

CLAIMS

1. A method of producing proliferated cells, the method comprising: culturing cells, which have been seeded
5 at a cell density of 0.002 to 2000 cells/cm², through adhesion culture in a proliferation culture medium in a presence of a culture substrate selected from a laminin fragment having integrin-binding activity and a modified form thereof, thereby proliferating the cells.

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2. The method according to claim 1, wherein the culturing is performed in a proliferation culture medium containing the culture substrate.

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3. The method according to claim 1 or 2, wherein the cells are stem cells.

4. The method according to any one of claims 1 to 3, wherein the cells are mesenchymal stem cells.

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5. The method according to any one of claims 1 to 4, wherein the cells are umbilical cord-derived mesenchymal stem cells.

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6. The method according to any one of claims 1 to 5, wherein the cells are frozen cells.

7. The method according to any one of claims 1 to 6,
wherein the culturing is performed until the cells reach a
confluent state.

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8. The method according to any one of claims 1 to 7,
wherein the culturing is performed in a culture vessel
having a bottom area of 500 cm² or more.

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9. The method according to any one of claims 1 to 8,
wherein the culture substrate is a laminin fragment having
integrin-binding activity.

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10. The method according to any one of claims 1 to 8,
wherein the culture substrate is a modified form of a
laminin fragment having integrin-binding activity.

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~~11. A method of producing a cell product, the method
comprising:~~

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~~culturing cells in a proliferation culture medium
according to the method according to any one of claims 1 to
10, thereby obtaining proliferated cells in an adhered
state; and~~

~~culturing the proliferated cells in a production
culture medium while maintaining the adhered state, thereby
causing the cells to produce a cell product.~~

~~12. A method of producing a cell product, the method comprising:~~

~~culturing cells in a proliferation culture medium according to the method according to any one of claims 1 to 10, thereby obtaining proliferated cells in an adhered state;~~

~~culturing the proliferated cells in a production culture medium while maintaining the adhered state, thereby causing the cells to produce a cell product; and~~

~~culturing the proliferated cells in a recovery culture medium while maintaining the adhered state, after performing the culturing in the production culture medium, wherein the culturing performed in the production culture medium and the culturing performed in the recovery culture medium are alternately repeated while maintaining the adhered state of the cells.~~

~~13. The method according to claim 11 or 12, wherein the production culture medium is a culture medium free of a xenogeneic component.~~

~~14. The method according to any one of claims 11 to 13, wherein the production culture medium is a culture medium free of a cytokine or insulin.~~

~~15. The method according to any one of claims 11 to 14,
wherein the production culture medium is a protein-free
culture medium.~~

5 ~~16. The method according to any one of claims 11 to 15,
further comprising collecting a supernatant of the
production culture medium after performing the culturing in
the production culture medium.~~

10 ~~17. A method of producing a culture supernatant of
stem cells, the method comprising:~~

~~— culturing stem cells through adhesion culture in a
proliferation culture medium in a presence of a culture
substrate selected from a laminin fragment having integrin-
15 binding activity and a modified form thereof, thereby
obtaining proliferated cells in an adhered state;~~

~~— culturing the proliferated cells in a production
culture medium while maintaining the adhered state, thereby
causing the cells to produce a cell product; and~~

20 ~~— culturing the proliferated cells in a recovery culture
medium while maintaining the adhered state, after
performing the culturing in the production culture medium,~~

~~— wherein the culturing performed in the production
culture medium and the culturing performed in the recovery
25 culture medium are alternately repeated while maintaining
the adhered state of the cells, and the method further~~

~~comprises collecting a supernatant of the production culture medium after performing the culturing in the production culture medium.~~

5 ~~18. The method according to claim 17, wherein the collecting of the supernatant of the production culture medium is performed after the culturing in the production culture medium is performed for a second or subsequent time.~~

10 ~~19. The method according to claim 17 or 18, wherein the stem cells are mesenchymal stem cells.~~

20 ~~20~~11. A method of producing a mesenchymal stem cell population having a reduced HLA-ABC positive rate, the method comprising: culturing mesenchymal stem cells in a proliferation culture medium in a presence of a culture substrate selected from a laminin fragment having integrin-binding activity and a modified form thereof, thereby obtaining proliferated cells.

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21 ~~21~~12. A mesenchymal stem cell population, comprising HLA-ABC positive mesenchymal stem cells at a ratio of 70% or less.

2213. The mesenchymal stem cell population according to claim 2112, comprising CD105 positive mesenchymal stem cells at a ratio of 50% or less.

~~23. A culture supernatant of stem cells, the culture supernatant comprising 5000 pg/mL or more of HGF.~~

~~24. A culture supernatant of stem cells, the culture supernatant comprising 50 pg/mL or more of CD9/CD63 EC domain fusion protein.~~

~~25. The culture supernatant according to claim 23 or 24, further comprising 3000 pg/mL or more of MCP-1, 1000 pg/mL or more of GRO, and 5 µg/mL or more of fibronectin.~~

~~26. The culture supernatant according to any one of claims 23 to 25, further comprising 200 pg/mL or more of TGF-1b, 5 pg/mL or more of IL-4, and 8 pg/mL or more of IL-10.~~

~~27. The culture supernatant according to any one of claims 23 to 26, wherein the culture supernatant is free of at least one of insulin, transferrin, or albumin.~~

~~28. The culture supernatant according to any one of claims 23 to 27, wherein the culture supernatant is free of a recombinant protein.~~

5 ~~29. The culture supernatant according to any one of claims 23 to 28, comprising at least one of IL-1 α , IL-1 β , or TNF- α in an amount of 0 to 15 pg/mL.~~

10 ~~30. The culture supernatant according to any one of claims 23 to 29, comprising 20 or more types of cytokines, wherein each cytokine has a concentration of 10 pg/mL or more.~~

15 ~~31. The culture supernatant according to any one of claims 23 to 30, wherein the culture supernatant is a culture supernatant of mesenchymal stem cells.~~

20 ~~32. The culture supernatant according to any one of claims 23 to 31, wherein the culture supernatant is a culture supernatant of umbilical cord derived mesenchymal stem cells.~~

~~33~~14. A therapeutic agent comprising the mesenchymal stem cell population according to claim 21-12 or 22-13 or
25 ~~the culture supernatant according to any one of claims 23 to 32.~~