

Cell Culturing Device

Field of disclosure

The present disclosure lies in the field of cell and organoid culturing and relates to a cell culturing device, a cell accommodation unit and a method for cultivating cellular structures
5 in a culturing medium as well as a cellular structure having been obtained by such a method.

Background, prior art

Current protocols for spheroid/organoid culture make use of commercial well-plates and/or bioreactors. In many cases, an initial step requires well-plates containing a U or V-shaped bottom that promotes the sedimentation of cells in close proximity. The low-bind properties
10 of the bottom surfaces favor cell-cell adhesions. For example if stem-cells are employed, the cell-cell adhesion results in cell spheroids, referred to as embryoid body (EB). In situ addition of differentiation factors results in this EB differentiating into different organoid types, e.g. brain, retina, kidney or gastruloid. Continuous culture of this initial organoid results in its growth, but since nutrient and oxygen supply to the organoid depends on
15 diffusion, after a certain time point organoid growth halts, hindering proper development of the organoid and often forming necrotic cores at its center.

To solve this problem, protocols typically include transferring the organoids after a certain initial growth time to larger containers in dynamic conditions, such as spinner flasks or petri dishes placed on orbital shakers. The movement of the liquid in these vessels enhances
20 the liquid exchange, so that zones of depleted oxygen and nutrients around the organoid are not allowed to form. This improves the diffusion of these elements inside the organoid, allowing longer growth and viability. While such protocols are suitable to provide organoid and promote their growth, they present a series of problems.

For example, organoids need to be transferred to a different container from the original plate
25 used for organoid formation. This step is notoriously difficult to automate and can further

result in organoid damage/stress. Additionally, organoids floating in moving media are regularly in contact with each other, resulting in potential fusion, damage and uncontrolled inter-organoid cross-talk. Furthermore, shear stress conditions in a shaken solution or a bioreactor are not uniform, resulting in organoids seeing variable shear stress depending on time and position in the vessel. This contributes to non-reproducible organoid development and uneven growth. Besides this, culturing cells or organoids in shaken containers and bioreactors typically requires large media volumes and space. This limits upscaling and results in expensive media consumption. Lastly, for further testing, organoids often need to be transferred back to well plates or petri-dishes, e.g. for individual drug testing or for preparation for further readout (imaging, single-cell RNA sequencing). This is also a laborious manual process prone to organoid damage and loss

Summary of disclosure

It is the general object of the present disclosure to advance the state of the art in the field of cell and organoid culturing and preferably to overcome one or more of the disadvantages of the prior art fully or at least partially. In advantageous embodiments, the one or more of the above mentioned problems is avoided or at least decreased.

The general object is achieved by the subject-matter of the independent claims. Further advantageous embodiments follow from the dependent claims and the overall disclosure.

In a first aspect, the general object is achieved by a cell culturing device for culturing cells and/or organoids. The cell culturing device comprises a cell accommodation unit and a first pressure generator in particular a pump. The cell accommodation unit may comprise at least one cell culturing chamber, a pressure chamber and at least one channel which connects the at least one cell culturing chamber and the pressure chamber with each other. The at least one cell culturing chamber may be configured for accommodating a liquid cell culturing medium and a cellular structure. Further, the at least one cell culturing chamber may comprise a venting opening which is configured for venting the cell culturing chamber, i.e. to release pressure, in particular an overpressure, from the cell culturing chamber. The

pressure chamber may comprise a pressure chamber inlet, e.g. an inlet which allows to introduce a medium into the pressure chamber. The at least one cell culturing chamber, the pressure chamber and the at least one channel may further be configured such that the pressure pulse from the first pressure generator, in particular the pump, propagates from the pressure chamber through the at least one channel to the at least one cell culturing chamber and in particular into the liquid cell culturing medium. In other words, it may be the case that the at least one cell culturing chamber, the pressure chamber and the at least one channel may be configured such that the pressure pulse generated by the first pressure generator displaces the medium in the pressure chamber (e.g. the liquid cell culturing medium and/or gas) towards and through the at least one channel and into the cell culturing chamber.

In a second aspect, the general object is achieved by a cell accommodation unit. The cell accommodation unit may comprise at least one cell culturing chamber which is configured for accommodating a liquid cell culturing medium and a cellular structure. Furthermore, the cell accommodation unit may comprise a pressure chamber which comprising a pressure chamber inlet. The cell accommodation unit may further comprise at least one channel which connects the at least one cell culturing chamber and the pressure chamber with each other. The at least one cell culturing chamber may further comprise a venting opening which is configured for venting the cell culturing chamber, i.e. to release pressure, in particular an overpressure, from the cell culturing chamber. The at least one cell culturing chamber, the pressure chamber and the at least one channel may further be configured such that a pressure pulse provided into the pressure chamber, propagates from the pressure chamber through the at least one channel to the at least one cell culturing chamber and in particular into the liquid cell culturing medium. In other words, it may be the case that the at least one cell culturing chamber, the pressure chamber and the at least one channel may be configured such that a pressure pulse provided into the pressure chamber displaces the medium in the pressure chamber (e.g. the liquid cell culturing medium and/or gas) towards and through the at least one channel and into the cell culturing chamber.

One advantage of the disclosure is therefore that when a liquid cell culturing medium is present in the cell culturing chamber, then one or more pressure pulses displace the medium in the pressure chamber towards the channel and further displaces the medium in the channel towards the cell culturing chamber on its way to the cell culturing chamber.

- 5 Thereby, a movement of the liquid cell culturing medium and optionally also of the growing cellular structure, such as the organoid, within the cell culturing chamber is caused. This movement is not caused by a shaker or a mechanical stirrer and therefore, shear forces are essentially avoided or at least significantly reduced. Thus, the movement is effected in a very mild manner and the growing cellular structures are efficiently supplied with nutrients.
- 10 For example, the delivery of oxygen to the cellular structure is diffusion limited. The disclosure at hand allows to efficiently supply the cellular structures with sufficient oxygen, but also prevents damages due to high shear forces. Additionally, the disclosure allows to culture cellular structures with very little space and liquid medium demand. In general, the cell accommodation unit is not moved by itself to cause movement of the liquid cell culturing
- 15 medium, but this is only achieved by the pressure pulse provided.

- As used herein, a "pulse" represents a single disturbance which propagates and/or moves through a transmission medium, such as a gas or the liquid cell culturing medium. Such a pulse is therefore typically not a continuous application of a pressure. While the pulse is a single disturbance which is rapidly applied and released and thus represents a pressure
- 20 spike followed by return to the original (unforced) state, continuous pressure application is steady and sustained. A pulse may typically be a localized disturbance and thus not a steady and sustained flow of medium. For example, a pressure pulse may cause a propagating wave, while the continuous pressure application results in a steady-state flow. A propagating pressure pulse which propagates through a medium from a first position to a
- 25 different second position through a medium displaces the medium towards the second position while propagating.

As used herein, the term "cellular structure" may refer to any cellular element, such as cells, in particular stem-cells, an assembly of cells, organoids, or only parts of cells.

A "channel" as used herein refers to a tubular structure which is delimited by a channel wall and which has two opposing openings. These openings allow access to the channel. Further, a channel has a certain length, i.e. extension. Furthermore, in the cross-section, i.e. the cross-section along its direction of extension, namely the longitudinal direction, the channel wall preferably circumferentially completely surrounds and delimits the channel.

It is generally understood herein that the term "comprising" is interpreted as meaning that it includes those features following this term, but that it does not exclude the presence of other features, as long as they do not render the claim unworkable. On the other hand, if the wording "consist of" is used, then no further features are present in the corresponding apart from the ones following said wording.

It is further understood that if the cell accommodation unit comprises more than one cell culturing chambers each cell culturing chamber is connected by at least one channel to the pressure chamber. Thus, the number of channels between the cell culturing chambers and the pressure chamber(s) is typically at least equal or equal to the number of cell culturing chambers.

For example, it may well be possible that the cell accommodation unit comprises a plurality of cell culturing chambers and more than one, or even all, of these cell culturing chambers may be connected to one pressure chamber by a channel connected the corresponding cell culturing chamber with the pressure chamber. Thus, each of these cell culturing chambers is then individually connected by a channel, in particular only by a single channel, to the pressure chamber.

Vice versa, it may additionally or alternatively also be possible that the cell accommodation unit comprises a plurality of such pressure chambers and that more than one of these pressure chambers are each connected by a channel, in particular only by a single channel, to one cell culturing chamber.

In some embodiments, there may be only one channel between each pressure chamber and cell culturing chamber of the cell culturing device. That is, there may be only one channel between each pressure chamber and cell culturing chamber pair of the cell culturing device.

- 5 The channel connecting the at least one cell culturing chamber and the pressure chamber with each other may in some embodiments fluidically connect the at least one cell culturing chamber and the pressure chamber. However, it may in some embodiments as described herein be possible that a fluid blocking structure is arranged within the channel.

- 10 The pressure chamber typically comprises the pressure channel inlet and additionally a pressure chamber outlet which opens, particularly directly opens, into the channel connecting the at least cell culturing chamber and the pressure chamber. Otherwise however, the pressure chamber is closed, in particular hermetically sealed. Therefore, the pressure chamber may except for the pressure chamber inlet and the pressure chamber outlet(s) not comprise additional inlets and outlets.

- 15 In some embodiments, the cell culturing device is devoid of channels which are along a vertical direction arranged above one another. As understood by the skilled person, the vertical direction extends from the bottom section of the pressure chamber, respectively of the cell culturing chamber, towards the pressure chamber inlet, respectively the venting opening (see z-direction in Fig. 1c of the appended figures). The vertical direction may be perpendicular to a plane defining the venting opening of the cell culturing chamber. In
20 embodiments with more than one of such channels, the channels are therefore vertically not arranged above one another in the vertical direction but are offset to each other. In some embodiments with more than one of such channels, the channels may be arranged adjacent to one another, in particular they may be arranged in a single horizontal plane. The
25 horizontal plane may be perpendicular to the vertical direction (see x-y plane in Fig. 1c).

The first pressure generator may for example be a pump, such as a dynamic pump, peristaltic pump, a piston pump or the like. Examples of dynamic pumps may be a centrifugal pump, an axial pump, a peripheral pump.

The liquid cell culturing medium may typically comprise water and nutrients, such as sugar and oxygen. Thus, the liquid cell culturing medium is typically aqueous.

In some embodiments, the at least one cell culturing chamber, the pressure chamber and the channel are configured such that the pressure pulse from the first pressure generator propagates from the pressure chamber through the channel to the at least one cell culturing chamber and in particular into the liquid cell culturing medium and thereby transports liquid medium, in particular liquid cell culturing medium, from the pressure chamber through the channel into the cell culturing chamber.

In some embodiments, the at least one cell culturing chamber comprises a cell culturing chamber bottom section. The cell culturing chamber bottom section refers to the surface upon which the liquid cell culturing medium is arranged. Typically, the cell culturing chamber may further comprise a cell culturing chamber wall and/or a cell culturing chamber top section. The cell culturing chamber wall may be adjacently arranged to the cell culturing chamber bottom section and may form the lateral delimitation of the cell culturing chamber. In particular embodiments, the cell culturing chamber may comprise a cell culturing chamber inlet which opens, in particular directly opens, into the channel connecting the cell culturing chamber with the pressure chamber. In certain embodiments, the cell culturing chamber inlet may be comprised in, respectively defined by, the cell culturing chamber wall. It may be possible that an edge is formed by the cell culturing chamber wall and the cell culturing chamber bottom section. In some embodiments in which the cell culturing chamber comprises a cell culturing chamber top section, the venting opening may be comprised in, respectively defined by, the cell culturing chamber top section. The cell culturing chamber top section may typically be oppositely arranged and spaced apart from the cell culturing chamber bottom section. The cell culturing chamber wall may be arranged between the cell culturing chamber bottom section and the cell culturing chamber top section.

The cell culturing chamber bottom section may in some embodiments be flat, e.g. planar. However, it may also be possible that the cell culturing bottom chamber forms a concavity, i.e. a concavity when seen from the cell culturing chamber. Thus, in such embodiments, the cell culturing chamber bottom section as a concave shape. For example, the cell culturing
5 bottom chamber may have a U-shaped or V-shaped or bell-curve shaped cross-section. It may also be possible that the cell culturing bottom section may be dome shaped, cone shaped or have the shape of truncated cone or truncated dome or of a partial sphere, such as a half sphere.

In some embodiments, the cell culturing chamber bottom section and/or the cell culturing
10 chamber wall may comprise, be coated with, or consist of, a hydrophilic material, such as a hydrophilic hydrogel. Such a hydrophilic material acts as ultra-low attachment surface which prevents binding of the cellular structure, in particular organoids.

In some embodiments, the pressure chamber comprises a pressure chamber bottom section. The pressure chamber bottom section refers to the surface upon which a pressure
15 chamber medium, in particular the liquid cell culturing medium, is arranged. Typically, the pressure chamber may further comprise a pressure chamber wall and/or a pressure chamber top section. The pressure chamber wall may be adjacently arranged to the pressure chamber bottom section and may form the lateral delimitation of the pressure chamber. In particular embodiments, the pressure chamber may comprise a pressure
20 chamber outlet which opens, in particular directly opens, into the channel connecting the pressure chamber with the cell culturing chamber. In certain embodiments, the pressure chamber outlet may be comprised in, respectively defined by the pressure chamber wall. It may be possible that an edge is formed by the pressure chamber wall and the pressure chamber bottom section. In some embodiments in which the pressure chamber comprises
25 a pressure chamber top section, the pressure chamber inlet may be comprised in, respectively defined by, the pressure chamber top section. The pressure chamber top section may typically be oppositely arranged and spaced apart from the pressure chamber bottom section. The pressure chamber wall may be arranged between the pressure chamber bottom section and the pressure chamber top section.

In some embodiments, the pressure chamber bottom section is flat, e.g. planar.

In some embodiments, the cell accommodation unit comprises, or consists of, two parts, a top section and a bottom section being arranged on the top section. Thus, the cell accommodation unit may not be made from a single piece in such embodiments. In certain
5 embodiments, the top section defines, respectively delimits, the pressure chamber inlet. Furthermore, the top section and the bottom section may in some embodiments together define, respectively delimit, the at least one cell culturing chamber, the pressure chamber and the channel connecting the at least one cell culturing chamber and the pressure chamber. For example, it may be possible that the cell culturing chamber bottom section
10 may be defined by the bottom section of the cell accommodation unit. The bottom section of the cell accommodation unit may for example be a plate.

In some embodiments, the cell accommodation unit may be made from glass, a polymer material or a metal.

In some embodiments, the cell accommodation unit further comprises a lid which covers
15 the at least one cell culturing chamber and the pressure chamber. Such a lid is typically removable. The lid protects the cell culturing chamber and the pressure chamber from contamination. The lid is typically a continuously closed lid except for openings provided for venting the cell culture chamber and connecting the first pressure generator.

In some embodiments, the channel connecting the at least one cell culturing chamber and
20 the pressure chamber and/or the pressure chamber outlet is arranged closer to the pressure chamber bottom section than both the pressure chamber inlet and the venting opening. Thus, the channel connecting the at least one cell culturing chamber and the pressure chamber and/or the pressure chamber outlet is in particular along a vertical direction arranged closer to the pressure chamber bottom section than both the pressure chamber
25 inlet and the venting opening. In certain embodiments, the channel connecting the at least one cell culturing chamber and the pressure chamber and/or the pressure chamber outlet is arranged in flush, respectively on the same height, than the pressure chamber bottom

section. If the pressure chamber is at least partially filled with a liquid medium, in particular the liquid cell culturing medium, then it is ensured that the pressure pulse propagates in a controlled manner through the medium through the channel and that the pulse is accurately provided into the cell culturing chamber.

- 5 In some embodiments, the first pressure generator, respectively the pump, is configured for temporarily pressurizing the pressure chamber with a pulse by providing a gas pulse into the pressure chamber and in particular into the medium being arranged within the pressure chamber. Gas pulses can be very accurately defined in their intensity and duration and therefore are ideally suited to ultimately move the growing cellular structures inside the cell
10 culturing chamber in a controlled and constant manner.

- In some embodiments, the first pressure generator is configured to pressurize the pressure chamber with a pressure pulse of 0.5 to 5000 mbar, in particular 5 to 5000 mbar, more particular 20 to 2000 mbar, more particular 50 to 1000 mbar, more particular 100 to 500 mbar. It is understood that these pressure values are overpressure values with respect to
15 the pressure inside the pressure chamber or the environmental pressure. Thus, for example a pressure pulse of 0.5 mbar is a pulse which has an overpressure of 0.5 mbar with respect to the pressure in the pressure chamber before the pulse is provided or to the environmental pressure.

- In some embodiments, the first pressure generator is configured to pressurize the pressure
20 chamber such with a pressure pulse that the pressure pulse propagating into the at least one cell culturing chamber lifts a cellular structure of diameter of up to 10 mm from the cell culturing chamber bottom section. In particular embodiments, the cellular structure being lifted by the pressure pulse has a mass of up to 1 mg, in particular up to 0.5 mg, more particular of up to 1 mg, more particular of up to 10 mg, more particular of up to 50 mg,
25 more particular of up to 100 mg.

In some embodiments, the first pressure generator is configured to pressurize the pressure chamber in a pulsating mode. In such a pulsating mode, a plurality of sequential and

particularly also successive, pressure pulses is provided to the pressure chamber. During such a pulsating mode, the pressure is not increased over time, i.e. over a time interval > 10 ms, in particular > 5 ms, more particular > 1ms, but the pressure of the pressure pulse is provided instantaneously, e.g. < 10ms, in particular < 5 ms, more particular < 1ms. A
5 pulsating mode is highly advantageous, because this results in the ability to even lift larger organoids growing inside the cell culturing chamber. As an example organoids of a maximum diameter of 10 mm could readily be lifted and moved in the 3D space. Thus, such a device has the advantage that not only the cell culturing medium is moved, but also the growing organoids can be lifted and moved in the 3D space inside the cell culturing medium.
10 This is highly beneficial for the growth of the organoids as they are sufficiently supplied with nutrients and shear stress is largely or fully avoided. Thus, the pulsating mode allows to move the growing organoids in the 3D space within the cell culturing medium in a highly controlled and mild manner.

In certain embodiments, the first pressure generator, in particular the pump, is configured
15 such that every pulse of the plurality of sequential pulses is followed by a relaxation interval Δt^R in which no pressure pulse is provided. The relaxation interval may in some embodiments be selected such that the medium in the pressure chamber, the channel and the cell culturing chamber, e.g. the cell culturing medium, equilibrates. This means that for example if a medium has been displaced from the pressure chamber to the channel and/or
20 the cell culturing chamber due to the pulse causing a disequilibrium the relaxation interval is selected such that the amount of medium in the pressure chamber is restored to the status before the pulse. This not only allows to provide a controlled movement of the medium and the cellular structures, but also avoids accumulation of liquid in the cell culturing chamber and eventually an overflow. In certain embodiments, the relaxation
25 interval may for example be between 1 s and 2 min, in particular between 10 s and 1 min, more particular between 20 s and 40 s. In embodiments with a control unit (see below), the relaxation interval may be controlled and/or stored in the control unit.

In some embodiments, the first pressure generator may be configured to pressurize the pressure chamber with a pressure pulse having a pulse duration Δt^P of 5 to 500 ms, in particular 20 to 300 ms, more particular 50 to 200 ms, more particular 50 to 100 ms.

The relaxation interval Δt^R , in the pulsating mode is typically longer than the pulse duration Δt^P in particular at least 2x longer, more particular 10x longer, more particular 100x longer, even more particular 1000x longer.

In some embodiments, the first pressure generator may be configured to perform a varying pressure program.

In some embodiments, the varying pressure program may for example be an anti-sedimentation pulse program. The anti-sedimentation pulse program may for example consist of a standard pulse (e.g. a pulse as described in the embodiments above, which may have a pressure of 0.5 to 5000 mbar, in particular 5 to 5000 mbar, more particular 20 to 2000 mbar, more particular 50 to 1000 mbar, more particular 100 to 500 mbar, and/or a duration Δt^P of 5 to 500 ms, in particular 20 to 300 ms, more particular 50 to 200 ms, more particular 50 to 100 ms) directly followed by a pulse with a lower pressure than the standard pulse (such a pulse with a lower pressure is referred herein as a "gentle pulse"). For example, the pulse with a lower pressure may have a pressure being at most 10%, more particular at most 5%, more particular at most 2%, even more particular at most 1%, of the pressure of the standard pulse. In some embodiments, the pulse with a lower pressure (i.e. the gentle pulse) may have a pressure of 0.5 to 10 mbar, in particular 1 to 5 mbar, more particular 1 to 3 mbar. In some embodiments, the duration Δt^P of the pulse with a lower pressure may be longer than the duration Δt^P of the standard pulse. For example, the duration of the standard pulse may be at most 15%, more particular at most 10%, even more particular at most 5%, of the duration of the pulse with the lower pressure (i.e. the gentle pulse). For example, the duration of the pulse with the lower pressure may be 0.5 to 10 s, in particular 0.5 to 5 s, more particular 1 to 5 s. It is understood that the anti-sedimentation pulse program may comprise a plurality of consecutive cycles, each cycle consisting of a standard pulse followed by a gentle pulse. In embodiments in which such a

gentle pulse follows a standard pulse, the sedimentation of the organoid is slowed down and thus, the organoid is maintained longer in suspension. In some embodiments, the varying pressure program may be stored in the control unit as described with respect to some embodiments herein.

- 5 In some embodiments, the varying pressure program may consist of a standard pulse, (e.g. a pulse as described in the embodiments above, which may have a pressure of 0.5 to 5000 mbar, in particular 5 to 5000 mbar, more particular 20 to 2000 mbar, more particular 50 to 1000 mbar, more particular 100 to 500 mbar, and/or a duration Δt^P of 5 to 500 ms, in particular 20 to 300 ms, more particular 50 to 200 ms, more particular 50 to 100 ms)
- 10 directly followed by a pressure maintain period. The pressure maintaining period may be a period in which a reduced pressure as compared to the pressure of the standard pulse is continuously applied. For example, the pressure applied in the pressure maintaining period may be a pressure being at most 10%, more particular at most 5%, more particular at most 2%, even more particular at most 1%, of the pressure of the standard pulse. For example,
- 15 the pressure applied in the pressure maintaining period may be 0.1 to 1 mbar, in particular 0.5 to 1.0 mbar. In some embodiments, the duration of the pressure maintaining period (i.e. the duration of applying the continuous pressure) may be longer than the duration Δt^P of the standard pulse. For example, the duration of the standard pulse may be at most 15%, more particular at most 10%, even more particular at most 5%, of the duration of the pressure
- 20 maintaining period. For example, the duration of the pressure maintaining period may be 0.5 to 10 s, in particular 0.5 to 5 s, more particular 1 to 5 s. A standard pulse being followed by such a pressure maintaining period can be beneficial to prevent that liquid is immediately moving back into the pressure chamber, which may cause small organoids to be sucked into the channel. In some embodiments, this varying pressure program may be stored in
- 25 the control unit as described with respect to some embodiments herein.

In some embodiments, the first pressure generator may be configured to pressurize the pressure chamber in a quasi-steady mode. In such a quasi-steady mode, the first pressure generator continuously, in particular linearly, increases and decreases the pressure in the pressure chamber to pressurize the pressure chamber with one or more pressure pulses.

Thus, in such a quasi-steady mode, the pressure is repeatedly changed between a pressure maximum and a pressure minimum. Thus, the pressure maximum is not instantaneously reached, but over a certain time interval. For example, the time to reach the pressure maximum from the pressure minimum may be $> 1\text{ms}$, in particular $> 5\text{ms}$, more particular $> 10\text{ms}$, even more particular $> 100\text{ms}$, even more particular $> 500\text{ms}$. In some embodiments, the rate with which the pressure changes in the quasi-steady state may generally be 0.001 to 2mbar/s .

In certain embodiments, the maximum pressure of such a quasi-steady mode is 3mbar or less, in particular 2mbar or less, which helps to avoid overflow of the cell culturing chamber. Again, it is understood that these pressure values are overpressure values with respect to the pressure inside the pressure chamber or the environmental pressure. The pressure difference between the maximum pressure and the minimum pressure in the quasi-steady state may in some embodiments be up to 10^4mbar , in particular up to 10^3mbar . The pressure is generally selected such that movement of the liquid cellular culturing medium in the cell culturing chamber is caused.

In some embodiments, the at least one channel, or each of the channels, is configured such that when the liquid cell culturing medium is filled at least to a certain filling level into the cell culturing chamber, the liquid cell culturing medium is prevented from flowing into the pressure chamber, in particular is such that when the liquid cell culturing medium is filled into the cell culturing chamber, the liquid cell culturing medium is prevented from flowing into the pressure chamber by itself. Thereby, a faster equilibrium in the cell culturing chamber may be achieved and/or contamination may be avoided.

In certain embodiments it may be possible that a fluid blocking structure is arranged within the channel or comprised in the channel. Such a fluid blocking structure may be configured to prevent a fluid, in particular a liquid from flowing through the channel at least in one configuration. However, it is understood that the fluid blocking structure is configured to transmit a pressure pulse from the pressure chamber via the channel into the cell culturing chamber.

In some embodiments, the fluid blocking structure may be a valve which is transformable between an open position in which fluid, in particular liquid, can flow through the channel and a closed position in which fluid, in particular liquid, is prevented from flowing through the channel.

- 5 In some embodiments the fluid blocking structure may be a membrane which is impermeable for fluids, in particular liquids, such as the liquid cell culturing medium, wherein the membrane is configured such that the pressure pulse from the first pressure generator propagates from the pressure chamber through the channel and the membrane to the cell culturing chamber. The term "impermeable" means that during operation of the device, i.e.
- 10 for example at a pressure of between 0.5 to 5000 mbar, no fluid, in particular no liquid, flows through the membrane.

It may in some embodiments also be possible that the channel comprises a channel wall, which is preferably partially or fully made of a hydrophobic material. It may in some embodiments additionally or alternatively be possible that the pressure chamber wall and/or

15 the pressure chamber bottom section may comprise or be made of a hydrophobic material. Such a material further prevents the flow of hydrophilic, and thus aqueous, liquids from flowing into and/or through the channel and into the pressure chamber. In embodiments in which the channel wall is only partially made of a hydrophilic material, it is preferred that at least the channel bottom wall section and optionally also the channel side wall sections, is

20 fully made from the hydrophobic material. During filling of the at least one cell culturing chamber, the hydrophobic material at the channel bottom wall section prevents creeping of the liquid (and usually aqueous) cell culturing medium into the pressure chamber. It is understood that the channel bottom wall section refers to the channel wall section forming the bottom delimitation of the channel and being therefore along the vertical direction

25 closest arranged to the pressure chamber bottom section. If the channel has a rounded cross section, it may in cross-section perpendicular to the channel extension direction refer to the bottom half of the channel. As used herein, a hydrophobic material has typically a water/surface contact angle of greater than 90°.

In some embodiments in which the pressure chamber comprises a pressure chamber outlet which opens, in particular directly opens, into the at least one channel, the pressure chamber outlet may be arranged offset, in particular above and therefore vertically offset, to the pressure chamber bottom section. Such embodiments are advantageous, because

5 when liquid, such as the liquid cell culturing medium, is filled into the one or more cell culturing chambers, such embodiments prevent due to the vertical offset of the pressure chamber outlet that liquid flows from the one or more cell culturing chambers through the channel into the pressure chamber. This may be further improved by applying a pressure into the pressure chamber during filling the liquid into the cell culturing chamber. If the

10 device is dry (i.e. before filling), the offset and capillary forces within the channel prevent the flow of liquid into the pressure chamber. This is particularly advantageous for embodiments in which the cell culturing device comprises a plurality of cell culturing chambers and a plurality of channels, wherein more than one cell culturing chamber is each connected by a channel with the pressure chamber. Thus, during filling, the contents of the

15 individual cell culturing chambers can be kept separated. It is understood that in such embodiments, the pressure chamber may then have multiple pressure chamber outlets which each are offset to the pressure chamber bottom section as described above and which each open into a channel connecting the pressure chamber with one of the cell culturing chambers. Furthermore, even after filling of both the pressure chamber and the

20 cell culturing chambers the contents of the individual cell culturing chambers can be separated again by removing liquid from the pressure chamber. It is understood that separation means in this context that there is no liquid bridge between the contents of different cell culturing chambers.

In certain embodiments, the channel, or each channel, forms together with the pressure

25 chamber, in particular the pressure chamber wall, at the pressure chamber outlet an edge having an inner angle equal to or smaller than 130° , in particular equal to or smaller than 90° . The inner angle refers in particular to the angle between the channel and the pressure chamber wall section being arranged between the pressure chamber outlet and the pressure chamber bottom section. The term "inner" means that the angle does particularly

30 not extend through, respectively does not include, the pressure chamber. Such angles are

advantageous, as they prevent that the liquid cell culturing medium being present in the cell culturing chamber and the channel flows into the yet empty pressure chamber (i.e. the pressure chamber being not yet filled with a liquid medium).

In some embodiments, the pressure chamber outlet, respectively the pressure chamber
5 outlets, is spaced apart from the pressure chamber bottom section by at least 10 micrometers. Such a distance is sufficient to achieve the prevention of liquid flowing into the pressure chamber.

In some embodiments, the pressure chamber outlet, respectively the pressure chamber
outlets, is spaced apart from the pressure chamber bottom section by at most 1 cm, in
10 particular at most 0.1 cm, more particular at most 10 mm, more particular at most 1 mm, more particular at most 500 micrometers, even more particular at most 100 micrometers.

In some the pressure chamber outlet, respectively the pressure chamber outlets, is spaced
apart from the pressure chamber bottom section by a distance of 10 micrometers to 1 cm,
in particular 10 micrometers to 10 mm, more particular 10 micrometers to 1 mm, more
15 particular 10 micrometers to 500 micrometers, more particular 50 micrometers to 500 micrometers, more particular 100 micrometers to 500 micrometers.

In certain embodiments, the pressure chamber wall section between the pressure chamber
outlet and the pressure chamber bottom section extends perpendicularly to the pressure
chamber bottom section. In some embodiments, the pressure chamber wall section
20 between the pressure chamber outlet and the pressure chamber bottom section extends
such that it forms an inclination, in particular a linear or curved inclination, towards the
pressure chamber bottom section. The inclination may in some embodiments form together
with the pressure chamber bottom section a bell-curve shape. The inclination may form an
outer angle between 90° and 180°, in particular 120° and 180°, more particular 130° and
25 175°, with the pressure chamber bottom section. The term "outer angle" means that the
angle extends through the pressure chamber. Preferably, the channel wall and/or the
pressure chamber wall section forming the inclination and/or the pressure chamber bottom

section is made from a hydrophobic material. Thereby, creeping of the liquid (and usually aqueous) cell culturing medium into the pressure chamber during filling of the cell culturing chamber can be prevented. Further, separation of multiple cell culturing chambers of the same cell culturing device being connected to the same pressure chamber can be achieved
5 by removing liquid, in particular liquid cell culturing chamber from the pressure chamber even after filling of the pressure chamber and the cell culturing chambers. Thereby, any liquid bridges between the cell culturing chambers are removed.

In some embodiments, the pressure chamber and the at least one cell culturing chamber are adjacently arranged, i.e. next to each other. The channel may extend essentially
10 transversely, in particular in an angle of larger than 75° , in particular of 75° to 105° , more particular of 80° to 100° , more particular of 85° to 95° , to the pressure chamber (respectively the pressure chamber wall) and/or the cell culturing chamber (respectively the cell culturing chamber wall). In some embodiments, the pressure chamber inlet and the venting opening of the cell culturing chamber are both arranged in a horizontal plane and the axes
15 perpendicular to this horizontal plane and extending through the center of the venting opening, respectively the pressure chamber inlet, are essentially parallel to each other. Furthermore, the channel may preferably be arranged in an angle of larger than 75° , in particular of 75° to 105° , more particular of 80° to 100° , more particular of 85° to 95° , to these axes. In certain embodiments, the pressure chamber, the channel and the cell
20 culturing chamber form together essentially in cross-section a U-shape.

In some embodiments, the at least one channel has a maximum channel height of 10 micrometers to 5 mm, in particular 10 micrometers to 500 micrometers, more particular of 10 micrometers to 200 micrometers. The channel height refers to the direction being in the operative state aligned with the gravitational force vector, respectively being perpendicular
25 to the pressure chamber bottom section. The term "maximum" means that it refers to the maximum distance between opposing channel wall sections in this direction. Thus, if the channel is for example circular in cross-section, the height extends along an axis being perpendicular to the pressure chamber bottom section and extending through the center of the circular cross section of the channel. Such channel heights prevent growing organoids

from being transported into the pressure chamber and further provide the desired capillary forces.

In some embodiments, the cell culturing device comprises a control unit. In certain embodiments, the control unit may be configured to control the first pressure generator and
5 in particular the pressure pulses generated by the first pressure generator. In some embodiments, the control unit may comprise different pressure programs. A pressure program may for example include pressure pulse parameters, such as pulse pressure, mode (e.g. quasi-steady or pulsating mode), pulse duration and relaxation interval.

In some embodiments, the cell culturing device may comprise a monitoring unit, e.g. one or
10 more sensors, which monitors the status of the liquid cell culturing medium, for example its movement, equilibrium, filling level or composition. Preferably, the monitoring unit is configured to transmit the monitored status to the control unit. In certain examples the control unit may be configured to amend the operating mode of the first pressure generator based on the received monitored status. For example, it may be possible that the control
15 unit may extend the relaxation interval or decrease or increase the pulse pressure based on the status received from the monitoring unit.

In some embodiments, the control unit may comprise a memory unit for storing data, such as different pressure programs and/or the status received from the monitoring unit.

In some embodiments, the cell culturing device further comprises a medium replacement
20 unit. Such a medium replacement unit allows to replace the medium, for example the cell culturing medium. The medium replacement unit may for example comprise a pump assembly. The medium replacement unit may for example a medium reservoir in which the medium to be filled into the cell accommodation unit, in particular the cell culturing chamber, can be stored. The medium reservoir may for example comprise a waste disposal tube for
25 disposing medium, e.g. liquid cell culturing medium. The waste disposal tube may preferably be connected to a waste reservoir for collecting the disposed medium.

The medium replacement unit may in some embodiments comprise a 3-way valve which is connected to the waste disposal tube, the medium reservoir and the cell culturing chamber or the pressure chamber.

The medium replacement unit may in some embodiments be controlled by the control unit.

- 5 It may also be possible that the pump assembly may comprise a pump assembly control unit. Such a pump assembly control unit may be separate from the control unit described herein above with regard to the cell culturing device as such or it may be a part of it, respectively integrated into it.

- The control unit, respectively the pump assembly control unit, may for example control the
- 10 3-way valve and the pump assembly and thus trigger replacement the medium, e.g. the cell culturing medium. The control unit, respectively the pump assembly control unit, may for example comprise different medium replacement programs which include predetermined or fixed medium replacement intervals after which the medium, e.g. the liquid cell culturing medium, is replaced. It may also be possible that the monitoring unit is configured to monitor
- 15 the status of the medium, e.g. the liquid cell culturing medium. For example, the monitoring unit may be configured to monitor the compositions, such as the nutrient content or ionic strength, or physical properties, such as the refractive index of the medium, e.g. the liquid cell culturing medium. This status may then be transmitted to the control unit, respectively the pump assembly control unit. If the control unit, respectively the pump assembly control
- 20 unit, detects that the status exceeds or falls below a predetermined threshold (e.g. a nutrient content or a refractive index) then the control unit, respectively the pump assembly control unit, triggers medium replacement. During the medium replacement, the 3-way valve may be operated such that the medium within the cell accommodation unit, in particular the cell culturing chamber, is first disposed via the waste disposal tube. Then the 3-way valve is
- 25 operated such that new medium, in particular liquid cell culturing medium, is being provided from the medium reservoir to the cell accommodation unit, in particular the cell culturing chamber.

In some embodiments, in which the control unit, respectively the pump assembly control unit, is configured to switch the 3-way valve between a delivery connection in which only the medium reservoir is fluidically connected to the cell culturing chamber or the pressure chamber and a disposal connection in which only the waste disposal tube is fluidically
5 connected to the cell culturing chamber or the pressure chamber.

In some embodiments, the cell culturing device, respectively the cell accommodation unit, comprises a plurality of cell culturing chambers, e.g. more than one cell culturing chambers according to any of the embodiments described herein. In certain embodiments, each cell culturing chamber is configured for accommodating a liquid cell culturing medium and a
10 cellular structure and each cell culturing chamber comprises a venting opening, which is configured for venting the cell culturing chamber. Furthermore, at least some of the or each cell culturing chamber(s) of the plurality of cell culturing chambers is/are connected with the pressure chamber by a channel, in particular a separate channel. Such embodiments allow to culture various different cellular structures in parallel with only one pressure chamber.

15 In some embodiments, the cell culturing chambers circumferentially surround the pressure chamber.

In certain embodiments, each cell culturing chamber of the cell culturing chambers being connected to the same pressure chamber by a channel have the same distance to the pressure chamber and/or the channels with which they are connected to the pressure
20 chamber have the same length. Such embodiments ensure similar or even equal conditions within each cell culturing chamber.

In some embodiments, the cell culturing device, respectively the cell accommodation unit, comprises a plurality of pressure chambers, e.g. pressure chambers according to any of the embodiments as described herein.

25 In certain embodiments, each pressure chamber comprises a pressure chamber inlet. In certain embodiments of the cell culturing device, a first pressure generator, in particular a

pump, is connected to the pressure chamber inlet of each pressure chamber and is configured for temporarily pressurizing the pressure chamber with a pressure pulse. It may be possible that each pressure chamber is connected to a separate first pressure generator or that some, but not all, of the pressure chambers are connected to a common first pressure generator or that all of the pressure chambers are connected to the same first pressure generator.

In embodiments with a plurality of pressure chambers, it may be possible that the cell culturing device, respectively the cell accommodation unit, comprises only a single cell culturing chamber being connected to more than one pressure chamber as described in more detail in certain embodiments below, or it may be possible that the cell culturing device, respectively the cell accommodation unit, comprises a plurality of cell culturing chambers. In the latter embodiments, each cell culturing chamber is connected by a channel, in particular a separate channel, to at least one or only one, pressure chamber.

In some embodiments, at least some or all of the pressure chambers are connected by a channel, in particular a separate channel, to the same cell culturing chamber. In certain embodiments, each channel has a channel length and the channel length of all channels is the same. It may also be possible that the pressure chambers circumferentially surround the cell culturing chamber, in particular radially symmetrically.

In some embodiments, the cell culturing device, respectively the cell accommodation unit, comprises a plurality of cell culturing chambers, e.g. cell culturing chambers as described in any of the embodiments herein, wherein each cell culturing chamber is configured for accommodating a liquid cell culturing medium and a cellular structure. Further, each cell culturing chamber may comprise a venting opening that is configured for venting the cell culturing chamber. The cell culturing device, respectively the cell accommodation unit, may in certain embodiments comprise a pressure chamber inlet. In certain embodiments of the cell culturing device, each pressure chamber may be connected to at least one of the cell culturing chambers by a channel.

In some embodiments, the cell accommodation unit is a well plate. In some embodiments, the one or more cell culturing chambers, the one or more chambers and the one or more channels are part of a well plate.

5 In some embodiments of such a well plate, the cell accommodation unit comprises a plurality of cell culturing chambers. The cell culturing chambers may preferably be grouped into rows of cell culturing chambers, wherein each row comprises a portion of the cell culturing chambers of the cell accommodation unit. In particular, each row may comprise cell culturing chambers being arranged one after another.

10 In some embodiments of such a well plate, the rows of cell culturing chambers are separated from each other by a pressure chamber. In particular, a pressure chamber may always be arranged between two rows of cell culturing chambers. In certain embodiments, the pressure chamber may be connected to each cell culturing chamber of each row between which it is arranged by a channel, i.e. a separate channel.

15 In some embodiments, a second pressure generator is arranged within the at least one cell culturing chamber being configured to apply, in particular directly apply, a counter pressure to the at least one cell culturing chamber.

In some embodiments, each channel as a channel length and the channel length of each channel of the cell culturing device, respectively, the cell accommodation unit has the same length.

20 In some embodiments of the cell culturing device, respectively the cell accommodation unit, the channel has a channel length of at least 500 micrometers, in particular at least 1 mm, more particular at least 5 mm.

In some embodiments of the cell culturing device, respectively the cell accommodation unit, the channel has a channel width (being perpendicular to the channel length) of at least 50
25 micrometers, in particular at least 100 micrometers. In some embodiments of the cell

culturing device, respectively the cell accommodation unit, the channel has a channel width (being perpendicular to the channel length) of at most 10 cm, in particular at most 5 cm.

In some embodiments, the cell culturing device, respectively the cell accommodation unit, the channel has a channel height (being perpendicular to the channel length and channel
5 width) of 10 micrometers to 5 mm, in particular of 50 micrometers to 1 mm.

In some embodiments, the at least one cell culturing chamber has a volume of 50 to 1000 microliters, in particular 50 to 1000 microliters, more particular 100 to 500 microliters.

In some embodiments, the cell culturing device comprises a plurality of cell accommodation units as described herein. It may be possible that the pressure chamber inlet(s) of each of
10 the cell accommodation units is connected to the same first pressure generator or to different first pressure generators. In certain embodiments, the plurality of cell accommodation units are stacked on top of each other allowing for medium and/or large scale organoid culturing.

The general object is in a third aspect achieved by a method for cultivating cellular
15 structures, in particular cells and/or organoids, in a liquid cell culturing medium. In some embodiments the method for cultivating cellular structures comprises the step of providing a cellular structure, in particular a cell, such as a stem-cell or a cell assembly, such as a stem-cell assembly, into a cell culturing chamber of a cell culturing device. The cell culturing device may particularly be a cell culturing device as described in any of the embodiments
20 herein. Furthermore, a liquid cell culturing medium is provided in the cell culturing chamber and optionally the channel connecting the at least one cell culturing chamber with the pressure chamber.

In some embodiments, the liquid cell culturing medium may be provided such in the cell culturing chamber and optionally the channel that the liquid cell culturing medium does not
25 flow into the pressure chamber. As described herein above, this may be achieved by providing a channel comprising a channel wall being partially or fully made from a

hydrophobic material and/or by arranging the pressure chamber outlet vertically offset to the pressure chamber bottom section.

The method may further comprise the step of providing a liquid medium into a pressure chamber of the cell culturing device. In particular, the liquid medium may further be provided
5 into a channel connecting the cell culturing chamber and the pressure chamber. The liquid medium provided into the pressure chamber and optionally the channel may in some embodiments also be a liquid cell culturing medium, in particular the same liquid cell culturing medium which is provided into the cell culturing chamber of the cell culturing device. The liquid medium may for example be provided such into the pressure chamber
10 that it is at least partially, or fully filled with the liquid medium.

The method may further comprise the step of temporarily pressurizing the pressure chamber with one or more pressure pulses such that the one or more pressure pulse(s) propagate(s) from the pressure chamber and the liquid medium within the pressure chamber through the channel to the cell culturing chamber into the liquid cell culturing
15 medium, thereby inducing movement of the cellular structure in the cell culturing chamber and/or thereby inducing movement of the liquid cell culturing medium within the cell culturing chamber. In particular, the movement of the liquid cell culturing medium may be a movement relative to the cell culturing chamber and/or relative to the cellular structure in the cell culturing chamber.

20 In some embodiments, the pressure pulse propagating from the pressure chamber through the channel to the at least one cell culturing chamber and in particular into the liquid cell culturing medium transports liquid medium, in particular liquid cell culturing medium, from the pressure chamber through the channel into the cell culturing chamber.

In some embodiments, the one or more pressure pulse(s) with which the pressure chamber
25 is temporarily pressurized has a pressure of 0.5 mbar to 5000 mbar, in particular 20 to 2000 mbar, more particular 50 to 1000 mbar, more particular 100 to 500 mbar. It is understood that these pressure values are overpressure values with respect to the pressure inside the

pressure chamber or the environmental pressure. In some embodiments, the ratio between the pressure of the one or more pressure pulse(s) with which the pressure chamber is temporarily pressurized and the channel length (that is the channel length of the channel connecting the at least one cell culturing chamber and the pressure chamber) is between
5 20 mbar/mm to 1000 mbar/mm, in particular 50 mbar/mm to 500 mbar/mm.

In some embodiments, temporarily pressurizing the pressure chamber may comprise, or consist of, pressurizing the pressure chamber with a plurality of sequential and particularly also successive, pressure pulses. Each of these pressure pulses has a pulse duration. The pulse duration may be the same or different. Such embodiments may be referred to as the
10 pulsating mode, as disclosed herein above with respect to the first embodiment of the disclosure.

In some embodiments of such a pulsating mode, every pulse of the plurality of sequential pressure pulses is followed by a relaxation interval Δt^R in which no pressure pulse is provided.

15 The relaxation interval may in some embodiments be selected such that the medium in the pressure chamber, the channel and the cell culturing chamber, e.g. the cell culturing medium, equilibrates. This means that for example if medium has been displaced from the pressure chamber to the channel and/or the cell culturing chamber due to the pulse causing an disequilibrium the relaxation interval is selected such that the amount of medium in the
20 pressure chamber is restored to the status before the pulse. This not only allows to provide a controlled movement of the medium and the cellular structures, but also avoids accumulation of liquid in the cell culturing chamber and eventually an overflow. In certain embodiments, the relaxation interval may for example be between 1 s and 2 min, in particular between 10 s and 1 min, more particular between 20 s and 40 s. In embodiments
25 with a control unit, the relaxation interval may be controlled and/or stored in the control unit.

In some embodiments, the first pressure generator may pressurize the pressure chamber with a pressure pulse having a pulse duration Δt^P of 5 to 500 ms, in particular 20 to 300 ms, more particular 50 to 200 ms, more particular 50 to 100 ms.

The relaxation interval Δt^R , in the pulsating mode is typically longer than the pulse duration Δt^P in particular at least 2x longer, more particular 10x longer, more particular 100x longer, even more particular 1000x longer.

In some embodiments, temporarily pressurizing the pressure chamber with one or more pressure pulses may comprise, or consist of, continuously, in particular linearly, increasing and decreasing the pressure in the pressure chamber to pressurize the pressure chamber with the pressure pulse. Such embodiments may be referred to as the quasi-steady mode, as disclosed herein above with respect to the first embodiment of the disclosure.

In some embodiments of such a quasi-steady mode, the pressure is repeatedly changed between a pressure maximum and a pressure minimum. Thus, the pressure maximum is not instantaneously reached, but over a certain time interval. For example, the time to reach the pressure maximum from the pressure minimum may be > 1ms, in particular > 5ms, more particular > 10 ms, even more particular > 100 ms., even more particular > 500 ms. In some embodiments, the rate with which the pressure changes in the quasi-steady state may generally be 0.001 to 2 mbar/s.

In certain embodiments, the maximum pressure of such a quasi-steady mode is 3 mbar or less, in particular 2 mbar or less, which helps to avoid overflow of the cell culturing chamber. Again, it is understood that these pressure values are overpressure values with respect to the pressure inside the pressure chamber or the environmental pressure. The pressure difference between the maximum pressure and the minimum pressure in the quasi-steady state may in some embodiments be up to 10^4 mbar, in particular up to 10^3 mbar. The pressure is generally selected such that movement of the liquid cellular culturing medium in the cell culturing chamber is caused.

In some embodiments, the method further comprises the step of exchanging the liquid cell culturing medium, in particular by new, e.g. fresh, liquid cell culturing medium. In some embodiments, the liquid cell culturing medium is exchanged after a certain time interval and/or after a certain number of pressure pulses. In some embodiments, the liquid cell
5 culturing device comprises a medium replacement unit, in particular a medium replacement unit as described in any of the embodiments herein, which performs the step of exchanging the liquid cell culturing medium.

In some embodiments, the method comprises the step of removing the liquid, in particular the liquid cell culturing medium, from the pressure chamber being connected to a plurality
10 of cell culturing chambers by separate channels, whereby the cell culturing medium remains in the cell culturing chambers. Preferably, any liquid bridge between the cell culturing chambers is thereby removed.

In some embodiments, the liquid cell culturing medium is replaced if a status of the cell culturing medium falls below or exceeds a predetermined threshold. In particular, this status
15 may be monitored by a monitoring unit as described in some embodiments herein. In certain embodiments, a monitoring unit continuously or in an interval-manner monitors the status of the cell culturing medium and transmits the monitored status to a control unit, such as a control unit as described herein. The control unit may then compare the monitored status with the predetermined threshold of the status and if it exceeds or falls below the
20 predetermined threshold trigger exchanging the liquid cell culturing medium. As explained above, the status may be one or more properties of the liquid cell culturing medium, such as the nutrient content or ionic strength, or physical properties, such as the refractive index.

In some embodiments, exchanging the liquid cell culturing medium is performed by a medium replacement unit comprising a pump assembly, a medium reservoir, a waste
25 disposal tube being preferably connected to a waste reservoir, and a 3-way valve, wherein the 3-way valve is fluidically connected to the waste disposal tube, the medium reservoir and the cell culturing chamber or the pressure chamber.

In some embodiments, the liquid medium. e.g. the liquid cell culturing medium, is provided such into the pressure chamber that the pressure chamber is only partially filled with the liquid medium and the remainder of the pressure chamber is filled with a gas, in particular air.

- 5 In some embodiments, the liquid cell culturing medium is provided such into the one or more cell culturing chamber that the cell culturing chamber is only partially filled with the liquid cell culturing medium and the remainder of the cell culturing chamber is filled with a gas, in particular air.

- 10 In some embodiments, temporarily pressurizing the pressure chamber with one or more pressure pulses comprises the delivery of one or more pressurized gas pulses into the pressure chamber.

- 15 According to a fourth aspect, the general object is achieved by a cellular structure, in particular an organoid, having been obtained by a method according to any of the embodiments described herein, in particular with respect to the third aspect of the disclosure.

In the following, non-limiting examples of the disclosure are disclosed. The disclosure therefore further comprises:

In a 1. example, a cell culturing device for culturing cells and/or organoids comprising:

- a cell accommodation unit comprising:
 - 20 ○ at least one cell culturing chamber being configured for accommodating a liquid cell culturing medium and a cellular structure, wherein the cell culturing chamber comprises a venting opening being configured for venting the cell culturing chamber;
 - a pressure chamber comprising a pressure chamber inlet;

- a channel connecting the at least one cell culturing chamber and the pressure chamber;

- a first pressure generator, in particular a pump, being connected to the pressure chamber inlet and being configured for temporarily pressurizing the pressure chamber with a pressure pulse;

wherein the at least one cell culturing chamber, the pressure chamber and the channel are configured such that the pressure pulse from the first pressure generator propagates from the pressure chamber through the channel to the at least one cell culturing chamber and in particular into the liquid cell culturing medium.

- 10 In a 2. example, the cell culturing device according to the 1. example, wherein the cell culturing chamber comprises a cell culturing chamber bottom section and/or wherein the pressure chamber comprises a pressure chamber bottom section.

- 15 In a 3. example, the cell culturing device according to the 2. example, wherein the cell culturing chamber bottom section has a concave shape, in particular a U-shaped, bell-curve-shaped or V-shaped cross section.

In a 4. example, the cell culturing device according to the 2. or 3. example, wherein the channel is arranged closer to the cell culturing bottom section and/or closer to the pressure chamber bottom section than both the pressure chamber inlet and the venting opening.

- 20 In a 5. example, the cell culturing device according to any of the previous examples, wherein the first pressure generator is configured for temporarily pressurizing the pressure chamber with a pressure pulse by providing a pressurized gas pulse into the pressure chamber.

- 25 In a 6. example, the cell culturing device according to any of the previous examples, wherein the first pressure generator is configured to pressurize the pressure chamber with a pressure pulse of 0.5 to 5000 mbar, in particular 20 to 2000 mbar, more particular 50 to 1000 mbar, more particular 100 to 500 mbar.

In a 7. example, the cell culturing device according to any of the previous examples, wherein the first pressure generator is configured to pressurize the pressure chamber in a pulsating mode, in which a plurality of sequential pressure pulses is provided to the pressure chamber.

- 5 In a 8. example, the cell culturing device according to the 7. example, wherein the first pressure generator is configured such that every pulse of the plurality of sequential pressure pulses is followed by a relaxation interval in which no pressure pulse is provided.

- In a 9. example, the cell culturing device according to any of the previous examples, wherein the first pressure generator is configured to pressurize the pressure chamber with a
10 pressure pulse having a pulse duration of 5 to 500 ms, in particular 20 to 300 ms, more particular 50 to 200 ms, more particular 50 to 100 ms.

- In a 10. example, the cell culturing device according to any of the previous examples, wherein the first pressure generator is configured to pressurize the pressure chamber in a quasi-steady state mode, in which the first pressure generator continuously, in particular
15 linearly, increases and decreases the pressure in the pressure chamber to pressurize the pressure chamber with the pressure pulse.

- In a 11. example, the cell culturing device according to any of the previous examples, wherein the channel is configured such that when the liquid cell culturing medium is filled into the cell culturing chamber, the liquid cell culturing medium is prevented from flowing
20 into the pressure chamber.

In a 12. example, the cell culturing device according to the 11. example, wherein a fluid blocking structure is arranged within the channel; or wherein the channel comprises a channel wall which is at least partially or fully made of a hydrophobic material.

- In a 13. example, the cell culturing device according to the 12. example, wherein the fluid
25 blocking structure is a valve being transformable between an open position in which fluid,

in particular liquid, can flow through the channel and a closed position in which fluid, in particular liquid, is prevented from flowing through the channel, or wherein the fluid blocking structure is a membrane being impermeable for fluids, in particular liquids, such as the liquid cell culturing medium, wherein the membrane is configured such that the pressure pulse
5 from the first pressure generator propagates from the pressure chamber through the channel and the membrane to the cell culturing chamber.

In a 14. example, the cell culturing device according to any of the previous examples, wherein the channel is connected to a pressure chamber outlet opening into the channel being arranged offset to, in particular above, the pressure chamber bottom section, wherein
10 preferably the channel and the pressure chamber define together at the pressure chamber outlet an edge having an inner angle α equal to or smaller than 130° , in particular equal to or smaller than 90° .

In a 15. example, the cell culturing device according to the 14. example, wherein the pressure chamber outlet is spaced apart from the pressure chamber bottom section by at
15 least 10 micrometers.

In a 16. example, the cell culturing device according to any of the previous examples, wherein the channel has a maximum channel height of 10 micrometers to 5 mm, in particular 10 micrometers to 500 micrometers, in particular 10 micrometers to 300 micrometers.

In a 17. example, the cell culturing device according to any of the previous examples,
20 wherein the cell culturing device further comprises a control unit being configured to control the first pressure generator.

In a 18. example, the cell culturing device according to any of the previous examples, wherein the cell culturing device further comprises a medium replacement unit, the medium replacement unit comprising a pump assembly, a medium reservoir, a waste disposal tube
25 being preferably connected to a waste reservoir, and a 3-way valve, wherein the 3-way

valve is connected to the waste disposal tube, the medium reservoir and the cell culturing chamber or the pressure chamber.

In a 19. example, the cell culturing device according to the 18. example, wherein the pump assembly comprises a pump assembly control unit being configured to switch the 3-way valve between a delivery connection in which only the medium reservoir is fluidically connected to the cell culturing chamber or the pressure chamber and a disposal connection in which only the waste disposal tube is fluidically connected to the cell culturing chamber or the pressure chamber.

In a 20. example, the cell culturing device according to any of the previous examples, wherein the cell culturing device comprises a plurality of cell culturing chambers, each being configured for accommodating a liquid cell culturing medium and a cellular structure, wherein each cell culturing chamber comprises a venting opening being configured for venting the cell culturing chamber, and wherein each cell culturing chamber is connected by a channel, in particular separate channel, with the pressure chamber.

In a 21. example, the cell culturing device according to the 20. example, wherein the cell culturing chambers circumferentially surround the pressure chamber.

In a 22. example, the cell culturing device according to the 20. or 21. example, wherein each channel has a channel length and wherein the channel length of all channels is the same.

In a 23. example, the cell culturing device according to any of the previous examples, wherein the cell culturing device comprises a plurality of pressure chambers, each comprising a pressure chamber inlet, wherein a first pressure generator is connected to the pressure chamber inlet of each pressure chamber and is configured for temporarily pressurizing the pressure chamber with a pressure pulse.

In a 24. example, the cell culturing device according to the 23. example, wherein at least some or all of the pressure chambers are connected by a channel, in particular a separate channel, to the same cell culturing chamber.

5 In a 25. example, the cell culturing device according to the 24. example, wherein each channel has a channel length and wherein the channel length of all channels is the same; and/or wherein the pressure chambers circumferentially surround the cell culturing chamber.

10 In a 26. example, the cell culturing device according to any of the previous examples, wherein the cell culturing device comprises a plurality of cell culturing chambers, each being configured for accommodating a liquid cell culturing medium and a cellular structure, wherein each cell culturing chamber comprises a venting opening being configured for venting the cell culturing chamber, and wherein the cell culturing device further comprises a plurality of pressure chambers, each comprising a pressure chamber inlet, wherein each pressure chamber is at least connected to one of the cell culturing chambers by a channel.

15 In a 27. example, the cell culturing device according to any of the previous examples, wherein the one or more culturing chambers, the one or more pressure chambers and the one or more channels are part of a well plate.

20 In a 28. example, the cell culturing device according to any of the previous examples, wherein a second pressure generator is arranged within the at least one cell culturing chamber being configured to apply a counter pressure to the at least one cell culturing chamber.

25 In a 29. example, a cell accommodation unit comprising at least one cell culturing chamber being configured for accommodating a liquid cell culturing medium and a cellular structure, wherein the cell culturing chamber comprises a venting opening being configured for venting the cell culturing chamber, a pressure chamber comprising a pressure chamber inlet, and a channel connecting the cell culturing chamber and the pressure chamber.

In a 30. example a method for cultivating cellular structures in a liquid cell culturing medium, the method comprising the steps:

- 5 - Providing a cellular structure and a liquid cell culturing medium into a cell culturing chamber of a cell culturing device, in particular a cell culturing device according to any of the previous examples;
- Providing a liquid medium, in particular the same liquid cell culturing medium as provided into a pressure chamber and optionally into a channel connecting the cell culturing chamber and the pressure chamber;
- 10 - Temporarily pressurizing the pressure chamber with one or more pressure pulses, in particular by a first pressure generator being connected to the pressure chamber inlet, such that the one or more pressure pulse propagates from the pressure chamber and the liquid medium within the pressure chamber through the channel to the cell culturing chamber into the liquid cell culturing medium, thereby inducing movement of the cellular structure and/or of the liquid cell culturing medium within the cell culturing chamber.

15 In a 31. example, the method according to the 30. example, wherein the one or more pressure pulse with which the pressure chamber is pressurized has a pressure of 0.5 to 5000 mbar, in particular 20 to 2000 mbar, more particular 50 to 1000 mbar, more particular 100 to 500 mbar.

20 In a 32. example, the method according to the 30. or 31. example, wherein temporarily pressurizing the pressure chamber comprises pressurizing the pressure chamber with a plurality of sequential pressure pulses, each having a pulse duration.

In a 33. example, the method according to the 32. example, wherein every pulse of the plurality of sequential pressure pulses is followed by a relaxation interval in which no pressure pulse is provided.

In a 34. example, the method according to any of the 30. to 33. examples, wherein a pulse duration of each pressure pulse is 5 to 500 ms, in particular 20 to 300 ms, more particular 50 to 200 ms, more particular 50 to 100 ms.

5 In a 35. example, the method according to any of the 30. to 34. examples, wherein temporarily pressurizing the pressure chamber with one or more pressure pulses comprises continuously, in particular linearly, increasing and decreasing the pressure in the pressure chamber to pressurize the pressure chamber with the pressure pulse.

10 In a 36. example, the method according to any of the 30. to 35. examples, further comprising exchanging the liquid cell culturing medium, in particular by a new liquid cell culturing medium. after a certain time interval and/or after a certain number of pressure pulses.

15 In a 37. example, the method according to the 36. example, wherein exchanging the liquid cell culturing medium is performed by a medium replacement unit comprising a pump assembly, a medium reservoir, a waste disposal tube being preferably connected to a waste reservoir, and a 3-way valve, wherein the 3-way valve is fluidically connected to the waste disposal tube, the medium reservoir and the cell culturing chamber or the pressure chamber.

20 In a 38. example, the method according to any of the 30. to 37. examples, wherein the liquid medium is provided such into the pressure chamber that the pressure chamber is only partially filled with the liquid medium and the remainder of the pressure chamber is filled with a gas, in particular air.

In a 39. example, the method according to any of the 30. to 38. examples, wherein temporarily pressurizing the pressure chamber with one or more pressure pulses comprises the delivery of one or more pressurized gas pulses into the pressure chamber.

25 In a 40. example, a cellular structure having been obtained by a method according to any of the 30. to 39. examples.

Brief description of the figures

The herein described disclosure will be more fully understood from the detailed description given herein below and the accompanying drawings which should not be considered limiting
5 to the disclosure described in the appended claims. The drawings are showing:

- Fig. 1a a perspective view of a cell accommodation unit as it is used in some
 embodiments of the disclosure;
- Fig. 1b a top view onto the cell accommodation unit of Fig. 1a;
- Fig. 1c a sectional view of the cell accommodation unit of Fig. 1b along A-A
- 10 Fig. 2a a top view onto a cell accommodation unit as it is used in other embodiments
 of the disclosure;
- Fig. 2b a sectional view of the cell accommodation unit of Fig. 2a along B-B;
- Fig. 3a a top view onto a cell accommodation unit as it is used in other embodiments
 of the disclosure;
- 15 Fig. 3b a sectional view of the cell accommodation unit of Fig. 3a along C-C;
- Fig. 4a a top view onto a cell accommodation unit as it is used in other embodiments
 of the disclosure;
- Fig. 4b a sectional view of the cell accommodation unit of Fig. 4a along D-D;
- Fig. 5a a perspective view of a cell accommodation unit as it is used in other
20 embodiments of the disclosure;

- Fig. 5b a sectional view of the cell accommodation unit of Fig. 5a;
- Fig. 6 a schematic view of a cell culturing device 1 according to an embodiment of the disclosure;
- Fig. 7 a schematic detailed section view of a cell accommodation unit as it used in
5 some embodiments of the disclosure;
- Fig. 8a, b different modes of the first pressure generator;
- Fig. 9 the liquid level increase in different cell culturing chambers over 5 pulses;
- Fig. 10a the liquid level increase in a specific cell culturing chamber over 5 pulses;
- Fig. 10b the liquid level increase over eight different cell culturing chambers being
10 connected to the same pressure chamber;
- Fig. 11a the field of velocities of fluid flow at the cell culture chamber inlet;
- Fig. 11b time-dependent average fluid velocity in a channel;
- Fig. 11c fluid velocity on the channel of a cell accommodation unit being a well plate with 2x6 cell culturing chambers;
- 15 Fig. 12 a fluorescent mixing assay;
- Fig. 13a,b a. organoid growth (measured area) after 7 days and 21 days in the device according to the disclosure (constant pulse interval and varying pulse duration – Fig. 13a) vs. a control orbit shaker vs. a control static well plate;
b. organoid growth (measured area) after 7 days and 13 days in the device according to the disclosure (constant pulse duration and varying pulse intervals – Fig. 13b) vs. a control orbit shaker vs. a control static well plate
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- Fig. 14 mouse gastruloids at day 5 after ESC seeding in a device according to the disclosure ("platform") and a static control wellplate;
- Fig. 15 mouse gastruloids obtained in a device according to the disclosure ("platform") and a shaken control (orbital shaker); and a graph showing the elongation index difference between organoids obtained with a device according to the disclosure ("Pulsed"), a shaker ("Shaking"), and static well-plate ("Static");
- Fig. 16a,b mouse gastruloids obtained in a static control (ultra-low attachment plate) and in device according to the invention with either a u-shaped bottom or a flat cell culturing bottom chamber; and a graph showing the elongation index difference between organoids obtained with a device according to the invention ("Pulsed") and static well-plate ("Static")
- 17a,b the filling process of cell culturing chambers with a liquid cell culturing medium;
- Fig. 18a-c the influence of channel wall hydrophobicity and vertical offset arrangement of pressure chamber outlet on the filling of the cell culturing chamber and the channel with liquid cell culturing medium;
- Fig. 19a-c a mouse brain organoid being lifted in a cell culturing device according to the disclosure and its trajectory tracked over 10 pulses;
- Fig. 20 immunostaining of representative organoids either grown in static conditions or either pulsed (i.e. according to the disclosure) or shaken from days 7 to 28 (day of fixing and sectioning);
- Fig. 21 immunostaining of two representative gastruloids at day 7 of culture in device showing formation of different tissues marked by E-Cadherin, Gata6, Meox1, Sox1 and T/BRA;

Fig. 22 embryoid bodies from mouse embryonic stem cells formed within devices (day 4) and gastruloids obtained from these embryoid bodies (day 7);

Fig. 23 dependency of pulse duration on the height the organoid is lifted.

Exemplary embodiments

5 Fig. 1a to 1c show a cell accommodation unit 2 as it can be used in certain embodiments of the disclosure. The cell accommodation unit comprises a pressure chamber inlet 6 which provides access to pressure chamber 5 (see Fig. 1c) and which can be connected to a first pressure generator. Cell accommodation unit 2 further comprises a cell culturing chamber 3 which has venting opening 4 for venting off an overpressure from the cell culturing chamber. In the cross-sectional view of Fig. 1c which is taken along A-A in Fig. 1b, channel 10 7 is shown which connects pressure chamber 5 and cell culturing chamber 3 with each other. Pressure chamber 5, respectively the pressure chamber walls of pressure chamber 5, defines, respectively comprises pressure chamber outlet 13 which opens directly into channel 7. It is understood that the pressure chamber outlet 13 concomitantly represents 15 one of the inlets of channel 7. Except for pressure chamber inlet 6 and pressure chamber outlet 13, pressure chamber 5 is closed and hermetically sealed. It should be noted that the channel height (along direction z) is shown in exaggeration. The channel length and thus the direction of channel extension is along direction x and the channel width along direction y.

20 Fig. 2a and 2b show another embodiment of a cell accommodation unit 2. While the embodiment in Fig. 1a-c comprises one cell culturing chamber, one pressure chamber and one channel connecting the pressure chamber and the cell culturing chamber, this embodiment comprises a plurality (in this case 12) cell culturing chambers 3 (the same elements are referred to with the same reference sign and only two of the 12 cell culturing 25 chambers are referenced for clarity purposes) which are each connected by a separate channel 7 with a common and single pressure chamber 5. Thus, each cell culturing chamber 3 is connected by its own channel 7 to pressure chamber 5 and pressure chamber 5

comprises 12 pressure chamber outlets, which each open into a channel. Therefore, each cell culturing chamber is connected by a channel to a pressure chamber. This holds true for the embodiment shown, but may in some embodiments also be true for any of the other embodiments described herein. Further, if there is more than one cell culturing chamber, there may preferably be the same number of channels which connect each of the cell culturing chambers to a pressure chamber. Also, this holds true for the embodiment shown in Fig. 2a and b, but may in some embodiments also be the case for any of the other embodiments as described herein. In this embodiment, all cell culturing chambers 3 have the same distance from their common pressure chamber 5. As can be seen from Fig. 2a, the cell culturing chambers 3 circumferentially surround (in this case circularly) pressure chamber 5 and its pressure chamber inlet 6. Such embodiments are advantageous, as they allow to culture multiple and particularly different, cellular structures with a common first pressure generator. Thus, such units are both cost- and space-efficient.

Fig. 3a and b show another embodiment of a cell accommodation unit 2. In this case, there is also only one cell culturing chamber 3 which circumferentially surrounds pressure chamber 5. In this embodiment, cell culturing chamber 3 has four venting openings 4 (only one is referenced for clarity purposes). As can be seen from the cross-section in Fig. 3b along C-C also channel 7 is circumferentially surrounds pressure chamber 5.

Fig. 4a and b show yet another embodiment of a cell accommodation unit 2. In this embodiment, there is only a single cell culturing chamber 4. Cell culturing chamber 3 is centrally arranged and comprises venting opening 4. Further, cell accommodation unit 2 comprises in this (or may also comprise in any other embodiment as described herein) more pressure chambers than cell culturing chambers. In this embodiment, cell culturing chamber 3 is connected by four different channels 7 to four different pressure chambers 5, each having a pressure chamber inlet 6 for being connected to a first pressure generator. In such embodiments, pressure pulses can be provided uniformly from all four sides or in an alternating manner, thereby improving mixing efficiency. By using pressure pulses uniformly from four sides, the pressure of each pressure pulse can be reduced, which provides even milder mixing conditions.

Fig. 5a and b. show another embodiment of a cell accommodation unit 2 as it can be used in some embodiments of the disclosure. In this embodiment, cell accommodation unit 2 is a well plate. It contains 64 cell culturing chambers 3 and four pressure chambers 5 being connected to the cell culturing chambers by 64 channels. It should be noted that the channels 7 have a very small channel height and are thus only implied by bold solid lines. It can be seen that the plurality of cell culturing chambers can in such a well plate in general be grouped in one or more rows of cell culturing chambers. In the embodiment shown, there are eight rows of cell culturing chambers, each consisting of eight cell culturing chambers being arranged one after another. Each pressure chamber 5 is arranged between two rows of cell culturing chambers and is in this embodiment connected by separate channels to each of the cell culturing chambers of those two rows. Furthermore, it can be seen that cell accommodation unit 2 comprises of two parts, namely top section 25 which defines the venting openings of the cell culturing chambers and the gas chamber inlets, as well as bottom section 26 which comprises, respectively delimits the cell culturing chamber bottom sections. Top section 25 and bottom section 26 together define the cell culturing chambers 3, the channels 7 and the pressure chambers 5. Fig. 5b further shows that the cell culture chamber bottom sections 10 form a concavity (i.e. a concavity towards cell culturing chamber 3 and in this embodiment has in cross-section a bell-shape). Such embodiments are beneficial, for culturing of cellular structures and in particular during the initial growth phase.

Fig. 6 shows a schematic representation of a cell culturing device 1 according to an embodiment of the disclosure. The cell culturing device 1 comprises a cell accommodation unit (such as for example a cell accommodation unit 2 as shown in Fig. 1a-c). The cell accommodation unit comprises pressure chamber 5 with pressure chamber inlet 6 and pressure chamber outlet 7. Pressure chamber outlet 7 directly opens into channel 7 which connects pressure chamber 6 with cell culturing chamber 3. Cell culturing device 1, respectively channel 7, comprises a fluid blocking structure 12 which may in this or any other embodiment as described herein be at least in one configuration configured such that it is impermeable for fluids, in particular liquids, but may in this or in another configuration allow to transmit a pressure pulse from the pressure chamber through the channel to the

cell culturing chamber. Both the cell culturing chamber 3 and the pressure chamber 5 are partially filled with a liquid medium, while the residual volume is a gas, such as air. Cell culturing chamber 3 may be filled with a liquid cell culturing medium 9 and pressure chamber 5 may be filled with the same medium or with a different liquid medium. The latter may be beneficial in embodiments in which a fluid blocking structure, such as a membrane is present in channel 7.

Cell culturing device 1 further comprises first pressure generator 8 which is connected, i.e. directly connected, to pressure chamber inlet 6 of pressure chamber 5. Pressure generator 8 is configured such that it can temporarily pressurize the pressure chamber with one or more pressure pulses (indicated by the four dashed curved lines). As indicated by the arrows, the pressure pulse then propagates from the gas in pressure chamber 5 into the liquid medium in pressure chamber 5 and from there into channel 7 and further into cell culturing chamber 3 and in particular into the liquid cell culturing medium 9. A cellular structure 23 being present in cell culturing medium is therefore sufficiently and optimally supplied with cell culturing medium. The pressure pulse causes the liquid cell culturing medium to move with respect to cellular structure 23 and/or cell culturing chamber 3 and/or also may cause movement, e.g. lifting, of cellular structure 23. This mixing is practically devoid of significant shear forces and therefore a very mild method to ensure efficient growth and avoid damages to the growing cellular structure 23. Since the cell culturing chamber comprises a venting opening (not referenced for clarity purposes, see Fig. 1-5), the overpressure is vented to the environment. The cell culturing device 1 further comprises a lid 24 which protects both the pressure chamber and the cell culturing chamber from undesired contamination.

Cell culturing device 1 further comprises a second pressure generator 22 in cell culturing chamber 3 being in general different from the first pressure generator and being configured to apply a counter pressure to the at least one cell culturing chamber.

The cell culturing device 1 may further comprise control unit 14 which may for example be configured to control first pressure generator 8. Additionally, cell culturing device 1

comprises monitoring unit 28, such as a sensor, which is configured to monitor a status of liquid cell culturing medium 9 inside cell culturing chamber 3. This status may be transmitted to control unit 14 which may then depending on the status trigger a pressure pulse or alternate the pressure pulse, such as its mode, strength, relaxation interval, duration, etc.

- 5 This status may also be transmitted to pump assembly control unit 21 which may be a separate control unit as shown in Fig. 6 or which may be included in control unit 14.

- Cell culturing device 1 further comprises medium replacement unit 15, which itself comprises 3-way valve 20, waste disposal tube 18 being connected to waste reservoir 19, medium reservoir 17, pump assembly 16 and pump assembly control unit 21. Depending
- 10 on the configuration of the 3-way valve, cell culturing chamber 3 may be fluidically connected to waste disposal tube 18 and waste reservoir 19 upon which used liquid cell culturing medium 9 can be removed from cell culturing chamber 3 by pump assembly 16, or cell culturing chamber 3 may be fluidically connected to medium reservoir 17 which contains fresh liquid cell culturing medium, such that fresh liquid cell culturing medium can
- 15 be provided into cell culturing chamber 3 by pump assembly 16. Pump assembly 16 and also 3-way valve 20 may be controlled by pump assembly control unit 21. Further, as already outlined above, monitoring unit 28 may transmit a status of liquid cell culturing medium 9 to pump assembly control unit 21. This status may for example be the refractive index of liquid cell culturing medium. If pump assembly control unit detects that the status
- 20 exceeds or falls below a predetermined threshold, it may trigger an exchange of the liquid cell culturing medium (i.e. by removing the used liquid cell culturing medium and providing new cell culturing medium as described above). As an example, monitoring unit 28 may monitor the refractive index of liquid cell culturing medium. Since an increased cloudiness may indicate the necessity to exchange the medium, a change of refractive index beyond a
- 25 predetermined threshold may trigger the medium exchange. Monitoring unit 28 may also monitor the nutrient content or the general composition of the liquid cell culturing medium. If this falls below a predetermined threshold, the pump assembly control unit may trigger a medium exchange.

Fig. 7 shows only a detailed section of a cell accommodation unit according to some embodiments. Here it can be seen that the pressure chamber bottom section 11 of pressure chamber 5 is spaced apart by distance d from pressure chamber outlet 13 which opens into channel 7 connecting pressure chamber 5 with cell culturing chamber 3. This helps to prevent that liquid cell culturing medium 9 within cell culturing chamber 3 and channel 7 flows into the yet empty pressure chamber 5. Distance d may for example be at least 10 micrometers. Furthermore, channel 7, forms together with pressure chamber 5, in particular the pressure chamber wall, at the pressure chamber outlet 13 an edge having an angle α .

Fig. 8a shows the pulsating mode in which the first pressure generator can be operated. It can be seen that pulses are instantaneously provided, maintained for a certain pulse duration and each pulse is then followed by a relaxation interval. Fig. 8b shows in contrast the quasi-steady mode in which the first pressure generator continuously, in particular linearly, increases and decreases the pressure in the pressure chamber to pressurize the pressure chamber with one or more pressure pulses.

Fig. 9 shows the change in liquid level in eight different cell culturing chambers being connected to the same pressure chamber. It can be seen that the change of liquid level is very uniform over the eight different wells and over the pulses. Further, it can be seen that equilibration takes place before the next pulse is provided. In this embodiment five pulses were provided with a pressure of 250 mbar for a pulse duration of 70 ms and spaced apart by 30 s.

Fig. 10a shows the maximum liquid level increase in a cell culturing chamber over 5 pulses and Fig. 10b shows the variation in liquid level when the average of 5 pulses is performed for each of the eight cell culturing chambers. Also, here it can be seen that the conditions over multiple sequential pulses in a single cell culturing chamber are uniform and that the conditions over eight different single cell culturing chambers being connected to the same pressure chamber are uniform.

Fig. 11a shows a field of velocities of the liquid cell culturing medium at the cell culturing chamber inlet when a pulse of 250 mbar and a duration of 70 ms is provided. Fig. 11b shows the time dependent average fluid velocity in the channel and Fig. 11c. shows the fluid velocity in a device as shown, i.e. a device having two rows, each having six cell culturing chambers and one pressure chamber being arranged between the two rows and having 12 channels which connect every cell culturing chamber with the pressure chamber. It can be readily seen that the flows are uniform in all cell culturing chambers, even if the cell culturing chambers are not circularly (i.e. radially symmetric) arranged around the pressure chamber. Fig. 11 a further shows that the flow velocity is uniform across the channel length.

Fig. 12 shows the fluorescent signal of a fluorescent agent being present in the cell culturing chamber. It can be seen that the pulses applied (250 mbar pressure, duration of 100 ms, and every 30 s a new pulse) rapidly homogenize the fluorescent signal in only about 3 min. This shows that the liquid exchange in embodiments in which no fluidic blocking structure prevents the flow of liquid through the channel, between the cell culturing chamber and the pressure chamber is strong. This indicates that for example oxygen being present in the liquid cell culturing medium is sufficiently provided to the growing cellular structure.

Fig. 13a shows the measured area of a mouse brain organoid after 7 (D14) and 21 (D28) days of growth in the device (cells were transferred into the device on day 7 and have been initially created in an ultra low attachment standard well plate) under three different pulse durations, according to the disclosure, a control orbital shaker experiment and a control static experiment (no movement). In Fig. 13a, the interval between the pulses of the 3 different pulse duration experiments was maintained constant at 30s. Fig. 13b shows the measured area of a mouse brain organoid after 7 (D14) and 13 (D20) days of growth in the device (cells were transferred into the device on day 7 and have been initially created in an ultra low attachment standard well plate) under a pulse with constant pulse duration of 70 ms and varying pulse intervals (5s, 10s, 30 s) according to the disclosure, a control orbital shaker experiment and a control static experiment (no movement). It can be seen that the growth with a device according to the disclosure is significantly higher than under static

conditions and equivalent to orbital shaker conditions, which may cause organoids fusing with each other, may be reproducible and is not amenable to integrated automation.

Fig. 14 shows mouse gastruloids obtained after 5 days post ESC seeding in a device according to the disclosure (indicated herein as "platform") and a static control. In this example, seeding was not done in the platform but in a standard well plate, and the gastruloid was transferred on day 4, which is when pulses help with the elongation. Importantly, direct seeding in the device according to the disclosure is alternatively also possible. Model systems such as organoids frequently shed cells to its surrounding medium, either due to apoptosis or due to the migratory nature of some cell types. For example, apoptosis of progenitor cells is seen during neural tube closure to ensure proper brain development by apoptosis of morphogen-producing cell. Shed cells are generally not needed for the model system, so both dead and viable shed cells are removed during media change. For this, strong flows are often needed in prior art known devices and methods using a manual pipette at the right position and angle, requiring a certain degree of practice. It is often a challenge to automate this process. Fig. 14 shows gastruloids at day 5 post ESC seeding. On day 4 half of the gastruloids were transferred to the device and half stayed in the cell culturing chambers and cells removed manually. As seen, after 24 hours and static conditions the surface of the wells below the gastruloids was filled with shed cells. These cells were entirely removed in the device according to the disclosure.

Fig. 15 shows mouse gastruloids obtained in a device according to the disclosure (indicated herein as "platform") and on an orbital shaker ("shaken control") after transfer on day 4. The graph shows the calculation of the elongation index for shaker ("Shaking"), a static wellplate ("Static") and with the device according to the disclosure (indicated "Pulsed"). It can be seen that the device according to the disclosure can be used to obtain organoid development not observed in alternative shakers or bioreactors. For example, mouse gastruloids with a very high aspect ratio were obtained by applying 250 mbar and 50 ms, pulses every 30 s in the overnight period between days 4 and 5 of culture. Thus, the disclosure allows to achieve phenotype changes in 3d model systems that may be caused by the controlled shear stress generated.

Fig. 16a shows mouse gastruloids obtained in a static control (ultra-low attachment plate) and in device according to the disclosure with a cell culturing bottom chamber having either a u-shaped or a planar cross-section; Fig. 16b shows a graph illustrating the elongation index difference between organoids obtained with a device according to the disclosure ("Pulsed") and a static well-plate ("Static"). It can be seen that the device according to the disclosure can be used to obtain organoid development not observed under alternative static conditions.

Fig. 17a and b show a device according to the disclosure with two rows each having six cell culturing chambers being connected to one pressure chamber which is arranged between the two rows by individual channels. Both Fig. 17a and b show the filling process of the cell culturing chambers with a liquid cell culturing medium. Fig. 17b is a device as shown in and described for Fig. 7, in which the pressure chamber bottom section is spaced apart by a distance d of at least 10 micrometers from the pressure chamber outlet. In Fig. 17a, this is not the case. It can be seen that in contrast to the liquid cell culturing medium in Fig. 17b efficiently prevented from flowing into the yet empty pressure chamber.

Fig. 18a illustrates the influence of a hydrophobic channel wall section. If at least the channel bottom wall section is made from a hydrophobic material, filling of the cell culturing chamber 3 and the channel does not lead to creeping of the liquid aqueous cell culturing medium into the pressure chamber 5 (see right detail view). In contrast, if the channel is made from a hydrophilic material, the liquid cell culturing medium creeps into the pressure chamber (see left detail view). Thus, it can be seen that a hydrophobic material is advantageous, as it allows to maintain a plurality of cell culturing chambers separated during filling. However, once the cell culturing chamber(s), the channel(s) and the pressure chamber is filled with liquid medium, in particular liquid cell culturing medium, it is not possible to separate a plurality of cell culturing chambers from each other anymore. This is different in the embodiments shown in Fig. 18b and c. In these embodiments, the pressure chamber outlet 13 is arranged vertically offset to the pressure chamber bottom section 11. Fig. 18b differentiates between hydrophilic channel wall sections (left detail view) and hydrophobic channel wall section (right detail view). In both cases, the vertical offset

arrangement of the pressure chamber outlet 13 prevents that liquid cell culturing medium flows into the pressure chamber during filling of the cell culturing chamber, however the water contact angle is different. Inner angle α between the pressure chamber wall and the channel (in particular its channel wall bottom section) is equal to or smaller than 130° , in particular equal to or smaller than 90° . In Fig. 18c, the pressure chamber wall section between the pressure chamber outlet 13 and the pressure chamber bottom section 11 extends such that it forms an inclination 29, in particular a linear or curved inclination, towards the pressure chamber bottom section 11. The inclination forms an outer angle β between 90° and 180° , in particular 120° and 180° , more particular 130° and 175° , with the pressure chamber bottom section. In such embodiments, a hydrophobic channel wall, in particular channel bottom wall section, and/or pressure chamber wall prevents creeping of the liquid cell culturing medium into the pressure chamber (see right detail view), while a hydrophilic channel wall, in particular channel bottom wall section, leads to creeping of the liquid cell culturing medium into the pressure chamber (see left detail view). Further, in contrast to Fig. 18a, it is for the hydrophobic embodiment of Fig. 18c and for both embodiments of Fig. 18b possible to reverse the connected state of a plurality of cell culturing chambers each being connected by a channel as shown to the same pressure chamber to a separated state. This can be achieved by removing liquid from the pressure chamber and thereby removing any liquid bridges between the cell culturing chambers.

Fig. 19a shows a fixed mouse brain organoid being pulsed at 250 mBar for 50 ms in a cell culturing chamber equivalent in size to a well of a 96 wellplate and containing a U shaped-bottom section. In Fig. 19a a single pulse is provided which lifts the organoid such that it almost reaches the height of the liquid level, visible as a white meniscus. Fig. 19b indicates the trajectory of the organoid for 5 consecutive pulses with intermediate intervals of 30 seconds. Fig. 19c shows the trajectory of brain organoid in every well of a row of wells of the device tracked for 10 pulses

Fig. 20 shows immunostained representative organoids either grown in static conditions or either pulsed (i.e. according to the disclosure) or shaken from days 7 to 28 (day of fixing and sectioning). Pulsing conditions were 250 mBar, 150 ms every 30 seconds. The media

was IDM+A with 1% matrigel. All organoids were grown in the same ratio of volume per organoid. The results show that a device according to the disclosure can grow organoids at least equivalent to shaken conditions, with all the major cell types (neural progenitors, neurons, oligodendrocytes, astrocytes) present in equivalent abundance.

5 Fig. 21 shows immunostaining of two representative gastruloids at day 7 of culture in device showing formation of different tissues marked by E-Cadherin, Gata6, Meox1, Sox1 and T/BRA. The protocol can be found under: <https://www.protocols.io/view/protocol-to-generate-gastruloids-lscb-epfl-ewov18n4pgr2/v1>. Briefly, gastruloids are obtained from an embryoid body of mouse embryonic stem cells in media N2B27. At 48 hours after seeding
10 CHIR99021 is provided at 3 μ M in the same media and for 24 hours. Pulsing is started at 72 hours. These results show that beyond their more elongated phenotype, gastruloids formed in a device according to the present disclosure form the expected tissue types and tissue structures of healthy gastruloids.

Fig. 22 shows embryoid bodies from mouse embryonic stem cells formed within devices
15 according to the present disclosure (day 4) and gastruloids obtained from these embryoid bodies (day 7). These results show that u-shaped cell culturing bottom chamber of the device is adequate to aggregate stem cells with a preference for cell-cell attachment over surface-cell attachment. Thus, all steps from cell seeding to flow culture can be integrated.

Fig. 23 illustrates the dependency of the pulse duration on the height an organoid in a device
20 according to the present disclosure is lifted. It can be seen that an increased pulse duration results in brain organoids lifting higher within the device. The time in suspension is not affected except for a gentle pulse.

Claims

1. Cell culturing device (1) for culturing cells and/or organoids comprising:

– a cell accommodation unit (2) comprising:

i. at least one cell culturing chamber (3) being configured for accommodating a liquid cell culturing medium (9) and a cellular structure, wherein the cell culturing chamber (3) comprises a venting opening (4) being configured for venting the cell culturing chamber (3);

ii. a pressure chamber (5) comprising a pressure chamber inlet (6);

iii. a channel (7) connecting the at least one cell culturing chamber (3) and the pressure chamber (5);

– a first pressure generator (8), in particular a pump, being connected to the pressure chamber inlet (6) and being configured for temporarily pressurizing the pressure chamber (5) with a pressure pulse;

wherein the at least one cell culturing chamber (3), the pressure chamber (5) and the channel (7) are configured such that the pressure pulse from the first pressure generator (8) propagates from the pressure chamber (5) through the channel (7) to the at least one cell culturing chamber (3) and in particular into the liquid cell culturing medium (9).

2. The cell culturing device (1) according to claim 1, wherein the cell culturing chamber (3) comprises a cell culturing chamber bottom section (10) and/or wherein the pressure chamber (5) comprises a pressure chamber bottom section (11), wherein preferably the cell culturing chamber bottom section (10) has a concave shape, in particular a U-shaped, bell-curve-shaped or V-shaped cross section.

3. The cell culturing device (1) according to any of the previous claims, wherein the first pressure generator (8) is configured for temporarily pressurizing the pressure chamber (5) with a pressure pulse by providing a pressurized gas pulse into the pressure chamber (5).
- 5 4. The cell culturing device (1) according to any of the previous claims, wherein the first pressure generator (8) is configured to pressurize the pressure chamber (5) with a pressure pulse of 0.5 to 5000 mbar, in particular 20 to 2000 mbar, more particular 50 to 1000 mbar, more particular 100 to 500 mbar; and/or wherein the first pressure generator (8) is configured to pressurize the pressure chamber (5) with a pressure pulse having a pulse duration of 5 to 500 ms, in particular 20 to 300 ms, more particular 50 to 200 ms, more particular 50 to 100 ms.
10
5. The cell culturing device (1) according to any of the previous claims, wherein the first pressure generator (8) is configured to pressurize the pressure chamber (5) in a pulsating mode, in which a plurality of sequential pressure pulses is provided to the pressure chamber (5), wherein preferably the first pressure generator (8) is configured such that every pulse of the plurality of sequential pressure pulses is followed by a relaxation interval in which no pressure pulse is provided.
15
6. The cell culturing device (1) according to any of the previous claims, wherein the first pressure generator (8) is configured to pressurize the pressure chamber (5) in a quasi-steady state mode, in which the first pressure generator (8) continuously, in particular linearly, increases and decreases the pressure in the pressure chamber to pressurize the pressure chamber with the pressure pulse.
20
7. The cell culturing device (1) according to any of the previous claims, wherein the channel (7) is configured such that when the liquid cell culturing medium is filled into the cell culturing chamber (3), the liquid cell culturing medium is prevented from flowing into the pressure chamber (5).
25

8. The cell culturing device (1) according to claim 7, wherein the channel comprises a channel walls which are at least partially or fully made of a hydrophobic material or wherein a fluid blocking structure is arranged within the channel; wherein preferably the fluid blocking structure (12) is a valve being transformable between an open position in which fluid, in particular liquid, can flow through the channel (7) and a closed position in which fluid, in particular liquid, is prevented from flowing through the channel (7), or wherein preferably the fluid blocking structure (12) is a membrane being impermeable for fluids, in particular liquids, such as the liquid cell culturing medium, wherein the membrane is configured such that the pressure pulse from the first pressure generator (8) propagates from the pressure chamber (5) through the channel (7) and the membrane to the cell culturing chamber .
9. The cell culturing device (1) according to any of the previous claims, wherein the channel (7) is connected to a pressure chamber outlet (13) opening into the channel (7) being arranged offset to, in particular above, the pressure chamber bottom section (11), wherein preferably the channel (7) and the pressure chamber (5) define together at the pressure chamber outlet (13) an edge having an inner angle (a) equal to or smaller than 130° , in particular equal to or smaller than 90° .
10. The cell culturing device (1) according to any of the previous claims, wherein the cell culturing device (1) further comprises a control unit (14) being configured to control the first pressure generator (8).
11. The cell culturing device (1) according to any of the previous claims, wherein the cell culturing device (1) further comprises a medium replacement unit (15), the medium replacement unit (15) comprising a pump assembly (16), a medium reservoir (17), a waste disposal tube (18) being preferably connected to a waste reservoir (19), and a 3-way valve (20), wherein the 3-way valve (20) is connected to the waste disposal tube (18), the medium reservoir (17) and the cell culturing chamber (3) or the pressure chamber (5), wherein preferably the pump assembly (16) comprises a pump assembly control unit (21) being configured to switch the 3-way valve (20) between a delivery

connection in which only the medium reservoir (17) is fluidically connected to the cell culturing chamber (3) or the pressure chamber (5) and a disposal connection in which only the waste disposal tube (18) is fluidically connected to the cell culturing chamber (3) or the pressure chamber (5) .

5 **12.** The cell culturing device (1) according to any of the previous claims, wherein the cell
culturing device (1) comprises a plurality of cell culturing chambers (3), each being
configured for accommodating a liquid cell culturing medium and a cellular structure,
wherein each cell culturing chamber (3) comprises a venting opening (4) being
configured for venting the cell culturing chamber (3), and wherein each cell culturing
10 chamber (3) is connected by a channel (7), in particular separate channel, with the
pressure chamber (5), wherein preferably the cell culturing chambers (3)
circumferentially surround the pressure chamber (5).

15 **13.** A cell accommodation unit (2) comprising at least one cell culturing chamber (3) being
configured for accommodating a liquid cell culturing medium and a cellular structure,
wherein the cell culturing chamber (3) comprises a venting opening (4) being
configured for venting the cell culturing chamber (3), a pressure chamber (5)
comprising a pressure chamber inlet (6), and a channel (7) connecting the cell
culturing chamber (3) and the pressure chamber (5).

20 **14.** Method for cultivating cellular structures in a liquid cell culturing medium, the method
comprising the steps:

- Providing a cellular structure and a liquid cell culturing medium (9) into a cell
culturing chamber (3) of a cell culturing device (1), in particular a cell culturing
device according to any of the previous claims;
- Providing a liquid medium, in particular the same liquid cell culturing medium
25 as provided into a pressure chamber (3) and optionally into a channel (7)
connecting the cell culturing chamber (3) and the pressure chamber (5);

- 5 – Temporarily pressurizing the pressure chamber (5) with one or more pressure pulses, preferably by delivering one or more pressurized gas pulses into the pressure chamber (5), in particular by a first pressure generator (8) being connected to the pressure chamber inlet (6), such that the one or more pressure pulse propagates from the pressure chamber (5) and the liquid medium within the pressure chamber (5) through the channel (7) to the cell culturing chamber (3) into the liquid cell culturing medium(9), thereby inducing movement of the cellular structure (24) and/or of the liquid cell culturing medium (9) within the cell culturing chamber (3).
- 10 **15.** The method according to claim 14, wherein temporarily pressurizing the pressure chamber (5) comprises pressurizing the pressure chamber (5) with a plurality of sequential pressure pulses, each having a pulse duration, wherein preferably every pulse of the plurality of sequential pressure pulses is followed by a relaxation interval in which no pressure pulse is provided.
- 15 **16.** The method according to claim 14 or 15, wherein temporarily pressurizing the pressure chamber (5) with one or more pressure pulses comprises continuously, in particular linearly, increasing and decreasing the pressure in the pressure chamber (5) to pressurize the pressure chamber (5) with the pressure pulse.