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(54) **DEVICE TO EXPOSE CELLS TO FLUID SHEAR FORCES AND ASSOCIATED SYSTEMS AND METHODS**

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USPC ... **435/6.1**; 435/287.1; 435/287.2; 435/288.7;
435/7.1

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(57) **ABSTRACT**

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The present technology relates generally to devices to expose cells to fluid shear forces and associated systems and methods. In particular, several embodiments are directed toward devices to expose cells to fluid shear forces in order to measure changes in internal cell forces. In some embodiments, a fluidic device includes a flow unit configured to induce fluid flow through the device. The device further includes a fluid channel configured to accept a biological sample dispersed on an array of flexible structures. The flow unit can be configured to induce disturbed and/or laminar flow in the fluid channel. The device can further include optical or magnetic detection means configured to measure a degree of deflection of one or more flexible structures in the array.

Related U.S. Application Data

(60) Provisional application No. 61/552,052, filed on Oct. 27, 2011, provisional application No. 61/645,191, filed on May 10, 2012, provisional application No. 61/709,809, filed on Oct. 4, 2012.

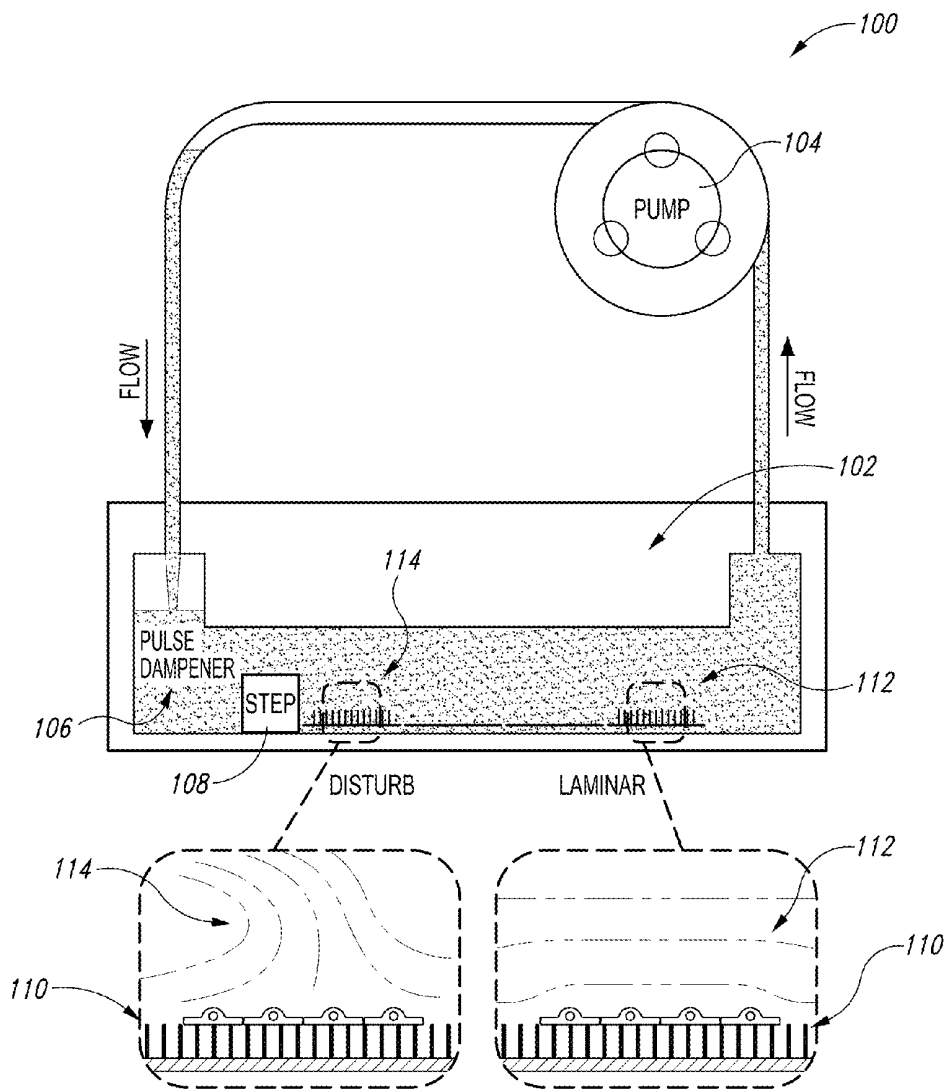


Fig. 1A

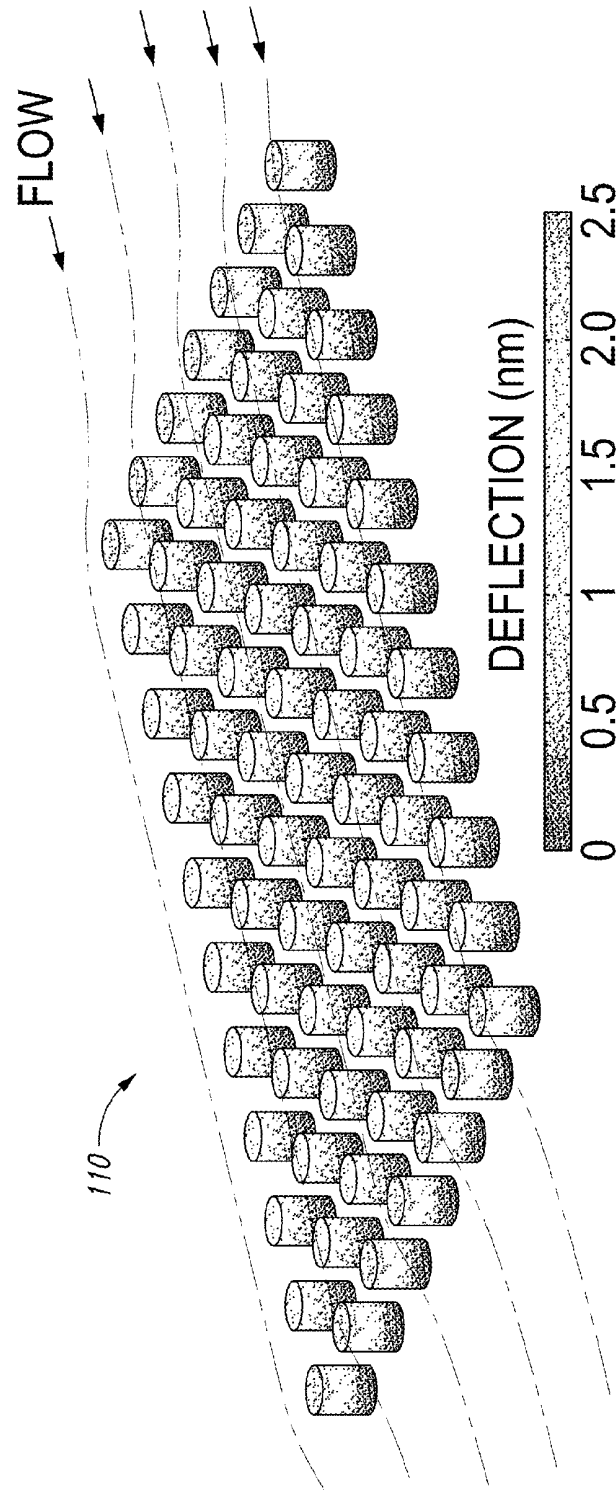


Fig. 1B

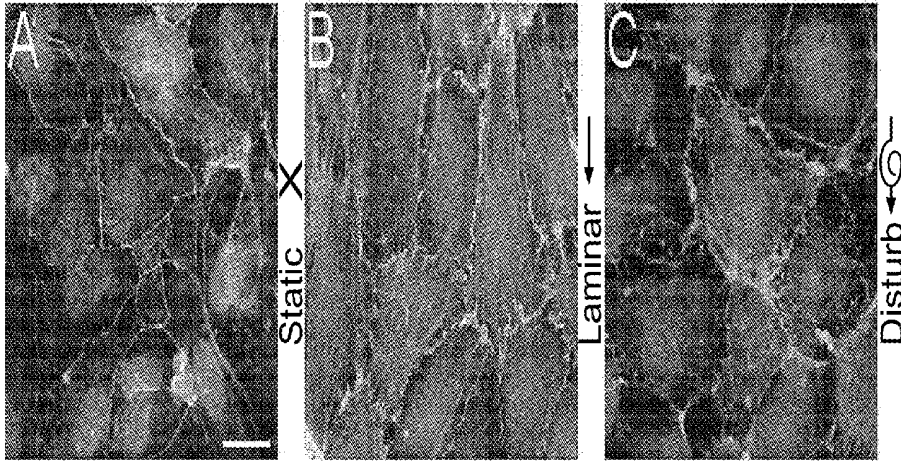


Fig. 2A

Fig. 2B

Fig. 2C

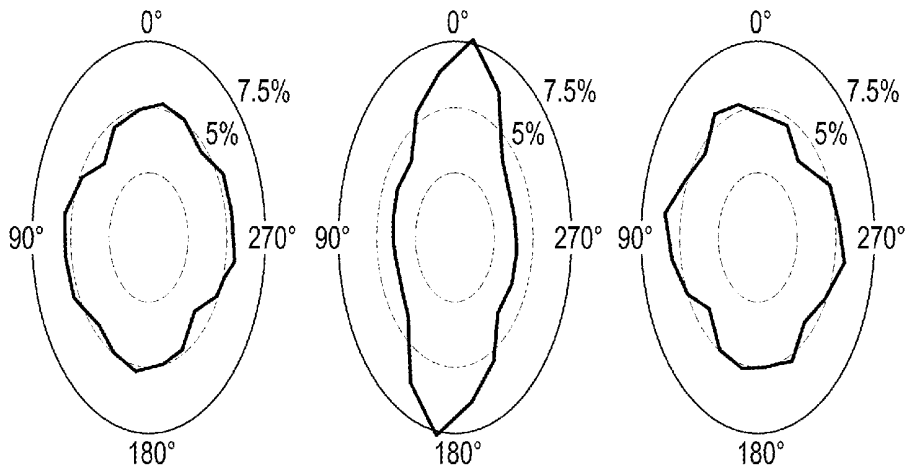


Fig. 2D

Fig. 2E

Fig. 2F



Fig. 3A



Fig. 3B



Fig. 3C

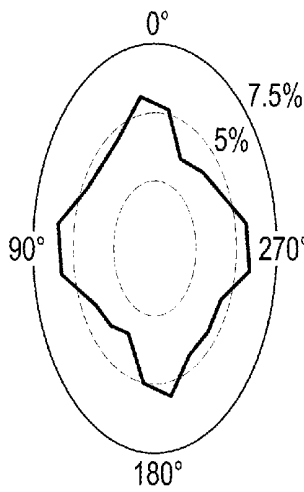


Fig. 3D

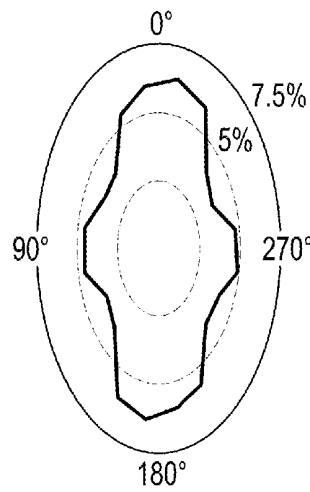


Fig. 3E

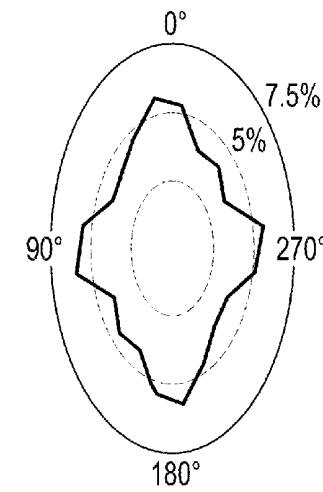


Fig. 3F

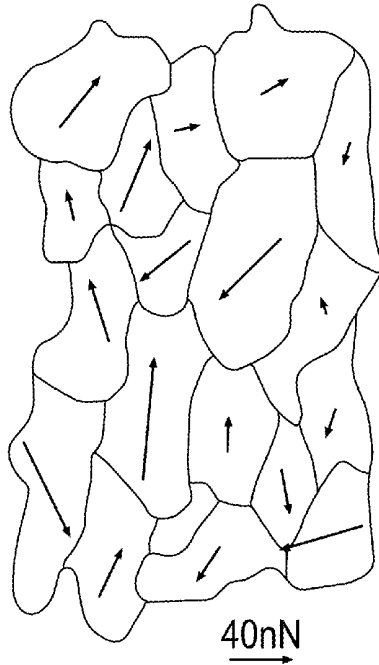


Fig. 4A

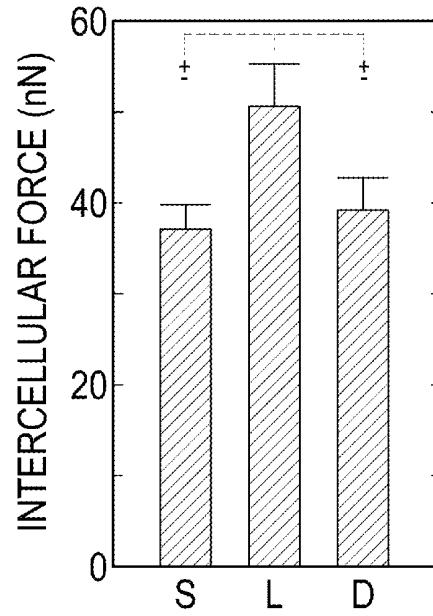


Fig. 4B

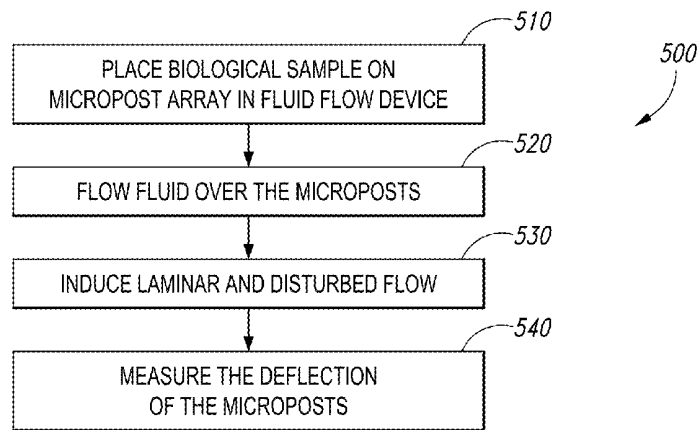


Fig. 5

DEVICE TO EXPOSE CELLS TO FLUID SHEAR FORCES AND ASSOCIATED SYSTEMS AND METHODS

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 61/552,052, filed Oct. 27, 2011; U.S. Provisional Patent Application No. 61/645,191, filed May 10, 2012; and U.S. Provisional Patent Application No. 61/709,809, filed Oct. 4, 2012, each of which is incorporated herein by reference in its entirety.

STATEMENT REGARDING FEDERALLY-SPONSORED RESEARCH

[0002] This invention was made with government support under 5T32EB001650 and 5R21HL097284 awarded by the National Institutes of Health, and N66001-11-1-4129 awarded by the Space and Naval Warfare Systems Center (SPAWAR). The government has certain rights in the invention.

TECHNICAL FIELD

[0003] The present technology relates generally to devices to expose cells to fluid shear forces and associated systems and methods. In particular, several embodiments are directed toward devices to expose cells to fluid shear forces in order to measure changes in internal cell forces.

BACKGROUND

[0004] Mechanical forces are important modulators of cellular function in many tissues. For example, mechanotransduction responses to laminar or disturbed flow can strongly affect the ability of endothelial cells to maintain the barrier between blood and the vessel wall. Laminar flow occurs in straight vessels and produces a steady shear stress on the cells. Disturbed flow forms downstream of obstructions, bends, or bifurcations, and produces a time-averaged, low shear stress due to eddies in the flow. These flows can activate mechanosensors in endothelial cells that lead to the activation of signaling pathways that affect cytoskeletal structures. In particular, laminar flow can initiate Rho GTPase pathways that cause alignment of actin filaments and assembly of adherens junctions. Conversely, disturbed flow leads to disorganized actin, disassembly of adherens junctions, and small gaps between adjacent endothelial cells. These structural changes in endothelial cells can strongly affect the integrity of the vascular barrier.

BRIEF DESCRIPTION OF THE DRAWINGS

[0005] FIG. 1A is a partially schematic illustration of a cellular flow chamber configured in accordance with embodiments of the technology.

[0006] FIG. 1B is an isometric, partially schematic illustration of an array of micropost substrates configured for placement in the cellular flow chamber of FIG. 1A in accordance with embodiments of the technology.

[0007] FIGS. 2A-2C are illustrations of cells cultured on flat substrates and placed under static, laminar, or disturbed flow conditions and configured in accordance with embodiments of the technology.

[0008] FIGS. 2D-2F are rose plots illustrating the orientations of the cells of FIGS. 2A-2C, respectively, in accordance with embodiments of the present technology.

[0009] FIGS. 3A-3C are illustrations of cells cultured on microposts and placed under static, laminar, or disturbed flow conditions and configured in accordance with embodiments of the technology.

[0010] FIGS. 3D-3F are rose plots illustrating the orientations of the cells of FIGS. 3A-3C, respectively, in accordance with embodiments of the present technology.

[0011] FIG. 4A is an illustration of vector fields of intercellular force in accordance with embodiments of the technology.

[0012] FIG. 4B is a graph illustrating intercellular forces under static, laminar, and disturbed flow conditions in accordance with embodiments of the technology.

[0013] FIG. 5 is a block diagram illustrating a method of applying shear force to a biological sample in accordance with embodiments of the technology.

DETAILED DESCRIPTION

[0014] The present technology relates generally to devices to expose cells to fluid shear forces and associated systems and methods. In particular, several embodiments are directed toward devices to expose cells to fluid shear forces in order to measure changes in internal cell forces. In some embodiments, a fluidic device includes a flow unit configured to induce fluid flow through the device. The device further includes a fluid channel configured to accept a biological sample dispersed on an array of flexible structures. The flow unit can be configured to induce disturbed and laminar flow in the fluid channel. The device can further include detection means configured to measure a degree of deflection of one or more flexible structures in the array.

[0015] Specific details of several embodiments of the technology are described below with reference to FIGS. 1-5. Other details describing well-known structures and systems often associated with cellular research tools have not been set forth in the following disclosure to avoid unnecessarily obscuring the description of the various embodiments of the technology. Many of the details, dimensions, angles, and other features shown in the Figures are merely illustrative of particular embodiments of the technology. Accordingly, other embodiments can have other details, dimensions, angles, and features without departing from the spirit or scope of the present technology. A person of ordinary skill in the art, therefore, will accordingly understand that the technology may have other embodiments with additional elements, or the technology may have other embodiments without several of the features shown and described below with reference to FIGS. 1-5.

[0016] FIG. 1A is a partially schematic illustration of a parallel plate fluid flow chamber 100 configured in accordance with embodiments of the technology. In several embodiments, the flow chamber 100 is sized for benchtop use to simulate fluid shear stresses on various cell types exposed to laminar and dynamic fluid flow in their natural, physiological environment. In some embodiments, at least a portion of the flow chamber 100 is constructed of clear acrylic or other suitable material.

[0017] The chamber 100 can include a main channel 102 through which pumped fluid flows. In one embodiment, the main channel 102 has a length of 100 mm, a width of 20 mm, and a depth of 0.5 mm. The main channel 102 can have other

dimensions in other embodiments. While FIG. 1A illustrates a single main channel 102, in further embodiments the chamber 100 can include a plurality of fluid channels. The main channel 102 is configured to accept one or more individual or arrays 110 of microposts. As discussed in further detail below with reference to FIG. 1B, in some embodiments, the array 110 comprises a biological sample that is cultured as monolayers on micropost substrates. In several embodiments, the sample comprises human pulmonary artery endothelial cells, or HPAECs. In further embodiments, the sample comprises whole blood, platelets, tumor cells, protein solutions, fibroblasts, smooth muscle cells, cardiomyocytes, white blood cells, red blood cells, or fragments thereof. The sample may comprise other types of biological material in still further embodiments. In embodiments with multiple fluid channels, a user can introduce different fluids, biological samples, and/or reagents in the different channels.

[0018] FIG. 1B is an isometric, partially schematic illustration of the array 110 of micropost substrates configured for placement in the fluid flow chamber 100 of FIG. 1A in accordance with embodiments of the technology. In some embodiments, the array 110 is created by casting a film of polydimethylsiloxane (PDMS) or similar material with a 0.25 mm thickness on a No. 2 glass coverslip. In other embodiments, other materials and/or thicknesses of materials can be used. In some embodiments, the individual microposts comprise silicon, polymers, metal, or ceramics. In some embodiments, the microposts or other portion of the chamber 100 is substantially coated in a non-fouling coating. In a particular embodiment, individual microposts have a diameter from about 1 nm to about 50 μm and/or an aspect ratio between about 1:1 and about 1:100. In further embodiments, the microposts can have other dimensions.

[0019] The arrays 110 of microposts can be micromolded into a desired arrangement. In order to prepare either flat substrates or microposts for cell attachment, a binding element (e.g., fibronectin) can be absorbed onto the surface of a PDMS stamp. The stamps can have no pattern ('flat stamp') or an array of positive relief patterns in the shape of a grid of squares ('square stamps'). Once the protein is adsorbed, the stamp can be placed into conformal contact with the substrate in order to transfer fibronectin onto the regions of contact. Afterwards, each substrate can be treated with 0.2% Pluronic F127 or other suitable material to ensure that cells adhere to regions where the fibronectin was printed.

[0020] As discussed above, in some embodiments, the substrates support HPAECs, which are seeded at confluent densities onto arrays of microposts or flat substrates. In some embodiments, the individual microposts are substantially coated with at least one binding element, such as proteins, glycans, polyglycans, glycoproteins, fibrinogen, fibronectin, von Willebrand Factor, collagen, vitronectin, laminin, monoclonal antibodies, polyclonal antibodies, and/or fragments thereof. Cells can be allowed to spread and reform their adherens junctions for a selected time period (e.g., two days) in culture before subjecting them to flow conditions. Microposts can act as elastic, cantilever beams which deflect in proportion to the force applied at their tips. Representative deflection caused by the Flow F applied to the array 110 is illustrated in FIG. 1B as a continuum from micropost base to tip.

[0021] Referring to FIGS. 1A and 1B together, in operation, the micropost arrays 110 are placed in the main channel 102 and exposed to static, laminar, and/or disturbed flow

conditions for a selected time period. For example, in some embodiments, the substrate arrays 110 with HPAEC monolayers can be placed inside the chamber 100 and shear force can be applied for the selected time period. In one embodiment, the shear force is applied continuously for 14 hours. In other embodiments, the force is applied intermittently or for more or less than 14 hours.

[0022] A flow rate (e.g., a steady flow rate of 2 mL) can be produced by a flow unit (e.g., a pump) 104 that is connected to the flow chamber 100 and configured to recirculate the media through the chamber 100 in the direction of the flow arrows. In a particular embodiment, the flow unit 104 is capable of introducing the shear stress from about -10 kPa to about 10 kPa. The flow unit 104 can comprise a positive displacement pump, a piezoelectric pump, a partial vacuum, a diaphragm pump, a peristaltic pump, a hydrostatic pump, or another device. The fluid passing through the chamber 100 can be any type of fluid configured to induce the desired shear stress on the array 110. In some embodiments, the fluid contains a drug, reagent, nucleotide, protein, or a material that can cause the cells bound to the microposts to contract.

[0023] A chamber of air 106 at the entrance of the channel 102 can damp the pulsatile flow so that a steady flow rate is produced in the channel 102. The air chamber 106 can be either upstream or downstream of the array 110. A backward-facing step 108 in the channel 102 can produce a region of disturbed flow 114 downstream from the step 108. In a particular embodiment, the step 108 is 0.25 mm tall. Flow in the disturbed flow region 114 can have separation in its fluid stream lines, stagnation points, and regions of reversal in the direction of flow. A laminar flow region 112 can occur further downstream from the region of disturbed flow 114. In further embodiments, the disturbed flow region 114 and laminar flow region 112 can be in other positions relative to one another or relative to the main channel 102. In still further embodiments, only one of disturbed flow or laminar flow is produced.

[0024] Changes in traction forces, intercellular forces, and adherens junctions can be measured to understand the role of tension at the cell-cell interface in regulating the endothelial barrier. For example, the degree of effect from the flow can be optically determined. More specifically, to measure the deflection of a micropost, the difference between the position of its tip and base can be analyzed from fluorescent images taken at the top and bottom of the arrays 110. The magnitude and direction of each traction force (F) can be computed from the deflection (δ) through the relationship:

$$F = \frac{3\pi ED^4}{64L^3} \delta$$

[0025] The length L and diameter D of the microposts in the array can be measured using a scanning electron microscope. Young's modulus of PDMS (E=2.5 MPa) can be determined by tensile testing. Cytoskeletal tension can be assessed by computing the average traction force per monolayer. Intercellular forces can be determined by the vector sum of the traction forces on a cell in a monolayer. Intercellular tension can be measured by the average intercellular force for cells within a monolayer.

[0026] In further embodiments, deflection can be determined by magnetic detection means. For example, magnetic nanowires can be embedded into microposts to apply external forces to cells. In some embodiments, the nanowires com-

prise cobalt or nickel wires grown by electrochemical deposition in the pores of a template. The magnetic microposts can be actuated by applying a uniform field (e.g., in a direction perpendicular to the long axis of the posts). The induced magnetic torque on the nanowire causes the magnetic posts to deflect; the field thereby applies nanonewtons of force to the biological sample. The nanowires can become rotated by the deflection of the posts. Micropost deflection can be detected using a Hall probe placed adjacent to (e.g., underneath) the magnetized array **110**.

[0027] FIGS. 2A-2C are representative immunofluorescent images of HPAECs cultured on flat substrates and placed under (A) static, (B) laminar, or (C) disturbed flow conditions for a selected time period (e.g., 14 hours). FIGS. 2D-2F are rose plots illustrating the orientations of the HPAECs of FIGS. 2A-2C, respectively. Endothelial cells can align their actin filaments in the direction of shear flow on flat substrates. HPAECs grown on flat substrates can align their actin filaments parallel to the direction of laminar flow, whereas under static and disturbed flow, their actin may have no preferential alignment.

[0028] FIGS. 3A-3C are representative illustrations of HPAECs cultured on microposts and placed under static, laminar, or disturbed flow conditions and configured in accordance with embodiments of the technology. FIGS. 3D-3F are rose plots illustrating the orientations of HPAECs of FIGS. 3A-3C, respectively. Similar to the cells described above with reference to FIGS. 2A-2F, actin filaments are oriented predominately in the direction of laminar flow. This does not hold true for static or disturbed flow. There is, however, a strong degree of actin alignment along the 0°, 90°, 180°, and 270° directions for all flow conditions, which can match the orthogonal arrangement of the microposts in the sample array (e.g., the array **110** illustrated in FIG. 1B). These alignment results are due to focal adhesions that can form at the microposts and therefore help to confine actin filaments between adjacent posts. However, laminar flow can create a stronger degree of alignment of actin in the direction of flow despite the strong influence of the arrangement of the microposts.

[0029] FIG. 4A is an illustration of vector fields of intercellular force for a representative HPAEC monolayer exposed to shear force for a selected time period (e.g., 14 hours) in accordance with embodiments of the technology. FIG. 4B is a graph illustrating representative intercellular forces calculated by the vector sum of the tugging forces acting on a cell under static (S), laminar (L), and disturbed (D) flow in accordance with embodiments of the technology. Referring to FIGS. 4A and 4B together, a shear force testing device (such as the fluid flow device) can provide significant information regarding intercellular force. For example, vector fields of intercellular force illustrate that cells with large intercellular forces acting on them are located not only at the perimeter, but within the interior of the monolayer as well. Cytoskeletal tension can be transmitted as traction forces at the microposts, but also as intercellular forces between adjacent cells within a monolayer. The average intercellular force may be higher for laminar flow as compared to static and disturbed flow conditions. Intercellular forces can increase under laminar flow and promote adherens junction assembly.

[0030] FIG. 5 is a block diagram illustrating a method **500** of applying shear force to a biological sample in accordance with embodiments of the technology. In some embodiments, the method **500** includes placing microposts in a fluid flow device, **510**. As discussed above, the microposts can have a

biological sample bound thereto. In particular embodiments, the biological sample comprises HPAECs.

[0031] The method **500** further includes flowing fluid over the microposts **520**. The fluid can be induced in movement by a pump or other device. This method of flowing fluid can be accomplished in a fluid flow chamber such as the chamber described above with reference to FIG. 1A. In several embodiments, flowing fluid over the microposts comprises inducing laminar and disturbed flow conditions. In some embodiments, these flow conditions can be induced by flowing the fluid over a backward-facing step in the fluid flow chamber, wherein the laminar and disturbed flow occur downstream of the step. In further embodiments, the method can include placing an air chamber in the fluid flow chamber to induce a steady flow rate. The method **500** can further include measuring the deflection of the microposts, **540**.

[0032] The technology disclosed herein offers several advantages over existing systems. For example, by using the approach of inducing shear flow over a micropost array, a quantitative image analysis can be performed to demonstrate that mechanotransduction of flow directly affects the intercellular tension in a monolayer, which coincides with the assembly of cell-cell contacts between adjacent cells. The systems disclosed herein can be used as a research tool to investigate numerous aspects of cellular structure and behavior in various environmental conditions. For example, the systems can be used to demonstrate that laminar flow can cause a rise in cytoskeletal tension that increases traction forces and intercellular forces and promotes the assembly of adherens junctions. Disturbed flow can be found to weaken cellular forces and cause adherens junction disassembly.

[0033] From the foregoing it will be appreciated that, although specific embodiments of the technology have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the technology. Further, certain aspects of the new technology described in the context of particular embodiments may be combined or eliminated in other embodiments. Moreover, while advantages associated with certain embodiments of the technology have been described in the context of those embodiments, other embodiments may also exhibit such advantages, and not all embodiments need necessarily exhibit such advantages to fall within the scope of the technology. Accordingly, the disclosure and associated technology can encompass other embodiments not expressly shown or described herein. Thus, the disclosure is not limited except as by the appended claims.

I/we claim:

1. A fluidics device, comprising:

a flow unit configured to induce fluid flow through the device;

at least one a fluid channel configured to accept a biological sample dispersed on an array of flexible structures, wherein the fluid channel is configured to induce at least one of disturbed or laminar flow in the fluid channel; and a detection means configured to measure a degree of deflection of one or more flexible structures in the array.

2. The device of claim 1, further comprising at least one of an air chamber or a step positioned in the fluid channel and configured to control a characteristic of fluid flow through the array.

3. The device of claim 1 wherein the biological sample comprises protein solutions, tissues, cells, or fragments of tissues or cells.

4. The device of claim 1 wherein the biological sample comprises at least one of whole blood, platelets, endothelial cells, tumor cells, fibroblasts, smooth muscle cells, cardiomyocytes, white blood cells, red blood cells, or fragments thereof

5. The device of claim 1 wherein the flexible structures comprise microposts made of silicon, polymers, metal, or ceramics.

6. The device of claim 1 wherein individual flexible structures have a diameter from about 1 nm to about 50 μm .

7. The device of claim 1 wherein individual flexible structures have an aspect ratio between about 1:1 and about 1:100.

8. The device of claim 1 wherein the flow unit is capable of introducing shear stress to the biological sample.

9. The device of claim 8 wherein the flow unit is capable of introducing the shear stress from about -10 kPa to about 10 kPa.

10. The device of claim 1 wherein the flow unit comprises a positive displacement pump, a piezoelectric pump, a partial vacuum, a diaphragm pump, a hydrostatic pump, or a peristaltic pump.

11. The device of claim 1 wherein individual flexible structures are substantially coated in a non-fouling coating.

12. The device of claim 1 where at least one of the flow unit or the fluid chamber is substantially coated in a non-fouling coating.

13. The device of claim 1 wherein the detection means comprises at least one of optical detection means or magnetic detection means.

14. The device of claim 1 wherein individual flexible structures are substantially coated with at least one binding element.

15. The device of claim 14 wherein the binding element is selected from group consisting of proteins, glycans, polyglycans, glycoproteins, fibrinogen, fibronectin, von Willebrand Factor, collagen, vitronectin, laminin, monoclonal antibodies, polyclonal antibodies, and fragments thereof

16. A shear flow device, comprising:

a fluid chamber configured to accept one or more flexible microposts, the microposts having a biological sample thereon;

a pump configured to induce fluid flow through the fluid chamber, the fluid having laminar flow characteristics in first region and disturbed flow characteristics in a second region; and

detection means configured to quantify a degree of deflection of individual microposts.

17. The device of claim 16 wherein individual microposts comprise a base and a tip, and wherein the detection means are configured to quantify a difference in position between the base and the tip.

18. The device of claim 16, further comprising at least one of a step or a pulse dampener positioned in the fluid chamber and configured to control a characteristic of fluid flow through the chamber.

19. An analytic method, comprising:

placing microposts in a fluid flow chamber, the microposts having a biological sample bound thereto;

flowing fluid over the microposts; and

measuring a deflection of the microposts.

20. The method of claim 19 wherein flowing fluid over the microposts comprises inducing laminar and disturbed flow to the biological sample.

21. The method of claim 20 wherein inducing laminar and disturbed flow comprises flowing fluid over a backward-facing step in the fluid flow chamber, wherein the laminar and disturbed flow occur downstream of the step.

22. The method of claim 19 wherein placing microposts having a biological sample bound thereto comprises placing microposts having human pulmonary artery endothelial cells bound thereto.

23. The method of claim 19, further comprising placing an air chamber in the fluid flow chamber to induce a steady flow rate.

24. The method of claim 19 wherein flowing fluid over the microposts comprises flowing a fluid containing at least one of a drug, reagent, nucleotide, protein, or a material configured to cause the biological sample to contract.

* * * * *

专利名称(译)	将细胞暴露于流体剪切力的装置和相关的系统和方法		
公开(公告)号	US20130109012A1	公开(公告)日	2013-05-02
申请号	US13/663339	申请日	2012-10-29
当前申请(专利权)人(译)	华盛顿通过其中心的商业化大学		
[标]发明人	SNIADOCKI NATHAN J TING LUCAS		
发明人	SNIADOCKI, NATHAN J. TING, LUCAS		
IPC分类号	G01N33/53		
CPC分类号	G01N33/5302 C12M35/04 G01N33/4905 B01L3/502746 C12M41/46 C12M23/20 C12M23/26 C12M29/00 G01N15/1056 G01N15/1404 G01N15/1459 G01N15/1463 G01N2015/1006		
优先权	61/709809 2012-10-04 US 61/645191 2012-05-10 US 61/552052 2011-10-27 US		
其他公开文献	US9140684		
外部链接	Espacenet USPTO		

摘要(译)

本技术一般涉及使细胞暴露于流体剪切力的装置和相关的系统和方法。特别地，若干实施例涉及将细胞暴露于流体剪切力以便测量内部细胞力的变化的装置。在一些实施例中，流体装置包括流动单元，该流动单元构造成引导流体流过装置。该装置还包括流体通道，该流体通道构造成接收分散在柔性结构阵列上的生物样品。流动单元可以配置成在流体通道中引起扰动和/或层流。该装置还可包括光学或磁性检测装置，其配置成测量阵列中一个或多个柔性结构的偏转程度。

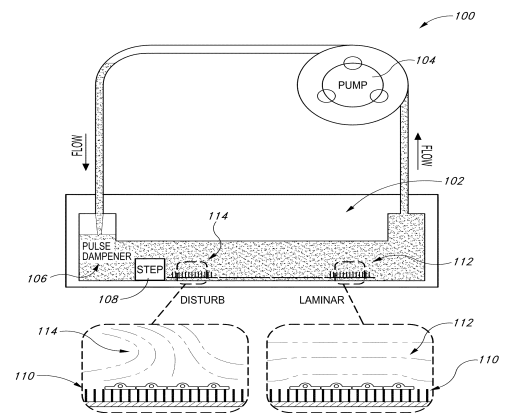


Fig. 1A