(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 765 498 B1

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant of the patent:
10.03.2010 Bulletin 2010/10

(21) Application number: **05753716.9**

(22) Date of filing: **16.06.2005**

(51) Int Cl.:
B01L 3/00 *(2006.01)* ***C12M 1/04*** *(2006.01)*
C12N 5/07 *(2010.01)*

(86) International application number:
PCT/IB2005/001691

(87) International publication number:
WO 2005/123258 (29.12.2005 Gazette 2005/52)

(54) **BIOREACTOR FOR STUDYING THE EFFECTS OF IMPOSED STIMULI ON CELLULAR ACTIVITY**

BIOREAKTOR ZUR UNTERSUCHUNG DER EFFEKTE VON ZWANGSSTIMULI AUF DIE ZELLAKTIVITÄT

BIOREACTEUR D'ETUDE DES EFFETS DE STIMULI IMPOSES SUR L'ACTIVITE CELLULAIRE

(84) Designated Contracting States:
AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IS IT LI LT LU MC NL PL PT RO SE SI SK TR

(30) Priority: **18.06.2004 IT PI20040046**

(43) Date of publication of application:
28.03.2007 Bulletin 2007/13

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Description

Field of the invention

[0001] The present invention relates to a bioreactor for studying the effects of stimuli of physical, chemical, mechanical and electromagnetic nature on cellular activity, for applications in many fields among which: in tissue engineering for development of biological constructs; in the industrial field for pharmacological "testing", and in the cosmetic field for studying allergologic reactions to the developed products.

Description of the prior art

[0002] It is well known that each biological tissue during its evolution and its normal activity is subject to physical and chemical stimuli that both determine its pathological and physiological status and affect its normal function. For this purpose, systems capable of reproducing physical or chemical stimuli have been sought in order to study its influence on the normal cellular activity.

[0003] Presently, real systems are known that reproduce a pressure stimulus, for studying the influence on the gangliar or endothelial cells. Other known systems reproduce a laminar flow, or turbulent flow, for simulating the permeation of nutrients through cellular membrane, as normally occurs in any biological tissue owing to blood flow.

[0004] Concerning isobar cell culture two systems are known:

- a first system studies the links between the increase of the pressure and apoptosis in gangliar cells. It consists of a special culture chamber that is brought to high pressures and is monitored by a mercury sphigmomanometer. The atmosphere in the chamber consists of a 5% mixture of CO_2 and the temperature is kept fixedly at 37°C;
- another system studies the links between the pressure variation and the release of endotheline 1. It consists of a cell culture plate with 24 chambers, coated by adhesive tape with which the upper edge of the plate is sealed, and at each chamber a hole is made where the predetermined pressure is applied and monitored by a pressure valve connected to a sphigmomanometer.

[0005] So called flow bioreactors also exist, which provide a chamber for cell culture that is arranged in series with a nutrient flow system. The applications of such bioreactors are various, such as the study of pathologies, the regeneration of cardio-muscular tissues, the development of hepatic functional substitutes, the regeneration and the testing of cartilage.

[0006] Flow bioreactors have been studied for high density cultures. In fact, the flow of nutrients that passes through a bioreactor allows a much easier perfusion of the same and a most effective removal of the cellular catabolites. These systems increase the growth speed of cellular mono-layers up to a confluence from 100% up to 200% and optimize the function, the morphology and the differentiation of the cells.

[0007] On the market, the many bioreactors differ from one another essentially for a variety of types of the culture chambers:

- "Rocking Culture System", consisting of a fixed base with an oscillating plate and a culture bag in which a gaseous mixture flows through special connectors on the upper part of the bag. The conveyance of the mass and of the gas, as well as the mixture of the culture medium, are achieved through the oscillation of the plate. The effects of the waves generated on the surface and of turbulence cause a considerable increase of the coefficient of volumetric transfer of nutrients with respect to a static culture.
- "Spinner Bioreactor System" having flasks with rotatable blades that cause the perfusion of oxygen and the distribution of the nutrients. For controlling the pH and the temperature it is necessary to put the system in an incubator.
- "Spinner-Air Lifted Bioreactor" that adopts a system for immobilizing the cells consisting of porous disks connected to each other by a stiff part made of steel.
- "Rotary Cell Culture System" also called rotating wall, having a rotating cylindrical chamber that contains a co-rotating cylindrical membrane, for exchanging the gas and for oxygenation.
- "Airlift Bioreactor", consisting of an elongated chamber arranged up to a vertical position, in whose lower part the mixture of gas is put in. The gas inserted from below causes the reduction of the density of the liquid contained in the tube. This causes a circulation of the culture medium through the inner tube towards the outermost zone of the tube.
- "Hollow-Fiber Bioreactor", having a network of artificial semipermeable capillaries that, once soaked by the culture medium for diffusive phenomena, it supplies oxygen to the cells, taking nutrients and removing the catabolites from the cellular metabolic paths as well as cleaning other inhibitors of the cell growth.
- "Flat Bed Perfusion System", comprising a flat chamber containing co-cultures of stroma, i.e. a mesh of connectival fibres normally of reticular nature. The cell is perfused by the culture medium.
- "Stirred Tank". This device has an electric motor that wheels some blades having a variable geometry and arrangement. They are very similar to the "Spinners" and they allow cultures with "microcarriers" or cultures in suspension. The blades are kept still by a steel part during a decantation step or during the intervention of an operator, to avoid to damage the cells or the "microcarriers".
- "Micro-Cell Culture Analog", consisting of a micro

culture chamber made by standard lithography and "etching" techniques and has micro-chambers that are arranged in series with different cellular cultures in order to analyse the effect of a same drug on different cells for studying its pharmacokinetiks.

[0008] The main limit of most of these systems is that they are not autonomous, since they require an incubator in order ensure required values of pH and temperature in the chamber. The presence of the incubator does not allow, in particular, the use of a computer for following in real time the progressive change of the parameters in order to adjust them during the experiment.

[0009] Bioreactors also exist where the presence of an incubator is not necessary; however, the structure of the chamber for cell culture does do not allow to follow the experiment in real time, by means of optical and/or fluorescence microscope, and then to determine the development of the cellular processes.

[0010] In conclusion, autonomous bioreactors do not presently exist that are at the same time capable of keeping the pressure the pH and the temperature in a culture chamber and to change it quickly in a controlled manner, as well as capable of generating in the cells a fixed flow of culture medium, with possibility of looking in real time at what is happening inside.

Summary of the invention

[0011] It is an object of the present invention to provide a device with the functions of a bioreactor that uses culture chambers that are easily modellable and conformable, and that allow the use of transducers and regulators, for monitoring in real time what happens in a culture chamber and for adjusting the parameters and the physical-chemical stimuli that are simulating physiological and/or pathological conditions.

[0012] Another object of the invention is to provide a device with the function of bioreactor where a flow of culture medium is present that allows a reduction of the amount of culture medium used for each experiment that is from 10 to 30 times less with respect to other devices present on the market, with considerable savings concerning both the amount of culture medium and the analysis of the substances in it contained.

[0013] A further feature of the present invention is to provide a bioreactor having means for conveying the culture medium in the culture chambers that do not damage the cells and cellular aggregates, do not have means for stirring, gas bubbling or other mechanical moving parts in the culture chamber.

[0014] These and other objects are achieved by a bioreactor for monitoring cellular activity in the presence of physical, chemical stimuli, comprising

- at least one culture chamber having an inlet and an outlet;
- a premixing chamber, separated from said at least one culture chamber, to prepare a culture medium;
- a circuit connected to said inlet and said outlet and comprising said premixing chamber;
- means for conveying in a controlled manner said culture medium through said circuit;
- means for generating at least one physical-chemical stimulus that has to be applied to the cells being tested, said stimulus being selected from the group of: temperature, pH, pressure or combination thereof;
- means for controlling said means for generating at least one physical-chemical stimulus, so that said or each stimulus reaches predetermined values.

[0015] Advantageously, said means for controlling comprise a specially developed software, which by a graphic interface easy to operate by each user allows both setting the parameters of the experiment and looking in real time at what happens to the cells.

[0016] This way an analysis in real time is allowed of a culture chamber without having the need to use an incubator surrounding the culture chamber.

[0017] In particular, said culture chamber is made of silicone rubber and is shaped in such a way that a desired laminar flow of the culture medium is created that can be outlined by a computer aided design program.

[0018] Preferably said cell is made of at least two parts that can overlap, where at least one has a recess in such a way that once overlapped to the other a passage for the culture medium is provided.

[0019] Advantageously, along said passage for the culture medium at least one of said parts that can overlap provides a glass slide for laboratories, in order to allow a microscope observation of the implanted cells. Said two parts that can overlap can be pressed on each other by two stiff plates kept together by releasable coupling means.

[0020] Preferably, said means for conveying in a controlled manner said culture medium through said culture chamber comprises:

- an inlet and outlet duct communicating with said culture chamber, to form a closed circuit with a separated premixing chamber;
- a peristaltic pump installed along said duct;
- an introduction point for drugs or other substances that boost or inhibit cellular activity arranged upstream from the culture chamber;
- a sample point downstream of the culture chamber for taking samples to analyse;
- a temperature sensor arranged upstream from the culture chamber.

[0021] In particular, said premixing chamber comprises:

- a container of inert material;
- a plug of inert material;
- means for operatively measuring the physiological

parameters of the culture medium.

[0022] Preferably, said container of inert material is shaped as a glass flask.

[0023] Advantageously the means for operatively measuring the physiological parameters of the culture medium can comprise:

- a pH sensor immersed in the culture medium present in said premixing chamber;
- a pressure sensor for measuring pressure in said premixing chamber;
- sensors for measuring chemical species such as O_2, CO_2, NO, etc.

[0024] Advantageously, in and at the bottom of the premixing chamber, a conical frustum concave structure is present where said pH sensor is arranged, in order to preserve it from a direct contact with possible bubbles of gas, which is introduced in said premixing chamber for adjusting the flow of culture medium and keeping it at a predetermined pH.

[0025] Preferably, means are provided for operatively adjusting the physiological parameters of the culture medium comprising:

- inlet/outlet ducts for a gas, for example air and CO_2, into/away from said premixing chamber for changing its pH and the pressure;
- a flow of thermostated fluid in a duct that surrounds said premixing chamber, for changing its temperature.

[0026] Preferably, the means for monitoring and controlling the physical-chemical stimuli applied to the cells in the culture chamber are selected from the group comprised of:

- an optical sensor for detecting bubbles in the cell culture chamber;
- a sensor for detecting deformation and mechanical stresses.

[0027] Advantageously, several culture chambers can be connected by means of ducts of predetermined length for simulating the behaviour of biological organs even complex, so that the cells contained in the chambers that are arranged upstream produce metabolites that, transported by the culture medium, feed the cells contained in the chambers that are arranged downstream.

[0028] Advantageously more chambers connected to each other are integrated on a single miniaturized support, in particular of stiff material, creating a circuit for the flow of culture medium that feeds, in a predetermine way and in succession, all the culture chambers.

[0029] Advantageously, said support of stiff material is made through a process of microforming, in particular, by photolithography and/or electroerosion.

[0030] Advantageously said support blocks the cells and the ducts with at least one glass slide of transparent material, allowing the microscope observation of the development of the cells contained in the chambers.

[0031] Advantageously, said inlet and outlet ducts of gas into/away from said premixing chamber can be operated by electrovalves driven by an electrical control unit.

[0032] Preferably, the bioreactor uses an electronic control unit for amplifying and filtering the electrical signals coming from the sensors, for measuring said physiological parameters of the culture medium, and located separately from said electrical drive control unit for the electrovalves, to avoid electromagnetic interferences.

Brief description of the drawings

[0033] The invention will now shown with the following description of an exemplary embodiment thereof, exemplifying but not limitative, with reference to the attached drawings wherein:

- figure 1 shows the bioreactor with the devices to it connected for measuring and adjusting from the outside all the biological and physical parameters of interest;
- figure 2 shows an exploded view of the premixing chamber for the culture medium;
- figures 3 and 4 show respectively an exploded perspective view and a cross sectional view of an exemplary embodiment of culture chamber;
- figure 4 shows a cross sectional view of the culture chamber;
- figures 5 and 6 show respectively an exploded view of the assembling arrangement and a cross section of another exemplary embodiment of culture chamber;
- figure 7 shows a succession of two culture chambers in series;
- figure 8 shows an application of a assembly of culture chambers connected in series and in parallel that simulate the operation of a biological apparatus.

Description of the preferred embodiments

[0034] In figure 1 an aggregate view is shown of a bioreactor for studying the effects of physical, chemical, mechanical and electromagnetic stimuli on cells activity.

[0035] In particular, the device uses a sensorized premixing chamber 1 that has the task of preparing a culture medium that is used for feeding the cells, which are arranged in a culture chamber 2, for eventually observing the development of the cells by a microscope 40. The signals at the outlet of the sensors are transmitted to a signal amplifying and filtering control unit 50, which transmits the treated signals to a computer 52 that saves them by an I/O data acquisition board.

[0036] Said computer is connected to a control unit 51

that operates electrovalves 20, 21 and 22, which adjust the introduction of air and carbon dioxide in the premixing chamber. Said control unit 51 is connected to control unit 50 by an electrical cable 53, in order to eliminate the interference of the electric supply network with the signal amplification and filtering system.

[0037] The culture medium is drawn from the premixing chamber 1 by duct 4 and its flow is adjusted by a peristaltic pump 30. The culture medium crosses then culture chamber 2 and continues its path through duct 3, returning again in the premixing chamber 1. At the outlet of culture chamber 2 the duct has a pick up point 23 for spilling out a sample of culture medium to analyse. Immediately before, along the duct, a temperature sensor is provided 24 that transmits a signal to the control unit 50 by electrical cable 9.

[0038] Premixing chamber 1 comprises a pH sensor 2, which transmits a signal to the control unit 50 by electrical cable 10. Anther parameter determined in the premixing chamber is pressure, through a pressure sensor 1 that transmits a signal to the control unit 50 by electrical cable 10.

[0039] The premixing chamber contains a controlled environment by a controlled introduction of air through duct 7 and of CO_2 through duct 8, which flow then in a duct 6. Such introductions are controlled respectively by electrovalves 21 and 22, operated by control unit 51. Premixing chamber 1 has also a gas outlet duct 5, which is also controlled by a servomechanism 20 operated by the same control unit 51, which allows keeping the pressure fixed in the bioreactor as imposed by the software.

[0040] The control of the physical-chemical and physiological parameters is carried out in order to follow the data imposed by the software through an algorithm of PID type, so that the system is steady, and this would not have happened if an ON/OFF control had been used, and corrections are made only when the system alone cannot turn back to the starting equilibrium, in order to reduce the effects coming from the outer environment and to simulate as far as possible an homeostasis of the cellular system.

[0041] The premixing chamber is described in more detail in figure 2. In particular, it comprises a glass container 60 or a container of other inert material, shaped for example as a flask, closed ermetically with a silicone plug 63 that allows to house the sensors and to arrange a plurality of inlet/outlet stiff ducts, to which flexible ducts are connected. In particular, the plug 63 is crossed: by a duct 68 for introducing gas (air or CO_2), which is connected to flexible duct 6; by duct 5, which has at one end a T-shaped sleeve to support pressure sensor 1 and at the other servomechanism 20 for the exit of the gas; by the inlet duct 65 and outlet duct 66, which are connected respectively to the flexible ducts 4 and 3 for the culture medium. Furthermore, plug 63 allows the movement of a stem for the pH sensor 64, which is connected to electrical cable 10.

[0042] On the bottom of container 60, a base is made of silicone rubber that has a frustum conical recess for reducing the space about pH sensor 64, to obtain a more accurate measure, avoiding that the added gas changes the pH.

[0043] The temperature of the culture medium 59 present in the container 60, is adjusted by a flow of liquid at a chosen temperature flowing in a duct 61 surrounding the container 60.

[0044] in figures from 3 to the 6 are shown some examples of embodiment of a culture chamber.

[0045] In particular, in figure 3 and 4 an exploded view and a cross sectional view are shown respectively of a particular type of cell where the shape of the duct is studied to ensure a laminar flow of culture medium. The cell comprises two parts of silicone material, a lower part 71 and an upper part 70 symmetric to each other. They comprise respective glass slides 74 and 75 that allow the observation by a microscope of the development of the cells previously deposited on the glass slides same. The two parts of silicone rubber are kept together by two stiff plates 72 and 73, for example of metal, kept together by screws 76.

[0046] Figures 5 and 6 show respectively an exploded view and a cross section of another exemplary embodiment of culture chamber, in which the flow of the culture medium is allowed by ducts 79 and 80. Even in the present example the cell comprises two parts of silicone rubber 70 and 71, comprising respective glass slides 74 and 75 for allowing the observation of the cells. The two parts of silicone rubber are then kept together by stiff plates 73 and 72 connected by screws 76.

[0047] As shown in figure 7, several culture chambers can be connected together in series or in parallel so that the products of the cells cultivated in the chambers arranged upstream feed the cells in the chambers arranged downstream, in order to simulate physiological systems, such as the respiratory system, the cardiovascular system, the metabolic system, the feeding system, etc. In the example treated chamber 160 and chamber 161 are located in series upstream and downstream with respect to the flow 149 of culture medium; on glass slide 150 are deposited for example "cells a" 156, which make "metabolites a" 154 and on glass slide 151 are deposited for example "cells b" 157 that make "metabolites b" 155 feed themselves with "metabolites a" 154.

[0048] Downstream from each cell a pick up point 152 and 153 can be provided for spilling out an amount of culture to analyse.

[0049] In figure 8 an example is described of combination of several culture chambers connected in series and in parallel by means of more or less long ducts (102, 103, 104, 105, 106, 107, 108, 109, 110) and integrated on a single support 100, executed by lithography on the body of the support same. The channels thus obtained in the support can be closed above with a glass slide 101 that covers the whole support 100, or alternatively with more glasses that close the single chambers. In particular, are used four chambers that contain respectively:

human or murine hepatic cells 220, endothelial cells 222, adipocytes 223, pancreatic cells 221, which completely simulate the metabolism of an organism. The biological processes of the cells is monitored individually by measuring the metabolites and the proteins in standard conditions, in sampling carried out near each chamber. The substances that can be detected are various, such as albumin, cholesterol, glucose, potassium, lactate, sodium, proteins total, triglycerides, urea and other.

[0050] In the example described a culture medium inlet channel is used 102 to feed the hepatic cells in chamber 220, from which, through ducts 109 and 110 the culture medium reaches respectively the adipocytes in chamber 223 and the cells endothelial in chamber 222, connected in parallel. From these chambers cells, through the ducts 107, 108 and 105, the culture medium reaches hepatic cells in chamber 220 through duct 106 and through duct 104 running through the pancreatic cells in chamber 221.

[0051] The foregoing description of a specific embodiment will so fully reveal the invention according to the conceptual point of view, so that others, by applying current knowledge, will be able to modify and/or adapt for various applications such an embodiment without further research and without parting from the invention, and it is therefore to be understood that such adaptations and modifications will have to be considered as equivalent to the specific embodiment. The means and the materials to realise the different functions described herein could have a different nature without, for this reason, departing from the field of the invention. It is to be understood that the phraseology or terminology employed herein is for the purpose of description and not of limitation.

Claims

1. Bioreactor for monitoring cellular activity in the presence of physical chemical stimuli, comprising:

    - at least one culture chamber (2) having an inlet and an outlet;
    - a premixing chamber (1), separated from said at least one culture chamber, to prepare a culture medium;
    - a circuit (4,3) connected to said inlet and said outlet and comprising said premixing chamber;
    - means for conveying in a controlled manner said culture medium through said circuit;
    - means for generating at least one physical-chemical stimulus that has to be applied to the cells being tested, said stimulus being selected from the group of temperature, pH, pressure or combination thereof;
    - means for controlling (51) said means for generating at least one physical-chemical stimulus, so that said or each stimulus reaches predetermined values.

2. Bioreactor for monitoring cellular activity, according to claim 1, wherein said culture chamber is made of silicone rubber and is shaped in such a way that through said culture chamber a laminar flow of the culture medium is achieved.

3. Bioreactor for monitoring cellular activity, according to claim 1, wherein said culture chamber is made of at least two parts that can overlap, where at least one part has a recess in such a way that once overlapped to the other part a passage for the culture medium is provided.

4. Bioreactor for monitoring cellular activity, according to claim 3, wherein, along said passage for the culture medium at least one of said parts that can overlap comprises a glass slide for laboratories arranged to operatively allow a microscope observation of cells implanted on it.

5. Bioreactor for monitoring cellular activity, according to claim 4, wherein said two parts that can overlap can be pressed on each other by two stiff plates kept together by releasable coupling means.

6. Bioreactor for monitoring cellular activity, according to claim 1, wherein said means for conveying in a controlled manner said culture medium through said culture chamber comprise:

    - an inlet and an outlet duct communicating with said culture chamber, to form a closed circuit with a separated premixing chamber;
    - a peristaltic pump installed along said duct;
    - an introduction point for drugs or other substances that boost or inhibit cellular activity arranged upstream from the culture chamber;
    - a sample point downstream of the culture chamber for taking samples to analyse;
    - a temperature sensor arranged upstream from the culture chamber.

7. Bioreactor for monitoring cellular activity, according to claim 1, wherein said premixing chamber comprises:

    - a container of inert material;
    - a plug of inert material;
    - means for operatively measuring the physiological parameters of the culture medium.

8. Bioreactor for monitoring cellular activity, according to claim 7, where the means for operatively measuring the physiological parameters of the culture medium comprise:

    - a pH sensor immersed in the culture medium present in said premixing chamber;

- a pressure sensor for measuring pressure in said premixing chamber;
- sensors for measuring chemical species, selected from the group of O_2, CO_2, NO, etc.

9. Bioreactor for monitoring cellular activity, according to claim 7, wherein, in and at the bottom of the premixing chamber, a frustum conical concave structure is present where said pH sensor is arranged, in order to preserve it from a direct contact with possible bubbles of gas, which is introduced in said premixing chamber for adjusting the flow of culture medium and for keeping it at a predetermined pH.

10. Bioreactor for monitoring cellular activity, according to claim 7, wherein means are provided for operatively adjusting the physiological parameters of the culture medium comprising:

- inlet/outlet ducts for a gas, such as in particular air and CO_2, flowing into/away from said premixing chamber for changing its pH and the pressure;
- a flow of thermostated fluid in a duct that surrounds said premixing chamber, for changing its temperature.

11. Bioreactor for monitoring cellular activity, according to claim 7, where the means for monitoring and controlling the physical-chemical stimuli applied to the cells in the culture chamber are selected from the group comprised of:

- an optical sensor for detecting gas bubbles in the cell culture chamber;
- a sensor for detecting deformation and mechanical stresses.

12. Bioreactor for monitoring cellular activity, according to claim 1, wherein several culture chambers are connected by means of ducts of predetermined length for simulating the behaviour of biological organs even complex, so that the cells contained in the chambers arranged upstream produce metabolites that, transported by the culture medium, feed the cells contained in the chambers that are arranged downstream.

13. Bioreactor for monitoring cellular activity, according to claim 12, wherein more chambers connected to each other are integrated on a single miniaturized support, in particular of stiff material, creating a circuit for the flow of culture medium that feeds, in a predetermined way and in succession, all the culture chambers.

14. Bioreactor for monitoring cellular activity, according to claim 13, wherein said support blocks the cells and the ducts with at least one glass slide of transparent material, allowing the microscope observation of the development of the cells contained in the chambers.

15. Bioreactor for monitoring cellular activity, according to claim 10, wherein said inlet and outlet ducts of gas flowing into/away from said premixing chamber, are associated to electrovalves driven by an electrical control unit.

Patentansprüche

1. Bioreaktor zur Überwachung von Zellaktivität in Anwesenheit physikochemischer Stimuli, umfassend:

- mindestens eine Kulturkammer (2) mit einem Einlass und einem Auslass;
- eine von der genannten mindestens einen Kulturkammer abgetrennte Vormischkammer (1) für die Zubereitung eines Kulturmediums;
- einen mit dem genannten Einlass und dem genannten Auslass verbundenen und die genannte Vormischkammer umfassenden Kreislauf (4, 3);
- Mittel zur gesteuerten Beförderung des genannten Kulturmediums durch den genannten Kreislauf;
- Mittel zur Erzeugung mindestens eines physikochemischen Stimulus, der auf die zu testenden Zellen anzuwenden ist, wobei der genannte Stimulus aus der Gruppe bestehend aus Temperatur, pH-Wert, Druck oder einer Kombination aus diesen ausgewählt wird;
- Mittel zur Steuerung (51) der genannten Mittel zur Erzeugung mindestens eines physikochemischen Stimulus, so dass der genannte oder jede Stimulus vorbestimmte Werte erreicht.

2. Bioreaktor zur Überwachung von Zellaktivität nach Anspruch 1, wobei die genannte Kulturkammer aus Silikongummi hergestellt ist und derart geformt ist, dass durch die genannte Kulturkammer eine Laminarströmung des Kulturmediums erreicht wird.

3. Bioreaktor zur Überwachung von Zellaktivität nach Anspruch 1, wobei die genannte Kulturkammer aus mindestens zwei Teilen hergestellt ist, die einander überlappen können, wobei mindestens ein Teil eine Aussparung aufweist derart, dass, sobald er den anderen Teil überlappt, ein Durchlass für das Kulturmedium geschaffen wird.

4. Bioreaktor zur Überwachung von Zellaktivität nach Anspruch 3, wobei entlang dem genannten Durchlass für das Kulturmedium mindestens einer der ge-

nannten Teile, die einander überlappen können, einen Objektträger für Laboratorien umfasst, welcher dazu eingerichtet ist, im Betrieb eine Mikroskopbeobachtung auf ihm implantierter Zellen zu ermöglichen.

5. Bioreaktor zur Überwachung von Zellaktivität nach Anspruch 4, wobei die genannten zwei Teile, die einander überlappen können, durch zwei steife Platten aufeinander gepresst werden können, welche durch lösbare Verbindungsmittel zusammengehalten werden.

6. Bioreaktor zur Überwachung von Zellaktivität nach Anspruch 1, wobei die genannten Mittel zur gesteuerten Beförderung des genannten Kulturmediums durch die genannte Kulturkammer Folgendes umfassen:

- einen Einlass- und einen Auslasskanal, welche mit der genannten Kulturkammer kommunizieren, um einen geschlossenen Kreislauf mit einer abgetrennten Vormischkammer zu bilden;
- eine entlang dem genannten Kanal angebrachte peristaltische Pumpe;
- einen vor der Kulturkammer eingebauten Einführungspunkt für Arzneimittel oder andere Substanzen, welche Zellaktivität verstärken oder hemmen;
- einen Probenpunkt hinter der Kulturkammer zur Entnahme zu analysierender Proben;
- einen vor der Kulturkammer eingebauten Temperatursensor.

7. Bioreaktor zur Überwachung von Zellaktivität nach Anspruch 1, wobei die genannte Vormischkammer Folgendes umfasst:

- einen Behälter mit inertem Material;
- einen Stopfen aus inertem Material;
- Mittel zur Messung der physiologischen Parameter des Kulturmediums im Betrieb.

8. Bioreaktor zur Überwachung von Zellaktivität nach Anspruch 7, wobei die Mittel zur Messung der physiologischen Parameter des Kulturmediums im Betrieb Folgendes umfassen:

- einen in das in der genannten Vormischkammer vorhandene Kulturmedium eingetauchten pH-Sensor;
- einen Drucksensor zur Messung des Drucks in der genannten Vormischkammer;
- Sensoren zur Messung chemischer Stoffe, ausgewählt aus der Gruppe bestehend aus O_2, CO_2, NO, etc.

9. Bioreaktor zur Überwachung von Zellaktivität nach Anspruch 7, wobei in und am Boden der Vormischkammer eine konkave Kegelstumpfstruktur vorhanden ist, wo der genannte pH-Sensor angebracht ist, um diesen vor einem direkten Kontakt mit möglichen Gasblasen zu schützen, welche in die genannte Vormischkammer eingeführt ist, um den Strom des Kulturmediums anzupassen und ihn auf einem vorbestimmten pH-Wert zu halten.

10. Bioreaktor zur Überwachung von Zellaktivität nach Anspruch 7, wobei Mittel zur Einstellung der physiologischen Parameter des Kulturmediums im Betrieb vorgesehen sind, welche Folgendes umfassen:

- Einlass-/Auslasskanäle für ein Gas, wie insbesondere Luft und CO_2, welches in die genannte Vormischkammer hinein und von dieser wegströmt, um ihren pH-Wert und den Druck zu ändern;
- einen Strom aus thermostatiertem Fluid in einem Kanal, welcher die genannte Vormischkammer umgibt, um ihre Temperatur zu ändern.

11. Bioreaktor zur Überwachung von Zellaktivität nach Anspruch 7, wobei die Mittel zur Überwachung und Steuerung der auf die Zellen in der Kulturkammer angewendeten physikochemischen Stimuli aus der Gruppe ausgewählt werden, welche besteht aus:

- einem optischen Sensor zum Erfassen von Gasblasen in der Zellkulturkammer;
- einem Sensor zum Erfassen von Verformung und mechanischen Belastungen.

12. Bioreaktor zur Überwachung von Zellaktivität nach Anspruch 1, wobei mehrere Kulturkammern mittels Kanälen vorbestimmter Länge verbunden sind, um das Verhalten sogar komplexer biologischer Organe zu simulieren, so dass die in den stromaufwärts eingebauten Kammern enthaltenen Zellen Metaboliten erzeugen, welche, transportiert von dem Kulturmedium, die Zellen versorgen, welche in den stromaufwärts eingebauten Kammern enthalten sind.

13. Bioreaktor zur Überwachung von Zellaktivität nach Anspruch 12, wobei weitere miteinander verbundene Kammern auf einer einzigen miniaturisierten Unterlage, insbesondere aus steifem Material, eingebaut sind, welche einen Kreislauf für den Strom des Kulturmediums schaffen, welcher auf vorbestimmte Weise und nacheinander sämtliche Kulturkammern versorgt.

14. Bioreaktor zur Überwachung von Zellaktivität nach Anspruch 13, wobei die genannte Unterlage die Zellen und die Kanäle mit mindestens einem Objektträger aus transparentem Material absperrt, welcher die Mikroskopbeobachtung der Entwicklung der in

den Kammern enthaltenen Zellen ermöglicht.

15. Bioreaktor zur Überwachung von Zellaktivität nach Anspruch 10, wobei die genannten Einlass- und Auslasskanäle für Gas, welches in die genannte Vormischkammer hinein und von dieser wegströmt, mit Elektroventilen verbunden sind, die von einer elektrischen Steuereinheit angetrieben werden.

Revendications

1. Bioréacteur pour surveiller l'activité cellulaire en présence de stimuli physico-chimiques, comprenant :

- au moins une chambre de culture (2) ayant une entrée et une sortie ;
- une chambre de prémélange (1), séparée de ladite ou desdites chambres de culture, pour préparer un milieu de culture ;
- un circuit (4,3) relié à ladite entrée et à ladite sortie et comprenant ladite chambre de prémélange ;
- des moyens de transport d'une manière commandée dudit milieu de culture à travers ledit circuit ;
- des moyens de génération d'au moins un stimulus physico-chimique qui doit être appliqué aux cellules devant être testées, ledit stimulus étant choisi dans le groupe de la température, du pH, de la pression ou d'une combinaison de ceux-ci ;
- des moyens de commande (51) desdits moyens de génération d'au moins un stimulus physico-chimique, de telle sorte que ledit ou chaque stimulus atteint des valeurs prédéterminées.

2. Bioréacteur pour surveiller l'activité cellulaire, selon la revendication 1, dans lequel ladite chambre de culture est faite de caoutchouc de silicone et est mise en forme de telle sorte qu'est obtenu, à travers ladite chambre de culture, un écoulement laminaire du milieu de culture.

3. Bioréacteur pour surveiller l'activité cellulaire, selon la revendication 1, dans lequel ladite chambre de culture est faite d'au moins deux parties qui peuvent se chevaucher, au moins une partie ayant une cavité de telle sorte qu'une fois qu'elle se chevauche avec l'autre partie, un passage pour le milieu de culture est fourni.

4. Bioréacteur pour surveiller l'activité cellulaire, selon la revendication 3, dans lequel, le long dudit passage pour le milieu de culture, au moins l'une desdites parties qui peuvent se chevaucher comprend une lame de verre pour laboratoires disposée pour permettre de manière fonctionnelle une observation au microscope de cellules implantées sur celle-ci.

5. Bioréacteur pour surveiller l'activité cellulaire, selon la revendication 4, dans lequel lesdites deux parties qui peuvent se chevaucher peuvent être pressées l'une contre l'autre par deux plaques rigides maintenues ensemble par des moyens d'accouplement libérables.

6. Bioréacteur pour surveiller l'activité cellulaire, selon la revendication 1, dans lequel lesdits moyens de transport d'une manière commandée dudit milieu de culture à travers ladite chambre de culture comprennent :

- un conduit d'entrée et un conduit de sortie communiquant avec ladite chambre de culture, pour former un circuit fermé avec une chambre de prémélange séparée ;
- une pompe péristaltique installée le long dudit conduit ;
- un point d'introduction pour médicaments ou autres substances qui activent ou inhibent l'activité cellulaire, disposé en amont de la chambre de culture ;
- un point de prélèvement d'échantillons en aval de la chambre de culture pour prélever des échantillons pour analyse ;
- un capteur de température disposé en amont de la chambre de culture.

7. Bioréacteur pour surveiller l'activité cellulaire, selon la revendication 1, dans lequel ladite chambre de prémélange comprend :

- un récipient en matériau inerte ;
- un bouchon en matériau inerte ;
- des moyens pour mesurer de manière fonctionnelle les paramètres physiologiques du milieu de culture.

8. Bioréacteur pour surveiller l'activité cellulaire, selon la revendication 7, dans lequel les moyens pour mesurer de manière fonctionnelle les paramètres physiologiques du milieu de culture comprennent :

- un capteur de pH immergé dans le milieu de culture présent dans ladite chambre de prémélange ;
- un capteur de pression pour mesurer la pression dans ladite chambre de prémélange ;
- des capteurs pour mesurer des espèces chimiques, choisies dans le groupe de O_2, CO_2, NO, etc.

9. Bioréacteur pour surveiller l'activité cellulaire, selon la revendication 7, dans lequel, dans et au niveau

du fond de la chambre de prémélange, une structure concave tronconique est présente, dans laquelle est disposé ledit capteur de pH, dans le but de la préserver d'un contact direct avec de possibles bulles de gaz, qui est introduit dans ladite chambre de prémélange pour ajuster l'écoulement du milieu de culture et pour le maintenir à un pH prédéterminé.

10. Bioréacteur pour surveiller l'activité cellulaire, selon la revendication 7, dans lequel des moyens sont prévus pour ajuster de manière fonctionnelle les paramètres physiologiques du milieu de culture, comprenant :

- des conduits d'entrée/sortie d'un gaz, tel qu'en particulier de l'air et du CO_2, s'écoulant dans/à l'opposé de ladite chambre de prémélange pour changer son pH et la pression ;
- un écoulement de fluide thermostaté dans un conduit qui entoure ladite chambre de prémélange, pour changer sa température.

11. Bioréacteur pour surveiller l'activité cellulaire, selon la revendication 7, dans lequel les moyens pour surveiller et commander les stimuli physico-chimiques appliqués aux cellules dans la chambre de culture sont choisis dans le groupe constitué par :

- un capteur optique pour détecter des bulles de gaz dans la chambre de culture de cellules ;
- un capteur pour détecter une déformation et des contraintes mécaniques.

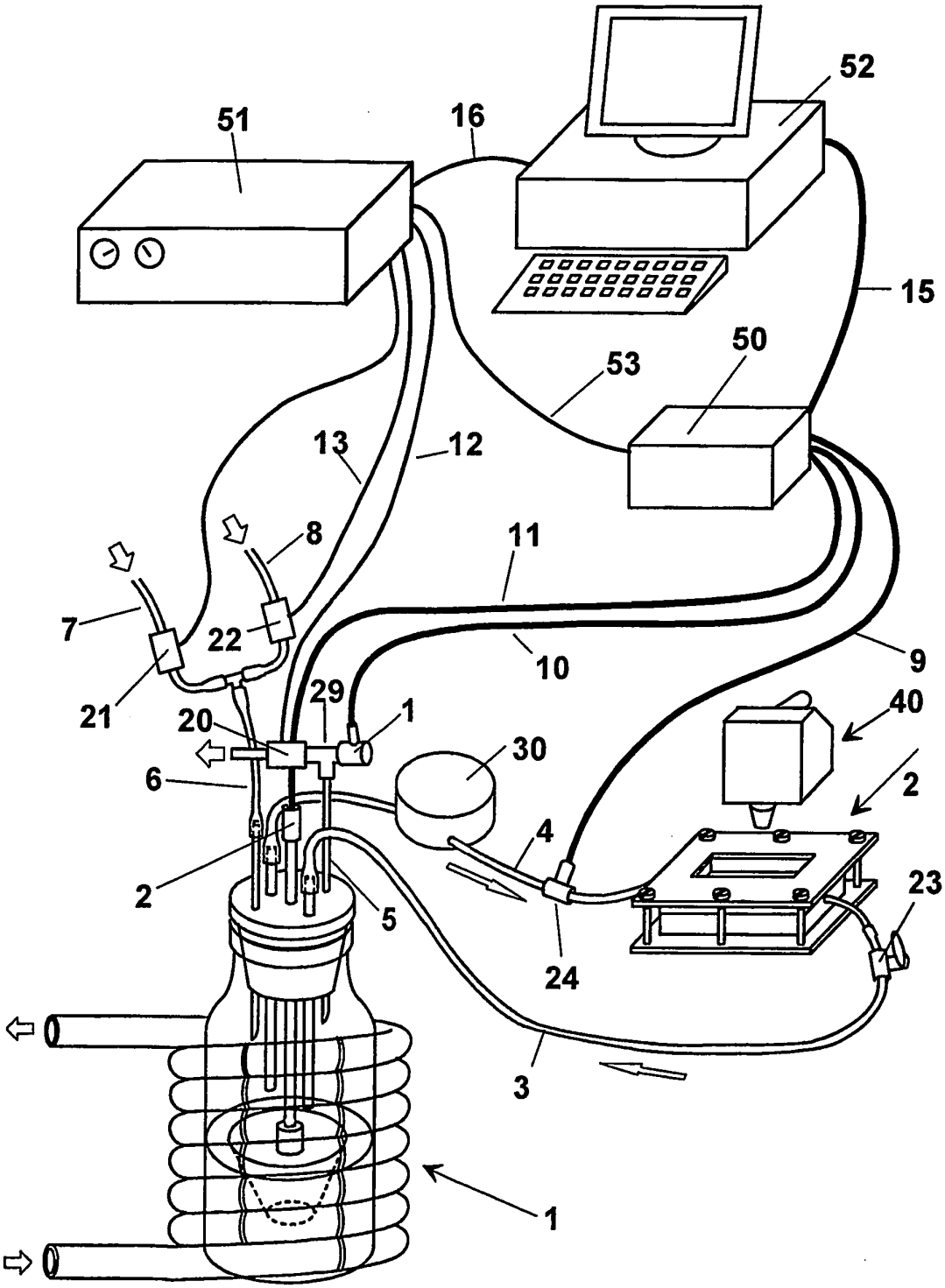
12. Bioréacteur pour surveiller l'activité cellulaire, selon la revendication 1, dans lequel plusieurs chambres de culture sont reliées au moyen de conduits de longueur prédéterminée pour simuler le comportement d'organes biologiques même complexes, de telle sorte que les cellules contenues dans les chambres disposées en amont produisent des métabolites qui, transportés par le milieu de culture, nourrissent les cellules contenues dans les chambres qui sont disposées en aval.

13. Bioréacteur pour surveiller l'activité cellulaire, selon la revendication 12, dans lequel plus de chambres reliées les unes aux autres sont intégrées sur un unique support miniaturisé, en particulier de matériau rigide, créant un circuit pour l'écoulement du milieu de culture qui nourrit, d'une manière prédéterminée et successivement, toutes les chambres de culture.

14. Bioréacteur pour surveiller l'activité cellulaire, selon la revendication 13, dans lequel ledit support bloque les cellules et les conduits avec au moins une lame de verre de matériau transparent, permettant l'observation au microscope du développement des cellules contenues dans les chambres.

15. Bioréacteur pour surveiller l'activité cellulaire, selon la revendication 10, dans lequel lesdits conduits d'entrée et de sortie de gaz s'écoulant dans/à l'opposé de ladite chambre de prémélange, sont associés à des électrovannes commandées par une unité de commande électrique.

Fig.1



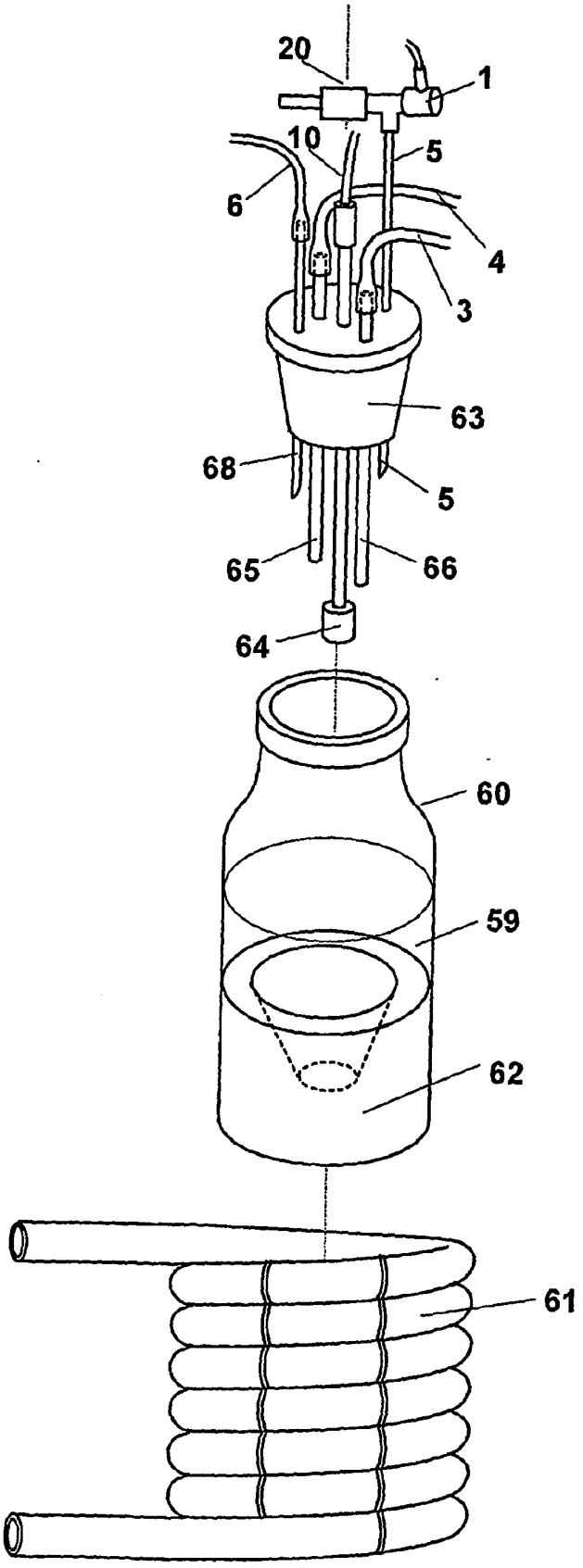


Fig.2

Fig.3

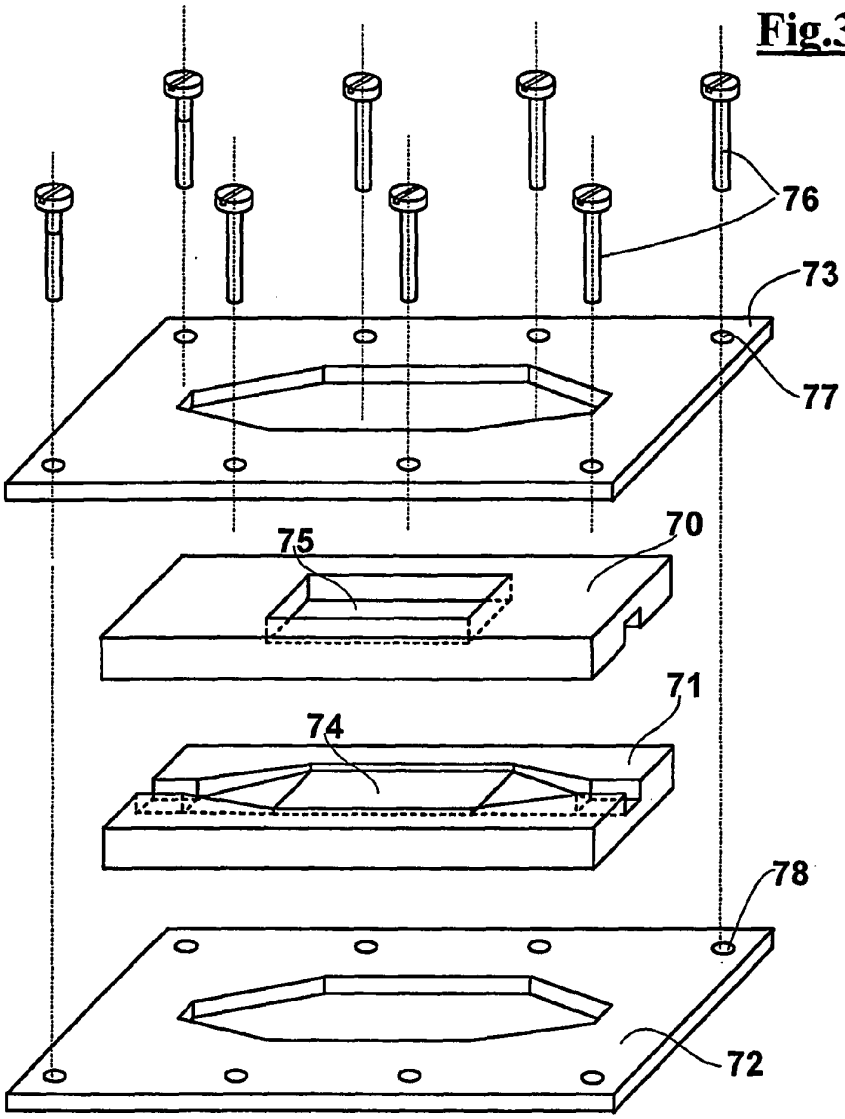


Fig.4

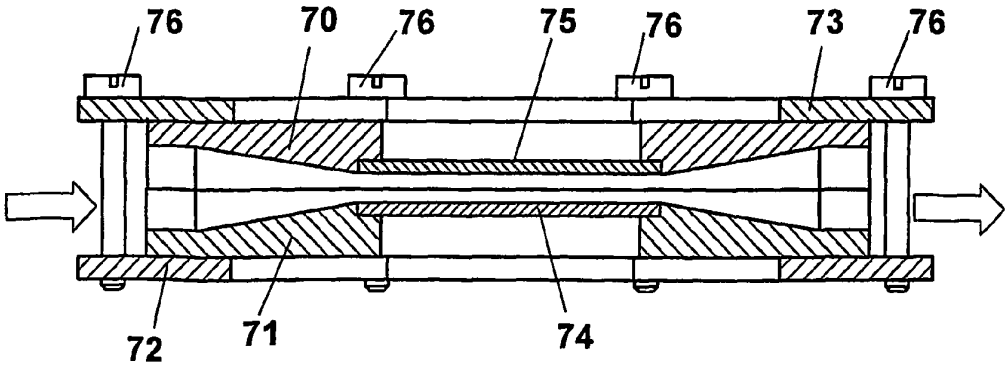


Fig.5

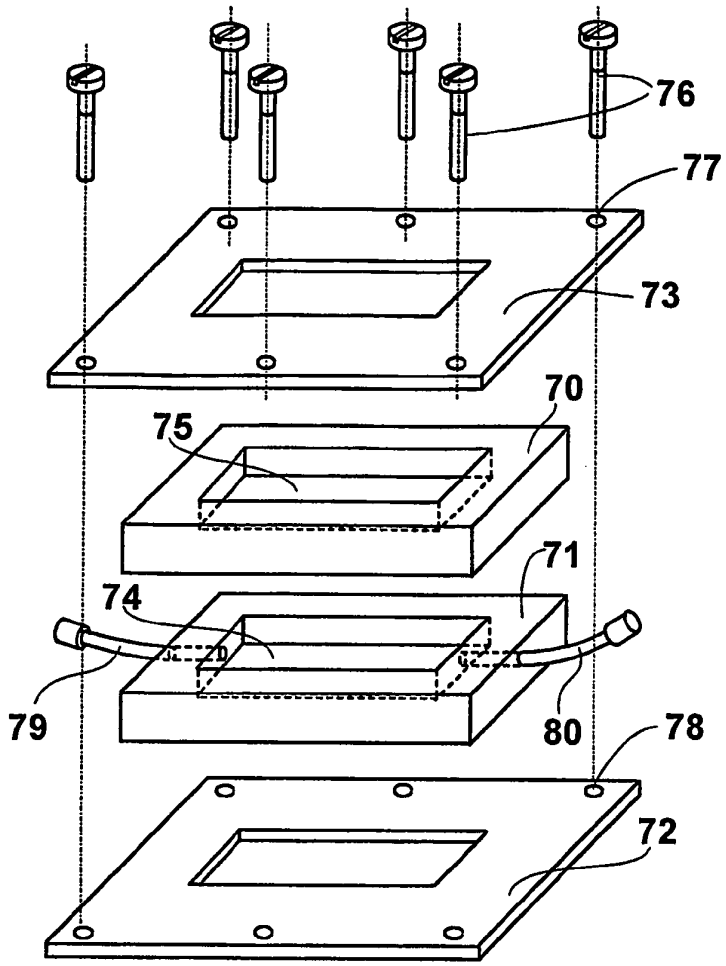
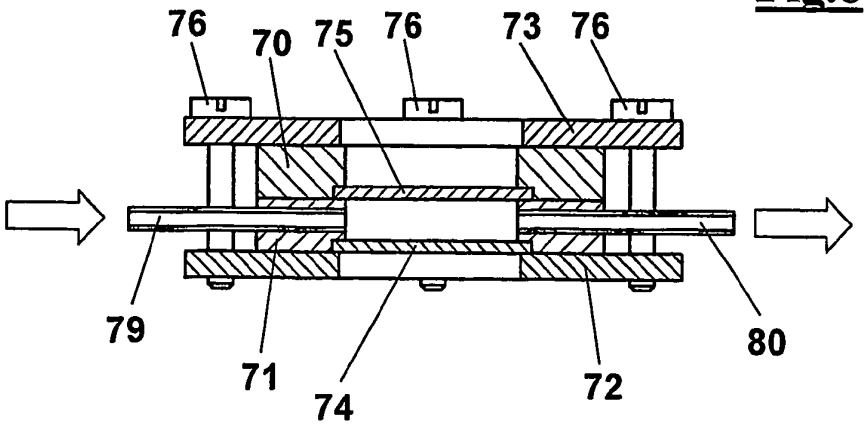


Fig.6



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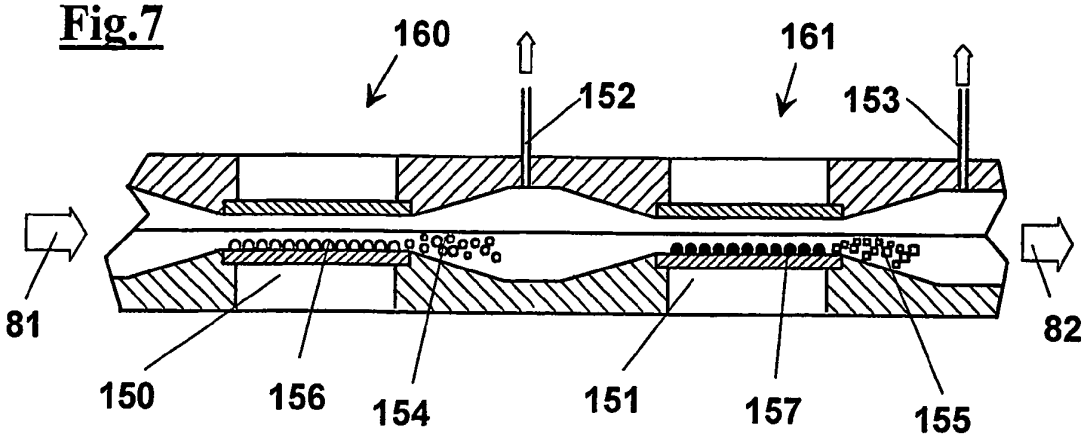
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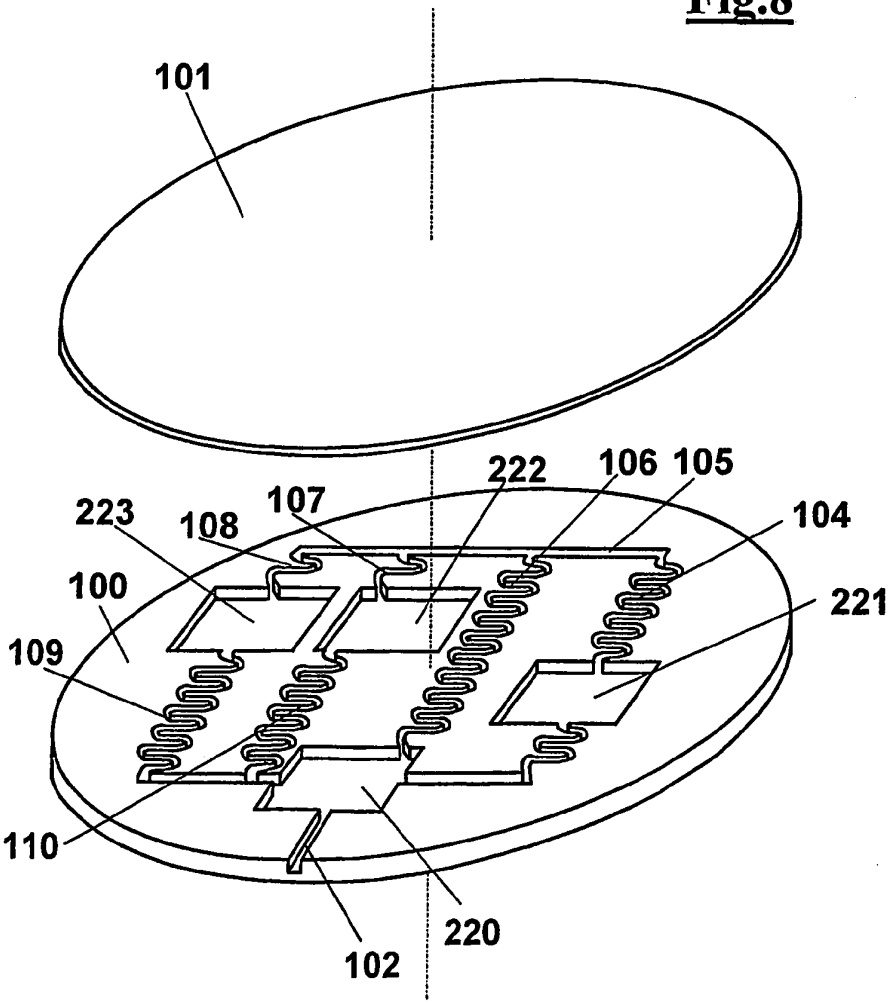
Fig.8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