push it out of a syringe by hand, which in turn has created a demand for separation solutions that enable easy recovering using a syringe and also enable white blood cells or mononuclear cells to be recovered at a high recovery rate.

[0011]

Patent Document 1: JP 2001-518792 T

Patent Document 2: WO 98/32840

[0012]

Non-Patent Document 1: TRANSFUSION, Vol. 45, pp. 1899-1908, 2005

SUMMARY OF THE INVENTION

[0013]

An object of the present ~~invention~~disclosure is to provide a method that can rapidly and easily separate a red blood cell-rich fraction, a white blood cell-rich fraction, and/or a platelet-rich fraction from a biological fluid containing these blood cell components without requiring centrifugation. A further object of the present ~~invention~~disclosure is to provide a separation material and a separation method that can recover, in particular, a mononuclear cell-rich fraction with low contamination of granular leukocytes, among white blood cells.

[0014]

The present inventors have intensively studied to achieve a method that enables blood cell components to be separated from a biological fluid without requiring centrifugation, which has been difficult to achieve, and have found that a red blood cell-rich fraction, a white blood cell-rich fraction, and/or a platelet-rich fraction can be separated from a biological fluid as follows: the red blood cell-rich fraction is obtained by capturing white blood cells and platelet on a blood cell separation material of a specific type; and then the white blood cell-rich fraction is obtained by recovering the white blood cells captured on the blood cell separation material using a separation solution. Thus, the present inventors have

completed the present invention.

[0015]

The present ~~invention~~disclosure relates to a method that can separate a red blood cell-rich fraction, a white blood cell-rich fraction, and/or a platelet-rich fraction from a biological fluid containing these blood cell components, by using a blood cell separation material that is able to capture white blood cells and platelets and allows separation and recovery of a white blood cell-rich fraction. Accordingly, the method of the present ~~invention~~disclosure is completely different from the conventional white blood cell capture and recovery methods in which a white blood cell removal filter is used or a material that substantially allows platelets to pass therethrough is used as disclosed in Patent Documents 1 and 2.

[0016]

The present inventors have also found that in particular a mononuclear cell-rich fraction which contains a high level of mononuclear cells among white blood cells can be efficiently separated by using a nonwoven fabric that has a specific density and a specific fiber diameter, as a blood cell separation material.

[0017]

Specifically, the present ~~invention~~disclosure relates to a method for separating blood cell components from a biological fluid, which includes:

(a) capturing white blood cells and platelets on a blood cell separation material by contacting the biological fluid with the blood cell separation material, thereby providing a red blood cell-rich fraction; and

(b) separating a white blood cell-rich fraction from the resulting blood cell separation material using a separation solution.

[0018]

In one aspect, there is provided a method for separating blood cell components from a biological fluid, comprising:

(a) capturing white blood cells and platelets on a blood cell separation material by contacting the biological fluid with the blood cell separation material, thereby providing a red blood cell-rich fraction whose white blood cell recovery rate is not more than 10%; and

(b) separating a white blood cell-rich fraction from the resulting blood cell separation material using a separation solution.

Preferably, the blood cell separation material is packed in a container having an inlet and an outlet for the biological

Preferably, the polyester fiber is a polyethylene terephthalate fiber or a polybutylene terephthalate fiber. Preferably, the nonwoven fabric includes a nylon fiber, a polypropylene fiber, or a polybutylene terephthalate fiber.

[0027]

Preferably, the nonwoven fabric has a density K of 2.0×10^4 to 1.9×10^5 and has a fiber diameter of 1 μm to 15 μm, and the white blood cell-rich fraction is a mononuclear cell-rich fraction.

[0028]

Preferably, the separation solution is a solution having a viscosity of from 1 mPa·s or higher to lower than 5 mPa·s.

[0029]

Preferably, a platelet recovery rate of the white blood cell-rich fraction or the mononuclear cell-rich fraction is not more than a white blood cell recovery rate of the white blood cell-rich fraction or the mononuclear cell-rich fraction. Preferably, a platelet recovery rate of the white blood cell-rich fraction or the mononuclear cell-rich fraction is not more than a mononuclear cell recovery rate of the white blood cell-rich fraction or the mononuclear cell-rich fraction.

[0030]

Preferably, a ratio of a mononuclear cell recovery rate to a granular leukocyte recovery rate of the white blood cell-rich fraction or the mononuclear cell-rich fraction is larger than 1.0.

[0031]

Preferably, the white blood cell-rich fraction or the mononuclear cell-rich fraction includes a hematopoietic stem cell, a mesenchymal stem cell, or a CD34-positive cell.

[0032]

The present ~~invention~~disclosure also relates to a red blood cell-rich fraction, a white blood cell-rich fraction, or a platelet-rich fraction separated by the above-described method.

In another aspect, there is provided a red blood cell-rich fraction whose red blood cell recovery rate is not less than 80%, white blood cell recovery rate is not more than 10%, and platelet recovery rate is not more than 40%;, a white blood cell-rich fraction whose white blood cell recovery rate is not less than 45%, red blood cell recovery rate is not more than 10%, and platelet recovery rate is not more than 50%, or a platelet-rich fraction separated by the method as defined above.

[0033]

The present ~~invention~~disclosure further relates to a blood cell separation material which includes a nonwoven fabric that allows separation of a mononuclear cell-rich fraction from a biological fluid, wherein the nonwoven fabric has a density K of 2.0×10^4 to 1.9×10^5 and has a fiber diameter of 1 μm to 15 μm.

[0034]

In yet another aspect, there is provided a blood cell separation material comprising

a nonwoven fabric that allows separation of a mononuclear cell-rich fraction from a biological fluid,

wherein the nonwoven fabric has a density K of 2.0×10^4 to 1.9×10^5 and has a fiber diameter of 1 μm to 15 μm, and wherein the nonwoven fabric comprises a polyethylene terephthalate fiber and/or a polybutylene terephthalate fiber.

Preferably, the blood cell separation material includes a nylon fiber, a polypropylene fiber, or a polybutylene terephthalate fiber.

[0035]

The present ~~invention~~disclosure further relates to a blood cell separation device including the above-described blood cell separation material, wherein a laminate of layers of the blood cell separation material is packed in a container having an inlet and an outlet for a biological fluid.

[0036]

In yet another aspect, there is provided a blood cell separation device comprising

the blood cell separation material as defined above,

wherein a laminate of layers of the blood cell separation material is packed in a container having an inlet and an outlet for a biological fluid.

The present ~~invention~~disclosure further relates to a blood cell separation device which includes:

the container packed with a laminate of layers of the blood cell separation material;

a flow-path opening and closing means located upstream of the inlet of the container;

a white blood cell-rich fraction recovery means connected to the flow-path opening and closing means; and

a separation solution introducing means located downstream of the outlet of the container.

[0037]

The present ~~invention~~disclosure further relates to a separation solution for recovering white blood cells captured on a blood cell separation material or a nonwoven fabric, which has a viscosity of from 1 mPa·s or higher to lower than 5 mPa·s. Preferably, the separation solution is free from dextran.

[0038]

The method of the present ~~invention~~disclosure can easily and rapidly separate a red blood cell-rich fraction, a white blood cell-rich fraction, and/or a platelet-rich fraction from a biological fluid such as whole blood, bone marrow, umbilical cord blood, menstrual blood, or a tissue extract. The method of the present ~~invention~~disclosure enables red blood cells, platelets, and white blood cells in a biological fluid to be separated and recovered although the conventional methods cannot separate and recover all these components, and cannot recover some of these. In addition, the use of the nonwoven fabric in the present ~~invention~~disclosure as a blood cell separation material provides a mononuclear cell-rich fraction.

[0039]

The method of the present ~~invention~~disclosure provides a red blood cell-rich fraction with remarkably low contamination of other blood cells. Such a fraction can be used for blood transfusion without being subjected to any processes. The method of the present ~~invention~~disclosure provides a white blood cell-rich fraction or a mononuclear cell-rich fraction with remarkably low contamination of red blood cells. Such a fraction will hardly receive bad influence caused by red blood cell hemolysis or the like even if it is cryopreserved until used. A filter constituted by a container packed with the separation material of the present ~~invention~~disclosure can be used in an aseptic closed system, which means that fractions can be aseptically recovered and used to amplify cells therein, without being subjected to any processes.

[0040]

The blood cell separation material of the present ~~invention~~disclosure can be used as a filter for the preparation of therapeutic cells for regenerative medicine, such as leukemia treatment, cardiac muscle regeneration, and blood vessel regeneration. The blood cell separation material of the present ~~invention~~disclosure is also very useful as a filter for the preparation of cell sources for regenerative medicine as well