(54) **MICROSCALE WHOLE BLOOD COAGULATION ASSAY PLATFORM**

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(57)   **ABSTRACT**

A blood clot retraction assay device and methods of measuring whole-blood clot retractive forces during coagulation are described. When placed in proximity to a micro-beam, a blood droplet attaches thereto, applying a force to the micro-beam during clot formation. Using Piezoresistive micro-beams or other Piezoresistive components disposed upon such micro-beams, the device measures retractive force of a blood droplet based upon changes in resistance. The sensitivity of such measurements allows use of small quantities of blood for measurement, between 15 microliters and 300 microliters. The device further permits continuous measurement of coagulation, allowing measurement of distinct reaction, contraction development, and fibrinolysis phases for each blood sample.
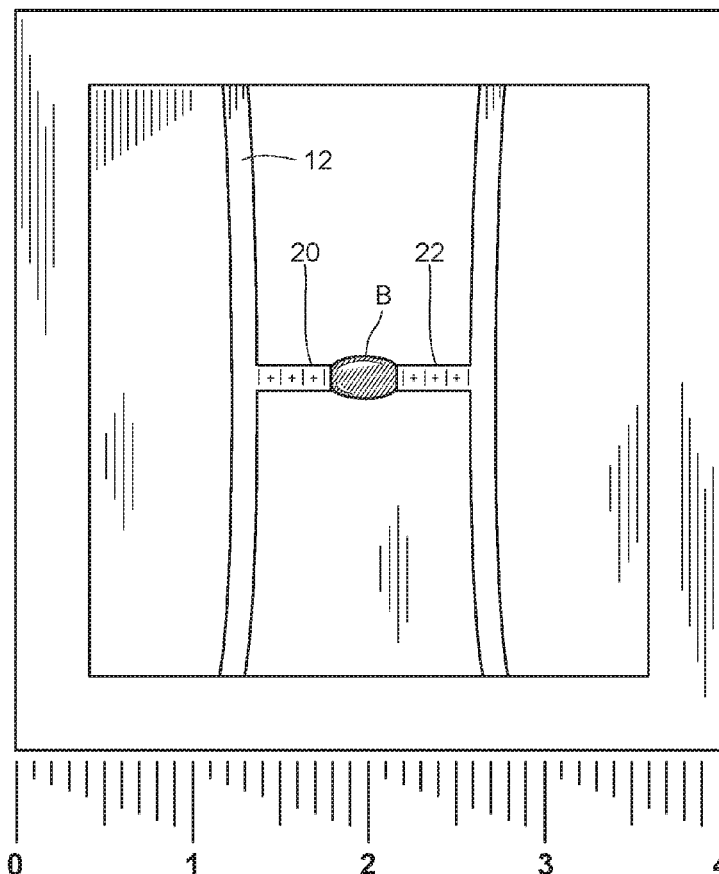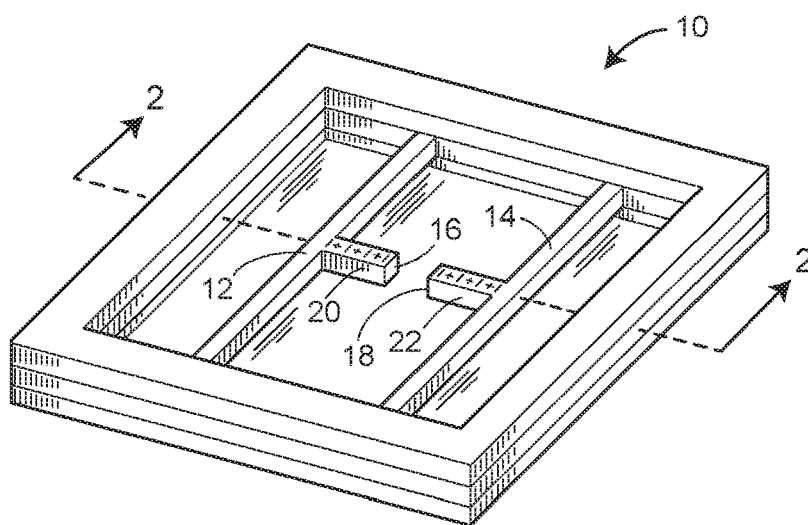
FIG. 1
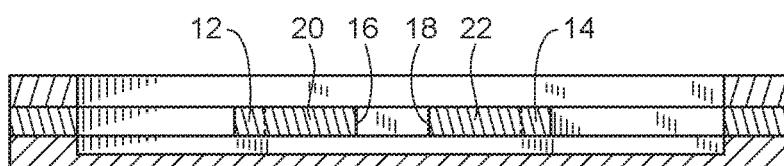
FIG. 2

FIG. 3

FIG. 4

FIG. 5A

FIG. 5B

FIG. 5C

110

116   112

115

118   B

FIG. 6

210

212a

216a

B

216b

212b

212a

216a

B

216b

212b

FIG. 7A   *FIG. 7B*

310

312b

312c

312a

B

312d

FIG. 8A

312b

312c

312a

B

312d

FIG. 8B

410

412b

413

412c

412a

B

FIG. 9

FIG. 10
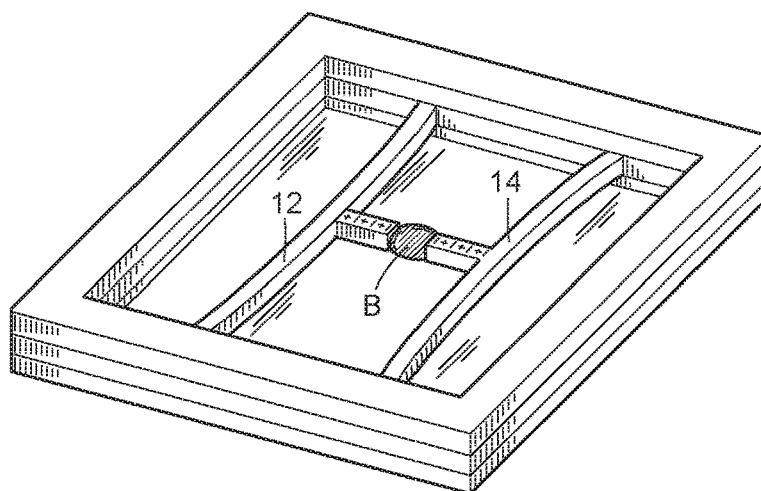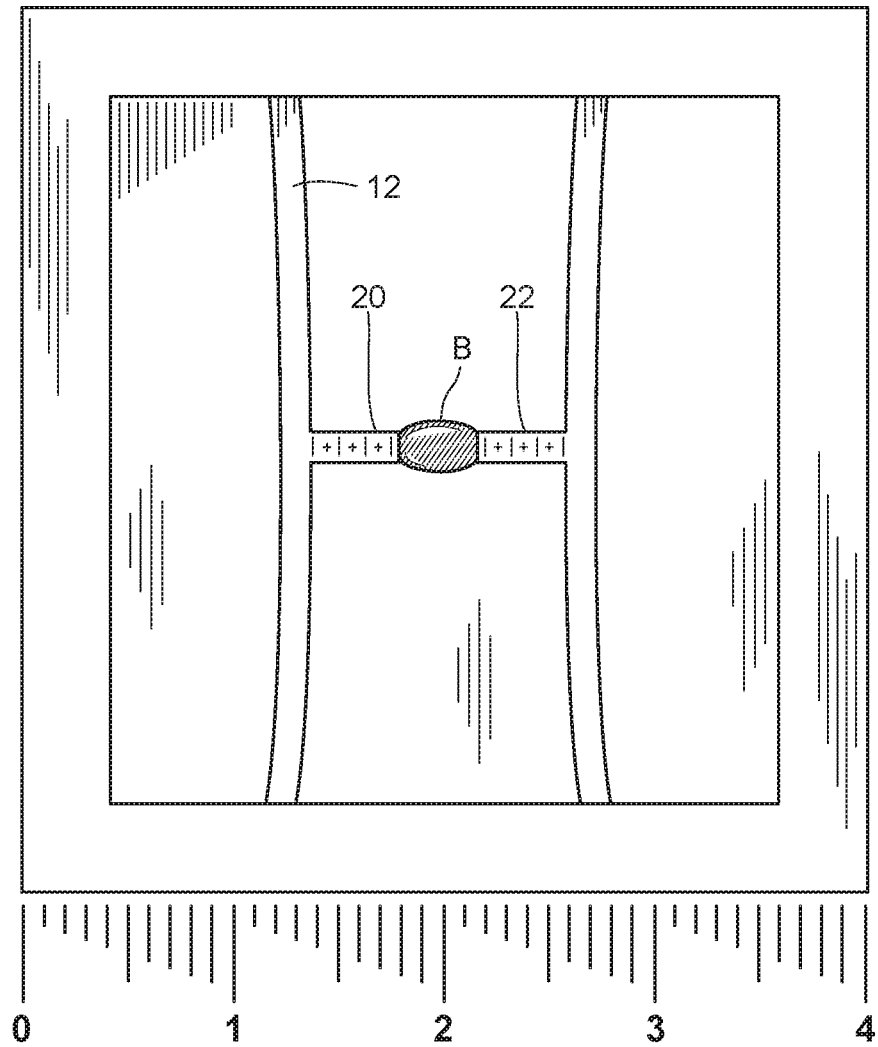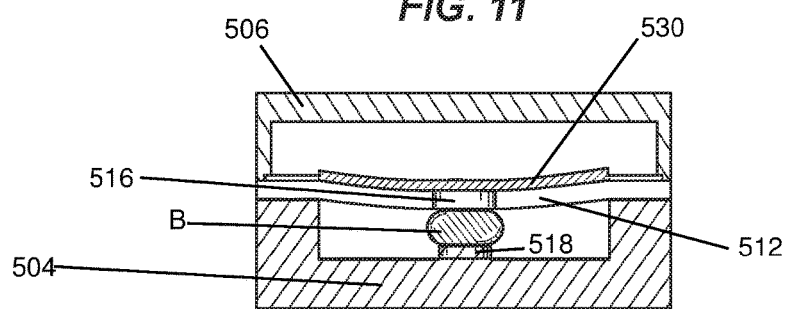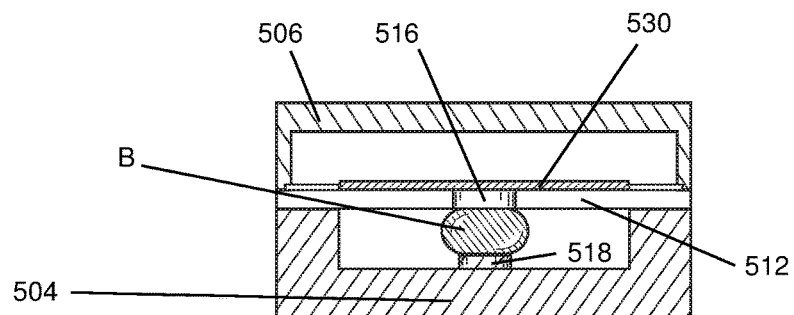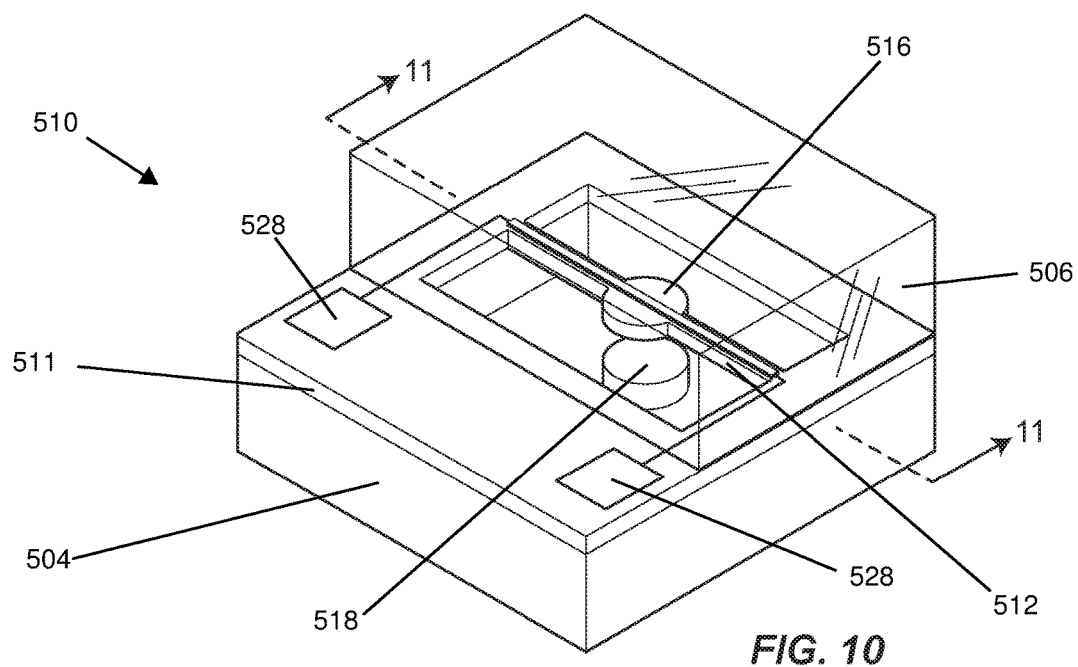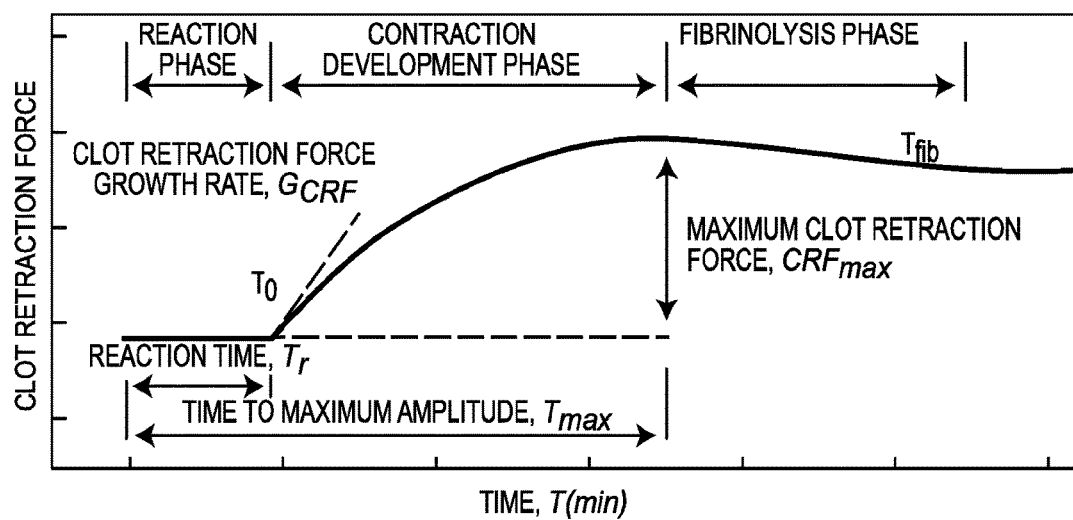

FIG. 11

FIG. 12
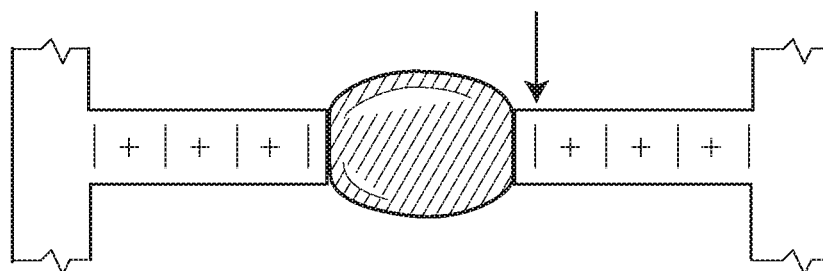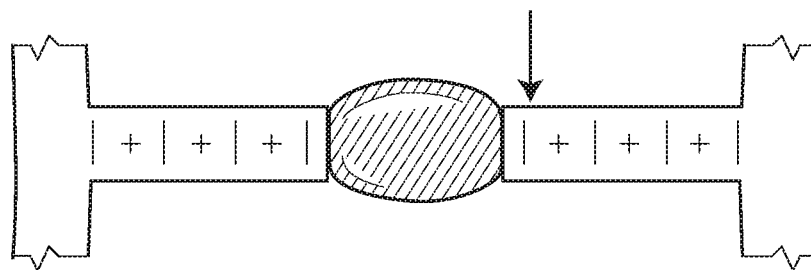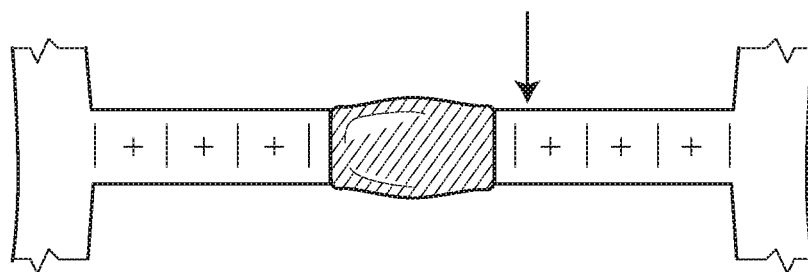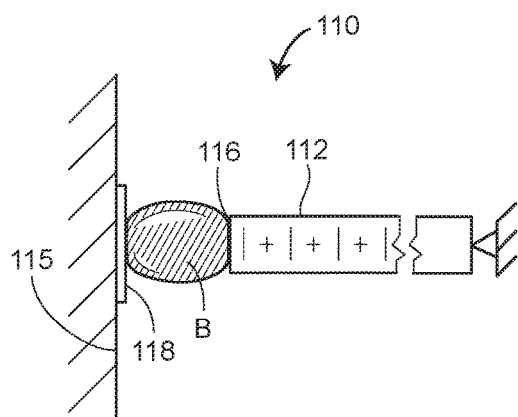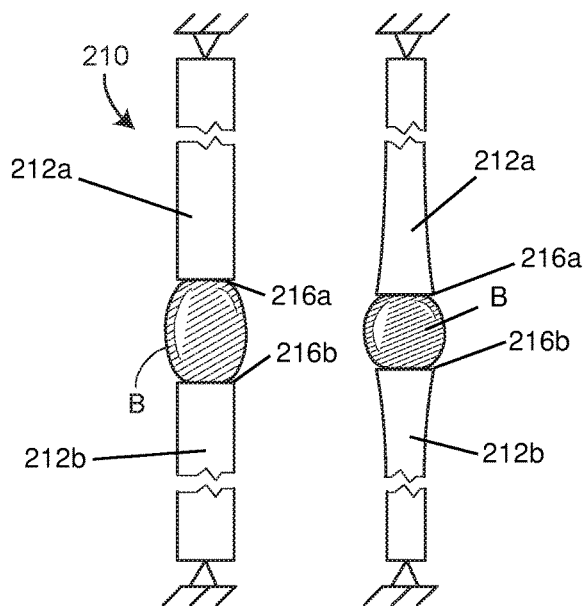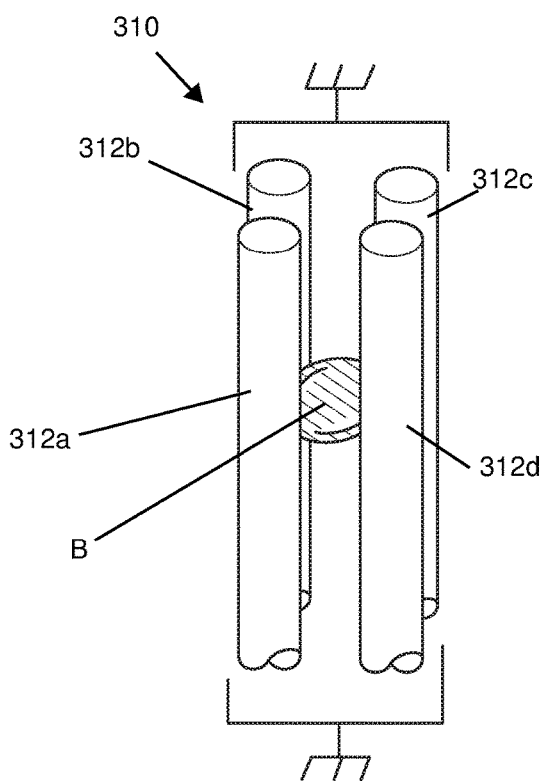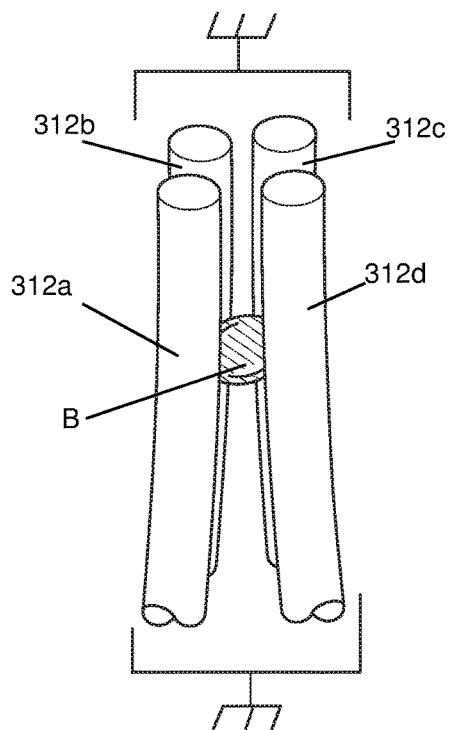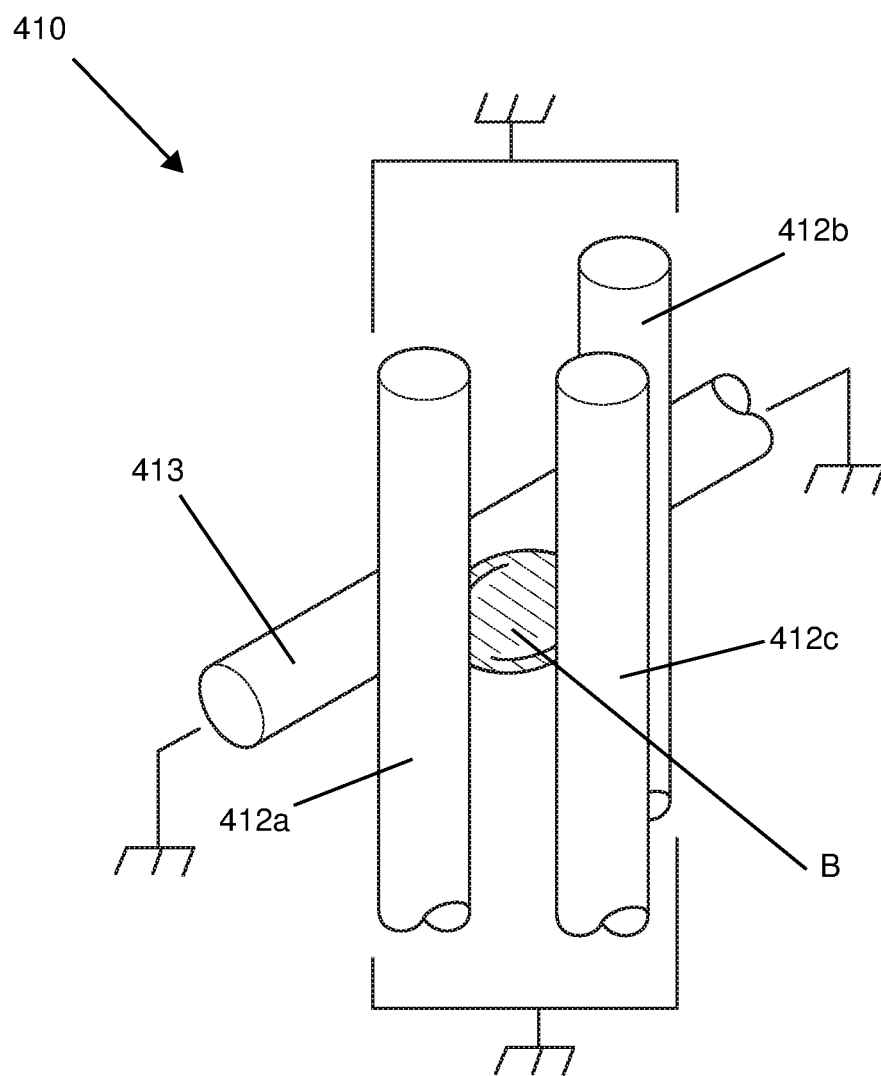
FIG. 13

MICROSCALE WHOLE BLOOD COAGULATION ASSAY PLATFORM

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of the filing date of U.S. Provisional Application No. 62/304,385, filed Mar. 7, 2016, which is incorporated herein by reference.

FIELD OF THE DISCLOSURE

[0002] This disclosure relates generally to monitoring of blood coagulation and, more specifically, to a system and method for continuous or periodic monitoring of blood to determine its coagulation status, in real time, using a microscale coagulation monitoring platform.

BACKGROUND

[0003] Blood coagulation is a critical hemostatic process involving an intricate interplay between cellular (red blood cells, white blood cells, and platelets) and acellular components (plasma proteins) of the blood. Key, N., et al., *Practical hemostasis and thrombosis.* 2009: Wiley Online Library; Tanaka, K. A., N. S. Key, and J. H. Levy, *Blood coagulation: hemostasis and thrombin regulation.* Anesthesia & Analgesia, 2009. 108(5): p. 1433-1446. Blood coagulation is normally initiated when a blood vessel is injured and the connective tissues (particularly collagen) underlying the endothelial lining of the blood vessel are exposed to blood flow, leading to platelet adhesion to connective tissues and their activation and aggregation, forming a plug at the site of vessel injury. Nuyttens, B. P., et al., Platelet adhesion to collagen. Thrombosis research, 2011. 127: p. S26-S29.

[0004] Blood coagulation is a critical hemostatic process that must be properly regulated to maintain a delicate balance between bleeding and clotting. Disorders of blood coagulation can expose patients to the risk of either bleeding disorders or thrombotic diseases, such as heart attack, stroke, deep venous thrombosis, or pulmonary embolism. Bennett, J. S., *Platelet-Fibrinogen Interactions.* Annals of the New York Academy of Sciences, 2001. 936(1): p. 340-354. Alternatively, misuse of medicines to slow coagulation for treating thrombotic disorders can place patients at risk for bleeding complications such as internal hemorrhage, excessive bruising, hemorrhagic strokes, etc. Therefore, the ability to rapidly determine the hemostatic profile of a patient is of great importance for providing timely and appropriate therapy in clinical settings such as perioperative hemostatic monitoring, transfusion therapies, thrombotic and hemophilic disease treatments, treatments for multisystem trauma, sepsis, and traumatic brain injury, therapeutic endpoints to anticoagulant medicines, and other acute diseases. Key, N., et al., *Practical hemostasis and thrombosis.* 2009: Wiley Online Library; Bombeli, T. and D. Spahn, *Updates in perioperative coagulation: physiology and management of thromboembolism and haemorrhage.* British journal of anaesthesia, 2004. 93(2): p. 275-287; Aggeler, P. M., *Physiological basis for transfusion therapy in hemorrhagic disorders: a critical review.* Transfusion, 1961. 1(2): p. 71-86.

[0005] Coagulation diagnostics using whole blood is very promising for assessing the complexity of the coagulation system and for global measurements of hemostasis. Compared to routine coagulation tests in the clinic (such as prothrombin time or PT, activated partial thromboplastin time or aPTT, and platelet count), whole-blood coagulation tests offer significant advantages of measuring the whole clotting process, starting with fibrin formation and continuing through to clot retraction and fibrinolysis, and allowing the plasmatic coagulation system to interact with blood cells including platelets, red blood cells, and white blood cells, thereby providing useful additional information on platelet function.

[0006] Among different whole blood coagulation tests, viscoelastic hemostasis assays including thromboelastography (TEG) and rotational thromboelastometry (ROTEM) have started to gain significant attention in the clinic. McMichael, M. A. and S. A. Smith, *Viscoelastic coagulation testing: technology, applications, and limitations.* Veterinary Clinical Pathology, 2011. 40(2): p. 140-153. In principle, TEG and ROTEM measure the changes in the shear elastic modulus of whole blood upon clotting. For example, TEG consists of a rotating cup and a pin which is connected to a rotation sensor. A blood sample is held in the cup and the pin is submerged in the blood sample during the assay. When the blood starts clotting, its viscoelasticity changes, leading to changes of rotational amplitudes of the pin when following the rotating cup. The rotational amplitude of the pin is recorded by the rotation sensor and reported for analysis of the viscoelasticity change of the blood sample. Such viscoelastic hemostasis assays have been shown to provide critical information on the interactivity of the major phases of clot formation and lysis and on the functional differences between different cellular and acellular components of the blood for differentiating the mechanism(s) related to clotting abnormalities. Despotis, G. J., et al., *Effect of heparin on whole blood activated partial thromoplastin time using a portable, whole blood coagulation monitor.* Critical Care Medicine, 1995. 23(10): p. 1674-79.

[0007] Besides viscoelastic hemostasis assays, whole-blood clot retraction assays using devices such as Hemodyne Hemostasis Analysis System (the HAS™ system was previously produced by Hemodyne, Inc.) also provide important information on blood coagulation function by reporting on clot elastic modulus and platelet contractile force (or clot retraction force). In the HAS™ system, whole blood samples are confined between two plates (either flat-flat or cone-cone surfaces), with the top plate attached to a strain-gage transducer. Clotting of blood results in a downward force pulling the top plate (or the clot retraction force), which is directly measured in real time by the strain-gage transducer. Using the HAS™ system, clot retraction force has been shown to be sensitive to platelet number and metabolic status, glycoprotein IIB/IIIa status, and the presence of antithrombin activities. Carr Jr, M. E., *Development of platelet contractile force as a research and clinical measure of platelet function.* Cell biochemistry and biophysics, 2003. 38 (1): p. 55-78. Importantly, clinical applications of the HAS™ system have demonstrated promising applications of such whole blood clot retraction assays for rapid assessment of global hemostasis and the response to a variety of procoagulant and anticoagulant medications. Krishnaswarmi, A., et al., *Patients with coronary artery disease who present with chest pain have significantly elevated platelet contractile force.* Thromb Haemostasis, 1999. 82(suppl. 1): p. 162; Greilich, P. E., et al., *Reductions in platelet force development by cardiopulmonary bypass are associated with hemorrhage.* Anesthesia & Analgesia,

1995. 80(3): p. 459-465; Carr, M., et al., *Batroxobin-induced clots exhibit delayed and reduced platelet contractile force in some patients with clotting factor deficiencies*. Journal of Thrombosis and Haemostasis, 2003. 1(2): p. 243-249; Carr, M., et al. *Anticoagulant and antiplatelet activities of heparin and low-molecular-weight derivatives*. in Blood. 1993. Wb Saunders Co Independence Square West Curtis Center, Ste 300, Philadelphia, Pa. 19106-3399; Carr, M., et al. *Dermatan sulfate suppresses platelet force as it prolongs the APTT*. in Blood. 1996. Wb Saunders Co Independence Square West Curtis Center, Ste 300, Philadelphia, Pa. 19106-3399; McCardell, K., S. Carr, and M. Carr. *Aprotinin augments protamine sulfate reversal of heparin antiplatelet effects*. in *Journal of Investigative Medicine*. 1996. Slack Inc 6900 Grove Rd, Thorofare, N.J. 08086.

[0008] Despite the clinical values that the existing whole blood coagulation tests (including TEG, ROTEM, and HAS™) have demonstrated through initial applications, such systems still have significant limitations in terms of costly expense to make and repair, limited sample throughput, large footprint, excessive sample size (>0.3 mL) that can be problematic for repetitive assays on pediatric patients, inter-assay variability, and significant user interventions, rendering them suboptimal or unsuitable for point-of-care applications. Furthermore, these limitations associated with the existing whole blood coagulation tests make it difficult for multiplexed blood assays to define specific coagulation disorders.

[0009] Some conventional blood coagulation assay approaches utilize the Piezoelectric effect to stimulate and measure resonance of a blood sample. However, this crude approach does not directly measure the contractive forces during coagulation and requires power regulation to stimulate the blood sample before determining the sample's response. When a Piezoelectric material is deformed under an external pressure or force, such deformation of the material induces an accumulation of electric charge within the material, resulting in a change of electric potential (i.e., voltage) within the material. Conversely, when such Piezoelectric material is applied with an external voltage, it will deform. This is known as the Piezoelectric effect and has known uses in micromechanical motors and voltage measurement.

SUMMARY OF THE DISCLOSURE

[0010] Suitable point-of-care coagulation devices that are simple, inexpensive, quick, high throughput, and can be widely available, may be fabricated using functional soft materials to develop a miniaturized clot retraction assay device termed mHemoRetractoMeter (mHRM). In a mHRM of the present disclosure, two doubly-clamped mechanical strain sensing beams made with soft elastomer polydimethylsiloxane (PDMS) are utilized for holding prescribed minute amounts of bloods samples while simultaneously measuring clot retraction force during blood clotting. Different major phases of clot formation and extraction of key blood clotting parameters can be analyzed using kinetic curves of clot retraction force obtained using the mHRM.

[0011] Unlike the Piezoelectric effect employed by various conventional blood coagulation assay approaches, the mHRM of the present disclosure employs Piezoresistive effect. The Piezoresistive effect is related to a change in the electrical resistivity of a material when it is deformed under an external pressure or force. The Piezoresistive effect is

completely different from the Piezoelectric effect, as this change in resistivity does not involve electric potential. In addition, applying an external voltage to a Piezoresistive material does not cause the material to deform. Using the Piezoresistive effect allows more detailed measurements of changes in the contractive forces that exist during blood coagulation.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWING

[0012] FIG. 1 is a perspective view of a clot retraction assay device of a first embodiment of the present disclosure;

[0013] FIG. 2 is a cross-sectional view, taken along lines 2-2 of FIG. 1, of the clot retraction assay device of the first embodiment of the present disclosure;

[0014] FIG. 3 is a perspective view of the clot retraction assay device illustrated in FIG. 1, with beams of the device deflected toward one another in response to coagulation of a droplet of blood provided between two sample platforms of the clot retraction assay device;

[0015] FIG. 4 is a plane view of the clot retraction assay device illustrated in FIG. 3, with graduation markings provided to aid in measuring deflection of the beams in response to coagulation of the droplet of blood provided between the two sample platforms of the clot retraction assay device;

[0016] FIG. 5A is an enlarged view, broken away, of the two sample platforms of the clot retraction assay device illustrated in FIG. 3, showing the droplet of blood in a first phase of coagulation, prior to measurable deflection of the beams of the clot retraction assay device;

[0017] FIG. 5B is an enlarged view, broken away, of the two sample platforms of the clot retraction assay device illustrated in FIG. 3, showing the droplet of blood in a second phase of coagulation, and illustrating some measurable deflection of the beams of the clot retraction assay device as a result of the progression of coagulation of the droplet of blood;

[0018] FIG. 5C is an enlarged view, broken away, of the two sample platforms of the clot retraction assay device illustrated in FIG. 3, showing the droplet of blood in a third phase of coagulation, and illustrating further measurable deflection of the beams of the clot retraction assay device beyond that illustrated in FIG. 5B as a result of the further progression of coagulation of the droplet of blood;

[0019] FIG. 6 is an enlarged, semi-schematic view of a portion of a clot retraction assay device in accordance with a second embodiment of the present disclosure, wherein a droplet of blood is received between a fixed wall (indicated schematically by a ground symbol) and a beam (much of which is broken away) running perpendicular to, but when in a non-deflected (i.e. non-stretched) rest or nominal condition, spaced away from the fixed wall a distance coinciding or substantially coinciding with a nominal diameter of a droplet of blood that has not yet experienced significant coagulation;

[0020] FIG. 7A is an enlarged view of a portion of a clot retraction assay device in accordance with a third embodiment of the present disclosure, featuring two elongate beams positioned coaxially and, in a non-deflected (i.e. non-stretched) rest or nominal condition, spaced away from one another a distance coinciding or substantially coinciding with a nominal diameter of a droplet of blood that has not yet experienced significant coagulation, with a droplet of blood received between the two elongate beams;

[0021] FIG. 7B is an enlarged view of the portion of the clot retraction assay device illustrated in FIG. 7A, showing the two beams stretched toward one another as a result of the progression of coagulation of the droplet of blood;

[0022] FIG. 8A is an enlarged perspective view of a portion of a clot retraction assay device in accordance with a fourth embodiment of the present disclosure, featuring four elongate beams positioned parallel to one another and spaced from one another in a non-deflected condition such that a droplet of blood that has not yet experienced significant coagulation, when suspended between the four elongate beams, is in contact with each of the four elongate beams, with a droplet of blood received between the four elongate beams;

[0023] FIG. 8B is an enlarged perspective view of the portion of the clot retraction assay device illustrated in FIG. 8A, showing the two beams deflected toward one another as a result of the progression of coagulation of the droplet of blood;

[0024] FIG. 9 is an enlarged perspective view of a portion of a clot retraction assay device in accordance with a fifth embodiment of the present disclosure, featuring four elongate beams, three of which are positioned parallel to one another and spaced from one another in a non-deflected condition such that a droplet of blood that has not yet experienced significant coagulation, when suspended between the three elongate beams, is in contact with each of the three elongate beams, and is in further contact with a fourth elongate beam extending perpendicularly to the three elongate beams, with a droplet of blood received between the four elongate beams;

[0025] FIG. 10 is a perspective view of a Piezoresistive clot retraction assay device of an additional embodiment of the present disclosure;

[0026] FIG. 11 is a cross-sectional view, taken along line 11-11 of FIG. 10;

[0027] FIG. 12 is a cross-sectional view, also taken along line 11-11 of FIG. 10, but illustrating the micro-beam in a deformed condition due to coagulation of a droplet of blood received in the assay device; and

[0028] FIG. 13 is a chart illustrating clot retraction force during coagulation.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0029] A microscale clot retraction assay device of a first embodiment of the present disclosure, also referred to herein as a mHemoRetractoMeter (mHRM) 10, is illustrated in FIGS. 1-5C. The mHRM 10 of this first embodiment features two opposing micro-beams 12, 14. The micro-beams 12, 14 are made of a deformable material that allows them to bend somewhat when an external force is applied. By way of example only, these micro-beams 12, 14 may each have a length of 2 cm, a width of 2 mm, and a thickness of 2 mm. Each of the micro-beams 12, 14 includes a sample platform 16, 18 defining a plateau to support a droplet of blood. Each sample platform 16, 18 is provided on a protrusion 20, 22, illustrated in FIG. 1 as a rectangular projection of, and extending perpendicular to, the respective micro-beam 12, 14 in a direction toward the opposing micro-beam 14, 12. In preferred embodiments, the micro-beams 12, 14 and protrusions 20, 22 are made of soft elastomers to allow plastic deformation in response to applied force from a sample. Such soft elastomer may be polydimethylsiloxane (PDMS),

which has advantageous properties relative to displacement in response to an external force load. By using PDMS components, the mHRM 10 is able to achieve a high degree of sensitivity in measuring contractive force of a sample based upon displacement of the micro-beams 12, 14.

[0030] The protrusions 20, 22 may be provided with indicia in the form of graduations, such as alternating dashes (–) and crosses (+), to aid in optical detection and measurement of deflection of each of the micro-beams 12, 14. Such optical detection and measurement are described below with respect to FIGS. 5A-C.

[0031] In use, a droplet of blood B having a volume as little as about 15 μL is deposited (such as by using a pipette) between the sample platforms 16, 18. The volume of the droplet of blood B is preferably between 15 μL and 300 μL to ensure accurate measurements. The micro-beams 12, 14 are spaced apart from one another such that when a droplet of blood that has not yet significantly coagulated is so deposited on the sample platforms 16, 18, the micro-beams 12, 14 are not in a deflected condition.

[0032] Upon platelet activation, the droplet of blood begins to clot, as discussed further below. The clot will contract, exerting a force on each of the micro-beams 12, 14 in the direction toward the opposing micro-beam 14, 12. This can be appreciated with reference to FIGS. 3 through 5C, which illustrate the micro-beams 12, 14 deflecting toward one another as clotting of the droplet of blood B progresses. To measure blood coagulation using the mHRM 10, the extent of micro-beam deflection is measured. As illustrated in FIG. 3, the contraction of droplet of blood B during coagulation exerts a force upon each of micro-beams 12 and 14 that pulls the beams together. The contractive force the droplet of blood B exerts is communicated to the micro-beams through the protrusions 20 and 22 to which the droplet of blood B is adheres at the sample platforms 16 and 18, respectively. As illustrated in FIG. 4, the contracting droplet of blood B pulls the protrusions 20 and 22 together, causing the micro-beams 12 and 14 to bend toward the droplet of blood B. FIGS. 5A-C illustrate is process over time. The stationary arrow in each figure is provided to indicate displacement of the micro-beam 14 caused during coagulation. The droplet of blood B is initially placed between the sample platforms 16 and 18 at FIG. 5A, at which point the micro-beams 12 and 14 are not significantly deformed. As coagulation begins and the droplet of blood B exerts a force on the micro-beams 12 and 14 in FIG. 5B, a small deformation of micro-beam 14 is seen by the displacement of the protrusion 22 (relative to the stationary arrow). After a longer period of time, the contractive force exerted by the droplet of blood B causes a greater deformation of the micro-beams 12 and 14, as illustrated in FIG. 5C by the further displacement of the protrusion 22 (relative to the stationary arrow). The indicia provided upon the protrusions 20, 22 aid in observation of the displacement caused by the contractive force exerted by the droplet of blood B.

[0033] In some embodiments, a camera may record time-lapse images of the mHRM 10 to determine displacement by comparison of the indicia upon the protrusions 20, 22 over time. By using indicia of known characteristics (e.g., distance between gradations), an observer may calculate the displacement of the protrusions 20, 22 at various times. Such displacement corresponds to the force exerted by the droplet of blood B. It should be understood that the relationship between such displacement and force depends upon the

specific configuration of the components of the mHRM, including the dimensions and composition of the micro-beams **12**, **14**. The relationship between displacement and force may likewise depend upon the temperature of the components. Therefore, the mHRM **10** may include components to measures or control temperature in some embodiments. In further embodiments, an attachment device may be used to securely connect the camera to the mHRM **10** for analysis, which may be of particular use when the camera is easily movable. For example, an attachment device (not shown) may be secured to a mobile computing device (such as a smart phone) having a camera to allow the camera to be used for analysis. In some such embodiments, the attachment device may be incorporated into or may form a part of the mHRM **10**. Similarly, the mHRM **10** may be divided into a disposable cartridge portion and a receiver portion containing a camera, ohmmeter, multimeter, or other measurement devices, as described further below.

[0034] Notably, the blood coagulation measurement protocol using the mHRM **10** is achieved without pre-functionalization of the device surface, such as through the use of adhesive proteins such as collagen, fibronectin, or other tissue factors. Such protein or lipid-based functionalization requires low-temperature storage of coagulation assay test devices, limiting their potential use for point-of-care applications in resource-limited settings. By utilizing the non-specific adhesion between whole blood and the plateau or surface of the sample platforms **16**, **18** of the protrusions **20**, **22**, protein or lipid-based functionalization is not necessary. Moreover, surface tension between the droplet of blood and the sample platforms **16**, **18** minimizes the surface area of the blood sample required for accurate coagulation measurements, resulting in a repeatable clot shape. This aids in increased accuracy of measurements to establish reliable control data, which facilitates the identification of variations from expected coagulation rates for a given test sample. The shape of this clot may form the basis for a new metric or clotting vital sign.

[0035] While indicia, such as the graduations described above, may be provided on the protrusions **20**, **22** to facilitate optical measurement of micro-beam deflection using a microscope of micro-beam deflection, electrical measurement is more reliable and desirable for point-of-care applications. Thus, the device may be further improved by means of additional features to facilitate electrical measurement of micro-beam deflection. Such electronic measurement may be facilitated by inclusion of conductive carbon black nanoparticles in PDMS components of the mHRM, particularly carbon black nanoparticles having an average size of approximately 50 nm. By percolating PDMS with conductive carbon black nanoparticles and mixing, the carbon black nanoparticles form dense aggregates with PDMS, which renders the carbon-PDMS composite (CPDMS) conductive. For example, at 22% (wt %) carbon black concentration, the CPDMS has an electrical resistivity of $0.2\Omega \cdot m$ in the absence of an external force. To be effective for measurement, the CPDMS should be between 10% and 26% (preferably between 18% and 26%) carbon by weight. Weight ratios below 18% result in limited conductivity (with weight ratios below 10% having particularly limited conductivity), while weight ratios above 26% prove difficult for mixing. In some embodiments, carbon nanotubes (such as multi-walled carbon nanotubes) may be mixed into the PDMS to produce a Piezoresistive CPDMS composite material. Although the

discussion herein refers to measurement of changes of resistance in CPDMS composite materials, it should be understood that any soft Piezoresistive material may be substituted. Thus, other conductive polymers that contain nanoscale inclusions of conductive materials (e.g., carbon nanotubes, graphene, or silver particles) may be used as an alternative to the CPDMS composite materials in the embodiments described herein. For example, a thin film of carbon nanotubes may be used as the Piezoresistive material.

[0036] To use the CPDMS or other Piezoresistive material for measuring coagulation based upon contractive force, one or more Piezoresistive components (such as CPDMS components) may be included within the mHRM. In particularly advantageous embodiments, the CPDMS components may comprise or be attached to one or more micro-beams (such as the micro-beams **12**, **14**), so that the CPDMS components are subjected to forces exerted by blood samples. The CPDMS components deform under external force load (such as force exerted by a blood sample during clot formation), and the changing gap size between carbon nanoparticles changes the resistance of the CPDMS components. This change in resistance can be measured using known means (e.g., by a digital or analog ohmmeter or multimeter). The measured change in resistance corresponds to the deformation of the CPDMS components, which further corresponds to the force exerted upon such components. Thus, the measured change in resistance of the one or more CPDMS components may be used to determine the coagulation of a blood sample. It should be appreciated that the relationship between the force exerted by the blood sample and the measured change in resistance of a CPDMS component will depend upon the specific configuration of the mHRM device, including the dimensions and composition of the CPDMS component.

[0037] For any particular configuration of an mHRM (such as those embodiments described herein which can measure coagulation in multiple dimensions), the Piezoresistivity of CPDMS can be quantified in order to calibrate the mHRM. As discussed above, the coagulation of a blood sample adhering to a portion of a micro-beam causes deformation of the micro-beam. If the micro-beam is made of CPDMS, such deformation will result in a change to the resistance across the micro-beam. Similarly, if a CPDMS layer is attached to the micro-beam (e.g., by deposition on the micro-beam surface or as a layer within the micro-beam), the CPDMS layer will deform along with the micro-beam, and such deformation will likewise result in a change to the resistance across the CPDMS layer. In either case, the change in resistance can be measured using known means of measuring resistance across the CPDMS component, viz. the CPDMS micro-beam or CPDMS layer. For example, measurements may be taken at a plurality of times using a multimeter or ohmmeter contacting portions of one or more CPDMS components. Alternatively, the mHRM (or a device to which it is connected) may include a dedicated Wheatstone bridge circuit and processing component to measure resistance across a portion of one or more CPDMS components.

[0038] The particular design and composition of the CPDMS components affect the resistance of the components. In addition to the geometry of the CPDMS components (e.g., length, cross-sectional area, cross-sectional shape), the specific composition of the CPDMS material

(e.g., type and amount of carbon added) also changes the resistance of the CPDMS components. Temperature also has an effect on the resistivity of the CPDMS material. The locations of connections to the resistance-measuring means will also affect the measurement. To accurately measure the force exerted by a blood sample, therefore, the mHRM must be calibrated based upon the particular CPDMS components used therein. Such calibration may be performed by calculating resistance from known characteristics of the component (e.g., resistivity of the particular CPDMS composite material used, length of the component, and cross-sectional area and shape of the component) or may be performed using measurements. Similar CPDMS components may be measured by application of known forces to the components, either in a laboratory testing configuration or in situ within an mHRM device. For example, a force probe may be attached to a micropositioner device, which force probe may then be used to apply various magnitudes of force to the CPDMS component to be tested. The resistance of the CPDMS component being tested may be recorded for each of a plurality of forces thus applied. In embodiments in which the CPDMS component is attached to a micro-beam, the entire micro-beam with the CPDMS component should be tested to accurately determine the relationship between the force applied to the micro-beam and the change in resistance of the CPDMS component. This is further described below with respect to the exemplary embodiment of FIGS. **10-12**.

[0039] Turning to FIG. **6**A, a portion of a second embodiment of the mHRM **110** is illustrated. In this version, a single micro-beam **112** is oriented perpendicularly to a fixed plane **115**, schematically illustrated by a ground symbol. The fixed plane may or may not comprise a conducting (or semiconducting) material. The micro-beam **112** is initially spaced from the fixed plane **115** such that when a droplet of blood B that has not yet significantly coagulated is deposited on sample platform **116**, the micro-beam **112** is not in a deflected condition. According to this embodiment, the fixed plane **115** does not deflect. To facilitate localization of the droplet of blood, the fixed plane **115** may include a circular depression or raised plateau **118**, but this does not deflect. Unlike the deflection of the micro-beams **12**, **14** of the first embodiment, which was deflection in a plane normal to the longitudinal axis of the micro-beams **12**, **14**, the deflection of the micro-beam **112** due to progression of coagulation of the droplet of blood B is in the form of stretching of the micro-beam **112** along the longitudinal axis, so that the sample platform **116** moves closer to the raised plateau **118**. The micro-beam **112** may be provided with indicia, such as graduations, to facilitate optical measurement of the deflection of the beam due to progression of coagulation of the droplet of blood B.

[0040] Alternatively, the micro-beam **112** may comprise a Piezoresistive material, such as CPDMS or other Piezoresistive materials described herein. In such embodiments, the resistance of the micro-beam **112** (or a portion thereof) may be measured, as described elsewhere herein. For example, an ohmmeter may be connected to portions of the micro-beam **112** near its connection to the fixed plane **115** and its sample platform **116**. As an alternative example, two points along the length of the micro-beam **112** between the fixed plane **115** and the sample platform **116** may be electrically connected (by electrodes, wires, etc.) to the ohmmeter. Wherever the measurement points connecting the micro-beam **112**

to the ohmmeter are located, the ohmmeter may be used to measure the change in resistance as the micro-beam **112** deforms in response to the contractive force of the droplet of blood B. Such measured changes in resistance may be used to calculate or otherwise determine the contractive force exerted by the droplet of blood B. For example, a lookup table may be used to determine the contractive force based upon previously performed experimental measurements and/or calculations. The contractive force thus determined may then be used for further analysis of the coagulation of the droplet of blood B, as described elsewhere herein.

[0041] Turning to FIGS. **7**A and **7**B, a portion of a third embodiment of the mHRM **210** is illustrated. In this embodiment, a pair of longitudinally coaxial micro-beams **212***a* and **212***b* are provided, and a droplet of blood B is placed between the micro-beams **212***a*, **212***b*. The micro-beams **212***a* and **212***b* are initially spaced apart from one another such that when a droplet of blood B that has not yet significantly coagulated is deposited between the micro-beams **212***a*, **212***b*, neither of the micro-beams **212***a*, **212***b* is in a deflected condition. Each of the micro-beams **212***a*, **212***b* is fixed at an end opposite of the blood sample receiving end or sample platform **216***a*, **216***b* of the respective micro-beam, as indicated schematically in FIGS. **7**A, **7**B by ground symbols. The deflection of the micro-beams **212***a*, **212***b* due to progression of coagulation of the droplet of blood B is in the form of stretching of the micro-beams **212***a*, **212***b* along each of their longitudinal axis, so that the sample platforms **216***a*, **216***b* of the micro-beams **212***a*, **212***b* move closer to one another, as illustrated by comparing FIG. **7**B to FIG. **7**A. Optical and/or Piezoresistive methodologies described herein may be employed to measure the deflection of the micro-beams **212***a*, **212***b* and assess the rate and/or phase of coagulation of the droplet of blood B.

[0042] A portion of a fourth embodiment of the mHRM **310** is illustrated in FIGS. **8**A, **8**B. In this embodiment, four micro-beams **312***a*, **312***b*, **312***c*, **312***d* are arranged in parallel, with a droplet of blood B disposed in contact with a platform, preferably located at a longitudinal mid-point, of each of the four micro-beams **312***a*, **312***b*, **312***c*, **312***d*. The micro-beams **312***a*, **312***b*, **312***c*, **312***d* are initially spaced apart from one another such that when a droplet of blood B that has not yet significantly coagulated is deposited between the micro-beams **312***a*, **312***b*, **312***c*, **312***d*, none of the micro-beams **312***a*, **312***b*, **312***c*, **312***d* is in a deflected condition. Each of the micro-beams **312***a*, **312***b*, **312***c*, **312***d* is fixed at both ends, as indicated schematically in FIGS. **8**A, **8**B by ground symbols. The deflection of the micro-beams **312***a*, **312***b*, **312***c*, **312***d* due to progression of coagulation of the droplet of blood B is in the form of bending of the micro-beams **312***a*, **312***b*, **312***c*, **312***d* toward one another, so that sample platforms (not shown) of the micro-beams **312***a*, **312***b*, **312***c*, **312***d* where the droplet of blood B contacts each of the micro-beams **312***a*, **312***b*, **312***c*, **312***d* move closer to one another, as illustrated by comparing FIG. **8**B to FIG. **8**A. As in the previous embodiments, optical and/or Piezoresistive methodologies described herein may be employed to measure the deflection of the micro-beams **312***a*, **312***b*, **312***c*, **312***d* and assess the rate and/or phase of coagulation of the droplet of blood B. An advantage of this approach is the clot formation and characteristics can be measured in multiple dimensions and, therefore, may be more indicative of how native clot formation occurs and interacts with blood vessel surfaces.

[0043] FIG. 9 illustrates a portion of a fifth embodiment of a mHRM 410, which is a variation of the fourth embodiment or a hybrid of the first and fourth embodiments. According to this fifth embodiment, three micro-beams 412a, 412b, 412c are arranged in parallel to one another, and a fourth micro-beam 413 is disposed perpendicularly to the other three micro-beams 412a, 412b, 412c. All four of the micro-beams 412a, 412b, 412c, 413 are fixed at both ends, as indicated schematically in FIG. 9 by ground symbols. The micro-beams 412a, 412b, 412c, 413 are initially spaced apart from one another such that when a droplet of blood B that has not yet significantly coagulated is deposited between the micro-beams 412a, 412b, 412c, 413, none of the micro-beams 412a, 412b, 412c, 413 is in a deflected condition. The deflection of the micro-beams 412a, 412b, 412c, 413 due to progression of coagulation of the droplet of blood B is in the form of bending of the micro-beams 412a, 412b, 412c, 413 toward one another, so that sample platforms (not shown) of the micro-beams 412a, 412b, 412c, 413 where the droplet of blood B contacts each of the micro-beams 412a, 412b, 412c, 413 move closer to one another. As in the previous embodiments, optical and/or Piezoresistive methodologies described herein may be employed to measure the deflection of the micro-beams 412a, 412b, 412c, 413 and assess the rate and/or phase of coagulation of the droplet of blood B.

[0044] Additionally, the orientation of the micro-beams 412a, 412b, 412c, and 413 along a plurality of axes facilitates measurement of clot formation in multiple dimensions. As illustrated, the primary axes of micro-beams 412a, 412b, 412c are substantially parallel to one another and orthogonal to the primary axis of micro-beam 413. Thus, the retractive forces of the droplet of blood B may be measured in at least two dimensions perpendicular to these primary axes. The total clot retraction force may be determined based upon the force components in each of a plurality of directions. Additional embodiments may include micro-beams arranged to measure contractive force in any number of dimensions, directions, or points. In such additional embodiments, the micro-beams may be orthogonal or non-orthogonal, and any number of micro-beams may be used to measure the contractive forces in a plurality of directions. By such configuration of a plurality of micro-beams, the regularity or irregularity of the clot may be measured. For example, a clot may be determined to be substantially spherical based upon approximately equivalent forces measured in a plurality of directions, or a clot may be determined to have substantially greater contractive forces in some directions than in others. Additionally, the shapes of clots may be determined based upon such multidimensional measurements. This may be computed from determined forces in each of a plurality of directions, or it may be determined by computing the deformation distances of each of the plurality of micro-beams in each of the directions. Both force and deformation may be determined based upon measurements of resistance across the Piezoresistive components, which may include the micro-beams.

[0045] In further embodiments, a multidimensional profile of a clot may be constructed based upon measured contractive forces in a plurality of dimensions. Such a multidimensional profile may indicate the shape or strength of the clot in each dimension at one or more times following the time clotting is induced. For example, the multidimensional profile may include measurements of contractive forces exerted by the droplet of blood B during clotting at regular time intervals (e.g., every quarter-second, every second, etc.) in each of a plurality of directions (e.g., three orthogonal directions, five non-orthogonal directions, etc.). In yet further embodiments, a four-dimensional model of the clot in three-dimensional space over time may be generated based upon, or as part of, the multidimensional model.

[0046] In a further particular embodiment, an exemplary Piezoresistive clot retraction assay device 510 as illustrated in FIGS. 10-12 may be provided. The exemplary device 510 is configured to measure coagulation of a droplet of blood B in a manner similar to those assay devices described above, but the exemplary device 510 illustrates a Piezoresistive component 530 layered upon a micro-beam 512. Thus, the principles of operation of Piezoresistive measurement may be more clearly described. FIG. 10 illustrates a perspective view of the Piezoresitive clot assay device 510, while FIGS. 11 and 12 illustrate cross sections of the device 510 along the line 11. FIG. 11 illustrates the cross section of the device 510 when the droplet of blood B is not exerting a measurable force upon the micro-beam 512. FIG. 12 illustrates the cross section of the device 510 when the droplet of blood B is exerting a contractive force upon the micro-beam 512, such as during platelet contraction.

[0047] As illustrated, the Piezoresistive clot retraction assay device 510 has a base 504, which may have a raised plateau 518 for localizing the droplet of blood B. The base 504 and plateau 518 may be formed as one integrated component or may be joined by adhesives or other known means, and each may be made of an acrylic or other rigid material. It is desirable that the base 504 and plateau 518 should not deform in response to the contractive force exerted by the droplet of blood B, so a rigid material (or substantial qualities of a less rigid material) should be used for these components. In some embodiments, a covering 506 may be included to cover the sample area that includes the micro-beam 512. It may be beneficial to cover the sample area in order to prevent external forces from interfering with the measurement. To permit introduction of the sample droplet of blood B, the cover 506 may be removable, or a sample introduction hole (not shown) may be included.

[0048] The base 504 supports the micro-beam 512, which is illustrated as one micro-beam 512 spanning the length of the base 504. In other embodiments, multiple micro-beams 512 may be used in a manner similar to that discussed elsewhere herein. The micro-beam 512 may be made in any convenient dimensions, but a width of approximately 2 millimeters (preferably between 1 mm and 3 mm) with a height of approximately 0.3 millimeters (preferably between 0.1 mm and 0.5 mm) is preferable. In further preferred embodiments, the micro-beam 512 preferably spans a distance of approximately 15 millimeters (preferably between 10 mm and 30 mm) between the sides of the base 504. The micro-beam 512 may be made of any flexible, non-conductive material, but PDMS is particularly advantageous for its spring properties. A PDMS micro-beam 512 of the dimensions set forth above will have a spring constant of approximately 0.06 µm. As perhaps best appreciated with reference to FIG. 10, the micro-beam 512 may be formed by cutting away sections of a plate 511 in some embodiments, which plate 511 may then be attached to the base 504.

[0049] Additionally, some embodiments may include a sample platform 516 on a portion of the micro-beam 512. For example, a circular sample platform 516 having a

diameter of 3 millimeters may be placed in the center of a micro-beam **512** that is 2 millimeters thick. Such sample platform **516** should align with the plateau **518** of the base **504** to localize the droplet of blood B in a particular position for measurement. The plateau **518** should be of similar (although not necessarily precisely the same) shape and area as the sample platform **516**.

[0050] The Piezoresistive component **530** is attached to the micro-beam **512**, such that the deformation of the micro-beam **512** due to force exerted by the droplet of blood B will cause the Piezoresistive component **530** to also deform. As illustrated, the Piezoresistive component **530** is layered upon the top of the micro-beam **512**, away from the bottom side of the micro-beam **512** that will contact the droplet of blood B. The Piezoresistive component **530** may be screen-printed onto the micro-beam **512** or otherwise deposited thereon. Such screen-printed Piezoresistive component **530** may have a thickness of approximately 50 micrometers (preferably between 25 μm and 100 μm). The width of the Piezoresistive component **530** may be the full width of the micro-beam **512** or less than the full width of the micro-beam **512**. For example, the Piezoresistive component **530** may be 15 mm long, 1 mm wide, and 50 μm thick. In particularly preferred embodiments, the Piezoresistive component **530** may comprise a CPDMS material, as described above. Specifically, the CPDMS material may have approximately 18% to 22% carbon concentration by weight (preferably approximately 22%). This will facilitate measurement by producing a substantial change in resistance of the Piezoresistive component **530** in response to deformation. Alternatively, the Piezoresistive component **530** may be another type of Piezoresistive material, such as other conductive polymers containing nanoscale conductive materials. For example, the Piezoresistive component **530** may be a carbon nanotube thin film deposited upon the micro-beam **512** via evaporation of the water from a carbon nanotube-in-water dispersion that contains at least water and carbon nanotubes. To facilitate electrical measurement, the Piezoresistive component **530** may be connected to electrodes **528** for further connection to an ohmmeter or multimeter (not shown). The electrodes **528** may also be screen printed on the plate **511** in some embodiments and may be made of any convenient conductive material (e.g., silver).

[0051] To measure coagulation of the droplet of blood B using the Piezoresistive clot retraction assay device **510**, a resistance measurement device (e.g., an ohmmeter, not shown) is connected to the electrodes **528**. The droplet of blood B is introduced to the sample area and placed between the plateau **518** and the sample platform **516**. This is illustrated in FIG. **11**, which shows the Piezoresistive clot retraction assay device **510** with a droplet of blood B that is not yet exerting a significant force upon the micro-beam **512**. In some embodiments, an initial reading may be taken to determine the magnitude of the minimal force exerted by the droplet of blood B upon the micro-beam **512** before coagulation has begun. The droplet of blood B may be treated with a reagent to stimulate or impede coagulation, either before or after being introduced to the device **510**. Alternatively, the surfaces may be coated with various agonists or antagonists of the cellular or acellular components of coagulation in order to identify the particular coagulation defect. As coagulation proceeds, the droplet of blood B will contract, which will exert a force upon the sample platform **516** and the plateau **518**. Because the

plateau **518** and base **504** are rigid relative to the micro-beam **512**, the sample platform **516** of the micro-beam **512** will be pulled toward the plateau **518** by this contractive force. The Piezoresistive component **530** will likewise be pulled toward the plateau **518** by the micro-beam **512**. The resulting deformation of the micro-beam **512** and the Piezoresistive component **530** is shown in FIG. **12**. As the Piezoresistive component **530** deforms in response to the contractive force of the droplet of blood B, the length of the Piezoresistive component **530** increases and part of the Piezoresistive component **530** become thinner. This deformation changes the geometry of the Piezoresistive component **530**, which results in a change in resistance of the Piezoresistive component **530**. The change in resistance may be measured in real time (either periodically or continuously) as the droplet of blood B coagulates.

[0052] From the readings of the changes in resistance of the Piezoresistive component **530**, information regarding the extent and timing of coagulation may be determined and analyzed to further determine a blood coagulation profile or characteristics of the patient from whom the sample was taken. Because the amount of blood required for each sample is so small (approximately 15 μL), multiple Piezoresistive clot retraction assay devices **510** may be used to simultaneously test the responses of the blood to different types or quantities of pro- or anti-coagulants. Thus, a fuller picture of the coagulation response of a patient may be obtained in real time with very little blood drawn. This is particularly important for pediatric, neonatal, or other patients for whom drawing large quantities of blood for testing would be dangerous or impractical.

[0053] As noted above, the mHRM **10** may be divided into two distinct parts: a removable cartridge that receives the droplet of blood B and a receiving unit that receives cartridges and performs measurements. Such embodiments are advantageous inasmuch as they separate the electronic and computing components (e.g., an ohmmeter for measuring resistance across the Piezoresistive components and a processor for determining force based upon changes in measured resistance) from the measurement structure (e.g., the micro-beams and base to which the droplet of blood B adheres). Because the electronic and computing components are relatively more durable and expensive than the measurement structure, the receiving unit may be used to measure coagulation of many blood samples. In contrast, the measurement structure must be cleaned between each sample, which may be time-consuming and may degrade the measurement accuracy over time. Because the components of the measurement structure can be manufactured relatively easily and cheaply, the measurement structure may be separated into a removable cartridge. In some embodiments, such cartridges may be configured to receive the droplet of blood B prior to insertion of the cartridge into the receiving unit.

[0054] As an example, the device **510** may be a removable cartridge in some such embodiments. Such cartridge may connect to a measurement device within the receiving unit by an electrical connection to the electrodes **528** when the cartridge is inserted within the receiving unit. The measurement device may be an ohmmeter, multimeter, or other similar devices, as described elsewhere herein. In operation, the droplet of blood B may be introduced into the sample area between the plateau **518** and the sample platform **516** prior to insertion of the cartridge into the receiving unit. The

covering 506 may then be moved into place to avoid contamination of the receiving unit by any portion of the blood sample to be assessed. The cartridge (the device 510) may then be inserted into the receiving unit (not shown). A chemical or other means of inducing coagulation may be introduced, either before or after the cartridge is inserted within the receiving unit. Following insertion of the cartridge, a measurement device of the receiving unit may begin measurement of the resistance across the Piezoresistive component 530 via the connections with the electrodes 528. In some embodiments, a plurality of resistance measurements may be taken to determine various metrics associate with the blood sample, as described further below. The measurement device may further be communicatively connected to a processing component of receiving unit, which may determine the various metrics based upon resistance measurements received from the measurement device, as described further below.

[0055] FIG. 13 illustrates change in blood clot retraction force over time during coagulation. The clotting process may be divided into three phases, as shown. First, a reaction phase exists from the time clotting is induced (T_0) until the onset of clot development (T_r), indicating a reaction time. Second, a contraction development phase exists from the onset of clot development at the reaction time (T_r) until the maximum clot retraction force is reached (T_{max}). Third, a fibrinolysis phase exists from the time the maximum clot retraction force is reached (T_{max}) until the time a steady-state plateau is reached (T_{fib}). Thus, the chart of FIG. 13 illustrates the changes of the clot retractive force of a whole blood sample over these three phases. The various embodiments of the measurement devices described above facilitate measurement of metrics associated with the phases, may be used to determine important aspects of hemostasis under different clotting agent and pro- and anti-coagulant treatments. Such metrics may include absolute or relative durations of each phase, magnitudes of clot retraction forces, or changes in magnitudes of clot retraction forces.

[0056] During the reaction phase, the coagulation cascade is underway for formation of the fibrin network needed for clot retraction force development. While this occurs, no discernible change to the clot retraction force is observed, viz. the clot retraction force remains at zero or a negligible value. Once the fibrin network is in place, the onset of clot retraction force development is observed, identifying the beginning of the contraction development phase. During the contraction development phase, interactions between activated platelets and fibrin strands lead to a rapid increase of clot retraction force and initiation of blood clot contraction. A growth rate of clot retraction force (G_{CRF}) may be measured as the rate of change of the clot retraction force at the reaction time (T_r), which may also be defined as the first derivative of clot retraction force with respect to time at the reaction time. Although the growth rate of clot retraction force (G_{CRF}) at the reaction time (T_r) is a particularly useful metric, other growth rates may be measured at other times during the contraction phase or fibrinolysis phase. The clot retraction force continues to increase during the contraction development phase, until the maximum clot retraction force (CRF_{max}) is reached, which value is also a particularly useful metric. Once the maximum clot retraction force (CRF_{max}) is reached, the fibrinolysis phase begins, during which clot retraction force decreases slowly from the maximum clot retraction force to a plateau or steady-state value

of the clot retraction force as the fibrin network disintegrates. In some embodiments, various metrics described above may be measured using clot retraction devices as described herein. Additionally, some embodiments may include one or more generated charts or plots, such as the chart illustrated in FIG. 13.

[0057] Aside from measurement and diagnostic purposes, the process described above would normally occur within a patient's body. It would thus be subject to variable conditions that can, in some instances, exert considerable influence upon the results. Two such conditions of particular interest are temperature within the patient's body and sheer forces from blood circulation. In some embodiments, therefore, the devices and methods described herein may be modified to control these conditions. In some such embodiments, temperature may be measured for the sample area where the droplet of blood B is placed for retractive force measurement, such as by thermocouples or digital thermometers. In further embodiments, heating or cooling elements may be included to control the temperature of the sample area. In additional embodiments, sheer forces caused by blood circulation may be mimicked by introducing vibrations to the measurement device. In some such embodiments, the sample area may be disposed within a hollow tube or sphere, configured to resemble a vein or artery. A vibrating element, such as a micromechanical vibrating motor, may be added to the device to generate sheer forces. Such vibrating element may be controlled to operate continuously or at intervals mimicking the flow of blood in response to a heartbeat. Alternatively, the sample area or the entire clot retraction assay device may be disposed within a fluidic channel supplied by a controllable fluid source. A fluid may thus be introduced to exert a controllable sheer stress on the droplet of blood B, thereby mimicking the sheer forces from blood circulation within a patient's circulatory system. Such temperature and/or sheer force conditions may be used, in some embodiments, to initiate clotting in response to these conditions.

[0058] As described herein, various devices and methods for measuring retractive forces exerted by blood clots and for using such measurements are disclosed. As discussed above, such forces are measured indirectly through their effects on deformation and displacement of micro-beams. Direct measurements of the displacement of the micro-beams may be optical, Piezoresistive, or by other techniques. When optical, image data may be automatically processed to determine the force associated with micro-beam displacement or deformation. When Piezoresistive, resistance measurements may be automatically processed to determine corresponding force, also based upon micro-beam displacement or deformation (as measured through a change in resistance). One or more processing components may be used to perform such processing and determine the corresponding retractive forces. The processing components may include one or more general-purpose computer processors configured by executable instructions to perform such processing, or the processing components may include one or more special-purpose processors permanently configured to perform such processing. The processing components may similarly include field programmable gate arrays or other similar components. The processing components may be disposed within the clot retraction assay devices described herein or may be communicatively connected thereto, such as via a wired or wireless communication connection. In

some embodiments, a general-purpose computer may be connected to the clot retraction assay device to process measurements of resistance caused by (or images showing) micro-beam displacement or deformation.

[0059] While various embodiments have been described herein, it will be understood that variations may be made thereto that are still within the scope of the appended claims. By way of example, and not limitation, the present disclosure contemplates at least the following aspects:

[0060] 1. A clot retraction assay device for assessing a blood sample, comprising: a micro-beam made of a deformable material, having at least one end connected to a support and extending away from the support in an axial direction; a Piezoresistive component disposed upon a surface of the micro-beam along the axial direction; a resistance measurement device connected to a plurality of points of the Piezoresistive component, the points separated by a distance in the axial direction, wherein the resistance measurement device is configured to measure a change in resistance across a portion of the Piezoresistive component resulting from deformation in response to a retractive force exerted upon the micro-beam by the blood sample.

[0061] 2. The clot retraction assay device of aspect 1, wherein the Piezoresistive component comprises a carbon-polydimethylsiloxane (CPDMS) composite.

[0062] 3. The clot retraction assay device of aspect 2, wherein the CPDMS composite is between 10% and 26% carbon by weight.

[0063] 4. The clot retraction assay device of aspect 1, wherein the Piezoresistive component comprises a carbon nanotube thin film.

[0064] 5. The clot retraction assay device of aspect 4, wherein the carbon nanotube thin film is disposed upon the surface of the micro-beam through deposition by evaporating water of a carbon nanotube-in-water dispersion.

[0065] 6. The clot retraction assay device of any of aspects 1-5, wherein: two ends of the micro-beam are connected to the support; the micro-beam is integrated into the support as a portion of one sheet of material; the Piezoresistive component extends between the two ends of the micro-beam; and the resistance measurement device is connected to the Piezoresistive component at each of the two ends of the micro-beam.

[0066] 7. The clot retraction assay device of any of aspects 1-6, further comprising a rigid base connected to the micro-beam, either directly or through the support, and wherein: the micro-beam includes a platform area having a platform width greater than a beam width of the micro-beam in a direction normal to the axial direction; the platform area is located a separation distance from a sample plateau of rigid base; and the size of each of the platform area and the sample plateau and the separation distance are configured to hold the blood sample between the platform area and the sample plateau by non-specific adhesion resulting from surface tension, the blood sample having a volume between 15 microliters and 300 microliters.

[0067] 8. The clot retraction assay device of any of aspects 1-7, further comprising a processing component configured to determine the retractive force exerted by the blood sample based upon the measured change in resistance across the portion of the Piezoresistive component.

[0068] 9. The clot retraction assay device of any of aspects 1-8, wherein the Piezoresistive component is a layer deposited upon the surface of the micro-beam.

[0069] 10. The clot retraction assay device of any of aspects 1-9, wherein the micro-beam and Piezoresistive component are disposed within a removable cartridge, and further comprising a receiving unit configured to receive the cartridge, the receiving unit comprising: the resistance measurement device; a plurality of conductive connections to the cartridge, wherein the conductive connections form conductive connections between the resistance measuring device and the plurality of points of the Piezoresistive component; a processing component communicatively connected to an output of the resistance measurement device, wherein the processing component is configured to determine the retractive force exerted by the blood sample based upon the measured change in resistance across the portion of the Piezoresistive component.

[0070] 11. A clot retraction assay device for assessing a blood sample, comprising: a micro-beam made of a deformable Piezoresistive material, having at least one end connected to a rigid support and extending away from the rigid support in an axial direction; a resistance measurement device connected to a plurality of points of the micro-beam, the points separated by a distance in the axial direction, wherein the resistance measurement device is configured to measure a change in resistance across a portion of the micro-beam resulting from deformation in response to a retractive force of the blood sample exerted upon the micro-beam.

[0071] 12. The clot retraction assay device of aspect 11, wherein the micro-beam is made of a carbon-polydimethylsiloxane (CPDMS) composite.

[0072] 13. The clot retraction assay device of aspect 12, wherein the CPDMS composite is between 10% and 26% carbon by weight.

[0073] 14. The clot retraction assay device of any of aspects 11-13, further comprising a sample plateau at a fixed position relative to the at least one end of the micro-beam connected to the rigid support, and wherein the sample plateau is disposed in proximity to the micro-beam at a distance sufficient to hold the blood sample between the sample plateau and the micro-beam by non-specific adhesion resulting from surface tension, the blood sample having a volume between 15 microliters and 300 microliters.

[0074] 15. The clot retraction assay device of any of aspects 11-14, further comprising one or more additional micro-beams made of the deformable Piezoresistive material, and wherein: the resistance measurement device is configured to measure, for each additional micro-beam of the one or more additional micro-beams, an additional change in resistance across an additional portion of the additional micro-beam resulting from deformation in response to the retractive force of the blood sample exerted upon the additional micro-beam; and the retractive force of the blood sample is determined based upon the change in resistance across the portion of the micro-beam and the one or more additional changes in resistance across the one or more additional micro-beams.

[0075] 16. The clot retraction assay device of aspect 15, wherein at least one of the one or more additional micro-beams is not parallel to the micro-beam.

[0076] 17. The clot retraction assay device of any of aspects 11-16, further comprising a processing component configured to determine the retractive force of the blood sample based upon the measured change in resistance across the portion of the Piezoresistive component.

[0077] 18. A clot retraction assay method, comprising: introducing a blood sample into a measurement area of a clot retraction assay device, the clot retraction assay device having at least one Piezoresistive component and a resistance measurement device configured to measure resistance across a portion of the Piezoresistive component; inducing blood clotting of the blood sample in the measurement area at a start time; determining a first resistance across the portion of the Piezoresistive component at the start time; determining a second resistance across the portion of the Piezoresistive component at a second time following the start time, wherein the second resistance is measured by the measurement device; determining, by a processor, a clot retraction force of the blood sample based upon the difference between the second resistance and the first resistance.

[0078] 19. The method of aspect 18, wherein the blood sample is less than 300 microliters.

[0079] 20. The method of either of aspect 18 or aspect 19, further comprising: determining a plurality of additional resistances across the portion of the Piezoresistive component at a plurality of times following the start time; and determining, by the processor, at least one of the following metrics associated with the blood sample based upon one or more differences between one or more of the plurality of additional resistances and the first resistance: a clot initiation time, a clot retraction force growth rate, a clot lysis onset time, a clot lysis rate, or a maximum clot retraction force.

[0080] 21. The method of any of aspects 18-20, further comprising: determining a plurality of additional resistances across the portion of the Piezoresistive component at a plurality of times following the start time; and determining, by the processor, at least one of the following metrics associated with the blood sample based upon one or more differences between one or more of the plurality of additional resistances and the first resistance: a reaction time associated with a reaction phase between the first time and a time associated with the beginning of a clot initiation and clot retraction force growth, a maximum clot retraction force time between the first time and a time associated with a maximum clot retraction force, a contraction development time of a contraction development phase between the time associated with the beginning of a clot retraction force growth and the time associated with the maximum clot retraction force, a plateau time between the first time and a time associated with a steady-state clot retraction force, or a fibrinolysis time between the time associated with the maximum clot retraction force and the time associated with the steady-state clot retraction force.

[0081] 22. The method of any of aspects 18-21, wherein: the clot retraction assay device includes a plurality of Piezoresistive components disposed to measure simultaneously a plurality of clot contraction forces of the blood sample in a plurality of directions; and determining the clot retraction force of the blood sample at the second time includes determining a plurality of forces in a plurality of directions based upon a plurality of resistances across the plurality of Piezoresistive components at the second time.

[0082] 23. The method of any of aspects 18-22, further comprising plotting: a reaction phase of the blood sample, including the clot retraction force of the sample from the start time at which clotting is induced (T_0) until an onset of clot development at a reaction time (T_r); a contraction development phase, including the clot retraction force of the sample from the onset of clot development at the reaction

time (T_r) until a time a maximum clot retraction force is reached (T_{max}); and a fibrinolysis phase, including the clot retraction force from the time the maximum clot retraction force is reached (T_{max}) until a time a steady-state plateau is reached (T_{fib}).

[0083] 24. The method of any of aspects 18-23, wherein the measurement area is disposed within a hollow chamber configured to resemble a vein or artery, which hollow chamber is connected to a vibrating element, and the method further comprising: operating the vibrating element to generate sheer forces within the chamber to simulate a heartbeat during blood clotting.

[0084] 25. A method of determining clot retraction force of a blood sample consisting of a droplet of blood, the method comprising: providing a clot retraction assay device including: a first micro-beam made of a deformable Piezoresistive material, having at least one end connected to a first rigid support and extending away from the first rigid support in an axial direction of the first micro-beam; a first resistance measurement device connected to a plurality of points of the first micro-beam, the points separated by a distance in the axial direction of the first micro-beam, wherein the first resistance measurement device is configured to measure a change in resistance across a portion of the first micro-beam resulting from deformation in response to a first retractive force of the blood sample exerted upon the first micro-beam; a second micro-beam made of a deformable Piezoresistive material, having at least one end connected to a second rigid support and extending away from the second rigid support in an axial direction of the second micro-beam; a second resistance measurement device connected to a plurality of points of the second micro-beam, the points separated by a distance in the axial direction of the second micro-beam, wherein the second resistance measurement device is configured to measure a change in resistance across a portion of the second micro-beam resulting from deformation in response to a second retractive force of the blood sample exerted upon the second micro-beam; a third micro-beam made of a deformable Piezoresistive material, having at least one end connected to a third rigid support and extending away from the third rigid support in an axial direction of the third micro-beam; and a third resistance measurement device connected to a plurality of points of the third micro-beam, the points separated by a distance in the axial direction of the third micro-beam, wherein the third resistance measurement device is configured to measure a change in resistance across a portion of the third micro-beam resulting from deformation in response to a third retractive force of the blood sample exerted upon the third micro-beam, primary axes of the second and third micro-beams being substantially parallel to one another and orthogonal to a primary axis of the first micro-beam; introducing a blood sample into a measurement area of the clot retraction assay device to be in contact with the first, second, and third micro-beams; and calculating a total clot retraction force based upon force components in each of a plurality of directions, the force components calculated based upon the measured changes in resistance from the first, second, and third resistance measurement devices.

[0085] 26. The method according to aspect 25, further comprising measuring regularity or irregularity of a clot formed by the droplet of blood by comparing at least one of (i) the retractive forces exerted by the droplet of blood along

the first, second, and third micro-beams or (ii) the deformation distance of each of the first, second, and third micro-beams.

[0086] 27. The method according to aspect 26, wherein: measuring the regularity or irregularity of the clot includes determining a multidimensional profile of the clot based upon the measured retractive forces exerted by the droplet of blood in a plurality of directions; and the multidimensional profile indicates at least one of the following in a plurality of dimensions: (i) the strength of the clot or (ii) the shape of the clot.

[0087] 28. The method according to any of aspects 25-27, further comprising: calculating in each of a plurality of directions a reaction phase of the blood sample, indicated by measured clot retraction forces of the sample from a time clotting is induced (T_0) until an onset of clot development at a reaction time (T_r); calculating in each of the plurality of directions a contraction development phase, indicated by measured clot retraction forces of the sample from the onset of clot development at the reaction time (T_r) until a time a maximum clot retraction force is reached (T_{max}); and calculating in each of the plurality of directions a fibrinolysis phase, indicated by measured clot retraction forces from the time the maximum clot retraction force is reached (T_{max}) until a time a steady-state plateau is reached (T_{fib}).

What is claimed is:

1. A clot retraction assay device for assessing a blood sample, comprising:
   a micro-beam made of a deformable material, having at least one end connected to a support and extending away from the support in an axial direction;
   a Piezoresistive component disposed upon a surface of the micro-beam along the axial direction;
   a resistance measurement device connected to a plurality of points of the Piezoresistive component, the points separated by a distance in the axial direction,
   wherein the resistance measurement device is configured to measure a change in resistance across a portion of the Piezoresistive component resulting from deformation in response to a retractive force exerted upon the micro-beam by the blood sample.

2. The clot retraction assay device of claim 1, wherein the Piezoresistive component comprises a carbon-polydimethylsiloxane (CPDMS) composite.

3. The clot retraction assay device of claim 2, wherein the CPDMS composite is between 10% and 26% carbon by weight.

4. The clot retraction assay device of claim 1, wherein the Piezoresistive component comprises a carbon nanotube thin film.

5. The clot retraction assay device of claim 4, wherein the carbon nanotube thin film is disposed upon the surface of the micro-beam through deposition by evaporating water of a carbon nanotube-in-water dispersion.

6. The clot retraction assay device of claim 1, wherein:
   two ends of the micro-beam are connected to the support;
   the micro-beam is integrated into the support as a portion of one sheet of material;
   the Piezoresistive component extends between the two ends of the micro-beam; and
   the resistance measurement device is connected to the Piezoresistive component at each of the two ends of the micro-beam.

7. The clot retraction assay device of claim 1, further comprising a rigid base connected to the micro-beam, either directly or through the support, and wherein:
   the micro-beam includes a platform area having a platform width greater than a beam width of the micro-beam in a direction normal to the axial direction;
   the platform area is located a separation distance from a sample plateau of rigid base; and
   the size of each of the platform area and the sample plateau and the separation distance are configured to hold the blood sample between the platform area and the sample plateau by non-specific adhesion resulting from surface tension, the blood sample having a volume between 15 microliters and 300 microliters.

8. The clot retraction assay device of claim 1, further comprising a processing component configured to determine the retractive force exerted by the blood sample based upon the measured change in resistance across the portion of the Piezoresistive component.

9. The clot retraction assay device of claim 1, wherein the Piezoresistive component is a layer deposited upon the surface of the micro-beam.

10. The clot retraction assay device of claim 1, wherein the micro-beam and Piezoresistive component are disposed within a removable cartridge, and further comprising a receiving unit configured to receive the cartridge, the receiving unit comprising:
    the resistance measurement device;
    a plurality of conductive connections to the cartridge, wherein the conductive connections form conductive connections between the resistance measuring device and the plurality of points of the Piezoresistive component;
    a processing component communicatively connected to an output of the resistance measurement device, wherein the processing component is configured to determine the retractive force exerted by the blood sample based upon the measured change in resistance across the portion of the Piezoresistive component.

11. A clot retraction assay device for assessing a blood sample, comprising:
    a micro-beam made of a deformable Piezoresistive material, having at least one end connected to a rigid support and extending away from the rigid support in an axial direction;
    a resistance measurement device connected to a plurality of points of the micro-beam, the points separated by a distance in the axial direction,
    wherein the resistance measurement device is configured to measure a change in resistance across a portion of the micro-beam resulting from deformation in response to a retractive force of the blood sample exerted upon the micro-beam.

12. The clot retraction assay device of claim 11, wherein the micro-beam is made of a carbon-polydimethylsiloxane (CPDMS) composite.

13. The clot retraction assay device of claim 12, wherein the CPDMS composite is between 10% and 26% carbon by weight.

14. The clot retraction assay device of claim 11, further comprising a sample plateau at a fixed position relative to the at least one end of the micro-beam connected to the rigid support, and wherein the sample plateau is disposed in proximity to the micro-beam at a distance sufficient to hold

the blood sample between the sample plateau and the micro-beam by non-specific adhesion resulting from surface tension, the blood sample having a volume between 15 microliters and 300 microliters.

15. The clot retraction assay device of claim 11, further comprising one or more additional micro-beams made of the deformable Piezoresistive material, and wherein:

the resistance measurement device is configured to measure, for each additional micro-beam of the one or more additional micro-beams, an additional change in resistance across an additional portion of the additional micro-beam resulting from deformation in response to the retractive force of the blood sample exerted upon the additional micro-beam; and

the retractive force of the blood sample is determined based upon the change in resistance across the portion of the micro-beam and the one or more additional changes in resistance across the one or more additional micro-beams.

16. The clot retraction assay device of claim 15, wherein at least one of the one or more additional micro-beams is not parallel to the micro-beam.

17. The clot retraction assay device of claim 11, further comprising a processing component configured to determine the retractive force of the blood sample based upon the measured change in resistance across the portion of the Piezoresistive component.

18. A clot retraction assay method, comprising:

introducing a blood sample into a measurement area of a clot retraction assay device, the clot retraction assay device having at least one Piezoresistive component and a resistance measurement device configured to measure resistance across a portion of the Piezoresistive component;

inducing blood clotting of the blood sample in the measurement area at a start time;

determining a first resistance across the portion of the Piezoresistive component at the start time;

determining a second resistance across the portion of the Piezoresistive component at a second time following the start time, wherein the second resistance is measured by the measurement device;

determining, by a processor, a clot retraction force of the blood sample based upon the difference between the second resistance and the first resistance.

19. The method of claim 18, wherein the blood sample is less than 300 microliters.

20. The method of claim 18, further comprising:

determining a plurality of additional resistances across the portion of the Piezoresistive component at a plurality of times following the start time; and

determining, by the processor, at least one of the following metrics associated with the blood sample based upon one or more differences between one or more of the plurality of additional resistances and the first resistance: a clot initiation time, a clot retraction force growth rate, a clot lysis onset time, a clot lysis rate, or a maximum clot retraction force.

21. The method of claim 18, further comprising:

determining a plurality of additional resistances across the portion of the Piezoresistive component at a plurality of times following the start time; and

determining, by the processor, at least one of the following metrics associated with the blood sample based upon one or more differences between one or more of the plurality of additional resistances and the first resistance:

a reaction time associated with a reaction phase between the first time and a time associated with the beginning of a clot initiation and clot retraction force growth,

a maximum clot retraction force time between the first time and a time associated with a maximum clot retraction force,

a contraction development time of a contraction development phase between the time associated with the beginning of a clot retraction force growth and the time associated with the maximum clot retraction force,

a plateau time between the first time and a time associated with a steady-state clot retraction force, or

a fibrinolysis time between the time associated with the maximum clot retraction force and the time associated with the steady-state clot retraction force.

22. The method of claim 18, wherein:

the clot retraction assay device includes a plurality of Piezoresistive components disposed to measure simultaneously a plurality of clot contraction forces of the blood sample in a plurality of directions; and

determining the clot retraction force of the blood sample at the second time includes determining a plurality of forces in a plurality of directions based upon a plurality of resistances across the plurality of Piezoresistive components at the second time.

23. The method of claim 18, further comprising plotting:

a reaction phase of the blood sample, including the clot retraction force of the sample from the start time at which clotting is induced (T_o) until an onset of clot development at a reaction time (T_r);

a contraction development phase, including the clot retraction force of the sample from the onset of clot development at the reaction time (T_r) until a time a maximum clot retraction force is reached (T_{max}); and

a fibrinolysis phase, including the clot retraction force from the time the maximum clot retraction force is reached (T_{max}) until a time a steady-state plateau is reached (T_{fib}).

24. The method of claim 18, wherein the measurement area is disposed within a hollow chamber configured to resemble a vein or artery, which hollow chamber is connected to a vibrating element, and the method further comprising:

operating the vibrating element to generate sheer forces within the chamber to simulate a heartbeat during blood clotting.

25. A method of determining clot retraction force of a blood sample consisting of a droplet of blood, the method comprising:

providing a clot retraction assay device including

a first micro-beam made of a deformable Piezoresistive material, having at least one end connected to a first rigid support and extending away from the first rigid support in an axial direction of the first micro-beam;

a first resistance measurement device connected to a plurality of points of the first micro-beam, the points separated by a distance in the axial direction of the first micro-beam,

wherein the first resistance measurement device is configured to measure a change in resistance across a portion of the first micro-beam resulting from

deformation in response to a first retractive force of the blood sample exerted upon the first micro-beam;

a second micro-beam made of a deformable Piezoresistive material, having at least one end connected to a second rigid support and extending away from the second rigid support in an axial direction of the second micro-beam;

a second resistance measurement device connected to a plurality of points of the second micro-beam, the points separated by a distance in the axial direction of the second micro-beam,

wherein the second resistance measurement device is configured to measure a change in resistance across a portion of the second micro-beam resulting from deformation in response to a second retractive force of the blood sample exerted upon the second micro-beam;

a third micro-beam made of a deformable Piezoresistive material, having at least one end connected to a third rigid support and extending away from the third rigid support in an axial direction of the third micro-beam; and

a third resistance measurement device connected to a plurality of points of the third micro-beam, the points separated by a distance in the axial direction of the third micro-beam,

wherein the third resistance measurement device is configured to measure a change in resistance across a portion of the third micro-beam resulting from deformation in response to a third retractive force of the blood sample exerted upon the third micro-beam, primary axes of the second and third micro-beams being substantially parallel to one another and orthogonal to a primary axis of the first micro-beam;

introducing a blood sample into a measurement area of the clot retraction assay device to be in contact with the first, second, and third micro-beams; and

calculating a total clot retraction force based upon force components in each of a plurality of directions, the force components calculated based upon the measured changes in resistance from the first, second, and third resistance measurement devices.

26. The method according to claim **25**, further comprising measuring regularity or irregularity of a clot formed by the droplet of blood by comparing at least one of (i) the retractive forces exerted by the droplet of blood along the first, second, and third micro-beams or (ii) the deformation distance of each of the first, second, and third micro-beams.

27. The method according to claim **26**, wherein:

measuring the regularity or irregularity of the clot includes determining a multidimensional profile of the clot based upon the measured retractive forces exerted by the droplet of blood in a plurality of directions; and

the multidimensional profile indicates at least one of the following in a plurality of dimensions: (i) the strength of the clot or (ii) the shape of the clot.

28. The method according to claim **25**, further comprising:

calculating in each of a plurality of directions a reaction phase of the blood sample, indicated by measured clot retraction forces of the sample from a time clotting is induced (T_0) until an onset of clot development at a reaction time (T_r);

calculating in each of the plurality of directions a contraction development phase, indicated by measured clot retraction forces of the sample from the onset of clot development at the reaction time (T_r) until a time a maximum clot retraction force is reached (T_{max}); and

calculating in each of the plurality of directions a fibrinolysis phase, indicated by measured clot retraction forces from the time the maximum clot retraction force is reached (T_{max}) until a time a steady-state plateau is reached (T_{fib}).

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