the second outflow port and performs a concentration process in which a liquid is removed from a mixture containing cells and the liquid accompanying the cells flowing out from the second outflow port. The first filter portion may include a tangential flow filter in the cell culture device according to the present invention.

[0011] The cell culture device according to the present invention may further comprise: a second storage container including a third outflow port and a third inflow port which is connected to the second inflow port; and a second filter portion which is provided within a fourth flow path connecting the third inflow port to the third outflow port and performs a concentration process in which a liquid is removed from a mixture containing cells and the liquid accompanying the cells flowing out from the third outflow port. In the cell culture device according to the present invention, the second filter portion may include a tangential flow filter. The cell culture device according to the present invention may alternately perform the concentration process using the first filter portion and the concentration process using the second filter portion.

[0012] The cell culture device according to the present invention may further comprise: a first pressure adjustment mechanism which adjusts a pressure within the first storage container; and a second pressure adjustment mechanism which adjusts a pressure within the second storage container.

[0013] In the cell culture device according to the present invention, the division processing portion may have a processing container for performing the division process and a third pressure adjustment mechanism which adjusts a pressure within the processing container.

[0014] The cell culture device according to the present invention may further comprise: a second stirring portion which is provided within a fifth flow path connecting the medium supply portion and the first flow path and stirs a fluid flowing in.

[0015] The cell culture device according to the present invention may further comprise: a cell supply portion which supplies cells cultured in the culture container to the first flow path.

[0016] The cell culture device according to the present invention may further comprise: a cell supply portion which is connected to a downstream side of the second stirring portion in the fifth flow path and supplies cells cultured in the culture container to the first flow path via the fifth flow path; and a third stirring portion which is provided on a downstream side of a region, to which the cell supply portion is connected, in the fifth flow path and stirs a fluid flowing in.

[0017] The cell culture device according to the present invention may further comprise: a freezing portion which is connected to a third region positioned between the second outflow

100 has an on-off valve V1, which is provided on a downstream side of the pump P1, of a pipe connecting the cell accommodating portion 101 to the pipe c1. The cells accommodated in the cell accommodating portion 101 are sent out into the flow path F3 using the pump P1 being driven and the on-off valve V1 entering an open state.

[0026] Cells to be cultured using the cell culture device 10 according to the present embodiment are not particularly limited, all cells such as animal cells, plant cells, fungal cells, bacterial cells, protoplasts, established cell lines, and genetically modified artificial cells can be cultured.

[0027] An example of cells to be cultured is stem cells. The stem cells are not particularly limited as long as the stem cells have self-replication potency and differentiation potency, and may be pluripotent stem cells or somatic stem cells.

[0028] The pluripotent stem cells are cells having a self-replication potency and multiple differentiation potency which enables the cells to be differentiated into all of an ectoderm, a mesoderm, and an endoderm. Examples of the pluripotent stem cells include Embryonic Stem cells (ES cells), induced Pluripotent Stem cells (iPS cells), Embryonic Germ cells (EG cells), Embryonal Carcinoma cells (EC cells), Multipotent Adult Progenitor cells (MAP cells), Adult Pluripotent Stem cells (APS cells), and Multi-lineage differentiating Stress Enduring cells (Muse cells).

[0029] Examples of the somatic stem cells include mesenchymal stem cells, hematopoietic stem cells, and neural stem cells.

[0030] Other examples of cells to be cultured include somatic cells constituting a living body and progenitor cells thereof. Specific examples thereof include lymphocytes, neutrophils, monocytes, megakaryocytes, macrophages, fibroblasts, basal cells, keratinocytes, epithelial progenitor cells, pericytes, endothelial cells, adipose progenitor cells, myoblasts, osteoblasts, chondrocytes, hepatic parenchymal cells, pancreatic β cells, and gliocytes.

[0031] Other examples of cells to be cultured include animal cells such as Chinese hamster ovary (CHO), COS, HeLa, HepG2, C127, 3T3, BHK, HEK293, and Bowes melanoma cells; insect cells such as Drosophila S2 and Spodoptera Sf9; fungal cells such as yeast and Aspergillus; bacterial cells such as Escherichia coli and Bacillus subtilis; and plant cells and callus. These cells may be cells into which a protein expression vector is introduced for the purpose of a mass expression of proteins.

[0032] In a case of starting the cell culture in a state where the cells and a medium are accommodated in advance within the culture container 20, it is possible to omit the cell supply

freezing portion 17.

[0059] <Control Portion>

A control portion 18 integrally controls operations of the pumps P1 to P5, the on-off valves V1 to V5, V11 to V16, V21, V22, V31, and V41, the gas supply mechanism 25, and the pressure adjustment mechanisms 26, 33, and 43. Accordingly, culturing of cells in accordance with a predetermined cell culture protocol is automatically performed without human intervention. The electrical connection wiring between the control portion 18 and each of the above-described components controlled by the control portion 18 is not shown in Fig. 1 from the viewpoint of avoiding complications of the drawing.

[0060] The culture container 20 is an example of a culture container of the present invention. The inflow port 21 of the culture container 20 is an example of a first inflow port in the present invention. The outflow port 22 of the culture container 20 is an example of a first outflow port in the present invention. The storage container 30 is an example of a storage container in the present invention. The inflow port 31 of the storage container 30 is an example of a second inflow port in the present invention. The outflow port 32 of the storage container 30 is an example of a second outflow port in the present invention. The circulation flow path F1 is an example of a first flow path in the present invention. The flow path F2 is an example of a second flow path in the present invention. The connection region X1 is an example of a first region in the present invention. The connection region X2 is an example of a second region in the present invention. The division processing portion 40 is an example of a division processing portion in the present invention. The processing container 42 is an example of a processing container in the present invention. The pressure adjustment mechanism 43 is an example of a third pressure adjustment mechanism included in the division processing portion in the present invention. The cell supply portion 100 is an example of a cell supply portion in the present invention. The medium supply portion 110 is an example of a medium supply portion in the present invention. The flow path F3 is an example of a fifth flow path in the present invention. The freezing portion 17 is an example of a freezing portion in the present invention.

[0061] Hereinafter, an example of a process which can be performed in the cell culture device 10 according to the present embodiment will be shown. The cell culture device 10 performs, for example, a subculture process, a medium exchange process, a division process, and a freezing process exemplified below. The subculture process, the medium exchange process, the division process, and the freezing process described below are realized by the control

[0096] In Step S44, the on-off valves V14, V16, and V41 are made to enter an open state. In addition, the atmosphere within the storage container 30 is pressurized by the pressure adjustment mechanism 33. Accordingly, the cells and the freezing liquid which have been staying in the pipe a5 are accommodated in the preservation container 17a of the freezing portion 17 via the flow path F5. The freezing portion 17 freezes the cells accommodated in the preservation container 17a together with the freezing liquid. The freezing process is completed through the process of the above-described Steps S41 to S44.

A method disclosed, for example, in JP4705473B may be applied as the method for cryopreserving cells. This method includes a step of quickly freezing the cells in a medium containing dimethyl sulfoxide (DMSO), propylene glycol, acetamide, and a medium in predetermined amounts. In addition, a method disclosed in JP4223961B may be applied. This method is a method for cryopreserving cells using a cryopreservation liquid containing at least one selected from the group consisting of dimethyl sulfoxide, glycerin, ethylene glycol, propylene glycol, and polyvinyl pyrrolidone as a cryoprotective agent at a predetermined concentration, and includes a step of suspending the cells in a cryopreservation liquid, a cooling step of cooling and freezing the cryopreservation liquid up to -80°C or lower at a predetermined cooling rate, and a preservation step of preserving the cryopreservation liquid at -80°C or lower.

[0097] In the subculture process, the medium exchange process, and the division process described above, a case of supplying two types of media A and B from the medium supply portion 110 has been exemplified. However, the type of medium to be used can be appropriately changed in accordance with a cell culture protocol. That is, the type of medium to be used may be one or may be greater than or equal to three in accordance with a culture protocol.

[0098] <Cell Culture Process>

The cell culture device 10 can automatically perform cell culture without human intervention using the control portion 18 performing a cell culture processing program exemplified below. Fig. 7 is a flowchart showing a flow of a process of the cell culture program executed by the control portion 18.

[0099] In Step S101, the control portion 18 performs the above-described subculture process to start cell culture by accommodating cells supplied from the cell supply portion 100 and a medium supplied from the medium supply portion 110 in the culture container 20.

[0100] In Step S102, the control portion 18 performs the above-described medium exchange

released into the air by the pressure adjustment mechanism 33. Accordingly, the air pressure within the second storage container 35 increases and a mixture containing the cells and the used medium which have been stored in the second storage container 35 flows into the second filter portion 61. A part of the used medium is removed from the mixture containing the cells and the used medium through the concentration process performed by the second filter portion 61 and is collected in the waste liquid collection container 19. Thereafter, a mixture containing the cells and the remaining used medium flows into the first storage container 30 via the pipe g1.

[0177] Thereafter, the process of Steps S52 and S53 is repeatedly performed as necessary. Accordingly, a used medium is removed from a mixture containing the cells and the used medium. The cells subjected to the concentration process are stored in the first storage container 30 in Step S54.

[0178] In the above-described description, the concentration process performed during a medium exchange process has been exemplified. However, the above-described concentration process can also be applied to a concentration process performed during a subculture process, a division process, and a freezing process.

[0179] According to the cell culture device 10C of the fourth embodiment, the concentration process is performed in the first filter portion 60 and the second filter portion 61, and therefore, a precipitation process is unnecessary in which cells are precipitated within a storage container. In addition, according to the cell culture device 10C, the concentration process is performed by alternately using the first concentration processing unit and the second concentration processing unit, and therefore, the transfer of cells, media, and the like between the first concentration processing unit and the second concentration processing unit can be performed through feeding pressure using the pressure adjustment mechanisms 33 and 38. Accordingly, it is possible to perform the concentration process without using a liquid contact pump and reduce the damage to cells as compared to the case of using a liquid contact pump. In the present embodiment, the case of alternately using the first concentration processing unit and the second concentration processing unit has been exemplified. However, the first concentration processing unit and the second concentration processing unit may be used at the same time. That is, in this case, concentration processes are performed side by side within the first concentration processing unit and the second concentration processing unit.

[0180] [First Modification Example]

Various modifications are possible in the configurations of the cell culture devices

according to the above-described first to fourth embodiments.

[0181] Fig. 21 is a view showing a configuration of a cell culture device 10D according to a first modification example. The cell culture device 10D has a pump P20 as means for transferring the cells and media to the storage container 30 from the culture container 20.

[0182] In a case where the culture container 20, for example, has a form of a plastic bag, it can be difficult to pressurize the atmosphere inside the culture container 20 using the pressure adjustment mechanism 26 due to pressure resistance performance of a container main body. The pump P20 can be applied to the case where it is difficult to pressurize the atmosphere inside the culture container 20 in this manner.

[0183] The pump P20 is preferably disposed between the connection region X2 and the outflow port 22 of the culture container 20 within the circulation flow path F1 as shown in Fig. 21. It is considered that cells receive damage from a division process in the division processing portion 40. In addition, if damage is further given to the cells immediately after the division process, there is a concern that cell viability may decrease. That is, there is a concern that damage may be accumulated within the cells passing through a pump after the division process, and therefore, cell viability may decrease. According to the cell culture device 10D of this modification example, the pump P20 is disposed between the connection region X2 and the outflow port 22 of the culture container 20, and therefore, the cells subjected to a division process in the division processing portion 40 are accommodated in the culture container 20 without passing through a pump.

[0184] A case where the pump P20 is applied to the cell culture device 10A of the second embodiment has been shown in Fig. 21. However, the pump P20 can also be applied to the cell culture devices 10, 10B, and 10C according to the first, third, and fourth embodiments.

[0185] [Second Modification Example]

Fig. 22 is a view showing a configuration of a cell culture device 10E according to a second modification example. The cell culture device 10E has a configuration in which the filter portion 60 in the cell culture device 10B according to the third embodiment is replaced by a centrifugal precipitation portion 70.

[0186] Similarly to the filter portion 60, the centrifugal precipitation portion 70 has a function of performing a concentration process of removing a liquid such as a used medium from a mixture containing the cells and the liquid. The centrifugal precipitation portion 70 centrifugally separates a liquid such as a medium from the cells using the difference in specific gravity between the cells and the liquid such as a medium, and discharges the liquid such as a

5. The cell culture device according to claim 4,

wherein the first filter portion includes a tangential flow filter.

6. The cell culture device according to claim 4 or 5, further comprising:

a second storage container including a third outflow port and a third inflow port which is connected to the second inflow port; and

a second filter portion which is provided within a fourth flow path connecting the third inflow port to the third outflow port and performs a concentration process in which a liquid is removed from a mixture containing cells and the liquid accompanying the cells flowing out from the third outflow port.

7. The cell culture device according to claim 6,

wherein the second filter portion includes a tangential flow filter.

8. The cell culture device according to claim 6 or 7,

wherein the concentration process using the first filter portion and the concentration process using the second filter portion are alternately performed.

9. The cell culture device according to any one of claims 6 to 8, further comprising:

a first pressure adjustment mechanism which adjusts a pressure within the first storage container; and

a second pressure adjustment mechanism which adjusts a pressure within the second storage container.

10. The cell culture device according to any one of claims 1 to 9,

wherein the division processing portion has a processing container for performing the division process and a third pressure adjustment mechanism which adjusts a pressure within the processing container.

11. The cell culture device according to any one of claims 1 to 10, further comprising:

a second stirring portion which is provided within a fifth flow path connecting the medium supply portion and the first flow path and stirs a fluid flowing in.