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(54) Title: METHODS AND SYSTEMS FOR CONSISTENT THROMBUS FORMATION AND MEASUREMENT THEREOF

(57) Abstract: Systems and methods to measure thrombus formation in a biological sample. The system can include at-least three reservoirs to enable gravity-based pressure head for sample flow, a microfluidic cartridge that contains at least one channel with a pre-measured flow rate, a conductive surface to enable deposition of biological coating via electrochemical process, and a light blocking mask that blocks light from selected regions in the microfluidic cartridge. Also provided are methods for using the system to measure thrombus formation.



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METHODS AND SYSTEMS FOR CONSISTENT THROMBUS FORMATION AND MEASUREMENT THEREOF

BACKGROUND OF THE INVENTION

1. Field of the Invention

[0001] The present invention relates generally to diagnostic systems and methods, and more specifically to the formation of thrombus in a biological sample and its characterization using an interrogation system.

2. Description of Related Art

[0002] Heart Attack is the leading cause of mortality all across the world. It results from the formation of blood clot inside a blood vessel, obstructing the flow of blood through the circulatory system. Plaque buildup and narrowing of arteries can take decades. However, once the constriction in the arteries becomes greater than fifty percent the risk of a major cardiac event becomes high. Arterial thrombosis - the process that involves going to full occlusion, only takes minutes. Therefore, the prevention of such an event with the optimal drug therapy becomes imperative.

[0003] Arterial thrombosis depends on two other key factors besides the greater than fifty percent stenosis in the artery. Firstly, presence of necessary bio-chemicals that is made available by a vascular injury in close proximity to the stenosis. The vascular injury can happen because of physical or emotional stress. Secondly, the blood of the patient plays a significant role. Some patient's blood clots faster, while others with von Willebrand disease as an example, takes a long time.

[0004] Cardiovascular doctors use anti-platelet drugs like Aspirin, Plavix®, Brillinta®, or Effient® for the treatment of arterial thrombosis. The challenge in creating an optimal therapy protocol is that not every patient responds to a given drug. Further, the drug dosage can be critical – too little drug may not make sufficient impact, while too much may lead to internal bleeding.

[0005] There are existing diagnostic devices that characterize blood clot or related bio-markers in an *in-vitro* setting to further guide physicians in their decision-making process for optimal drug and dosage. However, randomized clinical trials utilizing such devices have not led to any improvements in patient mortality, bringing into question the repeatability and reproducibility of test results with such devices.

[0006] The present invention has identified several areas to improve repeatability and reproducibility associated with thrombus formation. One of the target product quality requirements is to demonstrate low variability across multiple thrombi formed from a single patient blood draw.

[0007] An underlying principle of the present invention is to mimic arterial conditions necessary for thrombus formation, while avoiding or minimizing the noise sources. One of the sources of variance in thrombus characterization comes from the pump that is used to generate flow of biological sample. Pump flows rates can vary both spatially and temporally. Further, there is pump-to-pump variation adding to the noise when comparing results of different patients using multiple devices. The present invention uses gravity based pressure head to drive sample flow. Gravity provides the consistency that no pump can match.

[0008] Another source of variances comes from inherent limitations of the fabrication processes. A representative coronary artery is quarter of an inch in diameter. Reproducing a thrombus of that size under representative flow conditions would requires multiple liters of human blood. Realistic blood draw from a patient can only be in the order of milliliters. This requirement further dictates that the in-vitro arterial environment in the diagnostic device is scaled down via use of microfluidic elements. The challenge with using microfluidic elements is the associated part-to-part variation. The present invention accounts for this by measuring flow rates through each independent channel, and normalizing the final results appropriately.

[0009] An addition source of variance comes from the biological serums that are used to coat the microfluidic channels to simulate endothelial environment. Some of the typical serums include collagen, fibrin, and vWF. These serums are coated onto the channels using a liquid deposition method, wherein the serum is inserted into the channels and left for a sufficient amount of time. This process leads to random and non-uniform coating. The present invention uses an electrochemical process to deposit the serum inside the desired channel section.

[0010] The thrombus measurement approach also adds variance in final results. The conventional approach is to use pressure sensors or direct microscopy. Pressure sensors only provide integrated point measurements, and exhibit sensor-to-sensor variation issues. Direct microscopy is expensive, time consuming due to the associated post processing operation, and provides only surface measurements whereas thrombus formation is a volumetric phenomenon. Further, both pressure sensor and microscopy approaches don't scale well for making measurements at multiple locations simultaneously. The present invention uses an optical detection system involving laser light source and photodiodes.

[0011] The existing diagnostic devices focus on platelet activation. The present invention tests whole blood in a simulated stenosed artery that leads to lumen occlusion. The system quantifies the propensity of the blood of an individual to form an occlusive thrombus or self-lyse the clot, thus providing a prediction of future atherothrombotic occlusion in individual patients. In some scenarios like arterial thrombosis, pathological high shear flow is required to flow over collagen surface. In other scenarios low shear flow may be required to flow over alternate biochemical coating like fibrin. The present invention covers both high and low shear flows with any biochemical coating.

[0012] Thus, it would be beneficial to develop a reliable diagnostic system that can characterize a patient's blood for optimizing drug therapy, wherein such a system and methods can generate results with high repeatability and reproducibility. It is to such a diagnostic system and method that the present invention is directed.

SUMMARY OF THE INVENTION

[0013] The present invention includes systems and methods for testing a fluid sample in a simulated stenosed blood vessel that mimics conditions necessary for thrombus formation, while minimizing if not avoiding sources of error.

[0014] The present invention can quantify the propensity of the blood of an individual to form an occlusive thrombus or to self-lyse the clot, thus providing meaningful information on future atherothrombotic occlusion and enabling patient's optimized drug therapy.

[0015] In some scenarios like arterial thrombosis, pathological high shear flow is necessary to flow over collagen surface. In other scenarios low shear flow may be necessary to flow over

alternate bio-chemical coating like fibrin. The present invention can consider both high and low shear flows with a bio-chemical coating.

[0016] The present invention demonstrates low variability across multiple thrombi formed from a single patient blood draw. The present invention provides improved repeatability and reproducibility associated with thrombus formation.

[0017] The present invention minimizes if not eliminates noise sources that if left unmitigated, would present unacceptable error levels in reliably characterizing a patient's blood.

[0018] To overcome conventional pump issues, the present invention can use gravity-based pressure head for sample flow.

[0019] To overcome issues related to conventional fabrication tolerances, the present invention can measure flow rates through independent channels and normalize the final results using flow rate information.

[0020] To overcome issues related to conventional liquid deposition of biological serums, the present invention can use an electrochemical process to deposit the serum inside a desired channel section.

[0021] To overcome issues related to use of pressure sensors or direct microscopy, the present invention can use an optical detection system involving laser light source and photodiodes.

[0022] To overcome issues related to measuring a light transmission signal, the present invention can use a light blocking mask that allows light in selected regions of the channel where thrombus formation takes place.

[0023] In an exemplary embodiment of the present invention, a system useful in determining functional performance of a biological sample comprises a thrombosis unit in which thrombus formation takes place and an interrogation unit providing information related to the formation of the thrombus. The thrombosis unit can comprise a disposable cartridge and the interrogation unit can comprise a cartridge reader that characterizes thrombus formation in the disposable cartridge.

[0024] The disposable cartridge can comprise three reservoirs, a fluid interfacing reservoir used to insert the biological sample into the disposable cartridge, an inlet reservoir, and an outlet

reservoir, wherein the inlet and outlet reservoirs are used to provide identical gravity-based pressure head to multiple microfluidic channels of the disposable cartridge simultaneously.

[0025] The disposable cartridge can comprise a plurality of microfluidic channels, each inherently embodying a flow characteristic determined at least in part on the fabrication of each microfluidic channel, wherein the flow characteristic of each of the microfluidic channels is used in order to normalize final thrombus characterization results across each of the microfluidic channels upon operational use of the system.

[0026] The disposable cartridge can comprise a light blocking mask enabling precise characterization of thrombus formation where light passes through the thrombus and the transmission intensity is measured using a photo diode sensor.

[0027] The disposable cartridge can comprise a microfluidic cartridge and a cover slip for the microfluidic cartridge, wherein the cover slip comprises a conductive surface enabling the use of an electrochemical process for depositing a bio-chemical.

[0028] In another exemplary embodiment of the present invention, a system useful in determining functional performance of a biological sample comprises a thrombosis unit and an interrogation unit, wherein in the thrombosis unit, the propensity of the biological sample to form an occlusive thrombus is established, wherein the interrogation unit provides information related to the formation of the occlusive thrombus, wherein the information from the interrogation unit correlates to a prediction of future atherothrombotic occlusion, and wherein the thrombosis unit comprises one or more of a plurality of microfluidic channels, each inherently embodying a flow characteristic determined at least in part on the fabrication of each microfluidic channel, wherein the flow characteristic of each of the microfluidic channels is used in order to normalize final thrombus characterization results across each of the microfluidic channels upon operational use of the system, and/or three reservoirs, a fluid interfacing reservoir used to insert the biological sample into the thrombosis unit, an inlet reservoir, and an outlet reservoir, wherein the inlet and outlet reservoirs are used to provide identical gravity-based pressure head to multiple microfluidic channels of the thrombosis unit simultaneously, and/or a microfluidic cartridge and a cover slip for the microfluidic cartridge, wherein the cover slip comprises a conductive surface enabling the use of an electrochemical process for depositing a bio-chemical, and/or a light

blocking mask enabling precise characterization of thrombus formation where light passes through the thrombus and the transmission intensity is measured using a photo diode sensor.

[0029] In another exemplary embodiment of the present invention, a system useful in determining functional performance of a biological sample comprises a thrombosis unit comprising a plurality of microfluidic channels, each inherently embodying a flow characteristic determined at least in part on the fabrication of each microfluidic channel, wherein the flow characteristic of each of the microfluidic channels is used in order to normalize final thrombus characterization results across each of the microfluidic channels upon operational use of the system, three reservoirs, a fluid interfacing reservoir used to insert the biological sample into the thrombosis unit, an inlet reservoir, and an outlet reservoir, wherein the inlet and outlet reservoirs are used to provide identical gravity-based pressure head to the microfluidic channels simultaneously, a microfluidic cartridge and a cover slip for the microfluidic cartridge, wherein the cover slip comprises a conductive surface enabling the use of an electrochemical process for depositing a bio-chemical in a portion of the microfluidic channels, and a light blocking mask enabling precise characterization of thrombus formation where light passes through the thrombus and the transmission intensity is measured using a photo diode sensor, and an interrogation unit, wherein in the thrombosis unit, the propensity of the biological sample to form an occlusive thrombus is established, wherein the interrogation unit provides information related to the formation of the occlusive thrombus, and wherein the information from the interrogation unit correlates to a prediction of future atherothrombotic occlusion.

[0030] In another exemplary embodiment of the present invention, a method useful in determining functional performance of a biological sample comprises forming a thrombus in a thrombosis unit and interrogating the formation of the thrombus. The thrombosis unit can comprise a disposable cartridge, and interrogating the formation of the thrombus can be via a cartridge reader that characterizes thrombus formation in the disposable cartridge.

[0031] Thus, an object of the present invention is to provide thrombosis characteristics for a given biological sample, in exemplary embodiments, a blood fluid sample, and in further exemplary embodiments, a whole blood sample. This is achieved by providing gravity-based pressure head to multiple independent calibrated channels, each of them incorporating a stenosis

section. The thrombus formation is measured using a laser and photo-diode based optical system.

[0032] Another object of the present invention is to provide a method for characterizing thrombus formation using the system of the present invention.

[0033] These and other objects, features and advantages of the present invention will become more apparent upon reading the following specification in conjunction with the accompanying drawing figures.

BRIEF DESCRIPTION OF THE DRAWINGS

[0034] The accompanying figures, where like reference numerals refer to identical or functionally similar elements throughout the separate views, and which, together with the detailed description below, are incorporated in and form part of the specification, serve to further illustrate various embodiments and explain various principles and advantages, all in accordance with the present invention;

[0035] **FIG. 1** is an exploded view of the present invention according to an exemplary embodiment.

[0036] **FIG. 2** illustrates the invention of **FIG. 1** in another form, in an assembly of a cartridge reader containing a disposable cartridge, the cartridge reader designed to measure thrombus formation in the disposable cartridge.

[0037] **FIG. 3** is an exploded view of the cartridge of the present invention according to an exemplary embodiment showing it formed of a pumping cartridge, a cover slip for the pumping cartridge, a microfluidic cartridge, a cover slip for the microfluidic cartridge, and a light blocking mask.

[0038] **FIG. 4** is an isometric view of a pumping cartridge of **FIG. 3**.

[0039] **FIG. 5** shows isometric views of the pumping cartridge and the cover slip for the pumping cartridge of **FIG. 3**.

[0040] **FIG. 6** shows isometric views of the microfluidic cartridge of **FIG. 3**, and an enlarged view of a stenosis section of a channel of the microfluidic cartridge according to an exemplary embodiment of the present invention.

[0041] FIG. 7 shows isometric views of the microfluidic cartridge and the cover slip for the microfluidic cartridge of FIG. 3, the cover slip for the microfluidic cartridge having a bio-chemical coating according to an exemplary embodiment of the present invention.

[0042] FIG. 8 illustrates an exemplary way of depositing the bio-chemical coating on the cover slip of the microfluidic cartridge of FIG. 7.

[0043] FIG. 9 illustrates additional exemplary embodiments of the cartridge of the present invention.

DETAIL DESCRIPTION OF THE INVENTION

[0044] To facilitate an understanding of the principles and features of the various embodiments of the invention, various illustrative embodiments are explained below. Although exemplary embodiments of the invention are explained in detail, it is to be understood that other embodiments are contemplated. Accordingly, it is not intended that the invention is limited in its scope to the details of construction and arrangement of components set forth in the following description or illustrated in the drawings. The invention is capable of other embodiments and of being practiced or carried out in various ways.

[0045] As used in the specification and the appended Claims, the singular forms “a,” “an” and “the” include plural references unless the context clearly dictates otherwise. For example, reference to a component is intended also to include a composition of a plurality of components. References to a composition containing “a” constituent is intended to include other constituents in addition to the one named.

[0046] In describing exemplary embodiments, terminology will be resorted to for the sake of clarity. It is intended that each term contemplates its broadest meaning as understood by those skilled in the art and includes all technical equivalents that operate in a similar manner to accomplish a similar purpose.

[0047] Ranges may be expressed as from “about” or “approximately” or “substantially” one value and/or to “about” or “approximately” or “substantially” another value. When such a range is expressed, other exemplary embodiments include from the one value and/or to the other value.

[0048] Similarly, as used herein, “substantially free” of something, or “substantially pure”, and like characterizations, can include both being “at least substantially free” of something, or “at least substantially pure”, and being “completely free” of something, or “completely pure”.

[0049] As used herein, the term “identical” refers to values that are close to each other within the range of manufacturing tolerances.

[0050] “Comprising” or “containing” or “including” is meant that at least the named compound, element, particle, or method step is present in the composition or article or method, but does not exclude the presence of other compounds, materials, particles, method steps, even if the other such compounds, material, particles, method steps have the same function as what is named.

[0051] The characteristics described as defining the various elements of the invention are intended to be illustrative and not restrictive. For example, if the characteristic is a material, the material includes many suitable materials that would perform the same or a similar function as the material(s) described herein are intended to be embraced within the scope of the invention. Such other materials not described herein can include, but are not limited to, for example, materials that are developed after the time of the development of the invention.

[0052] In an exemplary embodiment of the present invention, the system includes a thrombosis unit providing sample flow using identical gravity-based pressure head to multiple independent calibrated microfluidic channels simultaneously. The thrombosis unit can comprise an inlet reservoir, an outlet reservoir, a fluid interfacing reservoir, and multiple unconnected channels that are in fluidic communication with either the top or the outlet reservoirs. The thrombosis unit can further include a microfluidic cartridge comprising multiple flow channels allowing a flow loop to be completed between inlet and outlet reservoir. One or more of the channels can feature a stenosis section that mimic narrowing of an artery due to plaque buildup.

[0053] In another exemplary embodiment of the present invention, a system for characterizing thrombus formation is disclosed. The system can include the thrombosis unit further comprising an integrated light blocking mask. The microfluidic cartridge of the thrombosis unit features the multiple channels where the thrombus formation takes place. The light blocking mask of the thrombosis unit enables precise characterization of thrombus formation in a dedicated setup where laser light passes through the thrombus and the transmission intensity is measured using a photo diode sensor.

[0054] In another exemplary embodiment of the present invention, a method for characterizing thrombus formation is disclosed. The method comprises delivering flow at identical gravity-based pressure head to multiple channels of the thrombosis unit simultaneously, and then using the light blocking mask that only allows light from the stenosis section present in the microfluidic channels.

[0055] In an exemplary embodiment of the present invention, thrombus formation of a fluid sample is investigated in a stenosis section of the invention. A portion of the fluid sample is presented to the stenosis section via a stenosis section flow, and thrombus formation in the stenosis section over time is interrogated.

[0056] A simplified schematic of the diagnostic system according to an exemplary embodiment of the present invention **100** is shown in **FIG. 1**, and comprises a thrombosis unit **200** and an interrogation unit **300**. The thrombosis unit **200** presents a system that mimics arterial conditions necessary for thrombus formation. The interrogation system **300** provides precise characterization of thrombus formation in the thrombosis unit **200**.

[0057] The thrombosis unit **200** can comprise a fluidic system capable of thrombosis formation of a patient's fluid sample in the stenosis section. As used herein, the term "fluid sample" refers to a biological sample whose thrombosis characteristics are tested using the present invention. For example, the fluid sample can be a blood fluid sample, the blood fluid being whole blood, or platelets, or a biological sample that is an unprocessed fluid directly from a patient, or it might be processed and treated with other chemicals including, but not limited to, anti-coagulants and anti-platelet agents.

[0058] The thrombosis unit **200** can comprise a cartridge **1000** (preferably a disposable cartridge) having reservoirs and channels. Only a portion of the cartridge **1000** is shown in **FIG. 1**. In an exemplary embodiment, the cartridge **1000** comprises a pumping cartridge **1100**, a cover slip **1200** for the pumping cartridge, a microfluidic cartridge **1300**, a cover slip **1400** for the microfluidic cartridge, and a mask **1500**. (See, **FIG. 3**).

[0059] In an exemplary embodiment, the cartridge **1000** comprises three reservoirs in fluidic communication – a fluid interfacing reservoir **1110** where the fluid sample is initially contained, an inlet reservoir **1120** where the fluid sample is staged at the beginning of system operation, and an outlet reservoir **1130** where the fluid sample is collected at the end of system operation. In an

exemplary embodiment, the cartridge **1000** comprises a plurality of inlet and outlet channels, for example, inlet channels **1121**, **1122**, **1123**, and **1124** in fluidic communication with the inlet reservoir **1120** and outlet channels **1131**, **1132**, **1132**, and **1134** in fluidic communication with the outlet reservoir **1130**. (See, **FIG. 4**).

[0060] The interrogation system **300** can comprise an energy source and a detector. In an exemplary embodiment, the energy source can comprise a light source **2000** and the detector can comprise a light detection system **6000**.

[0061] In operation, the present invention provides thrombus formation in the thrombosis unit **200** via fluid sample flow inside the cartridge **1000** from the inlet reservoir **1120** to the outlet reservoir **1130** through the independent and calibrated channels. One or more of the independent channels has a stenosis section where thrombus formation takes place. Thrombus formation is interrogated via interrogation system **300** preferably being measured using an optical detection system where light transmission intensity through the thrombus changes as thrombus formation evolves.

[0062] As used herein, the term independent channel refers generally to a channel whose flow performance is independent of another channel. Specifically, in the context of the present invention it also implies that the all the independent channels have their own output and input ports.

[0063] In an exemplary embodiment, the light source **2000** generates light **3000** that is passed through one or more optical elements **4000** to convert the light into a collimated sheet of light **5000**. Light transmission of the evolving thrombosis in the thrombosis unit **200** is measured using the light detection system that can be a photo sensing electronic circuit **6000**.

[0064] The interrogation system **300** can be arranged within a housing **9000**. As shown in **FIG. 2**, the light source **2000**, optical element **4000**, and light detection system **6000** can reside within cartridge reader **9000**. Cartridge reader **9000** measures thrombus formation within the disposable cartridge **1000**.

[0065] The cartridge **1000** can further comprise a reader alignment assembly to ensure proper alignment of the cartridge in the cartridge reader **9000**. In an exemplary embodiment, the reader alignment assembly comprises notch **1090** and a cooperative alignment assembly of the cartridge

reader **9000**. The notch **1090**, for example, a triangular notch, allows the cartridge **1000** to be aligned within the cartridge reader device via the cooperative alignment assembly of the cartridge reader, for example, two rods. One of the rods fits the triangular notch, while the second rod balances the cartridge on the other side of the cartridge bottom plane.

[0066] An exploded view of an exemplary embodiment of cartridge **1000** is shown in **FIG. 3** by way of illustration and not limitation. Cartridge **1000** can further comprise a cartridge alignment system to align the various components of the cartridge **1000**. The pumping cartridge **1100**, the cover slip **1200** for the pumping cartridge, the microfluidic cartridge **1300**, the cover slip **1400** for the microfluidic cartridge, and the light blocking mask **1500** are aligned via the cartridge alignment system, for example, alignment holes **1271**, **1371**, **1471**, **1571**, and corresponding diametrically opposite alignment holes. The cartridge alignment system can comprise alternative ways, and be achieved through other mechanical or optical means including computer vision and microscopy.

[0067] The thrombosis unit **200** enables thrombus formation in a fluid sample by providing at least one channel having a stenosis section with a gravity-based pressure head. In an exemplary embodiment, two or more channels having identical flow characteristics are presented with different portions of the fluid sample. Results of thrombus formation in more than one channel are then interrogated and results normalized.

[0068] In an exemplary embodiment of the pumping cartridge **1100** shown in **FIG. 4**, three reservoirs are incorporated, the fluid interfacing reservoir **1110** where the fluid sample is presented, the inlet reservoir **1120** where the fluid sample is staged at the beginning of operation, and the outlet reservoir **1130** where the fluid sample is collected at the end of operation. The inlet reservoir is in fluidic communication with one or more inlet channels, and the outlet reservoir is in fluidic communication with one or more outlet channels.

[0069] As used herein, while the pumping cartridge **1100** and the cover slip **1200** are separate components, at times the combination of **1100**, **1200** may be considered the pumping cartridge. The objective of the pumping cartridge is to provide identical gravity-based pressure head and identical flow resistance to multiple independent channels in the microfluidic cartridge **1300**. As shown, the element **1100** contains open channels, and the element **1200** acts as a cover slip to close the open channels. While as shown the pumping cartridge cannot function without its

cover slip, for the sake of clarity and exposing details conveniently, some illustrations may refer to just the element **1100** as the pumping cartridge.

[0070] In an exemplary embodiment shown in **FIG. 3**, inlet reservoir **1120** is in fluidic communication with multiple inlet channels – **1121**, **1122**, **1123**, and **1124**. The outlet reservoir **1130** is in fluidic communication with multiple outlet channels – **1131**, **1132**, **1132**, and **1134**. The inlet and outlet channels are discontinuous in the pumping cartridge **1100**. The fluidic continuity between inlet and outlet channels is established via the microfluidic cartridge **1300** as illustrated in **FIG. 3**.

[0071] The fluid sample is collected from the patient, for example, in a sample test tube with a stopper made of rubber or similar material. The fluid sample can be loaded, for example, via pouring or injecting, into the fluid interfacing reservoir **1110**. In another embodiment, the test tube filled with the fluid sample can be inserted into the fluid interfacing reservoir that can be cooperatively shaped as a cylindrical reservoir **1110** that houses at least one syringe needle which pierces through the rubber stopper into the fluid sample. The base of the fluid interfacing reservoir **1110** is in communication with an aspiration channel **1141**, such that the fluid sample can be routed from the reservoir **1110** to the inlet reservoir **1120** via the aspiration channel **1141**.

[0072] The exemplary embodiment of the pumping cartridge as shown in **FIG. 4** contains pressure channels to assist in routing of the fluid sample within the cartridge **1000**. A top pressure channel **1142** can be used to apply vacuum for routing fluid sample from the reservoir **1110** to the inlet reservoir **1120** via the aspiration channel **1141**. A bottom pressure channel **1145** can be used to apply vacuum to initiate fluid sample flow from the inlet reservoir **1120** to the outlet reservoir **1130** via the assembly of the pumping cartridge **1100**, the cover slip **1200** for the pumping cartridge, the microfluidic cartridge **1300**, and the cover slip **1400** for the microfluidic cartridge. Additional pressure channels, a second top channel **1143** and/or second bottom channel **1144** can be used to provide atmospheric pressure access to the top **1120** and outlet reservoir **1130**, respectively.

[0073] An exemplary embodiment of the pumping cartridge **1100** with its cover slip **1200** is shown in **FIG. 5**. The cover slip **1200** encloses otherwise open channels and open reservoirs of the pumping cartridge **1200** while providing inlet ports – **1221**, **1222**, **1223**, **1224** and outlet ports – **1231**, **1232**, **1233**, and **1234**. The ports interface on the first hand, with the inlet and outlet

ports of the pumping cartridge **1200**, and on a second hand, with inlet and outlet of channels of the microfluidic cartridge **1300** as shown in **FIG. 3**. The cover slip **1200** shown in **FIG. 5** provides top pressure ports **1242** and **1243** for fluidic communication with the inlet reservoir **1120**, and bottom pressure ports **1244** and **1245** for fluidic communication with the outlet reservoir **1130**. The pressure applied on these ports helps route the fluid sample within the cartridge **1000**.

[0074] As shown in **FIG. 5**, the pumping cartridge **1100** and the cover slip **1200** for the pumping cartridge are separate components. The split can be beneficial for fabricating closed channels. Alternatively, it is possible to form cartridge **1000** in a single fabrication process and from a unitary component.

[0075] If in two components, they can be bonded together via thermal bonding process. Alternative techniques like laser assisted bonding or solvent assisted bonding may also be utilized. Essential to proper operation, pre- and post-bonding treatment processes cannot leave appreciable particulate matter or debris that may interfere with the thrombus formation process.

[0076] Alignment of the two components **1100**, **1200** to be bonded can be accomplished via alignment holes – **1112**, **1113** on the pumping cartridge **1100** and their corresponding mating holes on the cover slip **1200**. Alternative alignment systems can be used, including another distinct feature on the pumping cartridge **1100** and a corresponding feature on the cover slip **1200** may be used for aligning. In an exemplary embodiment a triangular notch **1115** and the bottom plane of the cartridge maybe used to align cartridge **1100** with cover slip **1200**. The alignment may also be achieved through other means including computer vision and microscopy.

[0077] As used herein, while the microfluidic cartridge **1300** and the cover slip **1400** are separate components, at times the combination of **1300**, **1400** may be considered the microfluidic cartridge. The microfluidic cartridge contains multiple independent calibrated channels, each featuring their own stenosis section. The element **1300** contains open channels, and the element **1400** acts as a cover slip to close the open channels. While as shown the microfluidic cartridge cannot function without its cover slip, for the sake of clarity and exposing details conveniently, some illustrations may refer to just the element **1300** as the microfluidic cartridge.

[0078] Two views of an exemplary embodiment of the microfluidic cartridge **1300** are shown in **FIG. 6**. The microfluidic cartridge **1300** can comprise four independent calibrated channels

1310 that are flowed during device operation. Each of the channels **1310** incorporates an input port **1311** that is used to receive fluid sample from the inlet channel of the pumping cartridge **1100**, an output port **1317** that is used to send fluid sample back to the outlet channel of the pumping cartridge **1100**, and a stenosis section of the channel comprising a raised stenosis portion **1314** that together with sloping sections **1313** and **1315** mimics the arterial constriction because of plaque buildup. The stenosis section reduces the cross-sectional area of the channel **1310**. The channel can employ many shapes, including ovate and rectangular. In an exemplary embodiment, the depth of the channel at locations **1312**, **1315** can be 180 microns, while the depth at the stenosis portion **1314** can be 70 microns.

[0079] Due to the inherent limitations of the fabrication processes, there are variations in the depth of all channels, and importantly the stenosis section of the channel **1310** when compared from one channel **1310** to another. These variations can impact the final thrombus formation results. Thus, the present invention can comprise a normalization process, where each channel **1310** is measured for flow rate, and the flow rates used to normalize the thrombus formation results so imperfections between each channel **1310** are not as large a factor, or no factor, in results expected in each channel **1310**. In alternate embodiments, other methods like microscopy can be used to characterize microfluidic channel flow and used to normalize the thrombus formation results.

[0080] At least portions of the microfluidic channels **1310** as shown in **FIG. 7** have bio-chemicals to simulate the arterial environment post vascular injury. Bio-chemicals can include but are not limited to collagen and vWF. The bio-chemicals can be provided via a coating along the length of entire channel **1310**, or on only one or more portions of the channel. In one embodiment shown in **FIG. 7**, a bio-chemical coating **1410** is provided in the stenosis sections including the stenosis portion **1314** together with sloping sections **1313** and **1315** of each channel **1310** via the alignment of the cover slip **1400** for the microfluidic cartridge.

[0081] In another exemplary embodiment of the present invention, the cover slip **1400** for the microfluidic cartridge comprises a conductive surface. The conductive surface enables the use of an electrochemical process for depositing the bio-chemicals like collagen and vWF. A setup for electrochemical deposition of the bio-chemicals is shown in **FIG. 8**. The bio-chemicals to be coated are placed in a channel **8210** between two parallel conductive layers **1400** and **8100**. The

cover slip **1400** to be coated with the bio-chemical forms the anode. The opposing parallel surface **8100** forms the cathode. The cathode has an inlet port **8110** and an outlet port **8120** to allow bio-chemical to flow through the channel **8210** where deposition takes place. An insulating spacer **8200** separates the cathode from the anode. The deposition process can be controlled using the magnitude of electrical voltage and time of voltage applied. In an exemplary embodiment 12V was used over a period of 60 minutes.

[0082] Completing the assembly of cartridge **1000**, a laser light blocking mask **1500** is shown in **FIG. 3**. The mask only allows the light from the light source **2000** to pass through the stenosis portion **1314**, and the transition sections **1313** and **1315**. Blocking the light everywhere except these regions increases the signal-to-noise ratio. In an exemplary embodiment, the light blocking mask **1500** is an integrated part of the cartridge **1000**. In an alternate embodiment, the light blocking mask **1500** can be incorporated into the cartridge reader **9000**.

[0083] Returning to **FIG. 1**, the cartridge **1000** illustratively shows four vertical channels between the inlet reservoir **1120** and the outlet reservoir **1130**. The channels are designed to provide fluidic communication between the reservoirs **1120** and **1130**, and paths to the stenosis sections. Thus, these channels need not be vertically parallel in layout. **FIG. 9** shows alternative routes for the channels, where for example, the stenosis section is in a horizontal configuration. The cartridges in these embodiments provide identical gravity-based pressure head and identical flow resistance to the stenosis sections as the fully vertical channels. Further, the stenosis sections may exist as independent channels in a separate microfluidic cartridge similar to details provided in **FIG. 3**.

[0084] The design and functionality described in this application is intended to be exemplary in nature and is not intended to limit the instant disclosure in any way. Those having ordinary skill in the art will appreciate that the teachings of the disclosure may be implemented in a variety of suitable forms, including those forms disclosed herein and additional forms known to those having ordinary skill in the art.

[0085] While certain embodiments of this disclosure have been described in connection with what is presently considered to be the most practical and various embodiments, it is to be understood that this disclosure is not to be limited to the disclosed embodiments, but on the contrary, is intended to cover various modifications and equivalent arrangements included within

the scope of the appended claims. Although specific terms are employed herein, they are used in a generic and descriptive sense only and not for purposes of limitation.

[0086] This written description uses examples to disclose certain embodiments of the technology and to enable any person skilled in the art to practice certain embodiments of this technology, including making and using any apparatuses or systems and performing any incorporated methods. The patentable scope of certain embodiments of the technology is defined in the claims, and may include other examples that occur to those skilled in the art. Such other examples are intended to be within the scope of the claims if they have structural elements that do not differ from the literal language of the claims, or if they include equivalent structural elements with insubstantial differences from the literal language of the claims.

CLAIMS

What is claimed is:

1. A system useful in determining functional performance of a biological sample comprising:
 - a thrombosis unit in which thrombus formation takes place; and
 - an interrogation unit providing information related to the formation of the thrombus.
2. The system of Claim 1, wherein the thrombosis unit comprises a disposable cartridge; and
 - wherein the interrogation unit comprises a cartridge reader that characterizes thrombus formation in the disposable cartridge.
3. The system of Claim 2, wherein the disposable cartridge comprises three reservoirs:
 - a fluid interfacing reservoir used to insert the biological sample into the disposable cartridge;
 - an inlet reservoir; and
 - an outlet reservoir;
 - wherein the inlet and outlet reservoirs are used to provide identical gravity-based pressure head to multiple microfluidic channels of the disposable cartridge simultaneously.
4. The system of Claim 2, wherein the disposable cartridge comprises a plurality of microfluidic channels, each inherently embodying a flow characteristic determined at least in part on the fabrication of each microfluidic channel;
 - wherein the flow characteristic of each of the microfluidic channels is used in order to normalize final thrombus characterization results across each of the microfluidic channels upon operational use of the system.
5. The system of Claim 2, wherein the disposable cartridge comprises a light blocking mask enabling precise characterization of thrombus formation where light passes through the thrombus and the transmission intensity is measured using a photo diode sensor.

6. The system of Claim 2, wherein the disposable cartridge comprises:
- a microfluidic cartridge; and
 - a cover slip for the microfluidic cartridge;
- wherein the cover slip comprises a conductive surface enabling the use of an electrochemical process for depositing a bio-chemical.
7. A system useful in determining functional performance of a biological sample comprising:
- a disposable cartridge in which thrombus formation takes place comprising:
 - a pumping cartridge; and
 - a microfluidic cartridge; and
 - a cartridge reader that characterizes thrombus formation in the disposable cartridge.
8. The system of Claim 7, wherein the pumping cartridge comprises three reservoirs:
- a fluid interfacing reservoir used to insert the biological sample into the disposable cartridge;
 - an inlet reservoir; and
 - an outlet reservoir;
- wherein the inlet and outlet reservoirs are used to provide identical gravity-based pressure head to multiple calibrated microfluidic channels of the pumping cartridge simultaneously.
9. The system of Claim 7, wherein the pumping cartridge comprises a plurality of calibrated microfluidic channels each having a pre-use flow characteristic determined prior to operational use of the system via provision of identical initial flow conditions presented to each of the calibrated microfluidic channels;
- wherein the pre-use flow characteristic of each of the microfluidic channels are used in order to normalize final thrombus characterization results across each of the calibrated microfluidic channels upon operational use of the system.

10. The system of Claim 7, wherein the disposable cartridge further comprises a light blocking mask enabling precise characterization of thrombus formation where light passes through the thrombus and the transmission intensity is measured using a photo diode sensor.

11. A system useful in determining functional performance of a biological sample comprising:

a thrombosis unit; and

an interrogation unit;

wherein in the thrombosis unit, the propensity of the biological sample to form an occlusive thrombus is established;

wherein the interrogation unit provides information related to the formation of the occlusive thrombus;

wherein the information from the interrogation unit correlates to a prediction of future atherothrombotic occlusion; and

wherein the thrombosis unit comprises one or more of:

(i) a plurality of microfluidic channels, each inherently embodying a flow characteristic determined at least in part on the fabrication of each microfluidic channel, wherein the flow characteristic of each of the microfluidic channels is used in order to normalize final thrombus characterization results across each of the microfluidic channels upon operational use of the system; and/or

(ii) three reservoirs, a fluid interfacing reservoir used to insert the biological sample into the thrombosis unit, an inlet reservoir, and an outlet reservoir, wherein the inlet and outlet reservoirs are used to provide identical gravity-based pressure head to multiple microfluidic channels of the thrombosis unit simultaneously; and/or

(iii) a microfluidic cartridge and a cover slip for the microfluidic cartridge, wherein the cover slip comprises a conductive surface enabling the use of an electrochemical process for depositing a bio-chemical; and/or

(iv) a light blocking mask enabling precise characterization of thrombus formation where light passes through the thrombus and the transmission intensity is measured using a photo diode sensor.

12. The system of Claim 11, wherein determining functional performance of the biological sample comprises determining a patient's drug therapy from the biological sample of the patient.

13. The system of Claim 11, wherein determining functional performance of the biological sample comprises determining a patient's optimized drug therapy for the treatment of arterial thrombosis.

14. The system of Claim 11, wherein the thrombosis unit comprises at least element (i) - a plurality of microfluidic channels, each inherently embodying a flow characteristic determined at least in part on the fabrication of each microfluidic channel, wherein the flow characteristic of each of the microfluidic channels is used in order to normalize final thrombus characterization results across each of the microfluidic channels upon operational use of the system;

wherein a portion of the biological sample passes through each microfluidic channel, each microfluidic channel comprising a stenosis section that mimics narrowing of an artery due to plaque buildup by presenting a smaller cross-sectional area of the microfluidic channel than upstream of the stenosis section.

15. The system of Claim 14, wherein the stenosis section presents a smaller cross-sectional area of the microfluidic channel than downstream of the stenosis section.

16. The system of Claim 15, wherein each microfluidic channel has an inlet at a first pressure and an outlet at a second pressure;

wherein the stenosis section is located between the inlet and the outlet; and

wherein the pressure difference between the inlet and the outlet of each microfluidic channel at least partially imparts biological sample flow through the stenosis section prior to any occlusion at the stenosis section.

17. The system of Claim 16, wherein the thrombosis unit further comprises element (ii) - three reservoirs, a fluid interfacing reservoir used to insert the biological sample into the thrombosis unit, an inlet reservoir, and an outlet reservoir, wherein the inlet and outlet reservoirs are used to provide identical gravity-based pressure head to each microfluidic channel of the thrombosis unit simultaneously.

18. The system of Claim 14, wherein the thrombosis unit further comprises element (iii) - a microfluidic cartridge and a cover slip for the microfluidic cartridge, wherein the cover slip comprises a conductive surface enabling the use of an electrochemical process for depositing a bio-chemical;

wherein the bio-chemical is located at least in a portion of a stenosis section of the microfluidic channels.

19. A system useful in determining functional performance of a biological sample comprising:

a thrombosis unit comprising:

a plurality of microfluidic channels, each inherently embodying a flow characteristic determined at least in part on the fabrication of each microfluidic channel, wherein the flow characteristic of each of the microfluidic channels is used in order to normalize final thrombus characterization results across each of the microfluidic channels upon operational use of the system;

three reservoirs, a fluid interfacing reservoir used to insert the biological sample into the thrombosis unit, an inlet reservoir, and an outlet reservoir, wherein the inlet and outlet reservoirs are used to provide identical gravity-based pressure head to the microfluidic channels simultaneously;

a microfluidic cartridge and a cover slip for the microfluidic cartridge, wherein the cover slip comprises a conductive surface enabling the use of an electrochemical process for depositing a bio-chemical in a portion of the microfluidic channels; and

a light blocking mask enabling precise characterization of thrombus formation where light passes through the thrombus and the transmission intensity is measured using a photo diode sensor; and

an interrogation unit;

wherein in the thrombosis unit, the propensity of the biological sample to form an occlusive thrombus is established;

wherein the interrogation unit provides information related to the formation of the occlusive thrombus; and

wherein the information from the interrogation unit correlates to a prediction of future atherothrombotic occlusion.

20. A system useful in determining functional performance of a biological sample comprising:

a disposable cartridge comprising:

a pumping cartridge;

a cover slip for the pumping cartridge;

a microfluidic cartridge;

a cover slip for the microfluidic cartridge; and

a mask; and

a cartridge reader;

wherein the pumping cartridge comprises one or more of:

a plurality of open microfluidic channels, each inherently embodying a flow characteristic determined at least in part on the fabrication of each microfluidic channel, wherein the flow characteristic of each of the microfluidic channels is used in order to normalize final thrombus characterization results across each of the microfluidic channels upon operational use of the system; and/or

three open reservoirs, a fluid interfacing reservoir used to insert the biological sample into the disposable cartridge, an inlet reservoir, and an outlet reservoir, wherein the inlet and outlet reservoirs are used to provide identical gravity-based pressure head to multiple microfluidic channels of the disposable cartridge simultaneously.

21. The system of Claim 20, wherein the cover slip for the microfluidic cartridge comprises a conductive surface enabling the use of an electrochemical process for depositing a bio-chemical.

22. The system of Claim 20, wherein the mask comprises a light blocking mask enabling precise characterization of thrombus formation in the disposable cartridge where light passes through the thrombus and the transmission intensity is measured using a photo diode sensor.

23. The system of Claim 20, wherein the biological sample is staged at the beginning of system operation in the inlet reservoir;

wherein the biological sample is collected at the end of system operation in the outlet reservoir;

wherein each of the plurality of microfluidic channels is formed from a discontinuous inlet channel and outlet channel;

wherein each inlet channel is in fluidic communication with the inlet reservoir; and

wherein each outlet channel is in fluidic communication with the outlet reservoir.

24. The system of Claim 23, wherein the cover slip for the pumping cartridge comprises:

a plurality of inlet ports; and

a plurality of outlet ports;

wherein the cover slip for the pumping cartridge encloses otherwise open microfluidic channels and open reservoirs of the pumping cartridge while providing the inlet and outlet ports; and

wherein the inlet and outlet ports of the cover slip for the pumping cartridge are paired to an individual microfluidic channel of the pumping cartridge, such that an inlet port of the cover slip for the pumping cartridge interfaces with an attendant inlet channel of a microfluidic channel of the pumping cartridge, and an inlet port of the cover slip for the pumping cartridge interfaces with an attendant outlet channel of the same microfluidic channel of the pumping cartridge.

25. The system of Claim 24, wherein the microfluidic cartridge comprises a plurality of independent calibrated channels, each calibrated channel having an inlet and an outlet; and

wherein upon alignment and adjacent assembly of the pumping cartridge, the cover slip for the pumping cartridge, and the microfluidic cartridge, the inlet reservoir is in fluidic communication with the outlet reservoir.

26. The system of Claim 25, wherein upon alignment and adjacent assembly of the pumping cartridge, the cover slip for the pumping cartridge, and the microfluidic cartridge, the microfluidic channels of the pumping cartridge formed of a discontinuous inlet channel and outlet channel embody a continuous flow path from inlet reservoir to outlet reservoir via fluidic communication of the inlet reservoir to the inlet channel of the pumping cartridge, to the inlet port of the cover slip for the pumping cartridge, to the inlet of the independent calibrated channel of the microfluidic cartridge, to the outlet of the independent calibrated channel of the microfluidic cartridge, to the outlet port of the cover slip for the pumping cartridge, to the outlet channel of the pumping cartridge, to the outlet reservoir.

27. The system of Claim 25, wherein each calibrated channel of the microfluidic cartridge further comprises a stenosis section between the inlet and outlet, the stenosis section presenting a smaller cross-sectional area of the calibrated channel than upstream and downstream of the stenosis section.

28. The system of Claim 27, wherein each calibrated channel of the microfluidic cartridge further comprises an upstream sloping section and a downstream sloping section, the stenosis section located between the sloping sections, the sloping sections providing a gradual channel narrowing profile toward and away from the stenosis section.

29. The system of Claim 20, wherein the cartridge reader comprises:

a light source; and

a photo sensing electronic circuit.

30. The system of Claim 29, wherein the cartridge reader further comprises one or more optical elements.

31. The system of Claim 30, wherein the light source is capable of generating light that is passed through the one or more optical elements to convert the light into a collimated sheet of light that upon passing through one or more evolving thrombosis in the disposable cartridge is measured using the photo sensing electronic circuit.

33. A method useful in determining functional performance of a biological sample comprising:

forming a thrombus in a thrombosis unit; and

interrogating the formation of the thrombus.

34. The method of Claim 33, wherein the thrombosis unit comprises a disposable cartridge; and

wherein interrogating the formation of the thrombus is via a cartridge reader that characterizes thrombus formation in the disposable cartridge.

35. The method of Claim 34, wherein the disposable cartridge comprises three reservoirs:

a fluid interfacing reservoir used to insert the biological sample into the disposable cartridge;

an inlet reservoir; and

an outlet reservoir;

wherein the method further comprises using the inlet and outlet reservoirs to provide identical gravity-based pressure head to multiple microfluidic channels of the disposable cartridge simultaneously.

36. The method of Claim 34, wherein the disposable cartridge comprises a plurality of microfluidic channels, each inherently embodying a flow characteristic determined at least in part on the fabrication of each microfluidic channel;

wherein the method further comprises using the flow characteristic of each of the microfluidic channels in order to normalize final thrombus characterization results across each of the microfluidic channels upon operational use of the system.

37. The method of Claim 34, wherein interrogating the formation of the thrombus comprises:

using a light blocking mask of the disposable cartridge enabling precise characterization of thrombus formation;

passing light through the thrombus; and

measuring the transmission intensity using a photo diode sensor.

38. The method of Claim 34 further comprising using of an electrochemical process for depositing a bio-chemical;

wherein the disposable cartridge comprises:

a microfluidic cartridge; and

a cover slip for the microfluidic cartridge;

wherein the cover slip comprises a conductive surface enabling the use of the electrochemical process for depositing the bio-chemical.

39. A method useful in determining functional performance of a biological sample comprising:

examining the propensity of the biological sample to form an occlusive thrombus; and

determining information related to the formation of the occlusive thrombus.

40. A method of determining a patient's drug therapy comprising:

examining the propensity of a biological sample of the patient to form an occlusive thrombus;

determining information related to the formation of the occlusive thrombus;

correlating the information related to the formation of the occlusive thrombus to a prediction of future atherothrombotic occlusion; and

determining a patient's drug therapy based upon the correlation.

41. The method of Claim 40, wherein examining the propensity of a biological sample of the patient to form an occlusive thrombus comprises:

passing different portions of the biological sample through a plurality of microfluidic channels; and

mimicking narrowing of an artery due to plaque buildup by passing the portions of the biological sample through a stenosis section of each microfluidic channel presenting a smaller cross-sectional area of the microfluidic channel than upstream of the stenosis section.

42. A method of determining a patient's drug therapy comprising:

examining the propensity of a biological sample of the patient to form an occlusive thrombus;

determining information related to the formation of the occlusive thrombus;

correlating the information related to the formation of the occlusive thrombus to a prediction of future atherothrombotic occlusion;

minimizing error in the steps of determining and correlating; and

determining a patient's drug therapy based upon the correlation.

43. The method of Claim 42, wherein minimizing error in the steps of determining and correlating comprises providing gravity-based pressure head for biological sample flow.

44. The method of Claim 42, wherein examining the propensity of a biological sample of the patient to form an occlusive thrombus comprises:

passing different portions of the biological sample through a plurality of microfluidic channels; and

mimicking narrowing of an artery due to plaque buildup by passing the portions of the biological sample through a stenosis section of each microfluidic channel presenting a smaller cross-sectional area of the testing channel than upstream of the stenosis section;

wherein minimizing error in the steps of determining and correlating comprises:

assigning a flow characteristic to each microfluidic channel determined at least in part on the fabrication of each microfluidic channel; and

normalizing the results from the use of the microfluidic channels.

45. The method of Claim 42, wherein examining the propensity of a biological sample of the patient to form an occlusive thrombus comprises:

passing different portions of the biological sample through a plurality of microfluidic channels; and

mimicking narrowing of an artery due to plaque buildup by passing the portions of the biological sample through a stenosis section of each microfluidic channel presenting a smaller cross-sectional area of the testing channel than upstream of the stenosis section;

wherein minimizing error in the steps of determining and correlating comprises using an electrochemical process to deposit a bio-chemical inside a desired location of the microfluidic channels.

46. The method of Claim 42, wherein minimizing error in the steps of determining and correlating comprises:

using a light blocking mask enabling precise characterization of thrombus formation;

passing light through the thrombus; and

measuring the transmission intensity using a photo diode sensor.

47. A system as described herein.

48. The system of Claim 47, including each novel feature or combination of features disclosed herein.

49. A method as described herein.

50. The method of Claim 49, including each novel feature or combination of features disclosed herein.

51. A device as described herein.

52. The device of Claim 51, including each novel feature or combination of features disclosed herein.

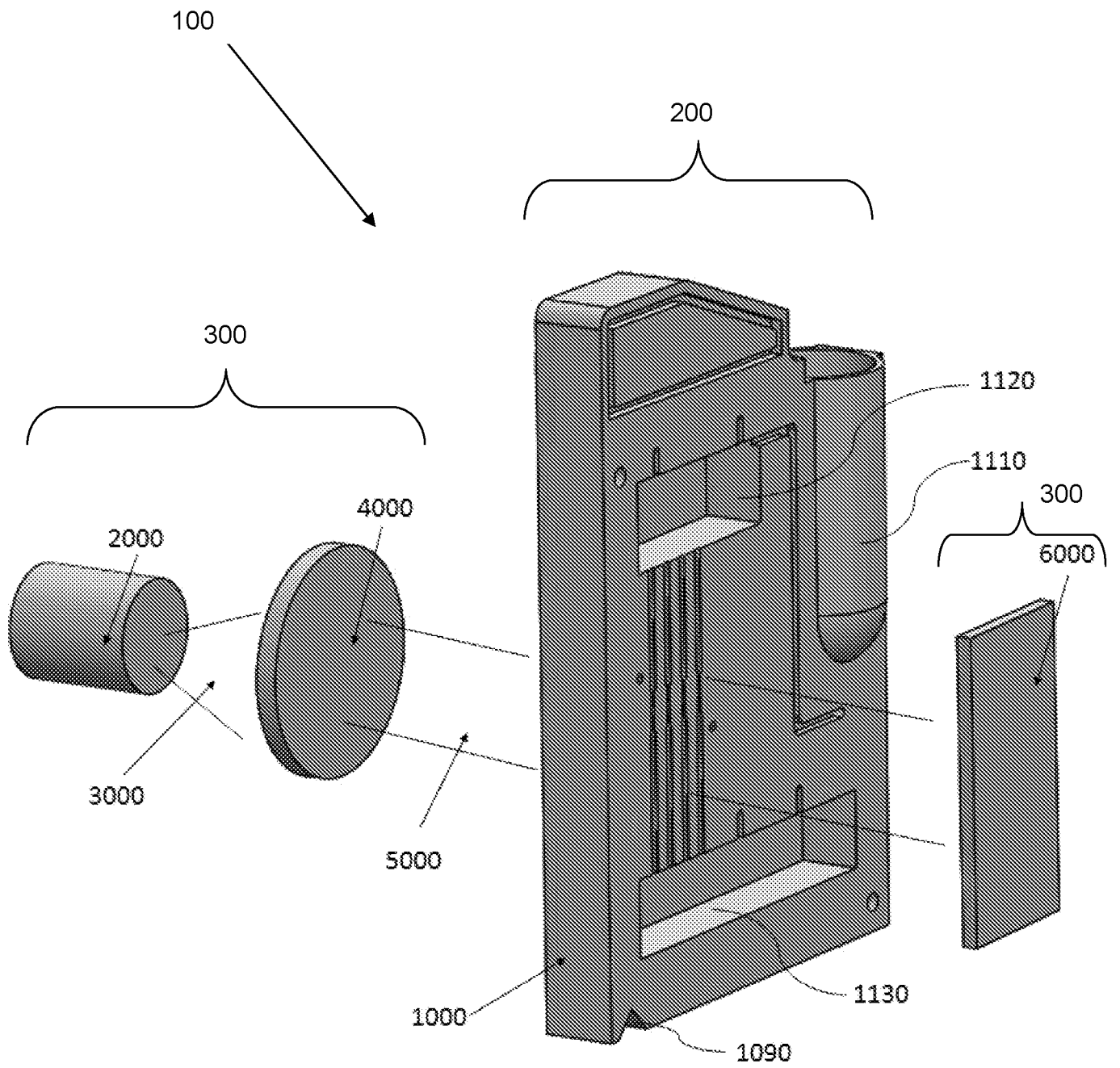


FIG. 1

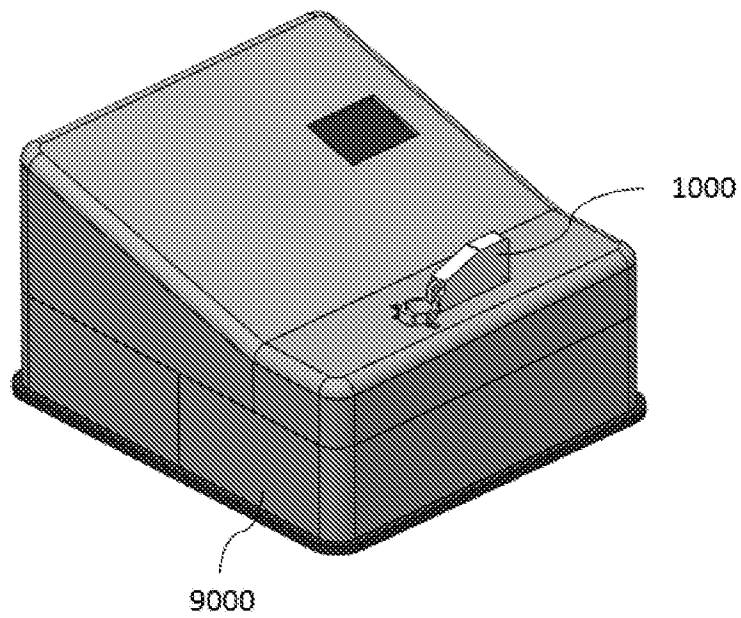


FIG. 2

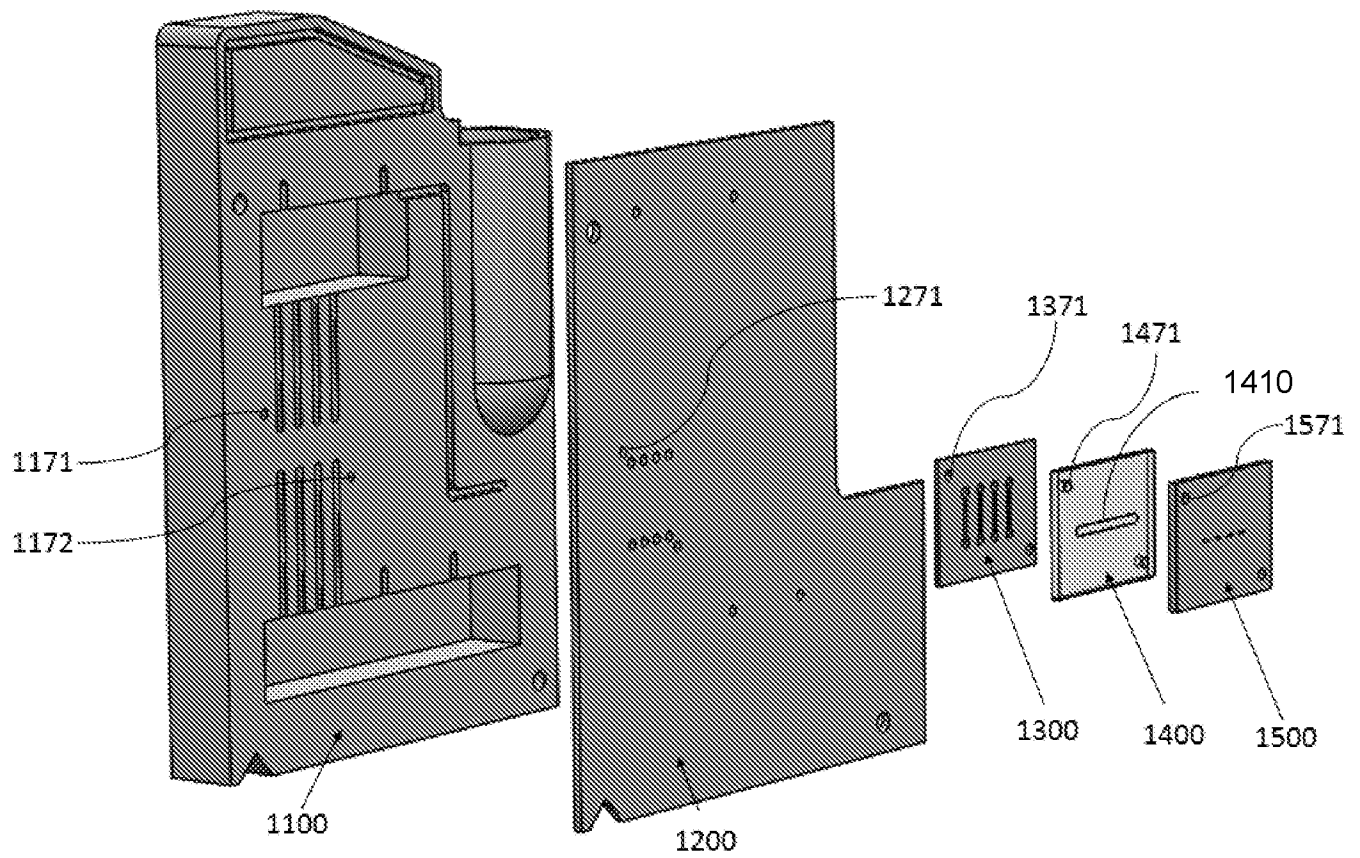


FIG. 3

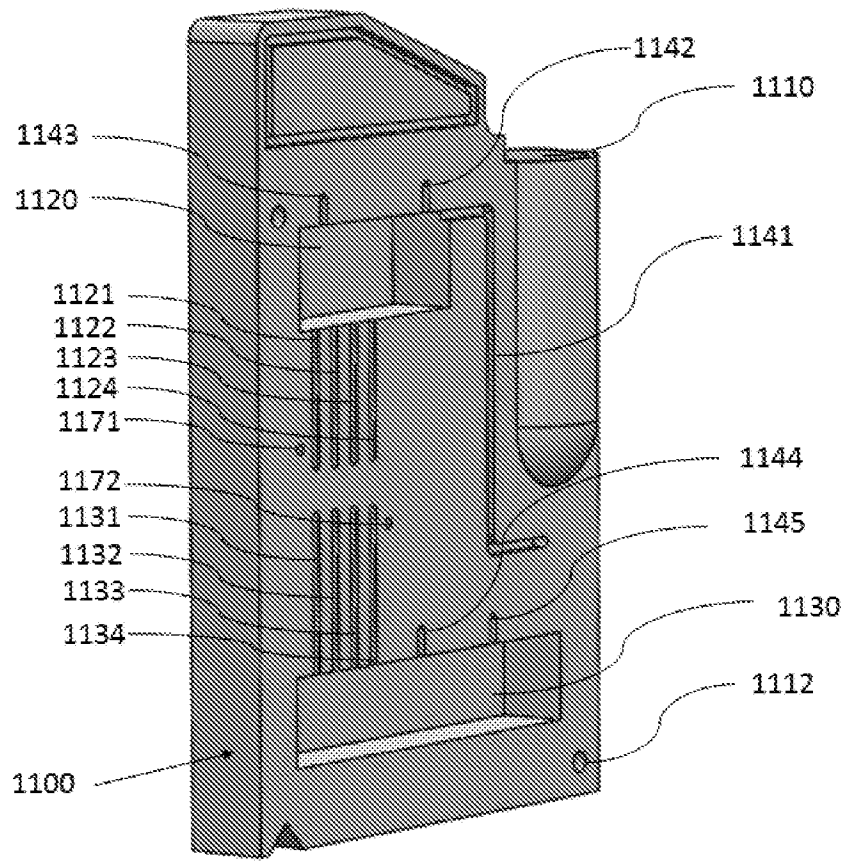
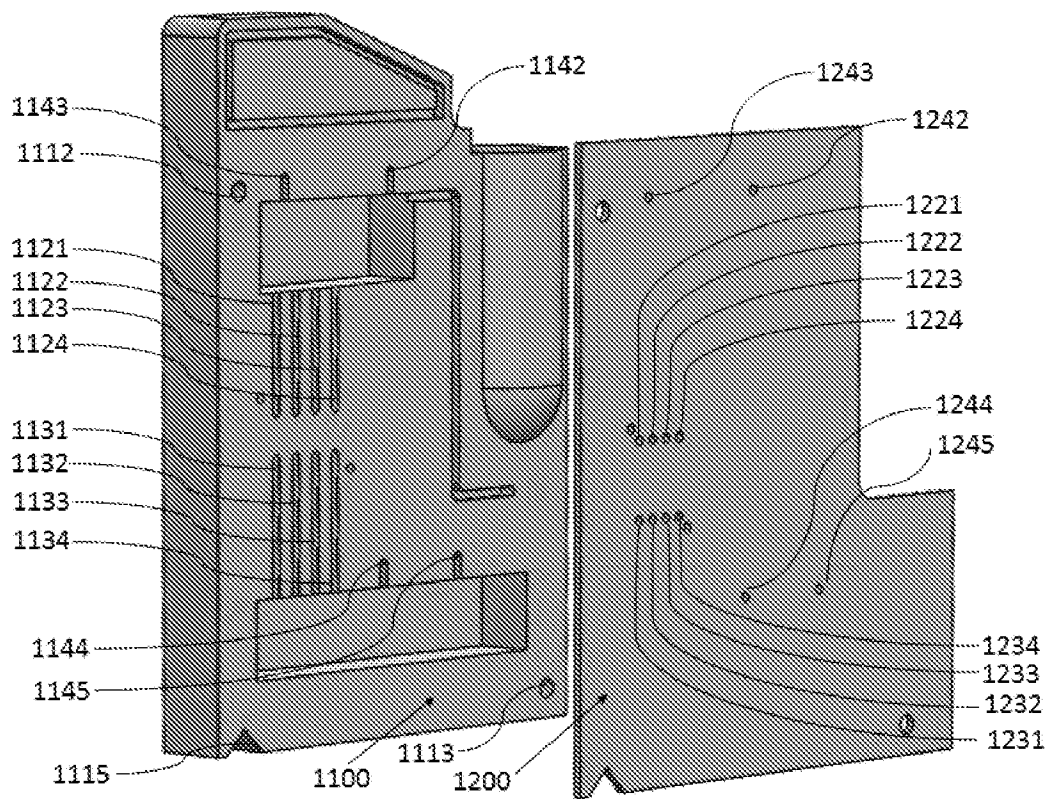


FIG. 4

**FIG. 5**

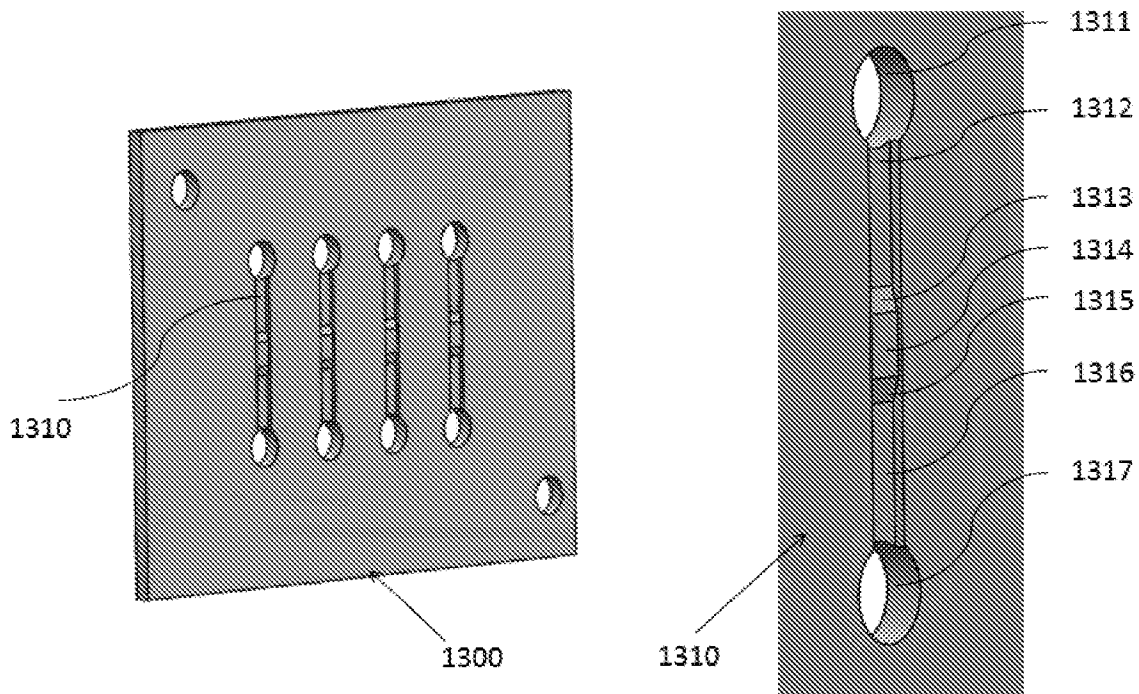
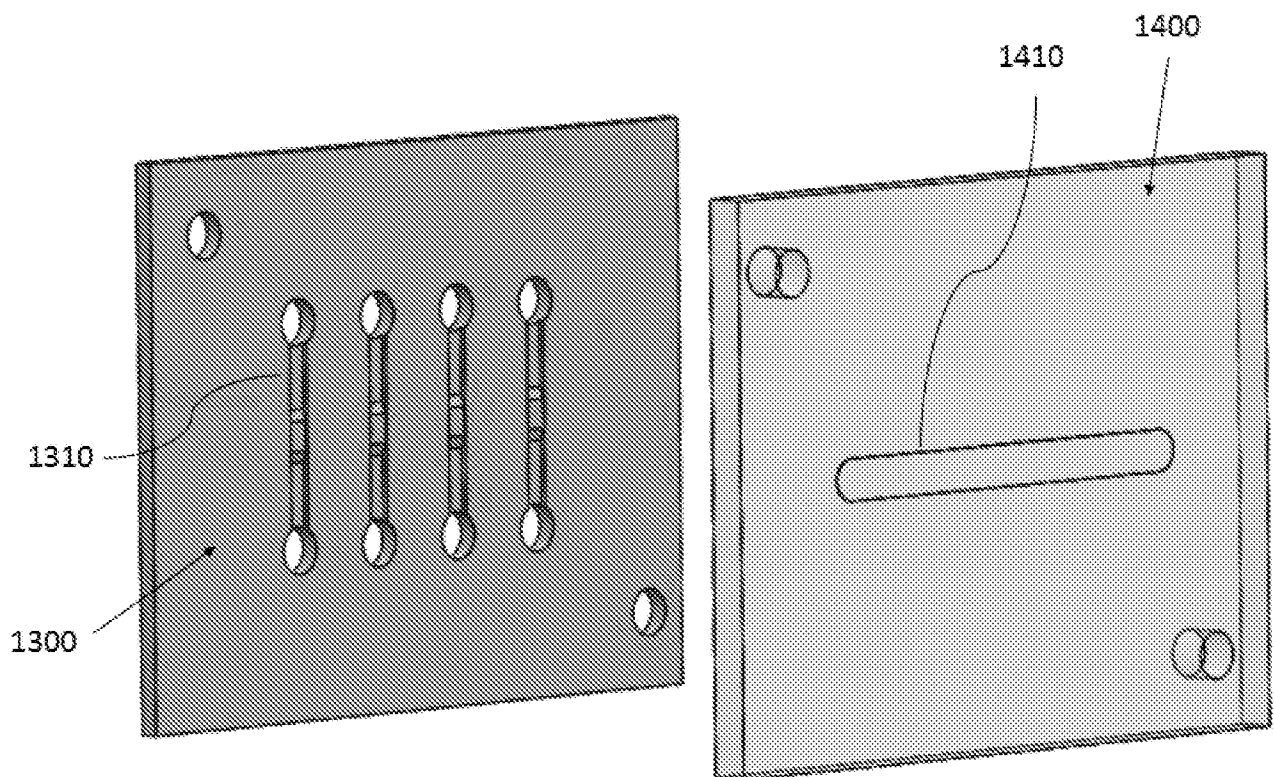


FIG. 6

**FIG. 7**

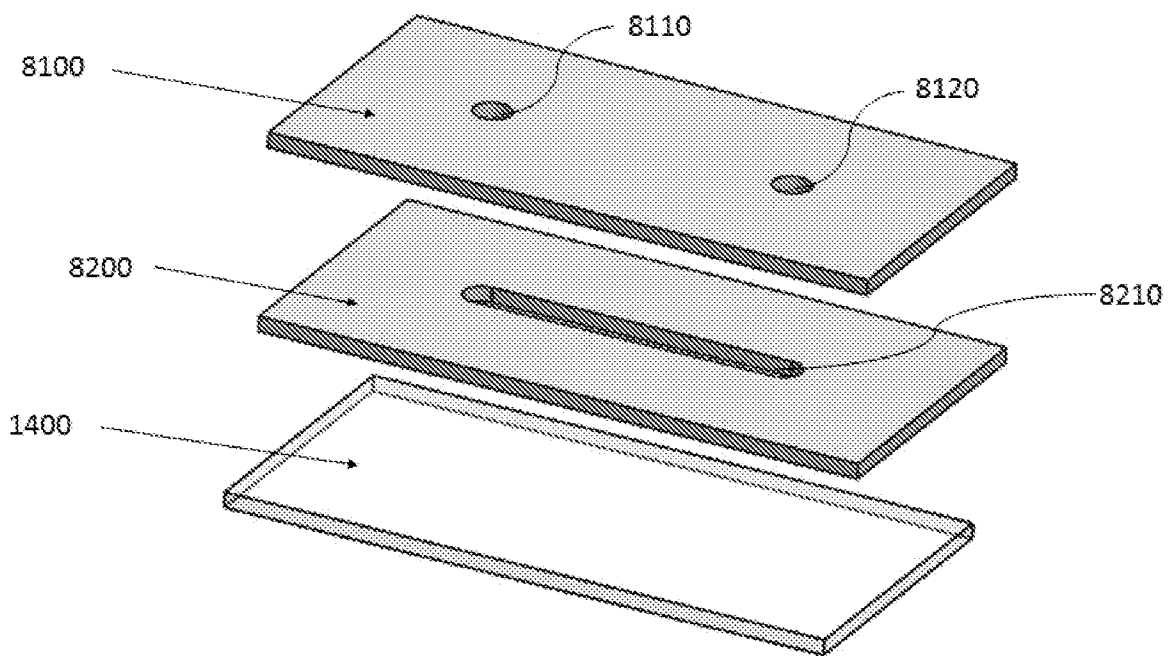


FIG. 8

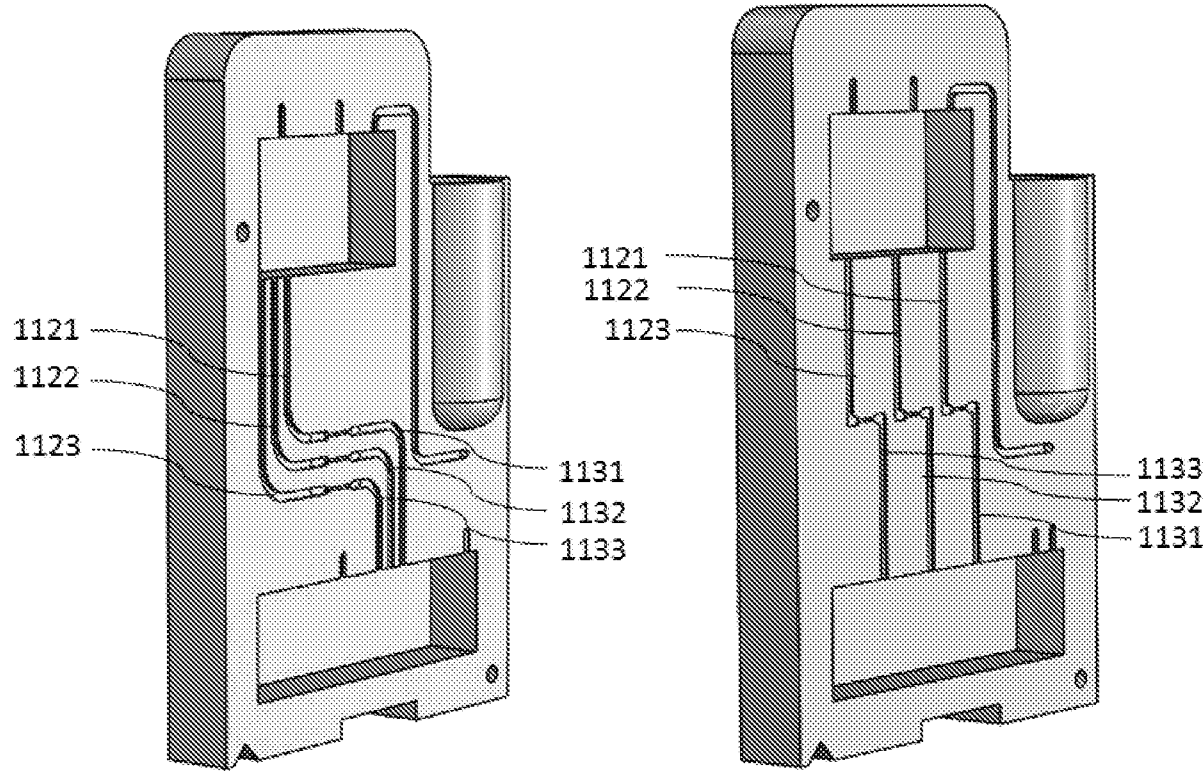


FIG. 9

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 18/60602

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C07K 14/705 (2019.01)

CPC - C07K 14/705, C07K 14/70503, C07K 16/4291

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

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

See Search History Document

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

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y --- A	US 2006/0211071 A1 (Andre et al.) 21 September 2006 (21.09.2006); entire document, especially abstract, [0016], [0074], [0062], [0077]-[0078], [0104], [0106]	1-2, 33-34 ----- 5, 11-13, 37 ----- 3-4, 6, 14-31, 35-36, 38
X --- Y --- A	US 2016/0069913 A1 (Perosphere Inc.) 10 March 2016 (10.03.2016); entire document, especially abstract, [0003], [0110], [0034]-[0035], [0037]	7 --- 10 --- 8-9, 19-31
Y	US 2013/0016335 A1 (Lo et al.) 17 January 2013 (17.02.2013); entire document, especially abstract, [0045]	5, 10-13, 37
A	Viles-Gonzalez, "Atherothrombosis: A widespread disease with unpredictable and life-threatening consequences", 27 April 2004 (27.04.2004), European Heart Journal (2004) 25, 1197-1207; entire document, especially abstract	1-38
A	US 2011/0045993 A1 (Kent et al.) 24 February 2011 (24.02.2011); entire document	1-38

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

28 February 2019

Date of mailing of the international search report

15 MAR 2019

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 18/60602

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 47-52
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
unsearchable because they are omnibus claims under Rule 6.2(a).

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I: Claims 1-31 and 33-38, directed to a system and method useful in determining functional performance of a biological sample.

Group II: Claims 39-46, directed to a method useful in determining functional performance of a biological sample and determining a patient's drug therapy.

The inventions listed as Groups I-II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

See supplemental box

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-31, 33-38

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☒ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 18/60602

Continued from Box III

Special Technical Features:

Group I requires a thrombosis unit, an interrogation unit, a disposable cartridge, a pumping cartridge, a microfluidic cartridge, a cartridge reader, and a light blocking mask, not required by group II.

Group II requires examining the propensity of the biological sample to form an occlusive thrombus, determining information related to the formation of the occlusive thrombus, correlating the information related to the formation of the occlusive thrombus to a prediction of future atherothrombotic occlusion, and determining a patient's drug therapy based upon the correlation, not required by group I.

Common Technical Features:

Groups I and II share the technical feature of determining functional performance of a biological sample relating to formation of thrombus. However, these shared technical features do not represent a contribution over prior art, because the shared technical feature is being anticipated by US 5,599,718 A to Gorog (hereinafter "Gorog"). Gorog teaches determining functional performance of a biological sample relating to formation of thrombus (abstract, "An in vitro method of monitoring thrombolysis of an occlusive thrombus formed under conditions which simulate in vivo is described... Formation of the thrombus is detected...").

As the shared technical features were known in the art at the time of the invention, they cannot be considered common technical features that would otherwise unify the groups. Therefore, Groups I-II lack unity under PCT Rule 13.

Note:

Claims 47-52 are held unsearchable because they are omnibus claims under Rule 6.2(a).

Claim 32 is missing from the application