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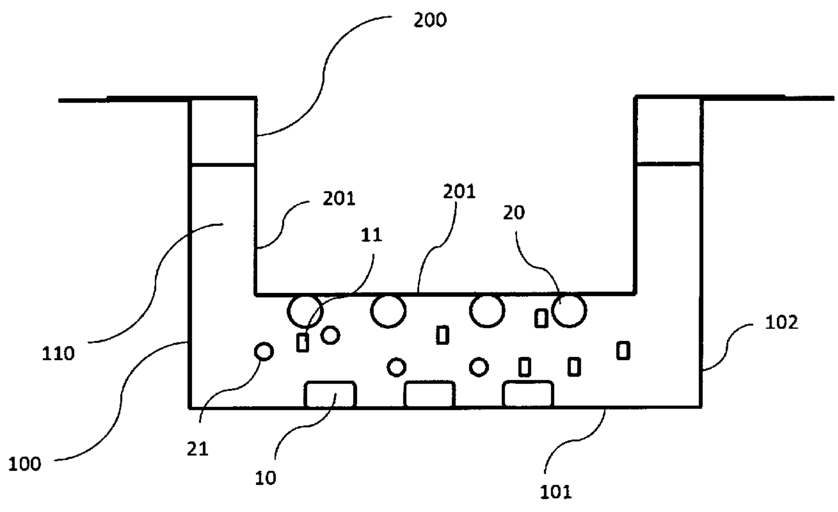

Figure 1

(57) **Abstract**: The invention relates to a cell culture device for culturing together at least a first type of cells and a second, different type of cells, wherein the first type of cells secretes at least a first type of signaling molecules which interacts with the second type of cells and the second type of cells secretes at least a second type of signaling molecules which interacts with the first type of cells, wherein the device comprises a first element having a first surface on which the first type of cells is present and a second element having a second surface on which the second type of cells is present and wherein the first surface and the second surface are separated only by a culture medium and wherein the first element and the second element are detachable.

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CELL CULTURE DEVICE

The present invention relates to a cell culture device for culturing together more than one types of cells. The present invention further relates to a method for culturing using such a device and a method for measuring intercellular interactions between more than one type of cells using such a device.

Growth characteristics of cells are affected by other types of cells. One way of determining the behavior of cocultured cells is by cell culture devices comprising a membrane as described in the art. For example, coculture of interacting endothelial cells (EC) and smooth muscle cells (SMC) have been studied e.g. by Fillinger et. al, Journal of surgical research 67, p. 169. This publication describes coculture of EC and SMC in bilayer and conditioned media models. In this publication, models were compared: SMCs cultured with ECs on the opposite side of the membrane in a bilayer coculture system that allows physical contact between ECs and SMCs (EC/SMC) and SMCs cultured in media continuously conditioned by ECs , without contact (conditioned media, CM).

WO2007/106868 discloses a cell culture device having an ultrathin porous membrane. The device includes first and second chambers separated by at least one nanoscale membrane positioned between the first and second chambers, the at least one nanoscale membrane having an average thickness of less than about 100 nm, and having a plurality of pores extending between opposite sides thereof.

While these cell culture devices can be used for certain types of cells, suitable membranes having an appropriate pore size have to be chosen for each combination of types of cells. There is hence a need in the art for a cell culture device that is simple and which can be applied for diverse cells which secretes various signaling molecules.

Accordingly, an object of the present invention is to provide a cell culture device for culturing different types of interacting cells, which solve the above and/or other problems. Further objects will be apparent from the description below.

According to a first aspect of the present invention, a cell culture device is provided for culturing together at least a first type of cells and a second, different type of cells, wherein the first type of cells secretes at least a first type of signaling molecules which interacts with the second type of cells and the second type of cells secretes at least a second type of signaling molecules which interacts with the

first type of cells,
wherein the device comprises a first element having a first surface on which the first type of cells is present and a second element having a second surface on which the second type of cells is present and
wherein the first surface and the second surface are separated only by a culture medium and
wherein the first element and the second element are detachable.

According to the device of the present invention, the first surface and the second surface are separated only by the culture medium. This is herein meant that the first surface and the second surface are present in the same culture medium. The signaling molecules are allowed to reach the cells with which they interact, without moving through e.g. pores of a membrane. The lack of membrane or other types of barrier between the first type of cells and the second type of cells allows free traveling of any signaling molecules between the first type of cells and the second type of cells. The movement of the signaling molecules is not restricted and thus the signaling molecules can be of any size. The fact that the first and the second surface are separated results in that the first and the second types of cells do not come in physical contact with each other, preventing the first and the second types of cells from attaching to each other. This avoids the risk of the first and the second types of cells influencing the secretion of signaling molecules by the presence of the first (second) type of cells next to the second (first) type of cells.

The first and the second surface are part of two detachable elements. This has an important advantage of allowing the two type of cells to be removed from the respective surface independently in separate environments after the culturing for any further measurements. In the case where the first and the second surface are not detachable, removing one type of cells by e.g. use of trypsin would usually result in the removal of the other type of cells, resulting in a mixture of two different types of cells. This is unsuitable when analysis of one specific type of cells is desired, e.g. when the amount of a certain molecule in the specific type of cells needs to be measured. Furthermore, this allows different types of cells to be subjected to different treatments or culturing before the first and the second elements are placed in their positions for culturing. For example, the first type of cells may be induced to secrete the first type of signaling molecules before the second element is placed in its position for culturing. This is especially advantageous in the cases where the second type of cells need to be triggered by the first type of signaling molecules to secrete the second type of signaling molecules. It is advantageously ensured that the co-culturing is started in the presence of the first signaling molecules.

It is noted that the device may comprise further types of cells in addition to the first and the second types of cells. For example, the first (second) type of cells may be present on the first (second) surface together with further different types of cells, i.e. two or more different types of cells may be present on the same surface. It is further noted that the cells may secrete further types of signaling molecules in addition to the first and the second types of signaling molecules. For example, first (second) type of cells may secrete not only first (second) type of signaling molecules, but may also secrete further different types of signaling molecules. These further signaling molecules may be of the type that may or may not interact with the other cells.

It is further noted that the culturing together of two or more types of cells is sometimes referred herein as co-culturing.

In some embodiments, the first element is a chamber containing the liquid medium and the first surface is the bottom of the chamber. The first type of cells remains at the bottom of the first chamber by gravity. In most cases movement (e.g. by diffusion) of the first type of cells is small so that it will not reach the second type of cells and make a physical contact therewith. In the cases where it is desired to ensure no contact between the first and the second type of cells, the first type of cells at the bottom of the first chamber may be attached to the bottom.

The second element may be suspended in the culture medium so that the second surface is in the liquid medium, i.e. under the surface of the culture medium. The second type of cells is preferably attached to the second surface of the second element. This is necessary e.g. when the second type of cells is present on the surface facing the bottom of the chamber. This is advantageous in that the first surface and the second surface face each other which makes it easy for the first (second) type of signaling molecules to reach the second (first) type of cells. In the cases where the second surface is facing up, i.e. where the second type of cells rests on a surface of the second element facing up, it is not necessary that the second type of cells is attached to the second surface of the second element.

The suspended second element may take any suitable shape as long as it can be inserted in the chamber. Further, the suspending of the second element in the culture medium may be implemented in a number of ways. For example, the suspended second element may e.g. be in a form of another chamber smaller than the chamber comprising the first type of cells at the bottom. The chamber to be inserted may have a rim that rests on the rim of the larger, receiving chamber. In another example, the suspended element may be a plate having a size that allows it to rest on the rim of the chamber. The plate may be provided with a protrusion protruding towards the bottom of the chamber, wherein the protrusion comprises the second surface to which the second type of cells is attached.

In embodiments where the second element is a chamber inserted in the chamber of the first element, the second surface may comprise a membrane. The membrane may be of any known type, e.g. PET, and may have pores of an average size of e.g. 0.1-10 µm, more typically 0.3-8 µm. The membrane may also be a membrane as disclosed in Fillinger et. Al, Journal of surgical research 67, p. 169 or WO2007/106868. The second element may in this case also contain a culture medium. This culture medium may be the same or different from the culture medium in the larger, receiving chamber.

The suspended second element may also be implemented as a plurality of separate objects, e.g. in the form of bars or plates. The second type of cells may be attached to the surface of the bars or may rest on the plates. The case where the suspended element comprises a plurality of separate or separable surfaces has an advantage that each of the surfaces may be removed from the culture medium at different time points for analysis so that time-dependent results can be obtained. The cells cultured in the same culture medium in the same way but attached to different surfaces may also be subjected to different analysis methods.

In further embodiments, both the first and the second elements are suspended in the culture medium. The suspended element may take any suitable form, for example as described above in relation to the second element. The first type of cells and the second type of cells are preferably attached to the first surface and the second surface, respectively.

It will be appreciated that only the surface on which the cells are attached needs to be in the culture medium and not the whole surface of the first and/or second element needs to be in the culture medium.

In further embodiments of the invention, more than two types of cells are cultured together. For example, all of said more than two types of cells are attached to different elements suspended in the culture medium. In other embodiments, one of said more than two types of cells is present at the bottom of the chamber containing cells and the rest of the cells are attached to different elements suspended in the culture medium.

A further aspect of the present invention relates to a method of culturing together more than one type of cells. The method comprises the steps of: providing the device according to the present invention and culturing the first type of cells at the first surface and the second type of cells at the second surface. The step of providing the device may comprise the sub-steps of:

a) providing the first element in the form of a first chamber and the second element in the form of a second chamber insertable in the first chamber with a space between the bottom of the first chamber and the lower surface of the bottom of the second chamber,

b) providing the first type of cells at the bottom of the first chamber,

c) providing the culture medium in the first chamber,

d) attaching the second type of cells at the lower surface of the bottom of the second chamber and

e) inserting the second chamber into the first chamber.

During the co-culturing, the device according to the present invention is typically maintained in a steady position. This ensures that the cells are maintained in their respective positions. In other cases, the device according to the present invention may be subjected to shaking during the culturing over part or the whole of the co-culturing process. This may facilitate the interaction between the different types of cells by inducing more movements of the signalling molecules.

An yet further aspect of the present invention relates to a method for measuring intercellular interactions between cells, comprising the steps of: performing the method of co-culturing according to the present invention; detaching the first element and the second element; and subjecting the first type of cells and/or the second type of cells to an analysis. The analysis may measure the amounts of the first and/or the second signaling molecules in the first and/or the second type of cells. Any known analysis methods for cell may be used, including e.g. flow cytometry, enzyme-linked immunosorbent assay (ELISA), liquid chromatography-mass spectrometry (LCMS), gas chromatography-mass spectrometry (GCMS) and immunocytochemistry. The culture medium which acted as the transporting medium for the signaling molecules may also be subjected to this analysis. Accordingly, the method may further comprise the step of separating the culture medium from the first type of cells and the second type of cells and subjecting the culture medium to an analysis.

Further, the analysis of the culture medium may be performed without stopping the co-culturing in the device according to the present invention, by taking a sample of the culture medium while the co-culturing is still performed.

In some embodiments of the method of the present invention for measuring intercellular interactions, the device used is a device according to the present invention where the second element is a chamber containing a culture medium inserted in the chamber of the first element and the second surface comprises a membrane. Sample may be taken from the culture medium in the chamber of the second element for analysis. This may be performed without stopping the co-culturing in the device according to the present invention. Accordingly, the method in these embodiments further comprises the step of subjecting the culture medium in the first chamber and/or the culture medium in the second chamber to an analysis while the method of co-culturing is performed

In a further embodiment, the method for measuring intercellular interactions between cells involves use of a plurality of devices according to the present invention. Co-culturing of the cells is started in a plurality of devices according to the present invention at the same time. The co-culturing is stopped at different time points for different devices for analysis of the cells therein, so that time-dependent data can be obtained. For example, co-culturing may be started for 10 devices according to the present invention. At the start of the co-culture (time=0), two of the devices may be subjected to an analysis. The rest of the group (8 devices) may go under the co-culturing for two hours, and two of them may be stopped for co-culturing and be subjected to an analysis. Similarly, after 6, 12 and 24 hours after the start of the co-culture, co-culturing may be stopped each time for two devices and they may be subjected to an analysis. Accordingly, there is provided a method comprising the steps of: providing a plurality of devices according to the present invention; co-culturing the cells in said plurality of devices according to the present invention and subjecting different sub-groups of the devices according to the present invention to an analysis at different predetermined times after the start of the co-culture.

An yet further aspect of the present invention relates to use of the device according to the present invention for measuring intercellular interactions between cells.

According to a further aspect of the invention, a system is provided comprising a plurality of the device according the present invention. Preferably, the first element of each of the devices is detachably connected to each other and/or the second elements are detachably connected to each other. In one embodiment of this system, a first substrate is provided comprising a plurality of holes each of which acts as the chamber for comprising the culture medium and a second substrate is provided comprising a plurality of protrusions each of which can be inserted to each of the holes of the first substrate. Each of the pairs consisting of the hole and the protrusion acts in the same way as the device of the present invention. Preferably, each of the protrusions can be detached from the substrate. This facilitates (cells on) each of the protrusions to be subjected to analysis at different times. Similarly, if each of the holes can be detached from the substrate, subjecting (cells in) each of the holes to analysis at different times will become easier.

The cells may be attached to the first and/or second surface in any known way. The ways to attach cells to a surface is well-known to the person skilled in the art. For example, collagen may be applied first and the cells may be subsequently applied. Another example is by providing a mechanical microstructure which is adapted so that cells can be attached thereto. Examples of such a mechanical microstructure of the surface include a textured microstructure and a porous microstructure having pores for accommodating the cells. Examples of ways of attaching cells to a surface is described e.g. in WO2007/106868. The description of WO2007/106868 is incorporated herein by reference with respect to the ways of attaching reagents to a membrane, which can be used in the present invention for attaching cells to the first and the second surfaces of the first and the second elements..

Tethering of the reagent to the substrate includes covalently bonding the reagent to the lower surface of the substrate, ionically associating the reagent with the lower surface of the substrate, adsorbing the reagent onto the lower surface of the substrate, or the like. Such association can also include covalently or non-covalently attaching the reagent to another moiety (of a coupling agent), which in turn is covalently or non-covalently attached to the lower surface of the substrate.

The surface may be first functionalized (i.e., primed) with a coupling agent that is attached to the surface thereof. This is achieved by providing a coupling agent precursor and then covalently or non-covalently binding the coupling agent precursor to the surface. Once the surface has been primed, the reagent is exposed to the primed surface under conditions effective to (i) covalently or non-covalently bind to the coupling agent or (ii) displace the coupling agent such that the reagent covalently or non-covalently binds directly to the membrane surface. The binding of the reagent to the substrate is carried out under conditions that are effective to allow one or more target-binding groups on the substrate to remain available for binding to a target reagent. Many of these materials and coupling agents are described in U.S. Patent Application Serial No. 10/082,634 to Chan et al, and U.S. Patent Application Serial No. 10/282,274 to Miller et al., each of which is hereby incorporated by reference in its entirety.

The distance between the first surface and the second surface should be chosen so that the first type of signaling molecules reaches the second type of cells and/or the second type of signaling molecules reaches the first type of cells. The distance is dependent on the type of cells and the type of culture medium and can be optimized by the person skilled in the art. The distance may e.g. be 0.3-4 mm. The distance of 0.5-1.2mm may be more preferred. In the cases where the distance between the first and the second surfaces vary, the smallest distance between the two surfaces is referred as the distance between the two surfaces. The adjustment of the distance optimizes the communication between the cells while minimizing the risk of the first and the second types of cells to have a physical contact with each other by diffusion of the detached cells.

The chamber used in the device according to the present invention can be formed by any suitable material and can have any desired configuration. For example, the chamber can be in the form of a dish, a centrifuge tube, a cell culture tube, or the like. Exemplary materials for the chamber include, without limitation, glass, ceramic, polycarbonate, vinyl, polyvinyl chloride (PVC), polydimethylsiloxane (PDMS), acrylic, polypropylene, polyethylene, polyethyl sulfone (PES), and metal.

Any combination of cells may be cultured in the culture device of the present invention. Animal cells are suitable, preferably mammalian cells. Particularly preferred are human cells. Other suitable cells include bacteria, cells of a fungus, yeast, algae and/or a plant. The cells may be genetically modified or altered cells. Preferred combinations of cells include combinations where at least one type of cells is human cells, such as human (primary) cells and bacteria cells. It is noted that human cells are commercially available.

Preferred examples of the types of cells that can be cultured in the device of the present invention include human primary cells, such as adipocytes / preadipocytes, bladder cells, brain cells, cardiac cells, endothelial cells, epidermal cells, epithelial cells, eye cells, fibroblasts, granulocytes, hepatic cells, human mesothelial cells, intestine and colon cells, lung and pulmonary cells, lymph cells, lymphocytes, mononuclear cells, neural nerve cells, phagocytes, renal cells, reproductive cells, skeletal cells, smooth muscle cells and umbilical cells. Further preferred examples of the types of cells that can be cultured in the device of the present invention include human stem cells such as mesenchymal stem cells, pericytes, CD34+ progenitor cells, CD133+ progenitor Cells, mononuclear cells and CD14+ Monocytes.

Particularly suitable cells for culturing together in the device of the present invention include human aorta endothelial cells (HAOEC) and human peripheral blood mono-nuclear cells (PBMC).

Once the co-culturing of cells has achieved its purpose, one or more types of cultured cells can be collected using any suitable collection techniques. For the purpose of separately analyzing the first type of cells and the second type of cells, the first element and the second element are detached. In the cases where the cells are attached to the surface, the cells can be removed by use of e.g. trypsin.

Any signaling molecule that possesses a natural or synthetic receptor can be studied in a device of the present invention, for example cytokines or cytokine related signaling cascades. Cell signaling molecules worthy of study include, without limitation, members of the Wnt family, members of the hedgehog family, notch family members, interferon family members, molecules that recognize toll-like receptors such as PAMPs, lymphokines, chemokines, cytokines, hormones, interleukins, molecules recognizing receptors of the Immunoglobulin super family (e.g., IL-I), molecules recognizing receptor tyrosine kinase receptors (e.g., FGF, HGF, etc.), molecules recognizing receptor serine/threonine kinase receptors (e.g., TGF-[beta], BMP, BMP inhibitor, etc.), and molecules recognizing seven transmembrane helix receptors (e.g., fMLP, IL-8, etc.). A particularly useful example include TNF-α (Tumor necrosis factor α) and PGE2 (prostaglandin E2).

Any suitable culture media can be employed in the culture device of the present invention. Suitable culture media need to support the growth and differentiation of cells of various tissues and (optionally) any accessory cells included therein. The culture media can be supplemented with any growth factors, metabolites, etc.

Generally, a cell culture medium for (mammalian) cells comprises salts, amino acids, vitamins, lipids, detergents, buffers, growth factors, hormones, cytokines, trace elements and carbohydrates. Examples of salts include magnesium salts, for example $MgCl_2.6H_2O$, $MgSO_4$ and $MgSO_4.7H_2O$ iron salts, for example $FeSO_4.7H_2O$, potassium salts, for example KH_2PO_4, KCl; sodium salts, for example NaH_2PO_4, Na_2HPO_4 and calcium salts, for example $CaCl_2.2H_2O$. Examples of amino acids are all 20 known proteinogenic amino acids, for example hystidine, glutamine, threonine, serine, methionine. Examples of vitamins include: ascorbate, biotin, choline.Cl, myo-inositol, D-panthothenate, riboflavin. Examples of lipids include: fatty acids, for example linoleic acid and oleic acid; soy peptone and ethanol amine. Examples of detergents include Tween 80 and Pluronic F68. An example of a buffer is HEPES. Examples of growth factors/hormones/cytokines include IGF, hydrocortisone and (recombinant) insulin. Examples of trace elements are known to the person skilled in the art and include Zn, Mg and Se. Examples of carbohydrates include glucose, fructose, galactose and pyruvate.

The pH, temperature, dissolved oxygen concentration and osmolarity of the cell culture medium are in principle not critical and depend on the type of cell chosen. Preferably, the pH, temperature, dissolved oxygen concentration and osmolarity are chosen such that it is optimal for the growth and productivity of the cells. The person skilled in the art knows how to find the optimal pH, temperature, dissolved oxygen concentration and osmolarity for the perfusion culturing. Usually, the optimal pH is between 6.6 and 7.6, the optimal temperature between 30 and 39°C, the optimal osmolarity between 260 and 400mOsm/kg.

The invention can in principle be used in any type of cell culture medium suitable for the culturing of (animal) cells. Guidelines for choosing a cell culture medium and cell culture conditions are well known and are for instance provided in Chapter 8 and 9 of Freshney, R. I. Culture of animal cells (a manual of basic techniques), 4th edition 2000, Wiley-Liss and in Doyle, A. , Griffiths, J. B., Newell, D. G. Cell & Tissue culture: Laboratory Procedures 1993, John Wiley & Sons.

Serum free media are preferred to media containing a serum source in the production of biopharmaceutical products as serum source media are frequently contaminated with viruses, present the risk of prionic infections, and can create a major obstacle in the downstream processing of the biopharmaceutical product (i. e. the further purification of the biopharmaceutical product from the cell culture medium).

Since compounds from a mammalian source also present an infection risk, preferably, the cell culture medium is not only serum-free, but also mammalian source free. More preferably the cell culture medium is not only mammalian source-free, but also animal source free.

Although the invention has been described in detail for purposes of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention as defined in the claims.

It is further noted that the invention relates to all possible combinations of features described herein, especially in the claims.

The invention is illustrated hereinbelow referring to the figures in which:

Figure 1 schematically illustrates an embodiment of the device according to the present invention;

Figure 2 schematically illustrates a further embodiment of the device according to the present invention;

Figure 3 schematically illustrates a further embodiment of the device according to the present invention;

Figure 4 shows a graph showing the amounts of signaling molecules during the co-culturing method according to the present invention and

Figure 5 shows a graph showing the amounts of signaling molecules during the co-culturing method according to a comparative example.

Referring to figure 1, the cell culture device according to the present invention is illustrated. A first element 100 in the form of a chamber comprises a bottom 101 and a side wall 102. The chamber 100 comprises a liquid culture medium 110. A first type of cells 10 secreting a first type of signaling molecules 11 rests on the bottom 101 (first surface) of the first chamber 100 by gravity. A second element 200 also in the form of a chamber is positioned within the first chamber 100. The second element 200 rests on the rim of the first element 100 by its rim. A second type of cells 20 secreting a first type of signaling molecules 21 is attached on the lower side (second surface) 201 of the bottom of the first chamber 200. The distance between the bottom 101 and the bottom 201 is such that the first type of signaling molecules 11 reaches the second type of cells 20 and the second type of signaling molecules 21 reaches the first type of cells 10. The first type of signaling molecules 11 may have an effect on the second type of cells 20 which increases or decreases the secretion of the second type of signaling molecules 21 by the second type of cells 20. The second type of signaling molecules 21 may have an effect on the first type of cells 10 which increases or decreases the secretion of the first type of signaling molecules 21 by the first type of cells 10.

The second chamber 200 in figure 1 may also contain a culture medium. In the case that the bottom of the second chamber 200 is a membrane, the second type of cells can have a contact also with the culture medium in the second chamber.

Figure 2 illustrates a further embodiment of the device according to the present invention. The first element 100 is identical to figure 1. A second element 200 is suspended in the culture medium 110 and hangs from a plate resting on the rim of the first element. The second element 200 is covered by a second type of cells attached thereto, which makes the whole surface of the second element 200 a second surface. The first and the second types of signaling molecules 11 and 21 work in the same was as described with respect to Figure 1.

Figure 3 illustrates a further embodiment of the device according to the present invention. The device comprises a chamber filled with a culture medium, as in Figures 1 and 2. However, in this embodiment, both the first element 100 and the second element 200 are suspended in the culture medium. The first and the second elements 100 and 200 in this embodiment are in the form of a bar, which is partly immersed in the culture medium. The portions immersed in the culture medium have cells attached thereto, i.e. the first element 100 has a first surface 101 to which first type of cells 10 are attached and the second element 200 has a first surface 201 to which first type of cells 20 are attached. The first and the second types of signaling molecules 11 and 21 work in the same was as described with respect to Figure 1. The first element 100 and the second element 200 are detachable from each other.

Many modifications of the device of the present invention are possible. For example, the embodiment illustrated in Figure 3 may be modified such that the first type of cells are present at the bottom of the chamber and the second type of cells are attached to the two bars suspended in the culture medium as shown. It will be appreciated that the number of bars may be modified to be more than two.

Examples

Example 1

The device according to the embodiment the present invention schematically illustrated in Figure 1 was used.

<u>Fixing cells on the inserting chamber</u>

A cylindrical cup for inserting in another cup was provided (hereinafter sometimes referred as an inserting chamber). The inserting chamber had a rim which can rest on another cup when inserted. The inserting chamber had a membrane as the bottom. The membrane had pores with a diameter of 0.4μm and a pore density of $2*10^6$pores/cm^2. (ThinCert, Greiner Bio-One)

The inserting chamber was placed upside down, i.e. the inserting chamber was placed so that the inside of the inserting chambers were facing downward.

500 μL of collagen coating solution (Cell Applications Inc) was placed on the outer surface of the membrane of the inserting chamber using a pipet. The entire outer surface of the membrane was covered and allowed to stand for 4 hours at room temperature in a flow cabinet. Subsequently, the collagen coating solution was washed away from the membrane with PBS. The inserting chambers were stored at 4 °C until use.

A suspension of human aorta endothelial cells (HAOEC) containing 50,000 cells/ml was prepared. 1ml of the cell suspension was coated on the outer surface of the membrane coated with the collagen coating solution. The inserting chamber was left for 2 hours in a flow cabinet to allow the cells to firmly attach to the outer surface of the membrane via the collagen.

A total of three inserting chambers having HAOEC attached on the outer surface of the membrane were made by the method described above, with 90% confluence.

Providing cells in the receiving chamber

Three chambers each having a larger diameter and depth (hereinafter sometimes referred as a receiving chamber) than the inserting chamber was provided.

Primary human peripheral blood mono-nuclear cells (PBMC) were seeded at the bottom of the receiving chamber comprising a culture medium RPMI (Sigma) with 10% FCS at 1,000,000ells/chamber. The PBMC were stimulated with 10 μg/ml lipid poly sagaride (LPS) from Sigma. After 1hour the culture medium was replaced by a culture medium without LPS and PBMC were left to secrete TNF-α for another 6 hours.

Culturing of cells

The inserting chambers with HAOEC were inserted in the receiving chambers with PBMC. The distance between the surface with HAOEC and the surface with PBMC was 0.7 mm. 3 ml of a culture medium was added to each of the receiving chambers and 1ml of the same culture medium was added to each of the inserting chambers. The three pairs of chambers were placed in an incubator at a temperature of 37°C and having an atmosphere of 5% CO_2.

The amounts of TNF-α and PGE2 in the culture medium in the inserting chambers and the receiving chambers were measured respectively at time points 0, 4 and 24 hours after start of the co-culture.

Results are shown in Figure 4.

Comparative example 1

The same device according to the embodiment of the present invention schematically illustrated in Figure 1 was used, except that the HAoEC were provided at the inner side of the inserting chamber. In this construction, HAoEC and PBMC are separated by a membrane.

The amounts of TNF-α and PGE2 in the inserting chambers and the receiving chambers were measured respectively at time points 0, 4 and 24 hours after start of the co-culture.

Results are shown in Figure 5.

It can be seen that, in the device according to the example (Table 1), the amount of TNF-α in the receiving chamber increased after the start of co-culturing, indicating that PBMC in the receiving chamber was releasing TNF-α. The amount of PGE2 in the receiving chamber increased with time. This indicates that HAOEC released PGE2, stimulated by TNF-α. It is therefore concluded that a good communication was established between HAOEC and PBMC in the receiving chamber in the device according to the present invention.

In comparison, the comparative example (Table 2) shows that the amount of PGE2 in the receiving chamber or the inserting chamber remained extremely low while the amount of TNF in the receiving chamber was very high. This shows that PBMC in the receiving chamber released a large amount of TNF but it did not reach HAOEC in the inserting chamber. There was a very low passage of molecules from the receiving chamber to the inserting chamber through the membrane. Also, the amount of TNF- α in the receiving chamber significantly increased over time in the comparative example, whereas the increase was small in the example according to the invention. This shows that the release of PGE2 has an effect on the release of TNF-α by PBMC, which effect was not seen in the comparative example.

Although not wishing to be bound by theory, the lack of communication in the comparative example may have been caused by the cells attached to the membrane blocking its pores for the signaling molecules to pass through.

CLAIMS

1. A cell culture device for culturing together at least a first type of cells and a second, different type of cells, wherein the first type of cells secretes at least a first type of signaling molecules which interacts with the second type of cells and the second type of cells secretes at least a second type of signaling molecules which interacts with the first type of cells,
wherein the device comprises a first element having a first surface on which the first type of cells is present and a second element having a second surface on which the second type of cells is present and
wherein the first surface and the second surface are separated only by a culture medium and
wherein the first element and the second element are detachable.
2. The device according to claim 1, wherein the first element is a chamber containing the culture medium and the first surface is the bottom of the chamber.
3. The device according to claim 2, wherein the second element is suspended in the culture medium in the chamber.
4. The device according to claim 2 or 3, wherein at least part of the second surface faces toward the first surface and the second type of cells is attached to the second surface.
5. The device according to claim 2, 3 or 4, wherein the second element is a chamber which is inserted in the chamber of the first element.
6. The device according to claim 5, wherein the chamber of the second element contains a culture medium.
7. The device according to claim 5 or 6, wherein the chamber of the second element comprises a membrane at its bottom and the second surface is the lower surface of the membrane.
8. The device according to claim 1, wherein the first element and the second element are both suspended in a culture medium.
9. The device according to claim 8, wherein the first type of cells is attached to the first surface and/or the second type of cells is attached to the second surface.
10. The device according to any one of claims 1-9, wherein the first type of cells and/or the second type of cells is human primary cells or human stem cells.

11. A method of culturing together at least a first type of cells and a second type of cells, comprising the steps of:
    - providing the device according to any one of claims 1-10 and
    - culturing the first type of cells at the first surface and the second type of cells at the second surface.
12. The method according to claim 11, wherein the step of providing the device comprises the sub-steps of:
    a) providing the first element in the form of a first chamber and the second element in the form of a second chamber insertable in the first chamber with a space between the bottom of the first chamber and the lower surface of the bottom of the second chamber,
    b) providing a first type of cells at the bottom of the first chamber,
    c) providing a culture medium in the first chamber,
    d) attaching the second type of cells at the lower surface of the bottom of the second chamber and
    e) inserting the second chamber into the first chamber.
13. A method of measuring intercellular interactions between cells, comprising the steps of:
    - performing the method of co-culturing according to claim 11 or 12;
    - detaching the first element and the second element; and
    - subjecting the first type of cells and/or the second type of cells to an analysis.
14. The method according to claim 13, wherein the device according to claim 6 is used and the method further comprises the step of subjecting the culture medium in the first chamber and/or the culture medium in the second chamber to an analysis while the method of co-culturing is performed.
15. Use of the device according to any one of claims 1-10 for measuring intercellular interactions between different kinds of cells.

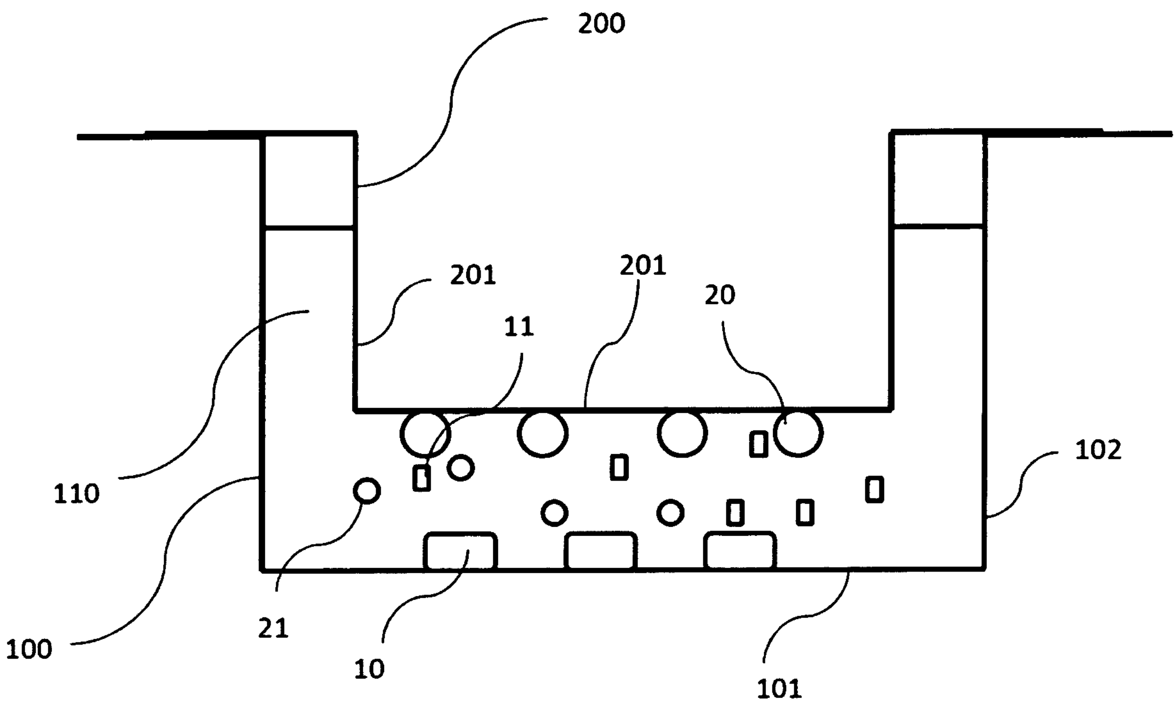

Figure 1

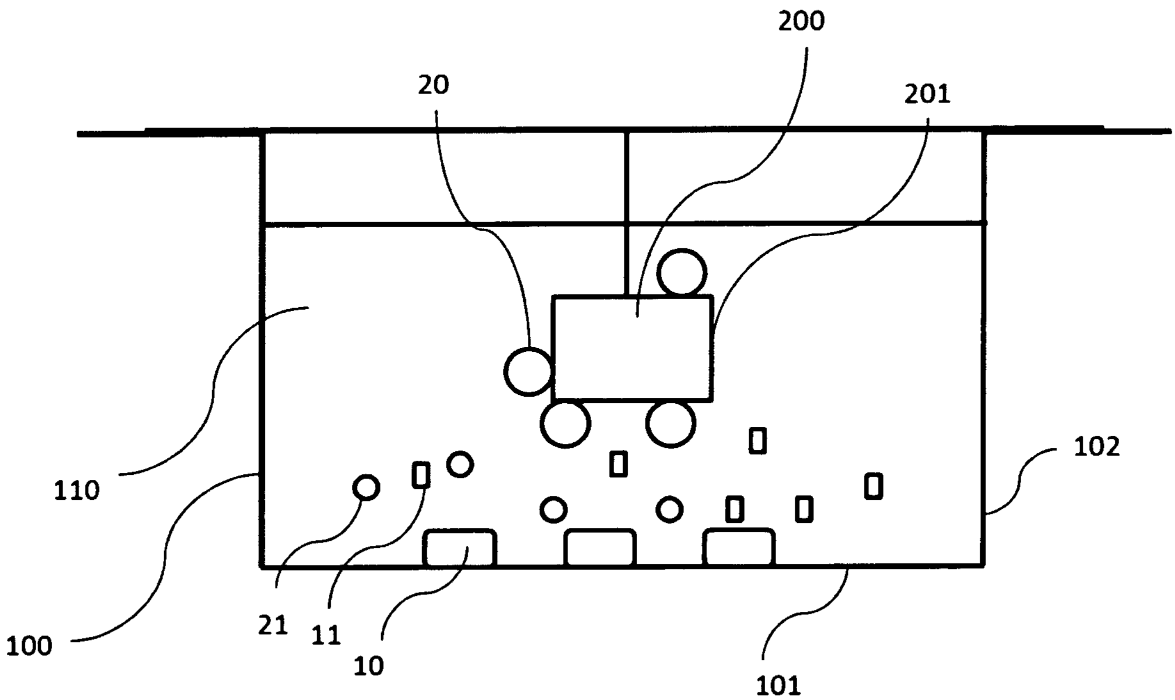

Figure 2

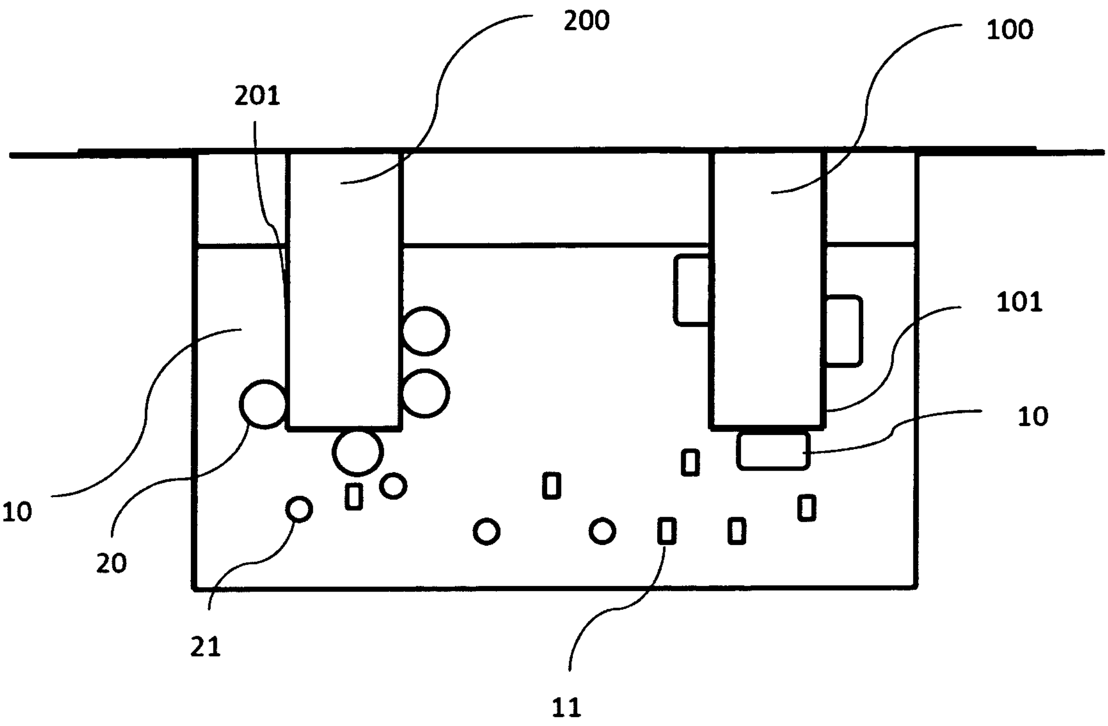

Figure 3

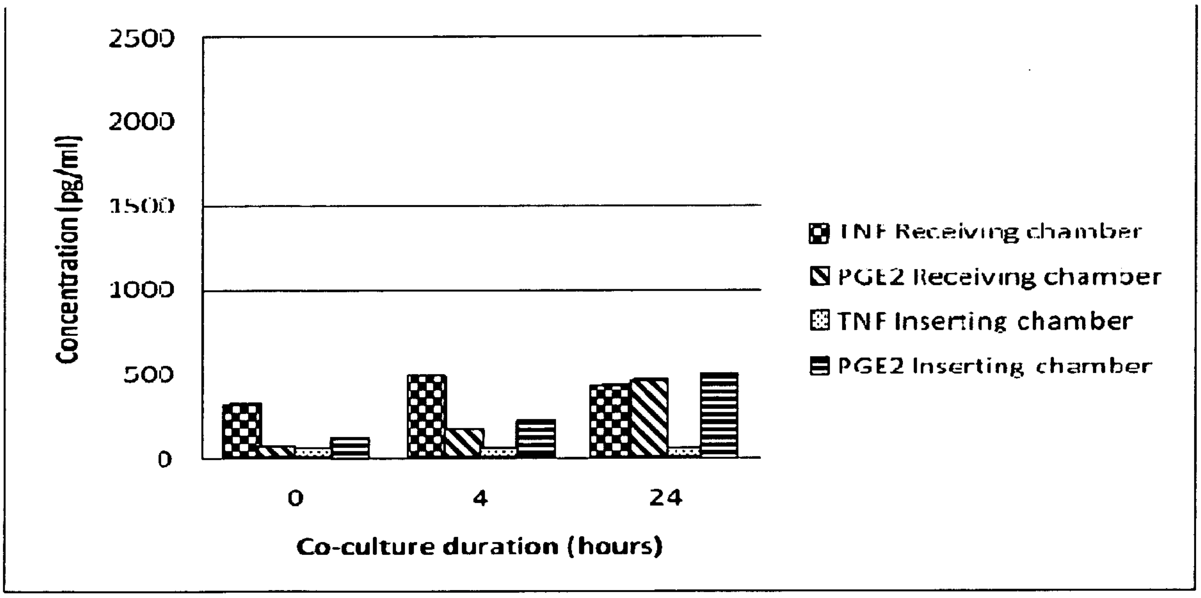

Figure 4

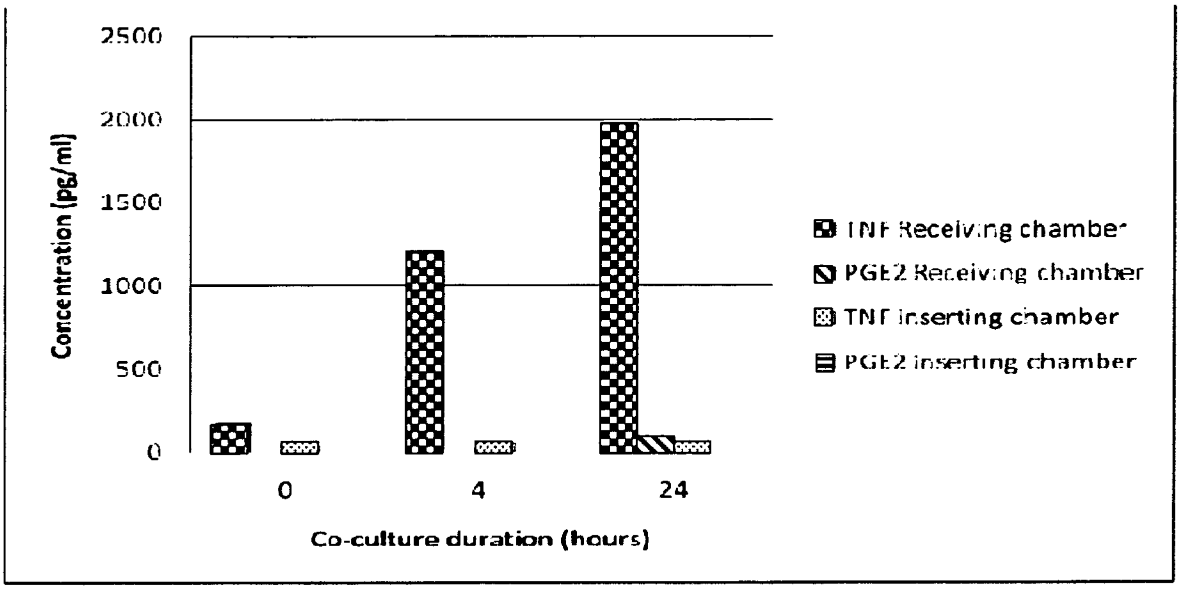
2500
2000
1500
1000
500
0
Concentration (pg/ml)
0
4
24
Co-culture duration (hours)
TNF Receiving chamber
PGE2 Receiving chamber
TNF inserting chamber
PGE2 inserting chamber

Figure 5