sides of the pressuring container in the supply path, and on the return path. The cell separator may further comprise a reflux path to send the liquid medium containing the relatively large cells concentrated from one of the at least two outlets of the hydrodynamic separation device to the culture tank. Such a configuration is possible that an exit-side end of the reflux path is connected near an entrance of the supply path so that the entrance of the supply path is also possibly used as exit of the reflux path.

[0020]

Moreover, according to an aspect of the present disclosure, a subject of a cell culture method is to comprise: a cell culture for culturing cells in a liquid medium; and a cell separation for separating, from the liquid medium, a liquid medium containing relatively large cells, the cell separation comprising: a separation process to separates the liquid medium containing the relatively large cells concentrated from the liquid medium containing the cells, by using a vortex flow generated by flow through a curved flow channel having a rectangular cross-section; and a pressure control to control a pressure environment of the liquid medium that flows in the separation process, so as to suppress a decrease in cell viability caused by pressure fluctuation in the liquid medium while flowing through the curved flow channel.

[0021]

The above-described cell culture method further comprises: a circulation process which returns the liquid medium containing the relatively large cells separated by the separation process to the cell culture, and circulates the liquid medium between the cell culture and the separation process. Continuous cell culture is possible by further comprising: a medium supplementation which replenishes the cell culture with a new liquid medium corresponding to an amount of the rest of the liquid medium

containing the cells of the culture tank 2 is sent to the hydrodynamic separation device 4 through the supply path 6. The cell culture system 1 of FIG. 1 has a pipe constituting a reflux path 7, and the reflux path 7 connects the hydrodynamic separation device 4 of the cell separator 3 and the culture tank 2 so that the liquid medium can be circulated between the culture tank 2 and the cell separator 3. That is, the cell culture system 1 has a circulation system composed of the supply path 6 and the reflux path 7.

[0031]

The fraction of the liquid medium containing the relatively large cells separated by the hydrodynamic separation device 4 of the cell separator 3 is returned to the culture tank 2 through the reflux path 7, and the cell culture is further continued. A fraction of the remaining liquid medium C' (fraction on the inner circumferential side) containing the relatively small cells is discharged from the hydrodynamic separation device 4 through a recovery path 8. Further, in order to replenish the culture tank 2 with a new liquid medium C0 corresponding to the amount of the remaining liquid medium C' discharged from the hydrodynamic separation device 4, a medium supplement unit 9 is provided. Then the liquid medium C in the culture tank 2 is maintained at a constant amount by replenishing the new liquid medium C0.

[0032]

The cell separation efficiency in the hydrodynamic separation device 4 varies depending on the Dean number and the pressure in the liquid supplied to the curved flow channel, and there are appropriate ranges of the Dean number and the pressure capable of suitable separation. The Dean Number (De)is expressed by the formula: $De = Re (D/2Rc)^{1/2}$ (Re: Reynolds number (-), D: representative length (m), Rc: turning radius of the flow channel (m)). Since the Dean number is proportional to the flow rate of

temporary container 50 and the hydrodynamic separation device 4a, it urges fluid pressure into the liquid medium instead of a pump. A residual pressure release valve V7 for releasing air to depressurize is installed in the gas line 51, and the liquid medium can be repeatedly delivered in the same manner as the pump by repeating pressurization and depressurization.

[0060]

When the cell culture operation is started, the on-off valves V1, V2, and V4 to V6 are opened. After the operation is stopped, it is confirmed that the liquid medium has been discharged from the flow paths to the culture tank 2 or the temporary container 50, or the recovery tank 20b. Then the on-off vales V1, V2, and V4 to V6 are closed and depressurization is performed by letting the air in the gas line escape from the residual pressure release valve V7.

[0061]

The cell culture system 1c of FIG. 9 has a configuration modified in the cell culture system 1 of FIG. 1 to perform gas pumping using the recovery tank 20. The gas pumping is configured so that the remaining liquid medium C' (fraction on the inner circumferential side) discharged from the hydrodynamic separation device 4 can be supplied again to the hydrodynamic separation device 4.

[0062]

In the cell culture system 1c, an on-off valve V1 and an on-off valve V2 are installed in the supply path 6 and the reflux path, respectively, and an on-off valve V5 is installed in the replenishment path 16 for replenishing a new liquid medium. When the on-off vales V1, V2, and V5 are opened to start the operation, the liquid medium C in the culture tank 2 is supplied to the hydrodynamic separation device 4. The liquid medium (fraction on the outer circumferential side) discharged from the outlet 42 of the hydrodynamic separation device 4 is refluxed to the culture tank 2.

separation device 4 from being supplied to the temporary container 50, and ensures recovery from the discharge path 53. Instead of the on-off valves V8 and V11, a switching valve may be installed at the confluence point where the return path 52 merges with the supply path 6c, to switch the connection of the flow path.

[0074]

Also in the cell culture system 1d', a fraction on the inner circumferential side having a low cell density can be obtained by repeating the hydrodynamic separation using a single hydrodynamic separation device 4. Therefore, as in the cell culture system 1a of FIG. 2, it is useful when the cell density of the liquid medium in the culture tank 2 is high and the cell density of the remaining liquid medium is not sufficiently reduced by one time of hydrodynamic separation.

[0075]

The multi-stage separation process in the cell culture system 1d' is started by closing the on-off valves V1, V8 and V9 and opening the on-off valves V2, V11 and V12 while the pressurizing container 54 holds the liquid medium C of the culture tank 2. Then the first hydrodynamic separation is performed. The fraction on the outer circumferential side is refluxed to the culture tank 2, and the fraction on the inner circumferential side is accommodated in the temporary container 50. The second hydrodynamic separation is started by closing the on-off valves V11 and V12 and opening the on-off valves V8 and V9. The fraction on the outer circumferential side is refluxed to the culture tank 2, and the fraction on the inner circumferential side is discharged from the discharge path 53. During standby without processing by the hydrodynamic separation device 4, the on-off valves V1, V2 and V9 are closed.

[0076]

The cell culture system 1e of FIG. 12 shows an

embodiment in which the on-off valves V1, V2, V8, V11 and V12 in the cell culture system 1d' of FIG. 11 are changed to check valves Va to Ve. This enables to reduce the complexity of the valve control operation.

[0077]

Specifically, a check valve Va is installed in the supply path 6b to prevent backflow from the pressurizing container 54 to the culture tank 2. A check valve Vb is installed in the supply path 6c to prevent the inflow from the hydrodynamic separation device 4 and the return path 52 into the pressurizing container 54. The return path 7 has a check valve Vc installed therein to prevent the flow from the culture tank 2 side to the hydrodynamic separation device 4 side. Therefore, the circulation of the liquid medium between the culture tank 2 and the hydrodynamic separation device 4 is determined in one direction. A check valve Vd is installed in the recovery path 8, and the supply destination of the liquid medium discharged from the outlet 43 of the hydrodynamic separation device 4 can be switched only by opening and closing the on-off valve V9. In the return path 52, a check valve Ve is installed to prevent inflow from the pressurizing container 54 and the hydrodynamic separation device 4 into the return path 52.

[0078]

Therefore, in the cell culture system 1e, the on-off valve V9 is closed and the separation process is started by gas pumping from the gas line 51'. The liquid medium in the culture tank 2 is supplied to the hydrodynamic separation device 4 via the pressurizing container 54 and separated. The fraction on the outer circumferential side is refluxed to the culture tank 2, and faction of the inner circumferential side is accommodated in the temporary container 50. Next, when the on-off valve V9 is opened and gas pressure feeding is performed from the gas line 51, the liquid medium C' of the temporary container 50 is supplied from the return path 52 to the hydrodynamic separation

device 4 and separated. The fraction on the outer circumferential side is refluxed to the culture tank 2, and the fraction on the inner circumferential side is discharged from the discharge path 53 and does not return to the temporary container 50 being pressurized.

[0079]

In the cell culture system 1e of FIG. 12, the liquid medium of the culture tank 2 is once housed in the pressurizing container 54 and then separated by the hydrodynamic separation device 4. That is, the flow of the liquid medium in the supply path 6b and the flow of the liquid medium in the reflux path 7 are alternately performed. Therefore, the supply path 6b and the reflux path 7 can be used by integrating their ends on the culture tank side into one. FIG. 13 shows an embodiment in which the end of the reflux path 7 on the culture tank side is integrated with the end of the supply path 6b in the cell culture system of FIG. 12.

[0080]

In the cell culture system 1f of FIG. 13, an on-off valve V1 is installed, instead of a check valve, in the supply path 6b' that supplies the liquid medium C from the culture tank 2 to the pressurizing container 54. The liquid medium C of the pressurizing container 54 is separated by the hydrodynamic separation device 4 in the same manner as described above, and the liquid medium containing the relatively large cells concentrated is discharged from the outlet 42. One end of the reflux path 7' for sending the liquid medium to the culture tank 2 is connected to the outlet 42, and a check valve Vc is installed. Since the other end of the reflux path 7', that is, the end on the exit side is connected near the entrance of the supply path 6b', the entrance of the supply path 6b' can also be used as the exit of the reflux path 7'.

[0081]

When the separation process is stopped in the cell

culture system, the liquid medium tends to stay in the pipes constituting the supply path for sucking the liquid medium from the culture tank and the reflux path for returning the liquid medium from the hydrodynamic separation device 4. The cells trapped in the pipe cannot breath, and if the cells are returned from the flow path to the culture tank by resuming the separation process, the proliferation may be adversely affected. In this regard, in the cell culture system 1f of FIG. 13, the operation is stopped by the return from the reflux path 7′ to the culture tank 2, and the on-off valve V1 and the check valve Vc of the supply path 6b and the reflux path 7′ prevent the backflow of the liquid medium from the culture tank 2. Therefore, it is difficult for the liquid medium to stay in the pipe after the process is stopped. Since it is the same as the cell culture system of FIG. 12 except that the supply path and the reflux path are integrated, description thereof will be omitted and the above will be referred to.
[0082]

In this way, utilizing the hydrodynamic separation technique, relatively large cells can be selectively concentrated and separated from the liquid medium being cultured. Therefore, by refluxing this together with a new liquid medium to the culture tank, cell culture can be continued while maintaining high proliferative power. The hydrodynamic separation can promote the concentration separation of cells much more efficiently than the centrifugation, and can concentrate and separate the cells with a high viability without damaging the cells as compared with the filter separation and the like. The fraction containing relatively small cells that have been separated and removed are purified after removing unnecessary substances such as cell debris, using a filter or the like. As a result, useful components can be recovered. In the hydrodynamic separation, the liquid medium is supplied to the curved flow channel at a

CLAIMS

[Claim 1] A cell culture system, comprising a culture tank that contains a liquid medium that cultures cells, and a cell separator, the cell separator comprising:

a hydrodynamic separation device having a curved flow channel having a rectangular cross-section, and separating relatively large cells from the cells contained in the liquid medium using a vortex flow generated by flow through the curved flow channel; and

a liquid feeding unit which flows the liquid medium through the hydrodynamic separation device in a pressure environment controlled so as to suppress decrease in cell viability caused by pressure fluctuation in the liquid medium during flowing through the hydrodynamic separation device.

[Claim 2] The cell culture system according to claim 1, wherein the hydrodynamic separation device has a single inlet for taking in the liquid medium and at least two outlets for discharging the separated liquid medium, wherein a liquid medium containing the relatively large cells concentrated is discharged from one of the at least two outlets, and the rest of the liquid medium containing relatively small cells is discharged from the other of the at least two outlets.

[Claim 3] The cell culture system according to claim 1 or 2, wherein the liquid feeding unit has an urging device that urges the liquid medium with a flow pressure for supplying the liquid medium to the hydrodynamic separation device.

[Claim 4] The cell culture system according to any one of claims 1 to 3, wherein the liquid feeding unit has a pressure control mechanism that controls the pressure environment so that a pressure difference between the

liquid medium introduced to the hydrodynamic separation device and the liquid medium derived from the hydrodynamic separation device is equal to or less than a predetermined value.

[Claim 5] The cell culture system according to claim 4, wherein the pressure control mechanism comprises:

a pressure monitoring unit to monitor a pressure of the liquid medium supplied to the hydrodynamic separation device; and

a pressure adjusting member that adjusts the pressure of the liquid medium supplied to the hydrodynamic separation device, based on the pressure monitored by the pressure monitoring unit.

[Claim 6] The cell culture system according to any one of claims 1 to 5, wherein the liquid feeding unit further has a flow rate-controlling mechanism for controlling a flow rate of the liquid medium supplied to the hydrodynamic separation device.

[Claim 7] The cell culture system according to claim 6, wherein the flow rate-controlling mechanism comprises:

a flow meter to monitor the flow rate of the liquid medium supplied to the hydrodynamic separation device; and

a flow rate-adjusting member to adjust the flow rate of the liquid medium supplied to the hydrodynamic separation device, based on the flow rate monitored by the flow meter,

wherein the flow rate of the liquid medium supplied to the hydrodynamic separation device is adjusted according to a size of the cells to be separated.

[Claim 8] The cell culture system according to any one of claims 1 to 7, further comprising:

a circulation system which returns a liquid medium containing the relatively large cells separated by the

hydrodynamic separation device of the cell separator to the culture tank, to circulate the liquid medium between the culture tank and the cell separator.

[Claim 9] The cell culture system according to claim 8, further comprising:

a medium supplement unit which replenishes the culture tank with a new liquid medium corresponding to an amount of the rest of the liquid medium.

[Claim 10] The cell culture system according to claim 9, wherein the medium supplement unit comprises:

a monitor that monitors an amount of the new liquid medium replenished in the culture tank; and

a flow rate-adjusting member that adjusts a flow rate of the new liquid medium replenished to the culture tank, based on the amount monitored by the monitor.

[Claim 11] A cell culture method, comprising:

a cell culture for culturing cells in a liquid medium; and

a cell separation for separating, from the liquid medium, a liquid medium containing relatively large cells,

the cell separation comprising:

a separation process to separates the liquid medium containing the relatively large cells concentrated from the liquid medium containing the cells, by using a vortex flow generated by flow through a curved flow channel having a rectangular cross-section; and

a pressure control to control a pressure environment of the liquid medium that flows in the separation process, so as to suppress a decrease in cell viability caused by pressure fluctuation in the liquid medium while flowing through the curved flow channel.

[Claim 12] The cell culture method according to claim 11, further comprising:

a circulation process which returns the liquid medium containing the relatively large cells separated by the separation process to the cell culture, and circulates the liquid medium between the cell culture and the separation process.

[Claim 13] The cell culture method according to claim 12, further comprising:

a medium supplementation which replenishes the cell culture with a new liquid medium corresponding to an amount of the rest of the liquid medium containing relatively small cells separated in the separation process.

[Claim 14] The cell culture system according to claim 2, wherein the cell separator further comprises:

a temporary container for accommodating the rest of the liquid medium discharged from the other outlet of the hydrodynamic separation device;

an additional hydrodynamic separation device; and

an additional liquid feeding unit which flows the liquid medium contained in the temporary container through the additional hydrodynamic separation device in a pressure environment controlled so as to suppress decrease in cell viability caused by pressure fluctuation in the liquid medium during flowing through the additional hydrodynamic separation device.

[Claim 15] The cell culture system according to claim 14, wherein the additional liquid feeding unit has an urging device that urges the liquid medium with a flow pressure for supplying the liquid medium to the additional hydrodynamic separation device, and the urging device comprises:

a pump that urges the liquid medium at a connection path connecting the temporary container and the additional hydrodynamic separation device; or

a pressurizing device that pressurizes the inside of

the temporary container and supplies the flow pressure to the contained liquid medium.

[Claim 16] The cell culture system according to claim 2, wherein the cell separator further comprises:

a temporary container for accommodating the rest of the liquid medium discharged from the other outlet of the hydrodynamic separation device;

a return path for resupplying the rest of the liquid medium contained in the temporary container to the hydrodynamic separation device; and

a switching mechanism for switching between supply of the liquid medium from the culture tank to the hydrodynamic separation device and supply of the rest of the liquid medium from the return path to the hydrodynamic separation device,

wherein, by switching of the switching mechanism, the liquid medium of the culture tank and the rest of the liquid medium of the temporary container are alternately supplied to the hydrodynamic separation device.

[Claim 17] The cell culture system according to claim 16, wherein the liquid feeding unit includes: a supply path connecting the culture tank and the hydrodynamic separation device; and an urging device that urges the liquid medium with a flow pressure for supplying the liquid medium to the hydrodynamic separation device, and the return path is connected to join the supply path,

wherein the urging device comprises:

a pump that urges the liquid medium at the supply path; or

a pressurizing container that temporarily stores the liquid medium in the supply path and pressurizes the stored liquid medium to apply the flow pressure.

[Claim 18] The cell culture system according to claim 2, wherein the liquid feeding unit includes: a supply path

connecting the culture tank and the hydrodynamic separation device; and a pressurizing container that temporarily stores the liquid medium in the supply path and pressurizes the stored liquid medium to apply a flow pressure for supplying the liquid medium to the hydrodynamic separation device, and wherein the cell separator further comprises:

a temporary container for accommodating the rest of the liquid medium discharged from the other outlet of the hydrodynamic separation device;

a return path that connects the downstream side of the pressurizing container in the supply path with the temporary container and resupplies the rest of the liquid medium contained in the temporary container to the hydrodynamic separation device; and

a switching mechanism for switching between supply of the liquid medium from the culture tank to the hydrodynamic separation device and supply of the rest of the liquid medium from the return path to the hydrodynamic separation device,

wherein, by switching the switching mechanism, the liquid medium of the culture tank and the rest of the liquid medium of the temporary container are alternately supplied to the hydrodynamic separation device.

[Claim 19] The cell culture system according to claim 18, wherein the switching mechanism has an on-off valve or a check valve provided on each of the upstream and downstream sides of the pressuring container in the supply path, and on the return path.

[Claim 20] A cell culture system, comprising a culture tank that contains a liquid medium that cultures cells, and a cell separator, the cell separator comprising:

a hydrodynamic separation device having a curved flow channel having a rectangular cross-section, to separate relatively large cells from the cells contained in the liquid medium, using a vortex flow generated by flow

through the curved flow channel; and

a liquid feeding unit which flows the liquid medium through the hydrodynamic separation device so as to suppress decrease in cell viability in the liquid medium during flowing through the hydrodynamic separation device.