CLAIMS

1. A method for manufacturing a culture product liquid, using a stem cell made from a bone marrow liquid collected from a donor, the method comprising the steps of:

separating a bone marrow liquid by separating in layers a bone marrow liquid collected from the donor;

extracting a bone marrow liquid by extracting an intermediate-layer bone marrow liquid positioned in an intermediate layer of the bone marrow liquid separated in layers by the step of separating a bone marrow liquid;

fixing a first stem cell by feeding the intermediate-layer bone marrow liquid extracted by the step of extracting a bone marrow liquid and a predetermined culture liquid into a first culture container having a predetermined capacity and a bottom surface of a predetermined area, allowing the first culture container to statically stand at a predetermined tilted angle for a predetermined period of time, and fixing a first stem cell contained in the intermediate-layer bone marrow liquid to a bottom surface of the first culture container;

culturing a first stem cell by discharging the culture liquid in the first culture container after fixing the first stem cell to the bottom surface of the first culture container by the step of fixing a first stem cell, feeding a new culture liquid into the first culture container, allowing the first culture container to statically stand at a predetermined tilted angle for a predetermined period of time, and culturing the first stem cell until the total area of the first stem cell reaches a first target ratio of the bottom-surface area of the first culture container area;

collecting a first stem cell by collecting the first stem cell from the first culture container when the total area of the first stem cell reaches the first target ratio in the process of culturing the first stem cell by the step of culturing a first stem cell;

separating a stem cell by centrifugally separating in layers the first stem cell collected by the step of collecting a first stem cell;

collecting a second stem cell by collecting the second stem cell positioned in the lowermost layer of the first stem cell separated in layers by the step of separating a stem cell;

fixing a second stem cell for feeding the second stem cell collected by the step of collecting a second stem cell and a predetermined culture liquid into a second culture container having a larger capacity and a bottom surface of a larger bottom-surface area than the first culture container, allowing the second culture container to statically stand at a predetermined tilted angle for a predetermined period of time, and fixing the second stem cell to a bottom surface of the second culture container;

culturing a second stem cell by discharging the culture liquid in the second culture container after fixing the second stem cell to the bottom surface of the second culture container by the step of fixing a second stem cell, feeding a new culture liquid into the second culture container, allowing the second culture container to statically stand at a predetermined tilted angle for a predetermined period of time, and culturing the second stem cell until the total area of the second stem cell reaches a second target ratio of the bottom-surface area of the second culture container; and

extracting a first culture product liquid by extracting from the second culture container a culture product liquid containing a predetermined metabolite secreted from the single type of second stem cell proliferated when the total area of the second stem cell reaches the second target ratio in the process of culturing in the step of second culturing a stem cell;

wherein the tilted angle of the first culture container ranges from 2 to 5° and the tilted angle of the second culture container ranges from 2 to 5°.

2. The method for manufacturing a culture product liquid according to claim 1, comprising the steps of:

collecting a second stem cell by collecting the second stem cell from the second culture container after extracting a culture product liquid from the second culture container by the step of extracting a first culture product liquid;

fixing a third stem cell by feeding the second stem cell collected by the step of collecting a second stem cell and a new culture liquid into a third culture container having a predetermined capacity and a bottom surface of a predetermined area, the capacity and the bottom-surface area being larger than the second culture container, allowing the third culture container to statically stand for a predetermined period of time, and fixing the second stem cell to a bottom surface of the third culture container;

culturing a third stem cell by discharging the culture liquid in the third culture container after fixing the second stem cell to the bottom surface of the third culture container by the step of fixing a third stem cell, feeding a new culture liquid into the third culture container, allowing the third culture container to statically stand for a predetermined period of time, and culturing the second stem cell until the total area of the second stem cell reaches a third target ratio of the bottom-surface area of the third culture container; and

extracting a second culture product liquid by extracting from the third culture container a culture product liquid containing a predetermined metabolite secreted from the single type of second stem cell proliferated when the total area of the second stem cell reaches the third target ratio in the process of culturing in the step of culturing a third stem cell.

3. The method for manufacturing a culture product liquid according to claim 2, wherein in the steps of fixing a third stem cell and culturing a third stem cell, the third culture container is allowed to statically stand at a predetermined tilted angle for a predetermined period of time.

4. The method for manufacturing a culture product liquid according to any one of claims 1 to 3, wherein in the step of separating a bone marrow liquid, 2 to 3cc of a bone marrow liquid is collected from the donor, 2 to 3cc of the bone marrow liquid is fed into a vertically elongating separation container, the separation container is allowed to statically stand at approximately the same temperature as the body temperature for a predetermined period of time, and the bone marrow liquid is vertically separated in layers in the separation container, and in the step of extracting a bone marrow liquid, the intermediate-layer bone marrow liquid positioned in the intermediate layer of the bone marrow liquid separated in layers in the separation container is extracted.

5. The method for manufacturing a culture product liquid according to any one of claims 1 to 4, wherein the capacity of the first culture container is 20 to 30cc, the initial plane shape of the first stem cell is an approximately circular shape, and the plane shape of the first stem cell deformed is primarily the approximately circular shape, and a flat shape when the first stem cell elongates in amorphous form in one direction, and in the step of fixing a first stem cell, the first culture container is allowed to statically stand at approximately the same temperature as the body temperature for a period of 12 to 24 hours, the deformation of the first stem cell from the initial plane shape in the first culture container is observed at an interval of 1 to 2 hours for the period of 12 to 24 hours,

and when the first stem cell is deformed from the initial plane shape to the amorphous flat shape, it is determined that the first stem cell is fixed to the bottom surface of the first culture container.

6. The method for manufacturing a culture product liquid according to any one of claims 1 to 5, wherein in the step of collecting a first stem cell, the first culture container is allowed to statically stand at approximately the same temperature as the body temperature for a period of 36 to 48 hours, and the total area of the first stem cell fixed to the bottom surface of the first culture container in relation to the bottom-surface area of the first culture container area is observed at an interval of 1 to 2 hours for the period of 36 to 48 hours.

7. The method for manufacturing a culture product liquid according to claim 6, wherein the first target ratio of the total area of the first stem cell is 70 to 80% of the bottom-surface area of the first culture container area.

8. The method for manufacturing a culture product liquid according to any one of claims 1 to 7, wherein in the step of separating a stem cell, the first stem cell is fed into a separation container, the separation container is placed in a centrifugal separator to centrifugally separate the first stem cell, and in the step of collecting a first stem cell, the second stem cell positioned in the lowermost layer of the first stem cell centrifugally separated in layers in the separation container is collected.

9. The method for manufacturing a culture product liquid according to any one of claims 2 to 8, wherein the capacity of the second culture container is 40 to 60cc, the capacity of the third culture container is 70 to 80cc, the initial plane shape of the second stem cell is an approximately

circular shape, the plane shape of the second stem cell deformed is primarily the approximately circular shape, and a flat shape when the second stem cell elongates in amorphous form in one direction, and in the steps of fixing a second stem cell and third fixing a stem cell, the second and the third culture containers are allowed to statically stand at approximately the same temperature as the body temperature for a period of 36 to 48 hours, the deformation of the second stem cell from the initial plane shape in the second and the third culture containers is observed at an interval of 1 to 2 hours for the period of 36 to 48 hours, and when the second stem cell is deformed from the initial plane shape to the amorphous flat shape, it is determined that the second stem cell is fixed to the bottom surfaces of the second and the third culture containers.

10. The method for manufacturing a culture product liquid according to any one of claims 2 to 9, wherein in the steps of collecting a second stem cell and collecting a third stem cell, the second and the third culture containers are allowed to statically stand at approximately the same temperature as the body temperature for a period of 36 to 48 hours, the total area of the second stem cell fixed to the bottom surface of the second and the third culture containers in relation to the bottom-surface area of the second and the third culture containers is observed at an interval of 1 to 2 hours for the period of 36 to 48 hours.

11. The method for manufacturing a culture product liquid according to any one of claims 2 to 10, wherein the second target ratio of the total area of the second stem cell is 88 to 92% of the bottom-surface area of the second culture container, and the third target ratio of the total area of the second stem cell is 88 to 92% of the bottom-surface area of the third culture container.

12. The method for manufacturing a culture product liquid according to any one of claims 1 to 11, wherein the first and second stem cells are mesenchymal stem cells.