

**Biochamber with a flow system designed for printing and culturing tissue models****Background of the invention**

**[0001]** The invention relates to a biochamber comprising a flow system and its use for bioprinting and culturing tissue models.

**State of the art**

**[0002]** Document CA2239815C relates to a cell storage device. This device includes a biochamber that contains windows in the top and bottom walls to provide an optical pathway for imaging. The culture medium is exchanged using a robotic system that automatically exchanges the medium using a pipette in a 96-well plate. This design of the biochamber does not allow the use of a flow system, making the exchange of the culture medium occur in a different way than in the solution according to the subject matter. In addition, the chamber according to CA2239815C does not contain luer lock connectors.

**[0003]** Document US9226494B2 relates to a modular bioreactor comprising screw-connected, parallel-connected component biochambers. The biochambers contain a substantially cylindrical matrix, which is connected to an input port and an output port. The output and input ports can include sealed adapters to prevent leakage of the culture medium. However, document US9226494B2 does not mention luer lock connectors as an alternative to adapters. The bioreactor according to US9226494B2 also does not include windows or transparent walls to allow optical observation.

**[0004]** Document AU2012200361B2 describes a bioreactor for preparing tissue for transplantation with a vascular design that differs significantly from the design according to the subject matter.

**[0005]** Document EP1325110A4 relates to an automated cell culture system. The system includes, but is not limited to, a biochamber suitable for use in a static cell culture or perfusion apparatus. It consists of a first chamber, a lid, a seal forming the first chamber, detachably connected to the lid and preventing contamination of the cell cultures in the biochamber, and at least one insert placed between the first chamber and the lid, thus forming a second chamber. The biochambers can be connected to each other or in series. In addition, the sampling interface included in the system includes a luer lock connector as an example of a connection.

**[0006]** Document AU2013206071B2 shows a biochamber whose upper part is attached to the base (preferably in a liquid-tight manner), for example by welding or by other fastening means

such as screws around the perimeter. The membrane is clamped between the top and the base and is stretched tight enough to divide the internal volume of the biochamber into upper and lower parts. The disk of the cell deposit is located at the bottom of the biochamber interior. It is generally disc-shaped with an upward projecting annular edge on its outer radial perimeter. After inoculation of the starting cells, cell growth takes place in the cell bed delineated between the upper surface of the disk, the lower surface of the membrane and the annular edge. The upper surface of the annular edge is preferably co-planar with the upper surface of the base flange when the disk is mounted in the base, so that the membrane can cooperate with the edge to seal the cell growth bed. In advantageous manufacturing examples, the upper part of the base and the cell bed disk are made of transparent, non-reactive plastic. The design of the biochamber is significantly different from the test subject. Airtightness is ensured by the use of a membrane, instead of the components fitting properly and being connected by screws. In addition, unlike the tested solution, the biochamber contains a bed for culturing the inoculated cells.

**[0007]** Document US60236703P0 relates to a bifunctional biochamber for providing a combination of traditional cell culture and liquid perfusion capabilities. The biocell according to US60236703P0 includes a static growth chamber with a removable lid to prevent contaminants from entering the chamber. In addition, the lid and base are color-coded to indicate the pressure provided by the threaded caps. The chamber ports allow culture medium to flow from the culture medium reservoir into the chamber. The publication does not mention 3D bioprinting, transparent biochamber walls or windows for microscopic observation.

**[0008]** Document WO2019200411A1 discloses a cell culture system. It includes a bioreactor designed as an open-loop system in which the culture medium flows from an inlet tank into the arterial side of mesenteric tissue and exits through the venous side into a collection tank. The bioreactor tubes were connected to a peristaltic pump and a three-way stopcock. According to one manufacturing example, the biochamber was assembled by first placing the PDMS base between the threaded rods of the acrylic bioreactor base. The collected mesenteric tissues were then transferred into the biochamber, and a 30G needle cannula connected to the bioreactor tube was inserted into the feeding artery. The remaining blood was removed from the network of microvessels in the mesenteric tissue by perfusion with approximately 3 ml of heparinized PBS (3 mg ml<sup>-1</sup>). When the microvascular effluent was clear, the mesenteric tissue was covered with a filter membrane, and then PDMS O-ring and acrylic top were added. The biochamber was closed with knurled caps and 5 ml of medium was added to the well of the chamber to immerse the tissue. Approximately 250 ml of medium was added to the inlet well, the biochamber was connected to the bioreactor tube and transferred to the incubator for perfusion culture.

WO2019200411A1 also suggests using a luer lock fitting for the fluid connection. The main goal of WO2019200411A1 is to provide a solution for microscopic observation of cultured cells and tissues. The chamber has a different design from that shown in the subject solution due to the fact that it is not sealed.

**[0009]** From document US20230257687A1, the nozzle for distributing fluid in the bioreactor is known. The design of the bioreactor is quite different from the solution in question (no leakage), while it is completely transparent.

**[0010]** The solution disclosed in EP4161671A1 relates to a system for monitoring and regulating the level of contents in a vessel. The vessel according to EP4161671A1 may be, among other things, a biocontainer for storing liquids, also flexible in the form of a bag. In some manufacturing examples, the biocointainer is made of stainless steel and includes two windows. The system is based on optical observation based on a light sensor and a laser-based light source.

**[0011]** Document US20220323300A1 relates to a biocontainer comprising a film with an inner and outer side, hinged elements, and a second film optionally containing hinged elements. The interconnected films form a container, and the articulating elements allow the biocontainer to expand and collapse.

#### **Subject matter of the invention**

**[0012]** The object of the invention is a biochamber that allows the bioprinting and culturing of models with a flow system, which includes a flow system, consisting of two modules: an inner module, containing a base 1 and a cover 4 of the biochamber, equipped with, respectively - the first window 2 and the second window 5; a seal and two luer lock connectors 10; an outer module, containing the first frame 7, the second frame 8 and fasteners 9.

**[0013]** Preferably, the fasteners 9 in the outer module are screws.

**[0014]** Preferably, the biochamber additionally contains a calibrator.

**[0015]** Preferably, the luer lock connectors 10 are made of polycarbonate.

**[0016]** Preferably, the dimensions of the biochamber are 12x40x3 mm, and the volume is 1.5ml.

**[0017]** Preferably, the biochamber is made of biocompatible materials meeting ISO 10993 standards.

**[0018]** Preferably, the biochamber is made by 3D printing technology from a biocompatible light-curable resin.

[0019] Preferably, the biocompatible light-curing resin is Formlabs Bimed Clear.

[0020] Also an object of the invention is a system for stable printing of liver tissue, comprising a biochamber according to the invention placed on a support 3.

[0021] Also an object of the invention is the use of a biochamber according to the invention for bioprinting and culturing tissue models.

#### **A brief description of the figures of the drawing**

[0022] The invention will be further illustrated in a preferred embodiment with reference to the attached drawing, in which:

[0023] Fig. 1 shows a schematic of the construction of the biochamber.

[0024] Fig. 2 shows the bioprinted model, including 3 flow systems, side view Fig. 2 a) and front view Fig. 2 b).

[0025] Fig. 3 shows a closed system for leak testing - point a) when static fluid pressure is applied; point b) when dynamic fluid pressure is applied.

[0026] Fig. 4 shows the bioreactor compatible with the biochamber (points a and b); the thermoblock dedicated for the bioreactor and biochambers supporting a set of three biochambers (point c); and the biochamber equipped with ports to the vascular system in the form of supportard luer lock laboratory connectors. The thermoblock dedicated to the bioreactor and biochambers can support a set containing one to three biochambers simultaneously.

[0027] Fig. 5 depicts the biochamber, specifically - point a) the upper base of the biochamber, equipped with laboratory glass walls, point b) the lower base of the biochamber, equipped with laboratory glass walls; point c) the support, point d) the holder that stabilizes the biochamber on the microscope table.

[0028] Fig. 6 shows a schematic of the construction of the biochamber in its assembled form.

[0029] Fig. 7 shows the parts of the biochamber - the base, the cover, and the oring-type seal.

[0030] In the accompanying figure, designations are used:

The attached figure uses the designations:

1 - Biochamber base

2- First window

- 3- Support
- 4- Biochamber cover
- 5- Second window
- 6- O-ring seal
- 7- First frame
- 8- Second frame
- 9- Fasteners in the form of screws
- 10- Luer lock connector

**Detailed description of the invention figures**

**[0031]** The biochamber shown in Fig. 1 consists of two modules - an inner and an outer module. The inner module consists of the base 1 and the lid 4 of the biochamber (shown in Fig. 7). The base 1 and lid 4 are equipped with a first window 2 and a second window 5, respectively, for microscopic observation. Windows 2 and 5 are bases that allow microscopic observations, and are made of glass, or other biocompatible polymer that allows real-time microscopic observations under a microscope.

**[0032]** The windows are adhered by means of silicone adhesives. The inner module also includes an oring-type seal 6 and two luer lock connectors 10. The luer lock connectors are made of polycarbonate and allow the biochamber to be connected to the flow circuit. The external module of the biochamber consists of a first frame 7 and a second frame 8, connected to each other by fasteners in the form of screws 9. The frame provides sufficient rigidity to seal the housing (at a preset pressure inside the chamber of 300 mmHg), the possibility of tight closure and easy immobilization of the biochamber on the microscope table, as well as adequate pressure causing the seal to fit the irregularities of the housing. It also prevents deformation during the sterilization process and conducts heat from the bioreactor body to the interior of the biochamber. The shape of the frames allows mounting in the bioreactor block as well as on the microscope table. The transparent bottom of the chamber allows microscopic observations; therefore, it is possible to assess the growth of tumor foci or the development of organoids and individual cells in the model, i.e. whether they proliferate or show viability after the administration of dyes. The structure of the assembled biochamber is shown in Fig. 6.

**[0033]** The chamber according to the invention has dimensions ranging from 5x15x1 mm to 25x70x10, and in a preferred embodiment the dimensions of the biochamber are 12x40x3 mm, and the preferred volume is 1.5ml.

**[0034]** A biochamber is used as a base for printing structures using 3D bioprinting technology. The device has the ability to be mounted on the worktable of a bioprinter, such as Cellink BioX during the bioprinting process. After the bioprinting process, the chamber is closed and its leak tightness, sterility, microscopic observation, and ability to feed and drain the culture medium are ensured. In addition, the biochamber can include a support 3, used during the bioprinting process, ensuring that the biochamber is non-deformable and fixed to the worktable of the bioprinter. In addition, the biochamber can include a calibrator, used to find the starting (zero) point, used at the beginning of the bioprinting process.

**[0035]** The body of the biochamber is made of biocompatible materials using any processing method, such as 3D printing, injection molding, milling or CNC machining. The frames that ensure the rigidity and tightness of the chamber are made of 316L stainless steel by CNC cutting technology. In a preferred manufacturing embodiment, the body of the biochamber is made using 3D printing technology from a biocompatible light-curing resin that meets ISO 10993 (inter alia for cytotoxicity, hemotoxicity tests, etc.) or FDA standards, preferably Formlabs Bimed Clear resin. In the context of the present invention, Formlabs Bimed Clear resin is to be understood as a biocompatible light-curing resin available under that trade name in the filing date of this application.

**[0036]** The biochamber provides insulation from the external environment by maintaining airtightness. It is possible to adjust the airtightness while maintaining a uniform compression on the biochamber, which reduces the risk associated with window cracking. Additionally, construction of the biochamber allows connecting the inlet and outlet channels for the culture medium, the providing space with specific geometric dimensions (up to 25x70x10 mm), allows printing the structures produced by 3D bioprinting inside the chamber, enabling the non-cytotoxicity of materials, and steam sterilization of every element of the biochamber. However, this is not a limiting embodiment since the biochamber according to the invention also enables the biomaterial to be poured (with or without cells) and cross-linked (with or without retaining) the flow channel. In such embodiments, the cell medium can flow through the created vascular channel or flow directly on the surface of the biomaterial (desirable preferably for skin or alveolar

tissue), or conduct 2D culturing in a flow involving plating the cells directly to the bottom (window 2 or 5) of the biochamber.

**[0037]** Easy access to the luer lock connectors improve the productivity and simplifies connection the circuit in the biochamber, enabling manual medium exchange, reagent administration or sample collection. It is also possible to easily disconnect from the perfusion circuit without breaching and losing sterility.

**[0038]** The biochamber can be easily and quickly closed without affecting its sealability. The construction of the biochamber also ensures that no deformation of components occurs during steam sterilization, no breaching during multi-day experiments, and provides visibility to the operator during 3D bioprinting.

**[0039]** The biochamber allows 3D printing of tissue models that comprise living cells. The biochamber allows for the conduction of continuous cultures. The model can comprise both normal cells and cancer cells.

**[0040]** Additionally, the biochamber allows the study of diffusion. For this purpose, the bioprinted model has at least 2-3 flow systems as shown in Fig.2. Evaluation of diffusion is possible by labeling molecules of a certain mass and thus, by observing the color propagation in the model or fluorescence visualization, the rate of substance diffusion from a flow system into the model can be evaluated.

**[0041]** The biochamber can be installed in the bioreactor.

**[0042]** Additionally, individual biochambers can be combined to form more complex tissue systems. In a preferred manufacturing embodiment, the biochambers are connected in series with each other in two alternatives: with returning the culture medium to the beginning of the system (to the bottle from which the culture medium is collected) or received at the output of the last biochamber (into a separate container/bottle). These connecting methods allow to create a “closed” or “open” system.

**[0043]** During 3D printing, the biochamber is mounted in a support 3, which is a metal component that protects the biochamber during printing and ensures that the chamber lies flat on the printer table. The first frame of the biochamber is directly connected to the support by the means of screws.

#### **EXAMPLE 1 Leak tightness of the chamber for bioprinting of bionic liver model**

[0044] Each unit was filled with a solution of similar viscosity to the culture medium, which was a solution of water containing phenol red dye at a concentration of 50 mg/L and UV-visible Errecom Brilliant Coolant dye at a concentration of 20%. The biochambers were subjected to leakage tests under static liquid pressure that was set to 100 kPa. The leak tightness of the biochambers was monitored by checking the presence of visible dyes under white light and UV light on the external part of the biochamber or on the liquid-absorbing surface. Using a hand pump with a valve, the system was pumped to achieve the liquid pressure above 100 kPa. A liquid flow rate of 10 mL/min was set using a peristaltic pump, and the test was conducted continuously for 7 days. Tests showed the leak tightness of all biochambers that were tested when static liquid pressure was applied with a value between 1 and 100 kPa, and when dynamic pressure was applied with a flow rate between 0.5 and 10 mL/min. Preferably, observations are conducted at pressures up to 40 kPa and with a flow rate of 0 to 10 mL/min.

#### **EXAMPLE 2 Thermal resistance of the chamber reaching 134°C**

[0045] In the course of the study, thermal resistance was tested by subjecting them to temperatures of 134°C and 121°C. To confirm the utility of the chambers for further testing, the biochambers were subjected once to leakage tests when static liquid pressure was applied to a value of 25 kPa and leakage tests when dynamic liquid pressure was applied, the duration of which was 7 days. The tests showed the chamber's thermal resistance reaching 134°C. The chambers have not been deformed, prove leak tightness and are therefore suitable for testing.

#### **EXAMPLE 3 Biocompatibility of the chamber.**

[0046] In order to verify the biocompatibility of the biochamber, the biochambers were sterilized, assembled and subsequently the cell suspension of line L929 was introduced, maintaining a density of  $0.01 \times 10^6 / 1 \text{ cm}^2$  of the culture surface. In order to populate the culture surface, the luer lock connectors of the biochamber were closed and the biochamber was placed in a cell culture incubator, where the culture was maintained for 5h at 37°C with 5% CO<sub>2</sub>. The closed system was assembled for culturing in the base of the chamber, taking into account the flow rate. Subsequently, the biochambers were connected to the system, after which, along with the bottle containing the culture medium, they were placed in the culture cell incubator. Subsequently, the peristaltic pump was activated. Control samples were also prepared in which cells were cultured on: a 100 mm "non-treated" cell culture plate, a glass window placed on a 100 mm "non-treated" cell culture plate, and a T25 cell culture bottle "treated", for each sample maintaining the same density of cells of the L929 line:  $0.01 \times 10^6 / 1 \text{ cm}^2$  of culture area. The culture was carried out for 3 days at 37°C with 5% CO<sub>2</sub>. During this time, >90% confluence was achieved. Tests showed no

loss of cell viability and functionality in the culture, with >90% confluence of the culture surface, indicating biocompatibility of the cells.

**[0047]** The chamber is compatible with the bioreactor developed in previous studies (Fig.4., a-b), which is a device that is universal to the population of cultured cells and allows control of biological parameters, regulation of pressure and flow rate in the vascular system of the printed tissues, as well as dispenses drugs and nutrients. This ensures appropriate culture conditions, including an appropriate supply of CO<sub>2</sub> to the cultured tissue. Compatibility of the chamber with the bioreactor is ensured by manufacturing a dedicated thermoblock for the bioreactor and biochambers (Fig.4., c) supporting a set of three biochambers, and equipping the biochambers with ports to the vascular system in the form of standard laboratory luer lock connectors (Fig.4., d). The luer lock connectors ensure compatibility of the chamber with the laboratory infrastructure and allow optimization of the method of delivering the culture medium to the printed model, ensuring adequate cell viability.

**[0048]** Specialized 3D designing software SOLIDWORKS was used to design the biochamber. The biochambers were designed and printed to fit the geometric dimensions of the printed tissue, to print the tissue directly in the biochamber base, and to enable microscopic observations during biological experiments by equipping the upper and lower bases of the biochamber with laboratory glass walls, as shown in Fig.5.a)-b). In order to enable stable printing of liver tissue directly in the base of the biochamber, a support 3 was developed to prevent the chamber from displacing during tissue bioprinting (Fig.5. point c). To enable microscopic observations during biological experiments, a holder was prepared to stabilize the biochamber on the microscope table (Fig.5. pt. d).

### Claims

1. A biochamber comprising a flow system, characterized in that it comprises two modules:
  - a) an inner module comprising a base (1) and a cover (4) of the biochamber, equipped with, respectively - a first window (2) and a second window (5); a seal (6) and two luer lock connectors (10);
  - b) an external module comprising a first frame (7), a second frame (8) and fasteners (9).
2. The biochamber according to claim 1, characterized in that the fasteners (9) in the external module are screws.
3. The biochamber according to claims 1 and 2, characterized in that it additionally comprises a calibrator.
4. The biochamber according to claims 1-3, characterized in that the luer lock connectors (10) are made of polycarbonate.
5. The biochamber according to claim 1-4, characterized in that its dimensions are from 5x15x1 mm to 25x70x10 mm.
6. The biochamber according to claim 1-5, characterized in that its dimensions are 12x40x3 mm and its volume is 1.5 ml.
7. The biochamber according to claim 1-6, characterized in that it is made of biocompatible materials meeting ISO 10993 standard.
8. The biochamber according to claim 1-7, characterized in that it is made using 3D printing technology from a biocompatible light-curing resin.
9. The biochamber according to claim 8, characterized in that it is a biocompatible light-curing resin is Formlabs Bimed Clear.
10. A system for stable printing of liver tissue, characterized in that the biochamber according to claim 1-9 is placed on a support (3).
11. Use of the biochamber according to claim 1-9 for bioprinting and culturing tissue models.