channel containing arrays of micropillars to separate and collect cells. However, contrary to the present invention, the object of such microfluidic device is not to trigger platelet shedding from megakaryocytes.

One object of the present invention is therefore to provide a fluidic device for producing platelets from a suspension of cells comprising megakaryocytes or their fragments, in particular suitable for high yield production while maintaining the functional qualities of the newly generated platelets. Another object of the invention is to provide a method for producing platelets suitable to large-scale production of high quality and standardized platelets.

SUMMARY OF THE INVENTION

In one aspect, the invention relates a fluidic device for producing platelets from a suspension of megakaryocytes, comprising:

- a production chamber comprising at least one channel delimited by non-porous walls, and at least one inlet opening at one end in which a suspension of cells comprising megakaryocytes or their fragments can be introduced and at least one outlet opening at the other end, in which platelets can be collected;

wherein at least one portion of the inner surface of the walls of said channel is textured with a plurality of obstacles.

Also disclosed herein is~~to~~ a fluidic device (1) for producing platelets from a suspension of megakaryocytes (5), comprising:

- a production chamber (3) comprising at least one channel (8) in which a suspension of cells comprising megakaryocytes or their fragments can be introduced to flow from its inlet (9) to its outlet (10);
- optionally a flow rate controller (7) for controlling the flow of said suspension in the channel (8);
- optionally a megakaryocyte sorter and/or a mixer (2) for enriching the suspension with megakaryocytes and homogenizing cell concentration of said suspension of megakaryocytes, upstream of the production chamber (3);

- optionally a platelet sorter (4) downstream from the production chamber (3) for purifying the outflow suspension (6) by sorting platelets from megakaryocytes or other cell residues;

wherein at least one portion (11) of the inner surface of the walls of said channel (8) is textured with a plurality of obstacles.

In a related aspect, the invention relates to an ex vivo method for producing platelets from megakaryocytes, said method comprising:

- introducing a suspension of megakaryocytes or their fragments into a fluidic device according to any one of Claims 1-8;
- subjecting said suspension to a flow under shear rate suitable for elongation, fragmentation of the megakaryocytes and platelet release in the channel of the production chamber; and

collecting platelets at the outlet of the channel.

The present disclosure further provides a fluidic device for producing platelets from a suspension of cells comprising megakaryocytes or their fragments, comprising:

- a production chamber (3) comprising at least one channel (8) delimited by non-porous walls, and at least one inlet opening (9) at one end of the channel, in which a suspension of cells comprising megakaryocytes or their fragments can be

introduced, and at least one outlet opening (10) at the other end of the channel, in which platelets can be collected;

- optionally a flow rate controller (7) for controlling the flow of said suspension in the channel (8);
- optionally a megakaryocyte sorter and/or a mixer (2) for enriching the suspension with megakaryocytes and homogenizing cell concentration of said suspension of megakaryocytes, upstream of the production chamber (3);
- optionally a platelet sorter (4) downstream from the production chamber (3) for purifying the outflow suspension (6) by sorting platelets from megakaryocytes or other cell residues;

wherein at least one portion (11) of the inner surface of the walls of said channel (8) is textured with a plurality of obstacles.

In specific embodiments, said plurality of obstacles are distributed over a two-dimensional surface, thereby forming a three-dimensional array of obstacles.

In another aspect, ~~the invention is directed to~~to the present disclosure provides an ex vivo method for producing platelets from megakaryocytes or their fragments, said method comprising:

- introducing a suspension of cells comprising megakaryocytes or their fragments into a fluidic device as defined above;
- subjecting said suspension to a flow under shear rate suitable for elongation, fragmentation of the megakaryocytes and platelet release in the channel of the production chamber; and
- collecting platelets at the outlet of the channel.

DESCRIPTION OF THE INVENTION

This invention will now be described in further details. In the following description, the expression “comprised between” should be understood to designate the range of values identified, including the lower and upper bounds.

The invention is directed to a fluidic device for producing platelets from a suspension of megakaryocytes, or their fragments.

As used herein, the term “platelets” denotes the anucleate cells that are involved in the cellular mechanisms of primary hemostasis leading to the formation of blood clots.

CLAIMS

1. A fluidic device ~~(1)~~ for producing platelets from a suspension of megakaryocytes ~~(5)~~, comprising:

- a production chamber ~~(3)~~ comprising at least one channel ~~(8)~~ delimited by non-porous walls, and at least one inlet opening ~~(9)~~ at one end in which a suspension of cells comprising megakaryocytes or their fragments can be introduced and at least one outlet opening ~~(10)~~ at the other end, in which platelets can be collected;

wherein at least one portion ~~(11)~~ of the inner surface of the walls of said channel ~~(8)~~ is textured with a plurality of obstacles.

2. The fluidic device according to claim 1, wherein said textured portion ~~(11)~~ of the inner surface of the channel ~~(8)~~ is further coated with a ligand with binding affinity for megakaryocytes, e.g., von Willebrand factor (VWF), its functional variants, or recombinant polypeptides comprising fragments of von Willebrand factor, or fibrinogen or fibronectin.

3. The fluidic device according to claim 1 or 2, wherein the density, size and shape of said obstacles are determined so as to enable the capture of megakaryocytes on said textured portion of the inner surface of the channel for platelet shedding.

4. The fluidic device according to any one of Claims 1-3, wherein the obstacles are posts ~~(12)~~ or beams ~~(15)~~.

5. The fluidic device of any one of Claims 1-4, wherein said channel ~~(8)~~ has a substantially square or rectangular section.

6. The fluidic device of any one of Claims 1-5, wherein the obstacles are posts ~~(12)~~ with a substantially circular section of a radius r, and said posts are arranged on the inner surface ~~(13)~~ of at least one portion of said channel to form a regular pattern with a hexagonal periodic structure such as:

(i) the radius of a post r is preferably between 50 nm and 15 mm, more preferably between 500 nm and 1.5 mm.

(ii) the closest distance p between two post centers is at least equal to 100 nm, preferably between 100 nm and 50 mm, preferably between 500 nm and 10 mm, even better between 5 µm and 1 mm.

(iii) the angle α, which is the smallest angle defined by the longitudinal direction of the channel ~~(14)~~ and one of the lattice vectors of the hexagonal Bravais lattice is such as α is comprised between 0 and 90° and preferably between 0 and 30°.

(iv) optionally said posts have a height h such as $0 < h \leq H$, wherein H refers to the smallest distance measured between two opposite walls in a section of the channel.

7. The fluidic device according to Claim 6, wherein said channel has a height H that is comprised between 5 µm and 1 mm.

8. The fluidic device according to any of Claims 1-7, wherein said production chamber (3) comprises at least one or a plurality of parallel channels with textured portion on their inner surfaces, for example between 2 channels and 10^6 channels.

9. An ex vivo method for producing platelets from megakaryocytes, said method comprising:

- introducing a suspension of megakaryocytes or their fragments into a fluidic device according to any one of Claims 1-8;
- subjecting said suspension to a flow under shear rate suitable for elongation, fragmentation of the megakaryocytes and platelet release in the channel of the production chamber; and
- collecting platelets at the outlet of the channel.

10. The method according to Claim 9, wherein the flow rate is fixed within a range value enabling to subject said megakaryocytes in the textured portion of the channel to wall shear rates varying from a minimum value to a maximum wall shear rate $\dot{\gamma}_{max}$, said maximum wall shear rate not exceeding 30000 s^{-1}, preferably 10000 s^{-1}, more preferably 8000s^{-1} and even more preferably 5000 s^{-1}.

11. The method according to Claim 9 or 10, wherein the collected platelets at the outlet of the channel further contains naked nuclei and/or intact megakaryocytes, said method further comprising the step of purifying, enriching or separating platelets from said suspension.

12. The method according to any one of Claims 9-11, wherein said suspension is a suspension obtained by the following steps: (i) providing megakaryocyte progenitor and/or stem cells, (ii) expanding said megakaryocyte progenitor and/or stem cells, and, (iii) differentiating the expanded cells into megakaryocytes.

13. The method of Claim 12, wherein the megakaryocyte progenitor and/or stem cells are selected from the group consisting of hematopoietic stem cells, embryonic stem cells and induced pluripotent stem cells.

14. The method according to any one of Claims 9-13, wherein said suspension of megakaryocytes is homogenized and/or purified prior to entering in the production chamber.

15. The method according to any one of Claims 9-14, wherein said platelets are sorted at the outlet of the channel by a method selected from the group consisting of cross flow filtration, laminar flow, dielectrophoresis, optical force, magnetic force, acoustic force, or inertial forces.

16. The method according to any one of Claims 9-15, wherein said platelets are functional platelets which can be activated like circulating blood platelets.