Cell Culturing Device

Field of disclosure

The present disclosure lies in the field of medicinal technology and relates in particular to devices for cell culturing and cellular assays. It relates to a cell culturing device, cell culturing systems, methods to produce a cell culturing device, a kit of parts, methods for culturing cells, cellular chips, the use of such a cellular chip, a cell culturing device blank and a cell culturing blank system.

Background, prior art

Biological research of living organisms goes side-by-side with attempts to apply this knowledge in building in vitro organ models, closely resembling in vivo counterparts. Organs-on-chips are in vitro organ models that hold great potential for enhancing the efficiency of drug development and reducing the reliance on animal testing. To ensure their successful integration in both research and industry, organ-on-chip must closely replicate the in vivo organ physiology and response to the treatments.

A comprehensive understanding of organ formation, functionality, and pathophysiology requires to study how cells and tissues interact within organs, which consist of various tissue types organized in three-dimensional structures with high dynamics, variability, mechanical properties, and biochemical microenvironment. However, most studies on cell and tissue biology have primarily relied on 2D cell-culture models, which fail to recreate the natural cellular microenvironment present in vivo. Efforts to address these limitations led to the development of 3D cell-culture models in which cells are grown within extracellular matrix (ECM) mimicking hydrogels. This approach enhances expression of differentiated functions and improves tissue organization.

Extensive research over the past decades has resulted in development of protocols for growing three-dimensional organ-like tissues from tissues specific stem cells, known as organoids. Organoids exhibit multiple histological and functional characteristics similar to real organs, providing unprecedented opportunities for studying tissue development, regeneration, and diseases in a laboratory setting without having to rely on animal testing. However, organoids have primarily relied on the spontaneous self-organization of stem cells, resulting in uncontrolled growth and variability in size and structure. Additionally, most epithelial stem cell-derived organoids possess a closed, cystic architecture, which limits their lifespan and poses challenges for experimental manipulation. Furthermore, organoids lack the ability to replicate essential features of organs, such as tissue-to-tissue interfaces (for instance, between epithelium and vascular endothelium), spatiotemporal gradients of chemicals and oxygen, and the mechanically dynamic microenvironment that plays a vital role in the functioning of nearly all living organs.

Devices known from the prior art can be divided into membrane-derived devices, which rely on a membrane separating two sides of the device as well as membrane-free devices with hydrogels.

In membrane-derived devices, a layer of epithelial cells is typically grown on a porous membrane inside a cell culture plate. Thereby access to the medium from both the apical and basolateral sides of the epithelial layer is allowed, making it a versatile tool for studying transport, absorption, and other metabolic activities in vitro. A common concept are membrane-based devices consisting of two perfusable channels separated by a thin polymer membrane. Human cells can be cultured in both channels, from both sides of the membrane, mimicking the epithelial barrier and physiological functions of living organs such as lung, intestine, kidney, skin, bone marrow, and blood-brain barrier.

The use of a 2D membrane in organ on chips systems precludes establishment of an in vivo-like 3D tissue architecture and makes it impossible to model complex multicellular interactions such as those emerging from non-parenchymal tissues. However, such systems have limitations, as they are unable to mimic the dynamic microenvironment of the

living tissues and the system hinders intrinsic cellular self-organization as cells are forced to grow on a synthetic 2D membrane. The 2D surface of the membrane flattens cellular self-organization and hampers interaction between the multiple cell types. Modelling various diseases often requires interaction of multiple cell types, including mesenchymal and immune cells. The polymer membranes used in such systems constitute a physical barrier between the epithelial and endothelial cells and preclude direct interaction between cells. Additionally, membrane-based systems hamper introduction of additional cell types, limiting their potential only to two tissues co-culture (e.g. epithelial/endothelial barrier). The only option for multiple tissues culture was suggested to connect various chips by tubing for media exchange, while direct interaction remains impossible. Limited lifespan of about 7-10 days of culture results in the absence of homeostasis and inability to model hallmarks of tissue physiology in long-term during health and disease.

Various limitations of the membrane-based systems has led to the development of the first generation of membrane-free, hydrogel-based organ-on-chip systems. There are for example membrane-free hydrogel organ-on-chip platforms which allow to culture epithelial cells in channels against an ECM compartment. While this system is specifically tailored for the high-throughput screening of the epithelium barrier integrity and absorption, flattening human three-dimensional organs to 2D topographies impairs in vivo physiology, decreases cell-type composition and limits their lifespan. Additionally, the closed microchannel design of the plate makes establishing an air-liquid interface difficult. Three-dimensional cellular architecture plays a crucial role in organ function, but the prior art is still lacking versatile micro-physiological systems allowing to recapitulate 3D ECM microenvironment, required for proper tissue homeostasis and function.

Summary of disclosure

It is the general object of the present disclosure to advance the state of the art in the field of medicinal technology, in particular organs-on-a-chip and preferably to overcome the disadvantages of the prior art, such as the ones discussed above, fully or at least partly. In

advantageous embodiments, the present disclosure presents ways to promote self-organization of growing cells within 3D tissues and to analyze tissues through imaging techniques in an easy manner. Further, multiplex co-culturing of cells can be achieved and complex networks of cells and cellular structures can be provided in a simple and reliable manner which provide further insights on the effect of a compound of interest on different cell types or different sides of a cell assembly. In further advantageous embodiments, the present disclosure allows to access multiple different sides of the hydrogel, i.e. to the mimicked ECM. In advantageous embodiments, devices are provided which can be easily used.

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

A first aspect of the present disclosure relates to a cell culturing device. The cell culturing device may comprise a hydrogel compartment which accommodates a hydrogel.

The cell culturing device may further comprise an inner reservoir compartment which may be delimited or defined by an inner reservoir wall structure. The inner reservoir compartment may comprise a top opening and a bottom opening being oppositely arranged to the top opening. The bottom opening of the inner reservoir compartment may open, e.g. directly open, into the hydrogel compartment. Thus, in certain embodiments, the inner reservoir compartment may be arranged above, in particular directly above the hydrogel compartment. It may thus be the case that the hydrogel is arranged adjacent the bottom opening. The top opening and the bottom opening of the inner reservoir compartment may be configured, respectively arranged, such that the hydrogel in the hydrogel accommodation compartment is accessible from the top opening. Accordingly, the hydrogel may be arranged such in the hydrogel compartment that it is directly accessible from the top opening, e.g. via the bottom opening. In certain embodiments, the hydrogel may be directly arranged below the bottom opening. That is, the bottom opening is arranged between the hydrogel and the inner reservoir compartment, respectively an inner reservoir compartment interior space. In particular embodiments, the top opening and the bottom

opening of the inner reservoir compartment and optionally the hydrogel, may be configured, respectively arranged, such that the hydrogel in the hydrogel accommodation compartment is accessible along a linear path from the top opening.

The cell culturing device may further comprise at least one pair, e.g. two, opposing lateral reservoir compartments. The opposing lateral reservoir compartments of the at least one pair may encompass the inner reservoir compartment and may therefore also at least partially be delimited by the inner reservoir wall structure. The inner reservoir wall structure may in particular separate each of the opposing lateral reservoir compartments from the inner reservoir compartment and/or may be arranged there between. Although it may in some embodiments be the case that the cell culturing device comprises only one pair of such opposing lateral reservoir compartments, it may in some embodiments also be possible that it comprises more than one pair of opposing lateral reservoir compartments as described herein. By virtue of the at least two opposing lateral reservoir compartments and the inner reservoir compartment a highly physiological environment is provided for tissue development.

Since the opposing lateral reservoir compartments encompass the inner reservoir compartment, the inner reservoir and the opposing lateral reservoir compartments may be arranged in a common 2D horizontal plane. Such a horizontal plane is arranged perpendicular to an axis extending, in particular perpendicularly, through the center of the bottom opening and/or the center of the top opening of the inner reservoir compartment. In some embodiments, the opposing lateral reservoir compartments may be arranged on the same height as the inner reservoir compartment. In particular embodiments, at least one or all of the lateral reservoir compartments may have a height which is larger than the height of the hydrogel compartment. In certain embodiments at least one or all of the lateral reservoir compartments may have a height which is equal to the sum of the height of the hydrogel compartment and a portion or the complete height of the inner reservoir compartment. It is understood that the height of a compartment is its extension in the vertical direction. The vertical direction may extend in the direction of the gravitational force vector and/or along the axis extending, in particular perpendicularly, through the center of the

bottom opening and/or the center of the top opening of the inner reservoir compartment. Since the pair of opposing lateral reservoir compartments encompass the inner reservoir compartment, a relatively large volume of the lateral reservoir compartments can be realized. Further, sufficient gas exchange is readily possible which is crucial for cell culturing.

In some embodiments, the cell culturing device may comprise a cell culturing device blank, as described in any of the embodiments herein and for example the hydrogel.

Each of the opposing lateral reservoir compartments has a height, i.e. extension between the bottom section and the opening of the lateral reservoir compartment of more than 1000 µm, in particular more than 3000 µm, more particular more than 4000 µm.

In some embodiments, the height of the hydrogel compartment and/or the hydrogel is at least 500 µm, in particular at least 600 µm, more particular at least 700 µm. The height of the hydrogel compartment and/or the hydrogel extends along the vertical direction and may for example be the distance between the bottom section and the bottom opening of the inner reservoir compartment.

In some embodiments, the volume of the hydrogel and/or the hydrogel compartment may be between 1 mm^3 to 500 mm^3, in particular 2 mm^3 to 100 mm^3.

In some embodiments, a contact area of the hydrogel being configured to be contacted by medium from the opposing lateral reservoir compartments is more than 40%, in particular more than 50%, more particular more than 65%, even more particular more than 75%, of the peripheral surface area of the hydrogel. The peripheral surface area of the hydrogel is the surface area of the peripheral surface, i.e. the surface extending essentially along the vertical direction and thus not in the horizontal direction.

In some embodiments, the hydrogel compartment and/or the hydrogel has an elongated horizontal cross-sectional shape in particular an elongated round horizontal cross-sectional

shape, such as an elliptic shape. An elongated shape means that the ratio of length : width is larger than 1, in particular larger than 1.5. In some embodiments, the hydrogel compartment and/or the hydrogel has an angular horizontal cross-sectional shape in particular a polygonal horizontal cross sectional shape having at least 3, in particular at least 4, more particular at least 5 sides and/or corners. In some embodiments, the polygonal horizontal cross sectional shape has at most 8, in particular at most 6, more particular at most 4 sides and/or corners. For example, the hydrogel compartment and/or the hydrogel may have a rectangular horizontal cross sectional shape. The horizontal cross section is perpendicular to the vertical direction and may for example extend in parallel to the bottom section and/or the bottom opening of the inner reservoir compartment. Polygonal hydrogel compartments or hydrogels are advantageous as compared to round, in particular circular, cross sectional shapes, because round cross sectional shapes are inefficient for medium diffusion. It has been observed that the diffusion rate through hydrogels having a round horizontal cross sectional shape is not high enough to support cells in the center of the hydrogel with enough nutrients from the fresh medium, slowly diffusing from the opposing lateral reservoir compartments. In contrast, polygonal, in particular trigonal, rectangular or pentagonal horizontal cross- sectional shapes show much higher diffusion rates due to their geometry. Therefore, cells being arranged in the center are provided with sufficient nutrients to allow ideal and constant cell growth over the hydrogel, such as on its surface.

In some embodiments, the maximum horizontal width (i.e. the maximum distance between two opposing peripheral points along the horizontal cross section) of the hydrogel compartment and/or the hydrogel is less than 4 mm, in particular less than 3 mm, more particular less than 2.5 mm. In some embodiments, the maximum horizontal width of the hydrogel compartment and/or the hydrogel is at least 0.5 mm, in particular at least 1 mm, more particular at least 1.5 mm.

In some embodiments, the opposing lateral reservoir compartments of each pair are configured such that they are not directly connected with each other. That is, they do not merge into each other. In certain embodiments, the opposing lateral reservoir compartments of each pair are configured such that they are only indirectly connected with

each other via the hydrogel, respectively the hydrogel compartment. In other words, the opposing lateral reservoir compartments of each pair are configured such that medium exchange between the opposing lateral reservoir is (with the exception of taking medium in and out via their openings) only possible via the hydrogel, or channels through the hydrogel, respectively the hydrogel compartment. This separation via the hydrogel allows to add different media into the opposing lateral reservoir compartments and allows to study their diffusion and distribution. For example, cells can be added in one reservoir, while a compound of interest acting as chemical stimulus added to another, opposite reservoir compartment.

The hydrogel may comprise a surface which faces and/or is exposed towards the inner reservoir compartment, in particular via the bottom opening. The surface may in some embodiment be even, respectively planar, or it may comprise, or in some embodiments consist of, a topography, such as described in some embodiments herein. The surface may face and/or may be exposed towards the inner reservoir compartment, in particular via the bottom opening. In other words, when viewing from the top opening onto the bottom opening, one views onto the visible surface.

In certain embodiments, the hydrogel may comprise a topography, in particular a microtopography, with a plurality of cavities and optionally a plurality of protrusions. For example, the surface of the hydrogel may comprise or consist of the topography, in particular microtopography. The microtopography may face and/or may be exposed towards the inner reservoir compartment, in particular via the bottom opening. In other words, when viewing from the top opening onto the bottom opening, one views onto the topography, in particular microtopography. The topography, particularly the microtopography, may be accessible from the inner reservoir compartment. A topography as described may describe a surface of the hydrogel which comprises a plurality of cavities. It may therefore be considered as an uneven or non-planar surface. The topography, in particular microtopography allows self-organization of cells within a 3D tissue. Further, the topography resembles closely in vivo tissue and its geometry and further allows to dispense with a membrane. These cavities have in the case of a microtopography an opening from

which the cavity starts and extends with a diameter or an open area in the micrometer range, e.g. in a range of less than 1000 µm, in particular 1 to 1000 µm, respectively less than 1000 μm^2, particularly 1 to 1000 μm^2.

In certain embodiments, the hydrogel fills the hydrogel compartment completely.

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.

The inner compartment may in some embodiments only comprise the top and bottom opening and thus no additional openings. For example, the inner reservoir wall structure may encompass the inner reservoir compartment, in particular laterally and completely. In certain embodiments, the top and bottom opening are arranged above one another and/or may be aligned with each other and/or may be coaxially arranged to each other.

The opposing lateral reservoir compartments may be laterally arranged to both the inner reservoir compartment and the hydrogel compartment. In some embodiments a height of each of the opposing lateral reservoir compartments is equal to the combined heights of the hydrogel compartment and the inner reservoir compartment.

In certain embodiments, the opposing lateral reservoir compartments and the hydrogel compartment may together form a U-shape in cross section, which preferably encompasses the inner reservoir compartment.

In some embodiments, the cell culturing device and/or the cell culturing device blank as described herein is devoid of an external membrane. For example, there is no membrane between the inner reservoir compartment and the hydrogel compartment, respectively the hydrogel.

In some embodiments, the cell culturing device comprises a bottom section which delimits the opposing lateral reservoir compartments and the hydrogel compartment. In certain embodiments, at least parts of the hydrogel are arranged between the inner reservoir wall structure and the bottom section. In particular, parts of the hydrogel may completely fill the space between the bottom section and the inner reservoir wall structure. In some embodiments, the bottom section is a plate, such as a common plate forming the bottom section of the opposing lateral reservoir compartments and the hydrogel compartment. It may also be possible that the bottom section is part of a laboratory vessel, such as for example a petri dish. The bottom section may for example be mounted to the inner reservoir wall structure and/or the outer wall structure, e.g. in a releasable or fixed connection, or they may only be in contact, e.g. loose contact, with each other, for example in that the inner reservoir wall structure and/or the outer wall structure rests on the bottom section. It may also be possible that the bottom section is not part of the cell culturing device. For example, a cell culturing device as described herein may form together with a bottom section a cell culturing assembly. As an example, a cell culturing device may be placed on a bottom section to form the cell culturing assembly.

In certain embodiments, the cell culturing device comprises an outer wall structure. The outer wall structure may form the peripheral lateral delimitation of the cell culturing device. In particular embodiments, the outer wall structure may be arranged such that each of the opposing lateral reservoir compartments are arranged between the outer wall structure and the inner wall structure.

The outer wall structure may in some embodiments be in contact with the bottom section of the cell culturing device. In particular, the bottom section may be connected, e.g. releasably connected, to the outer wall structure.

It is understood that each of the opposing lateral reservoir compartments (and thus also the lateral active and/or lateral passive reservoir compartments) may comprise an opening, in particular only one opening, which allows accessing the interior of the opposing lateral reservoir compartments from the outside environment. Such an opening may be considered

as a lateral reservoir compartment opening. The openings of the opposing lateral reservoir compartments may be arranged on the same side, and in particular on the same height, of the cell culturing device as the top opening of the inner reservoir compartment. In certain embodiments, the opening of each lateral reservoir compartment may have a larger open area than the bottom opening of the inner reservoir compartment. In some embodiments, each lateral reservoir compartment may be configured such that their bottom section is accessible, in particular along a linear pathway from their opening. Opposing lateral reservoir compartments comprising such openings have the advantage that medium exchange is facilitated and can even be performed automatically by robotic pipetting device. Further, the openings allow efficient gas exchange, which is crucial for cell culturing, thereby cell growth and viability is improved.

In certain embodiments, the open area of the opening of each lateral reservoir compartment may be between 10 mm^2 to 500 mm^2, in particular 20 mm^2 to 200 mm^2.

Openings as described above allow readily to access the lateral reservoir compartments.

In some embodiments, the opening of each lateral reservoir compartment is arranged above, e.g. vertically offset and in particular only vertically offset to, the bottom section of the lateral reservoir compartment. In particular, the opening of each lateral reservoir compartment is configured such that at least 50%, in particular at least 70%, more particular at least 90%, even more particular at least 95% of the bottom section of the lateral reservoir compartment are uncovered. That means that no wall portion is vertically arranged above these uncovered portions of the bottom section, and/or the open area defined by the opening of the lateral reservoir compartment is arranged vertically above these uncovered portions of the bottom section.

In some embodiments, the openings of the lateral reservoir compartments are arranged in parallel to the bottom section and/or are coaxially arranged with the bottom section (of the lateral reservoir compartment).

In some embodiments at least some or even all of the opening of the lateral reservoir compartments and/or the top opening of the inner reservoir compartment are uncovered and open to the environment.

In some embodiments, the opposing lateral side reservoirs have a cylinder shape. Such a cylinder shape may be a regular or irregular cylinder shape. A regular cylinder has a regular ground surface, such as a circle (circular cylinder) or a rectangle (prism), or an irregular shape, and it has a cylinder shell extending, in particular evenly and/or linearly, between the top and ground surface. In particular embodiments, the top surface of the cylinder shape is the opening of the corresponding lateral reservoir compartment and the ground surface is the bottom section of the corresponding lateral reservoir compartment.

The top opening and the bottom opening as well as optionally the openings of the lateral reservoir compartments may in some embodiments be, respectively may be considered as, a 2D plane.

In some embodiments, the open area defined by the top opening is equal or larger than the open area defined by the bottom opening.

In some embodiments, the top opening and/or the bottom opening may be round, in particular circular. In alternative embodiments, the top opening and the bottom opening may be angular.

In some embodiments, the hydrogel is arranged such in the hydrogel compartment that the opposing lateral reservoir compartments and the inner reservoir compartment are only indirectly in fluidic connection with each other via the hydrogel, that is, either through the hydrogel itself or through channels protruding the hydrogel. The hydrogel may in some embodiments separate the inner reservoir compartment and the opposing lateral reservoir compartments from each other. The term fluidic connection as used herein may in certain embodiments refer in particular to a liquid fluidic connection.

In some embodiments, the hydrogel completely fills the hydrogel compartment (optionally with the exception of channel protruding through the hydrogel).

In some embodiments, the inner reservoir wall structure comprises an inner ledge. In certain embodiments, the inner ledge delimits the bottom opening, in particular peripherally and completely. In certain embodiments, the inner ledge is arranged between the inner reservoir compartment and the hydrogel. In particular embodiments, the hydrogel contacts the inner ledge. An inner ledge is advantageous, because it maintains the hydrogel in place, in particular against undesired removal of the hydrogel towards the inner reservoir compartment. Thus, the inner ledge retains the hydrogel in the hydrogel compartment. This is particularly advantageous if a casting insert is used as it is described herein in certain embodiments of the methods for producing a cell culturing device. The inner ledge helps to maintain the hydrogel in place when the casting insert is removed. In addition, the inner ledge can serve as a resting surface onto which the casting insert can be rested during production of the cell culturing device as described herein. The inner ledge can improve the quality of device production and the device itself, because it ensures that the hydrogel has a constant thickness and that the hydrogel of multiple devices having been produced by the same method is constant and reproducible.

In some embodiments, the inner reservoir wall structure comprises a vertical inner wall section. The vertical inner wall section typically extends perpendicular to the 2D planes defined by the top opening and/or the bottom opening. The vertical inner wall section may have an inner surface which faces to, respectively delimits the inner reservoir compartment. The vertical inner wall section may also have one or more outer surfaces which each face to, respectively delimit at least one of the opposing lateral reservoir compartments.

It may for example be possible that the inner ledge is adjacently arranged to the vertical inner wall section and is angled thereto, in particular in an angle of <180° to 90°, in particular 150° to 90°.

In some embodiments, the inner reservoir wall structure comprises a vertical inner wall section and an adjacently thereto arranged inclined inner wall section. The vertical inner wall section and the inclined inner wall section may at least partially delimit at least one of the opposing lateral reservoir compartments. It may also be possible that each lateral reservoir compartment is at least partially delimited by such a vertical inner wall section and inclined inner wall section. Such an inclined inner wall section has the advantage that air being present in the corresponding lateral reservoir compartment can be easily vented out and is not entrapped in the lateral reservoir compartment. In some embodiments, the inner reservoir wall section does not contain a vertical inner wall section but it may still comprise an inclined inner wall section, which is inclined towards the vertical direction of the cell culturing device. As noted herein, the vertical direction may be aligned with the gravitational force vector and/or it may be perpendicular to the top opening of the inner reservoir compartment. For example, the inclined inner wall section may in cross section be arranged in an angle of between 10° to 80°, in particular 25° to 60°, more particular 30° to 50°, to the vertical direction.

It may in some embodiments also be possible that the cell culturing device comprises one or more venting channels. For example, at least one or all of the lateral reservoir compartments may be connected to such a venting channel. For example, an opening of the venting channel may open into the lateral reservoir compartments. Such a venting channel can be arranged such that during filling of the corresponding lateral reservoir compartment (for example with a liquid medium), any remaining gas in the lateral reservoir compartment is vented out of the lateral reservoir compartment through the venting channel. The venting channel may therefore open towards the outer environment.

In some general embodiments, the opposing lateral reservoir compartments may be configured such that during filling of the corresponding lateral reservoir compartments (for example with a liquid medium), any remaining gas in the lateral reservoir compartments is vented out of the lateral reservoir compartments. Thereby, entrapping air in the lateral reservoir compartments is avoided.

In some embodiments, the inclined inner wall section may form an inner angle (i.e. an angle encompassing a part of the inner reservoir wall structure) of between 90° and 180°, in particular between >90° and <180°, such as 100° to 170°, or 110° to 160°, or 120° to 150°.

In some embodiments, the inclined inner wall section is configured such that a cross-sectional open area of the at least one lateral reservoir compartment which is at least partially delimited by the inclined inner wall section decreases towards the hydrogel compartment. This means that the cross-section (i.e. a plane which is parallel to the vertical direction) decreases, in particular continuously decreases, towards the hydrogel compartment, respectively, the hydrogel.

It may for example be possible that the inclined inner wall section has a conical or frustoconical shape, e.g. a partial conical or partial frustoconical shape. It is understood that the tip of the cone or frustocone points towards the hydrogel compartment, respectively the hydrogel. Thereby a conical or frustoconical pathway of the lateral reservoir compartment towards the hydrogel compartment is provided. Thus, in some embodiments, at least one or all of the opposing lateral reservoir compartments may comprise a conical or frustoconical pathway or a partial conical or partial frustoconical pathway towards the hydrogel compartment. A partial conical shape is a section of a conus, in particular a section along a plane in parallel to or even along the rotational axis of the conus. The same applies to a partial frustoconical shape.

In some embodiments, the inclined inner wall section is arranged between the vertical inner wall section and the inner ledge. The inclined inner wall section may particularly be inclined to the inner ledge and the vertical inner wall section. In particular embodiments, the inner ledge may be angled with respect to the vertical inner wall section, e.g. by an angle of 80° to 100°, in particular 85° to 95°, more particular essentially 90°.

In some embodiments, the inner reservoir wall structure comprises one or more support structures which laterally encompass the hydrogel or parts of the hydrogel. These support structures may for example be a plurality of pillars. In certain embodiments, the support

structures may protrude from the inner ledge, in particular towards the bottom section. In some embodiments, the support structures may extend up to the bottom section and thus be in contact with the bottom section. In other embodiments, the support structures may extend towards the bottom section, but not up to the bottom section and therefore form a gap between themselves and the bottom section. In some embodiments, the one or more support structures may have a height (i.e. extension along the vertical direction, respectively from the inner reservoir wall structure towards the bottom section) of 90% or less of the height of the hydrogel compartment and/or the hydrogel.

In some embodiments, the inner reservoir wall structure comprises a plurality of support structures. The support structures may in particular be spaced apart from each other and define a gap or a plurality of gaps between each other. In certain embodiments, the gap between adjacent support structures may be at least 700 µm, in particular more than 800 µm. In some embodiments, the one or more support structures are arranged around the bottom opening. They may also be spaced apart from the bottom opening. Support structures as described are advantageous, as they provide further fastening of the hydrogel and help to maintain the hydrogel in place, in particular against lateral displacement towards the lateral reservoir compartments. This is particularly advantageous during filling the hydrogel precursor into the hydrogel compartment, as it allows to maintain the hydrogel precursor inside the hydrogel compartment. In certain embodiments, the one or more support structures are radially arranged around the bottom opening.

In some embodiments, the inner reservoir structure encompasses the periphery of the hydrogel. In certain embodiments, at least 50%, in particular at least 65%, more particular at least 75%, even more particular at least 80%, of the peripheral surface area of the hydrogel is exposed and therefore not covered (i.e. uncovered) by the one or more support structures. Such embodiments are advantageous, because although the hydrogel is efficiently maintained at its position, a relatively large surface area remains available for media and compound diffusion. The peripheral surface area is the surface area of the peripheral surface, i.e. the surface extending essentially along the vertical direction and thus not in the horizontal direction.

In some embodiments, the one or more support structures may define the lateral, i.e. peripheral, delimitation of the hydrogel compartment.

In embodiments in which the cell culturing device comprises a hydrogel delivery port (as described further below), the support structures are preferably arranged such that they encompass the hydrogel delivery port, respectively its delivery outlet into the hydrogel compartment.

In some embodiments, the inner reservoir wall structure separates the opposing lateral reservoir compartments from the inner reservoir compartment, in particular such that they are only fluidically indirectly connected via the hydrogel compartment and/or the hydrogel.

In some embodiments, the hydrogel compartment and/or the hydrogel is arranged between the opposing lateral reservoir compartments.

In certain embodiments, the hydrogel compartment and/or the hydrogel may be encompassed by and/or fluidically connected with the inner reservoir compartment and the opposing lateral reservoir compartments.

In some embodiments, the topography, in particular microtopography, is configured such that each cavity is devoid of an undercut. In some embodiments, the cavities may have the shape of a dome or a sectional sphere, or of a cylinder, in particular a circular cylinder.

In some embodiments, each cavity may have a cavity depth of 1 to 1000 µm. In some embodiments, each cavity may have a cavity volume of 1 to 100000 μm^3.

In some embodiments, each cavity may have a cavity opening, i.e. an opening from which the cavity extends, defining an open area of 1 to 100000 μm^2.

In certain embodiments, the topography, in particular the microtopography comprises at least 1 cavity, in particular at least 10 cavities, in particular at least 20 cavities, more particular at least 50 cavities.

In some embodiments, the cell culturing device further comprises a hydrogel delivery port which opens towards the hydrogel compartment. The hydrogel delivery port may be configured to deliver a hydrogel or a hydrogel precursor into the hydrogel compartment. In some embodiments, the hydrogel delivery port comprises a delivery outlet which opens directly into the hydrogel compartment. In some embodiments, the hydrogel delivery port has a port inlet via which the hydrogel or hydrogel precursor can be filled into the hydrogel delivery port. In some embodiments, the port inlet is arranged adjacent to the top opening of the inner reservoir compartment and/or is arranged on the same side of the cell culturing device blank as the top opening of the inner reservoir compartment. In certain embodiments, the port inlet may be offset, in particularly laterally offset to the top opening. In certain embodiments, the port inlet is delimited by the inner reservoir wall structure.

In some embodiments, the cell culturing device further comprises a hydrogel buffer compartment. The hydrogel buffer compartment may be configured to receive hydrogel and/or hydrogel precursor during filling of the hydrogel compartment. The hydrogel compartment may open, in particular directly open, into the hydrogel compartment. For example, the hydrogel buffer compartment may comprise a hydrogel buffer compartment opening via which the hydrogel buffer compartment is connected to the hydrogel compartment. In particular embodiments, the hydrogel buffer compartment is separate from the hydrogel delivery port. For example, it may not be directly connected with the hydrogel delivery port, but both the hydrogel delivery port and the hydrogel buffer compartment open into the hydrogel compartment. The hydrogel buffer compartment may in some embodiments additionally comprise a venting opening being configured to vent out air from the hydrogel buffer compartment to the outside environment during filling of the hydrogel compartment, respectively the hydrogel buffer compartment. Typically, the venting opening is different from the hydrogel buffer compartment opening. In certain embodiments, the hydrogel buffer compartment may be oppositely arranged to the hydrogel delivery port

and/or the delivery outlet of the hydrogel delivery port may be oppositely arranged to the hydrogel buffer compartment opening. The hydrogel buffer compartment may in certain embodiments have a volume of 1 to 100 µL, in particular 2 to 20 µL.

In some specific embodiments, a cell culturing device may comprise:

- a hydrogel compartment accommodating a hydrogel;

- an inner reservoir compartment being delimited by an inner reservoir wall structure, the inner reservoir compartment comprising a top opening and an oppositely arranged bottom opening, wherein the bottom opening opens into the hydrogel compartment and wherein the top opening and the bottom opening are configured such that the hydrogel is accessible from the top opening, wherein the inner reservoir wall structure comprises an inner ledge which delimits the bottom opening and/or which is arranged between the inner reservoir compartment and the hydrogel, wherein preferably the hydrogel (3) contacts the inner ledge (14);

- at least one pair of opposing lateral reservoir compartments, the opposing lateral reservoir compartments encompassing the inner reservoir compartment and each opening into the hydrogel compartment such that the hydrogel is in fluid communication with each lateral reservoir compartment;

- a hydrogel delivery port which opens towards the hydrogel compartment and being configured for delivering a hydrogel or a hydrogel precursor into the hydrogel compartment;

wherein the hydrogel comprises a surface which faces and/or is exposed towards the inner reservoir compartment, in particular via the bottom opening.

In some embodiments, the opposing lateral reservoir compartments of the at least one pair of opposing lateral reservoir compartments are lateral passive reservoir compartments. This

means that there may for example be no active transport of medium or compounds from these lateral passive reservoir compartments through the hydrogel, but that such medium or compounds can only passively diffuse through the hydrogel. Therefore, the hydrogel may for example be configured such that the transport of medium and/or compounds from any of the lateral passive reservoir compartments through the hydrogel may be controlled solely by their diffusion rate through the hydrogel. In some embodiments, the hydrogel may be devoid of channels which open into any of the lateral passive reservoir compartments, such that medium or any compounds can be exchanged between the lateral passive reservoir compartments only by diffusion through the hydrogel (with the exception of taking medium in and out via their openings). Since the hydrogel mimics the ECM, this can be an advantageous model system for studying the effect of a certain compound on the diffusion behavior for other compounds through the ECM (i.e. the hydrogel). In these embodiments, the cell culturing device comprises at least one pair of, or optionally only one pair of, opposing lateral reservoir compartments which are in this case lateral passive reservoir compartments.

In some embodiments, the opposing lateral reservoir compartments of the at least one pair of opposing lateral reservoir compartments are lateral active reservoir compartments. In contrast to lateral passive reservoir compartments, an active transport through the hydrogel may for example be possible, e.g. by means of channels protruding through the hydrogel. Such channels may for example connect the two lateral active reservoir compartments with each other. It may additionally or alternatively be possible that one or more channels connect one or two of the lateral active reservoirs with the inner reservoir compartment. Thus, such channels typically protrude through the hydrogel and open into at least two reservoir compartments, i.e. reservoir compartments selected from the two lateral active reservoir compartments and the inner reservoir compartment. In some embodiments, the hydrogel delimits one or more channels which extend between the two lateral active reservoir compartments and open in each of the two lateral active reservoir compartments. The channels may preferably configured for active medium exchange between the lateral active reservoir compartments. Active medium exchange means that in contrast to passive medium exchange being diffusion controlled, that the medium can flow between at least two

reservoir compartments being connected by such a channel. In certain embodiments, there may even be a pump unit which may be configured to actively pump medium through the one or more channels. It may also be possible to induce a gravity driven flow through these channels, for example by tilting the cell culturing device or by providing inclined channels. In the described embodiments, the cell culturing device comprises at least one pair of, or optionally only one pair or, opposing lateral reservoir compartments which are in this case lateral active reservoir compartments.

It may in some embodiments also be possible to have more than one pair of opposing lateral reservoir compartments. Typically two opposing lateral reservoir compartments form a reservoir compartment pair. In some embodiments there may for example be four lateral reservoir compartments, i.e. two pairs of opposing lateral reservoir compartments each consisting of two opposing lateral reservoir compartments. In certain embodiments, it may be possible that the cell culturing device has a pair of opposing lateral active reservoir compartments and a pair of opposing lateral passive reservoir compartments. Thus in such embodiments, the hydrogel mimicking the ECM can be accessed from 5 sides, namely from the inner reservoir compartment, the two lateral active reservoir compartments and the two lateral passive reservoir compartments. It may however also be possible that the cell culturing device has a first pair of opposing lateral passive reservoir compartments and a second pair of opposing lateral passive reservoir compartments. It may also be possible that the cell culturing device has a first pair of opposing lateral active reservoir compartments and a second pair of opposing lateral active reservoir compartments.

The one or more channels delimited by the hydrogel may in some embodiments have a cross-sectional open area of between 10 μm^2 to 1 mm^2, in particular 20 μm^2 to 0.8 mm^2.

In some embodiments, the one or more channels may from a network of channels. In particular embodiments, the network of channels may comprise one or more of channel crossings, recesses, protrusions and/or branchings. A channel having for example recesses and/or protrusions may be used to mimic certain internal organ structures. A network of channels can for example mimic a network of microvasculature and lymphatic vessels and

it is for example possible to observe compound migration or liquid flow through these vessels.

In some embodiments, the inner reservoir compartment may have a volume of 0.01 to 10 mL in particular 0.05 to 0.5 mL. In some embodiments, the opposing lateral reservoir compartments may each have a volume of 0.01 to 10 mL in particular 0.05 to 0.5 mL. Such large volume capacities are beneficial, because they allow prolonged use of the medium being present in the reservoir compartments without depletion and therefore decrease the frequency of required medium exchange operations.

In some embodiments, the hydrogel comprises, or consists of, a polymer selected from polysaccharides, gelatinous proteins, agarose, alginate, chitosan, dextran, gelatin, laminins, collagens, hyaluronan, fibrin, a crosslinked synthetic hydrophilic polymer, in particular being functionalized with an extracellular matrix (ECM)-derived protein or peptide, and mixtures thereof. The crosslinked synthetic hydrophilic polymer may in some embodiments be selected from the group comprising: poly(ethylene glycol), polyoxazoline, polyaliphatic polyurethane, polyether polyurethane, polyester polyurethane, polyethylene copolymer, polyamide, polyvinyl alcohol, poly(ethylene oxide), polypropylene oxide, polypropylene glycol, polytetramethylene oxide, polyvinyl pyrrolidone, polyacrylamide, poly(hydroxy ethyl acrylate), poly(hydroxyethyl methacrylate) and mixtures or co-polymers thereof. In some embodiments, the hydrogel comprises basement membrane-like matrix (Matrigel), a human-based extracellular matrix (such as Myogel).

A second aspect of the present disclosure relates to a cell culturing system. Such a cell culturing system may comprise a plurality of cell culturing devices as described in any of the embodiments herein, in particular with respect to the first aspect. For example, such a cell culturing system may comprise at least 2, at least 4, at least 10, at least 20, at least 50 at least 90 or at least 100 cell culturing devices. In particular embodiments, the cell culturing system may be configured as a well plate. Such a well plate may comprise all the cell culturing devices and allows for conducting several screens in parallel under different

conditions. In particular, the cell culturing devices may be arranged in a matrix of rows and columns.

A third aspect of the present disclosure refers to a method for producing a cell culturing device, in particular a cell culturing device as described with respect to any of the embodiments herein, such as the embodiments of the first aspect.

The method may comprise the step of providing a cell culturing device blank. The cell culturing device blank may particularly be a cell culturing device blank as described in any of the embodiments herein. It is understood that the cell culturing device blank differs from the cell culturing device in that it does not yet contain a hydrogel inside the hydrogel compartment. In some embodiments however, this may be the only difference between the cell culturing device and the cell culturing device blank. Thus, any of the embodiments described herein with respect to the cell culturing device may also apply to the cell culturing device blank. The cell culturing device blank may comprise a hydrogel compartment, an inner reservoir compartment being delimited by an inner reservoir wall structure, and a pair of opposing lateral reservoir compartments, the lateral reservoir compartments encompassing the inner reservoir compartment and each opening into the hydrogel compartment. The inner reservoir compartment may comprise a top opening and an oppositely arranged bottom opening. The bottom opening may open into the hydrogel compartment and the top opening and the bottom opening (as well as optionally the inner reservoir compartment) may both be configured such that the hydrogel compartment is accessible from the top opening, in particular along a linear path from the top opening to the bottom opening.

The method may also comprise the step of introducing a casting insert through the top opening into the inner reservoir compartment. The casting insert may be introduced such that it covers the bottom opening, in particular completely covers the bottom opening. In certain embodiments, the casting insert may comprise a casting surface. In particular embodiments, the casting insert may be introduced such into the inner reservoir

compartment that its casting surface is exposed towards, respectively faces, the hydrogel compartment.

In particular embodiments, the casting surface of the casting insert may comprise a topography, in particular a microtopography. which has a plurality of protrusions and optionally cavities. In some embodiments, the casting insert may be introduced such into the inner reservoir compartment that its topography, in particular microtopography, is exposed towards, respectively faces or protrudes even into, the hydrogel compartment. The casting insert may for example at least partially protrude through the bottom opening.

The casting insert may in particular comprise a base portion comprising the casting surface, particularly the topography, respectively microtopography, and a rod, in particular a straight rod, being mounted on a side of the base portion opposite of the casting surface. For example, the casting insert may be considered as a stamp. The casting surface may correspond to the surface of the hydrogel which faces and/or is exposed towards the inner reservoir compartment, in particular via the bottom opening. In other words, the casting surface of the casting insert serves as a negative for the surface of the hydrogel.

The method may further comprise the step of filling a hydrogel precursor into the hydrogel compartment. In particular, the hydrogel precursor may be filled such into the hydrogel compartment that the hydrogel precursor contacts the casting insert, in particular its casting surface. Thereby and after curing, the surface of the hydrogel corresponding to the casting surface is generated.

The method may further comprise the step of curing the hydrogel precursor to form a hydrogel which comprises a surface which faces and/or is exposed towards the inner reservoir compartment, in particular via the bottom opening. This surface of the hydrogel may correspond to the casting surface of the casting insert.

The method may further comprise the step of removing the casting insert through the top opening. Thereby, the cell culturing device may be provided.

In some embodiments, the casting surface may comprise or consist of a topography, in particular microtopography, with a plurality of protrusions. The topography, respectively microtopography, of the casting insert corresponds to the topography, respectively microtopography, of the hydrogel. In other words, the topography respectively microtopography, of the casting insert serves as a negative for the topography respectively microtopography, of the hydrogel. Therefore, each protrusion of the topography respectively microtopography, of the casting insert results in a cavity of the topography respectively microtopography, of the hydrogel and/or any cavity of the topography respectively microtopography, of the casting insert, results in a protrusion of the topography respectively microtopography, of the hydrogel.

The casting insert may in some embodiments be introduced through the top opening into the inner reservoir compartment such that the casting insert covers the bottom opening and such that the topography in particular, microtopography of the casting insert is exposed to and/or protrudes into the hydrogel compartment. When the hydrogel precursor is filled in the hydrogel compartment, the hydrogel precursor is filled such into the hydrogel compartment that the hydrogel precursor encompasses the protrusions of the microtopography of the casting insert. Curing of the hydrogel precursor may then form a hydrogel comprising a microtopography with a plurality of cavities.

Curing may in some general embodiments comprise a chemical transformation. For example, curing may comprise hardening and/or polymerizing. For example, curing can comprise, or consist of, light treatment, such as UV irradiation, heating and/or cooling.

In some embodiments, the inner reservoir wall structure of the cell culturing device blank comprises an inner ledge. The inner ledge may be an inner ledge, i.e. the same, as described with respect to the corresponding embodiments of the cell culturing device according to the first aspect of the disclosure. The inner ledge may delimit the bottom opening and/or may be arranged between the inner reservoir compartment and the hydrogel compartment. In particular embodiments, the inner ledge may form the peripheral delimitation of the bottom opening.

In some embodiments, the casting insert is introduced such into the inner reservoir compartment that it rests on the inner ledge of the inner reservoir wall structure. In particular the casting insert may be introduced such that it seals the bottom opening. It is understood that the casting insert and the cell culturing device blank are preferably configured such that the casting insert can rest on the inner ledge and completely cover the bottom opening. Further, the casting insert and the cell culturing device may be configured such that the casting surface, particularly topography or microtopography, of the casting insert faces and/or protrudes into the hydrogel compartment when the casting insert rests on the inner ledge.

In some embodiments, the hydrogel precursor may be filled in such a way into the hydrogel compartment that a central part of the hydrogel precursor after being filled into the hydrogel compartment is arranged below the casting insert and the bottom opening, and a peripheral part of the hydrogel precursor is arranged below the inner ledge of the inner reservoir wall structure. In particular embodiments, the hydrogel precursor may be filled in such a way into the hydrogel compartment that the inner ledge is arranged between the hydrogel precursor, in particular the peripheral part of the hydrogel precursor, and the inner reservoir compartment. The terms “peripheral part” and “central part” refer to areas of the hydrogel precursor after it has been filled into the hydrogel compartment. The peripheral part encompasses, in particular circumferentially encompasses the central part along its lateral periphery. The peripheral part and the central part may have a cylinder shape. The central part may typically be arranged directly below the bottom opening, respectively the casting insert and its topography, and the peripheral part may be arranged below the inner ledge.

In some embodiments, one or more support structures protrude from the inner ledge. The support structures may for example be the same as described herein with respect to the first aspect of the disclosure. During filling the hydrogel precursor into the hydrogel compartment, the one or more support structures may retain the hydrogel precursor in the hydrogel compartment.

In some embodiments, the cell culturing device blank comprises a hydrogel delivery port opening towards the hydrogel compartment. The hydrogel delivery port may for example be the same as described herein, e.g. with respect to the first aspect of the disclosure and/or the eleventh aspect. The hydrogel precursor may be provided into the hydrogel compartment via the hydrogel delivery port during filling. In particular, the hydrogel delivery port may have a port inlet from which the hydrogel precursor may be directly provided into the hydrogel compartment, e.g. via a delivery port outlet of the hydrogel delivery port. The delivery port outlet may be encompassed by support structures as described in the embodiments herein. Thereby it is ensured that the hydrogel precursor is introduced in the center and flows from there towards the support structures, which then prevent the hydrogel precursor from continuing to flow, in particular into the opposing lateral reservoir compartments.

In some embodiments, the opposing lateral reservoir compartments of the pair of opposing lateral reservoir compartments are lateral active reservoir compartments as described for example with respect to the embodiments of the first aspect. One or more channels may be formed which extend through the cured hydrogel and open into each of the two lateral active reservoir compartments. Forming the one or more channels can be performed after curing the hydrogel precursor. In particular, this may for example be achieved by depleting or degrading portions of the cured hydrogel. Suitable methods may for example be photo-degradation, such as laser ablation or light induced depolymerization, or chemical degradation. Alternatively, the channels may be formed already during the filling of the hydrogel precursor, for example by suitable mold inserts. For example, rods, such as micro-rods, may be introduced into the cell culturing device blank before the hydrogel precursor is filled into the hydrogel compartment. These may then be removed after curing to generate the channels.

In some embodiments, the hydrogel precursor comprises cells to be cultivated, i.e. before it is filled into the hydrogel compartment. This has the advantage that the behavior of cells under certain conditions can be monitored within the hydrogel. For example, their diffusion, growth and/or emission of signaling substances into thy hydrogel can be monitored. The

cells may for example be suspended in the hydrogel precursor prior to filling the hydrogel precursor into the hydrogel compartment.

In general, the cells used herein may be living cells. They may for example stem from human or animal donors. It may in some embodiments be possible that the cells are stem cells.

A fourth aspect of the disclosure relates to a kit of parts. The kit of parts may for example be used in the method according to any of the embodiments of the third aspect of the present disclosure.

The kit may comprise a cell culturing device blank. In particular a cell culturing device blank as described herein, such as with respect to the embodiments of the third or eleventh aspect. The kit may alternatively comprise a cell culturing blank system. In particular a cell culturing blank system as described herein, such as with respect to the embodiments of the twelfth aspect.

A cell culturing device blank may comprise a hydrogel compartment, an inner reservoir compartment being delimited by inner reservoir wall structure. Further, the cell culturing device blank may comprise at least one pair of opposing lateral reservoir compartments, the opposing lateral reservoir compartments encompassing the inner reservoir compartment and each opening into the hydrogel compartment. The inner reservoir compartment may comprise a top opening and an oppositely arranged bottom opening. The bottom opening may open into the hydrogel compartment. Further, the top opening and the bottom opening may be configured such that the hydrogel compartment is accessible from the top opening, in particular along a linear path from the top opening.

The kit may in some embodiments further comprise a casting insert. The casting insert may be a casting insert as described herein, for example with respect to the embodiments of the third aspect of the disclosure. The casting insert may comprise a casting surface. The casting insert may further be configured for being introduced through the top opening into

the inner reservoir compartment such that it covers the bottom opening. The casting insert and the cell culturing device blank may further be configured such that the casting surface topography, in particular microtopography, is exposed to the hydrogel compartment.

The kit of parts may optionally also contain a hydrogel precursor.

In some embodiments, the casting surface comprises or consists of a topography, in particular microtopography, with a plurality of protrusions. The casting insert may be configured for being introduced through the top opening into the inner reservoir compartment such that the casting insert covers the bottom opening and such that the topography, in particular microtopography, is exposed to and/or protrudes into the hydrogel compartment.

A fifth aspect of the present disclosure relates to a method for culturing cells with a cell culturing device, in particular with a cell culturing device as described herein, for example with respect to the embodiments of the first aspect. It may also be possible to perform the method with a cell culturing system as described herein, in particular with respect to the second aspect.

The method may comprise the step of providing a cell suspension comprising cells and a liquid medium in the inner reservoir compartment, in particular via the top opening.

The method may further comprise the step of allowing the cells to sediment onto the surface of the hydrogel, in particular into the plurality of cavities of the topography, in particular microtopography, of the hydrogel and culturing the cells. Sedimentation may be performed for a certain sedimentation time interval. The sedimentation time interval may for example be 1 to 15 min in particular 5 to 10 min. In some embodiments, the method may comprise centrifugation of the cell culturing device. Centrifugation may comprise sedimentation of the cells. By using such a centrifugation step, sedimentation can be accelerated.

The method may further comprise culturing the sedimented cells.

In some examples, the method according to the fifth aspect may resemble the formation of a cell barrier, such as an epithelial cell barrier, between an apical side (e.g. the top reservoir) and a basal side (e.g. the hydrogel and optionally the opposing lateral reservoir compartments). Thus, targeted exposure to compounds of interest, such as drugs can independently assessed on the basal and apical side.

In some embodiments, the majority (i.e. at least 50 vol.%, e.g. at least 80 vol.%, e.g. at least 90 vol.%, e.g. at least 95 vol.%) or all of the liquid medium of the cell suspension is removed from the inner reservoir compartment after the cells have sedimented, in particular via the top opening. This may for example be performed by a suitable cannula or syringe.

After the liquid medium has been removed, a different medium, e.g. a cell culturing medium, preferably a liquid cell culturing medium, can be added into the inner reservoir compartment and onto the surface, e.g. topography, respectively microtopography, of the hydrogel and the sedimented cells.

Alternatively, after the liquid medium has been removed, a hydrogel precursor can be filled into the inner reservoir compartment covering the sedimented cells. Subsequently, the hydrogel precursor can be cured, thereby forming a hydrogel. This hydrogel may be considered as an inner reservoir hydrogel, because it is present in the inner reservoir compartment. The hydrogel precursor can be the same as the hydrogel precursor as used in the embodiments of the third aspect, e.g. the production of the cell culturing device, or it can be different therefrom. In some embodiments, therefore, the inner reservoir hydrogel may be made of the same material than the hydrogel in the hydrogel compartment or it may be made of a different material. The inner reservoir hydrogel may thus completely enclose the sedimented cells (together with the hydrogel in the hydrogel compartment). This allows to establish a 3D tissue culture.

In some embodiments, one or more of the opposing lateral reservoir compartments can be filled with a medium. This medium may be the same for all opposing lateral reservoirs or it may be different. It is also possible that at least one of the lateral reservoir compartments

is not filled with a medium, i.e. it is left empty and may only comprise air. The term "medium" as used herein preferably relates to a liquid or solid medium. Thus, it is possible for example to observe the development of a medium gradient from one lateral reservoir compartment to its opposing other lateral reservoir compartment or from/to the inner reservoir compartment to one of the lateral reservoir compartments.

A sixth aspect of the present disclosure relates to another method for culturing cells with a cell culturing device, in particular with a cell culturing device as described herein, for example with respect to the embodiments of the first aspect. It may also be possible to perform the method with a cell culturing system as described herein, in particular with respect to the second aspect.

The method may comprise the step of providing a cell suspension comprising cells and a liquid medium in at least one or both of the two lateral active reservoir compartments and into the one or more channels. Providing the cell suspension into the channels may for example be performed by tilting the cell culturing device or by applying a pressure gradient between the two lateral active reservoir compartments into which the one or more channels open or by applying a pressure gradient between the at least one of the lateral active reservoir compartments into which the one or more channels opens and the inner reservoir compartment into which a channel may open.

The method may further comprise culturing the cells inside the one or more channels. This may generally comprise growing the cells inside and/or along the channels.

In some embodiments, prior to providing the cell suspension in at least one or both of the two lateral active reservoir compartments, medium being present in the lateral active reservoir compartment(s) may first be removed.

In some embodiments, it may also be possible that the cell suspension comprises as the liquid medium or also instead of the liquid medium, a hydrogel precursor in which the cells are suspended. Preferably then, this hydrogel precursor is cured after the cell suspension

has been provided inside the channels, thereby, a hydrogel is formed inside the channels, which encompasses the cells, optionally completely and/or together with the hydrogel being present in the hydrogel accommodation compartment. Thereby a 3D tissue culture is formed. The hydrogel precursor can be the same as the hydrogel precursor as used in the embodiments of the third aspect, e.g. the production of the cell culturing device, or it can be different therefrom. In some embodiments, therefore, the hydrogel formed inside the channels may be made of the same material as the hydrogel in the hydrogel compartment or it may be made of a different material.

After the cell suspension has been introduced into the channels or after or during culturing of the cells inside the one or more channels, the opposing lateral reservoir compartments and/or the inner reservoir compartment can be filled with a medium. This medium may be the same for all opposing lateral reservoirs and inner compartment or it may be different. It is also possible that at least one of the lateral reservoir compartments and the inner reservoir compartment is not filled with a medium, i.e. it is left empty and may only comprise air. The term “medium” as used herein preferably relates to a liquid or solid medium. Thus, it is possible for example to observe the development of a medium gradient from one lateral reservoir compartment to its opposing other lateral reservoir compartment or from/to the inner reservoir compartment to one of the lateral reservoir compartments.

A seventh aspect of the present disclosure relates to another method for culturing cells with a cell culturing device, in particular with a cell culturing device as described herein, for example with respect to the embodiments of the first aspect. It may also be possible to perform the method with a cell culturing system as described herein, in particular with respect to the second aspect.

The method may comprise the step of providing a cell suspension comprising cells and a liquid medium in at least one or both of the opposing lateral reservoir compartments of the at least one pair. The opposing lateral reservoir compartments may be lateral active reservoir compartments and/or lateral passive reservoir compartments, such as the ones described herein, e.g. with respect to the first aspect of the disclosure.

The method may further comprise the step of allowing the cells to sediment to a bottom section of the lateral reservoir compartment(s). Sedimentation may be performed for a certain sedimentation time interval. The sedimentation time interval may for example be 1 to 15 min in particular 5 to 10 min.

The cells, in particular the sedimented cells, may be cultured on the bottom section of the lateral reservoir compartment(s).

After the cell suspension has been introduced in at least one or both of the two opposing lateral reservoir compartments, any of the remaining opposing lateral reservoir compartments and/or the inner reservoir compartment can be filled with a medium. This medium may be the same for all opposing lateral reservoirs and inner compartment or it may be different. It is also possible that at least one of the lateral reservoir compartments and the inner reservoir compartment is not filled with a medium, i.e. it is left empty and may only comprise air.

In some embodiments, the majority (i.e. at least 50 vol.%, e.g. at least 80 vol.%, e.g. at least 90 vol.%, e.g. at least 95 vol.%) or all of the liquid medium of the cell suspension is removed after the cells have sedimented from the at least one or both of the two opposing lateral reservoir compartments of the at least one pair. This may for example be performed by a suitable cannula or syringe.

After the liquid medium has been removed, a different medium, e.g. a cell culturing medium, preferably a liquid cell culturing medium, can be added into the at least one or both of the two opposing lateral reservoir compartments of the at least one pair and onto the sedimented cells.

Alternatively, after the liquid medium has been removed, a hydrogel precursor can be filled into the at least one or both of the two opposing lateral reservoir compartments of the at least one pair covering the sedimented cells. Subsequently, the hydrogel precursor can be cured, thereby forming a hydrogel. The hydrogel precursor can be the same as the hydrogel

precursor as used in the embodiments of the third aspect, e.g. the production of the cell culturing device, or it can be different therefrom. In some embodiments, therefore, this hydrogel may be made of the same material than the hydrogel in the hydrogel compartment or it may be made of a different material. This hydrogel may thus completely enclose the sedimented cells (together with the hydrogel in the hydrogel compartment). This allows to establish a 3D tissue culture.

In some embodiments, it may also be possible that the cell suspension comprises as the liquid medium or also instead of the liquid medium, a hydrogel precursor in which the cells are suspended. Preferably then, this hydrogel precursor is cured after the cell suspension has been provided into the at least one or both of the two opposing lateral reservoir compartments of the at least one pair, thereby, a hydrogel, which encompasses the cells, optionally completely and/or together with the hydrogel being present in the hydrogel accommodation compartment. Thereby a 3D tissue culture is formed. The hydrogel precursor can be the same as the hydrogel precursor as used in the embodiments of the third aspect, e.g. the production of the cell culturing device, or it can be different therefrom. In some embodiments, therefore, the hydrogel formed in the at least one or both of the two opposing lateral reservoir compartments of the at least one pair may be made of the same material than the hydrogel in the hydrogel compartment or it may be made of a different material.

An eight aspect of the present disclosure relates to another method for culturing cells with a cell culturing device, in particular with a cell culturing device as described herein, for example with respect to the embodiments of the first aspect. It may also be possible to perform the method with a cell culturing system as described herein, in particular with respect to the second aspect.

The method may comprise the step of providing a cell suspension comprising a hydrogel precursor and cells in at least one or both of the two opposing lateral reservoir compartments of the at least one pair, in particular in at least one of the two lateral active reservoir compartment and/or the two lateral passive reservoir compartments.

The method may further comprise curing the hydrogel precursor to form a hydrogel.

In addition, the method may comprise culturing the cells inside the cured hydrogel.

The hydrogel precursor can be the same as the hydrogel precursor as used in the embodiments of the third aspect, e.g. the production of the cell culturing device, or it can be different therefrom. In some embodiments, therefore, this hydrogel may be made of the same material than the hydrogel in the hydrogel compartment or it may be made of a different material. This hydrogel may thus completely enclose the cells. This allows to establish a 3D tissue culture.

In some embodiments, the hydrogel precursor is either cured before the cells sediment to a bottom section of the lateral reservoir compartments. Alternatively, it may also be possible that the hydrogel is cured after the cells sediment to a bottom section of the lateral reservoir compartments.

In general in all embodiments of the methods described herein, in particular with respect to any of the embodiments of the fifth to eight aspect, the cells of the cell suspension may in certain embodiments be living cells. They may for example stem from human or animal donors. It may in some embodiments be possible that the cells are stem cells.

In a ninth aspect, the present disclosure relates to a cellular chip. Such a cellular chip may for example comprise a cell culturing device according to any of the embodiments as described herein, for example a cell culturing device according to the embodiments of the first aspect. The cellular chip may alternatively comprise a cell culturing system according to any of the embodiments as described herein, for example a cell culturing device according to the embodiments of the second aspect.

In addition, a cellular chip according to the ninth aspect may comprise cells being cultured in one or more selected from the hydrogel, onto the surface of the hydrogel, into the cavities of the topography, in particular microtopography, of the hydrogel, the inner reservoir

compartment, at least one of the opposing lateral reservoir compartments, in particular the lateral passive reservoir compartment and the lateral active reservoir compartments, and the one or more channels.

Such a cellular chip may therefore be ready to be used as a lab-on-a-chip device in a drug assay. Again, the cells of the cell suspension may in certain embodiments be living cells. They may for example stem from human or animal donors. It may in some embodiments be possible that the cells are stem cells.

In some embodiments, the cellular chip may be obtained by any of the method as described herein for culturing cells, such as the methods according to the fifth to eight aspect of the disclosure.

In a tenth aspect, the present disclosure relates to the use of a cellular chip, in particular a cellular chip as described in any of the embodiments herein, e.g. the embodiments of the ninth aspect. The cellular chip may be used for analyzing an interaction between the cultured cells and a compound of interest. The compound of interest may for example be a virus, a microorganism such as bacterium or fungus, or a drug such as a protein, peptide, antibody, nucleic acid, small molecule or larger molecule or antibody drug conjugates. AS used herein, a small molecule is a molecule having a molar mass of less than 1000 Da and a large molecule is a molecule having a molar mass of 1000 Da or more. Particular examples of large molecules are for example proteins and proteins aggregates, enzymes, antigens or antibodies.

In some embodiments, the compound of interest is provided into one or more selected from the hydrogel, onto the surface of the hydrogel, into the cavities of the microtopography of the hydrogel, the inner reservoir compartment, at least one of the opposing lateral reservoir compartments, in particular the lateral passive reservoir compartment and the lateral active reservoir compartments, and the one or more channels.

The use may further comprise monitoring cell status, such as vitality, monitoring cell metabolism, cell movement, cell growth, permeability, in particular permeability through cell barriers.

The use may further comprise monitoring cell signaling, such as paracrine, juxtacrine or endocrine signaling.

The use may further comprise the monitoring of the location, transport and/or movement of the compound of interest, for example, its change of location form the inner reservoir compartment to one of the opposing lateral reservoir compartments.

In an eleventh aspect, the present disclosure further relates to a cell culturing device blank. This cell culturing device blank may be one being used in the production of a cell culturing device, such as the embodiments described with respect to the third aspect. Vice versa, this cell culturing device blank may be used in the production of a cell culturing device.

The cell culturing device blank may comprise a hydrogel compartment being configured to be filled with a hydrogel. Typically, the hydrogel compartment may therefore be filled with air.

The cell culturing device blank may further comprise an inner reservoir compartment. The inner reservoir compartment may be delimited by an inner reservoir wall structure, The inner reservoir compartment may further comprise a top opening and an oppositely arranged bottom opening. The bottom opening may open into the hydrogel compartment. The top opening and the bottom opening (and optionally also the inner reservoir compartment) may be configured such that the hydrogel compartment is accessible from the top opening, in particular along a linear path from the top opening.

The cell culturing device blank may further comprise at least one pair of opposing lateral reservoir compartments, the opposing lateral reservoir compartments encompassing the

inner reservoir compartment. In particular, each of the opposing lateral reservoir compartments open, particularly directly, into the hydrogel compartment.

In some embodiments, the opposing lateral reservoir compartments may be arranged on the same height as the inner reservoir compartment. In particular embodiments, at least one or all of the lateral reservoir compartments may have a height which is larger than the height of the hydrogel compartment. In certain embodiments at least one or all of the lateral reservoir compartments may have a height which is equal to the sum of the height of the hydrogel compartment and a portion or the full height of the inner reservoir compartment. It is understood that the height of a compartment is its extension in the vertical direction. The vertical direction may extend in the direction of the gravitational force vector and/or along the axis extending, in particular perpendicularly, through the center of the bottom opening and/or the center of the top opening of the inner reservoir compartment. Since the pair of opposing lateral reservoir compartment encompass the inner reservoir compartment, a relatively larger volume of the lateral reservoir compartments can be realized. Further, gas exchange is readily possible which is crucial for cell culturing.

Each of the opposing lateral reservoir compartments has a height, i.e. extension between the bottom section and the opening of the lateral reservoir compartment of more than 1 mm, in particular more than 2 mm, more particular more than 3 mm.

In some embodiments, the height of the hydrogel compartment is at least 500 µm, in particular at least 600 µm, more particular at least 700 µm. The height of the hydrogel compartment extends along the vertical direction and may for example be the distance between the bottom section and the bottom opening of the inner reservoir compartment.

In some embodiments, the volume of the hydrogel compartment may be between 1 to 100 mm^3, in particular 2 to 10 mm^3.

In some embodiments, the hydrogel compartment has an elongated horizontal cross-sectional shape in particular an elongated round horizontal cross-sectional shape, such as

an elliptic shape. An elongated shape means that the ratio of length : width is larger than 1, in particular larger than 1.5. In some embodiments, the hydrogel compartment has an angular horizontal cross-section in particular a polygonal horizontal cross section having at least 3, in particular at least 4, more particular at least 5 sides and/or corners. In some embodiments, the polygonal horizontal cross section has at most 8, in particular at most 6, more particular at most 4 sides and/or corners. For example, the hydrogel compartment may have a rectangular horizontal cross section. The horizontal cross-section is perpendicular to the vertical direction and may for example extend in parallel to the bottom section and/or the bottom opening of the inner reservoir compartment.

In some embodiments, the maximum horizontal width (i.e. the maximum distance between two opposing peripheral points along the horizontal cross section) of the hydrogel compartment is less than 4 mm, in particular less than 3 mm, more particular less than 2.5 mm. In some embodiments, the maximum horizontal width of the hydrogel compartment and/or is at least 0.5 mm, in particular at least than 1 mm, more particular at least 1.5 mm.

In some embodiments, the opposing lateral reservoir compartments of each pair are configured such that they are not directly connected with each other. In certain embodiments, the opposing lateral reservoir compartments of each pair are configured such that they are only indirectly connected with each other via the hydrogel compartment. In other words, the opposing lateral reservoir compartments of each pair are configured such that medium exchange between the opposing lateral reservoir is (with the exception of taking fluid in and out of the openings) only possible via the hydrogel, respectively the hydrogel compartment. In certain embodiments, the opposing lateral reservoir compartments of each pair are configured such that they are only indirectly connected with each other via the hydrogel compartment.

In some embodiments, the inner reservoir wall structure comprises an inner ledge. In particular embodiments, the inner ledge may delimit the bottom opening. In some embodiments, the inner ledge may be arranged between the inner reservoir compartment and the hydrogel compartment.

In some embodiments, the inner reservoir wall structure comprises a vertical inner wall section and an adjacently thereto arranged inclined inner wall section. The vertical inner wall section and the inclined inner wall section may at least partially delimit at least one of the opposing lateral reservoir compartments. It may also be possible that each lateral reservoir compartment is at least partially delimited by such a vertical inner wall section and inclined inner wall section. Such an inclined inner wall section has the advantage that air being present in the corresponding lateral reservoir compartment can be easily vented out and is not entrapped in the lateral reservoir compartment. In some embodiments, the inner reservoir wall section does not contain an vertical inner wall section but it may still comprise an inclined inner wall section, which is inclined towards the vertical direction of the cell culturing device. As noted herein, the vertical direction may be aligned with the gravitational force vector and/or it may be perpendicular to the top opening of the inner reservoir compartment. For example, the inclined inner wall section may in cross section be arranged in an angle of between 10° to 80°, in particular 25° to 60°, more particular 30° to 50°, to the vertical direction.

It may in some embodiments also be possible that the cell culturing device blank comprises one or more venting channels. For example, at least one or all of the lateral reservoir compartments may be connected to such a venting channel, e.g. an opening of the venting channel may open into lateral reservoir compartments. Such a venting channel can be arranged such that during filling of the corresponding lateral reservoir compartment (for example with a liquid medium), any remaining gas in the lateral reservoir compartment is vented out of the lateral reservoir compartment through the venting channel. The venting channel may therefore open towards the outer environment.

In some general embodiments, the opposing lateral reservoir compartments may be configured such that during filling of the corresponding lateral reservoir compartment (for example with a liquid medium), any remaining gas in the lateral reservoir compartment is vented out of the lateral reservoir compartment. Thereby, entrapping air in the lateral reservoir compartment is avoided.

In some embodiments, the inclined inner wall section may form an inner angle (i.e. an angle encompassing a part of the inner reservoir wall structure) of between 90° and 180°, in particular between >90° and <180°, such as 100° to 170°, or 110° to 160°, or 120° to 150°.

In some embodiments, the inclined inner wall section is configured such that a cross-sectional open area of the lateral reservoir compartment which is at least partially delimited by the inclined inner wall section decreases towards the hydrogel compartment. This means that the cross-section (i.e. a plane which is parallel to the vertical direction) decreases, in particular continuously decreases, towards the hydrogel compartment.

It may for example be possible that the inclined inner wall section has a conical or frustoconical shape, e.g. a partial conical or partial frustoconical shape. It is understood that the tip of the cone or frustocone points towards the hydrogel compartment. Thereby a conical or frustoconical pathway of the lateral reservoir compartment towards the hydrogel compartment is provided. Thus, in some embodiments, at least one or all of the opposing lateral reservoir compartments may comprise a conical or frustoconical pathway or a partial conical or partial frustoconical pathway towards the hydrogel compartment. A partial conical shape is a section of a conus, in particular a section along a plane in parallel to or even along the rotational axis of the conus. The same applies to a partial frustoconical shape.

In some embodiments, the inclined inner wall section is arranged between the vertical inner wall section and the inner ledge. The inclined inner wall section may particularly be inclined to the inner ledge and the vertical inner wall section. In particular embodiments, the inner ledge may be angled with respect to the vertical inner wall section, e.g. by an angle of 80° to 100°, in particular 85° to 95°, more particular essentially 90°.

In some embodiments, the inner reservoir wall structure comprises one or more support structures which laterally encompass the hydrogel compartment or parts of the hydrogel compartment. The one or more support structures may the same as the one being described with respect to the corresponding embodiments of the first aspect of the disclosure. For

example in some embodiments, the one or more support structures may protrude from the inner ledge.

In some embodiments, the inner reservoir wall structure separates the two opposing lateral reservoir compartments from the inner reservoir compartment, in particular such that they are only fluidically indirectly connected via the hydrogel compartment.

In some embodiments, the hydrogel compartment is arranged between the two opposing lateral reservoir compartments.

In some embodiments, the hydrogel compartment is encompassed by and/or fluidically connected with the inner reservoir compartment and the two opposing lateral reservoir compartments.

In some embodiments, the cell culturing device blank further comprises a hydrogel delivery port. The hydrogel delivery port may open towards the hydrogel compartment and may be configured for delivering a hydrogel or a hydrogel precursor into the hydrogel compartment. In some embodiments, the hydrogel delivery port comprises a delivery outlet which opens directly into the hydrogel compartment.

In some embodiments, the hydrogel delivery port has a port inlet via which the hydrogel or hydrogel precursor can be filled into the hydrogel delivery port. The hydrogel delivery port may be arranged such that the hydrogel or hydrogel precursor can be directly delivered from the port inlet into the hydrogel compartment.

In some embodiments, the port inlet is arranged adjacent to the top opening of the inner reservoir compartment and/or is arranged on the same side of the cell culturing device blank as the top opening of the inner reservoir compartment. In certain embodiments, the port inlet may be offset, in particularly laterally offset to the top opening. In certain embodiments, the port inlet is delimited by the inner reservoir wall structure.

In some embodiments, the cell culturing device blank further comprises a hydrogel buffer compartment. The hydrogel buffer compartment may be configured to receive hydrogel and/or hydrogel precursor during filling of the hydrogel compartment. The hydrogel compartment may open, in particular directly open, into the hydrogel compartment. For example, the hydrogel buffer compartment may comprise a hydrogel buffer compartment opening via which the hydrogel buffer compartment is connected to the hydrogel compartment. In particular embodiments, the hydrogel buffer compartment is separate from the hydrogel delivery port. For example, it may not be directly connected with the hydrogel delivery port, but both the hydrogel delivery port and the hydrogel buffer compartment open into the hydrogel compartment. The hydrogel buffer compartment may in some embodiments additionally comprise a venting opening being configured to vent out air from the hydrogel buffer compartment to the outside environment during filling of the hydrogel compartment, respectively the hydrogel buffer compartment. Typically, the venting opening is different from the hydrogel buffer compartment opening. In certain embodiments, the hydrogel buffer compartment may be oppositely arranged to the hydrogel delivery port and/or the delivery outlet of the hydrogel delivery port may be oppositely arranged to the hydrogel buffer compartment opening. The hydrogel buffer compartment may in certain embodiments have a volume of 1 to 100 µL, in particular 2 to 20 µL.

In certain specific embodiments, a cell culturing device blank comprises:

- a hydrogel compartment being configured to be filled with a hydrogel;

- an inner reservoir compartment being delimited by an inner reservoir wall structure, wherein the inner reservoir compartment comprises a top opening and an oppositely arranged bottom opening, wherein the bottom opening opens into the hydrogel compartment and wherein the top opening and the bottom opening are configured such that the hydrogel compartment is accessible from the top opening; wherein the inner reservoir wall structure comprises an inner ledge which delimits the bottom opening and/or which is arranged between the inner reservoir compartment and the hydrogel compartment;

- at least one pair of opposing lateral reservoir compartments, the opposing lateral reservoir compartments encompassing the inner reservoir compartment and each opening into the hydrogel compartment;

- a hydrogel delivery port opening towards the hydrogel compartment and being configured for delivering a hydrogel or a hydrogel precursor into the hydrogel compartment.

In some embodiments, the cell culturing device blank comprises an additional pair of opposing lateral reservoir compartments which encompass the inner reservoir compartment and which each open into the hydrogel compartment such that the hydrogel compartment is in fluid communication with each of the opposing lateral reservoir compartments of the additional pair. Thus, it may in some embodiments also be possible to have more than two opposing lateral reservoir compartments. Typically two opposing lateral reservoir compartments form a reservoir compartment pair. In some embodiments there may for example be four lateral reservoir compartments, i.e. two pairs of opposing lateral reservoir compartments each pair consisting of two opposing lateral reservoir compartments. In certain embodiments, it may be possible that the cell culturing device has a first pair of opposing lateral reservoir compartments and a second pair of opposing lateral reservoir compartments. Thus in such embodiments, the hydrogel compartment can be accessed from 5 sides, namely from the inner reservoir compartment and the four lateral reservoir compartments.

In a twelfth aspect, the present disclosure relates to a cell culturing blank system. The cell culturing blank system may for example comprise a plurality of cell culturing device blanks as described in any of the embodiments herein, such as the embodiments of the eleventh aspect. For example, such a cell culturing blank system may comprise at least 2, at least 4, at least 10, at least 20, at least 50 at least 90 or at least 100 cell culturing device blanks.

In some embodiments, the cell culturing blank system may be configured as a well plate. Such a well plate may comprise all the cell culturing device blanks.

In a thirteenth aspect, the present disclosure relates to a method for producing a cell culturing device blank, in particular a cell culturing device blank as disclosed in any of the embodiments herein. The method may in particular comprise injection molding.

In some embodiments, the method may comprise the steps of:

- Providing a mold assembly;

- Injecting a molten material, in particular a molten polymer or a molten metal, into the mold assembly;

- Curing the injected molten material to form the cell culturing device blank and optionally removing the cured cell culturing device blank from the mold assembly.

In some embodiments, the mold assembly comprises a bottom portion and top portion, wherein the top portion comprises a mold top protrusion being configured to form the inner reservoir compartment of the cell culturing device blank; and wherein the bottom portion comprises at least on pair of opposing lateral mold bottom protrusions being configured to form the at least one pair of opposing lateral reservoir compartments of the cell culturing device blank.

It is understood that in this or any other embodiment described herein, the mold assembly and in particular the top portion and bottom portion of the mold assembly, is configured such that it forms a molding void having the shape of the cell culturing device blank. That is, the mold assembly may has a shape being inverted, respectively being the negative of the shape of the cell culturing device blank as described herein, in particular with respect to any of the embodiments of the eleventh aspect. The mold assembly may be configured to form the cell culturing device blank as described herein, in particular with respect to any of the embodiments of the eleventh aspect. The top portion and the bottom portion may be configured to be moved apart from each other and may be configured to form together the mold assembly.

In a fourteenth aspect, the present disclosure relates to a mold assembly as described herein, e.g. with respect to any of the embodiments of the thirteenth aspect.

In a fifteenth aspect, the present disclosure relates to a use of a mold assembly, in particular a mold assembly according to the fourteenth aspect, to manufacture a cell culturing device blank as described herein, in particular with respect to any of the embodiments of the eleventh aspect.

In the following, non-limiting examples of the present disclosure are provided:

A 1. example relates to a cell culturing device comprising:

- a hydrogel compartment accommodating a hydrogel;
- an inner reservoir compartment being delimited by an inner reservoir wall structure (5), the inner reservoir compartment comprising a top opening and an oppositely arranged bottom opening, wherein the bottom opening opens into the hydrogel compartment and wherein the top opening and the bottom opening are configured such that the hydrogel is accessible from the top opening;
- at least one pair of opposing lateral reservoir compartments, the opposing lateral reservoir compartments encompassing the inner reservoir compartment and each opening into the hydrogel compartment such that the hydrogel is in fluid communication with each lateral reservoir compartment;

wherein the hydrogel comprises a surface which faces and/or is exposed towards the inner reservoir compartment, in particular via the bottom opening.

A 2. example relates to the cell culturing device according to the 1. example, wherein the inner reservoir wall structure comprises an inner ledge which delimits the bottom opening

and/or which is arranged between the inner reservoir compartment and the hydrogel, wherein preferably the hydrogel contacts the inner ledge.

A 3. example relates to the cell culturing device according to the 1. or 2. example, wherein the inner reservoir wall structure comprises a vertical inner wall section and an adjacently thereto arranged inclined inner wall section, wherein the vertical inner wall section and the inclined inner wall section at least partially delimit at least one of the opposing lateral reservoir compartments.

A 4. example relates to the cell culturing device according to the 3. example, wherein the inclined inner wall section is configured such that a cross-sectional open area of the at least one lateral reservoir compartment being at least partially delimited by the inclined inner wall section decreases towards the hydrogel compartment.

A 5. example relates to the cell culturing device according to the 3. or 4. example, wherein the inclined inner wall section is arranged between the vertical inner wall section and the inner ledge.

A 6. example relates to the cell culturing device according to any of the previous examples, wherein each lateral reservoir compartment comprises an opening, wherein each lateral reservoir compartment is configured such that its bottom section is accessible along a linear pathway from its opening.

A 7. example relates to the cell culturing device according to any of the previous examples, wherein the inner reservoir wall structure comprises one or more support structures which laterally encompass the hydrogel or parts of the hydrogel.

A 8. example relates to the cell culturing device according to any of the 2. to 7. example, wherein the one or more support structures protrude from the inner ledge.

A 9. example relates to the cell culturing device according to any of the previous examples, wherein the inner reservoir wall structure separates the opposing lateral reservoir compartments from the inner reservoir compartment, in particular such that they are only fluidically indirectly connected via the hydrogel compartment and/or the hydrogel.

A 10. example relates to the cell culturing device according to any of the previous examples, wherein the hydrogel compartment is arranged between the opposing lateral reservoir compartments.

A 11. example relates to the cell culturing device according to any of the previous examples, wherein the hydrogel compartment and/or the hydrogel is encompassed by and/or fluidically connected with the inner reservoir compartment and the opposing lateral reservoir compartments.

A 12. example relates to the cell culturing device according to any of the previous examples, wherein the surface comprises a microtopography with a plurality of cavities, wherein the microtopography faces and/or is exposed towards the inner reservoir compartment, in particular via the bottom opening.

A 13. example relates to the cell culturing device according to the 12. example, wherein the microtopography is configured such that each cavity is devoid of an undercut.

A 14. example relates to the cell culturing device according to any of the previous examples, the cell culturing device further comprising a hydrogel delivery port opening towards the hydrogel compartment and being configured for delivering a hydrogel or a hydrogel precursor into the hydrogel compartment.

A 15. example relates to the cell culturing device according to any of the previous examples, wherein the cell culturing device further comprises hydrogel buffer compartment, the hydrogel buffer compartment opening into the hydrogel compartment and being in particular separate from the hydrogel delivery port.

A 16. example relates to the cell culturing device according to any of the previous examples, wherein the opposing lateral reservoir compartments of the pair of opposing lateral reservoir compartments are lateral passive reservoir compartments, wherein the hydrogel is devoid of channels which open into any of the lateral passive reservoir compartments such that compounds can be exchanged between the lateral passive reservoir compartments only by diffusion through the hydrogel.

A 17. example relates to the cell culturing device according to any of the examples 1 to 15, wherein the opposing lateral reservoir compartments of the pair of opposing lateral reservoir compartments are lateral active reservoir compartments, wherein the hydrogel delimits one or more channels extending between the two lateral active reservoir compartments and opening in each of the two lateral active reservoir compartments, wherein the one or more channels are configured for active medium exchange between the two lateral active reservoir compartments.

A 18. example relates to the cell culturing device according to the 17. example, wherein the cell culturing device additionally comprises a pair of opposing lateral passive reservoir compartments which encompass the inner reservoir compartment and which each open into the hydrogel compartment such that the hydrogel is in fluid communication with each lateral passive reservoir compartment, and wherein the hydrogel is devoid of channels which open into any of the lateral passive reservoir compartments such that compounds can be exchanged between the lateral passive reservoir compartments only by diffusion through the hydrogel.

A 19. example relates to the cell culturing device according to the 17. or 18. example, wherein the one or more channels have a cross-sectional open area of between 10 μm^2 to 1 mm^2, in particular 20 μm^2 to 0.8 mm^2.

A 20. example relates to the cell culturing device according to any of the 17. to 19. example, wherein the one or more channels form a channel network which in particular comprises one or more of channel crossings, recesses, protrusions and/or branchings.

A 21. example relates to a cell culturing system comprising a plurality of cell culturing devices according to any of the previous examples, wherein preferably the cell culturing system is a well plate.

A 22. example relates to a method for producing a cell culturing device, in particular a cell culturing device according to any of the 1. to 20. example, the method comprising the steps:

- Providing a cell culturing device blank, the cell culturing device blank comprising a hydrogel compartment, an inner reservoir compartment being delimited by an inner reservoir wall structure, and at least one pair of opposing lateral reservoir compartments encompassing the inner reservoir compartment and each opening into the hydrogel compartment, wherein the inner reservoir compartment comprises a top opening and an oppositely arranged bottom opening, wherein the bottom opening opens into the hydrogel compartment and wherein the top opening and the bottom opening are configured such that the hydrogel compartment is accessible from the top opening;

- Introducing a casting insert comprising a casting surface through the top opening into the inner reservoir compartment such that the casting insert covers the bottom opening and such that the casting surface is exposed to the hydrogel compartment;

- Filling a hydrogel precursor into the hydrogel compartment such that the hydrogel precursor contacts the casting surface of the casting insert;

- Curing the hydrogel precursor to form a hydrogel comprising a surface which faces and/or is exposed towards the inner reservoir compartment, in particular via the bottom opening;

- Removing the casting insert through the top opening, thereby providing the cell culturing device.

A 23. example relates to the method according to example 22, wherein the casting surface of the casting inserts comprises a microtopography with a plurality of protrusions and wherein the casting insert is introduced through the top opening into the inner reservoir compartment such that the casting insert covers the bottom opening and such that the microtopography is exposed to the hydrogel compartment; and wherein the hydrogel precursor is filled such into the hydrogel compartment that the hydrogel precursor encompasses the protrusions of the microtopography of the casting insert; and wherein curing the hydrogel precursor forms a hydrogel comprising a microtopography with a plurality of cavities.

A 24. example relates to the method according to examples 22 or 23, wherein the inner reservoir wall structure of the cell culturing device blank comprises an inner ledge which delimits the bottom opening and/or which is arranged between the inner reservoir compartment and the hydrogel compartment.

A 25. example relates to the method according to example 24 wherein the casting insert is introduced such into the inner reservoir compartment that it rests on the inner ledge of the inner reservoir wall structure.

A 26. example relates to the method according to example 24 or 25, wherein the hydrogel precursor is filled such into the hydrogel compartment that a central part of the hydrogel precursor is arranged below the casting insert and a peripheral part of the hydrogel precursor is arranged below the inner ledge of the inner reservoir wall structure.

A 27. example relates to the method according to any of examples 24 to 26, wherein one or more support structures protrude from the inner ledge and wherein during filling the hydrogel precursor into the hydrogel compartment, the one or more support structures retain the hydrogel precursor in the hydrogel compartment.

A 28. example relates to the method according to any of examples 22 to 27, wherein the cell culturing device blank comprises a hydrogel delivery port opening towards the hydrogel

compartment, wherein the hydrogel precursor is provided into the hydrogel compartment via the hydrogel delivery port during filling.

A 29. example relates to the method according to any of examples 22 to 28, wherein the opposing lateral reservoir compartments of the pair of opposing lateral reservoir compartments are lateral active reservoir compartments and wherein one or more channels are formed which extend through the cured hydrogel and open into each of the two lateral active reservoir compartments.

A 30. example relates to the method according to example 29, wherein the one or more channels are formed by depleting or degrading portions of the cured hydrogel, in particular by photo-degradation, such as laser ablation or light induced depolymerization, or by chemical degradation.

A 31. example relates to the method according to examples 22 to 30, wherein the hydrogel precursor comprises cells to be cultivated.

A 32. example relates to a kit of parts comprising:

- a cell culturing device blank, wherein the cell culturing device blank comprises a hydrogel compartment, an inner reservoir compartment being delimited by an inner reservoir wall structure, and at least one pair of opposing lateral reservoir compartments encompassing the inner reservoir compartment and each opening into the hydrogel compartment, wherein the inner reservoir compartment comprises a top opening and an oppositely arranged bottom opening, wherein the bottom opening opens into the hydrogel compartment and wherein the top opening and the bottom opening are configured such that the hydrogel compartment is accessible from the top opening; and

- a casting insert, wherein the casting insert comprises a casting surface and wherein the casting insert is configured for being introduced through the top opening into the

inner reservoir compartment such that the casting insert covers the bottom opening and such that the casting surface is exposed to the hydrogel compartment;

- optionally a hydrogel precursor.

A 33. example relates to the kit according to example 32, wherein the casting surface comprises a microtopography with a plurality of protrusions and wherein the casting insert is configured for being introduced through the top opening into the inner reservoir compartment such that the casting insert covers the bottom opening and such that the microtopography is exposed to the hydrogel compartment.

A 34. example relates to a method for culturing cells with a cell culturing device according to any of examples 1 to 20 or with a cell culturing system according to example 21, the method comprising the steps:

- Providing a cell suspension comprising cells and a liquid medium in the inner reservoir compartment, in particular via the top opening;

- Allowing the cells to sediment onto the surface of the hydrogel, in particular into the plurality of cavities of the microtopography of the hydrogel, and culturing the cells.

A 35. example relates to the method according to example 34, wherein after the cells have sedimented, the majority of the liquid medium is removed from the inner reservoir compartment and subsequently a hydrogel precursor is filled into the inner reservoir compartment covering the sedimented cells and wherein the hydrogel precursor is subsequently cured.

A 36. example relates to a method for culturing cells with a cell culturing device according to any of examples 1 to 20 or with a cell culturing system according to example 21, the method comprising the steps:

- Providing a cell suspension comprising cells and a liquid medium in at least one of the lateral active reservoir compartments of the pair of opposing lateral reservoir compartments and into the one or more channels, in particular by tilting the cell culturing device or by applying a pressure gradient between the lateral active reservoir compartments into which the one or more channels open;

- Culturing the cells inside the one or more channels.

A 37. example relates to a method for culturing cells with a cell culturing device according to any examples 1 to 20 or with a cell culturing system according to example 21, the method comprising the steps:

- Providing a cell suspension comprising cells and a liquid medium in at least one of the opposing lateral reservoir compartments;

- Allowing the cells to sediment to a bottom section of the at least one lateral reservoir compartment;

- Culturing the cells on the bottom section of the at least one lateral reservoir compartment.

A 38. example relates to a method for culturing cells with a cell culturing device according to any examples 1 to 20 or with a cell culturing system according to example 21, the method comprising the steps:

- Providing a cell suspension comprising a hydrogel precursor and cells in at least one of the opposing lateral reservoir compartments;

- Curing the hydrogel precursor to form a hydrogel;

- Culturing the cells inside the cured hydrogel.

A 39. example relates to the method according to example 38, wherein the hydrogel precursor is either cured before the cells sediment to a bottom section of the at least one lateral reservoir compartment or after the cells sediment to a bottom section of the at least one lateral reservoir compartment.

A 40. example relates to a cellular chip comprising a cell culturing device according to any of examples 1 to 20 or a cell culturing system according to example 21, the cellular chip further comprising cells being cultured in one or more selected from the hydrogel, on the surface of the hydrogel, in the cavities of the microtopography of the hydrogel, the inner reservoir compartment, at least one of the opposing lateral reservoir compartments, in particular the lateral passive reservoir compartments and/or the lateral active reservoir compartments, and the one or more channels.

A 41. example relates to the cellular chip according to example 40 having been obtained by any of the method according to any of examples 32 to 37.

A 42. example relates to a use of cellular chip according to examples 40 or 41 for analyzing an interaction between the cultured cells and a compound of interest, in particular a virus, a microorganism such as bacterium or fungus, or a drug such as a protein, peptide, antibody, nucleic acid, small molecule or antibody drug conjugates.

A 43. example relates to the use according to example 42, wherein the compound of interest is provided into one or more selected from the hydrogel, onto the surface of the hydrogel, into the cavities of the microtopography of the hydrogel, the inner reservoir compartment, at least one of the opposing lateral reservoir compartments, in particular the lateral passive reservoir compartments and/or the lateral active reservoir compartments, and the one or more channels.

A 44. example relates to a cell culturing device blank comprising:

- a hydrogel compartment being configured to be filled with a hydrogel;

- an inner reservoir compartment being delimited by an inner reservoir wall structure, wherein the inner reservoir compartment comprises a top opening and an oppositely arranged bottom opening, wherein the bottom opening opens into the hydrogel compartment and wherein the top opening and the bottom opening are configured such that the hydrogel compartment is accessible from the top opening;

- at least one pair of opposing lateral reservoir compartments encompassing the inner reservoir compartment and each opening into the hydrogel compartment.

A 45. example relates to the cell culturing device blank according to example 44, wherein the inner reservoir wall structure comprises an inner ledge which delimits the bottom opening and/or which is arranged between the inner reservoir compartment and the hydrogel compartment.

A 46. example relates to the cell culturing device blank according to examples 44 or 45, wherein the inner reservoir wall structure comprises a vertical inner wall section and an adjacently thereto arranged inclined inner wall section, wherein the vertical inner wall section and the inclined inner wall section at least partially delimit at least one of the opposing lateral reservoir compartments.

A 47. example relates to the cell culturing device blank according to example 46, wherein the inclined inner wall section is configured such that a cross-sectional open area of the at least one lateral reservoir compartment being at least partially delimited by the inclined inner wall section decreases towards the hydrogel compartment.

A 48. example relates to the cell culturing device blank according to example 46 or 47, wherein the inclined inner wall section is arranged between the vertical inner wall section and the inner ledge.

A 49. example relates to the cell culturing device blank according to any of examples 44 to 48, wherein the inner reservoir wall structure comprises one or more support structures

which laterally encompass the hydrogel compartment or at least parts of the hydrogel compartment.

A 50. example relates to the cell culturing device blank according to examples 45 and 49, wherein the one or more support structures protrude from the inner ledge.

A 51. example relates to the cell culturing device blank according to any of examples 44 to 50 wherein the inner reservoir wall structure separates the opposing lateral reservoir compartments from the inner reservoir compartment, in particular such that they are only fluidically indirectly connected via the hydrogel compartment.

A 52. example relates to the cell culturing device blank according to any of examples 44 to 51, wherein the hydrogel compartment is arranged between two opposing lateral reservoir compartments.

A 53. example relates to the cell culturing device blank according to any of examples 44 to 52, wherein the hydrogel compartment is encompassed by and/or fluidically connected with the inner reservoir compartment and the opposing lateral reservoir compartments.

A 54. example relates to the cell culturing device blank according to any of examples 44 to 53 wherein the cell culturing device blank further comprises hydrogel buffer compartment, the hydrogel buffer compartment opening into the hydrogel compartment and being in particular separate from the hydrogel delivery port.

A 55. example relates to the cell culturing device blank according to any of examples 44 to 54, the cell culturing device blank further comprising a hydrogel delivery port opening towards the hydrogel compartment and being configured for delivering a hydrogel or a hydrogel precursor into the hydrogel compartment.

A 56. example relates to the cell culturing device blank according to example 55, wherein the hydrogel delivery port has a port inlet and is arranged such that the hydrogel or hydrogel precursor can be directly delivered from the port inlet into the hydrogel compartment.

A 57. example relates to the cell culturing device blank according to example 55 or 56, wherein the port inlet is arranged adjacent to the top opening of the inner reservoir compartment(4) and/or is arranged on the same side of the cell culturing device blank as the top opening of the inner reservoir compartment.

A 58. example relates to the cell culturing device blank according to any of examples 44 to 57, wherein the cell culturing device blank comprises an additional pair of opposing lateral reservoir compartments which encompass the inner reservoir compartment and which each open into the hydrogel compartment such that the hydrogel compartment is in fluid communication with each of the opposing lateral reservoir compartments of the additional pair of opposing lateral reservoir compartments.

A 59. example relates to a cell culturing blank system comprising a plurality of cell culturing device blanks according to any of examples 44 to 58, wherein preferably the cell culturing blank system is a well plate.

A 60. example relates to a method for producing a cell culturing device blank according to any of examples 44 to 58, the method comprising the steps of

- Providing a mold assembly;
- Injecting a molten material into the mold assembly;
- Curing the injected molten material to form the cell culturing device blank.

A 61. example relates to the method according to example 60, wherein the mold assembly comprises a bottom portion and top portion, wherein the top portion comprises a mold top

protrusion being configured to form the inner reservoir compartment of the cell culturing device blank; and wherein the bottom portion comprises at least on pair of opposing lateral mold bottom protrusions being configured to form the at least one pair of opposing lateral reservoir compartments of the cell culturing device blank.

A 62. example relates to the method according to example 61, wherein the bottom portion further comprises at least one hydrogel delivery port protrusion being configured to form the hydrogel delivery port of the cell culturing device blank.

A 63. example relates to the method according to example 61 or 62, wherein the bottom portion further comprises one or more recesses being configured to form the one or more support structures of the cell culturing device blank.

A 64. example relates to a mold assembly forming a molding void having the shape of the cell culturing device blank according to any of examples 44 to 58.

Brief description of the figures

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

Fig. 1a a schematic perspective of a cell culturing device according to an embodiment of the invention;

Fig. 1b a sectional view of the cell culturing device of Fig. 1a along A-A;

Fig. 1c a sectional view of the cell culturing device of Fig. 1a along B-B;

Fig. 2a a perspective view of a cell culturing blank system according to an embodiment of the invention;

Fig. 2b a top view of the cell culturing blank system of Fig. 2a;

Fig. 2c a bottom view of the cell culturing blank system of Fig. 2a;

Fig. 3a a bottom view of a cell culturing device blank according to an embodiment of the invention and as it can be used in embodiments of the invention;

Fig. 3b a top view of the cell culturing device blank of Fig. 3a;

Fig. 3c a sectional view of the cell culturing device blank of Fig. 3b along C-C;

Fig. 4a a top view of a cell culturing device blank according to another embodiment of the invention and as it can be used in embodiments of the invention;

Fig. 4b a sectional view of the cell culturing device blank of Fig. 4a through lateral reservoir compartments 8 and 9;

Fig. 4c a sectional view of the cell culturing device blank of Fig. 4a through lateral reservoir compartments 10 and 11;

Fig. 4d a bottom view of the cell culturing device blank of Fig. 4a;

Fig. 5 a top view of a cell culturing system according to an embodiment of the invention;

Fig. 6a a top view of a cell culturing device blank according to another embodiment of the invention and as it can be used in embodiments of the invention;

Fig. 6b a sectional view along D-D of the cell culturing device blank of Fig. 6a;

Fig. 7 a perspective view of a cell culturing device blank according to another embodiment of the invention and as it can be used in embodiments of the invention;

Fig. 8a a perspective view of a mold bottom portion of a mold assembly as it can be used to produce a cell culturing device blank as described herein;

Fig. 8b a perspective view of a mold top portion of a mold assembly as it can be used to produce a cell culturing device blank as described herein;

Fig. 9 an illustrative process scheme of a method of preparing a cell culturing device and a cellular chip according to embodiments of the invention;

Fig. 10 a schematic sectional view of the cell culturing device according to another embodiment of the invention.

Exemplary embodiments

Figs. 1a to 1c show a cell culturing device 1 according to an embodiment of the present disclosure. Cell culturing device 1 comprises a centrally arranged inner reservoir compartment 4 which is laterally delimited by inner reservoir wall structure 5. As can be best seen from the sectional views of Fig. 1b and 1c, inner reservoir compartment 4 comprises a top opening 6 and an oppositely arranged bottom opening 7. Both the top opening and the bottom opening may be defined, respectively delimited, by inner reservoir wall structure 5. It can be seen that top opening 6 has a larger open area than bottom opening 7. Bottom opening 7 opens directly into hydrogel compartment 2 from inner reservoir compartment 4. Both top opening 6 and bottom opening 7 as well as inner reservoir compartment 4 are configured such that hydrogel compartment 2 is accessible from the top opening. This

means that for example a linear rod, such as a casting insert (see Fig. 9) can be inserted through top opening 6 and it is possible to access bottom opening 7 from there.

Hydrogel compartment 2 is arranged directly below the inner reservoir compartment 4. Further, bottom opening 7 directly opens into the hydrogel compartment. Hydrogel 3 is arranged inside the hydrogel compartment and fills it completely. It can further be seen from Fig. 1a and 1b that the hydrogel comprises a microtopography with a plurality of cavities, such as cavities 12 and 13 (it is noted that only two cavities are referenced and that the microtopography is not shown in Fig. 1a for clarity purposes). The cavities form recesses within hydrogel 3. It can further be seen that the microtopography faces the inner reservoir compartment 4 and is exposed towards inner reservoir compartment 4. Thus, cells can for example be introduced as a cell suspension into inner reservoir compartment 4. Allowing the cells to sediment will then provide such cells into the cavities of the microtopography.

Cell culturing device 1 further comprises four lateral reservoir compartments 8, 9, 10 and 11. Lateral reservoir compartment 8 and 9 form a first pair and are opposing each other and encompass inner reservoir compartment 4 as well as hydrogel compartment 2 and hydrogel 3. As can be seen from the sectional view shown in Fig. 1b, it is possible to access, i.e. contact, hydrogel 3 from both opposing lateral reservoir compartments 8 and 9. That is, a medium can for example be inserted into these opposing lateral reservoir compartments and this medium then comes in contact with hydrogel 3. It can be seen that the hydrogel 3 is devoid of any channels which open into any of opposing lateral reservoir compartments 8 and 9. Therefore, these two lateral reservoir compartments 8 and 9 are considered as lateral passive reservoir compartments. A medium which is introduced into any of these two lateral passive reservoir compartments can only be transported through hydrogel 3 by diffusion thereto. Thus, these two opposing lateral passive reservoir compartment 8 and 9 allow to observe the diffusion of a compound of interest through hydrogel 3 which may mimic an ECM. If in an assay a cell barrier is cultured on the surface of the hydrogel being exposed towards one of the lateral reservoir compartment 8 or 9, and this exposure leads to destruction of the barrier and leakage, a faster diffusion may for example be observed than if the barrier was intact.

It can be seen from both sectional view in Fig. 1b and 1c that the two opposing lateral reservoir compartments 8 and 9 together with hydrogel compartment 2 form a U-shape in cross section which encompasses inner reservoir compartment 4. Vice versa, also the two opposing lateral reservoir compartments 10 and 11 form together with hydrogel compartment 2 a U-shape in cross section which encompasses inner reservoir compartment 4. Opposing lateral reservoir compartments 10 and 11 form a second pair of opposing lateral reservoir compartments.

Lateral reservoir compartments 10 and 11 are also opposing each other and also encompass inner reservoir compartment 4, hydrogel compartment 2 and hydrogel 3. In contrast to lateral passive reservoir compartments 8 and 9 however, hydrogel 3 delimits channel 18 which opens into both lateral reservoir compartments 10 and 11 and provides a direct fluidic connection between them. In other words, channel 18 connects both lateral reservoir compartments 10 and 11 and penetrates through hydrogel 3. Therefore, lateral reservoir compartments 10 and 11 are lateral active reservoir compartments, because they enable active transport of a medium through the hydrogel via channel 18.

Lateral reservoir compartments 8, 9, 10 and 11 are each partially delimited by outer wall structure 29. Further, each lateral reservoir compartments 8, 9, 10 and 11 is arranged between outer wall structure 29 and inner reservoir wall structure 5. Cell culturing device 1 further comprises bottom section 25, which forms the base delimitation of the opposing lateral reservoir compartments and hydrogel compartment 2. It can be seen that hydrogel 3 extends between inner reservoir wall structure 5 and bottom section 25. Inner reservoir wall structure 5 further comprises inner ledge 14. Inner ledge 14 is angled with respect to a vertical inner wall section of inner reservoir wall structure 5 and peripherally surrounds and delimits bottom opening 7. It can be seen that inner ledge 14 is configured such that it decreases the open area of bottom opening 7 as compared to top opening 6. Inner wall structure 5 further comprises support structures 15 and 16, which laterally encompass a portion and in this case the majority (i.e. more than 50 vol.%) of hydrogel 3. Support structures 15 and 16 protrude from inner ledge 14 into hydrogel compartment 2. Such support structures are beneficial as they help to maintain the hydrogel and/or a hydrogel

precursor inside hydrogel compartment 2 and prevent its delocalization. Thus, the one or more support structures may generally form a form-locking engagement with the hydrogel. A peripheral section of hydrogel 3 is arranged below and in contact with inner ledge 14. Further, a central section of the hydrogel 3 including the microtopography (or in some embodiments in other words surface with cavities) is arranged below bottom opening 7.

Fig. 2a to 2c show a cell culturing blank system 101 according to an embodiment of the invention and as it can be used in some embodiments of the invention. While Fig. 2a shows a perspective view, Fig. 2b shows a top view in which the viewer views through the top openings of the corresponding cell culturing device blanks 19, 19' and 19'', and Fig. 2c shows a bottom view along the vertical direction. It is noted that the bottom section is removed to allow a view inside the system. Cell culturing blank system 101 includes three cell culturing device blanks 19, 19' and 19''. While reference signs are only provided for cell culturing device blank 1' for clarity purposes, the three cell culturing device blanks are identical in their features but differ only in their location within cell culturing blank system 101. Each cell culturing device blank comprises an inner reservoir compartment 4 being delimited by inner reservoir wall structure 5. The latter comprises inner ledge 14 which peripherally delimits bottom opening 7 which opens directly into hydrogel compartment 2 being configured for accommodating a hydrogel. Each cell culturing device blank further comprises a hydrogel delivery port 17 which allows to introduce a hydrogel or a hydrogel precursor into hydrogel compartment 2. As can be best seen from Fig. 2b, hydrogel delivery port 17 comprises a port inlet 27 which is arranged adjacent to the top opening of inner reservoir compartment 4. Port inlet 27 is further arranged on the same side of the cell culturing device blank as the top opening of inner reservoir compartment 4. That is, it is not arranged on the same side than the bottom opening 7. In this embodiment, port inlet 27 is further laterally offset to the top opening and it is delimited, respectively defined, by inner reservoir wall structure 5. As can be best seen from the bottom view shown in Fig. 2c, inner reservoir wall structure 5 further comprises six support structures, such as support structures 15 and 16 (only two are referenced for clarity purposes), which protrude from inner ledge 14 into hydrogel compartment 2. The support structures are radially arranged around bottom opening 7. They may in this or any other embodiments as described herein

be spaced apart from the bottom opening, in particular by a radial distance. The plurality of support structures are also spaced apart from each other such that they define gaps between them. Fig 2c further shows additional details of hydrogel delivery port 17. As can be seen, hydrogel delivery port 17 further comprises delivery outlet 28. Delivery outlet 28 is arranged such that it allows to provide the hydrogel or the hydrogel precursor directly into the hydrogel compartment 2. Further, it is arranged such that it opens into the area being encompassed by the support structures. The hydrogel delivery port can be L-shaped in cross-section as it is the case for the embodiment shown in Fig. 2c.

Fig. 3a to 3c show a cell culturing device blank 19 according to an embodiment of the invention and as it can be used in some embodiments of the invention. The bottom view shown in Fig. 3a shows bottom opening 7 of inner reservoir compartment 4 which opens from inner reservoir compartment 4 into hydrogel compartment 2. Inner reservoir compartment 4 and hydrogel compartment 2 are laterally encompassed by the two opposing lateral reservoir compartment 8 and 9 which are fluidically connected with hydrogel compartment 2 and indirectly with inner reservoir compartment 4 via hydrogel compartment 2. Again, and as it has been described with reference to Fig. 2a-c, cell culturing device black 19 comprises inner reservoir wall structure 5 with inner ledge 14 and support structures 15 and 16 protruding from inner ledge 14. Fig. 3c shows a sectional view along C-C in Fig. 3b. It can be seen that inner reservoir compartment 4 may be dome shaped. Further it can be seen that inner ledge 14 narrows the open area of bottom opening 7 as compared to top opening 6 of inner reservoir compartment 2. It is shown that the two opposing lateral reservoir compartments 8 and 9 are each arranged between inner reservoir wall structure 5 and outer wall structure 29. Delivery outlet 28 of hydrogel delivery port directly opens into hydrogel compartment 2 such that the hydrogel or hydrogel precursor is upon entering hydrogel compartment 2 encompassed by the support structures, such as support structure 15.

Fig. 4a shows a top view of a cell culturing device blank 19 according to another embodiment of the invention or as it can be used in other embodiments of the invention, e.g. for producing a cell culturing device according to embodiments of the invention. As it is

the case for cell culturing device blank 19 shown in Fig. 3a-c, cell culturing device blank 19 shown in Fig. 4a comprises an inner reservoir compartment 4 being delimited by inner reservoir wall structure 5 and comprising a top opening 6 and an oppositely thereto arranged bottom opening 7. Further, it comprises a hydrogel compartment into which bottom opening 7 opens and the inner reservoir compartment 4 is laterally encompassed by the two opposing lateral reservoir compartments 8 and 9 of a first pair of opposing lateral reservoir compartments. In addition however, cell culturing device blank 19 shown here comprises an additional pair of opposing lateral reservoir compartments 10 and 11. Thus, in total, the cell culturing device blank 19 comprises four lateral reservoir compartments. Opposing lateral reservoir compartments 8 and 9 form a first pair of opposing lateral reservoir compartments and lateral reservoir compartments 10 and 11 form a second pair of opposing lateral reservoir compartments. Such cell culturing device blanks are particularly advantageous as they allow to produce cell culturing devices with a hydrogel which can be accessed from at least 5 different sites, namely from the inner reservoir compartment and from each of the four lateral reservoir compartments 8, 9, 10 and 11. Further, it allows to use opposing lateral reservoir compartments as lateral passive reservoir compartments and concomitantly opposing lateral reservoir compartments 10 and 11 as lateral active reservoir compartments.

Fig. 4b is a sectional view through the center of inner reservoir compartment 4 of the cell culturing device blank shown in Fig. 4a and through the pair of opposing lateral reservoir compartments 8 and 9. It can be seen that both opposing lateral reservoir compartments 8 and 9 and also inner reservoir compartment 4 are in direct fluid communication with hydrogel compartment 2. Fig. 4c is a sectional view through the center of inner reservoir compartment 4 of the cell culturing device blank shown in Fig. 4a and through the pair of opposing lateral reservoir compartments 10 and 11. As can be seen, also these two opposing lateral reservoir compartments are in direct fluid connection with hydrogel compartment 2. Fig. 4d shows a bottom view of cell culturing device blank 19. In this embodiment, inner reservoir wall structure 5 comprises 4 support structures encompassing at least a portion of hydrogel compartment 2. Further, cell culturing device blank 19 comprises hydrogel delivery port 17. In addition, it can be seen that venting channels 32

and 33 are connected with lateral active reservoir compartments 10 and 11 and are configured to vent out remaining air from lateral active reservoirs 10 and 11 to the outside environment.

Fig. 5 shows a cell culturing system which comprises a 6x4 matrix of cell culturing devices, such as cell culturing devices 1, 1' and 1'' (only three are referenced for clarity purposes). The cell culturing system is designed as a well plate. In this well plate, the rows are designated with reference codes A, B, C and D and the columns are designated with codes 1, 2, 3, 4, 5, 6 to differentiate and identify the different cell culturing devices.

Fig. 6a shows a top view of a cell culturing device blank 19 according to another embodiment of the invention or as it can be used in other embodiments of the invention, e.g. for producing a cell culturing device according to embodiments of the invention. Cell culturing device blank 19 shown in Fig. 6a comprises an inner reservoir compartment (see Fig. 6b) being delimited by inner reservoir wall structure 5 and comprising a top opening 6 and an oppositely thereto arranged bottom opening 7. Bottom opening 7 opens into a hydrogel compartment (see fig. 6b). Inner reservoir compartment 4 is laterally encompassed by the two opposing lateral reservoir compartments 8 and 9 of a first pair of opposing lateral reservoir compartments. Cell culturing device blank 19 comprises an additional pair of opposing lateral reservoir compartments 10 and 11. Thus, in total, the cell culturing device blank 19 comprises four lateral reservoir compartments. Opposing lateral reservoir compartments 8 and 9 form a first pair of opposing lateral reservoir compartments and lateral reservoir compartments 10 and 11 form a second pair of opposing lateral reservoir compartments.

Fig. 6b shows a sectional view along D-D of cell culturing device blank 19 shown in Fig. 6a. It can be seen that the inner reservoir wall structure 5 which delimits and separates inner reservoir compartment 4 and opposing lateral reservoir compartments 8 and 9 comprises a vertical inner wall section 30 and inclined inner wall section 31. Inner reservoir wall structure 5 further comprises inner ledge 14 as described herein. Inclined inner wall section 31 is connected to both inner ledge 14 and vertical inner wall section 30. Furthermore, it is

inclined in an angle of less than 180 ° and more than 90° to both inner ledge 14 and vertical inner wall section 30. Inner ledge 14 and vertical inner wall may be arranged in an angle of essentially 90° to each other as shown in Fig. 6b. When a liquid medium is filled into lateral reservoir compartment 9, for example with a pipette, the medium level slowly rises and air being entrapped adjacent the hydrogel can be easily vented out by means of the inclined inner wall section 31. Thereby a conical or frustoconical pathway of the lateral reservoir compartments towards the hydrogel compartment is provided

Fig. 7 shows a perspective view with onto the bottom side of a cell culturing device blank 19 with the bottom section being removed. Cell culturing device blank 19 comprises a pair of opposing lateral reservoir compartments 8 and 9 and hydrogel compartment 2 which opens into an inner reservoir compartment via bottom opening 7. It can be seen that the inner reservoir wall structure 5 comprises inner ledge 14, vertical inner wall section 30 and inclined inner wall section 31 which allows to remove air during filling of lateral reservoir compartments with media. Additionally, cell culturing device black 19 comprises hydrogel delivery port 17 which opens via its delivery outlet 28 into hydrogel compartment 2. Additionally, cell culturing device blank 19 shown here comprises hydrogel buffer compartment 34 which also opens into hydrogel compartment 2. However, as can be seen, it is separated from hydrogel delivery port 17 and is thus another element. In this embodiment, hydrogel buffer compartment 34 is oppositely arranged of hydrogel delivery port 17 and its delivery outlet 28. Hydrogel buffer compartment 34 may comprise hydrogel buffer compartment opening 35 which opens into hydrogel compartment 2. Hydrogel buffer compartment opening 34 is different from delivery outlet 28. When a hydrogel or hydrogel precursor is filled into hydrogel compartment 2 via hydrogel delivery port 17, filling the hydrogel compartment completely can be difficult. It may happen that the hydrogel compartment is not filled yet completely and/or that the hydrogel or hydrogel precursor starts already to flow out of the hydrogel compartment. Filling the hydrogel compartment can be improved by providing such a hydrogel buffer compartment. Upon loading, excess of hydrogel or hydrogel precursor is filled into the hydrogel compartment and can flow into hydrogel buffer compartment instead of flowing out of hydrogel compartment 2. Thereby

filling of the hydrogel compartment is improved and more accurate and overloading the hydrogel compartment can be avoided.

Fig. 8a shows bottom portion 35 of the mold assembly which can form together with top portion 36 of the mold assembly shown in Fig. 8b together the mold assembly. The top portion and the bottom have a shape corresponding to the cell culturing device blank as described herein. That is, when the top portion and the bottom portion are arranged on top of each other, the form a mold assembly having a shape being a negative of the cell culturing device blank. It can for example be seen that bottom portion 35 of the mold assembly comprises at least one pair of opposing lateral mold bottom protrusions 37 and 38 which will form the at least one pair of opposing lateral reservoir compartments of the cell culturing device blank. Furthermore, bottom portion 35 of the mold assembly comprises at least one hydrogel delivery port protrusion 40 being configured to form the hydrogel delivery port of the cell culturing device blank when the molten material is injected into the mold. Top portion 36 of the mold assembly comprises a mold top protrusion 39 which will form the inner reservoir compartment. It is understood that in this or any other embodiment described herein, the mold assembly and in particular the top portion and bottom portion of the mold assembly, is configured such that it forms a void having the shape of the cell culturing device blank.

Fig. 9 shows a process scheme of an embodiment of the method to produce a cell culturing device 1 and a cellular chip 26 according to some embodiments of the present disclosure. In a first step a. a cell culturing device blank 19 (which may also be part of a cell culturing blank system) is provided. Cell culturing device blank 19 may for example be the one shown and described with reference to Fig. 3a-c or 4a-d. In a subsequent step b. casting insert 20 is provided. Casting insert 20 comprises a base portion having a microtopography with a plurality of protrusions, such as protrusions 21 and 22 (only two are referenced for clarity purposes) and further a rod which protrudes from the base portion. Casting insert 20 and the top opening 6 of the cell culturing device blank 19 are configured such that the base portion, respectively the casting insert 20 can be inserted into inner compartment 4. Casting insert 20 is introduced into inner compartment 4 as shown in step b. It rests and contacts

inner ledge 14 of cell culturing device blank 19 and its microtopography and in particular the protrusions face towards, respectively into the hydrogel compartment through bottom opening 7. In other words, the microtopography of casting insert 20 is exposed to hydrogel compartment 2. Then, a hydrogel precursor is inserted into hydrogel compartment 2, for example via a hydrogel delivery port as described herein. The hydrogel precursor is filled in such a manner into the hydrogel compartment 2 that it encompasses the protrusions of the microtopography of casting insert 20. It may also fill any cavities of casting insert 20, respectively of its microtopography. Thus, the microtopography of casting insert 20 serves as a negative for the microtopography of the hydrogel. The hydrogel precursor is then cured, for example by thermal treatment, such as heating and/or cooling or by irradiation, e.g. with UV light. After the hydrogel precursor has been cured to form a hydrogel 2, casting insert 20 is removed again via top opening 6 of cell culturing device 1 which is thereby formed (see step c).

In a next step or in a separate method, the thus obtained cell culturing device 1 which comprises cell culturing device blank 19 and a hydrogel 3 being accommodated in hydrogel compartment 2, can be used to produce a cellular chip 26. For example, in the embodiment shown, a cell suspension comprising cells 23 in a liquid medium 24 can be introduced into inner reservoir compartment 4. Since hydrogel 3 and its microtopography with the plurality of cavities, such as cavities 12 and 13 that correspond to the protrusions of casting insert 20, is directly arranged underneath bottom opening 7, the cells can either be selectively provided into the cavities by means of micropipettes, or adding the cell suspension into the inner reservoir compartment and allowing the cells to sediment. Thereby, a cellular chip 26 is formed which comprises the cell culturing device 1 and a plurality of cells 23. The cells can then be cultured for example such that they form 3D shapes and structures. For example, the cells may form a cell barrier on the hydrogel surface, respectively the microtopography. It may further be possible to add the same or different media into opposing lateral reservoirs 8 and 9 as it is indicated by the wave line.

Fig. 10 shows a cell culturing device 1 according to another embodiment of the invention. As it is the case for the cell culturing device shown and described with reference to Fig. 1a-

c, cell culturing device 1 shown here comprises inner reservoir compartment 4 being delimited by inner reservoir wall structure 5 which comprises inner ledge 14. Inner reservoir compartment 4 comprises also top opening 6 and bottom opening 7 which opens directly into hydrogel compartment 2 being filled with hydrogel 3. Further, cell culturing device 1 comprises a pair of, i.e. two, opposing lateral reservoir compartments 8 and 9, which encompass inner reservoir compartment 4 and hydrogel compartment 2. In contrast to Fig. 1a-c however, the inner reservoir wall structure 5 comprises next to vertical inner wall section 30 extending along vertical direction V, inclined inner wall section 31 which forms an angle α with vertical inner wall of for example between 120° to 150°. It can further be seen, that both lateral reservoir compartments 8 and 9 are partially delimited by such an inclined inner wall section. This may in certain embodiments be valid for every lateral reservoir compartment. When a liquid medium is filled into lateral reservoir compartment 9, for example with a pipette, the medium level slowly rises and air being entrapped adjacent the hydrogel can be easily vented out by means of the inclined inner wall section 31. Inclined inner wall section is further angled with respect to inner ledge 14.

CLAIMS

1. A cell culturing device blank (19) comprising:

- a hydrogel compartment (2) being configured to be filled with a hydrogel (3);
- an inner reservoir compartment (4) being delimited by an inner reservoir wall structure (5), wherein the inner reservoir compartment (4) comprises a top opening (6) and an oppositely arranged bottom opening (7), wherein the bottom opening (7) opens into the hydrogel compartment (2) and wherein the top opening (6) and the bottom opening (7) are configured such that the hydrogel compartment (2) is accessible from the top opening (6);
- at least one pair of opposing lateral reservoir compartments (8, 9, 10, 11) encompassing the inner reservoir compartment (4) and each opening into the hydrogel compartment (2).

2. The cell culturing device blank (19) according to claim 1, wherein the inner reservoir wall structure (5) comprises an inner ledge (14) which delimits the bottom opening (7) and/or which is arranged between the inner reservoir compartment (4) and the hydrogel compartment (2).

3. The cell culturing device blank (19) according to claim 1 or 2, wherein the inner reservoir wall structure (5) comprises a vertical inner wall section and an adjacently thereto arranged inclined inner wall section, wherein the vertical inner wall section (30) and the inclined inner wall section (31) at least partially delimit at least one of the opposing lateral reservoir compartments (8, 9, 10, 11).

4. The cell culturing device blank (19) according to claim 3, wherein the inclined inner wall section (31) is configured such that a cross-sectional open area of the at least

one lateral reservoir compartment (8, 9, 10, 11) being at least partially delimited by the inclined inner wall section (31) decreases towards the hydrogel compartment (2).

5. The cell culturing device blank (19) according to claim 3 or 4, wherein the inclined inner wall section (31) is arranged between the vertical inner wall section and the inner ledge (14).

6. The cell culturing device blank (19) according to any of the previous claims, wherein the inner reservoir wall structure (5) comprises one or more support structures (15, 16) which laterally encompass the hydrogel compartment (2) or at least parts of the hydrogel compartment (2), wherein preferably the one or more support structures (15, 16) protrude from the inner ledge (14).

7. The cell culturing device blank (19) according to any of the previous claims wherein the inner reservoir wall structure (5) separates the opposing lateral reservoir compartments (8, 9, 10, 11) from the inner reservoir compartment (4), in particular such that they are only fluidically indirectly connected via the hydrogel compartment (2).

8. The cell culturing device blank (19) according to any of the previous claims, wherein the hydrogel compartment (2) is arranged between two opposing lateral reservoir compartments (8, 9, 10, 11); and/or wherein the hydrogel compartment (2) is encompassed by and/or fluidically connected with the inner reservoir compartment (4) and the opposing lateral reservoir compartments (8, 9, 10, 11).

9. The cell culturing device blank (19) according to any of the previous claims, the cell culturing device blank (19) further comprising a hydrogel delivery port (17) opening towards the hydrogel compartment (2) and being configured for delivering a hydrogel or a hydrogel precursor into the hydrogel compartment (2), wherein preferably the hydrogel delivery port (17) has a port inlet (27) and is arranged such that the hydrogel

or hydrogel precursor can be directly delivered from the port inlet (27) into the hydrogel compartment.

10. The cell culturing device blank (19) according to any of the previous claims, wherein the cell culturing device blank (19) comprises an additional pair of opposing lateral reservoir compartments (8, 9, 10, 11) which encompass the inner reservoir compartment (4) and which each open into the hydrogel compartment (2) such that the hydrogel compartment (2) is in fluid communication with each of the opposing lateral reservoir compartments (8, 9, 10, 11) of the additional pair of opposing lateral reservoir compartments (8, 9, 10, 11).

11. The culturing device blank (19) according to any of the previous claims, wherein the cell culturing device blank further comprises hydrogel buffer compartment (34), the hydrogel buffer compartment (34) opening into the hydrogel compartment (2) and being in particular separate from the hydrogel delivery port (17).

12. Cell culturing device (1) comprising:

- a hydrogel compartment (2) accommodating a hydrogel (3);
- an inner reservoir compartment (4) being delimited by an inner reservoir wall structure (5), the inner reservoir compartment (4) comprising a top opening (6) and an oppositely arranged bottom opening (7), wherein the bottom opening (7) opens into the hydrogel compartment (2) and wherein the top opening (6) and the bottom opening (7) are configured such that the hydrogel (3) is accessible from the top opening (6);
- at least one pair of opposing lateral reservoir compartments (8, 9, 10, 11), the opposing lateral reservoir compartments encompassing the inner reservoir compartment (4) and each opening into the hydrogel compartment

(2) such that the hydrogel (3) is in fluid communication with each lateral reservoir compartment (8, 9, 10, 11);

wherein the hydrogel (3) comprises a surface which faces and/or is exposed towards the inner reservoir compartment (4), in particular via the bottom opening (7).

13. The cell culturing device (1) according to claim 12, wherein the surface comprises a microtopography with a plurality of cavities (12, 13), wherein the microtopography faces and/or is exposed towards the inner reservoir compartment (4), in particular via the bottom opening (7).

14. The cell culturing device (1) according to claim 12 or 13, wherein the opposing lateral reservoir compartments (8, 9) of the pair of opposing lateral reservoir compartments (8, 9) are lateral passive reservoir compartments (8, 9), wherein the hydrogel (3) is devoid of channels which open into any of the lateral passive reservoir compartments (8, 9) such that compounds can be exchanged between the lateral passive reservoir compartments only by diffusion through the hydrogel.

15. The cell culturing device (1) according to claim 12 or 13 wherein the opposing lateral reservoir compartments (10, 11) of the pair of opposing lateral reservoir compartments (10, 11) are lateral active reservoir compartments (10, 11), wherein the hydrogel (3) delimits one or more channels (18) extending between the two lateral active reservoir compartments (10, 11) and opening in each of the two lateral active reservoir compartments (10, 11), wherein the one or more channels (18) are configured for active medium exchange between the two lateral active reservoir compartments (10, 11).

16. The cell culturing device (1) according to claim 15, wherein the cell culturing device (1) additionally comprises a pair of opposing lateral passive reservoir compartments (8, 9) which encompass the inner reservoir compartment (4) and which each open into the hydrogel compartment (2) such that the hydrogel (3) is in fluid communication

with each lateral passive reservoir compartment (8, 9), and wherein the hydrogel (3) is devoid of channels which open into any of the lateral passive reservoir compartments (8, 9) such that compounds can be exchanged between the lateral passive reservoir compartments (8, 9) only by diffusion through the hydrogel (3).

17. Method for producing a cell culturing device (1), in particular a cell culturing device (1) according to any of claims 12 to 16, the method comprising the steps:

- Providing a cell culturing device blank (19), in particular a cell culturing device blank (1) according to any of claims 1 to 11, the cell culturing device blank comprising a hydrogel compartment (2), an inner reservoir compartment (4) being delimited by an inner reservoir wall structure (5), and at least one pair of opposing lateral reservoir compartments (8, 9, 10, 11) encompassing the inner reservoir compartment (4) and each opening into the hydrogel compartment (2), wherein the inner reservoir compartment (4) comprises a top opening (6) and an oppositely arranged bottom opening (7), wherein the bottom opening (7) opens into the hydrogel compartment (2) and wherein the top opening (6) and the bottom opening (7) are configured such that the hydrogel compartment (2) is accessible from the top opening (6);

- Introducing a casting insert (20) comprising a casting surface through the top opening (6) into the inner reservoir compartment (4) such that the casting insert (20) covers the bottom opening (7) and such that the casting surface is exposed to the hydrogel compartment (2);

- Filling a hydrogel precursor into the hydrogel compartment (2) such that the hydrogel precursor contacts the casting surface of the casting insert (20);

- Curing the hydrogel precursor to form a hydrogel (3) comprising a surface which faces and/or is exposed towards the inner reservoir compartment (4), in particular via the bottom opening (7);

- Removing the casting insert (20) through the top opening (6), thereby providing the cell culturing device (1).

18. Method for culturing cells with a cell culturing device (1) according to any of claims 12 to 16, the method comprising the steps:

- Providing a cell suspension comprising cells (23) and a liquid medium (24) in the inner reservoir compartment (4), in particular via the top opening (6);

- Allowing the cells (23) to sediment onto the surface of the hydrogel, in particular into the plurality of cavities (12, 13) of the microtopography of the hydrogel (3), and culturing the cells (23).

19. Method for culturing cells with a cell culturing device (1) according to any of claims 15 or 16, the method comprising the steps:

- Providing a cell suspension comprising cells and a liquid medium in at least one of the lateral active reservoir compartments (10, 11) of the pair of opposing lateral reservoir compartments (10, 11) and into the one or more channels (18), in particular by tilting the cell culturing device (1) or by applying a pressure gradient between the lateral active reservoir compartments (10, 11) into which the one or more channels (18) open;

- Culturing the cells inside the one or more channels (18).

20. Method for culturing cells with a cell culturing device (1) according to any claims 12 to 16, the method comprising the steps:

- Providing a cell suspension comprising cells and a liquid medium in at least one of the opposing lateral reservoir compartments (8, 9, 10, 11);

- Allowing the cells to sediment to a bottom section (25) of the at least one lateral reservoir compartment (8, 9, 10, 11);

- Culturing the cells on the bottom section (25) of the at least one lateral reservoir compartment (8, 9, 10, 11).

21. Method for culturing cells with a cell culturing device (1) according to any claims 12 to 16, the method comprising the steps:

- Providing a cell suspension comprising a hydrogel precursor and cells in at least one of the opposing lateral reservoir compartments (8, 9, 10, 11);

- Curing the hydrogel precursor to form a hydrogel (3);

- Culturing the cells inside the cured hydrogel (3).

22. Use of cellular chip (26) for analyzing an interaction between the cultured cells (23) and a compound of interest, in particular a virus, a microorganism such as bacterium or fungus, or a drug such as a protein, peptide, antibody, nucleic acid, small molecule or antibody drug conjugates, the cellular chip (26) comprising a cell culturing device (1) according to any of claims 12 to 16, the cellular chip (26) further comprising cells (23) being cultured in one or more selected from the hydrogel (3), on the surface of the hydrogel, in the cavities (12, 13) of the microtopography of the hydrogel (3), the inner reservoir compartment (4), at least one of the opposing lateral reservoir compartments (8, 9, 10, 11), in particular the lateral passive reservoir compartments (8, 9) and/or the lateral active reservoir compartments (10, 11), and the one or more channels (18).

23. Method for producing a cell culturing device blank (19) according to any of claims 1 to 11, the method comprising the steps of

- Providing a mold assembly;
- Injecting a molten material into the mold assembly;
- Curing the injected molten material to form the cell culturing device blank.

24. The method according to claim 23, wherein the mold assembly comprises a bottom portion (36) and top portion (37), wherein the top portion (37) comprises a mold top protrusion (40) being configured to form the inner reservoir compartment of the cell culturing device blank; and wherein the bottom portion comprises at least on pair of opposing lateral mold bottom protrusions (38, 39) being configured to form the at least one pair of opposing lateral reservoir compartments of the cell culturing device blank.

25. A mold assembly forming a molding void having the shape of the cell culturing device blank according to any of claims 1 to 11.