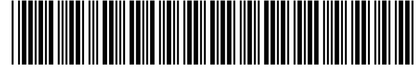
(19)



(11) **EP 2 741 083 A1**

(12) **EUROPEAN PATENT APPLICATION**

(43) Date of publication:
11.06.2014 Bulletin 2014/24

(51) Int Cl.:
G01N 33/50 *(2006.01)* ***B01L 3/00*** *(2006.01)*

(21) Application number: **13154001.5**

(22) Date of filing: **05.02.2013**

(84) Designated Contracting States:
AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR
Designated Extension States:
BA ME

(30) Priority: **07.12.2012 TR 201214253**

(71) Applicant: **Pesen Okvur, Devrim**
35320 Izmir (TR)

(72) Inventor: **Pesen Okvur, Devrim**
35320 Izmir (TR)

(54) **Three dimensional microfluidic device that determines metastatic capacity and homing choices**

(57) The invention provides a device that mimics the in vivo tumor microenvironment comprising different cell types, matrices, biological molecules and chemicals. All steps of metastasis, namely, angiogenesis, matrix invasion, cell migration, intravasation, circulation, extravasation and new tumor formation, in addition to homing choices of cancer cells can simultaneously and jointly be investigated using the said microfluidic device. The design of the device with multiple adjacent channels comprising 3D cell-laden or cell free matrices(24, 25, 26, 27, 28, 29) neighboring a flow channel (30) allows determination of metastatic capacity and homing choices of cancer cells.

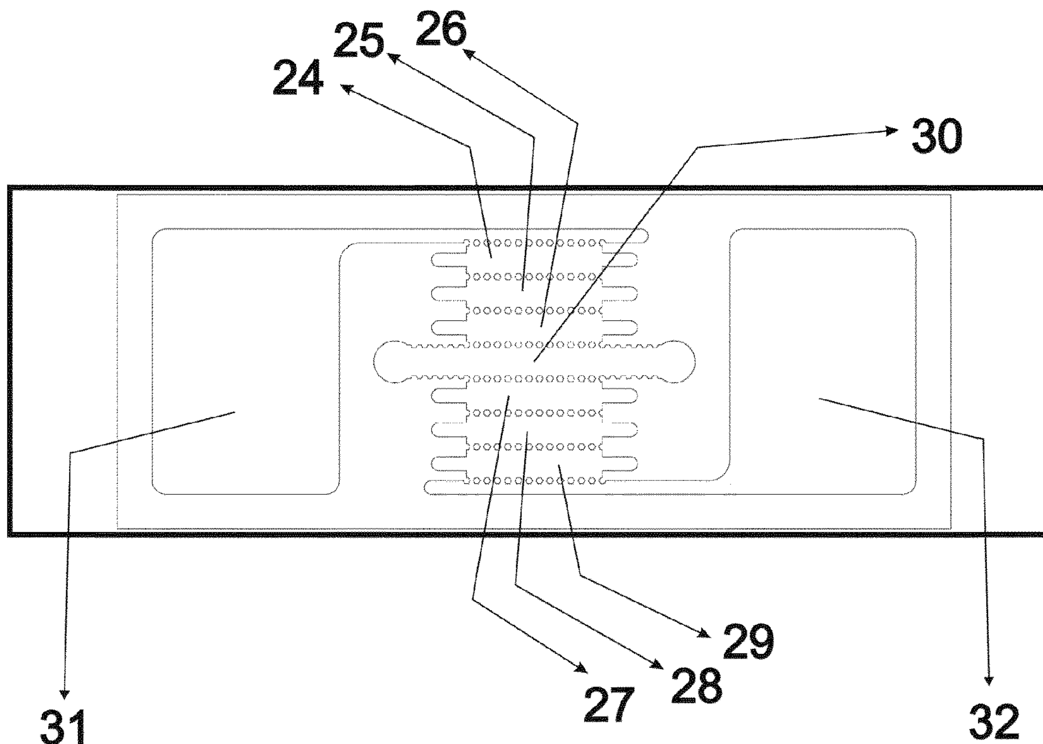


FIGURE 5

EP 2 741 083 A1

Description

Field of the Invention

[0001] The invention concerns a microfluidic device that determines metastatic capacity and homing choices. The device mimics the in vivo tumor microenvironment comprising different cell types, matrices, biological molecules and chemicals. All steps of metastasis, namely, angiogenesis, matrix invasion, cell migration, intravasation, circulation, extravasation and new tumor formation, can be simultaneously and jointly investigated using the said microfluidic device.

Background of the Invention

[0002] The leading cause of death for cancer patients is metastasis. Even if the primary tumor is surgically removed, cells that could have spread from the the primary tumor can cause formation of new tumors and recurrence of cancer. There is no test that can show whether cancer will recur or nor. In addition, as cancer spreads in the body, the tissues where new tumors form can be various. It is not known which type of tissue, cancer cells from each patient will prefer, for new tumor sites. Therefore, it is not possible to determine patient-specific therapies. In short, the desired points in diagnosis and therapy are not reached.

[0003] Metastasis of cancerous epithelial cells in the body comprises of steps occuring in a certain order: Cancer cells that have multiplied in an uncontrolled fashion induce angiogenesis, invade tissue matrix (invasion), migrate in tissue matrix towards blood vessels (migration), enter blood vessels (intravasation), move through blood vessels (circulation), exit blood vessels (extravsation) and form tumor in a new tissue. During these molecular events, cancer cells interact with various extracellular molecules (collagen, fibronectin, laminin, growth factors, free radicals, metal ions, etc.) and various cells (macrophages, fibroblasts, endothelial cells, epithelial cells, bone cells, etc.).

[0004] Important knowledge on cancer cell biology has been gained due to 2 dimensional and in vitro experiments. However, cells in vivo are in 3D (3 dimensional) matrices. Research shows that cell shape, adhesion, motility, response to growth factors and resistance to drugs are different in two and three dimensional settings [1-3]. Almost all experiments in vitro and even some animal models can not provide the in vivo orthotopic environment of cancer [4]. Today, only 8 out 100 clinical trials give effective results [5]. 3D cell culture systems have shown that they are a very necessary step between in vitro, in vivo and clinical experiments [6,7]. Therefore, to achieve a comprehensive understanding of the interactions of cancer with its microenvironment, new cell culture systems are needed.

[0005] There are some 3D and some co-culture examples [8,9]. However,mimicking the in vivo microenvironment is far beyond completion: In vivo, different cell and tissues types exist at certain locations with respect to each other, such as connective tissue being around blood vessels. In addition, cancer metastasis is composed of steps that occur in a certain order. Therefore, a system that will investigate these steps has to mimic the in vivo microenvironment.

[0006] Needle biopsy is used to predict metastatic capacity. It is also proposed that gene signatures will be useful [10]. However, functional tests at the cell and/or tissue levels do not exist. Needle biopsy is a structural test because it determines the organization of cells taken from the patient checking whether cells are connected to eacah other or are dispersed. On the other hand, a functional test would check whether cells carry out the steps of metastasis: angiogenesis, matrix invasion, cell migration, intravasation, circulation, extravasation and new tumor formation, or not.

[0007] Microfluidic technology provides precise spatial and temporal control, high-throughput analysis, low fabrication costs ve portability. Used material and waste volumes can be as low as picoliters. Using small volumes of unknown or toxic materials provides safe experimental study. Moreover, microfluidic technology can provide means to mimic physiological microenvironments. This feature can help us more realistically study cells in both health and disease states and improve drug testing approches. It can also help reduce animal testing.

[0008] With microfluidic technology based set-ups, some steps of cancer cell metastasis have been studied to certain degree. Research on breast cancer cells and fibroblast cells together in 3D collagen [11], angiogenesis due to growth factors in microfluidic channels and interactions of cancer cells with endothelial cells [12,13], effects of drugs in microfluidic channels on cancer cells in 3D cell culture [14], hepatocyte cell culture in microfluidic systems [15], interactions of breast cancer cells and macrophages in 3D microfluidic channels [16], interactions of breast cancer cells and fibroblasts in 3D microfluidic channels [17], interactions of breast cancer cells, macrophages and endothelial cells in 3D microfluidic channels [18] have been perfomed. However, there is neither a set-up nor a device that can simultaneously and jointly investigate all steps of cancer cell metastasis and that can mimic the required features of the in vivo microenvironment.

[0009] The patent which has the closest content to the submitted application here is the patent by Roger Kamm, titled "Device for High Throughput Investigations of Cellular Interactions" [19]. The basic unit in the mentioned patent, is composed of various flow channels around one microfluidic channel/area comprising 3D cell-laden matrix. By repeating the basic unit high-throughput is achieved. Since there is only one microfluidic channel/area comprising 3D cell-laden matrix in each basic unit, it is not possible for different cell types to be studied to be in neighboring but separate microfluidic channels/areas comprising 3D cell-laden matrices. Thus, cells to be stud-

ied together are mixed in 3D matrix and loaded into the microfluidic channel/area comprising 3D cell-laden matrix. In vivo, there can be more than one type of cell in one region, for example both macrophages and fibroblasts can be found in connective tissue; however different cell types are ofund in different tissues and organs: For example lung epithelial cells are found in lungs, breast epithelial cells are found in breast. For example in breast cancer, cancer epithelial cells are next to normal epithelial cells when they first form a tumor. When cancer epithelial cells pass into connective tissue, they come next to macrophages and fibroblasts that can be found together in connective tissue. As cancer epithelial cells spread in the body, they interact with different cell types such as endothelial cells, lung epithelial cells, live epithelial cells, bone cells found in different regions in the body. To be able to study cancer metastasis outside the organism, it is necessary to mimic the in vivo organization of different cell types in different regions, outside the organism. Therefore, the device in Kamm's patent, due to having only one 3D cell-laden matrix channel/area in its basic unit, is limited in mimicking the in vivo microenvironment. Hence, at most three different cell types could have been simultaneously studied. In addition, the mentioned device does not make it possible to investigate all steps of cancer metastasis simultaneously and jointly. Another deficiency is that the device cannot determine homing choices of cancer cells. For example, it cannot determine whether breast cancer cells that have entered blood flow will form new tumors in regions with 3D lung epithelial cell-laden matrix or 3D liver epithelial cell-laden matrix or 3D bone cell-laden matrix.

[0010] On the other hand, the device I present in this patent application, due to its basic unit comprising at least one flow channel and at least 3 on each side, at least 6 in total, channels, comprising 3D cell-free or cell-laden matrices neighbouring each flow channel, achieves (i) simultaneous investigation of at least five different cell types, (ii) simultaneous investigation of all steps of cancer metastasis and thus determination of metastatic capacity, and (iii) determination of homing choices of cancer cells.

[0011] To determine metastatic capacity, all steps of metastasis should be investigated simultaneously and jointly because each step is connected to others. Cancer cells less successful at one or more steps can still metastasize. The important question is whether new tumors form or not as a result of the sum of all steps. This is the question the drugs that will be used against cancer are expected to answer. More important than which metastatic step the drug affects is whether the drug prevents new tumor formation or not. Side effects of anti-cancer drugs on normal cells is an unwanted situation. It is ideal to use cell culture devices comprising 3d matrices, multiple and different cell types and thus best mimicking the in vivo conditions, to investigate side effects.

Summary of the invention

[0012] The purpose of the invention is to determine metastatic capacity of cancer cells.

[0013] Another purpose of the invention is to determine homing choices of cancer cells.

[0014] Another purpose of the invention is to simultaneously and jointly investigate all steps of metastasis, namely, angiogenesis, matrix invasion, cell migration, intravasation, circulation, extravasation and new tumor formation under conditions mimicking the in vivo microenvironment.

[0015] Another purpose of the invention is to test anti-metastasis drugs.

[0016] The microfluidic device with the desccribed features is shown in the drawings below.

[0017] The drawings are not necessarily to scale.

Brief description of the drawings

[0018]

Fig. 1 shows a drawing of a longitudinal outside view of a device and presentation of peripheral units
Fig. 2 shows a drawing of a longitudinal, top outside section view of a device
Fig. 3 shows a drawing of a longitudinal, top inside section view of a device with references 21 and 22
Fig. 4 shows a drawing of a longitudinal, top inside section view of a device with references 23
Fig. 5 shows a drawing of a longitudinal, top inside section view of a device with references 24, 25, 26, 27, 28, 29, 30, 31 and 32
Fig. 6 shows a drawing of the places of cross-sections at the longitudinal, top inside section view of a device
Fig. 7 shows a drawing of the cross-sections taken at places noted in Fig. 6
Fig. 8 shows a drawing of a longitudinal, top inside section view of a device and the 3D view of selected region of the device including a fluid reservoir
Fig. 9 shows a drawing of a longitudinal, top inside section view of a device and the 3D view of selected region of the device including two partial rows of posts.

Brief description of the references in the drawings

[0019] Parts in the figures are numbered and their explanations are given below:

1: The optically transparent surface that forms the base of the device
2: The structure of the device
3: Channel inlet or outlet
4: Channel inlet or outlet
5: Channel inlet or outlet
6: Channel inlet or outlet

7: Channel inlet or outlet
8: Channel inlet or outlet
9: Channel inlet or outlet
10: Channel inlet or outlet
11: Channel inlet or outlet
12: Channel inlet or outlet
13: Channel inlet or outlet
14: Channel inlet or outlet
15: Channel inlet or outlet
16: Channel inlet or outlet
17: Fluid reservoir inlet
18: Fluid reservoir outlet
19: Fluid reservoir inlet
20: Fluid reservoir outlet
21: Curved interior corner
22: Border area that prevents direct mixing of fluids in fluid reservoirs
23: Post
24: 3D cell-free or cell-laden matrix channel
25: 3D cell-free or cell-laden matrix channel
26: 3D cell-free or cell-laden matrix channel
27: 3D cell-free or cell-laden matrix channel
28: 3D cell-free or cell-laden matrix channel
29: 3D cell-free or cell-laden matrix channel
30: Flow channel
31: Fluid reservoir
32: Fluid reservoir
33: Connector
34: Tubing
35: Storage reservoir
36: Tubing
37: Tubing
38: Air bubble trap
39: Tubing
40: Flow regulating pump

Detailed description of the invention

[0020] The invention is a microfluidic device that can be used to simultaneously and jointly investigate all steps of metastasis and homing choices of cancer cells. The device mimics the in vivo tissue level organization of tumor microenvironments comprising various tissues and blood vessels. Different tissues are mimicked by at least six different channels comprising cell-laden or cell-free 3D matrices (24, 25, 26, 27, 28, 29). Blood vessels are mimicked by at least one flow channel (30) comprising endothelial cells. Channels comprising cell-laden or cell-free 3D matrices (24, 25, 26, 27, 28, 29) are arranged along each long side of the flow channel (30) and one of the channels on one side of the flow channel (30) comprises cancer cell laden matrix and all others comprise cell free or normal cell laden matrices.

[0021] In one embodiment, the structure (2) on the optically transparent surface has the following parts: at least one flow channel (30) comprising endothelial cells on its walls, at least three adjacent channels comprising cell-laden or cell-free 3D matrices, neighboring each flow channel (30) on each long side of it, with same or different lengths as the flow channel (30), in total at least six channels comprising cell-laden or cell-free 3D matrices (24, 25, 26, 27, 28, 29), at least two fluid reservoirs (31, 32) each neighbouring one of the channels comprising cell-laden or cell-free 3D matrices (24, 29) furthest from the flow channel (30), at least two border areas that prevent direct mixing of different fluids in the fluid reservoirs (22), borders comprising at least twelve posts (23) that separate neighboring channels (24, 25, 26, 27, 28, 29, 30) from each other, and channels (24, 29) from neighboring fluid reservoirs (31, 32), curved interior corners (21) that minimize air bubble formation.

[0022] There are at least three 3D cell-free or cell-laden matrix comprising channels adjacent to eac other and to each side of each flow channel (30). Cells and/or molecules from at least six 3D cell-free or cell-laden matrix comprising channels can reach each flow channel through spacings between posts. If the number of flow channels (30) is increased, number of 3D cell-free or cell-laden matrix containing channels will increase accordingly. If there are 2 flow channels (30), there will be at least 12 of 3D cell-free or cell-laden matrix containing channels.

[0023] Fluid reservoirs (31, 32) provide biological molecules and chemicals to cells furthest away from the flow and mimic interstitial fluid.

[0024] One end of the flow channel (30) is connected via connector (33) and tubing (39) to air bubble trap (38) connected via tubing (37) to flow regulating pump (40) and the other end of the flow channel is connected via connector (33) and tubing (34) to a storage reservoir (35) where flow passes through and cancer cells and other biological molecules and chemicals that have entered flow can be collected for analysis. The storage reservoir (35) is connected via tubing (36) to the flow regulating pump (40).

[0025] All channels (24, 25, 26, 27, 28, 29, 30) and fluid reservoirs (31,32) in the device have each one inlet for loading culture media, physiological buffer solution, biological molecules or chemicals to be tested, cell-free matrix, cell-laden matrix or a combination thereof and each one outlet for inside air or preloaded fluid to exit during loading (3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20). If the number of flow channels (30) is increased, numbers of inlets, outlets (15, 16), connectors (33), inlet and outlet tubings (34, 39), and if desired number of storage reservoirs (35), inlet and outlet tubings (34, 36) of storage reservoirs will be increased accordingly.

[0026] If the border areas that prevent direct mixing of fluids in the fluid reservoirs (22) are not used, the fluids in the two fluid reservoirs can have the same composition.

[0027] The fact that the interior corners are curved can reduce air bubbles that can form inside the device during fluid loading.

[0028] All channels (24, 25, 26, 27, 28, 29, 30) in the device are separated from each with rows of posts (23).

Fluid reservoirs (31, 32) are also separated from the outermost channels (24, 29) with rows of posts (23). There are at least 12 posts (23) in each row. Since the separation of channels and fluid reservoirs is realized with rows of posts instead of solid walls, cells, biological molecules and/or chemicals in one channel and/or reservoir can pass to other channel and/or reservoirs.

[0029] All channels (24, 25, 26, 27, 28, 29, 30), fluid reservoirs (31,32) and posts (23) are of the same height. Their height can be between 50 micrometers and 5 millimeters.

[0030] Width and length of each channel (24, 25, 26, 27, 28, 29,30) can be the same as or different from the widths and lengths of other channels. Widths of channels can be between 100 micrometers and 25 milimeters.

[0031] The horizontal cross-section of each post (23) can have the shape of a hexagon, a circle or an ellipse. The width of each post can be between 50 micrometers to 3 millimeters.

[0032] The spacing between two consecutive posts (23) is shorter than the width of the post (23) which has the smaller width of the two consecutive posts (23).

[0033] The diameters of inlet and outlets in the device (3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20) can be between 50 micrometers and 10 milimeters.

[0034] When more than one device is used, the devices can have separate optically transparent surfaces (1) or their structures (2) can be organized on one common optically transparent surface.

[0035] The material of the optically transparent surface (1) can be glass, polydimethylsiloxane (PDMS) or polystyrene (PS).

[0036] The material of the structure (2) on the optically transparent surface (1) can be polydimethylsiloxane (PDMS) or polystyrene.

[0037] The structure (2) on the optically transparent surface (1) can be fabricated by polymerizing PDMS on silicon or SU-8 masters prepared with standard lithography techniques, or by polymerizing PS on PDMS masters prepared with standard lithography techniques, or by injection molding PS. The optically transparent surface (1) and the structure can be bonded using UV/ozone treatment, plasma treatment and/or heating.

[0038] Cell lines and/or cancer patient biopsy cells such as cancer cells, macrophages, fibroblasts, endothelial cells, normal breast epithelial cells, myoepithelial cells, normal liver epithelial cells, hepatocytes, normal lung epithelial cells, normal bone cells can be used in the device. At least five different cell types can be simultaneously and jointly investigated in the device. Normal epithelial cells of type same as the cancer type or different from the cancer type can be used. For example, if breast cancer cells will be studied, breast cancer epithelial cells and normal breast epithelial cells and/or normal lung epithelial cells can be used.

Example 1: 1. Breast cancer epithelial cells 2. normal breast epithelial cells 3. fibroblasts 4. macrophages 5. endothelial cells.

Example 2: 1. Breast cancer epithelial cells 2. normal breast epithelial cells 3. fibroblasts 4. macrophages 5. endothelial cells 6. bone cells

Example 3: 1. Breast cancer epithelial cells 2. normal breast epithelial cells 3. fibroblasts 4. macrophages 5. endothelial cells 6. liver cells

Example 4: 1. Breast cancer epithelial cells 2. normal breast epithelial cells 3. fibroblasts 4. macrophages 5. endothelial cells 6. normal lung epithelial cells

Example 5: 1. Breast cancer epithelial cells 2. normal breast epithelial cells 3. fibroblasts 4. macrophages 5. endothelial cells 6. normal lung epithelial cells 7. bone cells

[0039] A sample placement of cells in the device for the Example 5 above: Breast cancer epithelial cells in matrix in relevant channel (24), normal breast epithelial cells in matrix in relevant channel (25), fibroblasts and macrophages in matrix in relevant channel (26), endothelial cells in flow channel (30), fibroblasts and macrophages in matrix in relevant channel (27),normal lung epithelial cells in matrix in relevant channel (28), bone cells in matrix in relevant channel (29).

[0040] Blood of cancer patient can be used as fluid passing through the flow channel (30). In this case, metastatic capacity of cancer cells that have already entered blood circulation can be investigated. Blood of healthy individual can be used as control.

[0041] A sample application is as follows:

Cell-free matrix is loaded into relevant channels (24, 29),

Cancer cell laden matrix is loaded into relevant channel (25),

Macrophage and fibroblast laden matrix is loaded into relevant channels (26, 27),

Normal epithelial cell laden matrix is loaded into relevant channel (28).

After matrices polymerize, endothelial cells are loaded into the flow channel (30) and culture media are loaded into fluid reservoirs (31, 32).

The storage reservoir (35) is filled with physiological buffer.

The flow regulating pump (40), air bubble trap (38), storage reservoir (35) and tubings (34, 36, 37, 39) are connected to each other.

The flow channel (30) is connected to flow via connectors (33) and tubings (34, 39). Tubings (34, 39) can be directly placed in inlet and outlet (15, 16) without using connectors (33) if desired.

Device is placed on microscope stage. Device is kept at 37°C. Device is also kept at 5% CO_2 atmosphere unless CO_2 independent culture media are used.

[0042] After endothelial cells form a monolayer, flow is started. Microscope images are taken at regular time in-

tervals at predefined positions in the device. Cell behavior is observed, recorded and analyzed. Or at a predetermined time point, for example 3 days, 7 days, 14 days, cells in the device are labeled using standard immunohistochemistry to determine locations of cancer cells and other normal cells of interest, for example, endothelial cells, macrophages, normal epithelial cells, etc.

[0043] If cancer cells reach normal epithelial cell laden matrix comprising channel (28) and form new tumors, they are classified as cancer cells with high metastatic capacity.

[0044] The device can be used to determine whether a biological molecule or a chemical or a combination thereof prevents metastasis or not. A sample application is as follows:

Cancer cell laden matrix is loaded into relevant channel (24),
Fibroblast laden matrix is loaded into relevant channels (25, 28),
Macrophage laden matrix is loaded into relevant channels (26, 27),
Normal epithelial cell laden matrix is loaded into relevant channel (29).

[0045] After matrices polymerize, endothelial cells are loaded into the flow channel (30) and culture media are loaded into fluid reservoirs (31, 32).

[0046] The storage reservoir (35) is filled with physiological buffer and biological molecule, chemical or a combination thereof to be tested.

[0047] The flow regulating pump (40), air bubble trap (38), storage reservoir (35) and tubings (34, 36, 37, 39) are connected to each other.

[0048] The flow channel (30) is connected to flow via connectors (33) and tubings (34, 39). Tubings (34, 39) can be directly placed in inlet and outlet (15, 16) without using connectors (33) if desired.

[0049] Device is placed on microscope stage. Device is kept at 37°C. Device is also kept at 5% CO_2 atmosphere unless CO_2 independent culture media are used.

[0050] After endothelial cells form a monolayer, flow is started. Microscope images are taken at regular time intervals at predefined positions in the device. Cell behavior is observed, recorded and analyzed. Or at a predetermined time point, for example 3 days, 7 days, 14 days, cells in the device are labeled using standard immunohistochemistry to determine locations of cancer cells and other normal cells of interest, for example, endothelial cells, macrophages, normal epithelial cells, etc.

[0051] If new tumors form in the normal epithelial cell laden matrix (29) comprising channel when controls of biological molecule, chemical or a combination thereof is used and new tumors do not form in the normal epithelial cell laden matrix comprising channel (29) when the biological molecule, or chemical or a combination thereof tested is used, then this indicates the biological molecule, or chemical or a combination thereof tested can be used against cancer metastasis.

[0052] The device can be used to determine the homing choices of cancer cells. For example to determine whether breast cancer cells will metastasize to lungs or bones:

in one device breast cancer cell-laden matrix can be loaded into one channel on one side of the flow channel (30) and normal bone cell-laden matrix into another channel on the other side of the flow channel (30) and in another device breast cancer cell-laden matrix can be loaded into one channel on one side of the flow channel (30) and normal lung epithelial cell-laden matrix into another channel on the other side of the flow channel (30) or

[0053] in one device breast cancer cell-laden matrix can be loaded into one channel on one side of the flow channel (30) and normal lung epithelial cell-laden matrix into one half of another channel and normal bone cell-laden matrix into the other half of the channel on the other side of the flow channel (30) or in one device breast cancer cell-laden matrix can be loaded into one channel on one side of the flow channel (30), normal lung epithelial cell-laden matrix and normal bone cell-laden matrix into other channels on the other side of the flow channel (30).

[0054] Then where breast cancer cells migrate and where they form new tumors in each device can be determined.

[0055] For example to determine whether breast cancer cells will metastasize to lungs or bones an application is as follows:

Normal epithelial cell laden matrix is loaded into relevant channel (24),
Cancer cell laden matrix is loaded into relevant channel (25),
Macrophage and fibroblast laden matrix is loaded into relevant channels (26, 27),
Normal lung epithelial cell laden matrix is loaded into relevant channel (28).
Normal bone cell laden matrix is loaded into relevant channel (29).

[0056] After matrices polymerize, endothelial cells are loaded into the flow channel (30) and culture media are loaded into fluid reservoirs (31, 32).

[0057] The storage reservoir (35) is filled with physiological buffer.

[0058] The flow regulating pump (40), air bubble trap (38), storage reservoir (35) and tubings (34, 36, 37, 39) are connected to each other.

[0059] The flow channel (30) is connected to flow via connectors (33) and tubings (34, 39). Tubings (34, 39) can be directly placed in inlet and outlet (15, 16) without using connectors (33) if desired.

[0060] Device is placed on microscope stage. Device is kept at 37°C. Device is also kept at 5% CO_2 atmos-

phere unless CO_2 independent culture media are used.

[0061] After endothelial cells form a monolayer, flow is started. Microscope images are taken at regular time intervals at predefined positions in the device. Cell behavior is observed, recorded and analyzed. Or at a predetermined time point, for example 3 days, 7 days, 14 days, cells in the device are labeled using standard immunohistochemistry to determine locations of cancer cells and other normal cells of interest, for example, endothelial cells, macrophages, normal epithelial cells, etc.

[0062] If cancer cells reach normal lung epithelial cell laden matrix comprising channel (28) and form new tumors, they are classified as cancer cells that home to lung tissue. If cancer cells reach normal bone cell laden matrix comprising channel (29) and form new tumors, they are classified as cancer cells that home to bone tissue.

[0063] To distinguish cancer cells from other cells, they can be labelled with fluorescent dyes before loading into the device. Another approach is to label cells other than the cancer cells to be loaded into the device with fluorescent dyes. Or these cells can express various fluorescent proteins.

[0064] Effect of different flow rate on cancer cell metastasis can be invesitgated by changing the flow rate generated with the flow regulating pump (40).

[0065] The matrix can be collagen, matrigel, laminin, hydrogel or a combination thereof. In addition, extracellular matrix proteins such as fibronectin, entactin can be added to the matrix.

[0066] Drugs, growth factors, biological molecules and/or chemicals to be tested can be added to one or more of the reservoirs (31, 32, 35). Intended effects on cancer cells and side effects on normal cells can be simultaneously and jointly studied.

[0067] When cells from cancer patient biopsies are used in the device, better informed choices can be made for personalized therapy.

[0068] The device can be fabricated using standard lithography, hot embossing, micro-injection molding and/or laser micromachining techniques.

[0069] Loading of materials to the device, change of fluids, microscopic observations and analyses can be automized with a computerized robotic set-up.

REFERENCES

[0070]

1 Cukierman, E., Pankov, R., Stevens, D. R. & Yamada, K. M. Taking cell-matrix adhesions to the third dimension. Science 294, 1708-1712 (2001).

2 Faute, M. A. D. et al. Distinctive alterations of invasiveness, drug resistance and cell-cell organization in 3D-cultures of MCF-7, a human breast cancer cell line, and its multidrug resistant variant. Clinical & Experimental Metastasis 19, 161-168 (2002).

3 Kim, H. D. et al. Epidermal Growth Factor-induced Enhancement of Glioblastoma Cell Migration in 3D Arises from an Intrinsic Increase in Speed But an Extrinsic Matrix- and Proteolysis-dependent Increase in Persistence. Mol Biol Cell 19, 4249-4259, doi:10.1091/mbc.E08-05-0501 (2008).

4 McMillin, D. W. et al. Tumor cell-specific bioluminescence platform to identify stroma-induced changes to anticancer drug activity. Nature Medicine 16, 483-U171, doi:10.1038/nm.2112 (2010).

5 Woodcock, J. & Woosley, R. The FDA critical path initiative and its influence on new drug development. Annual Review of Medicine 59, 1-12, doi:10.1146/annurev.med.59.090506.155819 (2008).

6 Debnath, J. & Brugge, J. S. Modelling glandular epithelial cancers in three-dimensional cultures. Nature Reviews Cancer 5, 675-688, doi:10.1038/nrc1695 (2005).

7 Hutmacher, D. W. et al. Can tissue engineering concepts advance tumor biology research? Trends in Biotechnology 28, 125-133, doi:10.1016/j.tibtech.2009.12.001 (2010).

8 Olumi, A. F. et al. Carcinoma-associated fibroblasts direct tumor progression of initiated human prostatic epithelium. Cancer Res 59, 5002-5011 (1999).

9 Weaver, V. M. et al. Reversion of the malignant phenotype of human breast cells in three-dimensional culture and in vivo by integrin blocking antibodies. J Cell Biol 137, 231-245 (1997).

10 van't Veer, L. J. et al. Gene expression profiling predicts clinical outcome of breast cancer. Nature 415, 530-536 (2002).

11 Bauer, M., Su, G., Beebe, D. J. & Friedl, A. 3D microchannel co-culture: method and biological validation. Integr Biol-Uk 2, 371-378 (2010).

12 Chung, S., Sudo, R., Vickerman, V., Zervantonakis, I. K. & Kamm, R. D. Microfluidic Platforms for Studies of Angiogenesis, Cell Migration, and Cell-Cell Interactions. Ann Biomed Eng 38, 1164-1177 (2010).

13 Shin, Y. et al. Microfluidic assay for simultaneous culture of multiple cell types on surfaces or within hydrogels. Nat Protoc 7, 1247-1259 (2012).

14 Elliott, N. T. & Yuan, F. A microfluidic system for investigation of extravascular transport and cellular uptake of drugs in tumors. Biotechnol Bioeng 109, 1326-1335 (2012).

15 Goral, V. N. et al. Perfusion-based microfluidic device for three-dimensional dynamic primary human hepatocyte cell culture in the absence of biological or synthetic matrices or coagulants. Lab Chip 10, 3380-3386 (2010).

16 Huang, C. P. et al. Engineering microscale cellular niches for three-dimensional multicellular co-cultures. Lab Chip 9, 1740-1748 (2009).

17 Sung, K. E. et al. Transition to invasion in breast cancer: a microfluidic in vitro model enables examination of spatial and temporal effects. Integr Biol-

Uk 3, 439-450 (2011).
18 Zervantonakis, I. K. et al. Three-dimensional microfluidic model for tumor cell intravasation and endothelial barrier function. P Natl Acad Sci USA 109, 13515-13520 (2012).
19 Kamm, R. D. *et al.* Device for High Throughput Investigations of Cellular Interactions. PCT/US2011/054029 (2011).

Claims

1. A microfluidic device comprising a structure (2) on an optically transparent surface (1) where the structure (2) comprises

(i) at least one flow channel (30) comprising endothelial cells,
(ii) at least three adjacent channels comprising cell-laden or cell-free 3D (3 dimensional) matrices, neighboring each flow channel on each long side of it, in total at least six channels comprising 3D cell-laden or cell free matrix (24, 25, 26, 27, 28, 29),
(iii) at least two fluid reservoirs (31, 32) each neighbouring one of the channels comprising 3D cell-laden or cell free matrix (24, 29) furthest from the flow channel (30),
(iv) borders comprising at least twelve posts (23) that separate neighboring channels (24, 25, 26, 27, 28, 29, 30) from each other, and channels (24, 29) from neighboring fluid reservoirs (31, 32).
(v) at least five different cell types in total.

2. The device of claim 1 wherein one end of the flow channel (30) is connected via connector (33) and tubing (39) to air bubble trap (38) connected to a flow regulating pump (40) and the other end is connected via connector (33) and tubing (34) to a storage reservoir (35) where the flow passes through and cancer cells, biological molecules and chemicals that have entered the flow can be collected for analysis.

3. The device of claim 1 wherein there are at least two border areas (22) that prevent mixing of different fluids in the fluid reservoirs (31, 32),

4. The device of claim 1 wherein the interior corners (21) are curved to minimize air bubble formation.

5. The device of claim 1 wherein the fluids in the reservoirs (31, 32, 35) and the flow channel (30) comprise culture media, physiological buffer solutions, biological molecules, chemicals to be tested or a combination thereof.

6. The device of claim 1 wherein all channels (24, 25, 26, 27, 28, 29, 30) and fluid reservoirs (31,32) comprise each one inlet for loading culture media, physiological buffer solutions, biological molecules, chemicals, cell-free matrix, cell-laden matrix to be tested or a combination thereof and each one outlet for the inside air or preloaded fluid to exit during loading (3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20).

7. The device of claim 1 wherein channels (24, 25, 26, 27, 28, 29, 30), fluid reservoirs (31,32) and posts (23) are of the same height.

8. The device of claim 1 wherein channels comprising 3D cell-laden or cell free matrix (24, 25, 26, 27, 28, 29) are the same or different lengths as the flow channel (30),

9. The device of claim 1 wherein all channels (24, 25, 26, 27, 28, 29, 30) are parallel to each other.

10. The device of claim 1 wherein all channels (24, 25, 26, 27, 28, 29, 30) each have a length between 500 micrometers and 20 centimeters.

11. The device of claim 1 wherein the horizontal cross-section of each post (23) is hexagonal, circular or elliptical.

12. The device of claim 1 wherein the horizontal cross-section of each post (23) is between 10 micrometers and 1 millimeter wide.

13. The device of claim 1 wherein the material of the optically transparent surface(1) is glass, polydimethylsiloxane or polystyrene.

14. The device of claim 1 wherein the material of the structure (2) is polydimethylsiloxane or polystyrene.

15. The device of claim 1 wherein the cells comprise a combination of cancer cells, macrophages, fibroblasts, endothelial cells, normal breast epithelial cells, myoepithelial cells, normal liver epithelial cells, hepatocytes, normal lung epithelial cells, normal bone cells.

16. The device of claim 15 wherein the cells are cell lines and/or cancer patient biopsy cells.

17. The device of claim 1 wherein at least one of the channels (24, 25, 26, 27, 28, 29) comprising 3D cell-laden or cell free matrix, comprises 3D cancer cell-laden matrix while the others comprise 3D cell-free matrix, 3D normal epithelial cell of type same as the cancer type-laden matrix, 3D normal epithelial cell of type different from the cancer type-laden matrix, 3D fibroblast-laden matrix, 3D macrophage-laden

matrix, 3D myoepithelial cell-laden matrix, 3D normal liver epithelial cell-laden matrix, 3D hepatocyte-laden matrix, 3D normal bone cell-laden matrix or a combination thereof.

18. The device of claim 1 wherein the 3D matrix in six or fewer of the channels (24, 25, 26, 27, 28, 29) comprising 3D cell-laden or cell free matrix is matrigel, collagen, laminin or a combination thereof.

19. The device of claim 1 wherein the flow channel (30) is a channel where

(i) Cancer cells that contact endothelial cells in the flow channel (30),
(ii) Cancer cells that pass endothelial cells in the flow channel (30) and enter the flow,
(iii) Cancer cells that enter the flow on one side of the flow channel and contact the endothelial cells on the other side of the flow channel (30) are observed with a microscope.

20. The device of claim 1 wherein the structure comprises channels (26, 27) neighboring the flow channel (30) and comprising 3D cell-laden or cell free matrix where migration of cancer cells in 3D cell-laden or cell free matrix towards the flow channel (30) and exit of cancer cells from the flow channel (30) into 3D cell-laden or cell free matrix can be observed.

21. The device of claim 1 wherein any of the channels (24, 25, 26, 27, 28, 29) comprising 3D normal cell of type same as the cancer type or different type-laden matrix is a channel where formation and/or presence of new tumors by cancer cells that have passed through the flow channel (30) and/or not, can be observed with a microscope.

22. The device of claim 1 wherein the fluid in the flow channel (30) is blood from cancer patient.

23. The device of claim 1 wherein the fluid in the flow channel (30) is blood from healthy individual.

24. A method of determining whether a biological molecule or a chemical or a combination thereof prevents metastasis or not comprising

a. adding the biological molecule, chemical or a combination thereof to be tested into one or more of the fluid reservoirs (31, 32) and/or storage reservoir (35) of the device in claim 1 wherein at least one of the channels (24, 25, 26, 27, 28, 29) comprising 3D cell-laden or cell free matrix comprises 3D cancer cell-laden matrix while the others comprise 3D cell-free matrix, 3D normal epithelial cell of type same as the cancer type-laden matrix, 3D normal epithelial cell of type different from the cancer type-laden matrix, 3D fibroblast-laden matrix, 3D macrophage-laden matrix, 3D myoepithelial cell-laden matrix, 3D normal liver epithelial cell-laden matrix, 3D hepatocyte-laden matrix, 3D normal bone cell-laden matrix or a combination thereof, and
b. determining whether new tumors form in a channel other than the one seeded with cancer cells to begin with or not
wherein if new tumors do not form in a channel other than the one seeded with cancer cells to begin with, then this iindicates that the biological molecule, chemical or a combination thereof tested can be used against metastasis compared to a suitable control biological molecule or chemical.

25. A method of determining the homing choices of cancer cells comprising

a. The device of claim 1 wherein at least one of the channels (24, 25, 26, 27, 28, 29) comprising 3D cell-laden or cell free matrix comprises 3D cancer cells to be tested-laden matrix while the others comprise 3D cell-free matrix, 3D normal epithelial cell of type same as the cancer type-laden matrix, 3D normal epithelial cell of type different from the cancer type-laden matrix, 3D fibroblast-laden matrix, 3D macrophage-laden matrix, 3D myoepithelial cell-laden matrix, 3D normal liver epithelial cell-laden matrix, 3D hepatocyte-laden matrix, 3D normal bone cell-laden matrix or a combination thereof, and
b. determining in which channels new tumors form or are present
wherein if a new tumor forms in a channel with normal lung epithelial cells, then this indicates that cancer cells tested will metastasize to the lungs, if a new tumor forms in a channel with bone cells, then this indicates that cancer cells tested will metastasize to the bones, if a new tumor forms in a channel with normal liver cells, then this indicates that cancer cells tested will metastasize to the liver.

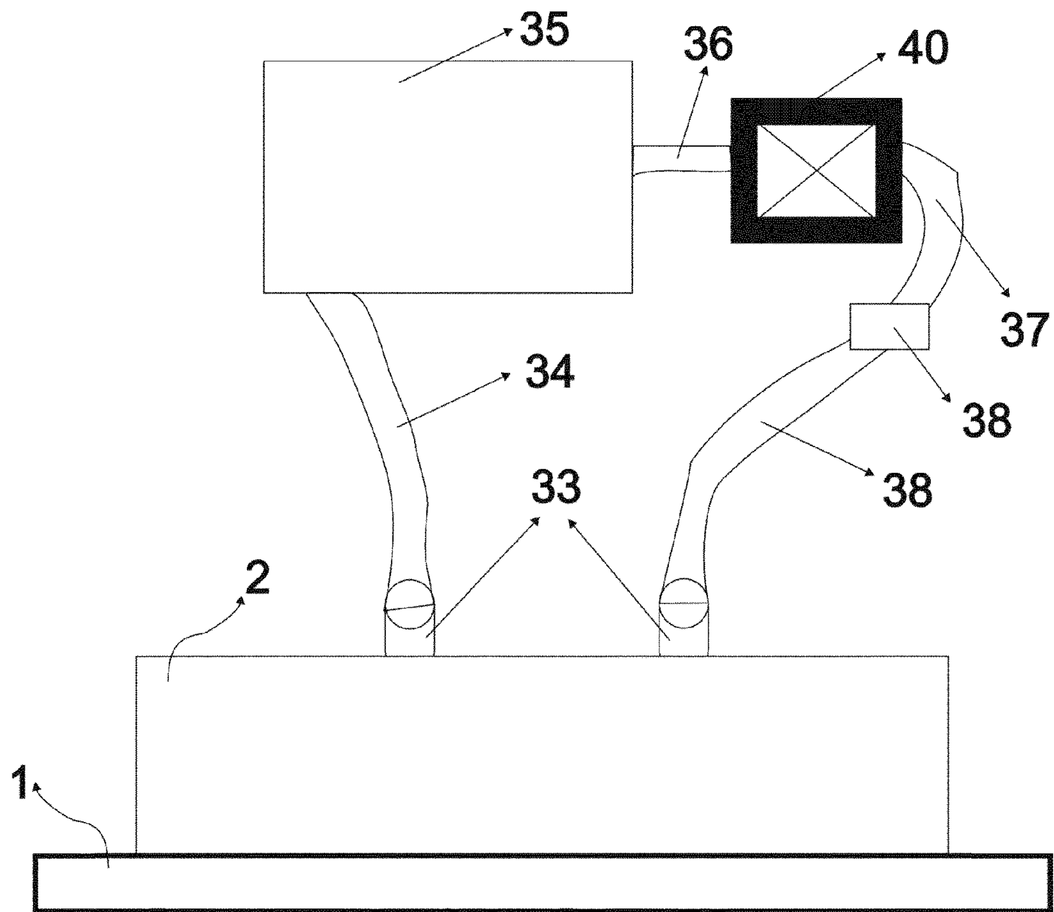


FIGURE 1

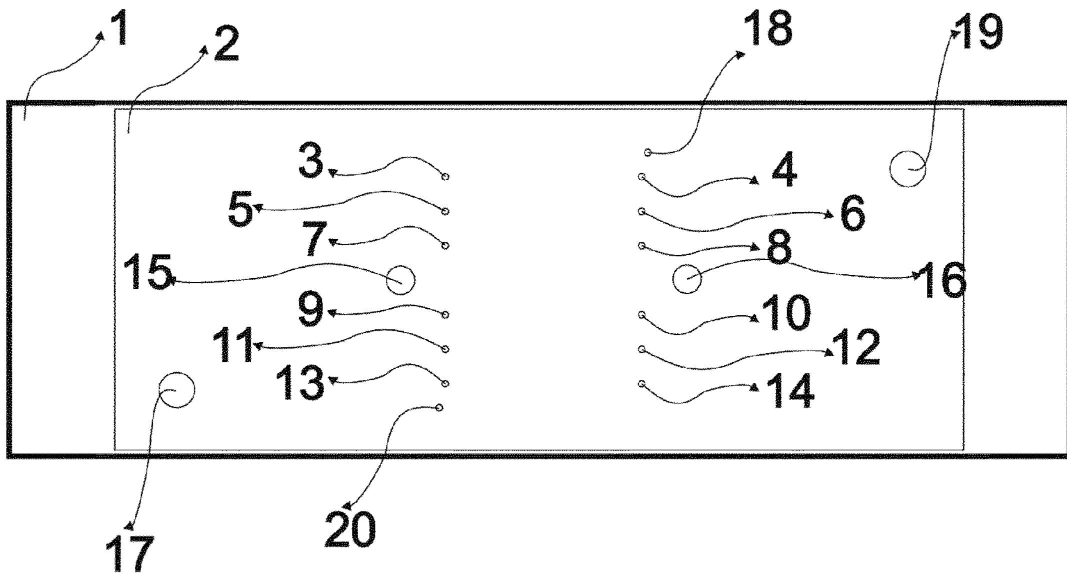


FIGURE 2

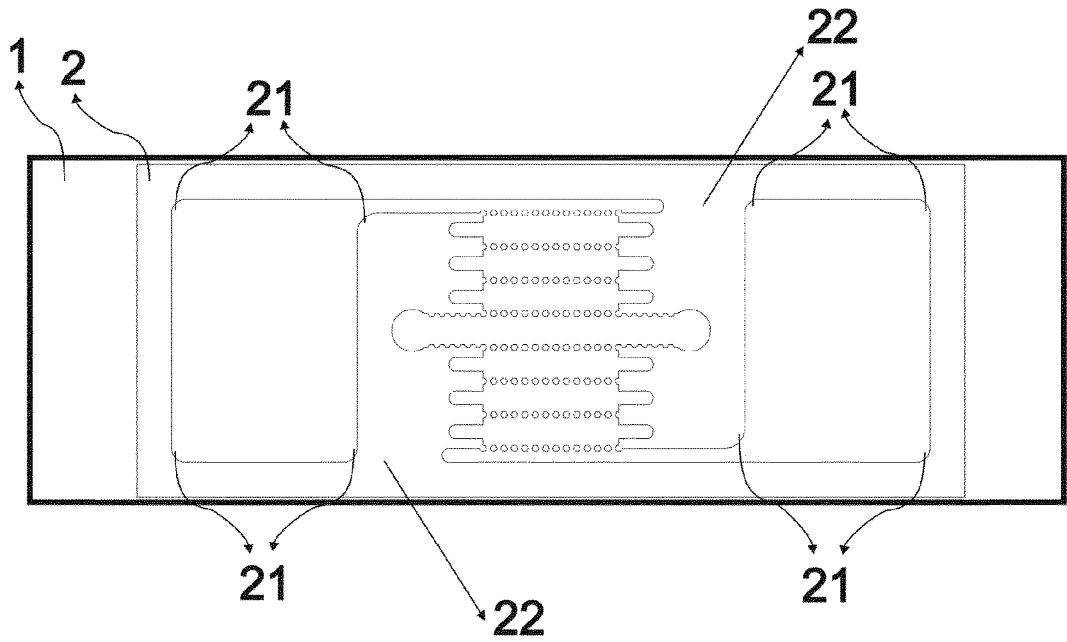


FIGURE 3

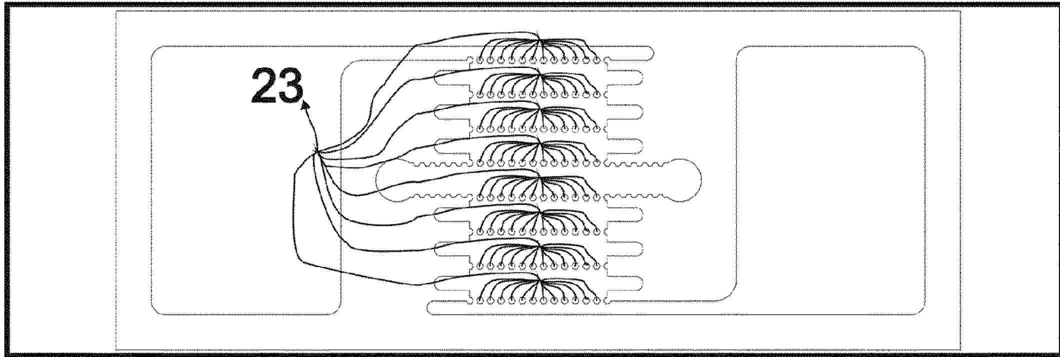


FIGURE 4

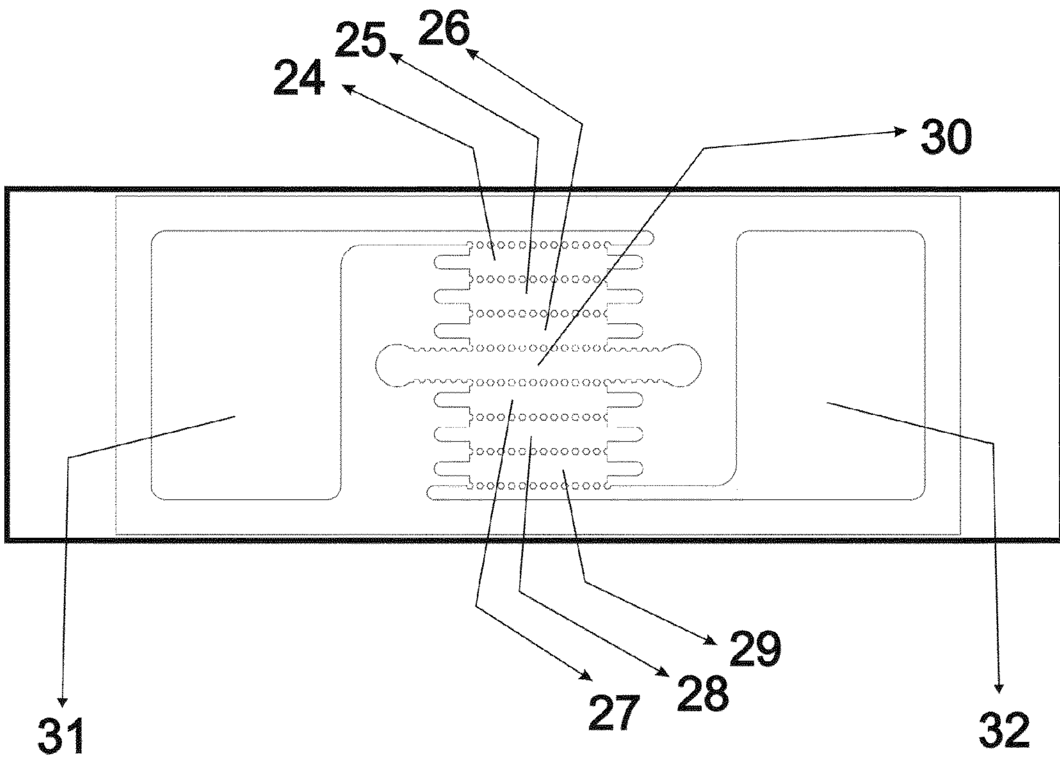


FIGURE 5

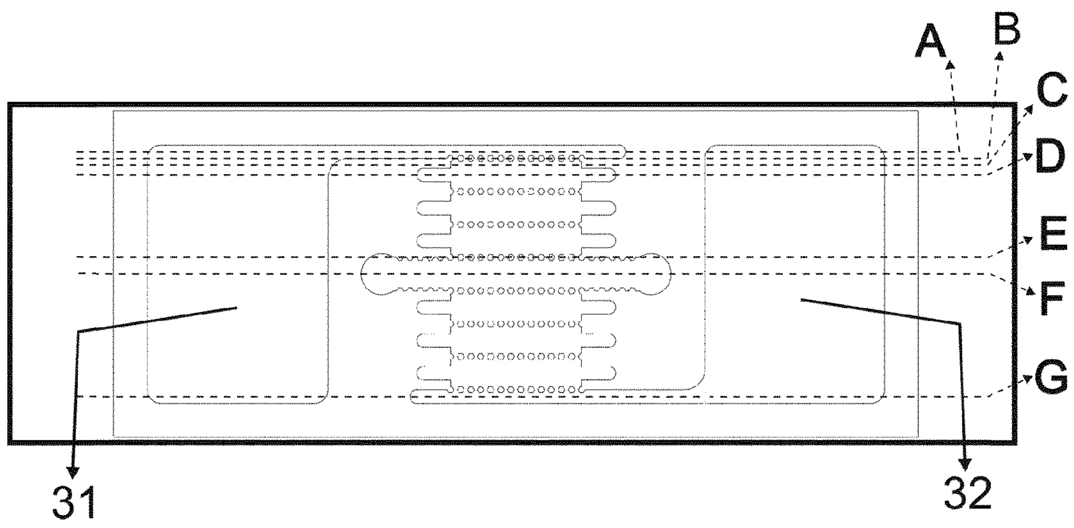


FIGURE 6

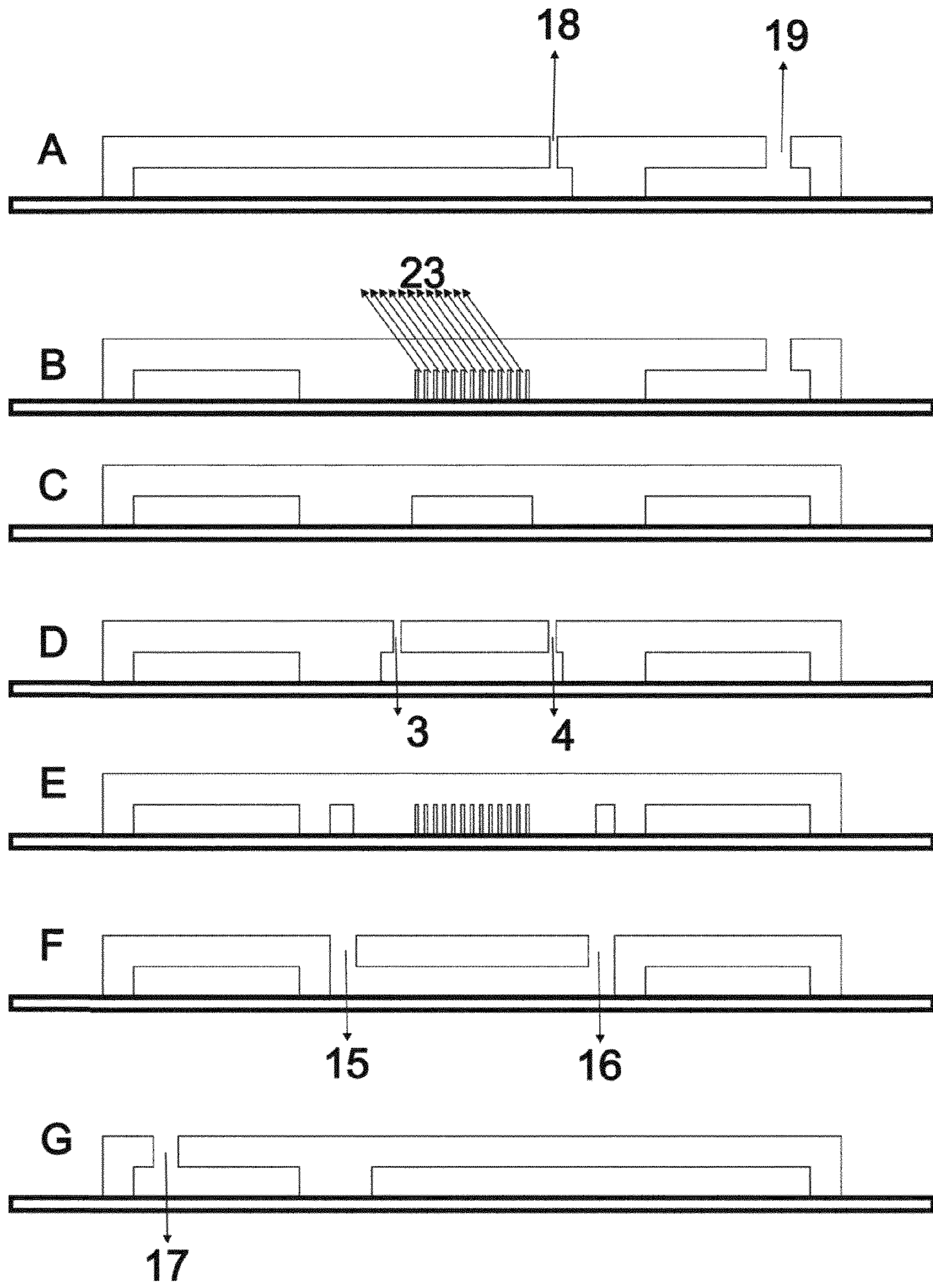


FIGURE 7

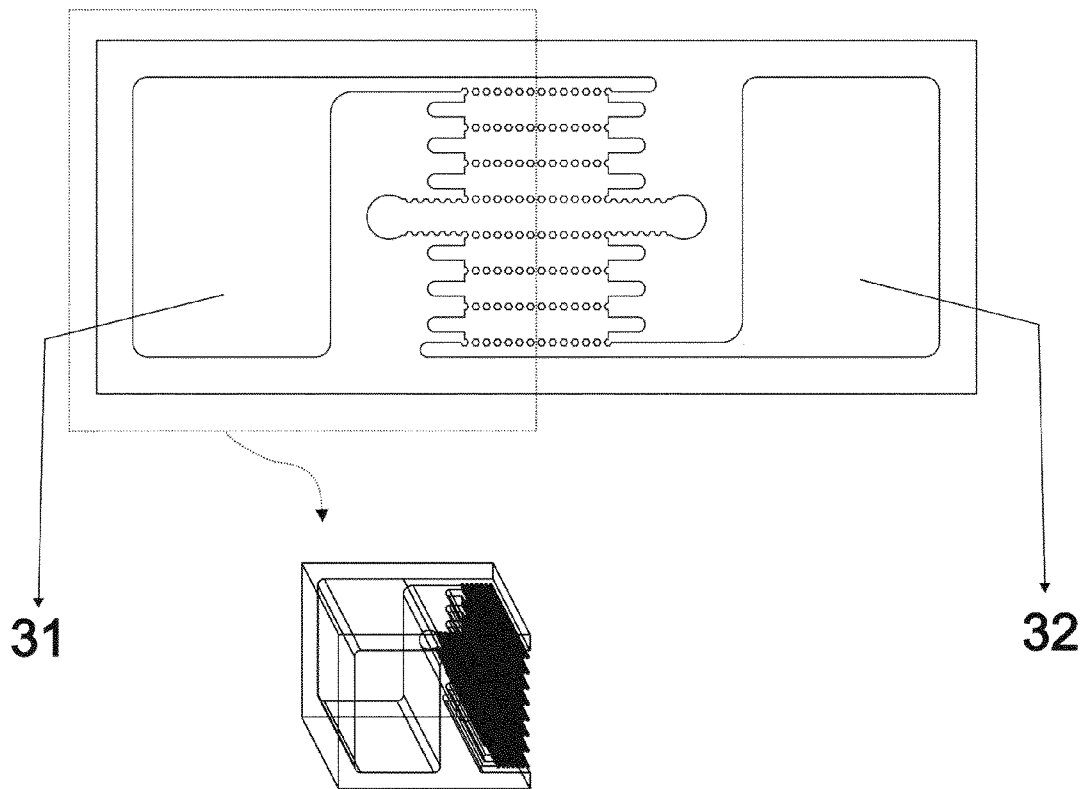


FIGURE 8

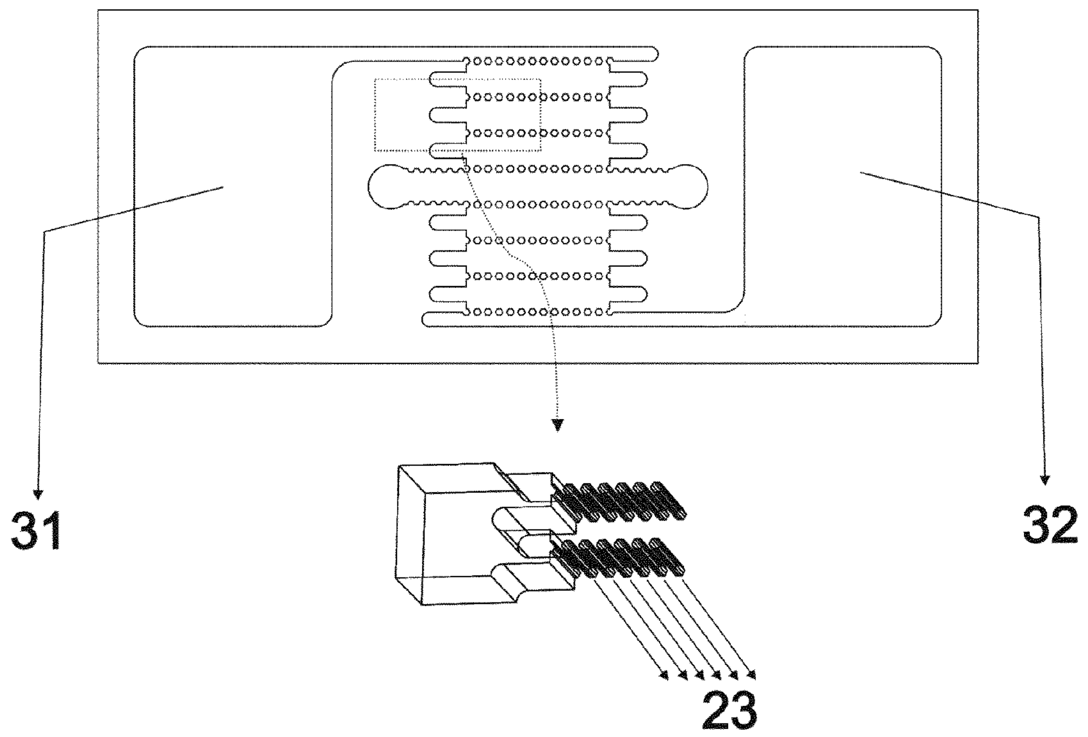
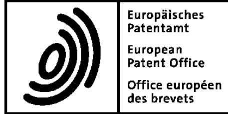


FIGURE 9



EUROPEAN SEARCH REPORT

Application Number
EP 13 15 4001

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
A	WO 2012/050981 A1 (MASSACHUSETTS INST TECHNOLOGY [US]; KAMM ROGER DALE [US]; ASADA HARUHI) 19 April 2012 (2012-04-19) * figure 25 * -----	1-25	INV. G01N33/50 B01L3/00
A	WO 2010/018499 A1 (KONINKL PHILIPS ELECTRONICS NV [NL]; CATTANEO STEFANO [NL]; GILLIES MU) 18 February 2010 (2010-02-18) * figure 6B * -----	1-25	
A	US 2012/083425 A1 (GEORGE STEVEN C [US] ET AL) 5 April 2012 (2012-04-05) * figures 5,8 * -----	1-25	
A	HUIPENG MA ET AL: "Characterization of the interaction between fibroblasts and tumor cells on a microfluidic co-culture device", ELECTROPHORESIS, vol. 31, no. 10, 22 May 2010 (2010-05-22), pages 1599-1605, XP055062929, * the whole document * -----	1-25	TECHNICAL FIELDS SEARCHED (IPC) G01N B01L
A	CAROLYN G. CONANT ET AL: "Use of Parallel Flow for Angiogenesis and Cancer Cell Invasion in Microfluidic Devices Using Real-Time Microscopy", BIOPHYSICAL JOURNAL, vol. 100, no. 3, 1 February 2011 (2011-02-01), page 316a, XP055062930, * abstract * -----	1-25	

The present search report has been drawn up for all claims

1

Place of search	Date of completion of the search	Examiner
The Hague	15 May 2013	Gunster, Marco

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons
....
& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C01)

ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 13 15 4001

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

15-05-2013

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2012050981 A1	19-04-2012	CA 2813211 A1	19-04-2012
		WO 2012050981 A1	19-04-2012
WO 2010018499 A1	18-02-2010	NONE	
US 2012083425 A1	05-04-2012	NONE	

EPO FORM P0459

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- US 2011054029 W **[0070]**

Non-patent literature cited in the description

- **CUKIERMAN, E. ; PANKOV, R. ; STEVENS, D. R. ; YAMADA, K. M.** Taking cell-matrix adhesions to the third dimension. *Science,* 2001, vol. 294, 1708-1712 **[0070]**
- **FAUTE, M. A. D. et al.** Distinctive alterations of invasiveness, drug resistance and cell-cell organization in 3D-cultures of MCF-7, a human breast cancer cell line, and its multidrug resistant variant. *Clinical & Experimental Metastasis,* 2002, vol. 19, 161-168 **[0070]**
- **KIM, H. D. et al.** Epidermal Growth Factor-induced Enhancement of Glioblastoma Cell Migration in 3D Arises from an Intrinsic Increase in Speed But an Extrinsic Matrix- and Proteolysis-dependent Increase in Persistence. *Mol Biol Cell,* 2008, vol. 19, 4249-4259 **[0070]**
- **MCMILLIN, D. W. et al.** Tumor cell-specific bioluminescence platform to identify stroma-induced changes to anticancer drug activity. *Nature Medicine,* 2010, vol. 16, 483-U171 **[0070]**
- **WOODCOCK, J. ; WOOSLEY, R.** The FDA critical path initiative and its influence on new drug development. *Annual Review of Medicine,* 2008, vol. 59, 1-12 **[0070]**
- **DEBNATH, J. ; BRUGGE, J. S.** Modelling glandular epithelial cancers in three-dimensional cultures. *Nature Reviews Cancer,* 2005, vol. 5, 675-688 **[0070]**
- **HUTMACHER, D. W. et al.** Can tissue engineering concepts advance tumor biology research?. *Trends in Biotechnology,* 2010, vol. 28, 125-133 **[0070]**
- **OLUMI, A. F. et al.** Carcinoma-associated fibroblasts direct tumor progression of initiated human prostatic epithelium. *Cancer Res,* 1999, vol. 59, 5002-5011 **[0070]**
- **WEAVER, V. M. et al.** Reversion of the malignant phenotype of human breast cells in three-dimensional culture and in vivo by integrin blocking antibodies. *J Cell Biol,* 1997, vol. 137, 231-245 **[0070]**
- **VAN'T VEER, L. J. et al.** Gene expression profiling predicts clinical outcome of breast cancer. *Nature,* 2002, vol. 415, 530-536 **[0070]**
- **BAUER, M. ; SU, G. ; BEEBE, D. J. ; FRIEDL, A.** 3D microchannel co-culture: method and biological validation. *Integr Biol-Uk,* 2010, vol. 2, 371-378 **[0070]**
- **CHUNG, S. ; SUDO, R. ; VICKERMAN, V. ; ZERVANTONAKIS, I. K. ; KAMM, R. D.** Microfluidic Platforms for Studies of Angiogenesis, Cell Migration, and Cell-Cell Interactions. *Ann Biomed Eng,* 2010, vol. 38, 1164-1177 **[0070]**
- **SHIN, Y. et al.** Microfluidic assay for simultaneous culture of multiple cell types on surfaces or within hydrogels. *Nat Protoc,* 2012, vol. 7, 1247-1259 **[0070]**
- **ELLIOTT, N. T. ; YUAN, F.** A microfluidic system for investigation of extravascular transport and cellular uptake of drugs in tumors. *Biotechnol Bioeng,* 2012, vol. 109, 1326-1335 **[0070]**
- **GORAL, V. N. et al.** Perfusion-based microfluidic device for three-dimensional dynamic primary human hepatocyte cell culture in the absence of biological or synthetic matrices or coagulants. *Lab Chip,* 2010, vol. 10, 3380-3386 **[0070]**
- **HUANG, C. P. et al.** Engineering microscale cellular niches for three-dimensional multicellular co-cultures. *Lab Chip,* 2009, vol. 9, 1740-1748 **[0070]**
- **SUNG, K. E. et al.** Transition to invasion in breast cancer: a microfluidic in vitro model enables examination of spatial and temporal effects. *Integr Biol-Uk,* 2011, vol. 3, 439-450 **[0070]**
- **ZERVANTONAKIS, I. K. et al.** Three-dimensional microfluidic model for tumor cell intravasation and endothelial barrier function. *P Natl Acad Sci USA,* 2012, vol. 109, 13515-13520 **[0070]**