cell culture medium.

[Mode 21]

30 The method according to any one of modes 1 to 20, wherein the porous polyimide film is:

- i) folded,
 - ii) wound into a roll,
 - iii) connected as sheets or fragments by a
 - 35 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 22]

A kit for use in the method according to any one of modes 1 to 21, including a porous polyimide film.

5 [Mode 23]

Use of a porous polyimide film for the method according to any one of modes 1 to 21.

[Mode 24]

10 A porous polyimide film for healing of a bone injury site.

[Mode 24a]

Use of a porous polyimide film in the manufacture of a medicament for healing of a bone injury site, wherein the porous polyimide film has a three-layer
15 structure consisting of an A-surface layer having a plurality of pores, a B-surface layer having a plurality of pores, and a macro-void layer sandwiched between the two surface layers,
a mean pore size in the A-surface layer is smaller than a
20 mean pore size in the B-surface layer, and the macro-void layer has a partition bonded to the A-surface layer and the B-surface layer, and a plurality of macro-voids surrounded by the partition, the A-surface layer, and the B-surface layer. [Mode 25]

25 The porous polyimide film according to mode 24, wherein the bone injury is a fracture.

[Mode 26]

The porous polyimide film according to mode 24, wherein the bone injury is bone loss.

30 [Mode 27]

The porous polyimide film according to any one of modes 24 to 26, which is to be used for transplantation into the body in contact with an affected area of bone injury.

35 [Mode 28]

The porous polyimide film according to any one of modes 24 to 27, which is a porous polyimide film

including a polyimide obtained from a tetracarboxylic dianhydride and a diamine.

[Mode 29]

5 The porous polyimide film according to mode 28, which 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.

10 [Mode 30]

 The porous polyimide film according to mode 28 or 29, which is a porous polyimide film with a multilayer structure, having two different surface layers and a macro-void layer.

15 [Mode 31]

 The porous polyimide film according to mode 30, which is to be used for transplantation into the body in such a manner that of the two different surface layers, the surface with the smaller mean pore size is in contact with the affected area of bone injury.

20 [Mode 32]

 The porous polyimide film according to any one of modes 24 to 31, wherein the film thickness of the porous polyimide film is no greater than 100 micrometers.

25 [Mode 33]

 The porous polyimide film according to any one of modes 24 to 32, wherein the surface of the porous polyimide film is treated by a step of modifying its physical properties.

30 [Mode 34]

 The porous polyimide film according to mode 33, wherein the step of modifying the physical properties of the surface of the porous polyimide film is selected from the group consisting of a step of alkali treatment of the surface of the porous polyimide film, a step of calcium treatment, a step of covering with a biocompatible material, and a combination of any of these steps.

[Mode 35]

The method according to mode 34, wherein the biocompatible material is selected from the group consisting of collagen, fibronectin, laminin, polylysine, 5 polylactide, polyglycolide, polycaprolactone, polyhydroxybutyrate, polylactide-co-caprolactone, polycarbonate, biodegradable polyurethane, polyether ester, polyesteramide, hydroxyapatite, collagen/ β -TCP complex, and combinations of the foregoing.

10 [Mode 36]

The porous polyimide film according to any one of modes 24 to 35 on which cells have been supported beforehand.

[Mode 37]

15 The porous polyimide film according to mode 36, wherein the cells are cells selected from the group consisting of mammal-derived pluripotent stem cells, tissue stem cells, somatic cells, and combinations of the foregoing.

20 [Mode 38]

The porous polyimide film according to mode 37, wherein the cells include mammal-derived bone marrow cells.

[Mode 39]

25 A kit for healing of a bone injury site, including a porous polyimide film according to any one of modes 24 to 38.

[Mode 40]

30 Use of a porous polyimide film according to any one of modes 24 to 38 for healing of a bone injury site.

[Mode 41]

A method for producing a porous polyimide film for healing of a bone injury site, the method including supporting bone marrow cells on a porous polyimide film.

35 [Mode 41a]

A method for producing a porous polyimide film for healing of a bone injury site, the method including

supporting bone marrow cells on a porous polyimide film,
wherein the porous polyimide film has a three-layer
structure consisting of an A-surface layer having a
plurality of pores, a B-surface layer having a plurality
of pores, and a macro-void layer sandwiched between the
two surface layers,

a mean pore size in the A-surface layer is smaller than a
mean pore size in the B-surface layer, and
the macro-void layer has a partition bonded to the A-
surface layer and the B-surface layer, and a plurality of
macro-voids surrounded by the partition, the A-surface
layer, and the B-surface layer. [Mode 42]

The method according to mode 41, wherein the bone
marrow cells are harvested from a target of healing of a
bone injury site.

[Mode 43]

A method of healing a bone injury site, including
applying a porous polyimide film according to any one of
modes 24 to 38 to a bone injury site.

Effect of the Invention

[0017]

The present invention utilizes the spatial structure

CLAIMS

1. A method of culturing bone marrow cells that
5 includes applying bone marrow cells to a porous polyimide film
and culturing them,
wherein the porous polyimide film has a three-layer structure
consisting of an A-surface layer having a plurality of pores, a
B-surface layer having a plurality of pores, and a macro-void
10 layer sandwiched between the two surface layers,
a mean pore size in the A-surface layer is smaller than a mean
pore size in the B-surface layer, and
the macro-void layer has a partition bonded to the A-surface
layer and the B-surface layer, and a plurality of macro-voids
15 surrounded by the partition, the A-surface layer, and the B-
surface layer.

2. The method according to claim 1, wherein the bone
marrow cells are marrow stromal cells.

3. The method according to claim 1, wherein the bone
20 marrow cells are bone marrow-derived blood cells.

4. The method according to any one of claims 1 to 3,
wherein the bone marrow cells are cells harvested from
mammalian bone marrow.

5. The method according to any one of claims 1 to 3,
25 wherein the bone marrow cells are primary cultured cells from
cells harvested from mammalian bone marrow.

6. The method according to any one of claims 1 to 5,
further including differentiation of bone marrow cells to
hematocytes by culturing.

7. The method according to any one of claims 1 to 6,
30 further including applying bone marrow cells to a porous
polyimide film and then adding a differentiation-inducing
accelerating substance and culturing, to accelerate
differentiation from the bone marrow cells to hematocytes in a
35 manner specific to the spatial structure of the porous
polyimide film.

8. A method of preparing hematocytes, including
recovering hematocytes obtained using a method according to
claim 6 or 7.

40 9. The method according to any one of claims 6 to 8,

wherein the hematocytes are erythroid progenitor cells or erythrocytes.

10. A method of culturing bone marrow cells including:

- 5 (1) a step of applying a first cell group to a porous polyimide film and culturing it, and
(2) a step of applying a second cell group to the porous polyimide film after the culturing in step (1), and culturing it,

10 wherein the second cell group consists of bone marrow cells, and

wherein the porous polyimide film has a three-layer structure consisting of an A-surface layer having a plurality of pores, a B-surface layer having a plurality of pores, and a macro-void layer sandwiched between the two surface layers,
15 a mean pore size in the A-surface layer is smaller than a mean pore size in the B-surface layer, and
the macro-void layer has a partition bonded to the A-surface layer and the B-surface layer, and a plurality of macro-voids surrounded by the partition, the A-surface layer, and the B-surface layer.
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11. The method according to claim 10, wherein the first cell group is selected from the group consisting of animal cells, insect cells, plant cells, yeast cells and bacteria.

25 12. The method according to claim 11, wherein the first cell group consists of bone marrow cells derived from a mammal.

13. The method according to any one of claims 10 to 12, further including differentiation of bone marrow cells to hematocytes by the culturing in step (2).

30 14. The method according to any one of claims 10 to 13, further including applying the second cell group in step (2) to a porous polyimide film and then adding a differentiation-inducing accelerating substance and culturing, to accelerate differentiation from the bone marrow cells, as the second cell group, to hematocytes in a manner specific to the spatial
35 structure of the porous polyimide film.

15. A method of preparing hematocytes, including recovering hematocytes obtained using a method according to claim 13 or 14.

40 16. The method according to any one of claims 13 to 15, wherein the hematocytes are erythroid progenitor cells or

erythrocytes.

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

18. The method according to claim 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
10 dianhydride and a diamine, and a coloring precursor, and then
heat treating it at 250°C or higher.

19. The method according to any one of claims 1 to 18,
using two or more porous polyimide films layered either above
and below or left and right in the cell culture medium.

20. The method according to any one of claims 1 to 19,
wherein the porous polyimide film is:

i) folded,
ii) wound into a roll,
iii) connected as sheets or fragments by a
20 filamentous structure, or
iv) bound into a rope,
and used by suspension or anchoring in the cell culture medium
in the cell culturing vessel.

21. A kit for use in the method according to any one of
25 claims 1 to 20, including a porous polyimide film.

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

23. Use of a porous polyimide film in the manufacture of
a medicament for healing of a bone injury site,
30 wherein the porous polyimide film has a three-layer structure
consisting of an A-surface layer having a plurality of pores, a
B-surface layer having a plurality of pores, and a macro-void
layer sandwiched between the two surface layers,
a mean pore size in the A-surface layer is smaller than a mean
35 pore size in the B-surface layer, and
the macro-void layer has a partition bonded to the A-surface
layer and the B-surface layer, and a plurality of macro-voids
surrounded by the partition, the A-surface layer, and the B-
surface layer.

40 24. The use according to claim 23, wherein the bone

injury is a fracture.

25. The use according to claim 23, wherein the bone injury is bone loss.

5 26. The use according to any one of claims 23 to 25, wherein the medicament is to be used for transplantation into the body in contact with an affected area of bone injury.

27. The use according to any one of claims 23 to 26, wherein the porous polyimide film is a porous polyimide film including a polyimide obtained from a tetracarboxylic dianhydride and a diamine.

10 28. The use according to claim 27, 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 29. The use according to any one of claims 23 to 28, wherein the medicament is to be used for transplantation into the body in such a manner that the A-surface layer is in contact with the affected area of bone injury.

20 30. The use according to any one of claims 23 to 29, wherein the film thickness of the porous polyimide film is no greater than 100 micrometers.

25 31. The use according to any one of claims 23 to 30, wherein the surface of the porous polyimide film is treated by a step selected from the group consisting of a step of alkali treatment of the surface of the porous polyimide film, a step of calcium treatment of the surface of the porous polyimide film, a step of covering the surface of the porous polyimide film with a biocompatible material, and a combination of any of these steps.

30 32. The use according to claim 31, wherein the biocompatible material is selected from the group consisting of collagen, fibronectin, laminin, polylysine, polylactide, polyglycolide, polycaprolactone, polyhydroxybutyrate, polylactide-co-caprolactone, polycarbonate, biodegradable polyurethane, polyether ester, polyesteramide, hydroxyapatite, collagen/ β -TCP complex, and combinations of the foregoing.

35 33. The use according to any one of claims 23 to 32, wherein the porous polyimide film is a porous polyimide film on
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which cells have been supported beforehand.

34. The use according to claim 33, wherein the cells are cells selected from the group consisting of mammal-derived pluripotent stem cells, tissue stem cells, somatic cells, and combinations of the foregoing.

35. The use according to claim 34, wherein the cells include mammal-derived bone marrow cells.

36. A kit for healing of a bone injury site, including a porous polyimide film according to any one of claims 23 to 35.

37. A method for producing a porous polyimide film for healing of a bone injury site, the method including supporting bone marrow cells on a porous polyimide film, wherein the porous polyimide film has a three-layer structure consisting of an A-surface layer having a plurality of pores, a B-surface layer having a plurality of pores, and a macro-void layer sandwiched between the two surface layers, a mean pore size in the A-surface layer is smaller than a mean pore size in the B-surface layer, and the macro-void layer has a partition bonded to the A-surface layer and the B-surface layer, and a plurality of macro-voids surrounded by the partition, the A-surface layer, and the B-surface layer.

38. The method according to claim 37, wherein the bone marrow cells are harvested from a target of healing of a bone injury site.