CLAIMS

1. A method for separating cells depending on sizes from a cell suspension containing two or more kinds of cells, which are targeted cells and untargeted cells, in a continuous fluid flow, the method comprising the steps of:

adding a target-capturing substance that recognizes a characteristic structure on cell surfaces of the targeted cells to the cell suspension to thereby produce complexes of the targeted cells and the target-capturing substance,

introducing the cell suspension containing the complexes into a cell-separating device including a separation area having a DLD microchannel structure, wherein a buffer is added and allowed to flow into a buffer inlet of the cell-separating device, and the cell suspension is added and allowed to flow into a sample inlet and to pass through a plurality of separation areas of the cell-separating device,

separating complexes having sizes not smaller than a determined threshold from the cell suspension, wherein cells having sizes smaller than the threshold move together with the cell suspension flow, and the complexes having the sizes not smaller than the threshold are obliquely displaced to move relative to the flow, thereby being separated, and

recovering the separated complexes from the outlet,

wherein the size of the target-capturing substance is 7 to 60 μm, the target cells are circulating tumor cells (CTCs), and

the target-capturing substance is composed of a combination body of a target-capturing molecule that recognizes the characteristic structure on the cell surfaces of the targeted cells and a substance carrying the target-capturing molecule, said substance carrying the target-capturing molecule has the gravity close to or slightly higher than the gravity of water.

2. A method for separating cells depending on sizes from a cell suspension containing two or more kinds of cells, which are

targeted cells and untargeted cells, in a continuous fluid flow, the method comprising the steps of:

adding a target-capturing substance that recognizes a characteristic structure on cell surfaces of the untargeted cells to the cell suspension to thereby produce complexes of the untargeted cells and the target-capturing substance,

introducing the cell suspension containing the complexes into a cell-separating device including a separation area having a DLD microchannel structure, wherein a buffer is added and allowed to flow into a buffer inlet of the cell-separating device, and the cell suspension is added and allowed to flow into a sample inlet and to pass through a plurality of separation areas of the cell-separating device,

separating complexes having sizes not smaller than a determined threshold from the cell suspension, wherein cells having sizes smaller than the threshold move together with the cell suspension flow, and the complexes having the sizes not smaller than the threshold are obliquely displaced to move relative to the flow, thereby being separated, and

recovering the separated targeted cells from the outlet, wherein the size of the target-capturing substance is 7 to 60 μm, the target cells are circulating tumor cells (CTCs),and

the target-capturing substance is composed of a combination body of a target-capturing molecule that recognizes the characteristic structure on the cell surfaces of the untargeted cells and a substance carrying the target-capturing molecule, said substance carrying the target-capturing molecule has the gravity close to or slightly higher than the gravity of water.

3. The method for separating cells according to claim 1 or 2, wherein the size of the targeted cells is similar to the size of the untargeted cells, or the sizes of the targeted cells and the untargeted cells overlap with each other.

4. The method for separating cells according to any one of claims 1 to 3, wherein the target-capturing molecule is an antibody,

a peptide aptamer, a lectin, an intercellular adhesion molecule, a sugar chain or a cell-recognizable polymer.

5. The method for separating cells according to any one of claims 1 to 4, wherein the substance carrying the target-capturing molecule is polystyrene or latex.

6. The method for separating cells according to any one of claims 1 to 5, wherein the determined threshold is 20 to 60 μm.

7. The method for separating cells according to any one of claims 1 to 6, wherein the cell suspension is blood.

8. The method for separating cells according to any one of claims 1 to 7, the height of the microchannel structure is set to a height at which two or more of the complexes cannot be simultaneously present in the height direction of the microchannel space.

9. The method for separating cells according to any one of claims 1 to 8, wherein

the cell suspension further contains the minute aggregates,

the step of removing minute aggregates from the cell suspension in the continuous fluid flow is further included after the step of producing the complexes and before the step of introducing the cell suspension into the cell-separating device,

the removal step is a step in which the cell suspension or a liquid mixture of the cell suspension and a buffer solution is allowed to flow into the minute aggregate-removing device including the removal structure having a microchannel structure and to pass through the microchannel in the minute aggregate-removing device, thereby capturing the minute aggregates.

10. The method for separating cells according to claim 9, wherein the microchannel structure is composed of pillars arranged at intervals wider than 30 μm;

wherein the microchannel structure is composed of two separate microchannel structure portions (structure portion α and structure portion β);

wherein the pillar arrangement interval in the microchannel structure portion α on the upstream side is wider than that in the microchannel structure portion β on the downstream side,

wherein the portion in the middle is narrower than the microchannel structure portions and has no pillar arranged, and

wherein a plurality of the pillars is provided at regular intervals parallel to the flow direction and the pillars are arranged with respect to the flow direction so that row is deviated.

11. The method for separating cells according to any one of claims 7 to 10, wherein the blood is added with an anticoagulant reagent.

12. The method for separating cells according to claim 11, wherein the anticoagulant is a thrombin inhibitor.

13. The method for separating cells according to claim 12, wherein the thrombin inhibitor is PPACK.

14. A minute aggregate-removing device comprising a microchannel structure, wherein the microchannel structure is composed of pillars arranged at intervals wider than 30 μm, and has a sample inlet at a front of the microchannel structure and a sample outlet at a back of the microchannel structure,

wherein the microchannel structure is composed of two separate microchannel structure portions (structure portion α and structure portion β), and

the minute aggregate-removing device has a portion that converges a width of the flow in a middle between the two separate microchannel structure portions,

wherein the pillar arrangement interval in the microchannel structure portion α on the upstream side is wider than that in the microchannel structure portion β on the downstream side,

wherein the portion in the middle is narrower than the microchannel structure portions and has no pillar arranged, and

wherein a plurality of the pillars is provided at regular intervals parallel to the flow direction and the pillars are arranged with respect to the flow direction so that row is deviated.

15. The minute aggregate-removing device according to claims 14, wherein the pillar arrangement interval in the microchannel structure portion β on the downstream side is set to 80 μm to 250 μm.

16. An integrated cell-separating device continuously comprising a minute aggregate-removing portion having the microchannel structure and a cell-separating portion separating the cells, wherein

the microchannel structure is composed of pillars arranged at intervals wider than 30 μm, and has a sample inlet at a front of the microchannel structure and a sample outlet at a back of the cell-separating portion,

the microchannel structure is composed of two separate microchannel structure portions (structure portion α and structure portion β),

the minute aggregate-removing device has a portion that converges a width of the flow in a middle between the two separate microchannel structure portions and

the cell-separating portion is continuously provided at a back of the minute aggregate-removing portion,

wherein the pillar arrangement interval in the microchannel structure portion α on the upstream side is wider than that in the microchannel structure portion β on the downstream side,

wherein the portion in the middle is narrower than the microchannel structure portions and has no pillar arranged, and

wherein a plurality of the pillars is provided at regular intervals parallel to the flow direction and the pillars are arranged with respect to the flow direction so that row is deviated.

17. The integrated cell-separating device according to claims 16, wherein the pillar arrangement interval in the microchannel structure portion β on the downstream side is set to 80 μm to 250 μm.

18. The integrated cell-separating device according to claims 16 or 17, wherein the cell-separating portion is a separation structure according to size fractionation based on Deterministic Lateral Displacement (DLD).