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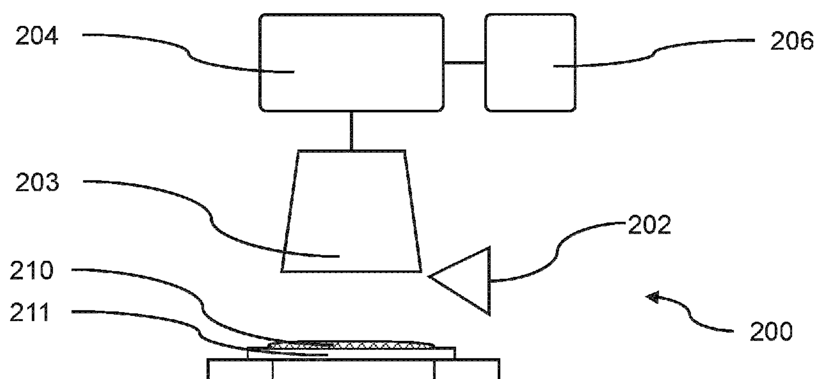
(54) **Title:** DEVICES AND METHODS FOR PREDICTING BLEEDING AND THROMBOSIS DIATHESIS



(57) **Abstract:** Methods and apparatus for determining procoagulant potentials and categorising blood samples according to bleeding or clotting tendency are disclosed. The method comprises obtaining an image of a blood sample. The blood samples are mounted on a two- dimensional substrate, and labelled with two or more colour indicators. The image is analyzed for different colour ranges, and then segmented based on one or more criteria. Features are then identified in the segmented image which are then used to determine platelet activation, proaggregation and procoagulant potential. These methods and apparatus may provide a more accurate determination of bleeding or clotting tendency with a small volume blood sample.

WO 2024/259537 A1

Devices and Methods for Predicting Bleeding and Thrombosis Diathesis

TECHNICAL FIELD

[0001] The invention relates to apparatus and methods for the assessment, quantification, and categorisation of bleeding and clotting tendencies.

BACKGROUND

[0002] In general, people vary from those who bleed too easily through to those at risk of thrombosis, where blood clots too readily, blocking blood vessels and leading to conditions such as deep vein thrombosis, stroke, or heart attack. Several assays of platelets function are currently available for laboratory and clinical use.

[0003] Thromboelastography (TEG) is a non-invasive test that quantitatively measures the ability of whole blood to form a clot. The principle of this *in vitro* test is to detect and quantify dynamic changes of the viscoelastic properties of a blood sample during clotting under low shear stress. The test is performed in a specially designed system called a thromboelastograph. The system uses a disposable cup in which a blood sample is placed, and a detection pin suspended in its center. The cup oscillates around the detection pin. Induced pin movement is recorded, and changes are measured as a function of time. Initially, there is little movement of the pin since liquid blood possesses minimal viscosity, and the oscillations of the cup are not transmitted to the pin. As the blood coagulates, it begins to adhere to both the cup and the pin, and movement of the cup induces motion on the pin.

[0004] Thromboelastometry (TEM), also named rotational thromboelastography (ROTEG) or rotational thromboelastometry (ROTEM), is an established viscoelastic method for hemostasis testing in whole blood. TEM investigates the interaction of coagulation factors, their inhibitors, anticoagulant drugs, blood cells, specifically platelets, during clotting and subsequent fibrinolysis. The rheological conditions mimic the sluggish flow of blood in veins.

[0005] Blood (~300 µl) is placed into the disposable cuvette using an electronic pipette. A disposable pin is attached to a shaft which is connected with a thin spring and slowly oscillates back and forth. The signal of the pin suspended in the blood sample is

transmitted via an optical detector system. The instrument measures and graphically displays the changes in elasticity at all stages of the developing and resolving clot.

[0006] The DiaPharma™ Atlas Platelet Strength Test (PST) uses a single-use microfluidic cartridge which incorporates an array of block and post microsensors. Analysis is based on the principle that upon successful aggregation and activation, platelets exert a contractile force upon the incorporated fibrin strands to remodel the platelet plug to minimize vascular disruption. The test card is connected directly to a blood collection syringe at its inlet and blood is automatically flowed over the block and post arrayed micro force sensors to produce platelet activating shear stress forces. Once properly adhered, platelets aggregate on the sensor blocks and exert a pulling force on the posts.

[0007] US 2020/292562 discloses a device for monitoring the spatial and temporal dynamics of thrombin, comprising a temperature-controlled sealed chamber with a transparent window and a light trap, said chamber being filled with a fluid medium and being designed to be capable of accommodating a cuvette containing a study sample of blood plasma, and into which a clot activating insert is introduced having a substance which initiates the clotting process applied to the lower end thereof, at least one means for lighting the sample, which is designed to be capable of receiving a light scattering signal from the sample, and at least one first irradiating means designed to be capable of exciting a fluorescence signal of a special marker which forms in the sample during the cleavage of a fluorogenic substrate.

[0008] US 2022/187292 discloses a diagnostic device which enables measurement of fibrinogen concentration in a blood sample. The device comprises; a wettable testing substrate including viewing indicators which allow determination of the status of a test. The substrate has a first end and second end and intermediate therebetween a flow receiving zone, a flow path zone, and a reaction zone; the reaction zone pre charged with at least one reagent. A blood sample to be tested is deposited near or in either of said flow receiving zone or said reaction zone, the sample reacting with the reagents inducing clotting of the sample. Water added to a dye added to said reaction zone, advances a distance along said substrate. The distance travelled along the substrate by the dye and through the sample is indicative of a measure of concentration of fibrinogen in said blood sample under test.

[0009] WO 2020/180662 discloses systems and methods for imaging and tracking fibrin formation via interaction of a test sample with a clotting agent or for imaging and tracking fibrin removal by an anti-clotting agent. In certain embodiments, the systems comprise a planar reflective substrate comprising one or more capture agents and/or one or more fibrin reference regions; a mount for holding the substrate; an illumination light source for directing illumination light toward a top surface of the substrate with fibrin formed thereon; an image detector aligned with respect to the mount for detecting a portion of the illumination light that is scattered by the fibrin, and/or reflected by the reflective substrate, thereby obtaining a label-free image of fibrin formation or fibrin removal; a processor of a computing device; and a memory having instructions stored thereon, wherein the instructions, when executed by the processor, cause the processor to: receive and/or access data corresponding to the one or more label free images, and use the one or more label-free images to determine one or more measures of fibrin formation or fibrin removal.

SUMMARY

[0010] In accordance with the present disclosure, there is provided a method for assessing, quantifying and categorising blood samples according to bleeding (or clotting) tendency, the method comprising:

obtaining an image of a blood sample labelled with one or more coloured indicators, and being mounted on a two-dimensional matrix;

segmenting and extracting features from the obtained image based on one or more criteria, wherein at least one of the criteria is based on colour; and

determining a measure of bleeding (or clotting) tendency based on the extracted features.

[0011] The blood sample may be labelled with two or more coloured indicators.

[0012] The method may comprise analyzing the obtained image in multiple different colour ranges. E.g., the image may be analyzed within various wavelength ranges, such as one or more of: violet: 380–450 nm; blue: 450–495 nm; green: 495–570 nm; yellow: 570–590 nm; orange: 590–620 nm; and/or red: 620–750 nm. Each coloured indicator range may include a characteristic frequency of one of the colour indicators and exclude characteristic features of other coloured indicators present, so that light detected in that range corresponds to that coloured indicator in order to help distinguish it from light emitted

from another coloured indicator that may be present. Each colour range may be a narrower range than the range of light detected by the imaging device.

[0013] The image may comprise a coloured image including pixels which span the multiple different colour ranges. The image may comprise multiple sub-images, each sub-image corresponding to a different colour range. E.g., the imager may take a sub-image of light emitted in the red spectrum, a sub-image of light emitted in the blue spectrum, and a sub-image of light emitted in the green spectrum. Each sub-image may be monochromatic (e.g., where the brightness of the pixel corresponds to the amount of light emitted within the corresponding colour ranges for that portion of the image).

[0014] The method may comprise imaging the blood sample over a period of time and monitoring the how the extracted features change over time.

[0015] The method may comprise obtaining multiple temporally spaced-apart images.

[0016] The segmentation and/or feature extraction of a previously obtained image may be used to segment and/or extract features from a subsequently obtained image to monitor the development of individual features over time. For example, the system may be configured to monitor how an individual platelet moves through a series of coagulation stages over time. E.g., if platelets are activated, but then do not go on to be ready to support clotting or actually clot, this can be used to determine what kind and dose of anticoagulant to administer.

[0017] The two-dimensional matrix may comprise a glass surface.

[0018] The blood sample may be deposited as a layer on the two-dimensional matrix.

[0019] A procoagulant agonist may be distributed on the two-dimensional matrix.

[0020] A procoagulant agonist may be added to the blood sample.

[0021] The procoagulant agonist may comprise a mixture of two or more of: collagen, fibronectin, and fibrinogen (e.g., in specific or predetermined ratios).

[0022] The segmentation and/or feature extraction comprises identifying one or more of: platelet membrane ballooning, procoagulant-spreading, microvesiculation, phosphatidylserine (PS) exposure, membrane thrombin and fibrin formation, and the differentiation of platelets into functionally distinct phenotypes.

[0023] The colour indicator may be a fluorescent or non-fluorescent labelled indicator.

[0024] The one or more coloured indicators may bind to proteins (or other materials) on an outer membrane of platelets and other blood cells such as red blood cells and/or

neutrophils within the blood sample. The one or more coloured indicators may selectively bind to platelets based on the stage of clotting (e.g., activated, pre-clotting, and/or actively clotting platelets). The one or more coloured indicators may selectively bind to different components in the blood (e.g., platelets, red blood cells, white bloodcells).

[0025] The controller may be configured to determine a measure of bleeding or clotting tendency based on one or more of: the ratio of colours in the image, the distribution of colours in the image, and the presence of particular cell shapes within the image.

[0026] The image may be obtained using a microscope and/or fluorescent viewer.

[0027] The blood sample may comprise isolated plasma platelets. Isolated plasma platelets may be a blood sample with other major components removed. The other major components may include neutrophils, red blood cells and/or white blood cells.

[0028] The blood sample may comprise whole blood.

[0029] The imager may have a resolution of less than 0.1 micron. The imager may have a resolution of less than 0.5 micron. The imager may have a resolution of less than 1 micron.

[0030] The step of determining a measure of bleeding or clotting tendency is based on previous associations between a bleeding or clotting tendency and the extracted features of images of blood samples.

[0031] The method may comprise using machine learning to determine a measure or category of bleeding or clotting tendency.

[0032] The method may comprise receiving feedback on the clot image of the person from whom the blood sample was taken, using the feedback to train or update a machine learning model, and using the trained or updated machine learning model to determining the measure of bleeding or clotting tendency based on extracted features of subsequently obtained images. Feedback on the clot image of the person may comprise personal information, such as age, biological sex, height, weight etc. Feedback on the person may comprise medical information, such as one or more of: medical history (e.g., a recent stroke or heart attack, or diseases which may affect haemostasis or clotting such as Covid-19), medical measurements (e.g., blood pressure, heart rate), family history (e.g., relatives who have had clotting or bleeding issues). Feedback on the person may comprise associating the blood sample with a measure of bleeding or thrombosis observed in that person (e.g., during a subsequent surgical operation).

[0033] The method may comprise receiving feedback on the person from whom the blood sample was taken and on interventions or mitigation measures administered to the person, and using the feedback to train or update a machine learning model, and using the trained or updated machine learning model to predict the effectiveness of mitigation measures based on extracted features of subsequently obtained images. Feedback on the interventions or mitigation measures may include the drug administered dose and timing of medication given to the person.

[0034] The determination of bleeding or clotting tendency may comprise one or more of the following: a diagnosis of platelet dysfunction or functioning; a procoagulation determination; a prediction of haemostatic or thrombotic response; and the categorisation of patients according to the tendency to form clots or bleed disproportionately during and after surgery or injury.

[0035] The determination of bleeding or clotting tendency may comprise categorising individuals according to the tendency to form clots or bleed disproportionately after drug administration.

[0036] The determination of bleeding or clotting tendency may comprise categorising individuals according to the tendency to form clots or bleed disproportionately due to inherited or acquired platelet dysfunction.

[0037] According to a further aspect, there is provided a method for categorising blood samples according to bleeding or clotting tendency, the method comprising:

obtaining an image of a blood sample mounted on a two-dimensional matrix;

segmenting and/or extracting features from the obtained image based on one or more criteria; and

determining a measure of bleeding or clotting tendency based on the extracted features.

[0038] According to a further aspect, there is provided an apparatus or device for categorising blood samples according to bleeding or clotting tendency, the method comprising:

an imager is configured to take a blood sample labelled with one or more colour indicators, and being mounted on a two-dimensional matrix; and

a controller, the controller configured to:

analyze the obtained image for different colour ranges;

segmenting and extracting features from the obtained image based on one or more criteria, wherein at least one of the criteria is based on colour; and

determining a measure of bleeding or clotting tendency based on the extracted features.

[0039] According to a further aspect, there is provided a method for categorising blood samples according to bleeding or clotting tendency, the method comprising:

obtaining an image of a blood sample comprising platelets, the blood sample being labelled with one or more colour indicators, and being mounted on a two-dimensional matrix;

processing the obtained image (e.g., electronically) to identify features based on one or more criteria, wherein at least one of the criteria is based on colour; and

determining a measure of bleeding or clotting tendency based on the identified features.

[0040] Processing the obtained image may comprise segmenting the image and/or extracting features from the image. The processing may be carried out using a processor, which may, for example, comprise electronic memory and/or comprise computer program code.

[0041] A measure of bleeding (or clotting) tendency may encompass a spectrum of bleeding tendencies including a tendency to bleed profusely, through a normal amount of bleeding, to a tendency to clot. A measure of bleeding tendency may encompass determining and/or quantifying platelets functionalities during the steps of haemostasis through the clotting stages (e.g., quiescent, preactivated, activated, or pro-aggregatory and procoagulant).

[0042] The blood sample may comprise platelets. Platelets are important for hemostasis. Activated platelets may be classified into two types, according to their agonist response: aggregating and procoagulant platelets. Aggregating platelets stretch out pseudopods to further attract platelets to the site of injury by connecting with fibrinogen. Aggregating platelets may release adenosine diphosphate (ADP). Procoagulant platelets promote the formation of thrombin and fibrin by interacting with coagulation factors and can thus be considered as the connector between primary and secondary hemostasis. In addition to their functions in blood coagulation, procoagulant platelets may release platelet microparticles and inorganic polyphosphate.

[0043] The coagulation agonist may comprise collagen. Collagen is the most abundant procoagulant agonist exposed during blood vessel injury. To arrest bleeding or during thrombosis, platelets adhere to themselves and to 2D matrices such as collagen and then undergo shape and size changes needed for their functioning.

[0044] Critical to the platelets' haemostatic response to arrest bleeding, is the surface exposure of inner aminophospholipids, particularly phosphatidylserine (PS), which promotes the assembly of the tenase and prothrombinase complexes on the platelet surface to facilitate the generation of thrombin and the localisation of coagulation.

[0045] Platelets adhering to subendothelial procoagulant agonists undergo retractable membrane blebbing, membrane evagination and PS externalization, irreversible membrane ballooning, focal-membrane adhesion, procoagulant membrane-spreading, membrane thrombin formation, aggregation as well as membrane shedding/ microvesiculation. Together, these events are known as procoagulant membrane dynamics (PMD). Moreover, we have shown that elements of platelet PMD are important amplifiers of blood clotting and sensitive monitors of haemostasis or thrombosis.

[0046] The apparatus may measure blood cell interactions and platelet PMD.

[0047] The colour indicator may comprise one or more of: $DIOC_6$ (3,3′-dihexyloxacarbocyanine iodide (green)); Alexa568-Annexin-V (red), 488 Dylight conjugated anti-GPIbβ antibody (green), conjugated anti-Pselectin antibody, conjugated anti-nitrotyrosine, conjugated anti-platelet glycoprotein antibody, conjugated anti-phosphatidylserine protein/antibody, conjugated anti-thrombin antibody, conjugated anti-platelet integrins antibody, conjugated anti-superoxide proteins/antibody MQAE (6-methoxyquinolinium derivative). The colour indicator may be configured to bind to platelet membranes.

[0048] The apparatus and method may require very small volumes of blood. Each blood sample may be less than 500 microlitres. Each blood sample may be less than 50 microlitres. Each blood sample may be less than 5 microlitres.

[0049] The apparatus may analyse results in less than 10 minutes. The imager may monitor the blood sample on the substrate for a period of time. The period of time may be less than 10 minutes. The period of time may be at least 10 minutes (e.g., between 15-50 minutes).

[0050] The imager may comprise a camera. The imager may comprise a video camera. The imager may comprise a CCD camera. The spectral range of the camera may span the characteristic frequencies emitted by the colour indicator. The spectral range of the illumination source may span the characteristic excitation frequencies of the colour indicator. The imager may record images at intervals of less than 1 minute. The imager may record at least 10 images over the imaging period of time.

[0051] The illumination source may be arranged to illuminate the surface of the sample facing the imager. The illumination source may be arranged to transmit light through the sample from the opposite side of the sample.

[0052] The apparatus may comprise a microscope for focusing an image onto the imager. The microscope may be a confocal microscope. The confocal microscope may be a spinning-disc confocal microscope. The microscope may be a video microscope. The microscope may be an epifluorescence microscope.

[0053] The image may be a two-dimensional image. The image may be a three-dimensional image.

[0054] The methods and apparatus may employ artificial intelligence (AI) techniques such as machine learning and iterative learning. Examples of such techniques include, but are not limited to, expert systems, case-based reasoning, Bayesian networks, behavior-based AI, neural networks, fuzzy systems, evolutionary computation (e.g., genetic algorithms), swarm intelligence (e.g., ant algorithms), and hybrid intelligent systems (e.g., Expert inference rules generated through a neural network or production rules from statistical learning). The methods and apparatus may use reinforcement learning, deep neural networks and/or recurrent neural networks.

[0055] The machine learning may be configured to examine one or more of: the spatial distribution of colour (e.g., fluorescence); and the morphometric characterization of platelets (e.g., size, shape, spreading).

[0056] The artificial intelligence involves segmenting the images to identify particular features. The particular features may include one or more of: membrane ballooning, procoagulant-spreading, microvesiculation, phosphatidylserine (PS) exposure, membrane thrombin and fibrin formation, membrane expression of granular releasates and regulatory proteins, and the differentiation into functionally distinct platelet phenotypes.

[0057] The machine learning may use supervised learning which comprises learning a function that maps an input to an output based on example input-output pairs. It may involve inferring a function from labeled training data consisting of a set of training examples. In supervised learning, each example is a pair consisting of an input object (e.g., features identified in the image) and a desired output value (e.g., bleeding tendency). A supervised learning algorithm may be configured to analyze the training data and produces an inferred function, which can be used for mapping new examples.

[0058] The apparatus may comprise an image data processor having a segmenter and identifier. A feature quantifier is configured to perform feature quantification. The image data are processed by image data processor which, using the segmenter and identifier, automatically segments and identifies relevant structures, features or materials from the image, then feature quantifier quantifies the relevant identified structures, features, material or combinations thereof.

[0059] Segmenting and extracting features in the image may comprise, or consist of, identifying platelets at different stages of coagulation, based on, for example, colour, morphology, size, shape and/or distribution. Segmenting and extracting features may comprise, or consist of, processing the image to identify features within the image (e.g., based on colour).

[0060] The segmentation may comprise semantic segmentation involves arranging the pixels in an image based on semantic classes. In this model, every pixel belongs to a specific class, and the segmentation model does not refer to any other context or information. For example, semantic segmentation performed on an image with multiple platelets, will provide a mask that categorizes all platelets into classes such as quiescent platelets, blebbed platelet, ballooned platelet, lamellipodial spread platelet, coalesced platelet etc.

[0061] The segmentation may comprise instance segmentation involves classifying pixels based on the instances of an object (as opposed to object classes). Instance segmentation algorithms do not know which class each region belongs to—rather, they separate similar or overlapping regions based on the boundaries of objects. This type of segmentation may provide a measure of how the platelet types are distributed across the substrate.

[0062] The segmentation may comprise panoptic segmentation. Panoptic segmentation combines semantic and instance segmentation. Like semantic segmentation, panoptic

segmentation is an approach that identifies, for every pixel, the belonging class. Unlike semantic segmentation, panoptic segmentation distinguishes different instances of the same class.

[0063] The segmentation may use edge-based segmentation; threshold-based segmentation; region-based segmentation; and/or Watershed segmentation.

[0064] The technique of extracting the features from the obtained image may help reduce the number of resources without losing any important or relevant information. Feature extraction helps to reduce the amount of redundant data from the data set. Feature Extraction may be used to identify particular objects within the image. Feature extraction may be used to identify platelets from other components in the blood (e.g., red blood cells). Feature extraction may be used to distinguish components in the blood from the background.

[0065] Feature extraction may be used to identify platelets at different stages of coagulation (e.g., distinguishing between quiescent platelets, activated platelets, pro-coagulated platelets and/or platelets actively engaged in clotting). Training the feature extraction may involve images of particular features being provided to the processor in associating with their type. This will allow machine learning to identify characteristic features (e.g., based on morphology, size, shape and/or colour). Feature extraction may be used to determine the total number of platelets in the image, the distribution of platelets in the image and/or the number of platelets at each stage in coagulation.

[0066] The device/apparatus may be bench top, hospital bedside operated or handheld.

BRIEF DESCRIPTION OF THE DRAWINGS

[0067] In the Detailed Description section below, one or more embodiments of the present technology are described in relation to the attached figures. These embodiments are intended to provide a better understanding of the invention, how the invention may be put into practice, and to demonstrate some of the advantages of the invention. The drawings are not necessarily to scale, emphasis instead being placed upon illustrating the principles of various embodiments of the invention. Similar reference numerals indicate similar components.

Figure 1a is a schematic diagram showing various phases in a platelets development during clotting.

Figures 1b-e are microscope images corresponding to the stages depicted in figure 1a.

Figure 2 is a schematic diagram of the apparatus used to determine tendency of a patient to bleed or clot.

Figure 3 is a flowchart showing the method of determining the clotting or bleeding tendency of a patient.

Figure 4a is a microscope image of a blood sample.

Figures 4b and 4c are zoomed in regions of the image of figure 4a which include features which would be coloured differently using coloured indicators.

DETAILED DESCRIPTION

Introduction

[0068] As noted above, there is a range of bleeding and clotting tendencies, from people who bleed too easily through to those at risk of thrombosis, where blood clots too readily, blocking blood vessels and leading to conditions such as deep vein thrombosis, stroke, or heart attack. Blood platelets are the central cell involved in this process; and although several assays of platelets function are currently available for laboratory and clinical use (as described in the background), none provide estimates accurate enough to:

1. understand platelet functioning or dysfunction; and
2. predict whether a patient tends to bleed or is at high risk of thrombosis.

This is largely because conventional in-vitro thromboelastometric tests such as TEG, ROTEM, or aggregometry are 'platelet in suspension' based, and fail to capture pivotal *in-vivo* platelet procoagulation mechanisms.

[0069] *In-vivo*, platelets adhere to matrixes such as the vessel wall where unique morphological transformations can occur that are integral to platelet procoagulant and haemostatic functions. These processes cannot be readily assessed by conventional *in vitro* platelet function tests which rely on surrogate measures of platelets ability to aggregate. Therefore, screening to assess a patient's innate bleeding or thrombosis risks remains an unmet clinical need for precision medicine, especially before, during and after surgeries.

[0070] Similar need for accurate estimates of platelet function exists in acquired thrombo-haemorrhagic conditions such as in preeclampsia, surgeries utilising the cardiopulmonary

bypass machine and in severe COVID-19 infection. Furthermore, many clinicians would not use current platelet tests on young children, because it requires too much blood, and only assesses a facet of haemostasis.

[0071] There is, therefore, a need in several clinical settings, for a reliable and small volume coagulation test system. The present technology may enable users to perform one or more of the following:

1. reliably estimate haemostatic function
2. diagnose platelet dysfunction, and/or
3. categorise patients according to risks of thrombosis or bleeding.

[0072] The present technology is based on the membrane changes that occur in human platelets when they change shape, size and membranes ('skin') properties to arrest bleeding or stick together to form clots that eventually block blood vessels, and lead to stroke, heart attack or deep vein thrombosis.

[0073] The inventor has identified, developed and tested several specially made 'coloured dyes' which bind to platelets 'skin' proteins, and which can be used to visualise and track specific aspects of these changes. The inventor has also identified features during the clotting process using these dyes. E.g., highly visible features (shining brightly) or changed features (dimmed or patchy) that indicate normal or abnormal blood clotting. Also, the inventor has identified features around the appearance and distribution of the dyes on the platelet 'skin', relating to whether the platelet is active and able to support clotting, and by how much it will, or whether the platelet is fatigued or inactive and hence likely to allow bleeding.

[0074] The present technology brings together Procoagulant Membrane Dynamics (PMD) data linked to bleeding or thrombosis (e.g., including how platelets develop over time during clotting) and an Artificial Intelligence (AI) powered PMD analysis that assesses coagulation in images of primary haemostatic plugs *in-vitro*, and categorizes patients based on the risk of thrombosis or bleeding. These indicators may be available quickly by uploading into a web application (or another remote computer) a specially prepared image of patient's blood sample.

[0075] Various aspects of the invention will now be described with reference to the figures. For the purposes of illustration, components depicted in the figures are not necessarily drawn to scale. Instead, emphasis is placed on highlighting the various contributions of

the components to the functionality of various aspects of the invention. A number of possible alternative features are introduced during the course of this description. It is to be understood that, according to the knowledge and judgment of persons skilled in the art, such alternative features may be substituted in various combinations to arrive at different embodiments of the present invention.

Procoagulant Membrane Dynamics (PMD)

[0076] To arrest bleeding or during thrombosis, platelets adhere to themselves and to 2D matrices such as collagen and then undergo shape, size and structural changes needed for their functioning. These pathways are shown schematically in figure 1a. Images of various stages depicted in figure 1a are shown in figures 1b-e.

[0077] During a clotting process, platelet undergo membrane ballooning, procoagulant-spreading, microvesiculation, phosphatidylserine exposure, membrane thrombin and fibrin formation, membrane expression of granular releasates and regulatory proteins, and the differentiation into functionally distinct platelet phenotypes.

[0078] Procoagulant Membrane Dynamics (PMD) analysis uses imaging technology to quantify these prothrombotic/procoagulant changes after specific stimulation. Assays like this which monitor haemostatic plug formation and PMD over solid matrixes are more likely to predict coagulability better than volumetric techniques like ROTEM. The inventor has characterised the spatiotemporal dynamics and drivers of the dramatic morphological transformation human platelets undergo during haemostasis or thrombosis.

[0079] Figure 1a is a schematic diagram of the Procoagulant Membrane Dynamics (PMD) of a Human Platelet.

[0080] Upon collagen activation, quiescent platelet (A), will experience a rise in cytosolic calcium (Ca^{2+}), and the opening of nonspecific and Ca^{2+} activated chloride channels 123. The platelet may undergo blebbing and filopodial (B) and or lamellipodial spreading (C) or undergo membrane ballooning (D-H, D-E).

[0081] Ballooning is induced by salt/water entry (facilitated by water channel AQP1 121) and lead to stretching of the plasma membrane 120 (as shown in the left zoomed-in diagram), the opening of mechanosensitive cation (MSC, TRPC6 122) channels, additional influx of extracellular Ca^{2+}, and a sustained rise in cytosolic Ca^{2+}. The result is PS externalization and the distinct phenotypic transformation to D, E, F, G and H.

[0082] It is the combination of internal hydrostatic pressure and external osmotic pressure which lead to irreversible membrane ballooning (D-H, D-E). Membrane ballooning is facilitated by microtubule disruption and may lead to the formation of an expansive PS-rich procoagulant surface and microvesicles through multiple coalescences (G–H). Some platelets will not undergo the ballooning process after activation (B, C) while others will balloon without proceeding to procoagulant-spread phenotype (E).

[0083] Figure 1b-e are scanning electron microscope images of various platelet phenotypes associated with the graphic depiction of the stages of platelet procoagulant remodelling as shown in figure 1a (labelled with corresponding letters.) In each of figures 1b-e, a scale bar is provided. the scale par corresponds to 1 μm for figures 1b and 1d, and 3 μm in figures 1c and 1e.

[0084] In the present technology, optical images are used to identify whether a blood sample is associated with a tendency to clot or a tendency to bleed.

Apparatus and Method

[0085] Figure 2 is a schematic of the apparatus used to perform the method. A user follows a protocol to generate a clot image (CLiM) from pinprick volume (e.g., less than 5 microliters) of a patient’s blood. The clot image is then processed (e.g., after being uploaded into a web application) to obtain one or more of:

1. a measure of platelet dysfunction or functioning;
2. procoagulation assessment and quantification;
3. a prediction of haemostatic or thrombotic response; and
4. the categorisation of patients according to the tendency to form clots or bleed disproportionately during and after surgery or injury.

[0086] The apparatus 200 shown in figure 2 comprises one or more illumination sources 202 configured to provide a spectrum of illumination across the spectral range of the colour indicators (e.g., to allow the colour indicators to be excited), an imager 203 configured to detect a two-dimensional image of the blood sample 210 on the substrate 211. The imager is also configured to detect the characteristic frequencies of the colour indicators. The imager is connected to a controller 204 which in turn is connected to a user interface 205. In fluorescence microscopy, the excitation frequencies/spectrum of a colour indicator may be different from the characteristic emission frequencies/spectrum.

[0087] The user would take a blood sample from a person (or animal) and place it on a two-dimensional substrate 211. The blood sample is mixed with fluorescently labelled indicators either before or after being placed on the substrate. In this case, the substrate 211 comprises a glass plate coated with a preset mixture of procoagulant agonists pre-spread in a layer on the substrate surface. The procoagulant agonists in this case comprises a mixture of collagen type I, IV, fibronectin, laminin, and/or fibrinogen. The procoagulant agonists mix may comprise at least 10% wt. of each of the components. The collagen may comprise a mix of collagen type 1 and collagen type 4 (e.g., in a 50:50 ratio by weight).

[0088] As described above, to arrest bleeding or during thrombosis, platelets adhere to themselves and to 2D matrices such as collagen and then undergo shape, size and structural changes needed for their functioning. During this process, platelet undergoes membrane ballooning, procoagulant-spreading, microvesiculation, phosphatidylserine (PS) exposure, membrane thrombin and fibrin formation, membrane expression of granular releasates and regulatory proteins, and the differentiation into functionally distinct platelet phenotypes.

[0089] The substrate and mixture of procoagulant agonist mimic the conditions that blood would experience at the surface of a wound or broken blood vessel.

[0090] The imager 203 monitors the blood cells as it undergoes changes on the substrate to quantify these prothrombotic/procoagulant changes after specific stimulation. The blood sample may be incubated throughout the image gathering. The image may be acquired from blood cells allowed to adhere to the substrate for 15-50 min with or without flow over the substrate.

[0091] A flowchart of the method used to determine a measure of bleeding tendency 306 is shown in figure 3. As described above, one or more images are obtained 301 of the blood sample mounted on a two-dimensional substrate and labelled with colour indicators. From these images the image is segmented 302. From the segmented image, features are extracted 303 from the image. In this embodiment, this allows the controller to determine the number, size and distribution of various types of platelet (e.g., quiescent platelet, microvesicles, ballooned platelet etc.) over time. The machine learning 304 then examines the extracted features to come to a decision on the particular parameter of interest (e.g., bleeding tendency or procoagulant potential). This decision is

communicated to the user by providing an output 306 at the user interface. Feedback on the output and/or the decision may be fed into an adaptive learning algorithm 308 to either train or improve the machine learning algorithm 304.

Image Processing

[0092] The apparatus monitors changes in the field of view over time using artificial intelligence (AI). In this case, images will be captured from multiple points (e.g., 5 points, or smaller areas of the sample surface) along the surface or channel of substrate at 0.5 - 1 min intervals for 15-50 min.

[0093] In this case, colour indicators are added to the sample to perform various functions. In a sample comprising isolated plasma platelets (e.g., a blood sample with red and white blood cells removed), colour indicators may be used to mark different stages in procoagulation and cellular haemostasis. For example, a first (e.g., green) dye may be used to measure the degree of activation to distinguish between quiescent and activated platelets. A second (e.g., red) dye may be used to indicate those platelets which are ready to support clotting (e.g., associated with phosphatidylserine, PS). PS is important in regulating the production of thrombin, the central regulatory molecule of blood coagulation. PS is normally located on the cytoplasmic face of the resting platelet membrane but can appear on the plasma-oriented surface of activated platelets and discrete membrane vesicles derived from activated platelets. A third (e.g., magenta) dye may be used to indicate thrombin generation on platelet and microvesicles membranes (e.g., platelets which are actively aggregating). A fourth (e.g., cyan or blue) dye may be used to indicate superoxide generation within the platelets. Platelets become permeable when activated, allowing the dye to stain the cytosol. The inventor has previously demonstrated this phenomenon. It will be appreciated that other colours may be used. In some embodiments, there may only be two dyes.

[0094] Clotting would typically move through all three stages, so typically a platelet would be marked with the first dye, then the second, and then the third. In practice, the second and third dyes may appear in quick succession or almost simultaneously.

[0095] The artificial intelligence involves segmenting the images to identify particular features. The segmentation may use the colours of various portions of the image, and shapes of various features within the image. Figure 4a is a clot image. Using the coloured indicators, different responses of the platelets are coloured differently. In this case, the

circular platelets are coloured red (as shown in zoomed-in portion in figure 4b), and the more diffuse platelets are coloured green (as shown in zoomed-in portion in figure 4c). These different coloured regions correspond to platelets at different stages of the haemostatic response and blood clotting.

[0096] The artificial intelligence may look at one or more of: the ratio of colours in the image, the distribution of colours in the image, and the presence of particular shapes within the image.

[0097] In this way, each patient's unique primary haemostatic plug or clot formation pattern is captured on microscope images. In this embodiment, images are analyzed in a series of segmentation and feature extraction techniques for different spectra including morphological image processing techniques for image analysis. For example, the colour distribution of the sample indicates the proportion of platelets at different stages in the clotting process. A morphological analysis of the platelets may also allow the image to be analysed to determine how the platelets are responding to the procoagulant agonist.

[0098] Features from the resulting images are extracted. Different types of general-purpose and deep learning algorithms are utilized in building and validating models. The models are evaluated, and parameters tuned to improve performance. The final models are deployed and integrated into a user interface to categorise patients according to thrombosis or bleeding risk.

[0099] In some embodiments, the machine learning may be configured to identify particular issues with the haemostatic process. For example, using the coloured indicators described above, if a large proportion of the platelets are being dyed with the first activated dye, indicating that the platelets are being activated, but then are not subsequently being marked with the second or third dye (i.e., they are not actually procoagulant), this may indicate a problem with the clotting mechanism for this particular individual. The machine learning may then recommend a low dose of an antiplatelet (e.g., aspirin) in order to mitigate the effects of microaggregation which may damage small diameter blood vessels. In contrast, if a large proportion of the platelets were procoagulant and pro-aggregatory, this may correspond to an individual who was likely to experience significant clotting. The machine learning may then recommend a full dose of a stronger antiplatelet (e.g., Clopidogrel and other P_2Y_{12} blockers) to mitigate the risk of a major clot forming.

[0100] In other embodiments, the dyes may also be used to identify particular components in the blood. For example, in a whole blood sample, a dye may be added to bind selectively on to the platelets. Then this dye may be used by the machine learning to identify the platelets, and then the other dyes can be used to determine the procoagulant features of the platelets. E.g., a platelet dye (e.g., blue) may be configured to dye to bind to glycoprotein V (GP5), glycoprotein IX (GP9), platelet integrin **α2β**1 or another material specific to the surface of the platelets under examination.

Categorization

[0101] Initially, the apparatus is configured to categorise patients into one of a plurality of distinct patient groups. For example, based on known volumes of blood loss after the same surgery, the categories may include:

1. normal or controls (NoM);
2. minor bleeders (MiB); and
3. minor clotters (MiC).

[0102] The apparatus may be configured to identify a MiB and MiC patients based on diminished and enhanced platelet PMD respectively, compared to controls. The apparatus may also be used to identify mega bleeders (MgB) or mega clotters (MgC).

[0103] The AI powered technology solution will analyse the morphometric characteristic (intensity and distribution) of the coloured indicators (e.g., Red, Blue and Green) colours over a contrast backgrounds (e.g., black) in our clotting image. The algorithm will determine the various relationships between various colours (distribution and intensity) in clotting images of specific patient groups. These relationships will then be linked to real-life clinical outcomes (blood loss, clot formation). In this embodiment, the AI analysis output determines a procoagulant index (Prog-Index), e.g., normalised to numbers ranging from 1 to 100 and used to categorise patients into groups.

Training/Feedback

[0104] To train the algorithm, a blood sample may be analysed by the apparatus and associated with known information about the quantity of bleeding during a particular surgery. This known information may be quantitative (e.g., a volume collected from the operating theatre) or qualitative (e.g., feedback provided by the medical staff based on their experience).

[0105] Another source of information which may be used to train the apparatus would be benchmark the apparatus against known tests. For example, a blood sample may be analysed by the apparatus and associated with known information from another blood coagulation test (e.g., ROTEM, TEG). This initial training can then be refined based on feedback from particular patients undergoing surgery.

[0106] Another source of information which may be used is for the apparatus to be provided with images of platelets at different coagulation stages, with the coagulation stage identified. This may allow the system to identify platelets within images as belonging to particular coagulation stages (e.g., based on a combination of colour, size, and/or morphological shape)

[0107] After the apparatus has been set up, the apparatus may continue to learn based on feedback. For example, the apparatus may provide a measure of bleeding tendency before surgery, and the user may provide feedback on the actual bleeding after the surgery is complete.

[0108] The feedback may comprise information on the quantity of bleeding during a particular surgery, and also possibly on any mitigation measures taken. For example, if the initial test indicates that the patient is likely to clot, the patient may be given an anticoagulant (e.g., apixaban, dabigatran, edoxaban, rivaroxaban, warfarin etc.) to mitigate the effects of their natural tendency to clot. This information should be provided to the apparatus so that, in this case, a higher-than-expected bleeding tendency can be ascribed to the mitigation measures and not to errors in the determination of bleeding tendencies.

[0109] Based on feedback comprising information on mitigation measures, the apparatus may be configured to determine the effect of mitigation measures on particular patients, and propose suitable mitigation measures for particular patients in order to move the patient into a safe zone of bleeding tendency for a particular surgery.

[0110] As categorizations are made, a feedback loop is created to feed the patient’s outcome back to the models to improve its performance. Various algorithms are used to orchestrate the interactions between the patient's image information and models, this way, the models are trained continuously from experience to improve its decision making.

[0111] In this way, the apparatus may be used to categorise patients on admission, into subgroups of prothrombotic or bleeding phenotypes based on their predicted haemostatic response, and thus enable individualised care or precision medicine.

[0112] The technology may also be adapted through further research to monitor anticoagulation therapy and inform dose titration of antithrombotic agents.

Advantages

[0113] At present, coagulation assays have failed to correlate with clinical outcomes such as clotting and bleeding diathesis; this is mainly because conventional in-vitro thromboelastometric tests such as TEG, ROTEM, or aggregometry are ‘platelet in suspension’ based, and do not quantify in-vivo platelet procoagulation mechanisms. In-vivo, platelets adhere to matrixes such as the vessel wall where unique morphological transformations can occur that are integral to platelet procoagulant and haemostatic functions. These processes cannot be readily assessed by conventional in vitro platelet function tests which rely on surrogate measures of platelets ability to aggregate or are based mainly on the number and responses of platelets in suspension.

[0114] The present technology monitors the spatiotemporal dynamics and drivers of the dramatic morphological transformation human platelets undergo during haemostasis or thrombosis. Accordingly, by assessing platelet adhesion, the primary haemostatic plug and platelet procoagulant dynamics over solid matrixes is more likely to predict coagulability better.

[0115] The present technology assesses clotting under more physiologically relevant conditions and predicts procoagulation better. It is the only product with predictive capability for whether a patient tends to bleed or clot. The innovation is AI-powered, minute-volume based and offers reliable results in 15-50 minutes. Notably, since our innovation utilises just a pinprick volume of blood for tests, it is very suitable for use in the paediatric population.

[0116] The present technology is less affected by platelet numbers in the blood than other blood clotting tests. For example, TEG or ROTEM require a certain level of platelets in order to provide an accurate result. Imaging the platelets means that, for example, in patients with diseases like cancers or others in which platelet count is affected, the present technology can still accurately quantify platelet function and procoagulant potential.

Other Options

[0117] The imaging apparatus may be provided as a handheld portable device. The sample may be taken as with a pinprick (e.g., similar to home blood sugar level tests for diabetics) and added to a disposable slide for imaging. The processing may occur within the device, or the image may be transferred to a remote computer for processing.

[0118] The system may be used to monitor the blood health of women during pregnancy. It is well known that women are at higher risk for a blood clot during pregnancy, childbirth, and up to 3-months after delivering a baby. This risk may be monitored using the present technology to determine the risk of thrombosis.

[0119] Although the present invention has been described and illustrated with respect to preferred embodiments and preferred uses thereof, it is not to be so limited since modifications and changes can be made therein which are within the full, intended scope of the invention as understood by those skilled in the art.

CLAIMS

1. A method for categorising blood samples according to bleeding or clotting tendency, the method comprising:

obtaining an image of a blood sample comprising platelets, the blood sample being labelled with one or more colour indicators, and being mounted on a two-dimensional matrix;

processing the obtained image to extract features based on one or more criteria, wherein at least one of the criteria is based on colour; and

determining a measure of bleeding or clotting tendency based on the extracted features.

2. The method according to claim 1, wherein the method comprises imaging the blood sample over a period of time and monitoring the how the extracted features change over time.

3. The method according to any one of claims 1-2, wherein the method comprises obtaining multiple temporally spaced-apart images.

4. The method according to claim 3, wherein the processing of the obtained image comprises segmenting the obtained image, and wherein the segmentation of a previously obtained image is used to segment a subsequently obtained image to monitor the development of individual features over time.

5. The method according to any one of claims 1-4, wherein the two-dimensional matrix comprises a glass surface.

6. The method according to any one of claims 1-5, wherein a procoagulant agonist mixture is distributed on the two-dimensional matrix.

7. The method according to any one of claims 1-5, wherein a procoagulant agonist mixture is added to the blood sample.

8. The method according to any one of claims 1-7, wherein the procoagulant agonist mixture comprises two or more of: collagen type I, collagen type IV, fibronectin, laminin, and fibrinogen.

9. The method according to any one of claims 1-8, wherein one of criteria uses the morphology of platelets within the sample to identify one or more of: platelet membrane ballooning, procoagulant-spreading, microvesiculation, phosphatidylserine (PS) exposure, membrane thrombin and fibrin formation, and the differentiation into functionally distinct platelet phenotypes.

10. The method according to any one of claims 1-9, wherein the colour indicator is a fluorescent labelled indicator.

11. The method according to any one of claims 1-10, wherein multiple colour indicators are used, each colour indicator being configured to mark platelets at different stages of procoagulation and haemostasis or thrombosis.

12. The method according to any one of claims 1-11, wherein the one or more colour indicators bind to proteins on an outer membrane of platelets within the blood sample.

13. The method according to any one of claims 1-12, wherein the controller is configured to determine a measure of bleeding or clotting tendency based on one or more of: the ratio or mathematical computation of colours in the image, the distribution of colours in the image, and the presence of particular shapes within the image.

14. The method according to any one of claims 1-13, wherein the image is obtained using a microscope or fluorescent imager.

15. The method according to claim 1, wherein the blood sample comprises isolated plasma platelets.

16. The method according to any one of claims 1-15, wherein the blood sample comprises whole blood.

17. The method according to any one of claims 1-16, wherein the step of determining a measure of bleeding or clotting tendency is based on previous associations between a bleeding or clotting tendency and the extracted features of images of blood samples.

18. The method according to any one of claims 1-17, wherein the method comprises using machine learning to determine a measure of bleeding or clotting tendency or to categorise according to bleeding or clotting tendencies.

19. The method according to any one of claims 1-18, wherein the method comprises receiving feedback on the person from whom the blood sample was taken, and using the feedback to train or update a machine learning model, and using the trained or updated machine learning model to determining the measure of bleeding or clotting tendency based on extracted features of subsequently obtained images.

20. The method according to any one of claims 1-19, wherein the method comprises receiving feedback on the person from whom the blood sample was taken and on mitigation measures administered to the person, and using the feedback to train or update a machine learning model, and using the trained or updated machine learning model to predict the effectiveness of interventions or mitigation measures based on extracted features of subsequently obtained images.

21. The method according to any one of claims 1-20, wherein the determination of bleeding or clotting tendency comprises one or more of the following: a determination of platelet dysfunction or functioning; a procoagulation determination; a prediction of haemostatic or thrombotic response; and a categorisation of individuals according to the tendency to form clots or bleed disproportionately during and after surgery or injury.

22. The method according to any one of claims 1-21, wherein the determination of bleeding or clotting tendency comprises categorising individuals according to the tendency to form clots or bleed disproportionately after drug administration.

23. The method according to any one of claims 1-22, wherein the determination of bleeding or clotting tendency comprises categorising individuals according to the tendency to form clots or bleed disproportionately due to inherited or acquired platelet dysfunction.

24. An apparatus for categorising blood samples according to bleeding or clotting tendency, the method comprising:

an imager configured to take blood sample comprising platelets, the blood sample being labelled with one or more colour indicators, and being mounted on a two-dimensional matrix; and

a controller, the controller configured to:

process the obtained image to extract features based on one or more criteria, wherein at least one of the criteria is based on colour; and

determining a measure of bleeding or clotting tendency based on the extracted features.

Figure 1a

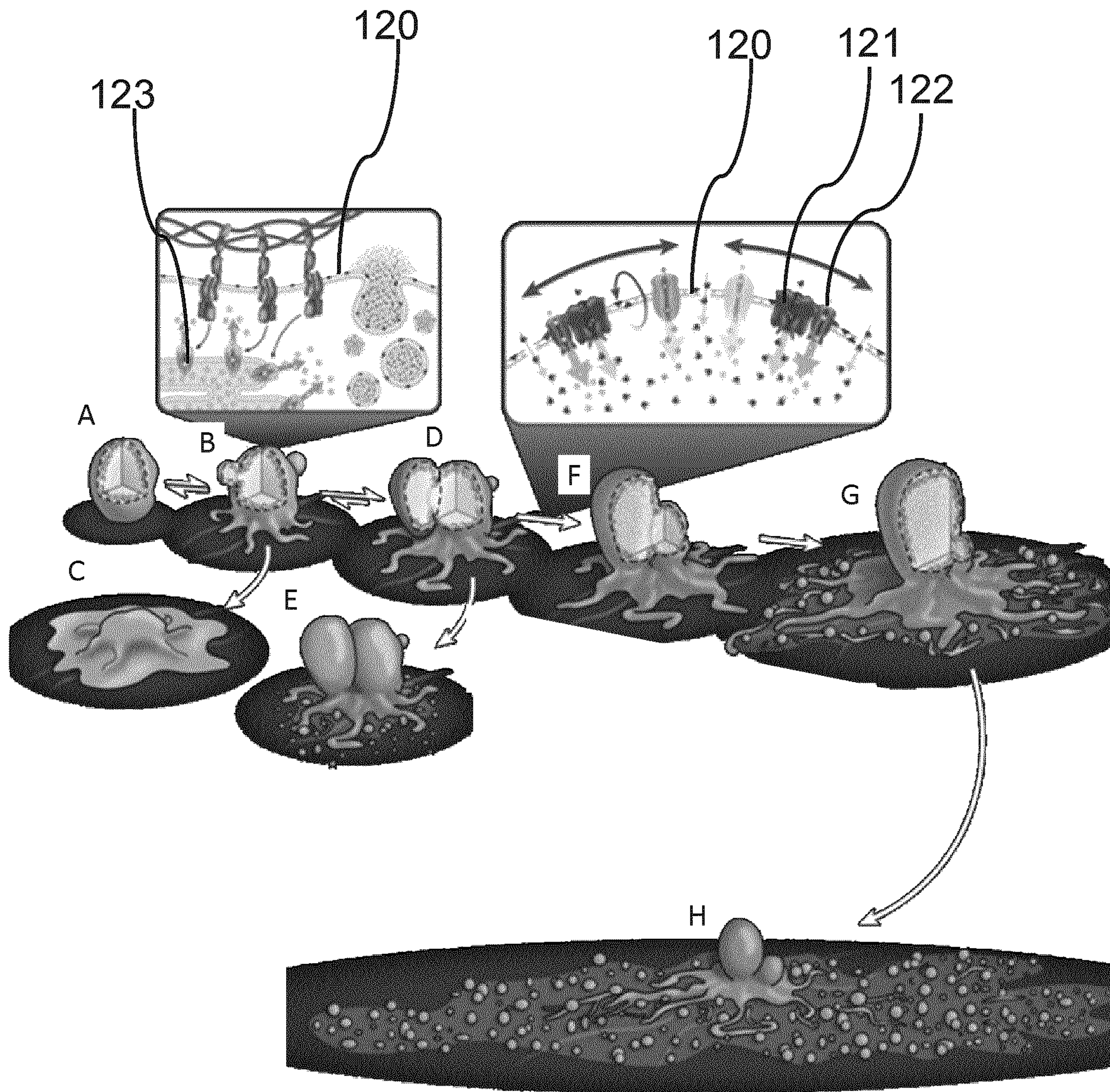


Figure 1b

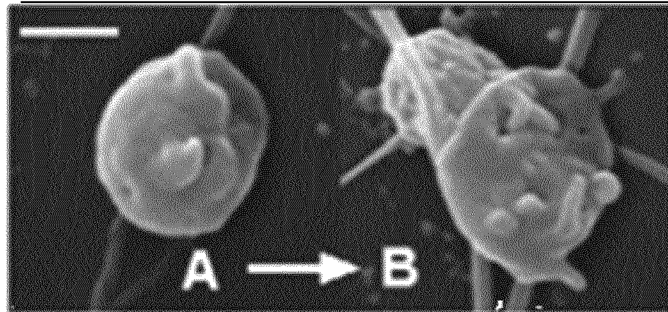


Figure 1c

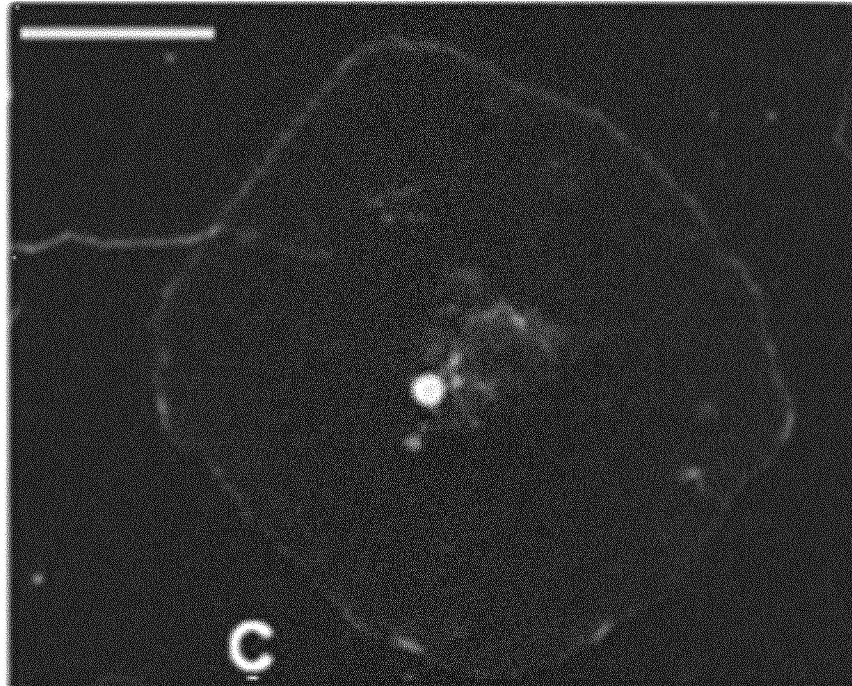


Figure 1d

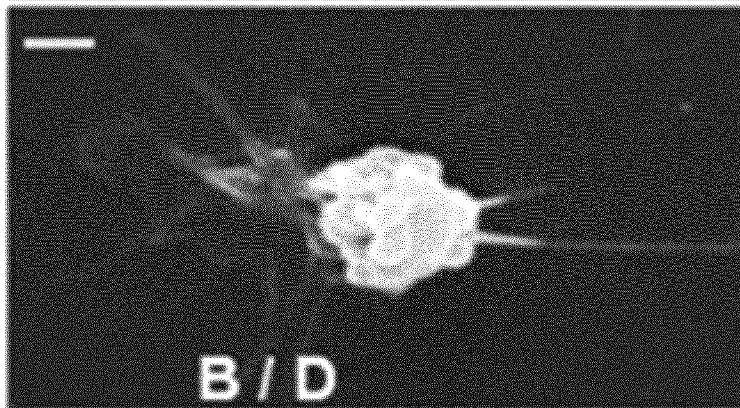


Figure 1e

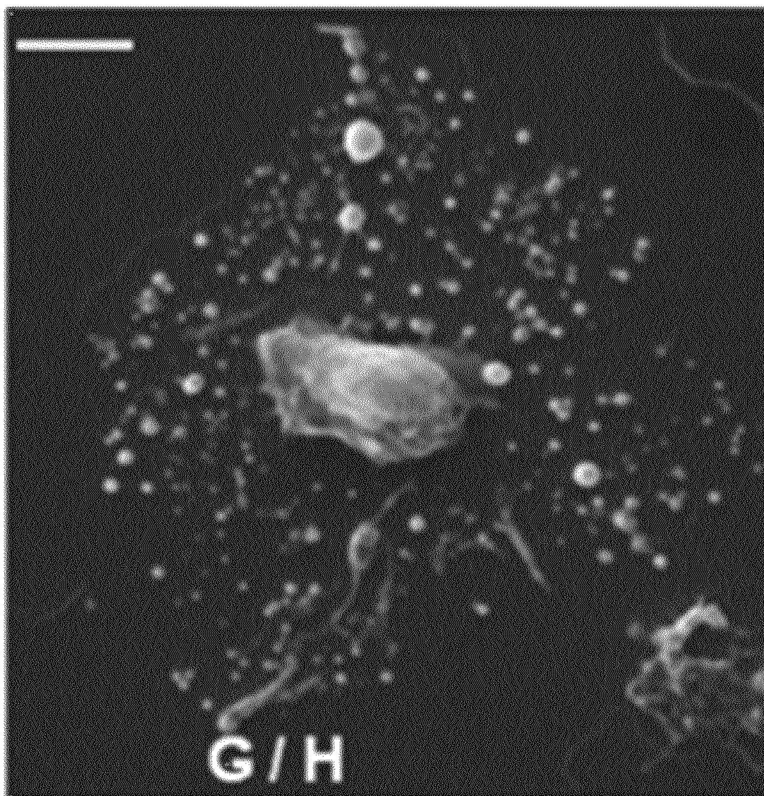


Figure 2

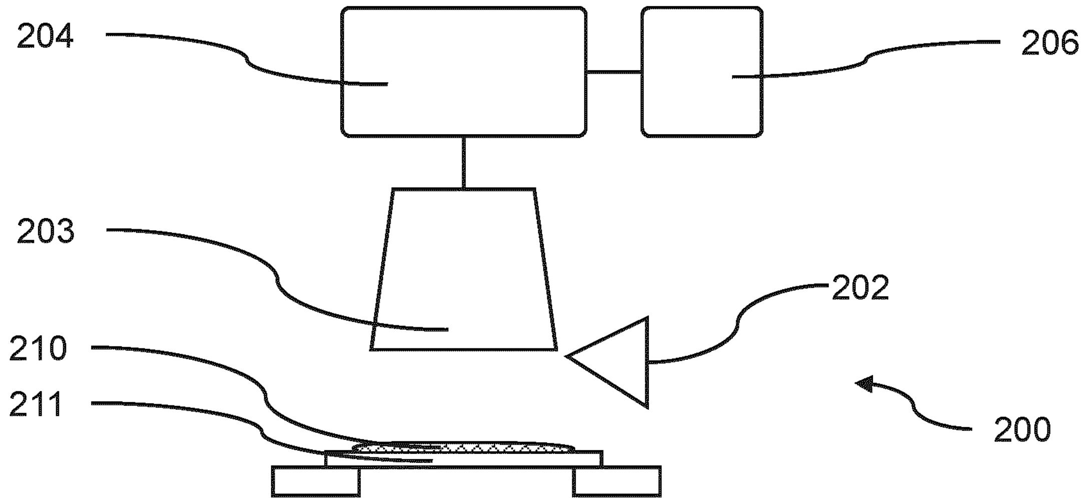


Figure 3

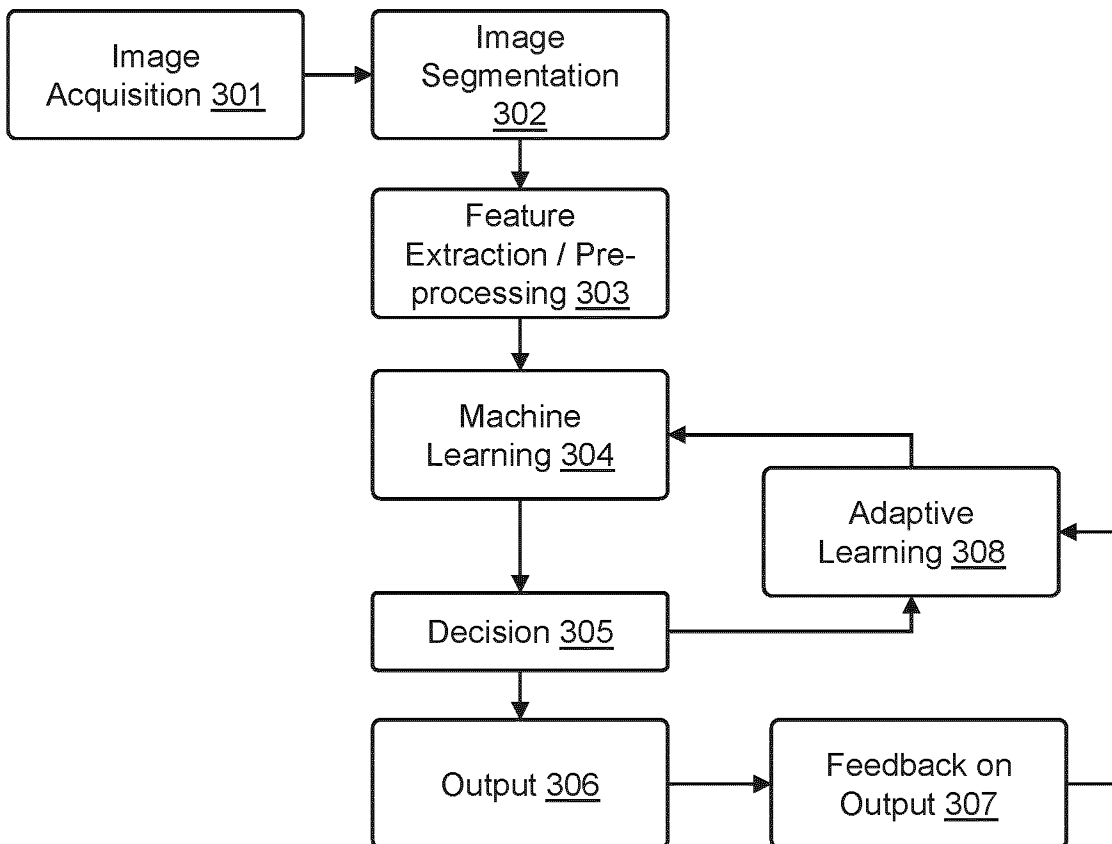


Figure 4a

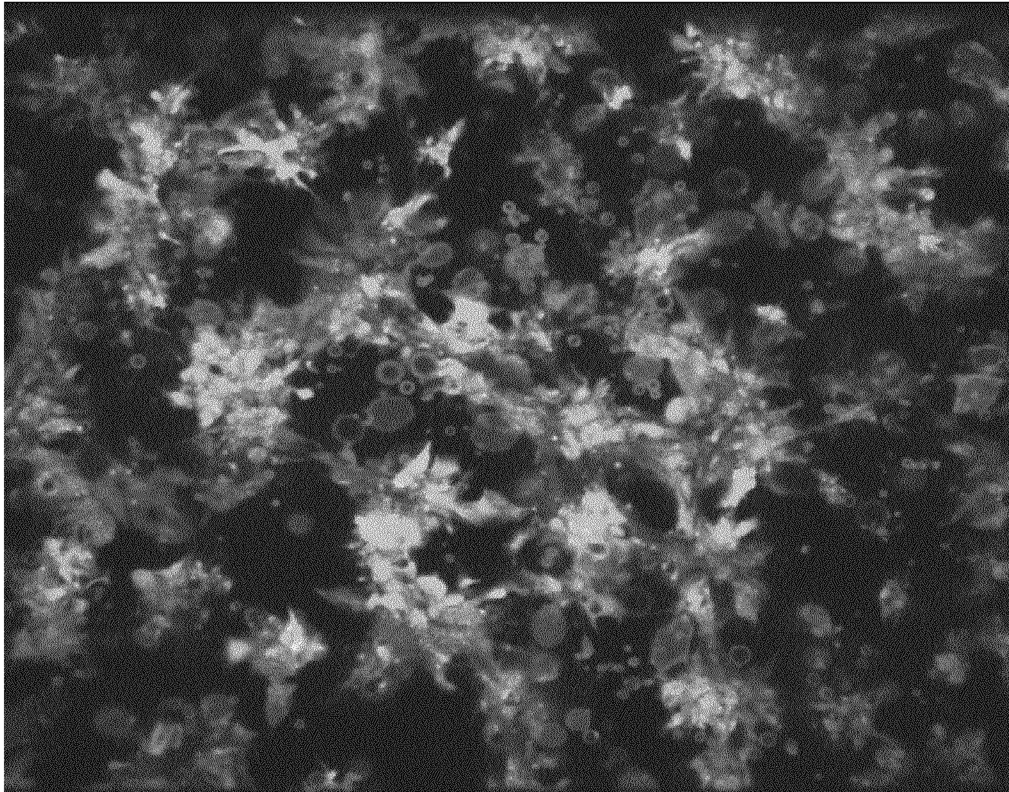


Figure 4b

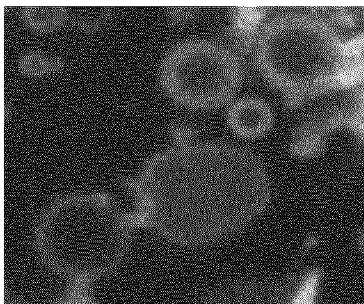
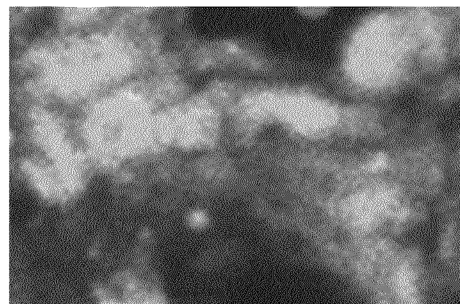


Figure 4c



INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2024/050840

A. CLASSIFICATION OF SUBJECT MATTER

IPC: ***G01N 15/01*** (2024.01)

CPC: **G01N 15/01** (2024.01), G01N 15/018 (2024.01)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
Keywords used across the whole IPC

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

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Questel-Obrit (FamPat) keywords: bleed+, clott+, imag+, color+, colour+, hemostasis, hemosta+, blood, platelet+, thromb+, fluorescen+, procoagulant, mount+, matrix, machine learn+, artificial intellig+, neural network.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	US 2021/0018489 A1 (RAZAVI, J. et al.) 21 January 2021 (21-01-2021) * See Abstract; paragraphs [0012], [0014]-[0015], [0064], [0066]-[0067], [0069]-[0071], [0077]-[0088], [0096], [0106]-[0110]; Figs. 1A, 2A. *	1 to 8, 11 and 13 to 23 9, 10 and 12
Y	US 2022/0193272 A1 (DeANGLIS, A. et al.) 23 June 2022 (23-06-2022) * See paragraphs [0014], [0018], [0067]-[0069], [0075]-[0083], [0094]-[0095], 0103]-[0107], [0109]-[0124]. *	9, 10 and 12
A	US 2023/0003658 A1 (BULL, B. S. et al.) 05 January 2023 (05-01-2023) * See entire document. *	

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

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"D"	document cited by the applicant in the international application	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"E"	earlier application or patent but published on or after the international filing date	"&"	document member of the same patent family
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)		
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
04 September 2024 (04-09-2024)

Date of mailing of the international search report
09 September 2024 (09-09-2024)

Name and mailing address of the ISA/CA
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INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2024/050840

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
US2021018489A1	21 January 2021 (21-01-2021)	None	
US2022193272A1	23 June 2022 (23-06-2022)	CN116635532A	22 August 2023 (22-08-2023)
		EP4262884A1	25 October 2023 (25-10-2023)
		JP2024502748A	23 January 2024 (23-01-2024)
		KR20230124023A	24 August 2023 (24-08-2023)
		WO2022137002A1	30 June 2022 (30-06-2022)
US2023003658A1	05 January 2023 (05-01-2023)	CA3159293A1	03 June 2021 (03-06-2021)
		EP4065956A1	05 October 2022 (05-10-2022)
		EP4065956A4	20 March 2024 (20-03-2024)
		JP2023504451A	03 February 2023 (03-02-2023)
		WO2021108811A1	03 June 2021 (03-06-2021)