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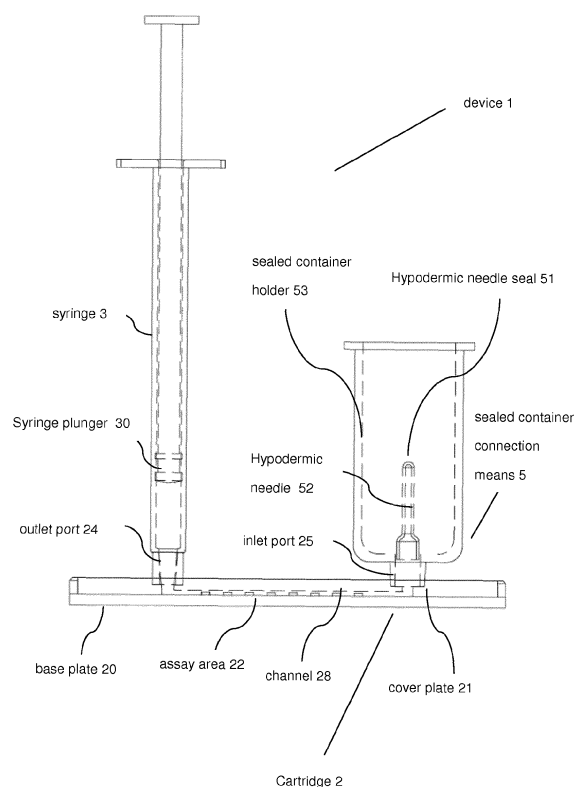
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(54) **Device and method for assessing the status of cells in a biological fluid**

(57) A device for assessing the status of a fluid containing cells of mammalian origin, the device comprises a cartridge (2) having an enclosed fluidic channel (28), an assay area (22) disposed within the fluidic channel (28) which is covered by at least one agent capable of selectively recruiting at least one type of cell contained within the mammalian fluid, an inlet port (25) for the fluidic channel, an outlet port (24) for the fluidic channel and a piercing means (51) adapted for connecting to a sealed container (4) containing the cell containing fluid by puncturing the container. The inlet port and piercing means are adapted to provide fluid communication between the fluidic channel and the sealed container when the sealed container is punctured by the piercing means. The outlet port is adapted for fluid communication with a syringe (3) such that actuation of the syringe provides controlled flow of fluid over the assay area to achieve desired physiological shear stress conditions for the type of cell being recruited at the assay area. The cartridge is adapted for integration with a read out instrument for acquiring optical signal at the assay area of the channel.

Figure 8



Description

Technical Field

[0001] The invention provides a device for work with cell-containing fluids comprising a cartridge having an enclosed fluidic channel with an assay area upon which or upon some fraction of which is disposed an agent capable of selectively recruiting at least one type of cells contained within the fluid.

Background of the invention

[0002] The invention is a direct improvement on prior inventions described in US6770434 and US7122301. The inventions in US6770434 and US7122301 describe the biological assay method for accessing biological cell adhesion in microfluidic biochips. The prior-art inventions US6770434 and US7122301 further describe a biological assay method of coating the internal bore of microchannel by ligand and delivering the cell containing sample through the microchannel at the steady flow rate and observing cell rolling, adhesion, migration of cell caused by a specific ligand coating the microchannel. These assays replicate physiological shear-stress environment found in the human circulatory system. However, the microchannel biochips described in US6770434 and US7122301 are not suitable for applications in a hospital environment and clinical research, as they does not provide easy-to-use disposable device and the handling of a sample by a user is complicated.

[0003] It is an object of the invention to overcome at least one of the above-referenced problems.

Statements of Invention

[0004] Broadly, the cartridge of the invention includes an inlet port and outlet port in fluid communication with the fluidic channel such that a flow of the fluid can be established through the channel. The outlet port comprises a piercing means capable of piercing a cover of a container for the fluid and establishing direct fluid communication between the container and the fluidic channel. Thus, when the container is a vacutainer (which receives blood directly from a patient), the blood is transferred directly from a patient to a vacutainer and then directly into the device for analysis. Thus, risk of contamination of the blood is minimized. The inlet port is adapted for fluid communication with a syringe, which can be actuated to provide flow of fluid across the assay area in both directions. Thus, a diagnostic assay can be carried out on a cell-containing fluid in a simple, safe and practical manner that involves directly engaging the container (for example, a vacutainer) with the piercing means, directly engaging a conventional syringe with the inlet port, and actuating the syringe to establish a flow of cell-containing fluid across the assay area. Further, the provision of the piercing means within a well adapted to receive

the container facilitates engagement between the container and the piercing means, and additionally prevents a user injuring themselves.

[0005] Accordingly, the invention provides a planar cartridge suitable for performing diagnostic studies of a fluid containing cells of mammalian origin, the cartridge comprising:

- an enclosed fluidic channel (28);
- an assay area (22) disposed within the fluidic channel and at least partially covered by at least one agent capable of selectively recruiting at least one type of cell contained within the fluid;
- an inlet port (25) for the fluidic channel, the inlet port comprising a well (5) disposed on a top of the cartridge for receipt of a sealed container (4) containing the cell containing fluid and piercing means (52) adapted to pierce the sealed container when it is inserted into the well and provide fluid communication between the sealed container and the enclosed fluidic channel; and
- an outlet port (24) for the fluidic channel disposed on the top of the planar cartridge, the outlet port adapted to engage with a fluid outlet of a syringe in a fluidically tight manner.

[0006] Typically, the piercing means (52) is a hypodermic needle, although other forms of piercing means are envisaged. Ideally, the piercing means (52) is located within the well (5). This ensures that the piercing means is not exposed and therefore avoids accidental injury to users caused by the piercing means.

[0007] Suitably, the fluidic channel comprises a liquid.

[0008] Suitably, the planar cartridge comprises a base plate coupled to a cover plate in a fluidically tight manner such that the fluidic channel and assay area is defined therebetween, wherein the inlet port and outlet port are provided on the cover plate.

[0009] Suitably, a seal is formed between the base plate and the cover plate of the cartridge, which seal is suitably achieved by means of a gasket, ideally an elastomeric gasket, positioned between the two plates.

[0010] Ideally, the cover plate and/or the base plate, or at least the portion of the cover and/or base plates which comprise the assay area, are optically transparent plate. Suitably, the cover and/or base plate have a thickness of 0.05 mm to 10 mm.

[0011] Typically, the fluidic channel is 20 micron to 10 mm wide. Generally, the fluidic channel is 10 micron to 1 mm deep.

[0012] In one embodiment, the planar cartridge of the invention is disposable.

[0013] In a preferred embodiment, the outlet port comprises a seal adapted to be broken when the outlet port engages with a fluid outlet of a syringe.

[0014] Generally, the device includes a barrier separating the buffer liquid from the fluidic channel that is capable of being broken immediately before the start of the

assessment of the fluid. The barrier may be, for example, a membrane or film of polymer material which is disposed in the device in such a manner such that upon actuation of the syringe the barrier is breached. For example, the barrier could be a membrane disposed on the cartridge at the mouth of the inlet port.

[0015] The container for the cell-containing fluid is a container of the type having an opening closed with a pierceable cover (i.e. a cover which can be pierced with a piercing means such as a needle), and ideally is a vacuum suction container suitable for collection of blood from a mammal. An example of such a container is a vacuum container. The device includes a piercing means in fluid communication with the outlet port. The piercing means generally comprises a hollow shaft with a sharp tip; an example of a piercing means is a hollow needle, ideally a hypodermic needle. The piercing means is typically sealed with a cover (for example a polymer cap) to prevent the leakage of buffer liquid from the interior of the cartridge.

[0016] Suitably, assay area provides an essentially tortuous for the flow of fluid, thus being capable of altering the characteristics of cell recruitment at the assay area.

[0017] In a preferred embodiment, the agent (the agent capable of selectively recruiting at least one type of cell contained within the mammalian fluid) is a ligand capable of inducing specific binding to the type of cells to be recruited. Generally, the agent may be a chemical, a compound, a metal, or a biological material such as a protein, a nucleic acid, a complex (i.e. a proteosome), a steroid hormone, or a cell. Examples of proteins include cell surface receptors, antibodies or antibody fragments, or antigens or antigenic determinants. Examples of chemicals include drugs, agonists or antagonists of cell surface receptors, and the like.

[0018] Suitably, the assay area contains at least one electrode incorporated into it for the detection of the cell recruited from the cell containing fluid.

[0019] The invention also provides a kit comprising a planar cartridge of the invention and a syringe having an outlet, wherein the outlet of the syringe and outlet port of the cartridge are optionally adapted for relative engagement in a fluidically tight manner.

[0020] Typically, the syringe comprises a buffer liquid, that optionally includes a normalizing agent for reversing the action of an anti-coagulant on a blood product, and/or a fluorescent agent.

[0021] The invention also provides a kit of the invention in an assembled form in which the outlet of the syringe is coupled with the outlet port of the cartridge in a fluidically tight manner.

[0022] Suitably, the syringe is coupled to a shaft of an automated driver thus forming a pump.

[0023] Optionally, the kit of the invention includes a syringe displacement actuator capable of providing a controlled flow of fluid through the fluidic channel. Examples of suitable syringe displacement actuators include a servo-motor or a stepper motor which can provide me-

chanical displacement of the syringe plunger in a controlled manner and preferably aided by electronic micro-controller. Typically, the actuator can provide for the plunger movement in both, forward and reverse directions, the timing and the speed of the plunger advancement are controlled. There are numerous practical embodiments of this actuator possible as will be appreciated by those skilled in the art. Other types of mechanical actuators are also possible, including e.g. a solenoid actuator.

[0024] The invention also provides a method for performing diagnostic studies of a cell-containing fluid of mammalian origin, which method employs a planar cartridge of the invention, the method comprising the steps of:

- coupling of a sealed container (4) containing the cell-containing fluid with the inlet port (25) of the cartridge (2) by means of the piercing means (51) puncturing the container;
- coupling a syringe having a fluid outlet to the outlet port of the planar cartridge such that a fluid tight seal is established between the outlet port and the syringe fluid outlet;
- actuating the syringe (3) to establish a flow of cell-containing fluid from the sealed container through the fluidic channel (28) over the assay area (22); and
- monitoring the binding of the at least one type of cell from the cell containing fluid to the assay area.

[0025] The invention also provides a method for performing diagnostic studies of a fluid containing cells of mammalian origin, which method employs a kit of the invention, the method comprising the steps of:

- coupling of a sealed container (4) containing the cell-containing fluid with the inlet port (25) of the cartridge (2) by means of the piercing means (51) puncturing the container;
- coupling the syringe to the outlet port of the planar cartridge such that a fluid tight seal is established between the outlet port and the syringe fluid outlet;
- actuating the syringe (3) to establish a flow of cell-containing fluid from the sealed container through the fluidic channel (28) over the assay area (22); and
- monitoring the binding of the at least one type of cell from the cell containing fluid to the assay area.

[0026] Suitably, the syringe comprises a liquid (prior to start of the assay), and wherein the step of actuating the syringe comprising the sequential steps of:

- actuating the syringe to pump the liquid from the syringe into the sealed container to mix with the cell-containing fluid; and
- actuating the syringe to withdraw a mixture of liquid and cell-containing fluid from the sealed container and establish flow of the mixture through the fluidic

channel.

[0027] Suitably, the liquid in the syringe is a buffer liquid, that optionally contains a fluorescence agent, and/or a normalizing agent for reversing the action of an anticoagulant on a blood product.

[0028] The term "diagnostic studies" should be understood to mean quantitative and qualitative studies of the cells within the fluid, and of the fluid itself. The term includes for example determining the type and/or amount of cells present in the fluid, the phenotype of the cells (for example whether the cells express certain receptors), the activity of the cells (for example the reactivity of platelets), or the response of the cells to certain stimuli. Other diagnostic studies of the cells or the fluid will be apparent to those skilled in the art.

[0029] Ideally, the syringe is adapted for actuation by a syringe displacement actuator. Thus, in one embodiment of the method of the invention, the syringe is actuated by a syringe displacement actuator.

[0030] Typically the plunger of the syringe is actuated to provide the desired level of shear stress at the assay area.

[0031] Suitably, the monitoring measures the cell binding for various values of the shear stress at the assay area.

[0032] Generally, the monitoring is achieved by an optical microscope.

[0033] Ideally, prior to the test, the syringe contains buffer liquid and the said buffer liquid is injected into the sealed container. Typically, the buffer liquid injected into the sealed container contains fluorescence staining agent. Typically, the fluorescence signal within the assay area is measured. Suitably, the fluorescence agent can bind specifically to a particular type of cells. Typically, fluorescence agent can only bind to cells in a specific physiological condition. Preferably, the physiological condition is activation of specific cell receptors, for example, platelet cell surface 2b3a integrin receptors that are activated during thrombosis.

[0034] Suitably, the buffer liquid contains a normalizing control agent that can counteract effects of anticoagulants present in the cells containing fluid thus allowing the fluid to regain normal coagulation characteristics. Preferably, the anticoagulant is present in the sealed container at the time of cell sample collection, whilst the normalizing agent is present in the syringe or in the cartridge or both. Suitably, actuation of the syringe plunger enables the repetitive movement of the fluid between the sealed container and the syringe.

[0035] Typically, the time required for achieving the desired level of cell recruitment is monitored.

[0036] Generally, a number of cells recruited within the assay area is measured.

[0037] Preferably, an area covered by the cells recruited within the assay area is measured.

[0038] Suitably, the movement of the syringe plunger is slow enough to ensure that the flow of fluid within the

channel is proportional to the linear velocity of the plunger.

[0039] Generally, the maximum plunger velocity is defined taking into account the dimensions of the channel and the ability of the fluid to flow.

[0040] Suitably, a cavity free from fluid is formed in the syringe and the flow velocity of fluid in the channel is measured by taking into account movement of the syringe plunger and/or the increase in the volume of the cavity.

Brief Description of Drawings

[0041] The invention will be more clearly understood for the following description of some embodiments thereof given by way of example only with reference to the accompanying drawings in which:

Figure 1. Isometric view of the device comprising cartridge, syringe and sealed container.

Figure 2. Isometric exploded view of the cartridge.

Figure 3. Side exploded view of the cartridge.

Figure 4. Top view of the cartridge base plate with an example of assay area layout.

Figure 5. Top view of the cartridge base plate with an example of torturous channel and assay areas.

Figure 6. Top view of the cartridge base plate with an example of flow restrictors in the channel and assay areas.

Figure 7. Side view of the cartridge base plate with an example of flow restrictors in the channel and assay areas.

Figure 8. Device with details of the means for coupling of sealed container and syringe to the cartridge.

Figure 9. Isometric view of the cartridge, syringe and the sealed container connection means.

Figure 10. Side view of the device, microscope objective and syringe actuator.

Figure 11. Details of readout device monitoring volume of cavity above the syringe plunger.

Figure 12. Details of the means for coupling of sealed container and syringe to the cartridge containing polymer membrane.

Description of the preferred embodiments

[0042] The invention can be best understood from the

description of the following drawings showing a number of embodiments. The embodiments given do not form an exhaustive list but rather are examples.

[0043] Fig 1 shows an embodiment of the device indicated by a numeral (1). It comprises the cartridge (2), the syringe (3) and the sealed container (4). The cartridge (2) comprises the base plate (20) and the cover plate (21). In this embodiment, the cartridge further comprises the syringe connection means (6) and sealed container connection means (5) connected in fluid-tight manner to the cover plate (21). The syringe connection means (6) is designed in the way that it is capable of receiving and of providing a fluid-tight seal with the syringe (3). In one embodiment the sealed container connection means (5) is equipped with the piecing means such as hypodermic needle capable of fluidic coupling and providing fluid tight seal with sealed container (4). We may use the terms needle or hypodermic needle throughout this specification to signify practical examples of piercing means. Other embodiments of piercing means can be also used with the invention as will be appreciated by those skilled in the art.

[0044] Embodiments with multiple sealed container or multiple syringes are also possible. They are not presented in the specification for brevity.

[0045] Fig 2 shows an exploded view and fluidic connections of cartridge (2) and also the design of its interior and exterior. The base plate (20) is a plate of polymer such as polystyrene or glass, preferably transparent in the optical frequency range. The base plate could be in the thickness range of some 0.05 to some 2 mm. The cover plate (21) is also a plate of polymer such as polystyrene or glass preferably transparent in the optical frequency range. The cover plate could be in the thickness range from 0.05 mm to 5 mm. The cartridge sealing means (23) could comprise gasket of soft polymer material such as PDMS (Polydimethylsiloxane) or other suitable compressible materials. The gasket defines the channel (28) terminated at two ports: inlet port (25) and outlet port (24). Cartridge sealing means (23) could be also arranged using embodiments other than those based on compressible gasket. The outlet port (24) is terminated in connector such as Luer connector or another fluid-tight connector capable of receiving syringe. The inlet port (25) is terminated in sealed container connection means (5). In a typical embodiment the sealed container connection means comprises a hypodermic needle (piercing means) covered by a hypodermic needle seal. In some embodiments the hypodermic needle (piercing means) could be placed inside the sealed container holder (53) that is also forming a part of sealed container connection means (5). The sealed container holder (53) can securely accept the sealed container containing blood sample. In a typical embodiment, the sealed container containing blood sample is a vacuumed blood sample collection container as commonly used in hospitals for collection of blood samples from patients. Typically such blood collection containers are equipped with

a polymer cap that is punctured by a hypodermic needle (piercing means) during the blood collection procedure.

[0046] Embodiments with multiple inlet ports (24) and outlet ports (25) are possible. They are not presented in the specification for brevity.

[0047] The width of the channel (28) can vary along the length of the channel. This embodiment is not shown for brevity. The change in the width of the channel leads to change in the flow velocity of the cell containing fluid as it moves along the channel and this in turn leads to change in the cell recruitment characteristics.

[0048] The height of the channel (28) can vary along the length of the channel. This embodiment is not shown for brevity. The change in the height of the channel leads to change in the flow velocity of the cell containing fluid as it moves along the channel and this in turn leads to change in the cell recruitment characteristics.

[0049] One could device embodiments with more than two plates, or embodiments where the base plate or the cover plate is an aggregate plate consisting of multiple layers.

[0050] Fig. 3 shows side exploded view of the cartridge. In this embodiment the outlet port (24) is a Luer connector firmly mounted on the exterior side of the cover plate (21). The outlet port (24) is coupled to the interior side of the cover plate (21) via the outlet port fluidic coupling (26). Fig. 3 also shows the cartridge sealing means (23) and base plate (20). The assay area (22) is shown schematically on the surface of the base plate (20) facing the cover plate (21).

[0051] Fig. 4 shows schematically layout of assay area in one possible embodiment of the base plate. It shows the inner surface of the base plate (20) and assay areas (22a), (22b), (22c), (22d) preferably located within the channel. The areas are preferably coated by different agents capable of recruiting different cell types. Alternatively, the areas could be covered by different concentrations of the same agent. The assay areas could be relatively flat or they could also be substantially non-flat. In this embodiment the assays area (22d) is substantially flat and the assay area (22a) is substantially non-flat. The assays area 22a is composed of protrusions, a part of which or the whole is covered by an agent capable of selectively recruiting cells. The non-flat assay area (22a) alters the flow of liquid in the channel that may be desirable the increasing recruitment of certain types of cells as the alteration in the flow leads to alteration in the shear stress that is important for the cell recruitment. In this particular embodiment there are also flow restrictors (23e). The flow restrictors are positioned in proximity to the assay area (22b). The flow restrictors (23e) may block part of the channel's cross-section and therefore may alter the recruitment characteristics of the areas proximal to the pillars. The shape of the flow restrictors, their size and numbers could vary for various embodiments of the device (1).

[0052] Fig. 5 shows the top view of the cartridge base plate representing example of tortuous channel and as-

say areas. The figure shows the base plate (20) with position (25a) of the inlet port and position (24a) of the outlet port. In this case the cartridge sealing means (23) defines a non-straight channel (28). The embodiment shown in Fig. 5 has two assay areas (22a) and (22b). They can be substantially flat or substantially non-flat as discussed in relation to the Fig. 4. They could be substantially continuous or composed of sub-areas. The embodiment shown in Fig. 4 presents each of the assay areas (22a) and (22b) composed of nine sub-areas of circular shape.

[0053] Fig. 6 shows top view of the cartridge base plate with an example of flow restrictors in the channel and assay areas. In this embodiment the arrays of flow restrictors 927a), (27b), etc are positioned in between the assay areas (2a), (22c), etc. or proximal to these.

[0054] Fig. 7 shows side view of the cartridge base plate with an example of flow restrictors in the channel and assay areas. Similar numerals are used as in previous figures.

[0055] Fig. 8 Shows details of the means for coupling of sealed container and syringe to the cartridge for a possible embodiment of the device. The numerals in the figure that are identical to the ones in previous figures, denote the same elements as above. Syringe (3) that has syringe plunger (30), is coupled to the outlet port (24) in a hermetically tight manner. Sealed container connection means (5) is comprised of a hypodermic needle (piercing means) (52), covered by a hypodermic (piercing means) seal (51) placed inside the sealed container holder (53). The interior of the hypodermic needle (piercing means) (52) is fluidically coupled to the interior of the channel (28) via inlet port (25). In this embodiment that hypodermic needle (piercing means) seal (51) is a polymer cup that can be punctured when the sealed container is inserted into the sealed container holder (53). The sealed container is not shown in Fig. 8.

[0056] Fig. 9 shows isometric view of the cartridge, syringe and the sealed container connection means. The numerals in Fig. 9 are similar to the ones in Fig. 8 and other prior figures.

[0057] Fig. 10 shows side view of the device, microscope objective and syringe actuator. The device is placed on XY translating stage (6) of a microscope. Preferably the XY stage (6) is motorized and controlled by a processor to increase the throughput of the system. The entire microscope is not shown in Fig. 10, the objective lense (8) is shown. The XY stage (6) allows positioning the objective (8) at a desired point along the channel (28). The syringe plunger is mechanically coupled to an actuator (32), that is also preferably controlled by a processor. Sealed container is shown in Fig. 10. There is space unfilled by the cell containing fluid (41) and other liquids at the upper portion of the sealed container (4). In Fig. 10 this space is marked as air bubble (42) but it should be kept in mind that this space does not have to contain air. It could be a vacuumed space or space filled with a particular gas. The pressure in that space is preferably below the atmospheric pressure or equal to it. The

hypodermic needle (piercing means) that penetrated the lower portion of sealed container (4), is not shown in Fig. 10.

[0058] Fig. 11 shows details of readout device monitoring volume of cavity above the syringe plunger. The numerals similar to the ones in Fig. 10, have the same meaning. One additional element in Fig. 11 is the air gap detector (36) comprising of air gap sensor light emitting diode (LED) (33) and the air gap sensor (34). There is also air gap between the surface of the cell containing fluid in the syringe (3) and the surface of the syringe plunger (30). This gap may change depending on the pressure within the sealed container (4), the hydraulic resistance to the flow in the channel (28) and the speed on movement of the syringe plunger (30). The air gap detector (36) detects movement of the surface of the cell containing fluid or buffer liquid within the syringe (3). The linear velocity of the cell containing fluid within the channel is measured from the linear velocity of the of the surface-gas liquid within the syringe. The shear stress is determined from the linear velocity of fluid within the channel and the dimensions of the channel using method familiar to those skilled in the art of microfluidics (see e.g. US patents No 7,122,301 to Shvets et al and No 6,770,434 to Shvets et al).

[0059] Fig. 12 shows details of the means for coupling of sealed container and syringe to the cartridge containing polymer membrane. In this embodiment there is a polymer membrane (28a) placed proximal to the outlet port. The membrane seals the channel (28). Depending on the type of test to be carried out, the syringe 93) could contain the buffer liquid before the start of the test or it could also contain no liquid. Likewise the channel (28) could contain liquid before the start of the test or it could be dry. The membrane could be punctured by pressurizing the liquid in the syringe or it could be punctured by fitting the syringe (3) into the connector of the outlet port (24).

[0060] In some embodiments the syringe (3) and the sealed container could be connected to the outlet port (24) and the inlet port (25) by means of conduits, e.g. by means of a flexible polymer tubing. These embodiments are not shown in the specification for brevity.

[0061] In some embodiments, the assay area could have electrodes incorporated into it. These electrodes could be used as means to monitor the cell binding. The layer of cells attached to the substrate could alter the resistance between such electrodes.

[0062] The following is the description of the sequence of steps that represents the typical method of testing cell containing fluid. These are carried out using the device described in Figures 1-12. Some of these steps are optional and could be modified depending of the type of test carried out and the specific embodiment of the device available.

1. Forming an array or a microarray within an assay area (22) on a biochip. The array is the array of one

or a number of agents of capable of selectively recruiting at least one type of mammal cells from the fluids. If required, this array could be formed at a manufacturing site and not at the laboratory site. In this case, laboratory practitioner can retrieve the device containing the array formed appropriately for the intended test, typically packed in a sealed package. The array could be formed by the device manufacturer and supplied to the laboratories. The array is formed on the inner surface of the base plate of the device.

2. Attaching the base plate (20) to a cover plate (21) preferably in a hydraulically tight manner using cartridge sealing means (23) e.g. elastic compression ring. This needs to be done in such a way that the assay area is located within the channel formed between the base plate (20) and the cover plate (21).

3. Taking blood from a patient using appropriate container (4), e.g. container for blood collection known in the industry as the Vacutaneous container or Vacutainer. Other types of blood collection containers could also be used. In a typical embodiment, the cover plate of the device has two female Luer connectors. Piercing means, such as hypodermic needle can be connected to one of them, the inlet port (25) using male Luer connector. Typically, the sharp end of the hypodermic needle (piercing means) is protected by a rubber sleeve.

4. Connecting syringe (3) filled with buffer liquid or compensating liquid to the Luer connector at the outlet port (24). The buffer is specifically selected for the assay to be performed. The volume of the syringe could be 0.1 ml-5 ml typically, but syringes with buffer volumes outside this range are also possible. Typically, the cartridge (2) has at least one channel, one inlet port (25) and one outlet port (24). Cartridges comprising more than one channel or more than one inlet port or more than one outlet port are also possible.

5. Connecting the sealed container (4) to the inlet port of the cartridge so that the hypodermic needle (piercing means) punctures through the rubber plug of the sealed container. In a typical embodiment the sealed container (4) is pushed into the sealed container holder (53) with the hypodermic needle (piercing means) (52) inserted in the middle of the holder. By doing so the soft cup of the sealed container is penetrated by the hypodermic needle (piercing means).

6. Injecting the contents of the syringe (3) into the sealed container (4). This could be done by hand or alternatively this could be done by automated actuator coupled to the syringe plunger (30). Shaking the contents of the sealed container could be done to

achieve homogeneous mixing of the contents following the injection of the contents of the syringe into the sealed container. For some assays there is no requirement for the syringe to be filled with any liquid before the start of the assay. For some tests dry syringe could be utilized.

7. By injecting the contents of the syringe (3) through the channel (28), the channel (28) gets wet which is essential for performance of certain agents capable of selectively recruiting at least one type of mammal cells from the fluids.

8. Then the plunger (30) of the syringe (3) is pulled back by taking the contents of the sealed container into the syringe through the channel (28). In a typical embodiment this is done slowly and the rate of the plunger retraction is controlled. It is advantageous to do it by means of syringe actuator controlled automatically and with the assistance of a computer processor.

9. As the contents of the sealed container passes through the channel (28) the attachment of the cells to the areas of the chip is monitored. For example, in some embodiments of the invention the number of cells could be counted for different values of the shear stress in the channel (28). The values of the shear stress could be controlled by means of the speed of the syringe plunger (30) movement. This monitoring can be achieved by placing the channel (28) within the field of view of a microscope. The objective of the microscope is facing into the assay area (22). In some embodiments the cartridge (2) could be placed on translation table of a microscope so that different part of the assay area could be monitored.

[0063] The invention is not limited to the embodiments hereinbefore described which may be varied in both construction and detail without departing from the spirit of the invention.

Claims

1. A planar cartridge suitable for performing diagnostic studies of a fluid containing cells of mammalian origin, the cartridge comprising:

- an enclosed fluidic channel (28);
- an assay area (22) disposed within the fluidic channel and at least partially covered by at least one agent capable of selectively recruiting at least one type of cell contained within the fluid;
- an inlet port (25) for the fluidic channel, the inlet port comprising a well (5) disposed on a top of the cartridge for receipt of a sealed container (4)

- containing the cell containing fluid and piercing means (52) adapted to pierce the sealed container when it is inserted into the well and provide fluid communication between the sealed container and the enclosed fluidic channel; and
 - an outlet port (24) for the fluidic channel disposed on the top of the planar cartridge, the outlet port adapted to engage with a fluid outlet of a syringe in a fluidically tight manner.
2. A planar cartridge of Claim 1, in which the piercing means (52) is a hypodermic needle.
 3. A planar cartridge of Claim 1 or 2 in which the fluidic channel comprises a liquid.
 4. A planar cartridge of Claim 1, 2 or 3 in which the piercing means (52) is located within the well (5).
 5. A planar cartridge of any preceding Claim, in which the planar cartridge comprises a base plate coupled to a cover plate in a fluidically tight manner such that the fluidic channel and assay area is defined therebetween, wherein the inlet port and outlet port are provided on the cover plate.
 6. A planar cartridge as claimed in any preceding Claim which is disposable.
 7. A planar cartridge as claimed in any preceding Claim in which the outlet port comprises a seal adapted to be broken when the outlet port engages with a fluid outlet of a syringe.
 8. A kit comprising a planar cartridge of any preceding Claim and a syringe having an outlet, wherein the outlet of the syringe and outlet port of the cartridge are adapted for relative engagement in a fluidically tight manner.
 9. A kit as claimed in Claim 8 in which the syringe comprises a normalizing liquid suitable for reversing the action of an anti-coagulant on a blood product.
 10. A kit as claimed in Claim 8 or 9 in an assembled form in which the outlet of the syringe is coupled with the outlet port of the cartridge in a fluidically tight manner.
 11. A kit as claimed in any of Claims 8 to 10 in which the syringe is coupled to a shaft of an automated driver thus forming a pump.
 12. A method for performing diagnostic studies of a cell-containing fluid of mammalian origin, which method employs a planar cartridge of any of Claim 1 to 7, the method comprising the steps of:
 - coupling of a sealed container (4) containing
- the cell-containing fluid with the inlet port (25) of the cartridge (2) by means of the piercing means (51) puncturing the container;
- coupling a syringe having a fluid outlet to the outlet port of the planar cartridge such that a fluid tight seal is established between the outlet port and the syringe fluid outlet;
 - actuating the syringe (3) to establish a flow of cell-containing fluid from the sealed container through the fluidic channel (28) over the assay area (22); and
 - monitoring the binding of the at least one type of cell from the cell containing fluid to the assay area.
13. A method for performing diagnostic studies of a fluid containing cells of mammalian origin, which method employs a kit of any of Claims 8 to 11, the method comprising the steps of:
 - coupling of a sealed container (4) containing the cell-containing fluid with the inlet port (25) of the cartridge (2) by means of the piercing means (51) puncturing the container;
 - coupling the syringe to the outlet port of the planar cartridge such that a fluid tight seal is established between the outlet port and the syringe fluid outlet;
 - actuating the syringe (3) to establish a flow of cell-containing fluid from the sealed container through the fluidic channel (28) over the assay area (22); and
 - monitoring the binding of the at least one type of cell from the cell containing fluid to the assay area.
 14. A method as claimed in Claim 12 or 13 in which the syringe comprises a liquid, and wherein the step of actuating the syringe comprising the sequential steps of:
 - actuating the syringe to pump the liquid from the syringe into the sealed container to mix with the cell-containing fluid; and
 - actuating the syringe to withdraw a mixture of liquid and cell-containing fluid from the sealed container and establish flow of the mixture through the fluidic channel.
 15. A method as claimed in Claim 14 in which the liquid in the syringe is a normalizing liquid suitable for reversing the action of an anti-coagulant on a blood product.

Figure 1

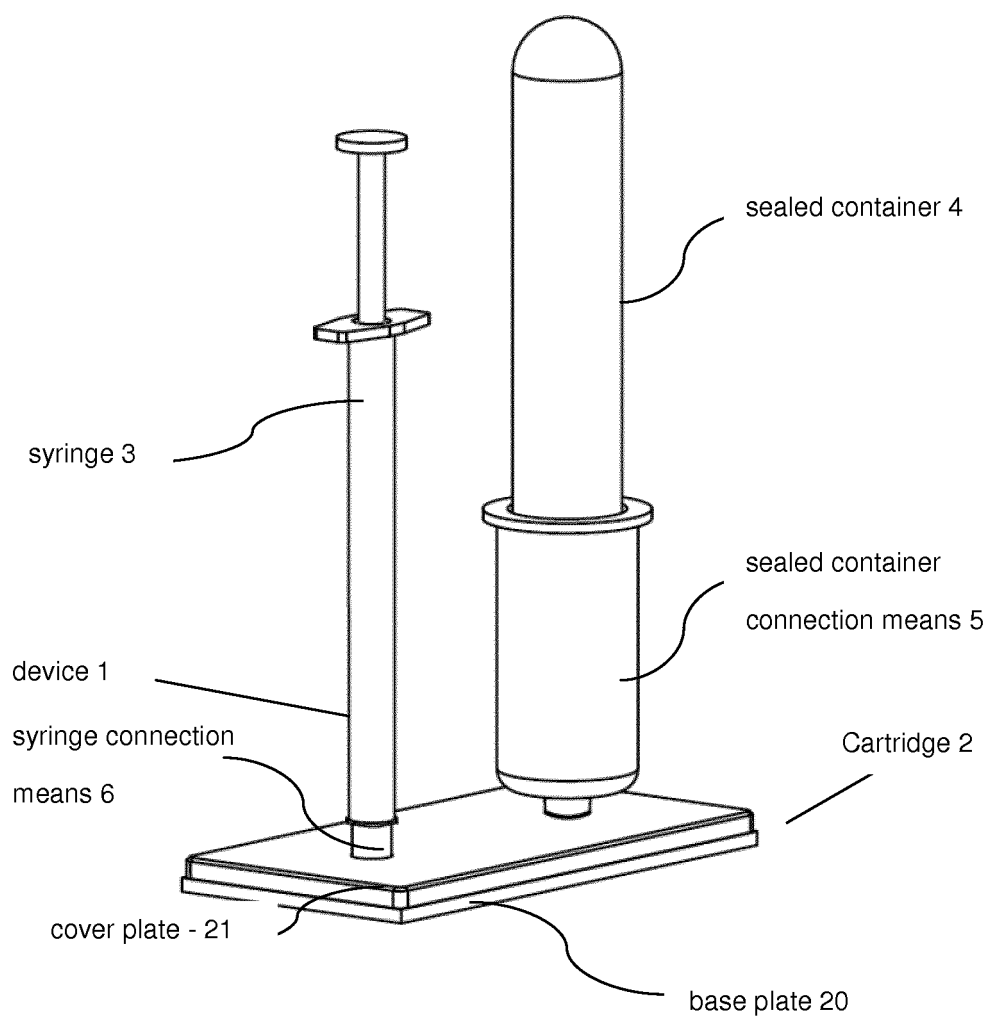


Figure 2

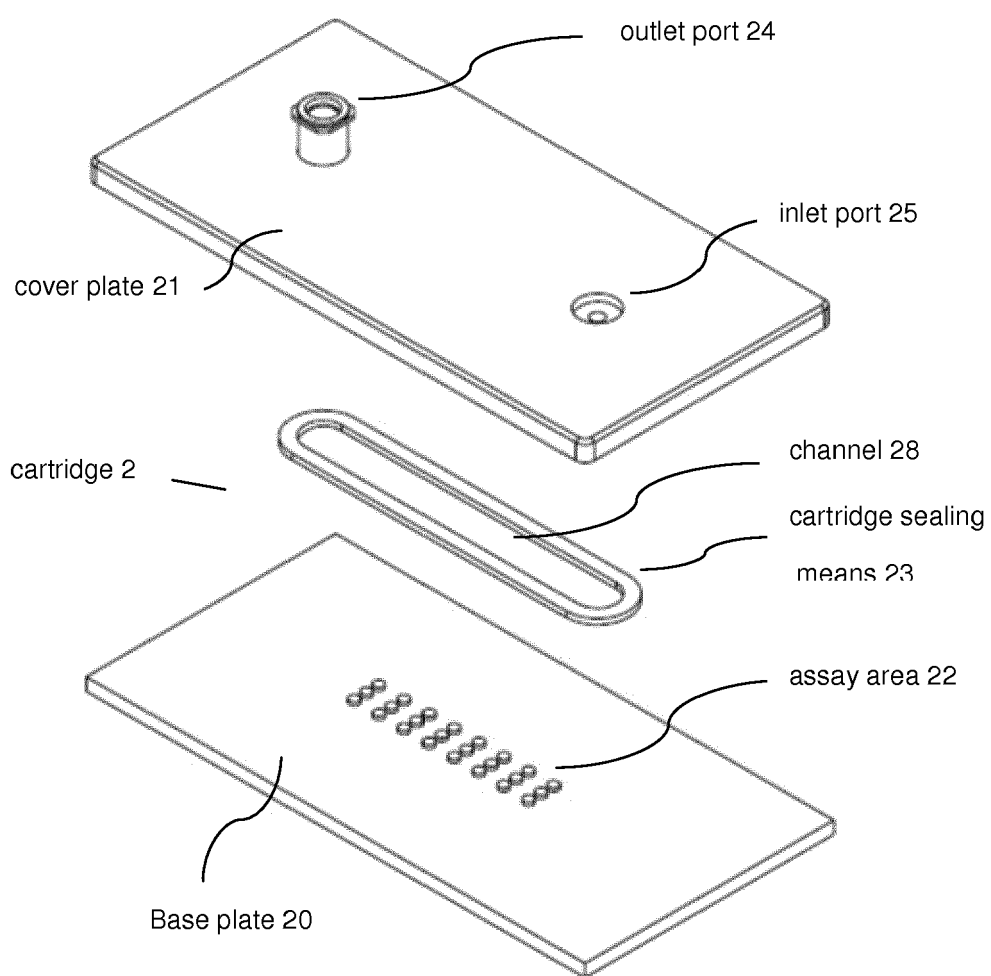


Figure 3

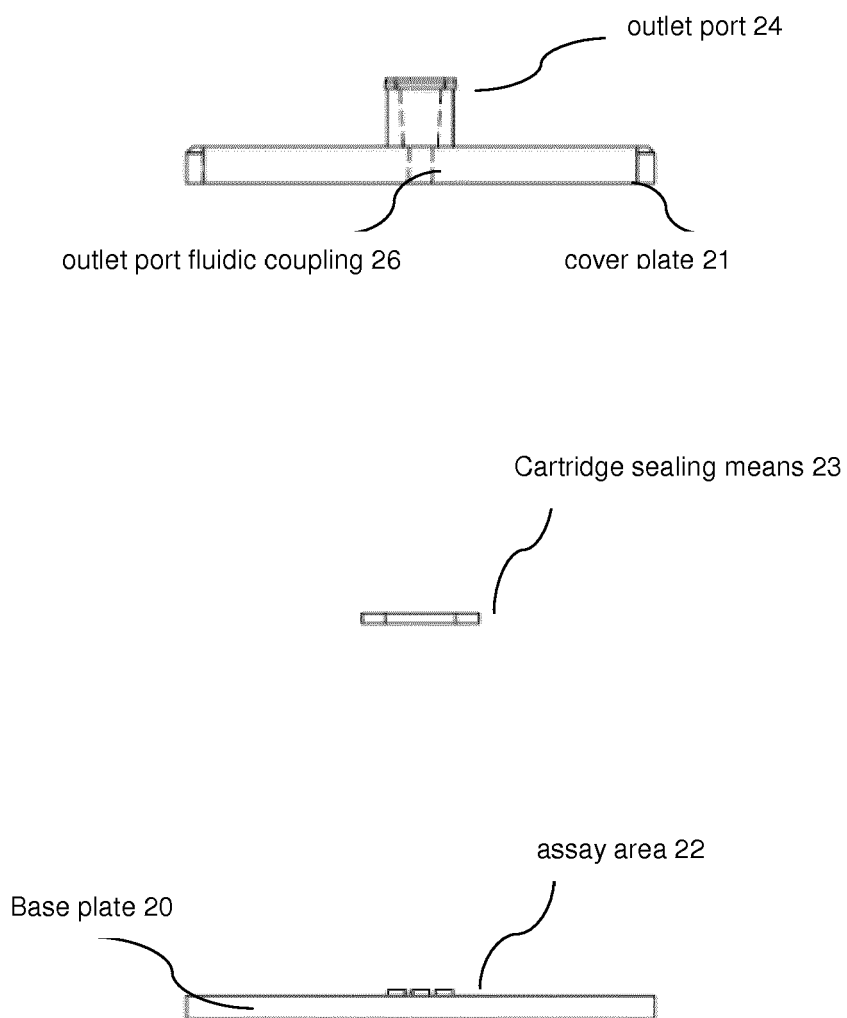


Figure 4

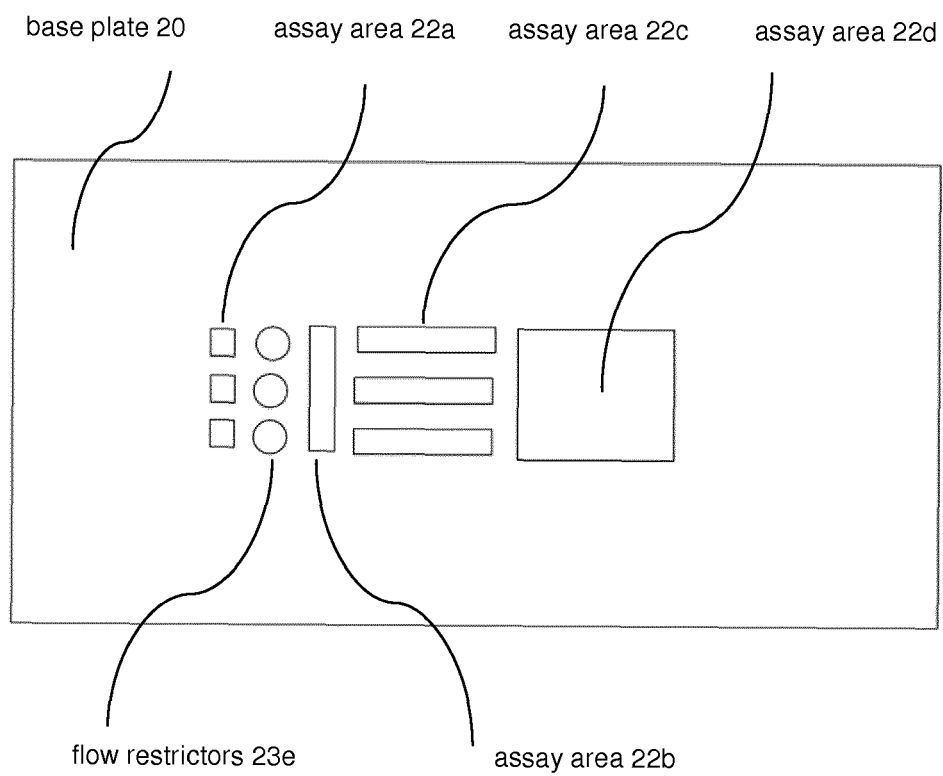


Figure 5

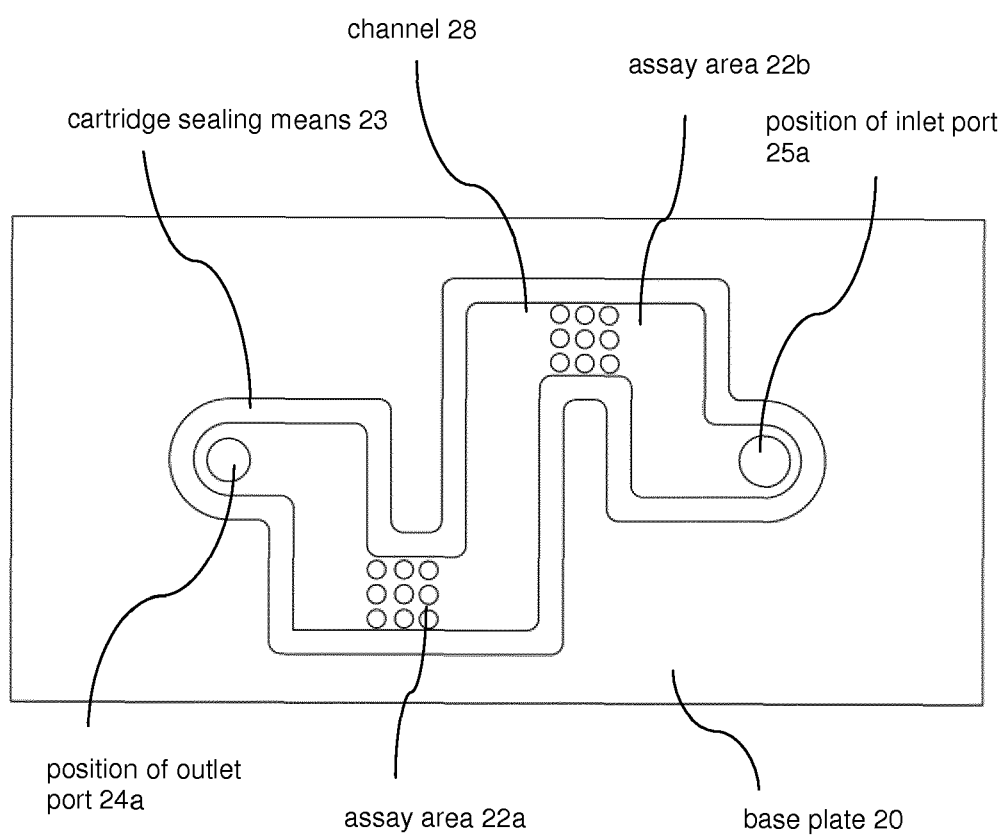


Figure 6

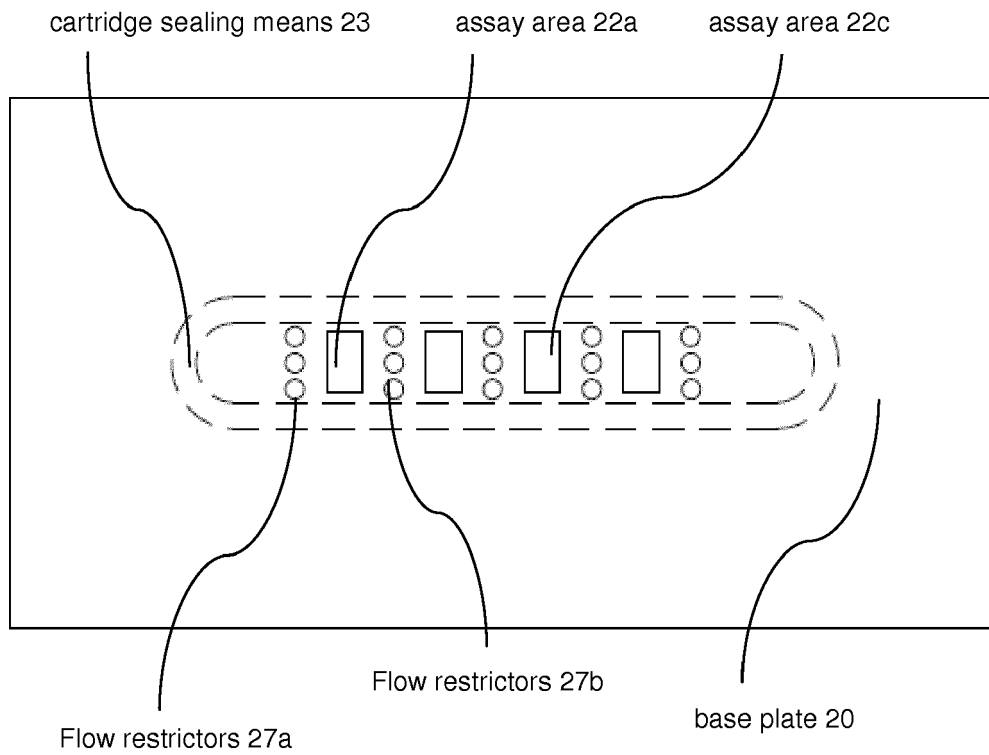


Figure 7

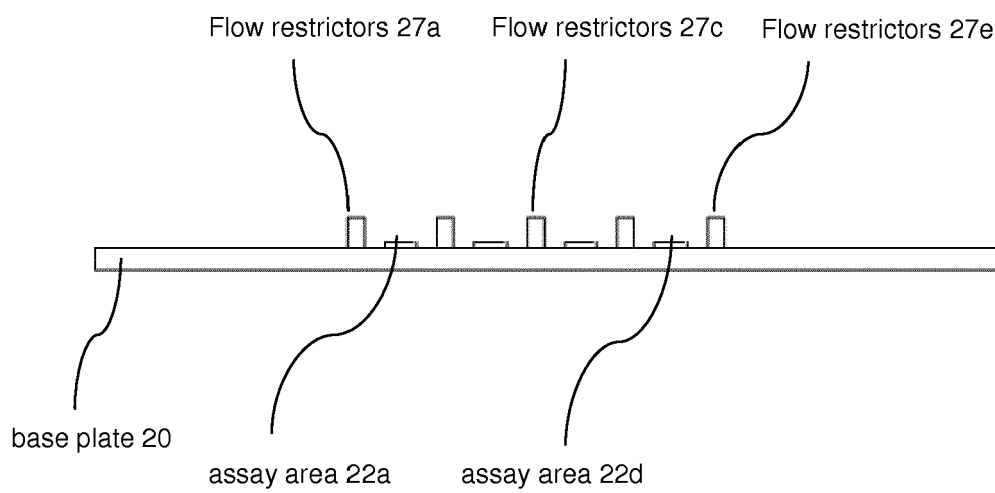


Figure 8

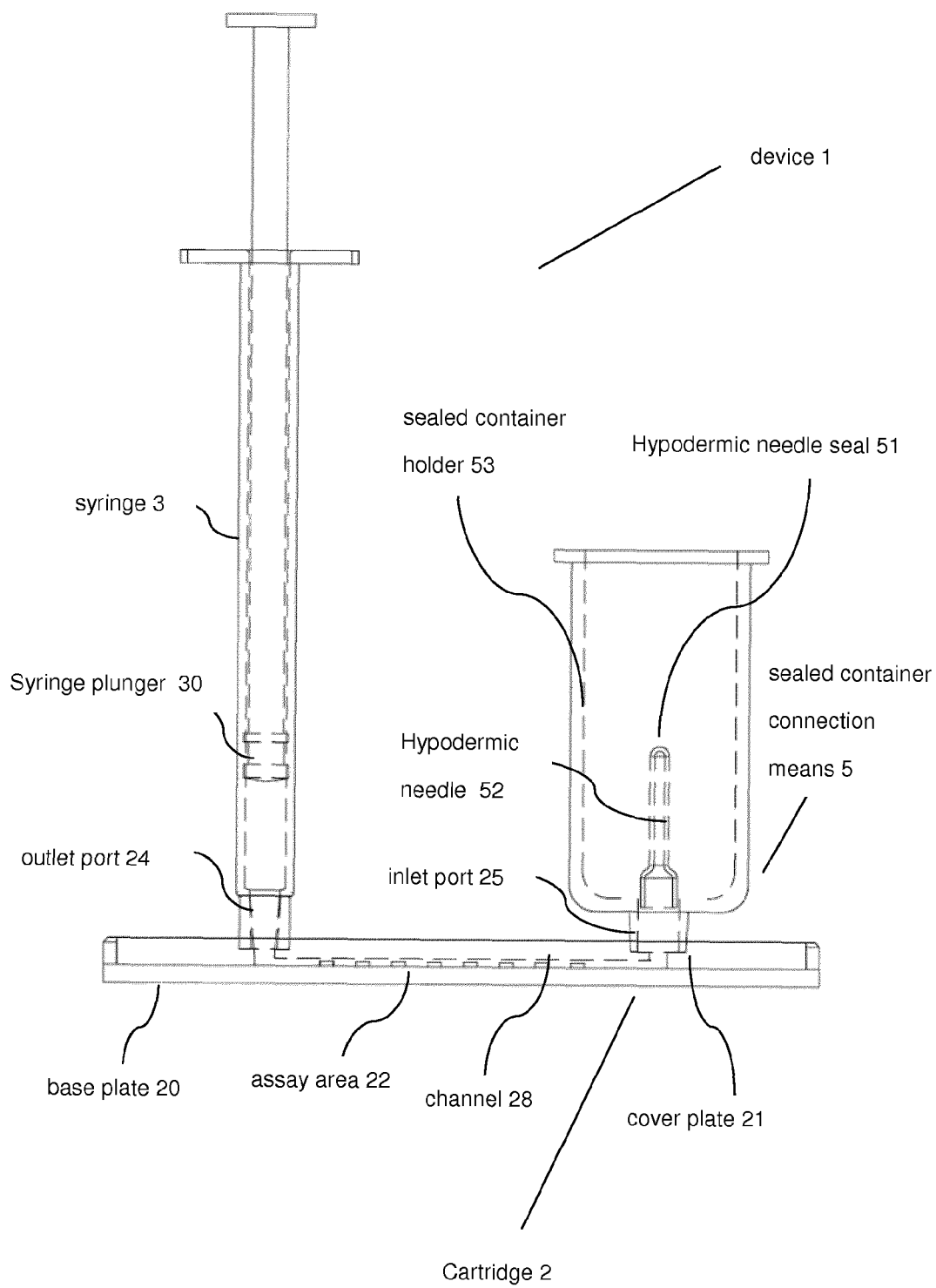


Figure 9

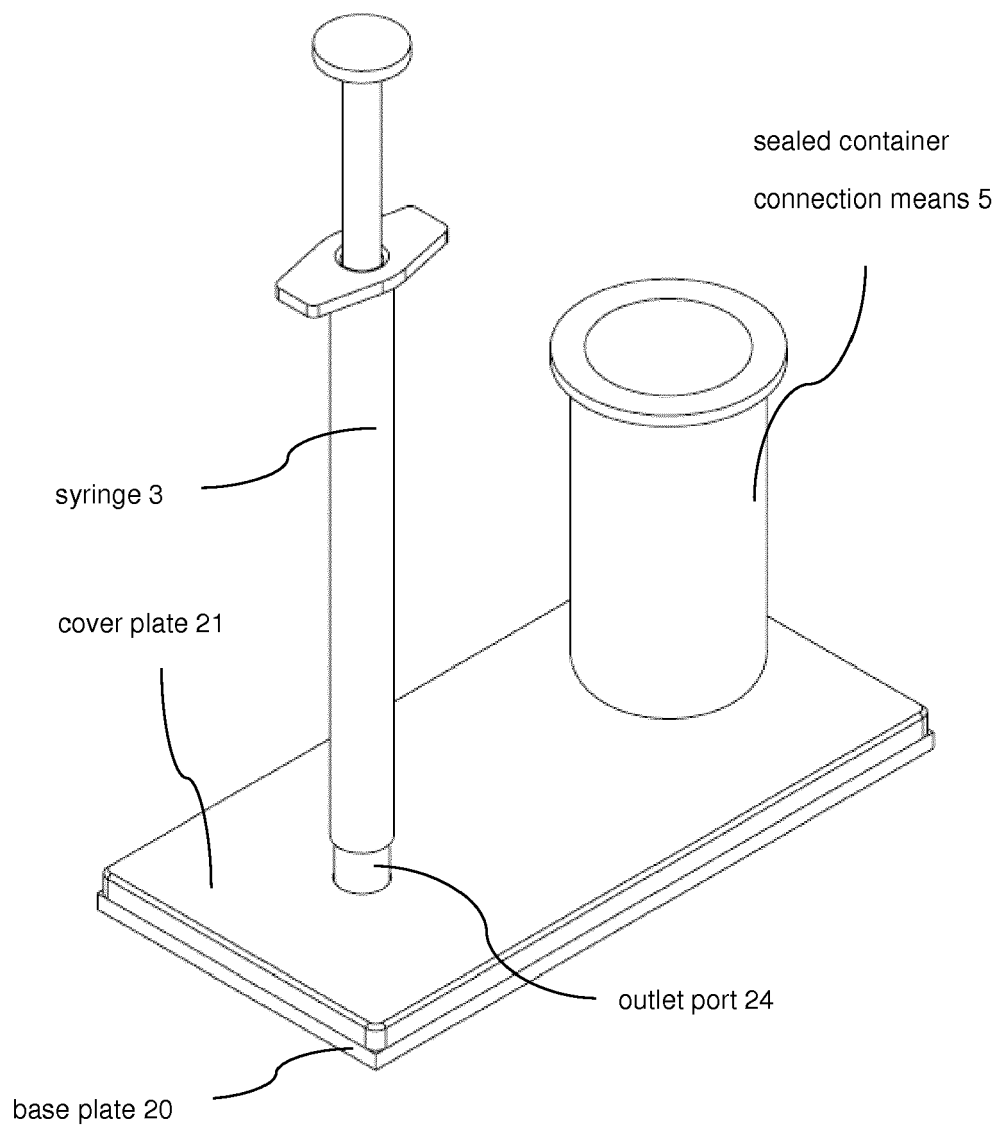


Figure 10

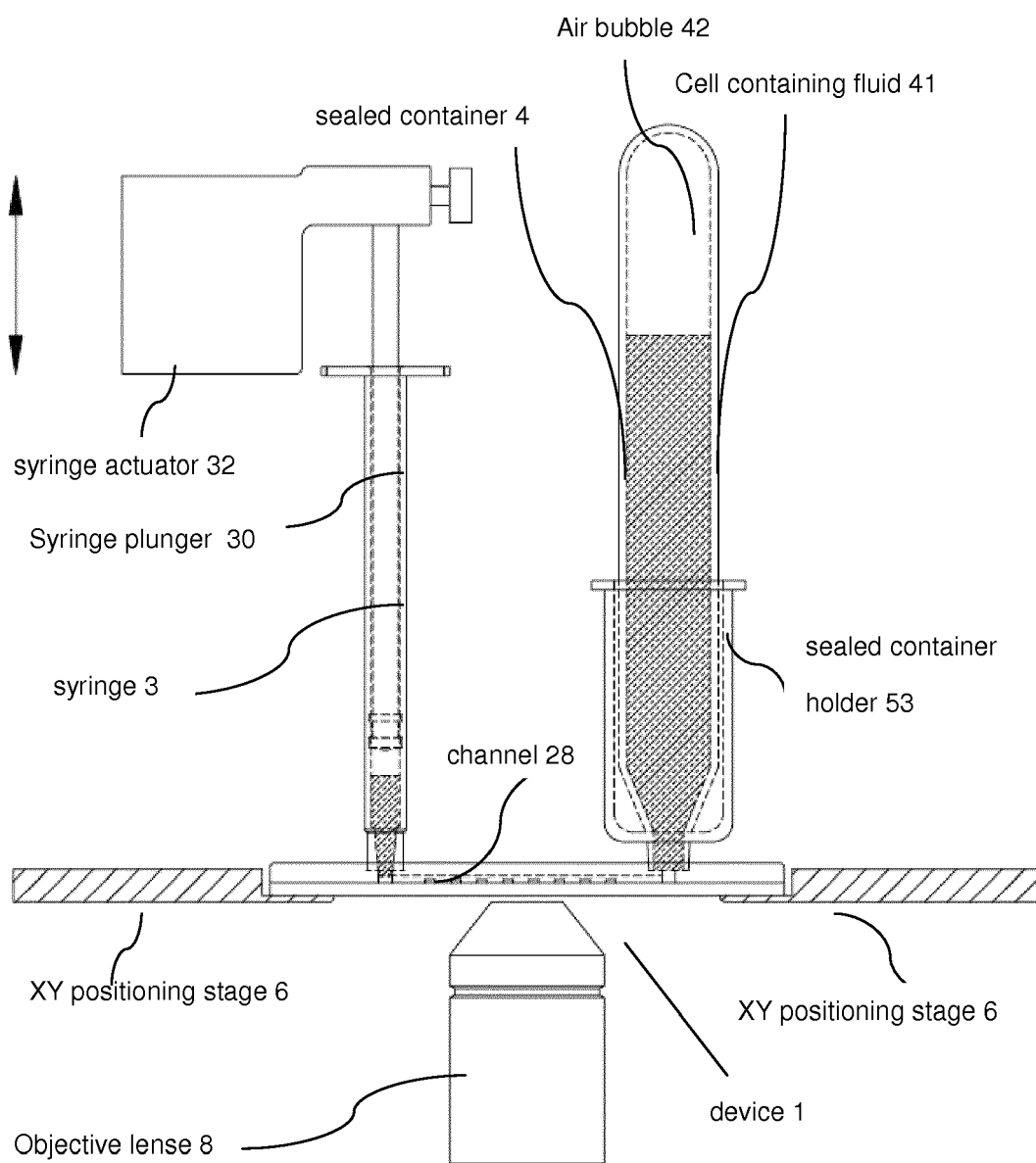


Figure 11

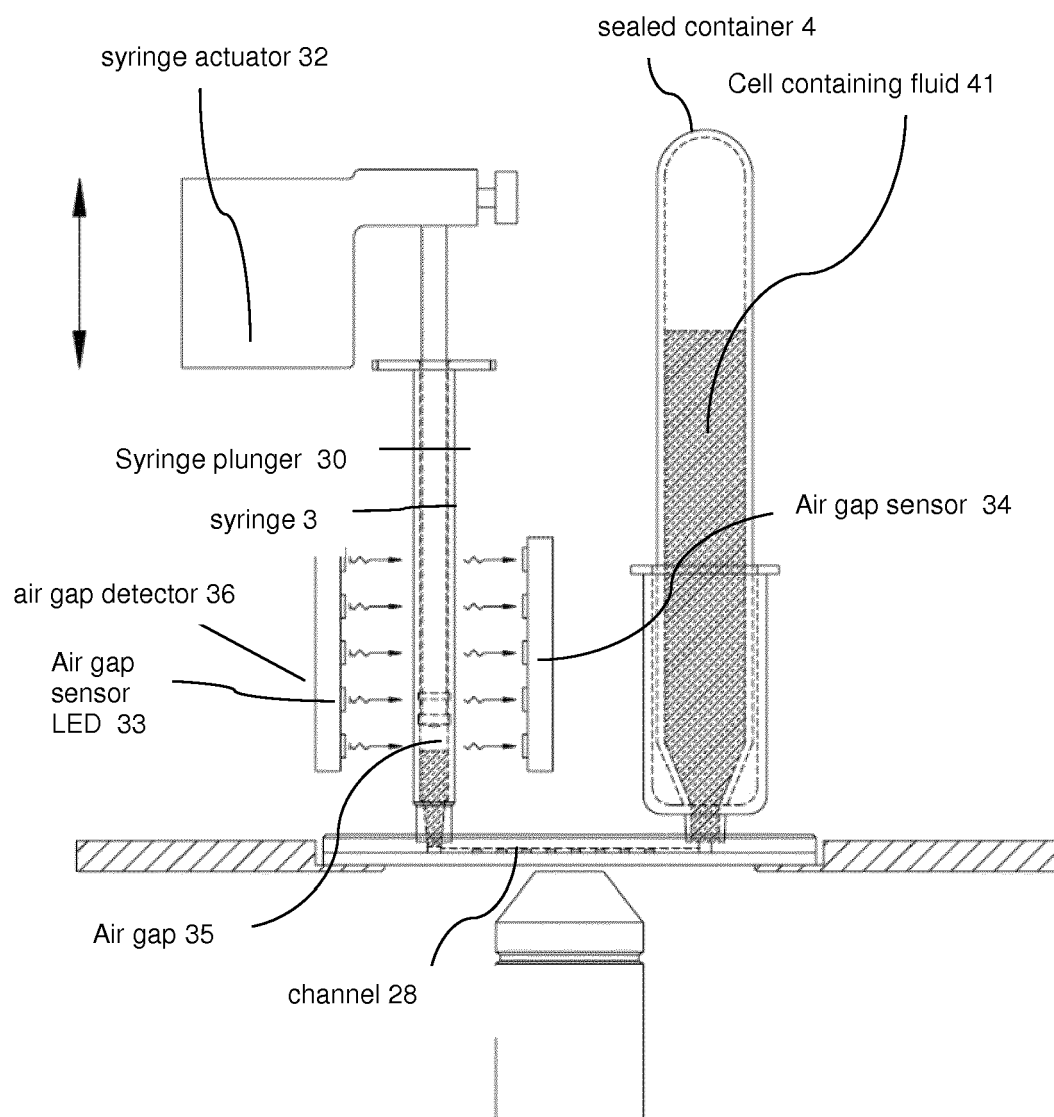
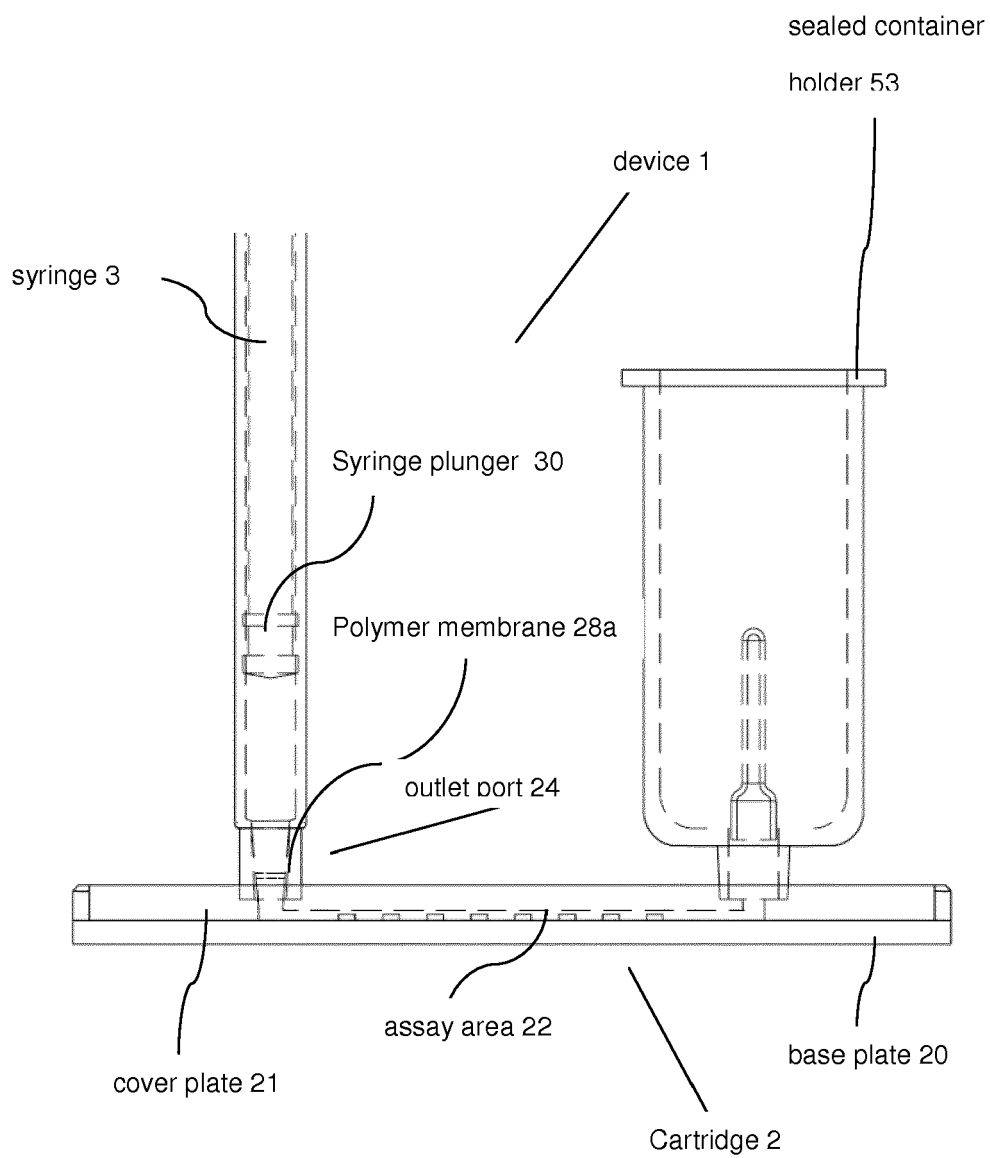


Figure 12





EUROPEAN SEARCH REPORT

Application Number
EP 12 16 4728

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X	WO 99/14595 A1 (ACCUMETRICS [US]) 25 March 1999 (1999-03-25) * page 10, line 20 - page 11, line 6; figures 1-6 * * page 16, line 22 - page 24, line 15 * * page 25, line 17 - page 30, line 26 *	1-8, 10-13	
A	USAMI S ET AL: "DESIGN AND CONSTRUCTION OF A LINEAR SHEAR STRESS FLOW CHAMBER", ANNALS OF BIOMEDICAL ENGINEERING, PERGAMON PRESS, OXFORD, GB, vol. 21, no. 1, 1 January 1993 (1993-01-01), pages 77-83, XP001028270, ISSN: 0090-6964 * abstract; figure 1 *	1-15	
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Place of search		Date of completion of the search	Examiner
The Hague		23 July 2012	Pessenda García, P
CATEGORY OF CITED DOCUMENTS		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	
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			

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