

MICRO-INCUBATION SYSTEMS FOR MICROFLUIDIC CELL CULTURE AND METHODS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of priority from provisional ~~provisional~~ patent application 61/566,651, filed December 3, 2011, which is hereby incorporated by reference for all purposes.

[0002] This application incorporates each of the following provisional patent applications by reference:

61/367,371 filed 07-23-2010

61/297,278 filed 01-21-2010

61/471,103 filed 04-01-2011

[0003] This application is related to material discussed in one or more of the following applications, each of which are incorporated herein by reference for all purposes: U.S. provisional patent application 61/037,297 filed 03-17-2008, U.S. provisional patent application 61/018,882 filed 01-03-2008, U.S. Application 11/994,997, filed 08-11-2008, which is a National Stage Entry of PCT/US06/26364, filed 07-06-2006 and which claims priority from U.S. provisional patent application 60/773,467 filed 02-14-2006 and from U.S. provisional patent application 60/697,449 filed 7 July 2005, U.S. Application 12/019,857, filed 01-25-2008, which claims priority to U.S. Provisional Patent Application No. 60/900,651 filed on 02-08-2007, U.S. Application Number: 11/648207, filed 12/29/2006, now issued as U.S. Pat. No. 8,257,964, which claims priority to U.S. Provisional Patent Application U.S. provisional patent application No. 60/756,399 filed on 01-04-2006, U.S. application 12/348907, filed 01-05-2009.

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FIELD OF THE INVENTION

[0005] The invention in various embodiments relates to assays, systems, and/or devices for culturing cells or other biologic material in controlled environments and that are applicable to related fields generally using microfluidic systems. Particular embodiments involve configurations that can be used with various standard automated handling systems, with active or passive loading and perfusion of medium and to provide high-throughput multi-assay automated systems for culturing, viewing, and analyzing cell growth, invasion, movement, chemotaxis or other properties. More specifically, the invention relates in specific embodiments to heat control systems for microfluidic culture plates and other automated systems for culture plates.

left-most line in the figure is for vacuum and connects to an outer vacuum ring around the manifold. This basic manifold design is modified using the teachings herein to produce the heated manifold.

FIG. 11 illustrates an example microfluidic perfusion system (ONIX™), microincubator controller and manifold (MIC) according to specific embodiments of the invention.

FIG. 12 illustrates an example microfluidic perfusion system (ONIX™), microincubator controller and manifold (MIC) and computer control system according to specific embodiments of the invention.

FIG. 13 shows NIH-3T3 mouse fibroblasts cultured using the microincubator system according to specific embodiments of the invention at t=0 (left) and after 15 hours (right) showing cell growth and viability. When no temperature or CO2 was controlled, the cells rapidly died within 2 hours.

FIGS. 14A-B illustrates one alternative of plate and culture unit design with an example culture unit filled with blue dye with the image taken from top according to specific embodiments of the invention.

FIG. 15 is a screenshot showing integration of the ONIX microfluidic perfusion system with an open-source microscopy application.

FIG. 16 illustrates a microincubation system integrated with a microscope system for cell analysis. The dimensions of the manifold in specific embodiments allow it to sit on a standard well plate stage, with a transparent optical path that does not interfere with light microscopy. This allows time-lapsed imaging of cells cultured in the micro-incubator.

FIG. 17 is a block diagram showing a representative example logic device in which various aspects of the present invention may be embodied.

FIG. 18A is a block diagram showing an automated piston driven system according to specific embodiments of the invention.

FIG. 18B illustrates perspective views of the automated piston driven system of FIG. 18A in the elected and sealed states.

FIG. 19 (Table 1) illustrates an example of diseases, conditions, or states that can be evaluated or for which drugs or other therapies can be tested according to specific embodiments of the present invention.

FIG. 20A-D are schematics of an example implementation of electronic control circuits for a manifold according to specific embodiments of the invention.

DETAILED DESCRIPTION OF SPECIFIC EMBODIMENTS

1. OVERVIEW

Definitions

[0030] A “particle” refers to biological cells, such as mammalian or bacterial cells, viral particles, or liposomal or other particles that may be subject to assay in accordance with the invention. Such particles have minimum dimensions between about 50-100 nm, and may be as large as 20 microns or more. When used to describe a cell assay in accordance with the invention, the terms “particles” and “cells” may be used interchangeably.

[0068] By repeatedly filling/aspirating the wells, cells can be maintained for long periods of time with minimal effort (e.g. compared to standard “bioreactors” which require extensive sterile preparation of large fluid reservoirs that cannot be easily swapped out during operation).

Example cell culture

[0069] Cells were cultured using the micro-incubation system to control temperature and gas atmosphere. In one example, human cancer cells (HT-1080, MCF-7, MDA-MB-231) were cultured at 37C and 5% CO₂ to monitor cell division over 24 hours. Additional cell types, including yeast, bacteria, primary cells, neurons, etc. have been successfully cultured using the microincubation system. As an example, FIG. 13 shows NIH-3T3 mouse fibroblasts cultured using the microincubator system according to specific embodiments of the invention at t=0 (left) and after 15 hours (right) showing cell growth and viability. When no temperature or CO₂ was controlled, the cells rapidly died within 2 hours.

Integrated Systems

[0022]

[0070] Integrated systems for the collection and analysis of cellular and other data as well as for the compilation, storage and access of the databases of the invention, typically include a digital computer with software including an instruction set for sequence searching and/or analysis, and, optionally, one or more of high-throughput sample control software, image analysis software, collected data interpretation software, a robotic control armature for transferring solutions from a source to a destination (such as a detection device) operably linked to the digital computer, an input device (e.g., a computer keyboard) for entering subject data to the digital computer, or to control analysis operations or high throughput sample transfer by the robotic control armature. Optionally, the integrated system further comprises valves, concentration gradients, fluidic multiplexors and/or other microfluidic structures for interfacing to a microchamber as described.

[0023]

[0071] Readily available computational hardware resources using standard operating systems can be employed and modified according to the teachings provided herein, e.g., a PC (Intel x86 or Pentium chip- compatible DOS,TM OS2,TM WINDOWS,TM WINDOWS NT,TM WINDOWS95,TM WINDOWS98,TM LINUX, or even Macintosh, Sun or PCs will suffice) for use in the integrated systems of the invention. Current art in software technology is adequate to allow implementation of the methods taught herein on a computer system. Thus, in specific embodiments, the present invention can comprise a set of logic instructions (either software, or hardware encoded instructions) for performing one or more of the methods as taught herein. For example, software for providing the data and/or statistical analysis can be constructed by one of skill using a standard programming language such as Visual Basic, Fortran, Basic, Java, or the like. Such software can also be constructed utilizing a variety of statistical programming languages, toolkits, or libraries.

{0024}

{0072} FIG. 17 shows an information appliance (or digital device) 700 that may be understood as a logical apparatus that can read instructions from media 717 and/or network port 719, which can optionally be

connected to server 720 having fixed media 722. Apparatus 700 can thereafter use those instructions to direct server or client logic, as understood in the art, to embody aspects of the invention. One type of logical apparatus that may embody the invention is a computer system as illustrated in 700, containing CPU 707, optional input devices 709 and 711, disk drives 715 and optional monitor 705. Fixed media 717, or fixed media 722 over port 719, may be used to program such a system and may represent a disk-type optical or magnetic media, magnetic tape, solid state dynamic or static memory, etc. In specific embodiments, the invention may be embodied in whole or in part as software recorded on this fixed media. Communication port 719 may also be used to initially receive instructions that are used to program such a system and may represent any type of communication connection.

~~{0025}~~

[0073] Various programming methods and algorithms, including genetic algorithms and neural networks, can be used to perform aspects of the data collection, correlation, and storage functions, as well as other desirable functions, as described herein. In addition, digital or analog systems such as digital or analog computer systems can control a variety of other functions such as the display and/or control of input and output files. Software for performing the electrical analysis methods of the invention are also included in the computer systems of the invention.

Auto-sealer Automated System

~~{0026}~~

[0074] FIG. 18 is a block diagram showing an automated piston driven system according to specific embodiments of the invention. According to further specific embodiments, an auto-sealer somewhat similar to a plate reader commonly used in biotechnology with the main difference is the design of the system component to allow automated handling of the microfluidic plates. In this implementation, the positive seal between manifold and microfluidic plate is still accomplished by applying vacuum to the interstitial areas, but the necessary initial downward force is applied mechanically. The area above the manifold is clear to allow access by an automated liquid handler such as the Tecan EVO. Vacuum and pressure sensors as well as plate presence and carriage position sensors allow for intelligent software based error handling. FIG. 18B is an image sequence showing how the automatic sealing device accepts and seals the manifold to a microfluidic plate. A single pneumatic linear actuator (e.g., a piston) provides horizontal and vertical motion. It has been found that this single operation allows more precise control of the manifold and plate and holds the plate in place during operation of the pneumatic manifold.

Other Embodiments

~~{0027}~~

[0075] Although the present invention has been described in terms of various specific embodiments, it is not intended that the invention be limited to these embodiments. Modification within the spirit of the invention will be apparent to those skilled in the art.

[0028]

[0076] It is understood that the examples and embodiments described herein are for illustrative purposes and that various modifications or changes in light thereof will be suggested by the teachings herein to persons

skilled in the art and are to be included within the spirit and purview of this application and scope of the claims.

~~{0029}~~

{0077} All publications, patents, and patent applications cited herein or filed with this submission, including any references filed as part of an Information Disclosure Statement, are incorporated by reference in their entirety.

WHAT IS CLAIMED:

1. A microincubator manifold comprising:
 - a pneumatic layer carrying one or more pneumatic channels for conveying pressure or gas to a culture plate;
 - a gasket layer for providing a removable air-tight seal to the culture plate;
 - a heat exchange module;
 - wherein said manifold seals to said culture plate thereby enclosing an incubation volume of gas; the incubation volume of gas circulating between a culture chamber or observation area of the plate and a heat exchange area of the heat exchange module; and
 - thereby providing a controlled temperature and gas composition above and in communication with the culture chamber or observation area.
2. The manifold of claim 1 further comprising:
 - one or more pneumatic connections at said pneumatic layer for interfacing with an external pneumatic controller;
 - one or more electrical connections to said heat exchange module for providing power and/or sensor and/or control connections between said heat exchange module and an outside controller.
3. The manifold of claim 1 and optionally also any combination of claim 2 to the preceding claim further comprising:
 - a transparent window that sits above said culture area when the manifold is sealed to the plate, thereby containing the incubation volume while allowing for observation of the cell culture chamber.
4. The manifold of claim 1 and optionally also any combination of claim 2 to the preceding claim further comprising:
 - cells are cultured in one or more microfluidic culture chambers in the plate that are separated from the incubation volume by one or more gas permeable layers.
5. The manifold of claim 1 and optionally also any combination of claim 2 to the preceding claim further comprising:
 - the heat exchange module having a circulating fan for circulating gases in the incubation volume.

6. The manifold of claim 1 and optionally also any combination of claim 2 to the preceding claim further comprising:

the heat exchange module having a second fan for cooling a portion of a heat exchanger.

7. A micro-incubator comprising:

a microfluidic plate comprising a plurality of wells and at least one culture chamber or observation area;

a manifold;

the manifold comprising a heat exchange module;

wherein said manifold seals to said plate thereby enclosing an incubation volume of gas;

the incubation volume of gas circulating between said culture or observation area of said plate and a heat exchange area of the heat exchange module;

thereby providing a controlled temperature and gas composition above and in communication with said culture or observation area.

8. A system for a microincubator comprising:

a manifold for connecting with a microwell culture system, said manifold comprising:

a heating element;

a gas circulation chamber;

wherein upon mating of the heating element and gas circulation chamber provides a controlled temperature and gas composition above and in communication with a cell culture or observation area.

9. An apparatus or system according to any combination of any of the above claims further wherein:

the incubation volume is less than about 10 milliliters.

10. An apparatus or system according to any combination of any of the above claims further wherein:

the incubation volume is maintained at about 37 C and about 5% CO₂.

11. An apparatus or system according to any combination of any of the above claims further wherein:

the microfluidic plate has the dimensions and configuration of a standard cell culture plate.

12. ~~An apparatus or system according to any combination of any of the above claims further wherein:~~

~~the manifold can be attached or removed thereby the incubation chamber can be created and removed without affecting the culture chamber integrity.~~

13. An apparatus or system according to any combination of any of the above claims further wherein:

the incubation contents can be controlled and changed independent of microchannel flow.

14. A manifold that is attachable to a microfluidic plate comprising:

an onboard circulating feedback heater;

a gas inlet to alter the composition of the circulated air; and

wherein circulating gas is exposed only to cell culture chambers of the microfluidic plate.

15. The manifold of claim 14 further comprising:

at least one vacuum connector allowing vacuum sealing between said manifold and said well plate.

16. The manifold of claim 14 or claim 15 further wherein:

temperature and gas control is independent of flow control.

17. The manifold of claim 14 and optionally also any combination of claim 15 to the preceding claim further wherein:

the manifold interfaces with a plate with the dimensions or outlook of a standard culture plate.

18. The manifold of claim 14 and optionally also any combination of claim 15 to the preceding claim further wherein:

the manifold is optically transparent over the cell culture region allowing microscopy.

19. The manifold of claim 14 and optionally also any combination of claim 15 to the preceding claim further wherein:

the temperature/gas control mechanism has no parts on the bottom side of the microfluidic plate.

20. The manifold of claim 14 and optionally also any combination of claim 15 to the preceding claim further comprising:

— a heat exchange module to control temperature and gas environment of the micro-incubation chamber;

an automatic sealing mechanism on one side of the manifold to provide an air-tight seal to a well plate;

one or more connectors to integrate with a flow control mechanism;

wherein the manifold integrates with optical imaging by microscopy or related methods;

wherein the manifold comprises one or more detectors that allow software to perform automated experiments.

21. A microfluidic cell culture system comprising:

at least one culture unit configured on a culture plate comprising:

a microfluidic culture chamber;

a perfusion barrier separating said chamber from one or more medium flow channels;

a plurality of independent inlet channels;

a perfusion well;

an outlet reservoir;

wherein the culture plate can be sealed to a pneumatic manifold, allowing pressure driven control of the independent inlet channels;

so that solutions can be quickly changed over the cells or can be multiplexed or combined in the culture chamber;

wherein the pneumatic manifold further comprises a heat exchange module for providing a circulating heat controlled gas in a contained gas volume over said culture chamber.

22. The system of claim 21 wherein a high fluidic resistance channel or perfusion barrier is used to reduce flow pressure from the flow inlets to the culture chamber.

23. The system of claim 21 or 22 wherein a high fluidic resistance channel or perfusion barrier is used to contain a gel media to the culture chamber.

24. The system of claim 21 and optionally also any combination of claim 22 to the preceding claim wherein a perfusion barrier mostly surrounds the chamber to separate the chamber from the flow channels.

~~25. The system of claim 21 and optionally also any combination of claim 22 to the preceding claim wherein gravity pressure is used to drive perfusion.~~

26. A method of culturing cells comprising:

placing cells into a culture chamber, the culture chamber connected to a plurality of microfluidic channels, the culture and channels configured into a culture unit on a standard well plate or a modified well plate;

placing media into different wells of the plate, said well fluidically connected to a plurality of microfluidic perfusion passages, said microfluidic perfusion passages providing perfusion passages through said cell invasion area;

allowing said cells to culture for an appropriate time;

observing and/or assaying cells and or media in said standard well plate by observing invasion of cells into said invasion region.

27. The method of claim 26 further comprising:

interfacing said standard well plate with a pneumatic heating manifold for providing pressure and gas as necessary to drive cells or media into said cell culture areas, said pneumatic manifold also providing a controlled circulating heated gas environment above said culture area.

28. The method of claims 26 and/or 27 further wherein said pneumatic manifold interfaces with said well plate using a vacuum seal.

29. The method of claim 26 and optionally also any combination of claim 27 to the preceding claim further comprising:

using a pipette to introduce and/or remove fluids from said reservoirs, said fluids passively perfusing through said cell culture areas as a result of gravity and/or differential fluid levels; and or surface tensions.

30. The method of claim 26 and optionally also any combination of claim 27 to the preceding claim further wherein said steps are performed by fully automated robotic equipment.

31. The method of any combination of claims 26 through the above claim further wherein said steps are performed by fully automated robotic equipment and further wherein said combination of pneumatic cell loading and passive perfusion allows simultaneous automatic culture of a large

number of plates using one pneumatic manifold and one automated pipettor because plates do not need to be attached to any equipment during perfusion fluid flow.

32. The micro-incubator of claim 1 and optionally also any combination of claims 2 to 6; further comprising a microfluidic device separable from the manifold, said microfluidic device consisting of:

one or more cell culture regions where cells and media are introduced by flow through one or more microfluidic channels;

one or more inlet wells or paths for introducing cells or media into said culture region;

one or more outlet wells or paths for removing cells or media into said culture region; and

further wherein one or more of the inlet wells or outlet wells are configured to provide gas or pressure or temperature control to the cell culture regions when used with a gas or temperature control device.

33. The system of claim 32 where the microfluidic device is formatted to a standard 96 well plate, with 24 independent units, where each unit occupies 4 well positions designated as flow inlet, cell/gel inlet, invasion chamber, and flow outlet.

34. The system of claim 32 or 33 where flow from the flow inlet to the flow outlet is driven by a gravity flow, e.g., a flow arising from a liquid height difference between the wells.

35. The system of claim 32 through the preceding claim and any combination thereof wherein the chamber materials are optically transparent to allow visualizing cells using light microscopy.

36. The system of claim 32 through the preceding claim and any combination thereof further comprising:

at least one culture unit, the culture unit comprising:

an inlet reservoir with a fluidic connection to the culture medium flow channel inlet;

an outlet reservoir with a fluidic connection to the culture medium flow channel outlet.

37. The system of claim 32 through the preceding claim and any combination thereof further wherein said system is integrated into a standard well plate.

38. The system of claim 32 through the preceding claim and any combination thereof further wherein said system is integrated into a standard well plate with a culture region situated within an open gas area.

39. The system of claim 32 through the preceding claim and any combination thereof further comprising:

a cell culture array on a standard N-well plate, where each culture unit consists of 3 to 16 well positions: where N equals any standard culture well plate, including 48, 96, 192, 384 well plates.

40. The system of any one or any combination of the above claims further wherein said system is integrated into a standard well plate.

41. The system of any one or any combination of the above claims further wherein said standard well plate is handled by robotic equipment allowing for automated cell culture and analysis, said robotic equipment being in part equipment designed for use with standard well plates.

42. The system of any one or any combination of the above claims further wherein said standard well plate is configured to pneumatically couple with a pneumatic manifold, said manifold providing pneumatic pressure to drive cells into said cell culture areas, said manifold further comprising at least one additional gas line for introducing a desired gas to said culture chambers.

43. The system of any one or any combination of the above claims further wherein: said cell culture areas are separated into at least three blocks, each block containing at least two cell culture areas; each block having an air channel adjacent thereto.

44. The system of any one or any combination of the above claims further wherein: cells are introduced into said cell culture areas using pneumatic pressure, and thereafter, cells are maintained by perfusion of media using a passive gravity driven fluid flow.

45. The system of any one or any combination of the above claims further comprising: a large fluidic opening directly connected to said cell culture channels to allow for easier cell flow.

46. The system of any one or any combination of the above claims further wherein: said perfusion passages have a height and/or width smaller than the diameter of cells, wherein said height is between 20-60 microns and said width is between 2-8 microns.

47. The system of any one or any combination of the above claims further wherein:

said perfusion passages have a height and/or width smaller than the diameter of cells, wherein said height is about 40 microns and said width is about 4 microns.

48. The system of any one or any combination of the above claims further comprising:
a plurality of cell culture units;
each of said units comprising at least two fluidic reservoirs; and
a plurality of said units being fluidically addressable in a cell culture array.
49. The device of claim 44 where each unit of the array is fluidically independent.
50. The device of claim 44 wherein the array complies with SBS microtiter plate standards.
51. The device of claim 44 wherein the array contains more than 16 addressable units.
52. The device of claim 44 wherein the array density is more than 2 units per square centimeter.
53. The device of claim 44 wherein the reservoir spacing is less than 5 mm.
54. The device of claim 44 wherein flow is driven by pneumatic pressure.
55. The device of claim 44 wherein flow is driven by a liquid height difference between one or more of the reservoirs.
56. The device of claim 44 wherein flow is driven by surface tension and/or capillary forces.
57. The device of claim 44 wherein the cells are cultured in a 3D matrix.
58. The device of claim 44 wherein the microfluidic passages are made of gas permeable materials.
59. The device of claim 44 wherein at least some portions of the microfluidic passages and the fluidic reservoirs are made of the same material.
60. An automatic handling system for sealing a pneumatic manifold to a culture plate comprising:
a pneumatic linear actuator cylinder (e.g., a piston);
one or more position sensors;
two arms for holding a manifold from the sides, without blocking a view through the top of a manifold;

a plate carriage and plate grippers for holding a culture plate;

said system configured so that movement of the cylinder causes the plate carriage to move horizontally to a position under said manifold;

said system configured so that movement of the cylinder causes the manifold to descend onto the plate.

61. The system of claim 60 further wherein:

the cylinder is attached to the arms so that the cylinder provides both horizontal and vertical motion.

62. The system of any one or combination of claim 60 to the preceding claim further wherein:

positive seal between the manifold and microfluidic plate is accomplished by applying vacuum through the manifold to the interstitial areas of the plate while the necessary initial downward force is applied mechanically.

63. The system of any one or combination of claim 60 to the preceding claim wherein:

the area above the plate remains clear prior to placement of the manifold to allow access by an automated liquid handler.

64. The system of any one or combination of claim 60 to the preceding claim wherein:

the area above the manifold remains clear during operation of the manifold to allow observation of culture areas during operation.

65. The system of any one or combination of claim 60 to the preceding claim further comprising:

one or more vacuum and pressure sensors as well as plate presence and carriage position sensors allow for intelligent software based control and error handling.

66. A automated plate handler generally as illustrated in **Error! Reference source not found.** and described herein.

67. A automated plate handler comprising:

a receiver for holding a well plate;

a pneumatic manifold with mechanisms for interfacing with at least one well of the well plate and providing one or more of a gas, a positive or negative pressure, temperature control;

an arm for providing an initial downward force pressing the manifold to the well plate;

~~one or more vacuum mechanisms for completing a seal between the manifold and the plate by applying vacuum to the interstitial areas of the plate between wells;~~

68. The automated plate handler according to claim 67 further wherein:
the automated plate handler operates as an auto-sealer and plate reader to allow automated handling of the microfluidic plates.
69. The automated plate handler according to claim 67 further wherein:
the area above the manifold is clear to allow access by an automated liquid handler.
70. The automated plate handler according to claim 67 further comprising:
vacuum and pressure sensors as well as plate presence and carriage position sensors.
71. The automated plate handler according to claim 67 further comprising:
a logic controller and tangibly recorder computer instructions to allow for intelligent software based software control and error handling.
72. The automated plate handler according to claim 67 further wherein the plate handler operates as shown in **Error! Reference source not found.B.**
73. The automated plate handler according to claim 67 further wherein a single pneumatic linear actuator (e.g., a piston) provides both horizontal and vertical motion.
74. The automated plate handler according to claim 67 further wherein a single pneumatic linear actuator (e.g., a piston) provides both horizontal and vertical motion and wherein the single operation provides highly precise control of the manifold and plate.
75. The automated plate handler according to claim 67 further wherein the handler holds the plate in place during operation of the pneumatic manifold.

WHAT IS CLAIMED:

1. A microfluidic cell culture system comprising:
 - at least one culture unit configured on a culture plate comprising:
 - a microfluidic culture chamber;
 - a perfusion barrier separating said microfluidic culture chamber from one or more medium flow channels;
 - a plurality of independent inlet channels in fluid communication with said one or more medium flow channels;
 - and an outlet reservoir in fluid communication with said microfluidic culture chamber;
 - and a pneumatic manifold;
 - wherein the culture plate is positively sealed to said pneumatic manifold, allowing pressure driven control of the plurality of independent inlet channels so that solutions can be quickly changed over the cells or can be multiplexed or combined in the microfluidic culture chamber;
 - wherein said pneumatic manifold further comprises a heat exchange module for providing a circulating heat controlled gas in a contained gas volume over said microfluidic culture chamber.
2. The system of claim 1 wherein said perfusion barrier is used to reduce flow pressure from said inlet channels to said microfluidic culture chamber.
3. The system of claim 1 wherein said perfusion barrier is used to contain a gel media to said microfluidic culture chamber.
4. The system of claim 1 wherein said perfusion barrier mostly surrounds the microfluidic culture chamber to separate the microfluidic culture chamber from the one or more medium flow channels.
5. The system of claim 1, further comprising an automatic handling system for sealing the pneumatic manifold to the microfluidic culture plate comprising: a pneumatic linear actuator cylinder; one or more position sensors; two arms for holding the manifold from the sides, without blocking a view through the top of the manifold; a plate carriage and plate grippers for holding the culture plate; said system configured so that movement of the cylinder causes the plate carriage to move horizontally to a position under said manifold; said system configured so that movement of the cylinder causes the manifold to descend onto the plate.

6. The system of claim 5 further wherein:
the cylinder is attached to the arms so that the cylinder provides both horizontal and vertical motion.
7. The system of claim 5 further wherein:
said positive seal between the manifold and microfluidic culture plate is accomplished by applying vacuum through the manifold to the interstitial areas of the microfluidic culture plate while the necessary initial downward force is applied mechanically.
8. The microfluidic cell culture system of claim 1, further comprising a medium well in fluid communication with said one or more medium flow channels.
9. The microfluidic cell culture system of claim 8, wherein said medium well is a gravity perfusion well.
10. The microfluidic culture system of claim 1, further comprising a cell inlet in fluid communication with said culture chamber.
11. The microfluidic cell culture system of claim 1, further comprising a gas circulation region formed by the positive seal of said pneumatic manifold to said microfluidic culture plate.
12. The microfluidic cell culture system of claim 11 wherein said heat exchange module further comprises a fan to circulate gas within said gas circulation region.
13. The microfluidic cell culture system of claim 1, wherein said heat exchange module comprises a convective heat exchanger.
14. The microfluidic cell culture system of claim 13, wherein said convective heat exchanger comprises heat exchange fins.
15. The microfluidic cell culture system of claim 14, wherein said heat exchange module further comprises a fan to circulate air over said heat exchange fins.

16. The microfluidic cell culture system of claim 1, wherein said pneumatic manifold further comprises a gas input.