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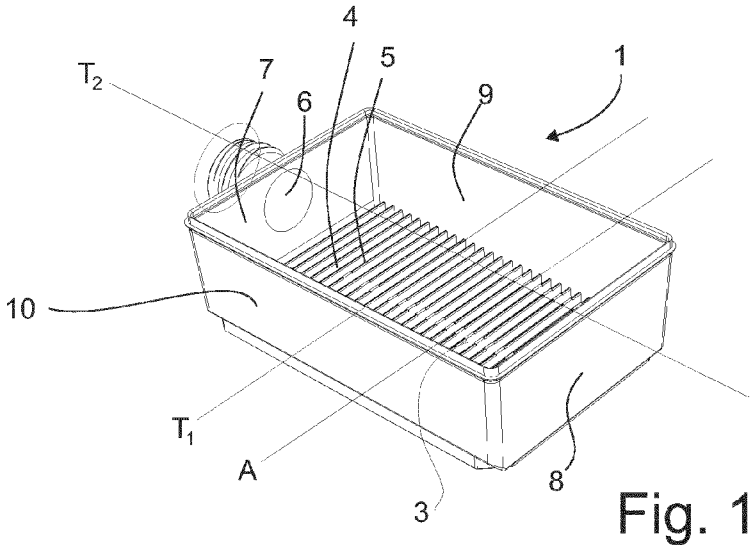

Fig. 1

(57) Abstract: A method of cultivation of cells comprises use of a vessel (1;1';11;17) having at least one interior surface (3;14;21) provided with a plurality of parallel grooves (4); said grooves being arrange for cultivation of cells. A simple tilting regime of the vessel provides the possibility of either exchanging the media or gently harvesting the cells, depending on the tilt direction of the vessel in relation to the longitudinal axis of the grooves.

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A cell cultivation method, a cell cultivation vessel, and uses thereof.

A method and a vessel for cultivating cells, in particular tissue cells, such as lymphoid cells, are provided.

The cultivation of cells is an essential procedure in a number of different bio-technologies, including cell therapy, tissue regeneration, as well as for basic research in e.g. biomedicine. However, the loss of the native physiological environment of the cells during cultivation results in the loss of contact to other cell types, the extracellular matrix and the specific mixture of growth regulatory factors and hormones essential for the cell, and will have dramatic effects on both the cellular morphology and the regulatory mechanisms of cell specific functions, often resulting in stopped or reduced cell growth.

It is accordingly important to ensure recruitment of both appropriate cells as well as suitable culture conditions during cultivation.

However, providing optimal culture conditions, i.e. ensuring that the cultivated cells maintain their growth characteristics, namely their metabolic activity and specific functions, is one of the most demanding tasks in cultivating cells. A problem which is especially important when using cultured cells for therapeutic treatment of among others cancer and infectious diseases.

Another problem in manufacturing cells for cell therapies is the ability to produce undamaged cells. Typically the cultivation process needs to be done carefully taking precaution not to damage the cells at any stages of the cultivation process.

Furthermore, in *ex vivo* cell cultivation of cells for cell therapies, particularly in a patient specific approach where the patient is treated with cells derived from his/her own cells (autologous) or from matched allergenic donor cells, it is a challenge to produce or manufacture the cell-based products in a clinically and commercially viable way. In many of theses cases, the process for generating each therapeutic dose of cells is relatively complex with multiple procedural steps and requires advanced cell manufacturing capabilities in order to be commercially successful.

Cultivation of lymphoid cells, used for adoptive immunotherapy is one example of cells that are highly sensitive to the growth conditions. Activated cytotoxic T-lymphocytes produce autocrine growth factors promoting their own growth, see e.g. Selleri *et al*: *CM-CSF/IL-3 receptor common β chain (CD131) expression as a biomarker of antigen-stimulated CD8+ T cells*; Journal of Translational Medicine, 2008 6:17. It is accordingly important to ensure that

said autocrine growth factors are not removed from the cell suspension, e.g. during media replacement in such amounts that it will affect cell growth.

Several methods and devices have been developed for overcoming these problems. For example, US 5,272,084 relates to a cell culture vessel wherein the bottom is provided with a plurality of grooves, configured and sized to be spannable by biological cells thereby emulating *in-vivo* growth conditions.

US 5,512,480 relates to a perfusion cell cultivating vessel for both adherent and non-adherent cells.

Thus, there is a demand for alternative devices and/or vessels for cultivation of cells, especially lymphoid cells, *ex vivo* at high yields, where the cells do not lose their metabolic activity and specific functions.

Accordingly, it is aimed to provide a method and a vessel for high yield cultivation of cells, especially lymphoid cells, for growing non-adherent cells and/or adherent cells, in particular non-adherent cells for undisturbed cultivation of cells and for cultivation of cells with minimum risk of contamination when adding and removing culture medium.

Suitably, the risk of cell-loss during medium exchange is reduced or eliminated, the cells can be grown with less mechanical interaction and less risk for damage, and still provide a means for simple and fast cell harvest and the cells are subjected to a minimum of environmental stress.

A cell cultivation method is provided, which comprises the subsequent steps of

(a) providing a cell culture vessel comprising at least one interior surface provided with a plurality of parallel grooves arranged for cultivation of cells,

(b) adding a cell culture and a culture medium to the vessel and distributing the cells and medium in the grooves,

(c) removing at least part of the medium from the vessel without disturbing the cells, by first tilting the vessel about a first tilting axis arranged parallel with the longitudinal axis of the respective grooves into a first tilting position, and then removing the medium, and

(d) harvesting the cultivated cells by tilting the vessel about a second tilting axis arranged perpendicular to the longitudinal axis of the grooves, and collecting the cultivated cells.

The cells used in the method can in principal be any kind of cells, including mammalian cells, plant cells, insect cells, and microorganisms, such as bacteria and yeast. However, since the method is especially suitable for ensuring cell-to-cell interaction it is preferred to use the method to cultivate cells which benefit from such interactions, e.g. lymphocytes to be used in cell therapy and/or research.

The term "vessel" means any kind of receptacle arranged for cultivation of cells and which has at least one surface provided with a plurality of parallel grooves. Accordingly, the term comprises flasks, bottles, and the like. Suitably, the surface upon which the plurality of parallel grooves is the bottom surface of the vessel; i.e. that surface upon which the vessel rests when in use.

Before the cultivation begins the cells and medium are distributed in the grooves, e.g. by carefully tilting the vessel into different positions until the cells and medium are evenly distributed in the grooves. The vessel may e.g. be carefully tilted one or more times about an axis parallel with the longitudinal axis of the grooves and/or one or more times in a direction perpendicular to said axis.

The cells and culture medium can be distributed in the vessel in separate steps, but it is preferred that they are distributed simultaneously as a single suspension, in order to reduce the number of handling steps. The exact choice will depend on e.g. the cell type, the medium, and the culture condition under which the cell cultivation are to take place.

When the cells are lymphocytes, said cells are preferably distributed as a monolayer in the respective grooves, such as a non-adherent monolayer of cells, before the cultivation begins, but this may be different for other types of cells.

In order to ensure that the cells have optimal growth conditions, it may be relevant at appropriate growth intervals to replace at least a part of the culture medium in order to provide sufficient nutrients and remove inhibitory metabolites. The relevant growth intervals depend on the cultivated cells, the medium used and the cultivation conditions. Therefore, the method may comprise the further step of: adding fresh medium to the vessel. Additionally, step (c) of the method may be repeated at intervals, until a desired cell yield has been achieved.

In order to ensure that the potential cell-to-cell interactions are not disrupted, it is relevant to ensure that the medium can be replaced and/or exchanged without disturbing the cells, or at least with as little disturbance as possible. Accordingly, the vessel to be used in the method comprises a number of parallel grooves arranged in such a way that substantially all cells and part of the growth medium will be retained in the grooves when the vessel is tilted about the first tilting axis, parallel with the longitudinal axis of the grooves, for collection and removal of the spent medium. It is in this way possible to remove a substantial part of the medium in the vessel in a single action without disturbing the cellular microenvironment areas in the grooves. This methodology allows for a medium exchange step that maintains a more tissue-like microenvironment, including e.g. the presence of accessory cells, paracrine and autocrine factors, while providing optimal access to nutrients and removal of metabolic by-products.

As the bottom of the vessel is typically rectangular, with grooves arranged in parallel with the edges of said rectangle, it is convenient to use the edges of the vessel as pivot points while tilting. In effect, one edge of the bottom of the vessel remains on a horizontal surface, while the remainder of the vessel is elevated. Tilting in this manner also reduces disturbance of the cells and medium.

It is preferred that the spent culture medium is collected at a side-wall or end-wall of the vessel when in the first tilted position. The spent culture medium can e.g. be removed from the vessel manually or automatically e.g. by a simple pipetting action. It is however preferred that the pipette does not come in contact with the cells in the grooves at any stage, and in an advantageous embodiment, removal of culture medium is performed from a location outside the grooves.

Fresh culture medium is preferably added carefully to the vessel in such a way that the cells are not, or only slightly disturbed when fresh medium is added, e.g. by placing the new medium along one end-wall or side-wall of the vessel. The medium can then easily be distributed by tilting the vessel carefully back and forth in a number of directions, e.g. about the first tilting axis and/or second tilting axis as described earlier. Alternatively, fresh medium can be distributed by moving a pipette across multiple grooves and tilting as required.

It is preferred that after fresh medium is distributed in the vessel, the fresh medium is carefully mixed with the cells retained in the grooves by carefully tilting the vessel back and forth about the second tilting axis. This will not only ensure that the all cells in the grooves are mixed with the fresh medium, but also that large cell clusters may be broken down into

smaller clusters (which can be preferred for continued cell growth) and/or that cells which unintentionally adhere to the side walls of the grooves, are carefully released.

The mechanical impact on the cells during the medium exchange step, i.e. the removal and addition steps, will - due to the unique construction of the vessel – be limited so that the cell growth is not affected, thereby preventing that the cells e.g. go into a lag-phase during/under and/or after medium exchange. Because of the unique medium exchange step, the method supports the growth of suspension cells, anchorage-dependent cells, or a mixture of both, and results in cultures with negligible shear stress, increased cell numbers and superior biological activity.

The method therefore provides culture conditions favouring close cell interactions, which is highly relevant when the cells are lymphoid cells e.g. T-lymphocytes. The tight interactions of the T-lymphocytes with for example antigen-presenting cells or with other population of lymphocytes are left undisturbed, and all or at least a significant part of the autocrine growth factors promoting their growth will not be removed, thereby providing better opportunities for both antigen-specific interactions and expansion of lymphocytes.

The steps of exchanging culture medium can be repeated as many times as needed until a satisfactory growth level and/or cell density is obtained. Since the cells have not, or only to a minimal degree, been disturbed during medium exchange, the desired cell yield can be achieved faster than hitherto known methods, and the cells' biological functions will be substantially intact.

At the end of the accumulated growth period the cells are harvested by a different tilting operation, which is provided by tilting the vessel about the second tilting axis perpendicular to the longitudinal axis of the grooves. This second tilting operation serves to collect the cells grown in any of the grooves at the same end of all grooves, wherefrom the collection of cells can be gently poured and/or removed by pipetting e.g. to another vessel for further processing.

Before the cells are harvested, part of the cultivation medium may be removed from the vessel as described above. However, a small amount of medium may be retained in the grooves before the second tilting operation is performed, to facilitate the harvesting procedure.

Accordingly, removal of culture medium and harvesting of cells is carried out via two different unique tilting regimes, which cannot be carried out in conventional multi-well plates or

culture flasks. Said tilting regime ensures that medium can be exchanged and cells harvested without significant cell loss, and without substantial deterioration of cells.

In some situations only a limited amount of precursor cells are available for the cultivation process. In order to ensure that said cells are in close proximity, also at the beginning of the cultivating process, the precursor cells and medium can initially be distributed in only some of the grooves. Since the grooves are distinct elements, the cell suspension will effectively be prevented from spreading to the remaining grooves. Thus, by placing the cells suspension in only some (e.g. a few) grooves of the vessel, a limited number of precursor cells can be given the optimal conditions for growing to a higher cell density. When the desired cell density is reached, the cell suspension, preferably with additional medium, can easily be distributed to the remaining grooves, where the cultivation process can continue as described earlier.

It is preferred that the interior surface comprising the grooves is divided into two or more sections, and wherein the cell culture and culture medium in step b) is first distributed in one section and then, after a cultivation period, distributed to one or more additional sections of the vessel. Preferably the precursor cells and medium is initially distributed in a section of the vessel comprising at least 10% of the elongated grooves, preferably at least 20% of said grooves. In a similar way, steps c) to d) can be performed in only one or more sections of the vessel, if desired.

Using the method it is possible to optimize both homotypic interaction in cultures of a single cell type and heterotypic contact in co-cultures. This methodology has potential applications for cell growth in tissue engineering, implant biology, and developmental biology, both in the arena of basic science and optimization of function for clinical and technological applications.

Also provided is a vessel, preferably for use in the method described above. Said vessel can in a preferred embodiment be a singled layered vessel e.g. in the form of a flask, box or bottle having a chamber defined by an outer circumferical wall and an interior surface, e.g. the bottom, provided with a plurality of grooves, separated by partition-walls. In order to ensure that medium and cells can be added and/or removed at desired intervals, said vessel preferably has at least one closable opening, which e.g. can be the entire top of said vessel, but it is preferred that only a small opening/inlet is provided, e.g. in one end-wall, side-wall or the top-wall of the vessel. For optimal access to the medium and cells, and to prevent cells and/or medium spilling during the tilting steps in the method, the at least one closable opening is preferably located at a location which does not lie along the axis of the parallel grooves, such as in one end-wall.

The partition-walls between the grooves are preferably lower than the circumferential wall of the singled layered vessel so that the circumferential wall can serve to stop and gather/collect culture medium when tilting about the first tilting axis and cells when tilting about the second tilting axis.

In a second embodiment of a singled layered vessel, the chamber is divided into a first and a second chamber part. The first chamber part comprises an interior surface, e.g. the bottom, provided with a plurality of parallel grooves, and the second chamber part comprises an interior surface (bottom) without grooves. The first and second chamber part are in fluid communication with each other, so that spent culture medium can be displaced from the grooves in the first chamber part, into the second chamber part by tilting the vessel about the first tilting axis. The second chamber part is in the vicinity of the opening, so collected spent culture medium can be decanted or pipetted from said second chamber part as described earlier. However, since the second chamber part does not comprise any grooves, and therefore not any cultured cells, less care needs to be observed when removing the spent medium.

The method can expediently comprise a modified step f) wherein the vessel after being tilted about the second tilting axis, where the cultured cells gather at one side wall of the vessel, is further tilted about the first tilting axis, to allow the cultured cells to move into the second chamber part, from where they can be harvested. So cultured cells can slide along the interior wall side of the vessel and into the second chamber part or towards an end-wall of said chamber part, due to the consecutive first tilting operation and second tilting operation.

The cells grown can for all embodiments be harvested carefully via the opening in the vessel. The opening is preferably located and dimensioned such that it allows both adding and removal of medium and cells, but still prevents contamination, spillage of culture medium, loss of cells, evaporation of medium etc.

In order to ensure that cells that potentially adhere to the side walls of the grooves are released before harvesting the cells, it is preferred that the vessel is tilted carefully back and forth about the second tilting axis a few times. If the cells are of the adhering kind, said cells may also be subjected to a detaching step, e.g. by addition of trypsin, before the cells are harvested.

In a still further embodiment, the vessel comprises at least two layers, each layer having an interior surface (bottom) provided with a plurality of grooves, i.e. corresponding to the above described single layered vessels. It is in this respect preferred that the top of one layer is

defined by the bottom of the layer directly above, as this will reduce the material requirements and proving a less expensive and lighter vessel.

The multilayered vessel can in principal comprise any number of layers, it is however relevant that the unique tilting operation, in order to exchange medium and harvest cells, can be provided without difficulties.

The use of a multilayered vessel, wherein each layer comprises a chamber having a plurality of grooves is a simple way of increasing the growing area of the vessel, and thereby cultivating more cells than by using a single layered vessel. Replacement of medium and harvest of cells can also be done rapidly in a multilayered vessel, substantially as a bulk operation.

Each layer of the vessel will preferably comprise a chamber, divided into a first and second chamber part as described above for the second embodiment of the single layered vessel. However, in order to ensure that the cultured cells are not disturbed during removal of the medium, the second chamber parts of each layer are in fluid communication with the second chamber parts of the other layers, and all said chamber parts are in fluid communication with the opening of the vessel. Preferably the second chamber parts define a single space, i.e. a space which is not divided into layers.

When the vessel comprises a chamber which is divided into a first and second chamber part, it is preferred that the groove located at the transition between the first chamber part and the second chamber part has an intermediate wall which is higher than the partition walls between the grooves, in order to ensure that the medium is not unintentionally displaced into the second chamber part during the tilting operation.

For vessels (both multilayered and single layered) having a first and second chamber part, it is further preferred that the intermediate wall is divided into a full intermediate wall extending from one of the side walls and a free intermediate wall extending from the opposite side wall, where the full intermediate wall extends all the way to the top of the layer and/or to the chamber top. The length of the full intermediate wall is preferably between 1/4 and 1/3 of the length of the intermediate wall. Said full intermediate wall can then effectively function as a backstop when adding culture medium and/or cells. In this way the medium and cells cannot flow into the second chamber part when the vessel is orientated so that the medium and/or cells are located in the vicinity of the full intermediate wall.

As described earlier, situations may occur where only a limited amount of precursor cells are available for the cultivation process. In order to ensure that said cells are in close proximity,

also at the beginning of the cultivating process, the interior surface comprising the grooves, can advantageously be divided into two or more sections. In this way, the limited amount of cells can be placed in one section at the beginning of the cultivation process, and then, after a larger amount of cells are obtained after a cultivation period, the cells can be distributed to one or more additional sections, where the cultivation process can continue.

Such sections can be provided equally well in the single layered and multilayered embodiments of the vessel. Furthermore, the number and size of the sections can vary, depending e.g. on the embodiment of the respective vessel, the amount of cells and/or the culture conditions, the only requirement being, that the section(s) has a size that will favor close cell interactions during cultivation.

Since the grooves are distinct elements, the section(s) can in one embodiment simply be provided by distributing the cell and medium in only some of the grooves. It is however preferred that the sections are divided by one or more dividing wall(s) which is/are higher than the partition walls. Using such dividing wall will ensure that the cell suspensions can be distributed in the grooves of a particular section, without having to take special precautions to prevent the cell suspension from flowing into the other sections e.g. when distributing the cells and/or medium.

The size and number of the section(s) of a particularly vessel, can be chosen depending on the actual amount of precursor cells provided. As an example, for a vessel comprising two sections, said sections may have a ratio of the grooves of 50:50 or 25:75 or 10:90. If said dividing wall(s) are releasably arranged in the vessel, it is possible to arbitrarily choose the size and/or number of the respective sections. This will reduce the manufacturing costs, as a single vessel easily can be altered and/or adjusted simply by adjusting the placement of the releasable dividing walls in the vessel.

The sections are preferably in fluid communication with each other, so that cells and culture medium can be displaced from one section to other sections e.g. by tilting the vessel about the first tilting axis. This can, in a preferred embodiment, be accomplished by using one or more dividing wall(s) which is slightly higher than the partition walls. Alternatively dividing wall(s) can be used, in which a section of said wall is slightly higher than the partition walls, e.g. about 25% - 100% higher, and the remaining section of the dividing wall(s) is much higher, e.g. extends all the way to the top wall of the vessel in order to function as an effective back stop. Finally, if the partition wall(s) are releasably arranged in the vessel, a fluid path can be provided, by simply removing the relevant partition wall(s).

That the sections are in fluid communication not only ensures an easy distribution between the sections, it also ensures that the cells are subjected to less stress, which is advantageous for continued cell growth and for automation of the cultivation process. Furthermore, the risk of contamination is reduced.

The grooves preferably have a configuration that allows for close cell interaction and provides sufficient room for the cultured cells. It is in this respect, it is preferred that the bottom of each groove is substantially concave, having a U-shaped cross-section.

Design features of the groove surfaces may in some embodiments and for some cultivation scenarios be particular important to avoid damaging the cells. It has been found that when the bottom of the grooves are concave, the lymphoid cells are neither disrupted, nor will the intracellular interactions and the autocrine growth factors be affected during medium removal and/or replacement, thereby ensuring that the cells constantly have optimal growth conditions.

Accordingly, grooves having bottoms of square or triangular cross-section are preferably excluded from the claimed invention since the cells tends to stick to the corners and/or gather at the lowest point (in the case of a triangular cross-section). Cells retained in the corners can only be released by applying an undesirable shear force to the cultivated cells, which significantly will reduce the cell yield and quality during harvest. Furthermore, optical analysis of cells in a vessel in which the grooves have bottoms of triangular cross-section is complicated by optical deflection caused by this shape.

In one embodiment the bottom of the grooves are in addition to the substantially concave cross-section, waved and/or undulated and/or further comprises one or more indentations along the length of the grooves. In this way the alternating valleys and peaks change the landscape of the bottom and defines small cavities wherein the cells can rest, and wherefrom the culture medium easily can be exchanged by the first tilting operation. The small cavities also provide better opportunities for fewer cells to be in close proximity thereby allowing them to interact and grow better.

When growing cells are subjected to replacement of culture medium they have a higher tendency to stays in the grooves due to the changing bottom landscape, and only when allowed to slide along the longitudinal axis of the grooves the cells, i.e. when the vessel is tilted about the second tilting axis, the cells will leave said groove. All embodiments of grooves work well with both adhering and non-adhering cells cultures and have minimum environmental stress caused as a general response to the media changes the cells are subjected to during growth.

The exact dimensions of the grooves may vary depending on the cultured cells, but for lymphocytes the inventors have found that a groove having a depth between 2 and 7 mm, preferably between 4 and 5 mm, e.g. about 4.5 mm, and a width between 1 and 4 mm, preferably between 2 and 3 mm, e.g. about 2.75 mm has proven highly advantageously for maintaining the cells growth characteristics, their metabolic activity and specific functions. The grooves may be dimensioned such that – when the vessel is tilted about the first tilting axis (T1) arranged parallel with the longitudinal axis A of the respective grooves such that said at least one interior surface lies at an angle of at least 45° to the horizontal – substantially all cells located in a groove are retained in said groove.

The length of the groove and the number of layers in the vessel, can be individually chosen in order to obtain the desired cell yield. In a simple manual vessel used for research and/or analysis, each groove may have a length between 60 and 100 mm, however said length can be larger for example when using the vessel and method in automated processes. The only requirement being, that the length of the groove may not negatively affect the cells during harvest, i.e. when the vessel is tilted about the second tilting axis, e.g. due to excessive or prolonged shear stress.

The present vessel is preferably a batch reactor. Vessels that are dependent on flow-through and/or re-circulation of culture medium, e.g. perfusion vessels, are specifically excluded due to the mechanical impact on the cells by the constant flow of culture medium, added oxygen etc.

By using the vessel and method it is possible to favor the level of homotypic interaction in cultures of a single cell type and the degree of heterotypic contact in co-cultures over a wide range. This methodology has potential applications in tissue engineering, implant biology, and developmental biology, both in the arena of basic science and clinical and industrial applications.

However, since the vessel and method provides superior conditions for continued close cell interactions the vessel is particularly advantageous as a batch reactor for cultivation of lymphoid cells e.g. T-lymphocytes. Thereby, the invention provides a manufacturing process that is not only robust and scalable, but highly suitable for product commercialization in adoptive T-cell immunotherapy for treatment of among others, oncologic and hematologic malignancies.

The method and vessel will be described in further details below with references to the accompanying drawings, in which

Fig. 1 is a perspective view of a first embodiment of a single layered vessel.

Fig. 2 is a cross-sectional view of the embodiment of fig. 1.

Fig. 3 is an enlarged view of a section of the cross-sectional view of fig. 2.

Fig. 4 is a perspective view of a second embodiment of a single layered vessel.

Fig. 5 is a perspective view of a multilayered vessel.

Figs. 6 shows steps b) or d) of the method.

Fig. 7 shows step c) of the method.

Fig. 8 shows step f) of the method.

Fig. 9 shows an alternative design of a groove to be used in a vessel.

Fig. 10 shows a further design of a groove to be used in a vessel.

Fig. 11. is a perspective view of a modified embodiment of the single layered vessel of fig. 1.

Fig. 12. shows the method using the vessel of fig. 11.

Fig. 13 shows microscope images of cultured cells according to the example, before (13A) and after (13B) tilting.

Fig. 14 shows the results of IFN-gamma production carried out in vessels according to the invention (MG) and commercially-available T75 vessels.

The method and vessel will be described below with the assumption that they are to be used for the cultivation of mammalian lymphoid cells. However, this assumption is not to be construed as limiting, and the method and vessel can just as easily be used for cultivation of other living cells, e.g. mammalian blood or tissue cells, insect cells, plant cells, or microorganisms.

Dimensions of the vessels and grooves are shown by way of example and modifications are foreseen within the scope of the present invention.

Fig. 1 shows, in perspective, a first embodiment of a single layered vessel 1.

Said vessel comprises a chamber 2 having an interior bottom surface 3 with a plurality of parallel substantially concave grooves 4 each defining a plurality of longitudinal cavities separated by partition walls 5. The top of the vessel is preferably closed by a top wall (not shown) so that the only opening in the vessel is an opening 6 formed in an end wall 7 of the vessel. During cultivation the opening 6 is closed by a lid and/or cap (not shown) ensuring that the environment inside the vessel is stable and contamination is prevented.

The grooves 4 are limited by a circumferical wall consisting of two end-walls 7,8 and two side-walls 9,10. These walls are higher than the partition walls 5, thereby ensuring that culture medium and cells are kept inside the vessel 1 during tilting.

The vessel defines a first tilting axis T_1, arranged parallel with the longitudinal axis A of the respective grooves 4, and a second tilting axis T_2, arranged perpendicular to said axis A of said grooves 4.

When the vessel 1 is tilted about the first tilting axis T_1, the spent medium above the grooves and part of medium in the grooves will be allowed to flow towards one of the end-walls 7,8, where the spent medium can be remove by a simple pipetting action through the opening 6 or by simply, but gently, pouring out through the opening 6. In contrast to the medium outside of the grooves and some medium in upper part of grooves, the cultivated cells together with some amount of medium will be retained in the grooves by the partition walls aided by surface tension forces. Thus by tilting about the first tilting axis T_1 it is possible to remove part of the spent medium from the vessel in a single action without disturbing the cellular microenvironment areas in the grooves, and at the same time reduce the concentration of potential inhibitory metabolic by-products.

In a similar way the cells can be harvested by tilting the vessel about the second tilting axis T_2 allowing the cells to be gathered close to one of the side-walls 9 or 10, where the cultured cells then can be harvested also through the opening 6, optionally by tilting the vessel about the first tilting T_1 axis after the cultured cells has been gathered at one of the side-walls 9,10.

It is understood that fresh medium easily can be added to the vessel e.g. by placing the medium via the opening 6 at one of the end walls 7,8 or at the side wall 9 or 10 and distributing it carefully in the grooves 4 by tilting the vessel 1 in different directions.

Because of the unique medium exchange and harvesting steps, the method supports the growth of suspension cells, anchorage-dependent cells, or a mixture of both, and results in

cultures that are subjected to negligible shear stress during cultivation, providing increased cell numbers and superior biologic activity.

In the embodiment shown the vessel has a substantial rectangular shape, however the vessel could just as easily have a quadratic shape or the like.

Fig. 2 is a cross sectional side view of the embodiment in fig. 1, and fig. 3 shows an enlarged section of the bottom surface 3 with grooves 4. Since the bottom of the grooves 4 have substantially U-shaped cross-section, the intracellular interactions between the lymphoid cells are not disrupted, and some medium containing autocrine growth factors will be retained during medium removal, thereby ensuring that the cells constantly have optimal growth conditions. Furthermore, the cell yield will be higher, since the grooves have no corners to which the cells can stick during harvesting.

Fig. 4 is a perspective view of a second embodiment of a single layered vessel 11 according to the invention. This embodiment is shown in a transparent view, to better illustrate the interior structure of the vessel. Non-transparent vessels are nevertheless also within the intended scope of the invention.

Said vessel comprises a chamber 2 divided into a first chamber part 12 and a second chamber part 13. The first chamber part 12 comprises an interior bottom surface 14 provided with a plurality of parallel grooves 4, and the second chamber 13 part comprises a bottom 14' without grooves. The vessel also comprises a top wall 16 to obtain a closed vessel except for a small opening 6, which is closed by a cap/lid (not shown) during the cultivation process.

The grooves 4 are limited by an end wall 8 and two side walls 9,10. These walls are higher than the partition walls 5, thereby ensuring that culture medium and cells are kept inside the vessel 1 during tilting.

The groove 4' located at the transition between the first and second chamber part has a free intermittent wall 15 of a larger height than the partition walls 5 between the grooves. Said free intermittent wall 15 ensures that when cells and/or medium are distributed inside the grooves, e.g. before cultivation or during addition of fresh medium, the medium and/or cells are maintained inside the first chamber part 12.

As for the first embodiment of a single layered vessel 1, the second embodiment 11 defines a first tilting axis T_1, arranged parallel with the longitudinal axis A of the respective grooves 4, and a second tilting axis T_2, arranged perpendicular to the axis A of said grooves 4.

Since the free intermittent wall 15 does not extend to the top-wall 16 of the vessel, the first and second chamber part are in fluid communication with each other, so that spent culture medium can be displaced from above the grooves 4 into the second chamber part 13 by tilting the vessel 11 about the first tilting axis T_1. The second chamber part 13 is in the vicinity of the opening 6 and spent culture medium can be pipetted from said second chamber part as described earlier. Since the second chamber part 13 does not comprise any grooves, and therefore not any cultured cells, less care needs to be observed when removing the spent medium. This is especially relevant when the method is performed automatically.

Fresh medium is added in a similar way as for the first single layered embodiment, i.e. by tilting the vessel in different directions until it is substantially evenly distributed in the grooves.

As the for the first embodiment shown in fig. 1, the cells can be harvested by tilting the vessel 11 about the second tilting axis T_2 allowing the cells to be gathered close to one of the side walls 9 or 10, and then optionally further tilting the vessel 11 about the first tilting axis T_1 to allow the cultured cells to move into the second chamber part 13 for easy collection.

Fig. 5 is a perspective view of a multilayered vessel 17 comprising three layers 18a,18b,18c. Each layer corresponds substantially to the second embodiment of a vessel 11 in that each layer is divided into a first and second chamber part, denoted 19,20 for the multilayered vessel, and are delimited by end walls 8a,8b,8c and 7; two side walls 9a,9b,9c and 10a,10b,10c, and a top wall top wall 16 with a small opening 6. Said opening can be closed by a cap/lid (not shown) during the cultivation process.

The respective first chamber parts 19a,19b,19c comprises an interior bottom surface 21a,21b,21c having a number of grooves 4. However the second chamber part 20 for each layer 18, forms a single and integrated space, i.e. there is only a single bottom surface 20' for all layers, making it very simple to distribute and remove e.g. medium to/from the respective layers.

The grooves 4a,4b,4c located at the transition between the first chamber parts 19a,19b,19c and the second chamber part 20 have a free intermittent wall 22a,22b,22c of larger height than the partition walls 5 between the grooves 4. In order to ensure that the medium and cells are retained in the first chamber parts during distribution, a part 23a,23b,23c of the intermittent wall, extends all the way to the top of the chamber, in order to effectively functioning as a backstop when exchanging culture medium. For the upper layer 18c the different heights of the free intermittent wall 22c and the full intermediate wall 23c is singled out by a bold line in fig. 5. A similar profile is found in the other levels 18a, 18b.

The multilayered vessel 17 allows for growth of very high numbers of cells under identical conditions. One single vessel needs to be filled with the suspension of cells and culture medium and only a single vessel needs to be handled in the method of the present invention. Since all cells are subjected to same growth condition with minimum disturbance and mechanical interaction, many cells having similar growth stages can be harvested. Thus by using the vessel and/or the method of the present invention healthy viable cell batches of high cell numbers can be achieved fast and effective.

It must be stressed that the number of layers in fig. 5 is only one embodiment, and there could just as easily have been e.g. two, five, eight or more layers. Higher number of layers or larger dimensions of the vessel, might be especially interesting in automatic processes where there is substantially no manual interference, and weight and dimensions of the vessel, including content, are less relevant.

Fig. 6 shows the steps of distributing a cell suspension, or the medium alone, in the vessel 17 shown in fig. 5.

Fig. 6a shows the step wherein a cell suspension 25 i.e. cells in culture medium has been added to the vessel though the opening 6, as indicated by arrow B. The vessel 17 is in tilted position during loading so that a suspension of cells flows towards the sidewall 10a,10b,10c of each of the layers 18a,18b,18c (i.e. the sidewall which is not adjacent to the full intermittent walls 23a,23b,23c), and into the first chamber parts 19a,19b,19c and the second chamber part 20, where it is allowed to be distributed evenly along the complete side wall 10, as shown in fig. 6b.

Fig. 6b, 6c and 6d show the vessel 17 placed on the respective side- and end-walls, but viewed through the top part of the vessel, and accordingly only the top layer 18c is shown.

Fig. 6c, and fig. 6d shows how the cell suspension 25 is evenly distributed in the grooves before the actual cultivation begins, by first erecting the vessel 17 to rest on end-wall 8 such that the suspension is allowed to gather along the end wall 8a,8b,8c of the respective layers 18a,18b,18c, as shown in fig. 6c, and then to rest on side wall 9 such that the suspension is gathered along the opposite side walls 9a,9b,9c of the respective layers 18a,18b,18c, where the full intermittent walls 23a,23b,23c prevent the suspension from entering the second chamber part 20, as shown in fig. 6d. The vessel is then placed in the position of use, where the suspension is allowed to be distributed along the grooves 4a,4b,4c. The vessel is in this respect preferably tilted back and forth about the second tilting axis T_2, i.e. the suspension is allowed to flow in the longitudinal direction of the grooves.

The tilting from one side wall 10 to the other side wall 9, via the end-wall 8 as shown in figs. 6b, 6c, and 6d, ensures that the suspension is distributed along the length of the grooves 4 in only the first chamber part 19. Cells are preferably distributed as a monolayer where each of the cells is surrounded by culture medium for future growth in a suitable environment.

It will be understood that addition of fresh medium is accomplished by a similar tilting scheme as described above for distributing the cell suspension. Even though this means that cells will be redistributed in the grooves together with the fresh medium during the tilting regime in e.g. fig. 6b, 6c, and 6d, the mechanical impact on the cells will be negligible, and redistributing the cells has the advantage that the cells and fresh medium will be gently mixed, ensuring that new medium is available for all cells.

Other ways of distributing the cell suspension or medium are contemplated within the scope of the appended claims.

The side view of fig. 6e, seen from side-wall 10, illustrate the position of the vessel during cell growth, and it can be seen that the suspension is distributed evenly in the plurality of grooves 4 in all three layers 18a,18b,18c, whereas the second chamber part 20 does not comprise any cells and/or medium. In this, horizontal, position of the vessel the cells are allowed to rest and grow e.g. in an incubator for a selected growth period. This growth period is determined based on the cultivated cells and the culture conditions, but it is normally preferred to keep the cells in the log-phase so that the culture medium must be replaced before either nutrient depletion or a build up of inhibitory metabolites occurs.

To replace part of the used culture medium with fresh culture medium the vessel 17 is tilted about the first tilting axis T_1 towards the second chamber part 20 to decant and gather culture medium in the second chamber part, as shown in fig. 7. After removal of at least part of the used medium from the second chamber part 20, replacement medium is then added, mixed with the cell suspension left in the grooves by tilting repeatedly in different directions and then distributed in the manner shown in figs. 6a – 6e. The alternating growth periods and replacement of culture media are repeated as many times needed in order to reach the desired growth level, propagation level and number of cells.

In the final harvesting steps shown in fig. 8, part of the spent culture medium may first be removed from the first and second chamber part 19,20, as described for fig 7. Then the vessel 17 is tilted about the second tilting axis T_2 to collect the cells from all grooves at one of the sidewalls 9,10. Once collection at the sidewall 9,10 has been completed the vessel 17 is tilted again but about the first tilting axis T_1 (not shown) which allows the collected resulting cells to slide on the sidewall 10 and be gathered in the second chamber part 20

before they are transferred to another vessel, vehicle, receptacle or recipient for further processing, storage or use.

The grooves of the vessels 11,17 shown in figs. 4,5,6,7 and 8 are shaped as the grooves of the vessel shown in figs. 1 – 3, all grooves are concave and have U-shaped cross-sectional bottoms, providing smooth bottoms with no sharp turns or edges.

Figs. 9 and 10 are perspective views of alternative designs of a groove 4 for a vessel 1,11,17 of the present invention.

The alternative groove 27 of fig. 9 has a plurality of indentions 28 in the curved groove bottom 29 towards the free groove opening 30. The indentions 28 are distributed equidistantly along the length of the groove 27. The indentions are three-dimensional structures starting from the curved groove bottom and may have various polyhedron shapes, such as pyramidal indentions, as shown in fig. 9 or truncated cone indentions.

In contrast the groove 31 of fig. 10 has a waved or undulated bottom 32. The alternating valleys 33 and peaks 34 delimit small reactor cavities in the groove.

It will be understood that the groove can have any design and construction as long as the cultivated cells will be retained in the grooves when the vessel is tilted about the first tilting axis for collection of the spent medium, and will flow along the groove when said vessel is tilted about the second tilting axis.

Fig. 11 is a perspective view of a modified embodiment of the single layered vessel of fig. 1, and for like parts same reference numbers are used. In the modified embodiment of the vessel 1', the interior bottom surface 3, with a plurality of parallel substantially concave grooves 4, has been divided into a first section 35 and a second section 36 by a partition wall 37. Said partition wall is divided into a first part 38a, having a limited height in order to allow the two sections 35 and 36 to be in fluid communication, and a second part 38b extending to the top of the vessel in order to function as an effective backstop for the cell suspension.

In this way, a limited amount of cells can be placed in e.g. the first section 35 at the beginning of the cultivation process, and then, after a larger amount of cells are obtained in said section, the cells can be distributed to the second section 36. Accordingly, the modified vessel 1' provides a possibility of allowing a lower amount of cells to be in close proximity, at the beginning of the cultivating process.

Fig. 12 shows how the cell are distributed and cultivated in the vessel shown in fig. 11. Fig 12a shows how the cell suspension 39 (cells and medium) is first added to the first section 35 when the vessel 1' is placed in the upright position resting on the wall 8. As shown in fig 12b, the vessel is then turned to rest on the sidewall 10, allowing the cell suspension 39 to be distributed evenly at one end of the grooves of the first section 35. Thereafter the vessel is been placed in the position of use, see fig. 12c, i.e. in the horizontal position allowing the cell suspension to be evenly distributed in the length of the grooves of the first section 35.

The dividing wall 37 prevents the cell suspension 39 from entering the second section 36 of the vessel. While growing the cells in the first section 35 media can easily be exchanged and/or added as described above, only using the first section 35 of the vessel, until the desired cell density in the first section has been reached.

In order to distribute the cells also in the second section 36, the vessel 1' is again placed in the upright position resting on the wall 8, where they are suspended into a larger amount of media, see fig. 12d. The cell suspension is then distributed evenly in the first and second section 35, 36 of the vessel, by then resting the vessel on the side wall 9, see fig. 12e, allowing the cell suspension to flow over the lower section 38a of the dividing wall, and then placing the vessel in the horizontal position, as shown in fig. 12f.

In this way cell growth is started in a small area and it is possible to continue the growth in a larger area without exchanging to a new vessel.

The design of the vessel and the method for cultivation of the cells enables the operator to keep the cells at an optimal density for continued growth and serves to stimulate further proliferation. The only thing needed to do is to replace part of the medium with fresh culture medium from time to time, which when using the method of the present invention, does not disturb or damage the cells so that growth is stopped temporarily or completely. A simple tilting regime of the vessel provides the possibility of either exchanging the media or gently harvesting the cells, depending on the tilt direction of the vessel in relation to the longitudinal axis of the grooves.

The vessel has a simple and inexpensive design, and can therefore be used equally well for both research or for large scale production, where existing vessels are too troublesome, expensive and complicated to use.

Modifications and combinations of the above principles and designs are foreseen within the scope of the present invention.

Example

Using the immunization protocol currently employed in the production of tumor-specific cytotoxic lymphocytes (as described in WO2008081035), generation of CTLs from healthy donors were compared, either in MG flasks (according to the invention) or in standard T75 flasks. In both cases, flasks were placed on their side.

At the initiation of culture, 10 ml of cell suspension is placed in the flask. In order to distribute cell suspension equally among the grooves, the flask is first tilted using the long edge as a pivot, and then placed in the horizontal position. During cultivation, cells are located on the bottom of grooves, forming characteristic spheroids. Simple tilting of the flask using the bottom short edge as a pivot, minimally disturbs cells.

Fig. 13 shows microscope images of cultured cells according to the example, before (13A) and after (13B) tilting in both directions, using the bottom edges of the flask as a pivot. As can be seen, the cells are minimally disturbed.

At days 2 and 5, 10 and 20 ml of new medium is added to the flask. At day 7, half of medium can be replaced by tilting of the flask using the bottom short edge as a pivot, removing medium collected in the corner of the flask (about 20 ml), and adding new medium. Harvesting of cells is performed by tilting the flask using the long edge as a pivot, and then removing cell suspension. In the end, the flask is turned over to help collect the last part of the cell suspension. If the amount of cells is not enough to seed to the whole flask, part of the flask could be used, and cells are distributed to the whole flask later.

After 9 days, cells were harvested, NK cells were removed by CD56+ beads, and interaction of the remaining cells with tumor cell line MDA-MB-231 was measured by IFN-gamma production in culture supernatants.

Figure 14 demonstrates experiments performed with blood from four different donors. Fig. 14 shows the results of IFN-gamma production carried out in vessels according to the invention (MG) and commercially-available T75 vessels.

First, a high heterogeneity of generation of CTL response against tumor cells among different healthy donors could be seen, reflecting a high heterogeneity of precursor frequencies to cancer/testis antigens. Donors 72/15 and 84/15 did not generate a CTL response neither in T75, nor in MG flasks. Donor 83/15 generated significant CTL response in both T75 and MG flasks, while donor 71/15 generated moderate CTL response that was twice as high in MG flasks than in T75 flasks. In conclusion, these experiments demonstrated that MG flasks

could be used for generation of tumor-specific CTL response at the same or higher efficiency than standard T75 flasks.

5

Claims

1. A cell cultivation method comprising the subsequent steps of

(a) providing a cell culture vessel (1;1';11;17) comprising at least one interior surface (3;14;21) provided with a plurality of parallel grooves (4) arranged for cultivation of cells,

(b) adding a cell culture and a culture medium to the vessel (1;1';11;17) and distributing the cells and medium in the grooves (4),

(c) removing at least part of the medium in the vessel without disturbing the cells, by tilting the vessel (1;1';11;17) about a first tilting axis (T_1) arranged parallel with the longitudinal axis A of the respective grooves (4) into a first tilting position, and then removing the medium, and

(f) harvesting the cultivated cells by tilting the vessel (1;1';11;17) about a second tilting axis (T_2) perpendicular to the longitudinal axis A of the grooves (4) and collecting the cultivated cells.

2. The method according to claim 1, further comprising the steps of

(d) adding fresh medium to the vessel,

(e) repeating step c) and d) at intervals, until a desired cell yield has been achieved.

3. The method according to claim 2, wherein step d) comprises adding fresh culture medium to the vessel (1; 1';11;17) and distributing it in the grooves (4) by tilting the vessel (1; 1';11;17) in different directions.

4. The method according to any one of the preceding claims, wherein the vessel (11;17) is divided into a first and second chamber part (12,13;19,20), and wherein

- the first chamber part (12;19) comprises the at least one interior surface (14;21) provided with the plurality of parallel grooves (4), and

- the second chamber part (13;20) is without grooves (4) and being in fluid communication with the first chamber part, and

wherein the culture medium in step c) is removed by tilting the vessel (11;17) about the first tilting axis (T_1) into a first tilting position, thereby allocating at least a part of the spent medium in the second chamber part (13;20).

5. The method according to any one of the preceding claims, wherein step f) is modified in first tilting the vessel (11;17) about the second tilting axis T_2 and then about the first tilting axis T_1 to allow the harvested cells to move into the second chamber part (13;20).

6. The method according to any one of the preceding claims, wherein the interior surface comprising the grooves is divided into two or more sections, and wherein the cell culture and culture medium in step b) is first distributed to one section and then, after a cultivation period, distributed to one or more sections of the vessel.

7. The method according to claim 6, wherein steps c) to f) are performed in one or more sections of the vessel.

8. A method according to any of claims 1 – 7 wherein the vessel is a multilayered vessel (17) and wherein each layer has a first (19) and second chamber part (20) and wherein the first chamber part comprising at least one interior surface (21) provided with the plurality of parallel grooves (4).

9. A vessel (1;1';11;17) for cell cultivation, wherein the vessel comprises at least one interior surface (3;14;21) provided with a plurality of parallel grooves (4), **characterised** in that the bottom of each groove is substantially concave.

10. The vessel (1;1';11;17) according to claim 9, wherein the bottom of the grooves (4) have a substantially U-shaped cross-section.

11. The vessel (1;1';11;17) according to claim 9 or 10, wherein the bottom of the grooves (4) are waved and/or undulated and/or comprise one or more indentations.

12. The vessel (11;17) according to any of claims 9-11, **characterised** in that the vessel (11;17) is divided into a first and second chamber part (12,13;19,20), and wherein the first chamber part (12;19) comprises at least one interior surface (14;21) provided with a plurality of parallel grooves (4), and the second chamber part (13;20) is without grooves (4) and being in fluid communication with the first chamber part (12;19).

13. The vessel (17) according to any one of claims 9-12, **characterised** in that the vessel is a multilayered vessel (17) and each layer (18) comprises at least one interior surface (21) provided with a plurality of parallel grooves (4).

14. The vessel (11;17) according to claim 12 or 13, **characterised** in that the groove (4';4a;4b;4c) located at the transition between the first chamber part (12;19) and the second chamber part (13;20) has an intermittent wall (15;22) of larger height than the partition walls (5) between the other grooves (4).

15. The vessel (11;17) according to claim 14, **characterised** in that said intermittent wall (22) comprises a free intermittent wall (22) and a full intermittent wall (23) which extends all the way to the top of the respective layer, the chamber or the top of the vessel.

16. The vessel (1') according to any one of claims 9-15, **characterised** in that the interior surface comprising the grooves is divided into two or more sections.

17. The vessel according to any one of claims 9-16, wherein the grooves are dimensioned such that – when the vessel is tilted about a first tilting axis (T_1) arranged parallel with the longitudinal axis A of the respective grooves such that said at least one interior surface lies at an angle of at least 45° to the horizontal – substantially all cells located in a groove are retained in said groove.

18. Use of the vessel (1;1';11;17) according to any of claims 9-17 as a batch reactor.

19. Use of the vessel (1;1';11;17) according to any of claims 9-17 in a method according to any of claims 1 – 8 for undisturbed cultivation of cells, especially lymphoid cells.

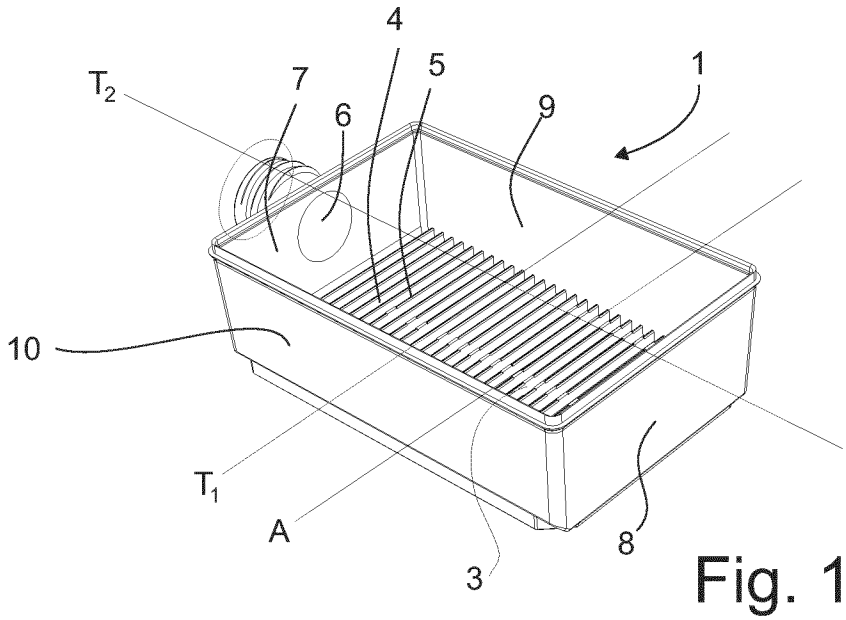


Fig. 1

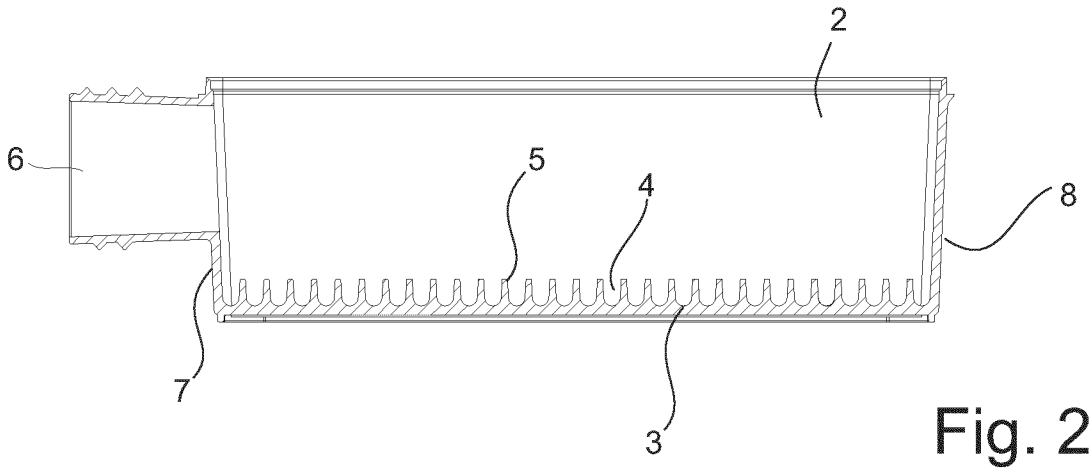


Fig. 2

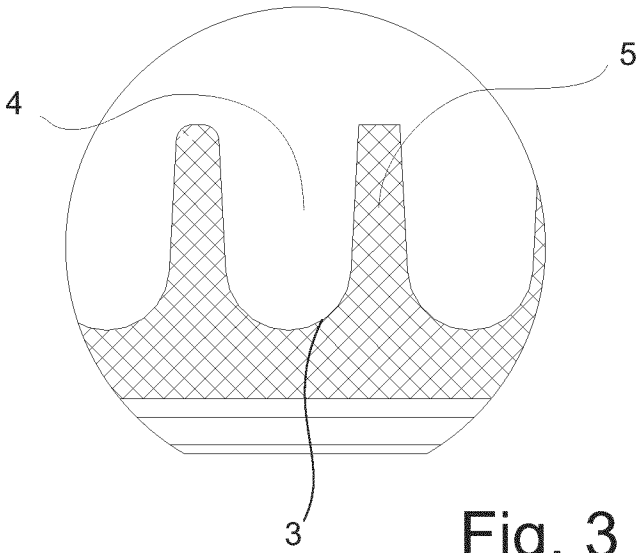


Fig. 3

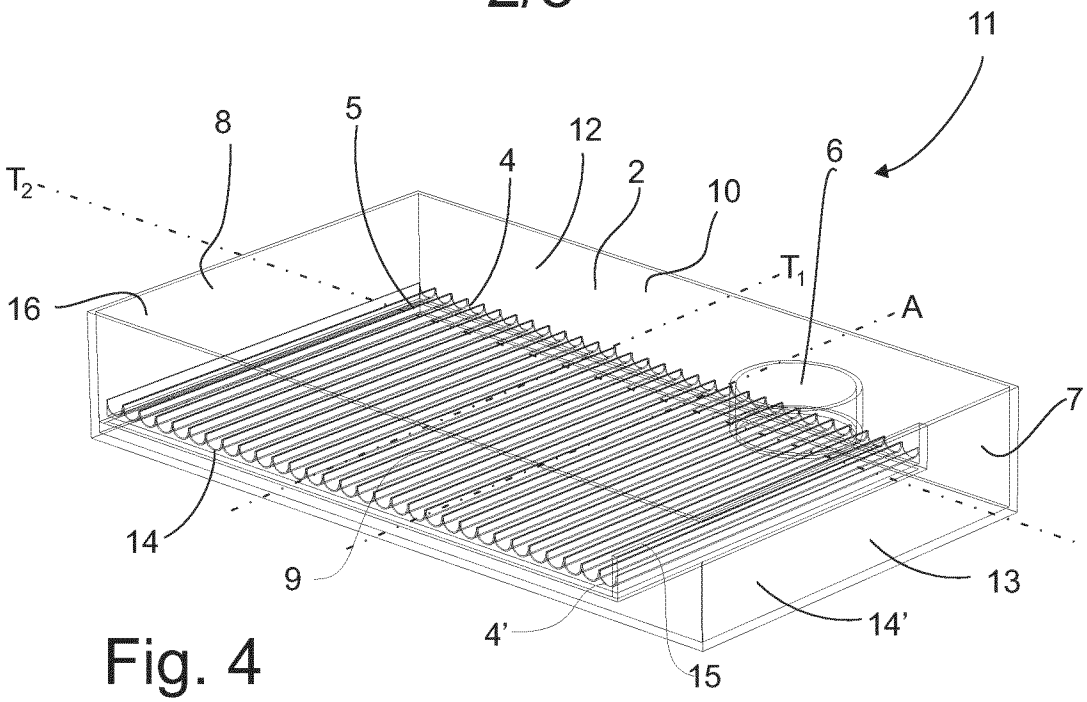
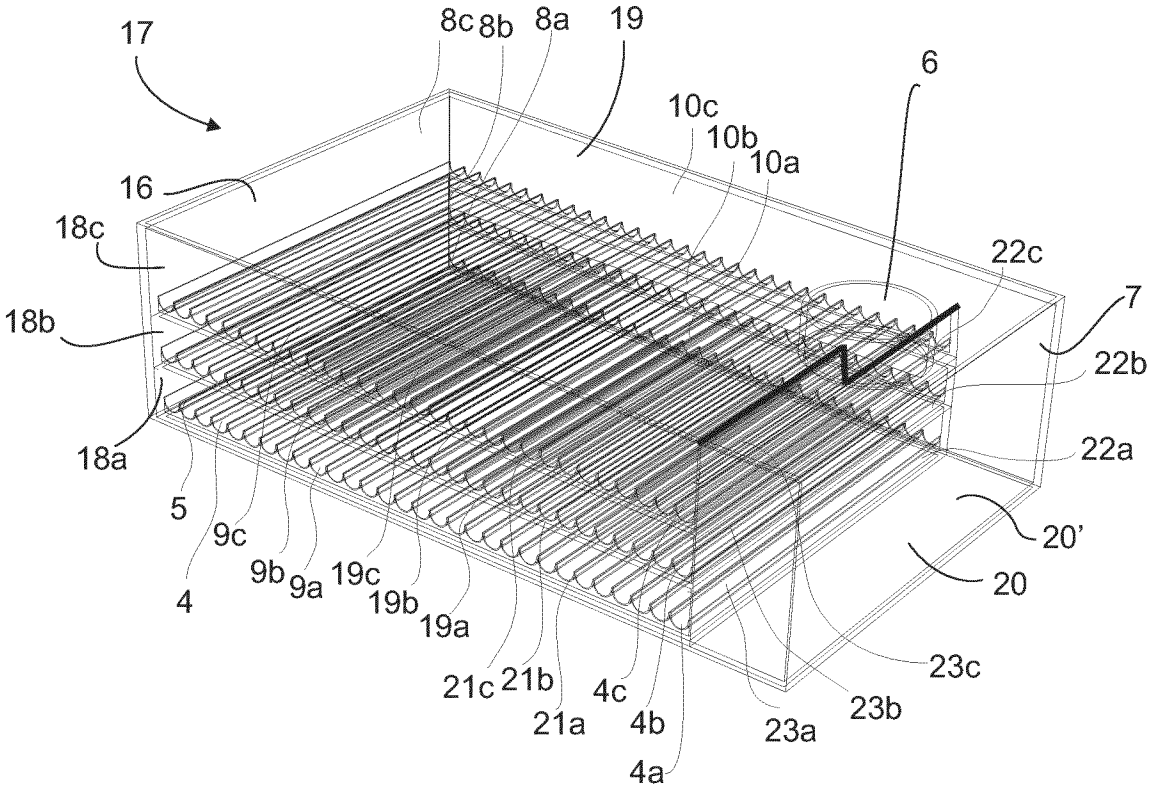

11
8
5
12
4
2
6
10
T_2
T_1
A
16
7
14
9
13
4'
14'
15

Fig. 4


17
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8b
8a
19
6
10c
10b
10a
16
18c
22c
7
18b
22b
18a
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5
20'
9c
9b
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23b
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4b
23a
4a

Fig. 5

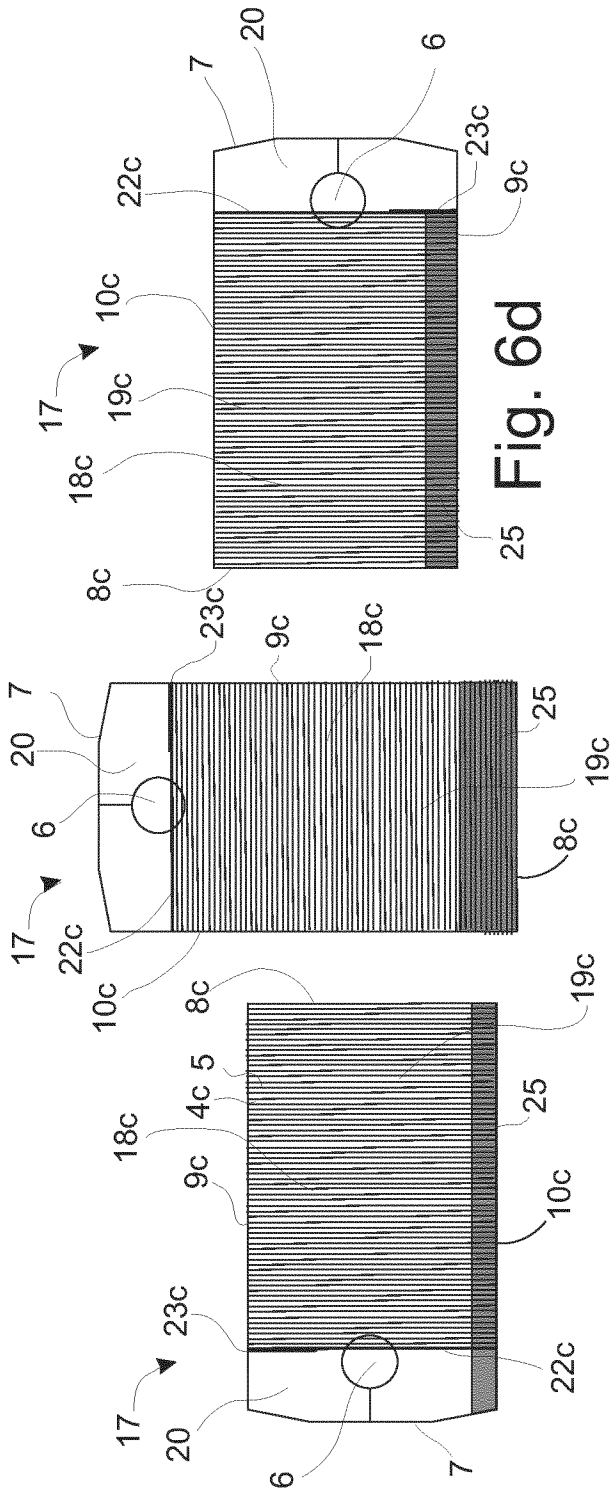


Fig. 6d

Fig. 6c

Fig. 6b

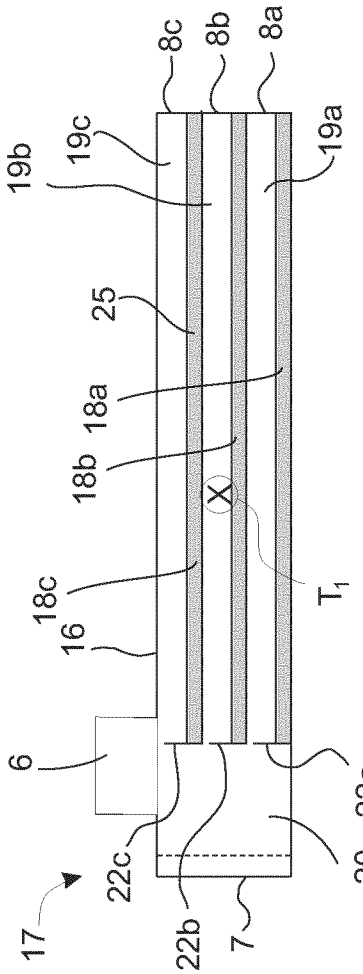


Fig. 6e

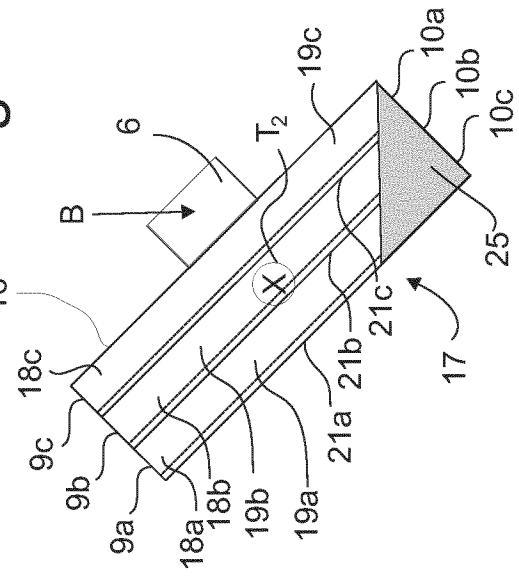
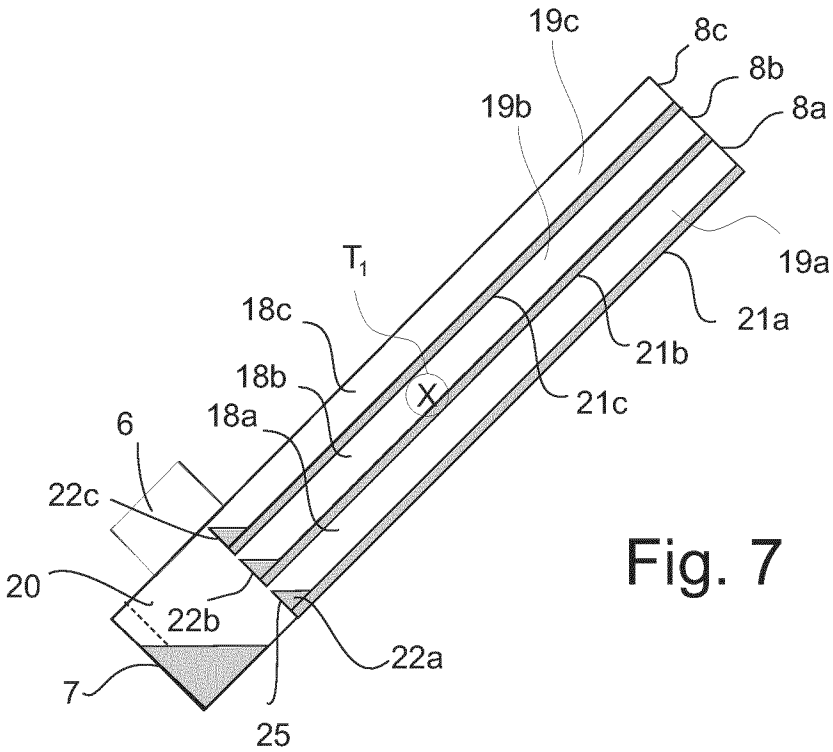


Fig. 6a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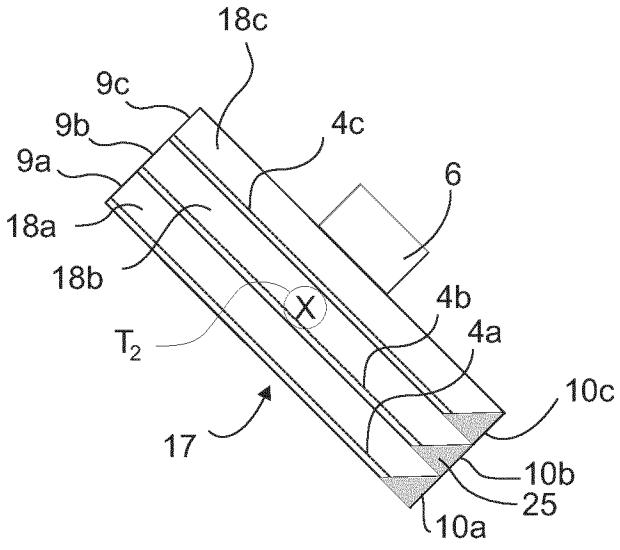
8c
8b
19b
8a
19a
T1
18c
21a
21b
18b
21c
18a
6
22c
20
22b
22a
7
25

Fig. 7


18c
9c
9b
4c
9a
6
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18b
4b
4a
T2
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17
10b
25
10a

Fig. 8

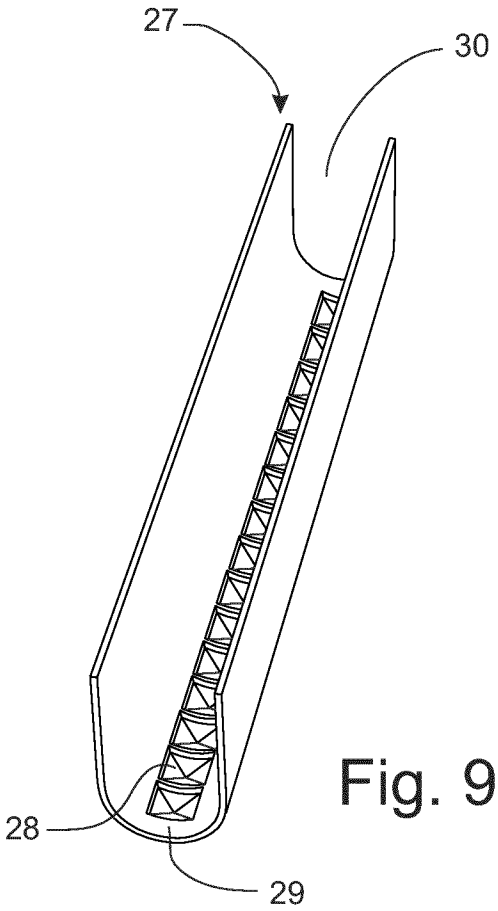

Fig. 9

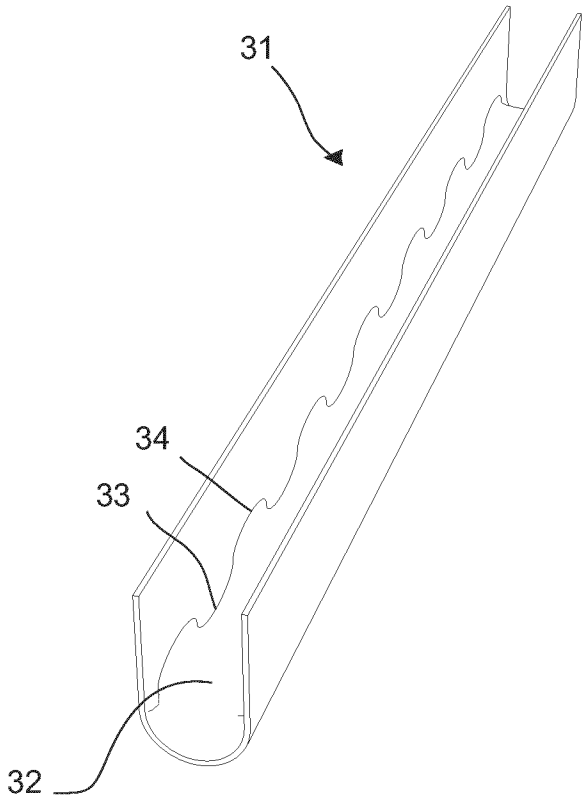

Fig. 10

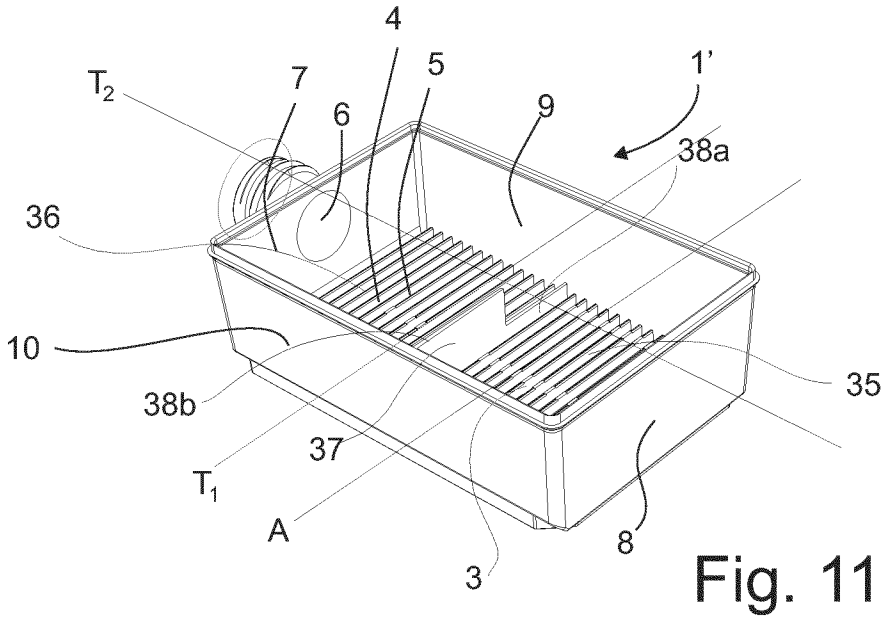

Fig. 11

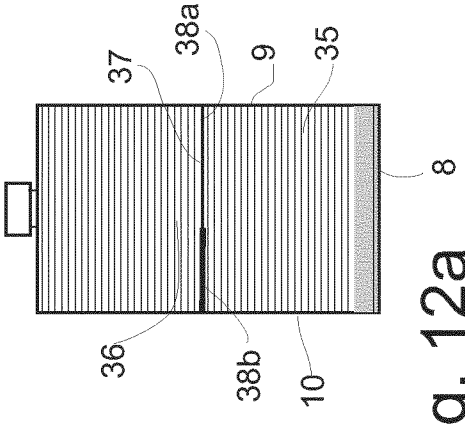

Fig. 12a

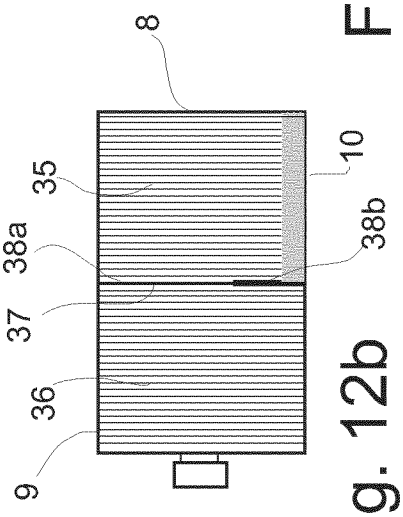

Fig. 12b

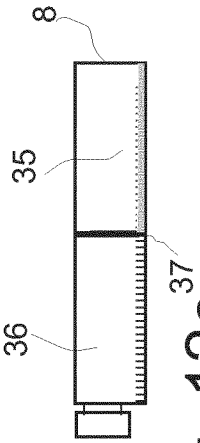

Fig. 12c

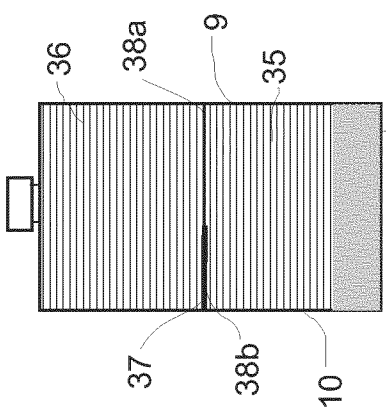

Fig. 12d

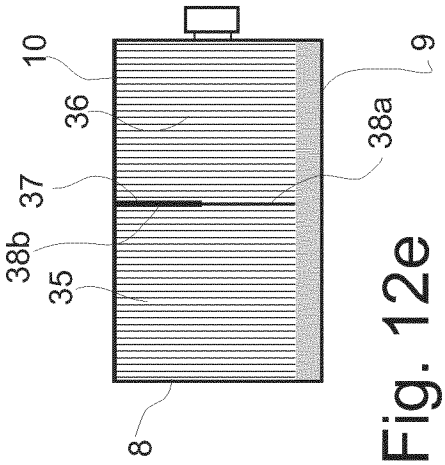

Fig. 12e

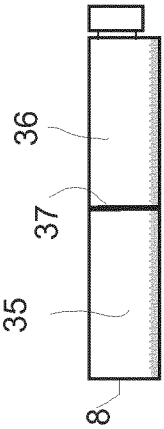

Fig. 12f

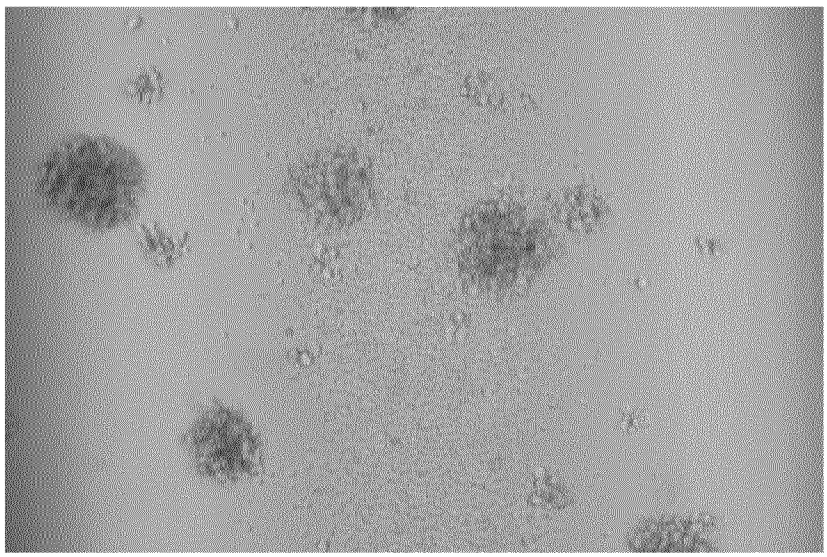

Fig. 13A

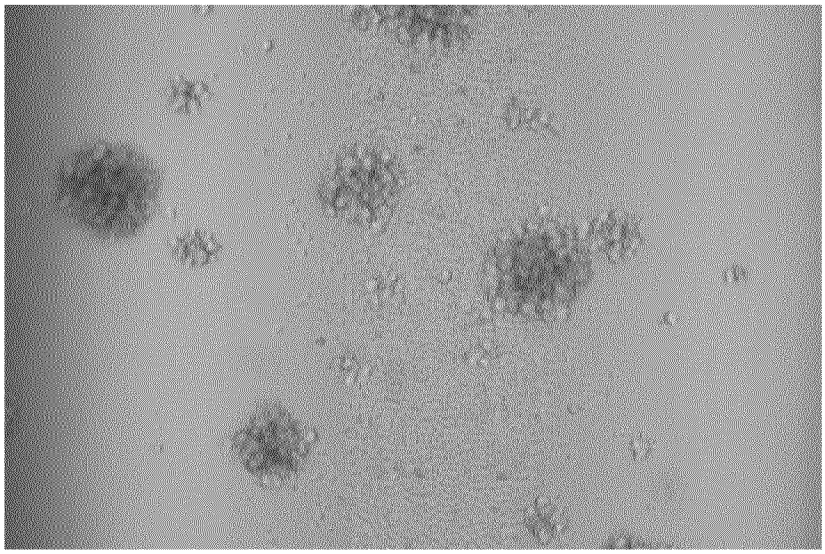

Fig. 13B

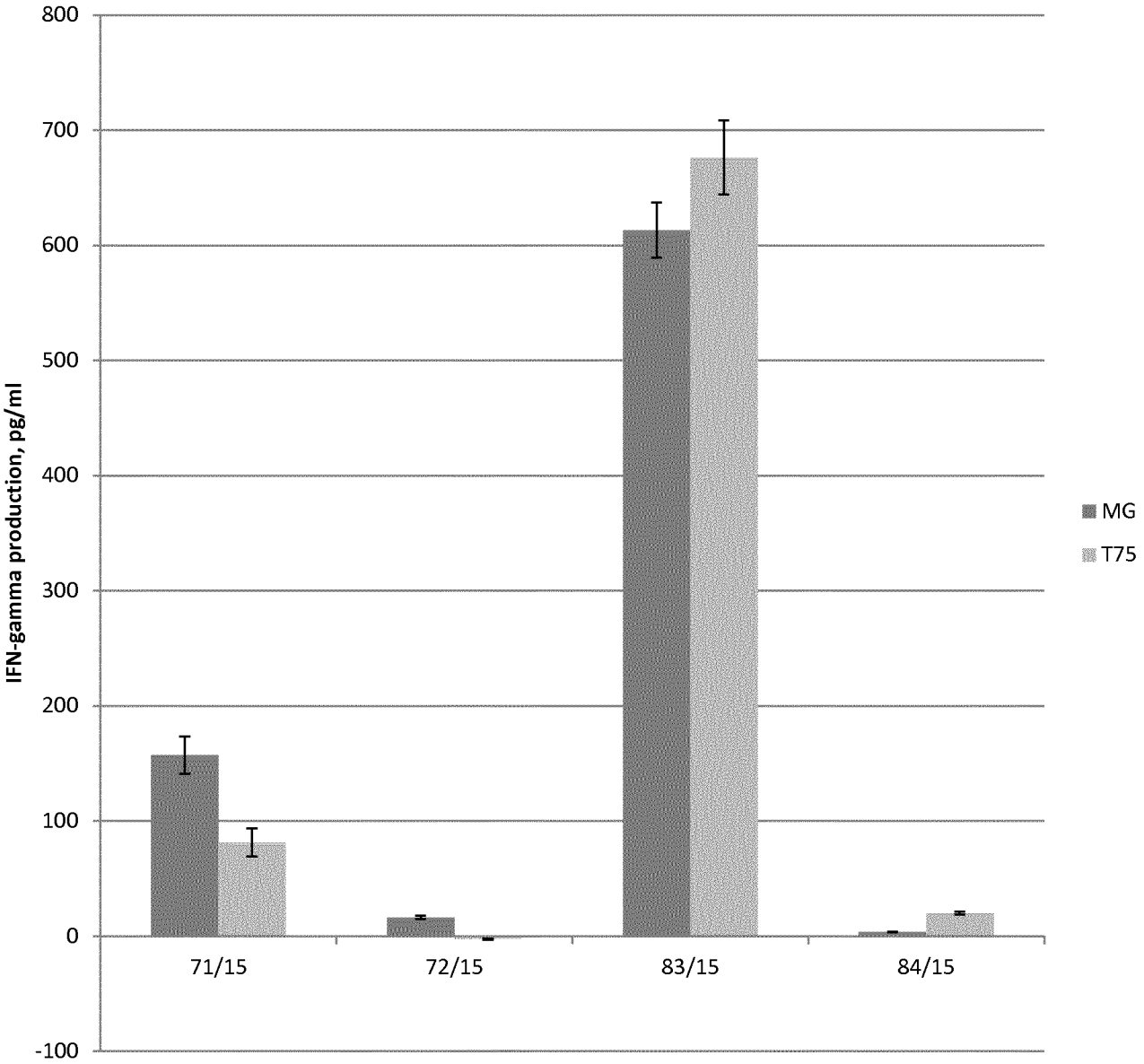
800
700
600
500
400
300
200
100
0
-100
IFN-gamma production, pg/ml
71/15
72/15
83/15
84/15
MG
T75

Fig. 14

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/069099

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12M1/24 C12M1/12
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12M

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	US 5 272 084 A (O'CONNELL DENNIS M [US] ET AL) 21 December 1993 (1993-12-21) figure 1 -----	9-11, 16-18 12-15,19
Y	WO 2010/008566 A2 (MILLIPORE CORP [US]; CLARK PHIL [US]; GREENIZEN KURT [US]) 21 January 2010 (2010-01-21) figure 1 ----- -/--	12-15,19

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 October 2016	07/11/2016

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Trommsdorff, Marion

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	Anonymous: "BD Falcon Cell Culture Multi-Flask", BD Biosciences , 1 January 2011 (2011-01-01), XP002762892, Retrieved from the Internet: URL:http://www.bdbiosciences.com/documents/multiflasks-data-sheet.pdf [retrieved on 2016-10-13] the whole document	12-15
A	US 2010/184182 A1 (HASE MASAHIKO [JP]) 22 July 2010 (2010-07-22) the whole document	1-19

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Information on patent family members

International application No
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