Claims

Claim 1

A method for producing a cell preparation containing mesenchymal stem cells, comprising the steps of:

(A) proliferating mesenchymal stem cells in a serum-free medium "A" containing an FGF, a PDGF, a TGF-β, an HGF, an EGF, at least one phospholipid, and at least one fatty acid; and

(B) screening mesenchymal stem cells whose immunosuppression ability is maintained or improved, from the mesenchymal stem cells thus proliferated in the step (A).

Claim 2

A method according to claim 1, further comprising, before the step (B), the step of:

(C) culturing, in a medium containing serum, the mesenchymal stem cells which have been subjected to the step (A).

Claim 3

A method according to claim 1 or 2, further comprising, before the step (A), the step of:

(D) pre-proliferating the mesenchymal stem cells in a serum-free medium B containing an FGF, a PDGF, an EGF, at least one phospholipid, and at least one fatty acid.

Claim 4

A method according to any one of claims 1 to 3, further comprising the step of:

(E) screening the mesenchymal stem cells which do not have tumorigenicity, from the mesenchymal stem cells proliferated in the step (A).

Claim 5

The method according to any one of claims 1 to 4, wherein

in the step (A), the mesenchymal stem cells are proliferated by use of a culture vessel suitable for proliferation of the mesenchymal stem cells.

Claim 6

A method according to any one of claims 1 to 5, wherein

the serum-free medium "A" further contains cell adhesion molecules in the step (A).

Claim 7

The method according to any one of claims 1 to 6, wherein:

the mesenchymal stem cells are subcultured at least once in the step (A).

Claim 8

The method according to claim 7, wherein:

in the step (A), the subculturing is performed in such a way that the mesenchymal stem cells are peeled off by use of a cell removing agent which does not contain a component derived from a mammal or a microorganism.

Claim 9

A method according to claim 5, further comprising, before the step (A), the step of:

selecting the culture vessel suitable for the proliferation of the mesenchymal stem cells.

Claim 10

The method according to any one of claims 1 to 9, wherein:

the phospholipid is selected from the group consisting of phosphatidic acid, lysophosphatidic acid, phosphatidylinositol, phosphatidyl serine, phosphatidyl ethanolamine, phosphatidyl choline, and phosphatidyl glycerol.

Claim 11

The method according to any one of claims 1 to 10, wherein:

the fatty acid is selected from the group consisting of linoleic acid, oleic acid, linolenic acid, arachidonic acid, myristic acid, palmito~~y~~leic acid, palmitic acid, and stearic acid.

Claim 12

A cell preparation comprising mesenchymal stem cells which have been produced by a method according to any one of claims 1 to 11.

Claim 13

A serum-free additive ~~for a medium for producing a cell preparation containing mesenchymal stem cells whose immunosuppression ability is maintained or improved,~~ comprising~~:~~

~~———~~an FGF;

~~———~~a PDGF;

~~———~~a TGF-β;

~~———~~an HGF;

~~———~~an EGF;

~~———~~at least one phospholipid; and

~~———~~at least one fatty acid when used for a method for producing a cell preparation containing mesenchymal stem cells whose immunosuppression ability is maintained or improved, wherein the method comprises the steps of:

(A) adding the serum-free additive to a serum-free medium "A";

(B) proliferating mesenchymal stem cells in the serum-free medium "A"; and

(C) screening mesenchymal stem cells whose immunosuppression ability is maintained or improved, from the mesenchymal stem cells thus proliferated in the step (B).

Claim 14

A~~n~~ serum-free additive for a medium according to claim 13, further comprising

~~———~~cell adhesion molecules.

Claim 15

A serum-free culturing medium when used for a method for producing a cell preparation containing mesenchymal stem cells whose immunosuppression ability is maintained or improved, wherein the method comprisesing the steps of:

(A) adding a serum-free additive comprising an FGF; a PDGF; a TGF-β; an HGF; an EGF; at least one phospholipid; and at least one fatty acid to the serum-free culturing medium;

(B) proliferating mesenchymal stem cells in the serum-free culturing medium; and

(C) screening mesenchymal stem cells whose immunosuppression ability is maintained or improved, from the mesenchymal stem cells thus proliferated in the step (B)~~an additive for a medium according to claim 13 or 14~~.

Claim 16

A culture method for producing a cell preparation containing mesenchymal stem cells whose immunosuppression ability is maintained or improved, comprising the step of:

(D~~F~~) culturing mesenchymal stem cells in the serum-free~~a~~ culturing medium according to claim 15.

Claim 17

A kit when used for a method for producing a cell preparation containing mesenchymal stem cells whose immunosuppression ability is maintained or improved, comprising at least:

an additive "A" for a medium according to claim 13 or 14 and ~~,~~

~~Claim 18~~

~~A kit according to claim 17, further comprising:~~

~~an additive B for a medium containing an FGF, a PDGF, an EGF, at least one phospholipid, and at least one fatty acid.~~