CLAIMS

1. A stem cell culturing method for culturing specific types of stem cells using a first bone marrow aspirate collected from a donor, wherein

the stem cell culturing method comprises a bone marrow aspirate separating step of separating the first bone marrow aspirate collected from a donor into layers, a bone marrow aspirate extracting step of extracting a second bone marrow aspirate positioned at an intermediate layer among the first bone marrow aspirate separated into layers in the bone marrow aspirate separating step, a deformation first observation step of observing deformation of first stem cells in a first culturing vessel from an initial planar shape while statically allowing it for a predetermined time statically in a state of inclining the first culturing vessel at an inclination angle of 2 to 5° with predetermined time intervals after injecting the second bone marrow aspirate extracted in the bone marrow aspirate extracting step and a predetermined culture medium into the first culturing vessel having a predetermined volume and a predetermined area of a bottom surface, a total surface area first observation step of injecting a new culture medium into the first culturing vessel while discharging the culture medium in the first culturing vessel by judging the first stem cells be fixed to the bottom surface of the first culturing vessel when the first stem cells are deformed from the initial planar shape to a predetermined planar shape as a result of observation in the deformation first observation step, and observing a total surface area of the first stem cells fixed to the bottom surface

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of the first culturing vessel with respect to the bottom surface area of the first culturing vessel while statically allowing it for a predetermined time in a state of inclining the first culturing vessel at an inclination angle of 2 to 5° with predetermined time intervals, a stem cell first extracting step of extracting the first stem cells from the first culturing vessel when the planar shape of the first stem cells is expanded by proliferating the first stem cells at the bottom surface area of the first culturing vessel and the total surface area of the first stem cells with respect to the bottom surface area of the first culturing vessel reached a first target ratio as a result of observation in the total surface area first observation step, a stem cell centrifugation step of centrifuging the first stem cells extracted in the stem cell first extracting step into layers, a stem cell second extracting step of extracting the second stem cells positioned at an undermost layer after separating the first stem cells into two layers in the stem cell centrifugation step, and a stem cell third extracting step of injecting the second stem cells extracted in the stem cell second extracting step and a predetermined culture medium into a second culturing vessel having a larger capacity and a larger bottom surface area of the bottom surface than those of the first culturing vessel, statically allowing the second culturing vessel for a predetermined time to fix the second stem cells to the bottom surface of the second culturing vessel and proliferating the second stem cells, and when a total surface area of the second stem cells with respect to the bottom surface of the second culturing vessel reached a second target ratio by expanding a planar shape of the second stem cells at the bottom surface area of the second culturing vessel, extracting the

second stem cells from the second culturing vessel.

2. The stem cell culturing method according to claim 1, wherein the stem cell culturing method comprises a deformation second observation step of injecting the second stem cells extracted in the stem cell second extracting step and the culture medium into the second culturing vessel, then, observing deformation of the second stem cells in the second culturing vessel from an initial planar shape while statically allowing it in a state of inclining the second culturing vessel at an inclination angle of 2 to 5° for a predetermined time statically with predetermined time intervals, and a total surface area second observation step of injecting a new culture medium into the second culturing vessel while discharging the culture medium in the second culturing vessel by judging the second stem cells be fixed to the bottom surface of the second culturing vessel when the second stem cells are deformed from the initial planar shape to a predetermined planar shape as a result of observation in the deformation second observation step, and observing a total surface area of the second stem cells fixed to the bottom surface of the second culturing vessel with respect to the bottom surface area of the second culturing vessel while statically allowing it for a predetermined time in a state of inclining the second culturing vessel at an inclination angle of 2 to 5° with predetermined time intervals, and in the stem cell third extracting step, when the planar shape of the second stem cells is expanded by proliferating the second stem cells and the total surface area of the second stem cells with respect to the bottom surface area of the second culturing vessel reached the second target ratio as a result of observation in the total surface area

second observation step, the second stem cells are extracted from the second culturing vessel.

3. The stem cell culturing method according to claim 1 or claim 2, wherein, in the bone marrow aspirate separating step, 2 to 3 cc of the first bone marrow aspirate are collected from the donor, the 2 to 3 cc of the first bone marrow aspirate are injected into a separating vessel extending in the vertical direction, and the separating vessel is statically allowed at substantially the same temperature as a body temperature for a predetermined time to separate the first bone marrow aspirate in the separating vessel into layers in the vertical direction, and in the bone marrow aspirate extracting step, the second bone marrow aspirate positioned at an intermediate layer in the first bone marrow aspirate separated into layers in the separating vessel is extracted.

4. The stem cell culturing method according to any one of claim 1 to claim 3, wherein a capacity of the first culturing vessel is 20 to 30 cc, an initial planar shape of the first stem cells is substantially circular, a planar shape of the first stem cells after deformation is a flat shape in which the first stem cells are extended in irregular in one direction with substantially the circular as a core, and in the deformation first observation step, deformation of the first stem cells in the first culturing vessel from an initial planar shape is observed during 12 to 24 hours with intervals of 1 to 2 hours while statically allowing the first culturing vessel at substantially the same temperature as a body temperature for 12 to 24 hours, and when the first stem cells are deformed in an irregular flat shape, the first stem

cells are judged to be fixed to the bottom surface of the first culturing vessel.

5. The stem cell culturing method according to any one of claim 1 to claim 4, wherein, in the total surface area first observation step, the total surface area of the first stem cells fixed to the bottom surface of the first culturing vessel with respect to the bottom surface area of the first culturing vessel is observed during 36 to 48 hours with intervals of 1 to 2 hours while statically allowing the first culturing vessel at substantially the same temperature as a body temperature for 36 to 48 hours.

6. The stem cell culturing method according to any one of claim 1 to claim 5, wherein the first target ratio of the total surface area of the first stem cells with respect to the bottom surface area of the first culturing vessel is 70 to 80%.

7. The stem cell culturing method according to any one of claim 1 to claim 6, wherein, in the stem cell centrifugation step, the first stem cells are injected into the separating vessel, the separating vessel is located in a centrifugal separator to centrifuge the first stem cells, and in the stem cell second extracting step, the first stem cells are centrifuged into layers in the separating vessel, and then, the second stem cells located at the undermost layer are extracted.

8. The stem cell culturing method according to any one of claim 1 to claim 7, wherein a capacity of the second culturing vessel is 40 to 60 cc, an initial planar shape of the second stem cells

is substantially circular, a planar shape of the first stem cells after deformation of the second stem cells is a flat shape in which the second stem cells are extended in irregular in one direction with substantially the circular as a core, and in the deformation second observation step, deformation of the second stem cells in the second culturing vessel from an initial planar shape is observed during 36 to 48 hours with intervals of 1 to 2 hours while statically allowing the second culturing vessel at substantially the same temperature as a body temperature for 36 to 48 hours, and when the second stem cells are deformed in an irregular flat shape, the second stem cells are judged to be fixed to the bottom surface of the second culturing vessel.

9. The stem cell culturing method according to any one of claim 1 to claim 8, wherein, in the total surface area second observation step, the total surface area of the second stem cells fixed to the bottom surface of the second culturing vessel with respect to the bottom surface area of the second culturing vessel is observed during the 36 to 48 hours with intervals of 1 to 2 hours while statically allowing the second culturing vessel at substantially the same temperature as a body temperature for 36 to 48 hours.

10. The stem cell culturing method according to any one of claim 1 to claim 9, wherein the second target ratio of the total surface area of the second stem cells with respect to the bottom surface area of the second culturing vessel is 88 to 92%.

11. The stem cell culturing method according to any one of claim 1 to claim 10, wherein the first and the second stem cells are

mesenchymal stem cells.