



- (51) International Patent Classification:
B01L 3/00 (2006.01)
- (21) International Application Number:
PCT/GB2015/051971
- (22) International Filing Date:
7 July 2015 (07.07.2015)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
1412043.0 7 July 2014 (07.07.2014) GB
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- (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.
- (84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

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(54) Title: CELL POSITIONING AND ANALYSIS DEVICE

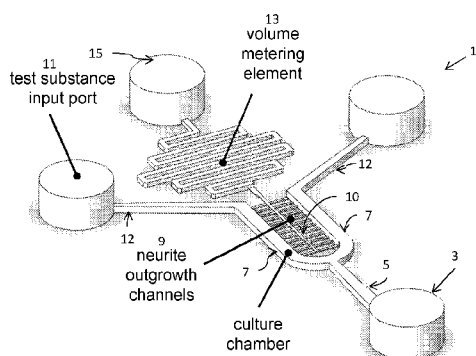


Figure 3

(57) Abstract: The invention relates to a microfluidic device suitable for generating a capillary action during loading comprising: one or more input channels; one or more compartments connected to the one or more input channels, and comprising one or more traps or analysis sites; a volume metering element connected to the traps or analysis sites of the one or more compartments via one or more microchannels; wherein the volume metering element has a larger volume, or substantially equal volume, than the one or more compartments, and is arranged to control capillary action through the one or more microchannels. The invention further relates to the use and fabrication of such device.

Published:

— *with international search report (Art. 21(3))*

CELL POSITIONING AND ANALYSIS DEVICE

This invention relates to a device for delivering a known sample volume and for positioning and analysing cells, such as neuronal cells.

The analysis of samples that contain (bio)chemicals and cells is highly dependent on the ability to manipulate sample volumes with high precision. The modern embodiment of a pipette comprises a plunger within a barrel for withdrawing and then dispensing a known sample volume. These can be used, for example, to transfer fluids to test vessels such as microcentrifuge tubes. Pipettes can either be operated by an individual or in more advanced systems can be part of a robotic dispensing scheme.

At point of care testing sites such as GP surgeries, hospitals, ambulances or in the field pipettes are usually replaced with other sample delivery methods such as displacement using a pad that can be depressed or by capillary wicking through a fibrous, paper-like matrix. The classical application is in urine analysis for the detection of hormones indicative of pregnancy. This is one of a vast number of applications, involving a variety of aqueous samples. For example, blood samples can be analysed for the presence of drugs, viruses, bacteria, parasites, biomarkers, platelets, white blood cells, microvesicles, exosomes and other (bio)chemical analytes of interest.

It has become well recognised by the pharmaceutical industry and the broader research community that there is a need for large volumes of high quality and higher resolution cell data. A major step in this direction is the simple, reliable and cost effective means to handle and culture single cells. Formats are typically parallelised, with contemporary examples including single cell confinement in chambers or droplets to analyse the genome and the transcriptome.¹⁻³ Other approaches involve the addition of cell samples onto a substrate, with the cells suitably dilute to separate individual cells, and following a period for replication to identify so-called colony forming units that will be clonal in nature. This is standard practice in bacteriology and virology, but the same principle can also be extended to the selection of mammalian cells with a key example being the selection of stem cells that, in addition to lineage commitment, have the capacity for self-renewal and expansion.

Other lines of investigation involve the preparation of *in vitro* tissue models. There are several motivations, including reduced complexity, the greater degrees of control (*e.g.* cell density and distribution) and manipulation as opposed to using live animals, the cost savings and the speed of the assay. To create more physiologically relevant models there has been interest in the development of scaffolds with micron and millimetre scale features, and also in the development of microfabricated systems to structure the cell culture or *ex vivo* tissue and, in some cases, introduce additional functionality such as the ability to locally sense analytes or the ability to extract sample volumes for off-chip analysis. The majority of micro-culture systems still rely on thousands to millions of cells, although there have been some efforts towards minimalism. The minimalism approach is inherently efficient and can deliver data with single cell resolution. Importantly, there is a general lack of suitable cell lines that

recapitulate the behaviour of the tissue-specific cell types. In many cases, cells harvested following the sacrifice of animals, typically rodents, are used as superior model systems which represent the gold standard. With the advent of minimalistic cell culture micro-culture systems there is the potential to greatly increase the number of experiments that can be undertaken using cells prepared from an animal dissection. For this to be achieved the micro-culture systems must be cost effective and scalable. The ability to interface with industry standard platforms, such as robotic pipetting stations and high content imaging systems, is a key issue towards assay scalability. The preparation of *in vitro* neuronal tissue models are proving especially useful.

Understanding the workings of the brain in healthy and diseased states remains a grand challenge. The brain is characteristically complex: It is highly compartmentalized, layered, and contains diverse cell types with plastic connectivity via axon and dendrite outgrowths. To better investigate the brain and the greater nervous system there is a need for more complex, yet well defined, neurobiology models which emulate the *in vivo* microenvironment. The interconnections within the brain are organized over micron length scales, dimensions which can be readily achieved using microfabrication techniques and replicated for high throughput analysis.

Patterning the cell adhesion microenvironment has been used to disentangle the connectivity of nervous tissue.⁴ This capability has been applied to signal transmission⁵⁻⁷ and growth dynamics⁸ research, and recently used for high throughput neurotoxicity screening.⁹⁻¹¹ More complex spatially defined models can be fashioned using compartmentalized co-culture microenvironments. These microfabricated refinements of the Campenot chamber¹² contain microchannels for guided neurite outgrowth.¹³ These tissue models can be used for studying pathology propagation following localized treatments with toxins, pathogens, mechanical damage or other insults and perturbations. Arrayed and disentangled neurite outgrowths provide a useful analytical display,⁹⁻¹¹ and the compartmentalized arrangement also brings the opportunity for selectively treating or isolating the soma or outgrowths for off-chip analysis.^{14,15} These systems have been used to great effect to study axon degeneration and regeneration following chemical^{15,16} or laser¹⁶⁻¹⁸ axotomy, tauopathy¹⁹ and viral^{20,21} dissemination, and mRNA localization in axons.¹⁴ The systems have also been adapted for engineering the polarity of the synaptic junction using geometric diodes²² or for synaptogenesis screening using HEK293 cells disguised as post-synaptic structures by recombinant decoration with neuroligin-1.²³

Despite the many benefits of compartmentalized neuron culture platforms there is still great scope for improvement. Tens of thousands of neurons are currently seeded into each millimeter-scale compartment to attain significant numbers of inter-compartment connections.¹⁴ Consequently, the neuron number, their positions and neurite interconnections are not controlled and the vast majority of the network is locally entangled. As a consequence only a small proportion of neurons (<5%) extend outgrowths between compartments. This limits the investigation of neurite-based (bio)material transport between the compartments and reduces the determinism of reconstructed neuronal networks. The inability to control cell seeding density also results in varied cell viability and responses, and the occurrence of

multiple outgrowths within a channel which prevents the analysis of single outgrowths. Moreover, extravagant cell usage (as with standard *in vitro* assays) is undesirable for high throughput experiments, especially with rare cells such as *substantia nigra* cells, dopaminergic cells suitable for Parkinson's research, or peripheral neurons. High throughput studies would require the sacrifice of multiple animals with data analysis complicated by inter-individual variance. Another major drawback is that pipetting sufficient neurons into the microfluidic culture compartment can be troublesome, not suitably user-friendly and limiting the scope for automation with robotic pipette systems that is necessary for high throughput screens.

Material transport between cells and especially within neuronal tissue is increasingly recognised as a central process occurring in the spread of infectious organisms (*e.g.* rabies, herpes, prions), toxic materials (*e.g.* manganese nanoparticles), 'dementia' diseases (*e.g.* amyloid plaques in Alzheimer's and Parkinson's disease) and pathology (*e.g.* arising from inflammation). Experiments are currently transitioning from research labs into pharmaceutical and toxicology laboratories. The experiments demand cell cultures (*e.g.* neuronal) to be separated via miniature interconnecting channels, one compartment being treated and with the analytical end-point being the transmission (or not) of the test substance, or resulting products or pathology to the second compartment. A commercial device (Xona Microfluidics LLC) exists, but has numerous limitations:

- [1] The device requires large cell numbers which reduces the capacity for high throughput screening. One of the major bottle necks is the need for rodent neurons that must be prepared by highly skilled technicians – maximising the number of experiments from a single neuronal preparation is of significant value.
- [2] The cell culture density in the device is not controlled which greatly impacts cell viability and behaviour. To ensure adequate cell seeding, current systems require the addition of dense cell suspensions (*e.g.* 10^7 cells/mL) that are prone to aggregate resulting in non-controlled seeding density and sometimes, device clogging.
- [3] The intercompartment cell-to-cell connection levels are low (~5%) as the cells are randomly positioned.
- [4] The number of outgrowths (axons or dendrites) per connection channel cannot be controlled, thereby either reducing the number of connections further or with the risk of multiple outgrowths per channel. Overall, this greatly prohibits single outgrowth imaging, the gold standard analytical end-point.
- [5] Devices are not particularly user-friendly and are relatively large, making experimental up-scaling difficult.

An improved microfluidic method was developed by Dinh *et al* (*Lab Chip*, 2013, 13, 1402)²⁴, which could be used to control the cell number and position more effectively. However, this method requires syringe pumps making it (i) not user-friendly, and (ii) not suitable for scale-up with industry standard robotic pipetting stations.

An aim of the present invention is to provide an improved microfluidic device which can address the drawbacks of currently available devices, and be extended to a wider range of applications requiring precision sample volume metering and precision particulate metering and positioning.

According to a first aspect of the invention, there is provided a microfluidic device suitable for generating a capillary action during loading comprising:

one or more input channels;

one or more compartments connected to the one or more input channels, and comprising one or more traps or analysis sites;

a volume metering element connected to the traps or analysis sites of the one or more compartments via one or more microchannels;

wherein the volume metering element has a larger volume, or substantially equal volume, than the one or more compartments, and is arranged to control capillary action through the one or more microchannels.

The invention provides an improved microfluidic device which can address the drawbacks of currently available devices, and be extended to a wider range of applications requiring precision sample volume metering and precision particulate metering and positioning. Specifically the microfluidic device is designed to deliver, by capillary action, and at a flow interruption point cause the flow to stop and thereby control the volume of liquid transported into the device and over an analysis site.

The microfluidic device can advantageously work without a syringe or other types of pump to introduce cells, such as neurons, and would further use minimal cell numbers as they would be positioned in the cell traps to maximise the probability of single outgrowths extending through the microchannels. In addition, the microfluidic cell arraying principle should ideally be gentle to avoid damaging the cells. For example, even small pressures used to drive cells into trap structures can damage the cells. Capillary flow microfluidic circuits may provide all the benefits of the known Xona system (compartmentalised neuron co-cultures with localised system perturbation (*i.e.* fluidically isolated)), while solving current problems with the Xona system. For example, the microfluidic device of the present invention can be used for the metered delivery and positioning of neurons to arrayed locations for high probability inter-compartment connectivity (e.g. ~70%). This system is inherently economical requiring only ~100 cells per device and <1 μL of test substance when necessary (this is especially relevant to rare and costly novel compounds). The microfluidic device and operating principle of the present invention can be appropriately reconfigured to match the demands of a wide variety of (bio)chemical analysis scenarios.

A further benefit is that the microfluidic device of the present invention is suitable for scale-up (no fluidic interconnection may be required) and use with industry standard robotic

pipetting stations. This scale of operation is matched by analytical imaging platforms that can achieve whole plate, high content imaging in a few minutes.

The ability to accurately position cells can be extended to a wide variety of applications, such as electrophysiology, cell counting, immune synapse preparation, scratch and migration assays (here a microfabricated insert is removed to initiate cell migration), or other applications requiring the precision metering and positioning of single or multiple cells with examples including genome, exome, transcriptome or proteomic analyses or other biomolecules such as lipids and carbohydrates. Indeed, cell metering and positioning is a broadly applicable feature for (bio)chemical sample analysis.

Reference to “connected” refers to a connection that is arranged to be a fluid connection between components of the microfluidic device. *i.e.* fluid (when present) is capable of flowing from one component connected to another component, for example in the presence of capillary action or active pumping. Such a term is not intended to exclude connections comprising control features such as valves, which may be arranged to control or shut off fluid flow.

Reference to “loading” refers to the delivery of cells or other analytes into the device, for example a suspension of cells will be placed into an input well, whereby the suspension flows into and through the device and the cells are arrayed in the cell traps of the cell culture compartment(s).

Reference to “particles” refers to analytes within the sample that may be loaded into the device. Particles may be small, *i.e.* less than 1000nm, such as small molecules/chemicals of 900Da or less, biomolecules, viruses or exosomes, or they may be larger (e.g. micrometre scale) for positioning at traps with sub-particle-sized apertures and may include cells such as bacteria, algae, fungi, mammalian cells or oocytes. Particles may also include organs or tissues such as brains or brain regions, or may include whole organisms such as worms (*e.g. C. elegans*), parasites, embryos or immature zebra fish.

Reference to use “without a syringe or other types of pump”, or similar, refers to the exclusion of a device such as a syringe or pipette, or a pump, to actively push or pull cells and/or fluids through the channels of the device. Such terms do not exclude the use of a device, such as a pipette, to add fluids, cells, and/or agents, such as test substances, to reservoirs, for example in an input well.

Reference to “direct” or “immediate” connection may refer to an immediate and direct connection that does not comprise fluid connection via other components of the microfluidic device. However, in some embodiments the connection may not be “direct/immediate” where fluid connection may be via additional channels, sample ports, chambers, wells, valves, or the like.

VOLUME METERING ELEMENT

The term “volume metering element” used herein may also be referred to as a “volume metering channel”.

The volume metering element may be an outlet channel. One end of the volume metering element may be open in order to allow passage of air, for example to prevent the build-up of pressure that would prevent capillary action effectively moving fluid through the device. The volume metering element may be arranged to influence, such as promote, a capillary pressure during loading of the microfluidic device. The volume metering element may be arranged to control the volume of liquid flowing through the microfluidic device during the loading of the microfluidic device. The volume metering element may be arranged to allow a specific volume of liquid to flow via capillary action. The volume metering element may be arranged to control the volume of fluid passing through the microfluidic device, in particular passing through the compartment and/or microchannels of the microfluidic device. In one embodiment, the volume metering element is arranged to control the volume of fluid passing through the cell culture compartment and microchannels of the microfluidic device. In another embodiment, the volume metering element is arranged to control the volume of fluid passing through the cell culture compartment, traps or analysis sites, and microchannels of the microfluidic device. The terminus of the volume metering element may substantially prevent flow.

The volume metering element may be arranged to control the flow rate of fluid passing through the microfluidic device, in particular passing through the compartment and/or microchannels of the microfluidic device. In another embodiment, the volume metering element may be arranged to control the flow rate of fluid passing through the microfluidic device, in particular passing through the cell culture compartment and/or microchannels of the microfluidic device. In another embodiment, the volume metering element may be arranged to control the flow rate of fluid passing through the microfluidic device, in particular passing through the cell culture compartment, traps or analysis sites, and microchannels of the microfluidic device.

The volume metering element may be arranged to control the flow rate and volume of fluid passing through the microfluidic device, in particular passing through the compartment and/or microchannels of the microfluidic device. In another embodiment, the volume metering element is arranged to control the flow rate and volume of fluid passing through the microfluidic device, in particular passing through the cell culture compartment and/or microchannels of the microfluidic device. In another embodiment, the volume metering element is arranged to control the flow rate and volume of fluid passing through the microfluidic device, in particular passing through the cell culture compartment, traps or analysis sites, and microchannels of the microfluidic device.

The control of the fluid by the volume metering element may be by capillary pressure, whereby a fluid front may travel through the volume metering element under capillary action, and the volumetric capacity, length, surface free energies and/or hydraulic diameter of the volume metering element are arranged to allow a specific volume of fluid and/or rate of fluid flow. The volume of the volume metering element may define the volume delivered through

the device. The volume of the volume metering element may define the volume delivered through the compartment, such as the cell culture compartment. The volume of the volume metering element may define the volume delivered through the cell culture compartment. The dimensions, arrangement and surface free energies may define the flow rate.

The volume metering element may comprise a capillary flow interruption point. The capillary flow interruption point may be an enlargement, such as a substantial enlargement in the hydraulic diameter or circumference of the volume metering element. The enlargement may be at least a 2-fold enlargement in the hydraulic diameter or circumference of the volume metering element. The enlargement may be at least a 3-fold enlargement in the hydraulic diameter or circumference of the volume metering element. The enlargement may be at least a 5-fold enlargement in the hydraulic diameter or circumference of the volume metering element. The enlargement may be at least a 10-fold enlargement in the hydraulic diameter or circumference of the volume metering element. In another embodiment, the enlargement may be at least a 20-fold enlargement in the hydraulic diameter or circumference of the volume metering element. In another embodiment, the enlargement may be at least a 50-fold enlargement in the hydraulic diameter or circumference of the volume metering element. In another embodiment, the enlargement may be at least a 100-fold enlargement in the hydraulic diameter or circumference of the volume metering element. The enlargement may be a sudden enlargement, such as a stepped enlargement in the wall of the volume metering element. The enlargement may be the sudden end of the volume metering element into an opening in a wall, chamber or well of the microfluidic device. Alternatively, the enlargement may be a tapering of the volume metering channel, whereby it would become increasingly difficult for capillary action to take place as the diameter of the volume metering element increases (i.e. the capillary force will be weaker as the channel enlarges to a point at which it slows the flow and potentially stops the flow of fluid). Alternatively, flow interruption may comprise a reduction in size of the hydraulic diameter such that the flow rate is greatly reduced and may be considered to effectively have a zero flow rate. The reduction in size may comprise a 10-fold reduction in size of the hydraulic diameter. Alternatively, the reduction in size may comprise a 50-fold reduction in size of the hydraulic diameter. Alternatively, the reduction in size may comprise a 100-fold reduction in size of the hydraulic diameter.

The capillary flow interruption point may be a change in surface properties of the volume metering element, for example, a section of the microchannel may be provided with a hydrophobic surface. The capillary flow interruption point may be the end of the volume metering element, for example as it emerges into an outlet well. The capillary flow interruption point may be a point along the volume metering element for which the volume of liquid supplied to the device is incorporated fully into the microfluidic device. For example, where the volume metering element comprises a volume capacity substantially larger than the microchannels, compartment and inlet channel, the fluid front may stop at a point in the volume metering element as the last of the fluid provided enters the microfluidic device, for example into the inlet channel. In another embodiment, where the volume metering element comprises a volume capacity substantially larger than the microchannels, cell culture

compartment, and inlet channel, the fluid front may stop at a point in the volume metering element as the last of the fluid provided enters the microfluidic device, for example into the inlet channel. In another embodiment, where the volume metering element comprises a volume capacity substantially larger than the microchannels, cell culture compartment, trap or analysis sites, and inlet channel, the fluid front may stop at a point in the volume metering element as the last of the fluid provided enters the microfluidic device, for example into the inlet channel.

The volume metering element may be a channel extending from the microchannel(s) to an outlet, such as an outlet well.

The start of the volume metering element may be defined as the point upstream at which all of the microchannels in the device contribute to the flow into the volume metering element. For example, the volume metering element may begin or connect at the last (most downstream) microchannel(s). The start of the volume metering element may be at the opposing end of the volume metering element relative to the capillary flow interruption point.

The volume of the volume metering element may be defined as the section of the volume metering element between the start of the volume metering element (e.g. where the channel connects to the microchannels (upstream in use)) and the capillary flow interruption point. For example, the volume metering element may comprise a smaller channel having a step change into a larger channel or chamber, which in use would be a capillary flow interruption point, and the volume of the volume metering element may be defined as the volume of the smaller channel, and not include the volume of any further channel or chamber after this capillary flow interruption point.

The volume metering element may have a volume of between about 1 nanolitre and about 1 millilitre. The volume metering element may have a volume of between about 1 nanolitre and about 1 microlitre. The volume metering element may have a volume of between about 1 microlitre and about 100 microlitres. The volume metering element may have a volume of between about 50 nanolitres and about 5,000 nanolitres. The volume metering element may have a volume of between about 50 nanolitres and about 2,000 nanolitres. The volume metering element may have a volume of between about 50 nanolitres and about 1000 nanolitres.

The volume metering element may have a larger (e.g. 2-fold to 10-fold) volume than the compartment, or in the case of multiple compartments, larger than the combined volume of the compartments. The volume metering element may have a larger (e.g. 2-fold to 10-fold) volume than the cell culture compartment, or in the case of multiple cell culture compartments, larger than the combined volume of the cell culture compartments. The volume metering element may have a larger volume than the combined volume of the microchannels and the compartment(s). The volume metering element may have a larger volume than the combined volume of the microchannels and the cell culture compartment(s). The volume metering channel may have a substantially equal volume to the combined volume of the microchannels and the compartment(s). The volume metering channel may

have a substantially equal volume to the combined volume of the microchannels and the cell culture compartment(s).

The volume metering element may have at least a 1-fold larger volume than the compartment, or in the case of multiple compartments, at least 1-fold larger than the combined volume of the compartments. The volume metering element may have at least a 1-fold larger volume than the cell culture compartment, or in the case of multiple cell culture compartments, at least 1-fold larger than the combined volume of the cell culture compartments.

The volume metering element may have at least a 2-fold larger volume than the compartment, or in the case of multiple compartments, at least 2-fold larger than the combined volume of the compartments. The volume metering element may have at least a 2-fold larger volume than the cell culture compartment, or in the case of multiple cell culture compartments, at least 2-fold larger than the combined volume of the cell culture compartments.

The volume metering element may have at least a 5-fold larger volume than the compartment, or in the case of multiple compartments, at least 5-fold larger than the combined volume of the compartments. The volume metering element may have at least a 5-fold larger volume than the cell culture compartment, or in the case of multiple cell culture compartments, at least 5-fold larger than the combined volume of the cell culture compartments.

The volume metering element may have at least a 10-fold larger volume than the compartment, or in the case of multiple compartments, at least 10-fold larger than the combined volume of the compartments. The volume metering element may have at least a 10-fold larger volume than the cell culture compartment, or in the case of multiple cell culture compartments, at least 10-fold larger than the combined volume of the cell culture compartments.

The volume metering element may have at least a 20-fold larger volume than the compartment, or in the case of multiple compartments, at least 20-fold larger than the combined volume of the compartments. The volume metering element may have at least a 20-fold larger volume than the cell culture compartment, or in the case of multiple cell culture compartments, at least 20-fold larger than the combined volume of the cell culture compartments.

The volume metering element may have at least a 50-fold larger volume than the compartment, or in the case of multiple compartments, at least 50-fold larger than the combined volume of the compartments. The volume metering element may have at least a 50-fold larger volume than the cell culture compartment, or in the case of multiple cell culture compartments, at least 50-fold larger than the combined volume of the cell culture compartments.

The volume metering element may have at least a 100-fold larger volume than the compartment, or in the case of multiple compartments, at least 100-fold larger than the combined volume of the compartments. The volume metering element may have at least a 100-fold larger volume than the cell culture compartment, or in the case of multiple cell

culture compartments, at least 100-fold larger than the combined volume of the cell culture compartments.

The volume metering element may be between about a 1-fold and about a 20-fold larger in volume than the compartment, or in the case of multiple compartments, between about 1-fold and about 20-fold larger than the combined volume of the compartments. The volume metering element may be between about a 1-fold and about a 20-fold larger in volume than the cell culture compartment, or in the case of multiple cell culture compartments, between about 1-fold and about 20-fold larger than the combined volume of the cell culture compartments.

The volume metering element may be between about a 1-fold and about a 200-fold larger in volume than the compartment, or in the case of multiple compartments, between about 1-fold and about 200-fold larger than the combined volume of the compartments. The volume metering element may be between about a 1-fold and about a 200-fold larger in volume than the cell culture compartment, or in the case of multiple cell culture compartments, between about 1-fold and about 200-fold larger than the combined volume of the cell culture compartments.

The volume metering element may be between about a 1-fold and about a 100-fold larger in volume than the compartment, or in the case of multiple compartments, between about 1-fold and about 100-fold larger than the combined volume of the compartments. The volume metering element may be between about a 1-fold and about a 100-fold larger in volume than the cell culture compartment, or in the case of multiple cell culture compartments, between about 1-fold and about 100-fold larger than the combined volume of the cell culture compartments.

The relative volume between the volume metering element (s) and the compartment(s)/cell culture compartment(s) are intended to be a reference to connected (e.g. fluidly connected) volume metering elements(s) and compartment(s)/cell culture compartment(s), (i.e. the relation between the volume of a volume metering element and the volume of the compartment(s)/cell culture compartment(s), for which flow is arranged to be controlled by the volume metering element).

Advantageously, the volume of the volume metering element relative to the compartment(s) or cell culture compartment(s) may be adjusted depending on the application of the device. For example, in some applications, additional non-arrayed cells may be desired in the cell culture compartment(s), for example for providing growth support. For example, an equivolume cell culture compartment(s) and volume metering element could provide one additional cell for every one arrayed cell. In other applications such helper cells may be undesirable, such that you want zero additional/non-arrayed cells or particles. In this regard the volume metering element may be significantly larger in volume, such as greater than 100-fold that of the compartment(s) or cell culture compartment(s) for use with low cell concentrations.

The volume metering element may be tapered, such that it has a narrower section graduating to a wider section. The volume metering element may increase in hydraulic diameter or circumference as it extends further away from the microchannels. The volume metering element may increase in hydraulic diameter or circumference as it extends downstream, for example during loading of the microfluidic device.

The volume metering element may comprise multiple channels. For example, a single outlet channel may connect to the microchannel(s), and comprise a split, such as a bifurcation, into multiple channels at a section downstream of a single volume metering channel.

The volume metering element may be branched, wherein each branch of the volume metering channel connects pairs or groups of microchannels. The volume metering element may comprise both branched sections at the microchannel-connected end, and multiple channels at the end distal to the microchannel-connected end. The volume metering element may comprise a branched section at the end connected to the microchannel(s), and multiple channels at the end that is distal to the end of the volume metering element connected to the microchannel(s). The volume metering element may comprise a channel incorporating a plurality of fin structures that act to produce multiple flow paths within the same channel. The fin structures may divide a channel of the volume metering element into multiple smaller channels. The fins may be tapered in shape, with the apex pointing in the opposing direction of the arranged flow path. The fin structures may increasingly divide a channel of the volume metering element into multiple smaller channels towards the outlet or capillary flow interruption point (e.g. the number of fins may increase from the start to the end of the volume metering element). Overlapping rows of fins may be provided in which the fins in a row are substantially aligned with each other with a subsequent row of aligned fins overlapping the previous row of fins (e.g. forming a configuration of overlapping rows of fins). The number of fins may increase by one fin for each row towards the outlet (i.e. along the arranged flow path) with the first row comprising one fin. For example in a direction along the arranged flow path a first fin may be provided followed by a row of two fins, then a row of three fins, etc.

The volume metering element may comprise a substantially circular, rectangular or square cross-section. The volume metering element may comprise a substantially rectangular cross-section.

The volume metering element may be non-linear. The volume metering element may be curved, or have multiple curves. The volume metering element may be arranged in a circuitous path. The volume metering element may be arranged in a spiral path. The volume metering element may be arranged in a serpentine path.

The volume metering element may form a T-junction with a microchannel, whereby the microchannel ends into the volume metering element. Alternatively, or additionally, the volume metering element may form a T-junction, where two aligned microchannels end, and the volume metering element leads away from the microchannels. Where two microchannels

end at the volume metering element, the two microchannels may be aligned opposite each other on either side of the connection to the volume metering element.

The microfluidic device may comprise multiple volume metering elements, such as two or more. Alternatively, the microfluidic device may comprise three or more volume metering elements. The microfluidic device may comprise two volume metering elements. The microfluidic device may comprise three volume metering elements. Each compartment, such as cell culture compartment, may be connected via the microchannel(s) to a separate volume metering element. For example, for heterogeneous co-cultures, the input wells and input channels and cell culture compartments may be separate for each cell culture and a volume metering element may be individually provided for flow control for each of these. However, in some embodiments, different compartments, such as cell culture compartments, may share flow control via the same volume metering element (e.g. as they are connected via the microchannels).

Where the microfluidic device comprises multiple volume metering elements, they may be equal in volume, size or diameter. Alternatively the volume metering elements may be different in volume, size or diameter, as required for example, for different cell cultures and/or different analysis. If multiple, different cell culture compartments are provided to create sequential co-cultures, the region of the volume metering element that connects with the microchannels may be substantially smaller (e.g. 10-20 microns or 1-20 microns) than the opposing (next) cell culture compartment (e.g. 100-200 microns or 20-200 microns). This provides that capillary flows from the first will only fill the microchannels (and travel up the volume metering element) and not extend into the adjacent cell culture compartment. The adjacent cell culture compartment would remain dry for subsequent capillary flow loading with new cells that could be different in nature.

MICROCHANNELS

The microchannel(s) can serve to connect the compartment(s) to the volume metering elements(s). The microchannel(s) can serve to connect the cell culture compartment(s) to the volume metering elements(s). In another embodiment, the microchannel(s) can serve to connect the traps or analysis sites of the compartment(s) to the volume metering elements(s). The microchannels may comprise an array of channels extending from the compartment(s). In another embodiment, the microchannels may comprise an array of channels extending from the cell culture compartment(s). In use, the microchannels may form a fluid connection between the compartment(s) and the volume metering element. Alternatively in use, the microchannels may form a fluid connection between the cell culture compartment(s) and the volume metering element.

In some embodiments, each microchannel may end into a volume metering element. In an embodiment comprising two or more compartments, such as cell culture compartments, the compartments may be connected to each other by the one or more microchannels. Adjacent compartments, such as cell culture compartments, may be interconnected by the

microchannel(s). Two or more microchannels may be arranged substantially parallel to each other. The microchannels may form a grid structure, with multiple microchannels traversed by one or more further microchannels. A microchannel may traverse across multiple microchannels and, in use, form a fluid connection between the multiple microchannels and the volume metering element. Groups of, or all, microchannels may connect into a single channel before connecting into a volume metering element of the microfluidic device.

Microchannels may be grouped, wherein each microchannel group connects to a different volume metering element relative to another group of microchannels.

The microchannels may be outgrowth channels, for example, arranged to allow outgrowth from a cell, such as a neuronal cell. The outgrowth may be a neurite. The outgrowth may be an axon or dendrite or filipodia or other cellular structure.

The microchannels may be arranged to block the passage of cells. In another embodiment, the microchannels may be arranged to block the passage of particles. The microchannels may be smaller than the cells/particulates of interest. For example, the microchannels may be too small in hydraulic diameter or circumference for the passage of a eukaryote cell. In the case of a nucleated cell the microchannels may be too small in hydraulic diameter or circumference for the passage of the nucleus, such as less than 10 microns, or less than 5 microns or less than 2.5 microns. For example, the microchannels may be too small in hydraulic diameter or circumference for the passage of a mammalian cell. The microchannels may comprise a sub-cell sized hydraulic diameter or circumference, for example, a sub-neuronal cell sized hydraulic diameter or circumference. The microchannels may form, at least in part, the cell traps, wherein the inlet to the microchannel from the cell culture chamber is a cell trap.

In one embodiment, the microchannels may be smaller than the cells to be studied/analysed, but larger than any other cells or particles in the sample to be loaded into the device. Such a configuration may help to filter out unwanted cells or particles from the analysis.

The microchannels may comprise a hydraulic diameter or circumference sized to allow cell outgrowths, such as a neurite. For example, the cell outgrowths may be axons or dendrites. The microchannels may comprise a hydraulic diameter or circumference of 10 microns or less, or 5 microns or less, or 2.5 microns or less. The microchannels may have a hydraulic diameter or circumference of 2.5, 5 or 10 microns substantially along its length. The microchannels may comprise a hydraulic diameter or circumference of 4 microns or less, or 3 microns or less. The microchannels may have a hydraulic diameter or circumference of 3 or 4 microns substantially along its length.

The length of the microchannels may be several tens to several hundred microns long, for example, for neuron applications. The microchannels may be at least about 10 μm in length. The microchannels may be at least about 20 μm in length. The microchannels may be at least about 50 μm in length. The microchannels may be at least about 100 μm in length. The microchannels may be at least about 150 μm in length. The microchannels may be between about 10 μm and 1000 μm in length. The microchannels may be between about 50 μm and

5000 μm in length. The microchannels may be between about 20 μm and 1000 μm in length. The microchannels may be between about 30 μm and 800 μm in length.

In alternative embodiments, cell traps of one cell culture compartment may be positioned close to opposing cell traps of another cell culture compartment, whereby the microchannels may be short enough to allow for cell-body to cell-body contact. The microchannels may be less than about 10 μm in length. The microchannels may be less than about 5 μm in length. The microchannels may be less than about 3 μm in length. The microchannels may be between about 1 μm and 10 μm in length. The microchannels may be between about 1 μm and 8 μm in length. The microchannels may be between about 1 μm and 5 μm in length. The microchannels may be between about 1 μm and 3 μm in length.

A microchannel may be in the form of an aperture, such as a sub-particle sized aperture, formed between a compartment, such as a cell culture compartment, and a volume metering element. A microchannel may be in the form of an aperture, such as a sub-cell sized aperture, formed between a compartment, such as a cell culture compartment, and a volume metering element. For example, the microchannel(s) may be minimal in length, and form an aperture in a partition between the compartment(s), such as the cell culture compartment(s), and the volume metering element(s). A microchannel may be in the form of an aperture in a partition between a compartment, such as a cell culture compartment and a volume metering element. The aperture and partition may form the cell trap.—The microchannel may be of a length corresponding to the thickness of the aperture.

The microchannels may be circular, rectangular, triangular, or square, in cross-section.

The microfluidic device may comprise two or more microchannels per cell trap. Alternatively, the microfluidic device may comprise multiple cell traps sharing an inlet to a single microchannel, for example to promote fasciculation in which neurite outgrowths may form bundles. This is useful to assess possible protective mechanisms or propagation promoting conditions that arise from growth in bundles.

The microfluidic device may comprise microchannels of varying lengths relative to each other, for example, for determining effects of distance/cell outgrowth length. In an embodiment comprising microchannels of different lengths, two or more cell culture compartments may be provided at different distances from a first cell culture compartment, and connected to the first cell culture compartment by microchannels of suitable length.

Agents, such as test substances or assay reagents may be delivered to the compartment(s), such as the cell culture compartment(s). Agents, such as test substances or assay reagents may be delivered directly, for example exclusively, to the microchannels by an additional inlet channel. The additional inlet channel may connect directly to one or more microchannels and/or compartments. The additional inlet channel may connect directly to a microchannel that traverses other microchannels, and is in fluid connection with the other microchannels. The additional inlet channel may form a path for an agent that connects directly to a traversing microchannel that traverses other microchannels, and is in fluid connection with the other microchannels, and the traversing microchannel ends into, and

connects with, the volume metering element. The microchannels may not permit, or facilitate, perfusion of directly delivered agents to the cell culture chamber. For example, the effect of an agent specifically on cell outgrowths may be studied by delivering an agent directly into one or more microchannels, or groups of microchannels, whereby, the agent would not be provided to the cell body in the cell culture compartment. In another example, different reagents may be delivered to different analysis sites for the detection of different analytes. In another example, different reagents may be delivered to different compartments for the detection of different analytes.

Advantageously just a single additional inlet channel connected to the cell culture chamber is sufficient to create a flow path of the agent or assay reagents that will be largely restricted to the inlet channel and compartments, such as cell culture compartments. Alternatively, an inlet channel connected and orthogonal to the microchannels can be used to create a flow path of the agent or assay reagents that will largely be restricted to the inlet channel and also the volume metering element, but to a much less extend the orthogonal channels connecting the compartments, such as the cell culture compartments.

The compartments can be occupied with particles and/or cells. At least one of the compartments may be without particles and/or cells.

OUTLET WELL AND CONNECTION

The microfluidic device may further comprise an outlet well or chamber connected to the volume metering element. The connection between the volume metering element and the outlet well may be direct/immediate. Alternatively, the connection between the volume metering element and the outlet well may be via additional channels, sample ports, chambers, wells, valves, or the like.

The outlet well may be arranged to be positioned downstream of the volume metering element, for example, downstream whilst loading the microfluidic device. The outlet well may be arranged to be positioned immediately downstream of the volume metering element, for example, immediately downstream whilst loading the microfluidic device.

The output well may have a substantially larger volume than the output channel. The outlet well may have a volume of a standard well of a standard 6-well, 24-well, 96-well or 384-well plate. The outlet well may be positioned and sized substantially equal to a standard 6-well, 24-well, 48-well, 96-well or 384-well plate, in order to facilitate automation with standard multiwell handling machinery. The outlet wells may be positioned to coincide with the well positions of 6-well, 24-well, 96-well or 384-well plates, but may have dissimilar sizes, for example with diameters of 8 mm or less, diameters of 5 mm or less, diameters of 3 mm or less, diameters of 2 mm or less and diameters of 1 mm or less.

The transition or connection from the volume metering element to the output well may be a sudden and substantial increase in size, such as the hydraulic diameter, for example, in use to

stop capillary action causing flow of fluid through the device as soon as the fluid front reaches the outlet well.

The microfluidic device may comprise multiple outlet wells. For example, in a device comprising multiple volume metering elements, each volume metering element may be connected to a separate outlet well.

INPUT WELLS AND CONNECTION

The microfluidic device may further comprise one or more input wells connected to the input channel. The connection between the input well and the input channel may be direct/immediate. Alternatively, the fluid connection between the input well and the input channel may be via additional channels, sample ports, chambers, wells, valves, or the like.

The input well may be arranged to be positioned upstream of the input channel, for example, upstream whilst loading the microfluidic device. The input well may be arranged to be positioned immediately upstream of the input channel, for example, immediately upstream whilst loading the microfluidic device.

The input well may have a substantially larger volume than the input channel. The input well may have a volume of a standard well of a standard 6-well, 24-well, 96-well or 384-well plate. The input well(s) may be positioned substantially equal to an arrangement of wells for a standard 6-well, 24-well, 48-well, 96-well or 384-well plate format, in order to facilitate automation with standard multiwell handling machinery. The input wells may be positioned to coincide with the well positions of 6-well, 24-well, 96-well or 384-well plates, but may have dissimilar sizes, for example with diameters of 8 mm or less, diameters of 5 mm or less, diameters of 3 mm or less, diameters of 2 mm or less and diameters of 1 mm or less.

The input well may have a volume of between about 1 μ l and about 16 ml. The input well may have a volume of between about 50 μ l and about 16 ml. The input well may have a volume of about 15.5 ml. The input well may have a volume of about 6 ml. The input well may have a volume of about 3.5 ml. The input well may have a volume of about 1.4ml. The input well may have a volume of about 350 μ l. The input well may have a volume of about 50 μ l. The input well may have a volume of about 10 μ l. The input well may have a volume of about 1 μ l.

Input well(s) may be arranged to receive cells for cell loading of the microfluidic device.

The microfluidic device may comprise multiple input wells, such as two input wells, or three, or more input wells. One input well may be for loading cells, whilst one or more additional input wells may be for loading agents. In embodiments comprising two or more compartments, such as cell culture compartments, the device may comprise two or more input wells capable of selectively supplying agents to particular compartments. For example, one or two, or more input wells may feed into multiple compartments, such as cell culture compartments, or each individually feed into different compartments, or groups of

compartments. Different cell types/cultures or samples may be loaded into separate compartments, such as cell culture compartments via two or more different input wells.

INPUT CHANNEL

The input channel(s) may be for sample, particle or cell loading. The input channel(s) may be for cell loading. The input channel(s) may be arranged to allow the passage of the sample, particles or cells into the compartment, such as the cell culture compartment. The input channel(s) may be arranged to allow the passage of the cells into the cell culture compartment.

Multiple input channels may be provided in the microfluidic device. For example, second or third input channels may connect to the compartment, such as the cell culture compartment, or to separate compartments. One or more input channels may be for loading cells, whilst one or more additional input channels may be for loading agents, or different types of cells. In one embodiment, the device may comprise a single input channel for loading cells or particles. In one embodiment, the device may comprise a single input channel for loading cells or particles and one or more additional input channels for loading agents.

Additionally, or alternatively an additional input channel may connect directly to the microchannels, for example to deliver agents locally to the microchannels. An input channel connecting directly to the microchannels may be connected to an additional input well. An input channel connected directly to the microchannels may not be connected directly to the compartment, such as the cell culture compartment. The additional input channels may have a volume arranged to be small enough to avoid significantly competing for fluid with the volume metering element. The additional input channels may have a smaller volume than the volume metering element. The additional input channels may have a substantially smaller volume than the volume metering element. Where an additional flow path is provided for delivering agents to particles, cells or cell outgrowths, the volume of the channels defining the flow path for the agents may be small enough to avoid significantly competing with the flow path to the volume metering element.

The ability to deliver agents locally to the microchannels enables the specific effects of the agent on cell outgrowths to be observed.

The additional input channel may connect from an additional input well. Additional input channel(s) may connect from the same input well.

The microfluidic device may comprise additional input and additional output wells that form a fluid path from one well to the other via the compartment, such as the cell culture compartment. For example, an agent may be deliverable to the cells in the cell culture compartment by flowing an agent through an additional input well, which connects to the cell culture compartment and to an additional output well connecting to the cell culture compartment. Such a feature may facilitate the selective treatment of cells in the cell culture compartment with an agent, and not the outgrowths in the microchannel(s).

The input channel(s) may be connected upstream of the compartment, such as the cell culture compartment, for example during loading of the microfluidic device. Input channel(s) may additionally be connected upstream of microchannels, for example during loading of the microfluidic device.

COMPARTMENT

In one embodiment, the compartment(s) is a cell culture compartment(s), for example arranged to contain cells. In another embodiment, the compartment(s) is a tissue, organ or organism culture compartment(s), for example arranged to contain tissue, an organ or an organism. The cell culture compartment is the chamber in which cells or other particles are captured and can be incubated to facilitate culture. The compartment may comprise a chamber having microchannels extending therefrom. The compartment may be arranged to receive and keep particles or cells upon loading of the microfluidic device.

The microfluidic device may comprise two compartments, such as two cell culture compartments. For example, where interaction between cells is to be studied, the cell culture compartment may be aligned alongside another cell culture compartment, which are connected to each other by one or more microchannels. For example, the input channel that leads from the input well may diverge to form two or more compartments that are interconnected by substantially smaller microchannels. The microfluidic device may comprise multiple compartments, and each compartment may be connected, via microchannel(s) to a separate volume metering channel.

The compartment may comprise one or more traps arranged along a wall of the compartment. In one embodiment, the cell culture compartment may comprise one or more cell traps arranged along a wall of the cell culture compartment. The compartment may comprise at least 2 traps. In one embodiment, the cell culture compartment may comprise at least 2 cell traps.

The compartment may comprise at least 3 traps. The compartment may comprise at least 4 traps. The compartment may comprise at least 5 traps. The compartment may comprise at least 8 traps. The compartment may comprise at least 20 traps. The compartment may comprise at least 50 traps. The compartment may comprise at least 100 traps. The compartment may comprise between about 2 traps and about 100 traps. The compartment may comprise between about 10 traps and about 100 traps. The compartment may comprise between about 100 traps and about 1,000 traps. The compartment may comprise between about 1 trap and about 1,000 traps. The compartment may comprise between about 1 trap and about 10,000 traps. The compartment may comprise between about 1 trap and about 100,000 traps.

In one embodiment, the cell culture compartment may comprise at least 3 cell traps. In another embodiment, the cell culture compartment may comprise at least 4 cell traps. In another embodiment, the cell culture compartment may comprise at least 5 cell traps. In

another embodiment, the cell culture compartment may comprise at least 8 cell traps. In another embodiment, the cell culture compartment may comprise at least 20 cell traps. In another embodiment, the cell culture compartment may comprise at least 50 cell traps. In another embodiment, the cell culture compartment may comprise at least 100 cell traps. In another embodiment, the cell culture compartment may comprise between about 2 cell traps and about 100 cell traps. In another embodiment, the cell culture compartment may comprise between about 10 cell traps and about 100 cell traps. In another embodiment, the cell culture compartment may comprise between about 100 cell traps and about 1,000 cell traps. In another embodiment, the cell culture compartment may comprise between about 1 cell trap and about 1,000 cell traps. In another embodiment, the cell culture compartment may comprise between about 1 cell trap and about 10,000 cell traps. In another embodiment, the cell culture compartment may comprise between about 1 cell trap and about 100,000 cell traps.

The cell culture compartment(s) may be arranged to accommodate an array of cells, such as mammalian cells, bacteria, fungi, algae, parasites, embryos, oocytes, worms, or brain cells. The cell culture compartment(s) may be arranged to accommodate an array of neurons. The cells may be individually supported in cell traps. In another embodiment, the compartment(s) may be arranged to accommodate an array of particle analysis sites.

The provision of two or more cell culture compartments may enable the formation of co-cultures with distinct cell types in the different cell culture compartments.

The compartment(s) may have a volume of about 1 nanolitre. Alternatively, the compartment(s) may have a volume of about 100 nanolitres. The compartment(s) may have a volume of at least about 1 nanolitres. The compartment(s) may have a volume of at least about 10 nanolitres. The compartment(s) may have a volume of at least about 20 nanolitres. The compartment(s) may have a volume of at least about 50 nanolitres. The compartment(s) may have a volume of less than about 300 nanolitres. The compartment(s) may have a volume of less than about 200 nanolitres. The compartment(s) may have a volume of less than about 120 nanolitres. The compartment(s) may have a volume of less than about 100 nanolitres. The compartment(s) may have a volume of less than about 1 microlitre. The compartment(s) may have a volume of less than about 10 microlitres. The compartment(s) may have a volume of less than about 100 microlitres. The compartment(s) may have a volume of less than about 1 millilitre.

In one embodiment, the cells may be arrayed in an area of the cell culture compartment of at least $100\ \mu\text{m}^2$ and provided at concentrations less than one cell per 10 picolitres, for example in the case of mammalian cells. In embodiments wherein the cells are bacterial cells, the cell may be provided at a concentration of less than 1 cell per 10 femtolitres. The volumetric fraction occupied by the cells may not be greater than 10%. The volumetric fraction occupied by the cells may not be greater than 1%. The volumetric fraction occupied by the cells may not be greater than 0.1%. The volumetric fraction occupied by the cells may not be greater than 0.01%. The volumetric fraction occupied by the cells may not be greater than 0.001%.

The compartment(s) may be any shape that facilitates continued flow through the microchannels channels and out through the volume metering element(s). The compartment may be formed by continuation or bifurcation of an inlet channel. For example, the compartment may be a section, such as a downstream section, of the input channel. The input channel may transition into the compartment without any substantial change in hydraulic diameter or circumference. Alternatively, the input channel may transition into the compartment with a substantial change in hydraulic diameter or circumference, such as an increase in hydraulic diameter or circumference. The transition from the input channel to the compartment may be marked by one or more of a change in hydraulic diameter or circumference; the presence of traps, such as cell traps, and inlets to microchannels; and a bifurcation, wherein the compartment starts at the point of bifurcation.

Multiple compartments may be connected to the same input channel, and optionally input well. Alternatively, multiple compartments may be connected by different/additional input channels, and optionally input wells. Each compartment may be connected to their own separate input wells, and optionally input channels. The device may comprise at least one input well and at least one input channel for each compartment. For example, a device comprising two compartments, each compartment may comprise an exclusive inlet channel, and optionally an exclusive input well.

TRAPS

The traps may comprise particle traps or cell traps. In one embodiment, the traps comprise cell traps. In another embodiment, the traps may comprise particle traps. The particle traps may be arranged to localise particles, such as small molecules, biomolecules, proteins, virus particles, or nanoparticles, and the like.

In one embodiment the traps may not be mechanical traps, for example the traps may be capable of trapping a cell, particle, tissue, organs, embryos, or organisms by affinity (such as biorecognition). The traps may comprise regions of affinity on a surface of the compartment. Such traps may be termed “affinity traps”. The affinity traps may comprise or consist of a region of affinity tags. The affinity tags may be biomolecules such as peptides, proteins, enzymes, antibodies, nucleic acid or nucleic acid analogues (such as DNA, PNA, RNA, LNA, PMO, or aptamer) which are capable of binding to the cell or particle to be trapped at the trap site in the compartment. The affinity tags may comprise biotin, streptavidin or neutravidin. The affinity tags may comprise any molecule capable of specifically or preferentially binding the cell or particle of interest.

The affinity traps may be planar (e.g. a flat surface), or may comprise 3D structure, which increases the surface area for trapping. In one embodiment, the affinity traps comprise a porous matrix. In another embodiment, the affinity traps may comprise projections, ridges, or pili structures with the affinity tags thereon.

In embodiments where an affinity trap is used to trap cells or particles, the microchannels and/or apertures in the traps may be larger than any cells or particles in the sample to be loaded into the device. Such a configuration advantageously allows the trapping of target cells or particles, whilst allowing other non-target cells or particles to be flushed away.

Secondary traps may be provided in addition to the traps. For example an affinity trap or a smaller-apertured trap may be arranged to be positioned in the vicinity of a cell trap for trapping and analysing secretions/products of the trapped cell, or within the cell trap itself. For example an affinity trap or a smaller-apertured trap may be arranged to be positioned downstream of a cell trap for trapping and analysing secretions/products of the trapped cell.

The particle or cell traps may be in the form of an inlet to the microchannels. The particle or cell traps may be connected to the volume metering element(s) via the microchannels.

Cell traps may be arranged to facilitate the positioning of cells. The cell traps may define sub-cell-sized apertures in the cell culture compartment. The cell traps may define sub-mammalian-cell-sized apertures in the cell culture compartment. The cell traps may define sub-eukaryote-cell-sized apertures in the cell culture compartment. The cell traps may define sub-nucleus-sized apertures in the cell culture compartment. Sub-nucleus-sized apertures enable the capture of cells that are deformable (for example the majority of mammalian cells are deformable). The cell traps may define apertures of less than about 10 microns in the cell culture compartment. The cell traps may define apertures of about, or less than about 5 microns in the cell culture compartment. The cell traps may define apertures of less than about 4 microns in the cell culture compartment. The cell traps may define apertures of about, or less than about 3 microns in the cell culture compartment. The cell traps may define apertures of about, or less than about 2.5 microns in the cell culture compartment. The cell traps may define apertures of less than about 100 microns in the cell culture compartment. The apertures in the compartment may be inlets to a microchannel, which connects to the volume metering element. The apertures in the compartment may form the microchannels.

The inlets to the microchannels from the cell culture compartment may be enlarged to form the cell-traps, for example the section of microchannel that connects to the cell culture compartment may increase in diameter or circumference as it enters the cell culture compartment, such that it may partially envelope and accommodate a cell.

The cell traps may comprise a recessed surface in the cell culture compartment. The cell traps may comprise a recessed surface in the cell culture compartment in addition to defining the sub-cell sized aperture (i.e. which is the inlet to a microchannel). The recessed surface may be provided in the base of the cell culture compartment or in a side wall of the cell culture compartment. The microchannel may connect to the recessed surface. The recessed surface may form a pocket arranged to accommodate a cell, such as a mammalian cell. The recessed surface may form a pocket arranged to accommodate a cell, such as a eukaryote cell. The recess may be substantially domed in shape. The recess may be substantially pyramidal in shape. The recess may be substantially square or rectangular in shape. The recess may be at least about 2 μm deep. The recess may be at least about 5 μm deep.

The recess advantageously presents more surface area to the cell, thereby encouraging outgrowth into the microchannel. The recess may also protect the cell from shear stress. The recess may be sufficiently deep to encourage cell entrapment, and potentially outgrowth into the microchannel.

Additionally, or alternatively, the cell traps may comprise pillars in the cell culture compartment, which may flank or partially surround the inlets to the microchannels. The pillars may be spaced apart sufficiently to allow a cell to enter the space between the pillars at the position of the aperture/inlet to the microchannel. The pillars may be spaced apart by a distance of between about 10 and 100 μm . The pillars may be joined, or extending from, the wall of the cell culture compartment. Alternatively the pillars may be positioned such that they define a space between the pillar and the wall of the cell culture compartment.

The cell traps may be positioned in the base of the cell culture compartment or in a side wall of the cell culture compartment.

SURFACE PROPERTIES

The input channel, compartment, such as the cell culture compartment, microchannel and outlet channel may comprise a hydrophilic surface. The volume metering element may comprise a hydrophilic surface. The input channel may comprise a hydrophilic surface. The microchannels may comprise a hydrophilic surface. The compartment may comprise a hydrophilic surface. The hydrophilic surfaces may have a contact angle of less than 90° . The hydrophilic surfaces may have a contact angle of less than 80° . The hydrophilic surfaces may have a contact angle of less than 60° . The hydrophilic surfaces may have a contact angle of less than 50° .

Advantageously, the hydrophilic surface can encourage capillary action of fluid through the device during loading.

In use during loading of the microfluidic device, fluid may be loaded into the input well(s), which would then be arranged to flow by capillary action through the input channel(s), the compartment(s), the microchannels, and the volume metering channel. The flow by capillary action would stop at the volume metering element, either part-way along the volume metering element, or at the connection/transition point of the volume metering element, into the outlet well. The flow by capillary action may stop at a capillary flow interruption point in the volume metering element.

MULTIWELL FORMAT

The microfluidic device may be arranged on a multiwell plate format. For example, a multiwell plate may comprise 1, 2, 3, 10, 20 or more microfluidic devices of the present invention arranged in a single multi-well plate. The multi-well plate may comprise a layout of

wells (e.g. input and output wells) substantially equal to a standard 6-well, 24-well, 48-well, 96-well or 384-well plate. For example, a 96-well plate may comprise 24 microfluidic devices, wherein some, or all, of the wells of the 96-well plate form the input or output wells of the microfluidic devices. The input channel(s), cell culture chamber(s), microchannel(s) and output channel(s) may be arranged in the space between the wells as provided in the format (i.e. position and size) of a standard 6-well, 24-well, 48-well, 96-well or 384-well plate.

OTHER FEATURES

The microfluidic device may comprise a polymer material. The microfluidic device may comprise a hydrogel. The polymer material may form a hydrogel. The polymer material may be substantially transparent. The polymer material may allow the exchange of gases, such as oxygen and carbon dioxide, for example in the instance of cell culture and analysis. The polymer material may be gas permeable. The polymer material may be gas permeable and liquid impermeable.

Various gas permeable polymeric materials are known in the art for use in microfluidic devices and are contemplated to be amenable for use in the microfluidic device described herein. For example, in certain embodiments, the gas permeable material is an organosilicone polymer (e.g., polysiloxane, PDMS variants such as MDX-4, and modified PDMS compositions that enhance gas (e.g., oxygen and carbon dioxide) permeability), polyethylene, or polyurethane. In certain particular embodiments, the gas permeable material is polydimethylsiloxane. The polymer may comprise any polymer selected from the group comprising polydimethylsiloxane (PDMS), perfluoropolyether (PFPE), ring-opening metathesis polymerization (ROMP) polymer, decylnorbornene (DNB), fluoronorbornene (FNB), hexylnorbornene (HNB), polytetrafluoroethylene (PTFE), polyvinylidene difluoride (PVDF), latex, and combinations thereof. The polymer material may comprise or consist of PDMS.

The microfluidic device may further comprise one or more sensors. The sensors may be in the form of biorecognition molecules such as, but not limited to, antibodies, nucleic acids (e.g. DNA, RNA or aptamers), peptide nucleic acids or molecularly imprinted polymers. The sensor sites may be used as part of an optical detection approach that may involve fluorescence. The sensors may comprise chemical sensors. The sensors may be arranged to provide electrochemical and/or electrophysiological recordings. The sensors may be arranged to be positioned and aligned with arrayed cells, and/or their outgrowths. The sensors may comprise electronic (e.g. metallised) circuits or patterns layered into the material of the microfluidic device. The metallisation may be gold or indium tin oxide (ITO). The use of indium tin oxide is advantageous as it is largely transparent enabling imaging data to be correlated with electrochemical and/or electrophysiological data. The sensors may be planar or three-dimensional in character. The sensors may be coated, for example by self-assembly, with electrochemical reporter molecules and/or cell adhesion molecules.

At least one surface of the microfluidic device may be coated with poly-lysine, poly-ornithine, an alternative polyamine, or an amino silane such as, but not limited to, 3-aminopropyl triethoxysilane (APTES), diethylenetriaminosilane (DETA), bis(trimethoxy silylpropyl)amine (BTMSPA) or 3-aminopropyldiisopropyl-ethoxysilane (APDIPES); or combinations thereof. The coating may be on the surface of the microchannels, such as the base of the microchannels.

Alternatively, or additionally, a surface of the microfluidic device may be coated with cell adhesion-promoting molecules. Cell adhesion-promoting molecules may comprise fibronectin, laminin, collagen, the tripeptide RGD motif or similar; or combinations thereof.

The surface may be coated or patterned by microspotting, inkjet printing, microcontact printing, by microfluidic writing methods, or by other micropatterning methods known to those in the field. Such coatings may be used to detect single analytes or multiple different analytes in the sample or produced by the arrayed cells. The coated or patterned surface may be at the cell trap site, and optionally may be restricted to the cell trap site. Alternatively, materials that prevent cell adhesion may be patterned around the cell trap sites to restrict cells to the trap sites.

A region of the microfluidic device may be treated with agents, such as active agents. Active agents may comprise any active agent selected from the group comprising a toxin; a virus; prion material; nanoparticles; a test substance; and a substance (whether known or unknown) to elicit an effect on the cell type cultured; or combinations thereof. Active agents may further comprise reagents as required for an analytical procedure, such as labels and washing solutions.

The combined volume of the compartment(s), the input channel(s), the volume metering element(s), and the microchannels may be about 100 μl . In another embodiment, the combined volume of the compartment(s), the input channel(s), the volume metering element(s), and the microchannels may be about 98 μl . The combined volume of the compartment(s), the input channel(s), the volume metering channel(s), and the microchannels may be less than about 200 μl . The combined volume of the compartment(s), the input channel(s), the volume metering element (s), and the microchannels may be less than about 150 μl . The combined volume of the compartment(s), the input channel(s), the volume metering element (s), and the microchannels may be less than about 100 μl . The combined volume of the compartment (s), the input channel(s), the volume metering element (s), and the microchannels may be less than about 10 μl .

FABRICATION AND USE

The microfluidic device may be fabricated by replica moulding, hot embossing, injection moulding, or other means from a master template. The master template may be fabricated by one or more photolithographic, grayscale lithography, or other microfabrication steps.

The master template may comprise a photoresist material, such as an epoxy-based negative photoresist. The photoresist material may comprise SU-8 or similar material.

The microfluidic device may be fabricated using 3-D printing methods, or by machining techniques such as laser machining.

The microfluidic device may be assembled by positioning on top of a substrate, such as glass or a polymer substrate. The substrate may be thin, for example, less than 200 microns to facilitate high magnification imaging. The microfluidic device may be assembled by bonding the device to the substrate by means including, but not limited to, plasma bonding, anodic bonding, thermal bonding, solvent-assisted bonding or ultrasonic welding. Alternatively, the microfluidic device may be placed on top of the substrate without bonding. This is advantageous for removing the device for immunostaining cell cultures and also to initiate migration assays. For a similar wound healing assay the device will be treated to promote cell adhesion, such that removal of the device damages the cells.

The microfluidic device may be used to transport a known sample volume over a detection site for the detection of (bio)chemicals, (bio)molecules, viruses, exosomes or microvesicles or other substantially small and/or colloidal analytes. A variety of detection modes exist and may include but be restricted to spectrophotometric or electroanalytical methods.

The microfluidic device may be pre-treated. The pre-treatment may comprise sterilisation. The pre-treatment may comprise autoclaving. Pre-treatment may comprise incubation of the device in media, such as cell media. Pre-treatment may comprise application of a solvent such as, but not limited to, ethanol, butanol, methanol and aqueous phase sodium hydroxide. The solvent may act to increase the hydrophilic character of the polymer or hydrogel microfluidic channels once already assembled on a substrate. The pre-treatment with solvent may comprise a time period for the solvent to evaporate in advance of adding cells to the device. Alternative treatments may involve derivatization with a coating that produces a hydrophilic character, such as but not limited to the co-polymer poly-*L*-lysine grafted with poly(ethylene glycol).

The microfluidic device may be used for establishing heterotypic co-cultures. For example, different cell types may be cultured in separate cell culture compartments, which may be loaded via separate input channels and wells. The loading of the heterotypic co-cultures may be each controlled separately by separate volume metering channels connected (via microchannels) to the respective cell culture compartments.

Cultures or co-cultures of cells may be treated with one or more agents. The cultures of co-cultured cells may be monitored or measured, for example periodically during and/or after treatment. Methods of monitoring include, but not limited to, bright field and phase contrast microscopy, fluorescence microscopy, Raman microscopy, coherent anti-Stokes Raman microscopy, second harmonic generation microscopy, or by electronic methods such as electrophysiological recordings, including patch clamping, and electrochemical analysis methods for the measurement of analytes such as neurotransmitters, including but not limited to serotonin, as well as reactive oxygen species.

Cultures or co-cultures of cells may be stained for analysis. The staining may comprise immunohistochemical staining. Staining may be provided by disassembling the microfluidic device, for example after an experiment, to treat the cells with staining reagents. Alternatively, staining reagents may be introduced through the inlet/outlet well(s), or other microwell ports.

Staining reagents may comprise antibodies, aptamers or similar as a biorecognition agent. Labels may be pre-attached or subsequently attached to such staining reagents. The labels may comprise distinct optical signatures, such as a distinct fluorescent emission maxima for imaging the cells. For example, fluorescence microscopy may be used for imaging.

In accordance with another aspect of the invention, there is provided an array of microfluidic devices according to the invention.

The array according to the invention, wherein the inlet/outlet wells are aligned to the layout of a standard 6-well, 24-well, 48-well, 96-well or 384-well plate.

CELL CULTURE DETAILS

The microfluidic device may be used to study and/or culture cells. The cells may comprise or consist of eukaryote cells. The cells may comprise or consist of mammalian cells. The cells may comprise any cell type selected from the group comprising neuronal cells; neuronal precursor cells; stem cells; other cells that produce outgrowths; astrocytes, muscle cells; immune cells; and fungal cells; or combinations thereof. The cells may comprise or consist of neuronal cells or pre-neuronal cells. In another embodiment, the cells may be selected from any for the group comprising bacterial cells, fungal cells, algae, whole parasites, oocytes, whole worms, and brain cells, or combinations thereof. In another embodiment, the cells may comprise a whole embryo, for example a non-human embryo. In another embodiment, the cells may comprise a whole embryo, for example a zebra fish embryo or a human embryo.

The culture or co-culture of cells may be heterogenous or homogeneous in cell type. The culture or co-culture of cells may be homogeneous in cell type. The culture or co-culture of cells may be heterogenous in cell type. A first cell culture compartment may comprise a cell type, and an opposing cell culture compartment may comprise the same or different cell type. Two or more cell culture compartments may contain the same or similar cell preparations. Two or more cell culture compartments may contain different, or substantially different, cell preparations.

The cell number or concentration used for loading the device may be sufficient to occupy all cell traps. The cell concentration used for loading the device may be about 1000 cells/ml, for example in the case of mammalian cells. The cells may be provided at a concentration of 1×10^7 cells per ml, or more, for example in the case of mammalian cells. The cells may be provided at a concentration of 5×10^6 cells per ml, or more, for example in the case of mammalian cells. The cells may be provided at a concentration of 1×10^6 cells per ml, or more, for example in the case of mammalian cells. The cells may be provided at a concentration of 1×10^6 cells per ml, or less, for example in the case of mammalian cells. The cells may be

provided at a concentration of 1×10^5 cells per ml, or less, for example in the case of mammalian cells. The cells may be provided at a concentration of 10,000 cells per ml, or less, for example in the case of mammalian cells. The cells may be provided at a concentration of 1000 cells per ml, or more, for example in the case of mammalian cells. The skilled person will recognise that other cell types, such as bacterial cells, may be studied and maintained in higher concentrations. Bacterial cells may be provided at a concentration of less than 1 cell per 10 femtolitres. Bacterial cells may be provided at a concentration of between about 1 cell per 10 femtolitres and 1 cell per 100 picolitres. The cells may be provided in an amount such that they occupy no more than 10% of the volumetric fraction. Alternatively, the cells may be provided in an amount such that they occupy no more than 1% of the volumetric fraction. Alternatively, the cells may be provided in an amount such that they occupy no more than 0.1% of the volumetric fraction. Alternatively, the cells may be provided in an amount such that they occupy no more than 0.01% of the volumetric fraction. Alternatively, the cells may be provided in an amount such that they occupy no more than 0.001% of the volumetric fraction.

In use, substantially all cell traps of the device may be occupied by a cell. In use, 90% of cell traps of the device may be occupied by a cell. In use, 80% of cell traps of the device may be occupied by a cell. In use, 70% of cell traps of the device may be occupied by a cell. In use, 50% of cell traps of the device may be occupied by a cell.

The microfluidic device may not comprise an evaporation bed, or otherwise rely on evaporation of fluids in use. The microfluidic device may not comprise an atmosphere-interfaced evaporation bed. The microfluidic device may not comprise the use of an external pumping device for loading cells from the input well into the cell culture chamber. The microfluidic device may not comprise the use of an external pumping device for flowing fluid through the cell culture chamber and microchannels. The microfluidic device may be used with manual or robotic pipetting, or pumping, to draw fluid through the device via the outlet well during loading. The microfluidic device may be used with manual pipetting, or pumping, to push fluid through the device via the input well during loading.

Agents, such as test substances and assay reagents, may be introduced into the microfluidic device, such as into the microchannels, by gravity-driven flow.

According to another aspect of the invention, there is provided a microfluidic device suitable for generating a capillary action during loading comprising:

- one or more input channels;

- one or more cell culture compartments connected to the one or more input channels, and comprising one or more cell traps;

- a volume metering element connected to the cell traps of the one or more cell culture compartments via one or more microchannels;

- wherein the volume metering element has a larger volume, or substantially equal volume, than the one or more cell culture compartments, and is arranged to control capillary action through the one or more microchannels.

According to another aspect of the invention, there is provided the use of the microfluidic device of the invention for study and/or culture of cells, optionally neuronal cells.

According to another aspect of the invention, there is provided the use of the microfluidic device of the invention for: biomarker detection or quantification; analyte analysis; cell selection; genome analysis; transcriptome analysis; proteome analysis; or *in vitro* tissue modelling.

The analyte may comprise drugs, virus, bacteria, parasites, biomarkers, platelets, white blood cells, microvesicles, exosomes, or other (bio)chemical analytes of interest. In another embodiment, the analytes may comprise nucleic acid, such as DNA or RNA, peptides, or proteins.

According to another aspect of the invention, there is provided a method of studying and/or culturing cells, such as neuronal cells, comprising the loading of the cells into the microfluidic device of the invention, occupying one or more cell traps with the cells, and incubating the microfluidic device.

Occupying one or more cell traps with the cells may comprise adding to an input well a cell suspension and allowing capillary action to flow the suspension of cells into the cell culture compartment, whereby a cell in the suspension would become trapped in a cell trap as the fluid flow, driven by capillary pressure, through the microchannels (e.g. neurite outgrowth channels) directs the cells to the trap site. Occupying one or more cell traps with the cells may not make use of a pump or pipette to flow a cell into the cell trap. In particular, the device itself may define the volume of liquid flow for arraying cells by use of the volume metering element, but a pipette or similar device may still be used to provide the liquid/cell suspension to the device. The pipette or similar device does not provide the driving force for arraying cells.

The skilled person will understand that optional features of one embodiment or aspect of the invention may be applicable, where appropriate, to other embodiments or aspects of the invention.

Embodiments of the invention will now be described in more detail, by way of example only, with reference to the accompanying drawings.

Figure 1: Capillary pressure P_c generates a capillary flow in small and wettable channels with a rectangular cross-section. Here the surface tension γ combined with the width a and height b dimensions of the channel and the contact angle θ of the PDMS microchannel walls and the glass substrate describe the capillary pressure.

Figure 2: Changes to the geometry and dimensions impact the characteristics of the flow rate (Q).

Figure 3: Neuronal co-culture circuit (top), and capillary action flow path from the input port (bottom).

Figure 4: *Top:* Video documentation of a red dye filling the microfluidic circuit by capillary action. *Bottom:* The small outgrowth channels greatly reduce the flow rate, while the volume metering element (VME) increases the flow rate, although with rate decay due to increased frictional areas (Washburn scaling (inset)). Flow terminates at the 3 upper outlet ports (not visible here).

Figure 5: *Left:* Particle arraying by capillary-driven flow through a volume metering element. 10- μ m-diameter polystyrene particles were used as cell models. In this example a 67 nL volume metering element was used with a particle concentration of 2×10^6 /mL to occupy nearly all of the traps (2×50). *Right:* Particle and neuron arraying results for given input concentrations, with white columns denoting the number of particles or cells at trap locations and grey columns denoting the number of particles or cells outside the trap locations.

Figure 6 illustrates cell traps. **Figure 6A** shows a cell trap with a recessed surface; and **Figure 6B** shows a cell trap with pillars flanking the inlet to the microchannel.

Figure 7: Co-culture circuit arrangements with a common volume metering element arranged as a serpentine (a), a tapered channel (b), bifurcating parallel channels (c), and radial tapering channels (d), that is further shown as a 3-D drawing (e) and during the capillary flow driven filling of a red dye (f).

Figure 8: Circuit varieties with different technical capabilities. For example: Arraying cells in a single culture chamber (a); arraying a homotypic co-culture in two connected culture chambers (b; see Figs 3-5); arraying a heterotypic co-culture using a common VME (c). A heterotypic co-culture circuit providing the option to treat or extract the neurite outgrowths (d). Multiple culture chambers each connected to a VME to prepare more complex co-culture arrangements (e). See Fig. 9 for further details.

Figure 9: *Top:* Sequential capillary flow circuit for the preparation of more complex co-cultures. This circuit has 4 chambers, with 3 connected to VMEs. *Bottom:* The microchannels leading to the VMEs connect via still smaller microchannels to a neighbouring chamber. The abrupt enlargement does not favour capillary flow, with the VME providing the preferred route. The neighbouring channel remains temporarily vacant, thereby enabling other chambers to be subsequently filled by capillary action.

Figure 10: The co-culture circuits can be fluidically isolated (*i.e.* treatment of a single chamber) by using gravity-driven feed. An upper flank port is filled with a test substance (green) and the other upper ports are filled with media (pink). The column height difference is achieved by removing media in the bottom port. In 35 and 55-mm-high devices isolation is achieved for >3 hours.

Figure 11 illustrates how the input microwells of the microfluidic circuit can be positioned to align with the wells of standard 96-well and 384-well microtitre plates.

Figure 12(a) illustrates a microfluidic device/circuit for arraying cells as a co-culture by capillary flow. **Figure 12(b)** provides an enlarged view of a section of the microfluidic surface. The directions of the flows are illustrated with arrows. Cells, represented as black spheres, are also illustrated with one arrayed and two more being introduced to the system by capillary flow.

Figure 13 illustrates an alternative volume metering element consisting of multiple fin structures which form channels therebetween. The arrow indicates the direction of flow.

Capillary flow microfluidic circuits

With reference to Figure 1, a capillary flow describes the filling of an empty channel and is driven by a capillary pressure within a wettable microchannel with a rectangular or square cross section. The material wetting properties (contact angle) produce a curvature that, in combination with the surface tension of the liquid, produces the capillary pressure. This fundamental feature can be used for many applications in biology by the innovative design of microchannel dimensions, geometries and material properties and sequential combinations of these.

With reference to Figure 2, different geometries and different dimensions produce different flow characteristics: A long channel, here arranged as a serpentine, has flows that progressively accumulate friction, resulting in velocity decay during channel filling. In radial channels that widen, either alone or in a branched format, the capillary pressure increases. By appropriate design can balance the increasing friction component to maintain the flow rate. Abrupt reduction in channel cross section results in a sudden drop in the flow rate. In contrast, arrival at a significantly larger channel or chamber, such as a port, causes the flow to stop.

With reference to Figures 3 and 12 a microfluidic circuit 1 is shown for arraying cells as a co-culture by capillary flow (a). The device comprises a cell input well 3 and test substance input wells 11 connected to input channels 5 which are downstream of the input wells 3, 11 during loading. The input wells 3, 11 each have a volume of 43 μL (or 35 μL in an alternative embodiment).

The input channels 5 each have a length of 4 mm, with a width of 300 μm and height of 50 μm . The input channels 5 feed into the cell culture compartments 7. In this example, there are two parallel cell culture compartments 7, which are connected to each other by an array of outgrowth channels (microchannels) 9, which are arranged to allow cell outgrowths to extend through. A single cell input well 3 feeds into both cell culture compartments 7 by an input channel 5. Each cell culture compartment is connected downstream from respective test substance input wells 11. The cell culture compartments 7 are each 2 mm in length, with a

width of 200 μm and height of 50 μm , and a volume of 20 nL. The cell culture compartments 7 each have an array of cell traps along one wall of the cell culture compartments 7. The cell traps form the inlet to the outgrowth channels (microchannels) 9. In this example, there are ten cell traps per cell culture compartment 7, which will accommodate a single cell per cell trap.

The array of microchannels 9 extending from the cell culture compartments 7 via the cell traps, have a combined volume of 7.5 nL. Each outgrowth channel (microchannel) 9 has a length of 250 μm , with a width of 5 μm and height of 3 μm , and a volume of 375 pL. In this example, there are twenty outgrowth channels (microchannels) 9, one for each cell trap. A traversing microchannel 10 runs centrally and transversely through the array of outgrowth channels (microchannels) 9. Each outgrowth channel (microchannel) 9 is aligned with another outgrowth channel (microchannel) 9 extending from the opposing cell culture compartment 7, thereby allowing outgrowth contact. The end of each outgrowth channel (microchannel) 9, that is the downstream end, is connected to the traversing microchannel 10, which provides a path to the volume metering channel (outlet channel) 13. The traversing microchannel 10 has a length of 2 mm, with a width of 20 μm and height of 50 μm , and a volume of 2 nL.

The volume metering channel (outlet channel) 13 starts at the downstream end of the traversing microchannel 10. The volume metering channel (outlet channel) 13 has a length of 38 mm, with a width of 200 μm and height of 50 μm , and a volume of 380 nL.

The volume metering channel (outlet channel) 13 follows a serpentine path and dictates the volume transported through the outgrowth channels (microchannels) 9 to dictate the number of cells (for a given cell density added to the cell (neuron) input well 3) arrayed in both cell culture compartments 7. The volume transported is dictated by the substantially larger volume of the volume metering channel (outlet channel) 13 relative to the outgrowth channels (microchannels) 9, the cell culture compartments 7, and input channel 5. The volume metering channel (outlet channel) 13 is connected downstream to an outlet well 15, which has a volume of 35 mL. The transition from the volume metering channel (outlet channel) 13 into the outlet well 15 is a sudden opening/ending of the distal/downstream end of the volume metering channel (outlet channel) 13 onto the side wall of the outlet well 15. The sudden opening/ending of the distal/downstream end of the volume metering channel (outlet channel) 13 onto the side wall of the outlet well 15 is a capillary flow interruption point.

Additional input wells 11 are provided to be able to add the same or different agents, such as test substances, into the device 1. This provides that cells in the cell culture compartments 7 can be selectively treated. This creates additional flow paths from the additional input wells 11 and channels 12 through to the volume metering channel 13. The additional input channels 12 may have a relatively smaller volume than the volume metering channel 13 in order to avoid competing with the volume metering channels for fluid, thereby delaying cell arraying.

The directions of the flows are illustrated with arrows (b). Cells 17, represented as black spheres are also illustrated, with one arrayed and two more being introduced to the system by capillary flow. These will subsequently be arrayed. Once an outgrowth channel (microchannel) 9 inlet/cell trap is occupied by a cell 17 the flow is diverted to neighbouring outgrowth channels (microchannels) 9 for arraying subsequent cells. Cell arraying is complete when all outgrowth channel (microchannel) 9 inlets/cell traps are occupied and/or when the capillary pressure driven liquid front arrives at the end of the volume metering channel (outlet channel) 13.

The homogeneous co-culture can extend outgrowths, such as axons, to connect the two cultures. Addition of a test substance to the input wells can be used to selectively treat one cell culture compartment and not (or at least minimally) the other. This example is a 2-layer circuit, with input microwells 3, 11 inserted through the device to access the microfluidic circuit using a pipette. Single layer circuits can also be fabricated, with the requirement that the cell trapping aperture dimension is less than 4 microns and preferably less than 3 microns.

With reference to Figure 4 the flow rate characteristics have been documented. The flow rate, in nL/s, within the co-culture arraying circuit is a function of the dimensions and geometries of the wettable microchannel. In this example, the entire 67 nL is filled in 68.3 s. Velocity decay due to the accumulation of a friction component (so-called Washburn scaling) is evident during filling of the serpentine volume metering element, and abrupt reduction in the flow rate is evident during passage through the micron-scale (3 μ m) neurite outgrowth channels.

With reference to Figure 5, the volume metering capacity of the volume metering can be used to deliver and array a known number of particles or cells provided the concentration of the particles or cells is known and is there are a similar or smaller number of arraying sites (*i.e.* entrances to the neurite outgrowth channels). In practice more particles or cells are needed than are described by the loading principle (*i.e.* the number of particles or cells in the volume described by the circuit and especially the volume metering element). This is a result of sedimentation and surface capture upstream of the arraying sites. In practice, and if required, this can be avoided by using density matched liquid, thereby giving absolute particle arraying efficiency.

With reference to Figure 6, two types of cell traps are shown. Figure 5A shows a cell trap with a recessed surface 31 to accommodate a cell 17, and a microchannel 9 (which is sub-cell sized in diameter) extending away from the cell culture compartment 7. The arrow indicates the direction of capillary flow during loading/arraying of the cells 17. Figure 5B shows a similar cell trap with pillars 33 flanking the aperture to the microchannel 9 instead of a recessed surface 31. A cell trap may comprise both pillars 33 and a recessed surface 31. A cell 17 may have an outgrowth 18, such as a dendrite or axon, which will extend down the microchannel 9 to potentially make contact with a cell outgrowth from an opposing cell culture compartment connected by the microchannel 9.

With reference to Figure 7, the volume metering channel (outlet channel) 13 can have different layouts: (a) shows a serpentine volume metering channel (outlet channel) 13, (b) shows a tapered volume metering channel (outlet channel) 13, (c) shows a volume metering channel (outlet channel) 13 branching by divergence before convergence; and (d) shows a volume metering channel (outlet channel) 13 of multiple parallel paths. These different layouts produce different loading characteristics in accordance with Washburn scaling; for a given channel cross-section as the length increases the flow rate decreases. Constant flow rates can be achieved by widening the volume metering channel (outlet channel) as illustrated in (b). Channel parallelisation acts to increase the flow rate multi-fold, albeit with each path subject to Washburn scaling. Branched channels that each progressively widen can be used to counteract Washburn scaling. A 3-D illustration of the device with this volume metering element is shown (e), along with a frame from video documentation of it being filled by capillary action (f).

With reference to Figure 8, the microfluidic circuit can have different embodiments with the requirement for a distal volume metering channel (outlet channel) 13. For example the circuit of the microfluidic device 1 can be provided for arraying single cells in one cell culture compartment 7 (a). Cells can be arrayed in two cell culture compartments 7 from a single input well 5 (b) or from two input wells 5 (c) that provides the option to create a heterogeneous co-culture. The volume metering channel (outlet channel) 13 can also be interfaced to a second input well 21 via additional input channel 22 (d) for the selective treatment and/or access to the cell outgrowths within the microchannels/outgrowth channels 9. The volume of the additional input channel 22 is significantly smaller than the volume of the volume metering channel 13 to avoid competing with the volume metering channel 13 for fluid, which can delay cell arraying. The circuit of the microfluidic device 1 can also be parallelised to investigate the probability of transport across multiple, sequential cell-cell contacts (e). Here the volume metering channels (outlet channels) 13 are identified as elongated triangles. The volume metering channels 13 may be the same volume, size or diameter, or they may be different volumes, sizes or diameter as required, such that they can allow selected flow paths to be favoured or controlled differently. This particular system has three input wells 3, 103 and respective channels 5 (depicted at the bottom), connecting via a grid of interconnected microchannels 9 to three (central) volume metering channels 13. The three paths, so defined paths, each include a distinct cell culture chamber 7 or area, such that this system has the potential to support the culture of a heterogeneous system containing three cell types (e.g. glial cells, neurons, and muscle cells). The inlets 11, 103 feeding into each corner of the microchannel grid 9, provide two paths, allowing the targeted delivery of substances to the different cell cultures in the cell culture compartments 7. One of these paths or both could be used in selective treatment experiments.

These are examples of many possible microfluidic circuit designs that implement the invention.

With reference to Figure 9, it is feasible to have multiple sequential capillary flow loading in different, yet connected, chambers. Each chamber is connected to a volume metering element. The connecting channels taper from large (10 microns wide), flow promoting channels to

small (3 microns wide), flow reducing channels before adjoining the neighbouring chamber. The preferred capillary flow path is to the volume metering such that flows are terminated on arrival at the neighbouring chamber. Flow termination is assisted by the abrupt change in height, from 3 microns to 50 microns. This represents a passive valve format, creating a time frame while the volume metering element is being filmed that can be used for the addition of a second test and/or cell sample, again by capillary action, to the second, neighbouring chamber. This process can be repeated for the construction of interconnected chambers that can have different cell types in different locations (*i.e.* spatially organised heterotypic co-cultures).

With reference to Figure 10, the microfluidic circuit design for the simultaneous arraying of a homotypic co-culture (see Figure 3) is also suitable for fluidic isolation, whereby one chamber can be treated with a test substance and not the other. This enables one of the cell cultures to be treated and not the other. This necessitates that there is zero to minimal transport of the test substance to the neighbouring chamber, either by passage through the neurite outgrowth or connecting channels or around the base of the U-shaped structure connecting the two culture chambers. In practice this requires a maintained flow and a pressure balancing between the two chambers. In this example, the upper left port was filled with the test substance (here the dye fluorescein), and the central and upper ports were filled with an equal volume and equal column height of liquid or media. Liquid or media is removed from the bottom port, producing a column height difference between the upper ports and thus establishing a gravity driven flow that is slow, balanced and can be maintained for >3 hours in this example. Using this flow method fluidic isolation can be satisfied. Flow maintenance is essential as static treatments allow test substances to diffuse throughout the entire microfluidic circuit and thereby contamination regions and/or cells or cell extensions that are not intended to be treated.

With reference to Figure 11, the input wells of the microfluidic device can be positioned to align with the wells of standard 96-well and 384-well microtitre plates. In this manner, an assay can be automated using, for example, robotic pipetting stations for the investigation of 24 test conditions on a single 96-well plate (top) and 96 test conditions on a single 384-well plate (bottom).

Use of the microfluidic device

A capillary pressure is generated in a rectangular cross-section microchannel that is generally hydrophilic (*i.e.* having a contact angle of $<90^\circ$). One device type consists of a poly-lysine coated glass base with a poly(dimethylsiloxane) (PDMS) microfluidic device pre-treated with ethanol or PLL-g-PEG, or similar hydrophilic coating, and assembled on top.

The capillary pressure P_c generated by this system can be described by; $P_c = g((2\cos\theta_{PDMS}/w) + (\cos\theta_{PDMS} + \cos\theta_{PDL})/h)$, where g is the surface tension of the liquid and θ is the contact angle of the liquid on the identified surface, and w is the width, with h the height. The capillary pressure increases as the channel dimensions become smaller. However, the

fluidic resistance is influenced by the surface area to volume ratio (as approximation, the fluidic resistance, $R_f = l/8(wh/w+h)^2$), increasing with diminishing dimensions such that overall transport velocities are slower at smaller dimensions. As liquid is transported by capillary flow along a microchannel the friction component increases to progressively reduce the flow rate and velocity. This is termed Washburn scaling, with the time t taken to travel a length l , being $t = (\eta/2P_c R_f) l^2$, where η is the viscosity of the liquid. This is an important consideration for designing the volume metering element (outlet channel) to fulfil different requirements, such as speed of filling, gentle handling of cells, and the like.

Microfluidic channels that are wettable can be filled by capillary action, a feature that can be exploited for the single-use self-propulsion of fluid along the microchannel. This eliminates complex microfluidic pumps and interfaces and makes the method suitable for interfacing with a pipette. Neurons are routinely cultured on poly-lysine or poly-ornithine coated glass slides that may further be coated with cell adhesion proteins such as laminin and fibronectin (these and similar coatings can be used for other cell types, including stem cells). These surface coatings are hydrophilic and therefore suitable for generating capillary pressure. PDMS is an excellent material for the replication of microfluidic systems for cell culture as it is gas permeable, providing a means for O₂ and CO₂ exchange during culture, and transparent to enable imaging. PDMS in its native form is hydrophobic (with a contact angle >90°) and will not be wetted by aqueous liquids. However, solvents such as ethanol will readily wet PDMS surfaces and microchannels and rapidly become adsorbed into the PDMS bulk and evaporate into the atmosphere. Alternatively the microchannels can be rendered hydrophilic by the addition of phospholipids in organic solvents, or single or multi-layer electrolyte assemblies such as the co-polymer poly-*L*-lysine grafted with poly(ethylene glycol). This transiently modifies the PDMS surface to a wettable, hydrophilic state (with a contact angle <90°).

A PDMS device with microfluidic structures moulded into its base can be contacted with the glass slides coated with neuron adhesion molecules to provide a suitable environment (small dimensions and low contact angle) for capillary pressure driven fluid pumping.

Through holes can be prepared in the PDMS using a biopsy punch for pipette access to deliver fluids. Of relevance to this invention, small (*e.g.* 1 µL) volumes of ethanol are used to prime the PDMS microfluidic surfaces (*i.e.* reduce the contact angle) with excess ethanol rapidly evaporating from the through-holes (termed microwells in Figure 1), rendering these surfaces hydrophobic. The PDMS surfaces contacting the biomaterial-coated glass substrate are not treated with ethanol or a hydrophilic coating and remain hydrophobic which serves to prevent aqueous fluids leaking from the microfluidic channels. Consequently, once the capillary pressure driven fluid front arrives at the outlet microwell the flow is stopped (this feature also prevents aqueous fluids leaking out from the microfluidic channel). Flow cessation is also aided from the sudden transition from micron-scale channels to the millimetre-scale fluidic ports (output wells). At this transition the change in the curvature of the liquid front reduces the capillary pressure. The microfluidic dimensions can be precisely controlled and thus the volumes delivered by capillary flow can also be precisely controlled (with sub-nanolitre resolution). The invention describes the design and use of a so-called

volume metering element (outlet channel) within a microfluidic culture system. By positioning a volume metering element downstream of sub-cell-sized microfabricated apertures a known volume of liquid can be transported through these. By the use of an appropriate cell seeding density a known number of cells can be positioned at the aperture sites. Capillary flow transport is subject to Washburn scaling, diminishing in velocity as the liquid front proceeds along the channel. Shown in Figure 2, the layout of the volume metering element can have various configurations to alter the flow properties. A serpentine element can be used for progressively slower cell arraying (Fig. 2(a)), while a tapering element can be used to maintain the flow rate (Fig. 2(b)), and for faster arraying (and ethanol evaporation), branching (Fig. 2(c)) or parallel elements (Fig. 2(d)) can be used.

Capillary flows for cell arraying impart only minor stresses on the cells, making this approach suitable for delicate cells such as murine embryonic neurons, neuronal precursor cells and stem cells. Dense mammalian cell suspensions (*e.g.* $\geq 10^6/\text{mL}$), especially neuron suspensions, are prone to aggregate which prohibits accurate single cell positioning. The volume metering element can be increased (length, parallelisation, width, etc) to accommodate a volume significantly larger than the culture compartments. Consequently, low density cell suspensions can be used to reduce aggregation and aid single cell arraying.

The layout of the microfluidic system can take various forms to match the requirements of different experiments: For example, a single array of cells can be prepared using the device illustrated in Figure 3(a), whereas homogeneous co-cultures can be prepared in two-compartment using the device illustrated in Figure 3(b). The device illustrated in Figure 3(c) can be used for the preparation of a two-compartment heterogeneous culture, with the device in Figure 3(d) further providing the control for the selective manipulation of the central channel. As illustrated in Figure 3(e) the co-culture systems can contain multiple distinct cell culture compartments, again with the cells loaded using transport through multiple volume metering elements (here indicated by a tapered line). This also illustrates the scope for increasing the complexity of the microfluidic systems.

Another consideration is the layout of the microwell inlets/outlets. For high throughput investigations it is desirable to have these aligned with the well layout of industry standard microtitre plates, notably 96-well and 384-well formats, both of which are illustrated in Figure 4. This layout is convenient for individual scientists, and especially suitable for interfacing with robotic pipetting workstations.

Protocol

Device Dimensions and Fabrication: A PDMS moulding master was fabricated by a two-layer photolithography process using the photoresist SU-8 on a silicon support wafer. The first layer contains the microscopic outgrowth channels that are 3 μm high and 5 μm wide, with an intercompartment length of 500 μm . The first layer also includes the larger microfluidic channels, the culture compartments and the volume metering element (VME). The second layer has all these structures with the exception of the outgrowth channels, and is

spin-coated to a height of 30-60 μm . In one example, a 100- μm -wide VME with a length of 33 mm was used to transport 100 nL of fluid through an array of apertures (defined by the inlets to the outgrowth channels). In this example (see Figure 1) the VME is arranged as a serpentine for progressively slower neuron arraying. In other embodiments (see Figure 2), parallel VMEs or tapered VMEs can be used to increase or maintain the cell arraying rate. There were 50 outgrowth channels connecting the two compartments. This was interfaced at the midpoint to the VME. PDMS (Sylgard® 184, Dow Corning) was thoroughly mixed as 9 parts pre-polymer with 1 part curing agent and degassed in a vacuum dessicator for 15 minutes. Delrin polymer frames were placed around the microfluidic circuit structures on the SU-8 wafer and used to contain PDMS (height of 5 mm) during thermal curing at 80°C for 1 hour. After cooling PDMS devices were removed from the wafer and a 3-mm-diameter biopsy punch was used to provide the through-holes that define the fluidic ports (input/output microwells).

Pre-Treatments, Assembly and Priming: PDMS can leach certain compounds that are toxic to delicate cells such as neurons. The release of these compounds can be reduced by autoclaving the PDMS devices. PDMS can also adsorb cell support factors (*e.g.* growth factors), rendering the microenvironment less suitable for maintaining the cells in a viable and healthy state. To reduce this effect, the PDMS surface can be passivated by incubation in media (*e.g.* neurobasal media with B-27® and glutamine supplements) overnight. Excess media is removed using a nitrogen stream and the PDMS devices were mounted (by gentle pressing) on coverslip glass that was pre-coated with poly-*D*-lysine (PDL, 100 $\mu\text{g}/\text{mL}$ overnight, followed by a rinse with distilled water). A 1 μL aliquot of ethanol (99%) was deposited in the neuron input microwell, filling the entire device by capillary action in seconds. This was left to evaporate, requiring 25 minutes. Ethanol wetting serves two functions: (i) surface priming to reduce the contact angle to enable capillary flow, and (ii) sterilization. Alternatively, the microchannel surfaces can be rendered sterile using an oxygen plasma treatment, then rendered hydrophilic by submersion in a 10 mM HEPES (4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid, pH 7.4) buffer containing 100 $\mu\text{g}/\text{mL}$ of PLL(20)-g[3.5]-PEG(2) for 1 h at room temperature. Substrates were then rinsed with a sequence of 1 x phosphate buffered saline (PBS), MilliQ water and a N_2 stream.

Neuron Preparation, Arraying and Culture: Hippocampal neurons were prepared from E16 mice embryos in Dulbecco's phosphate buffered saline. Cells were dissociated with 0.05 mM trypsin, and 10% foetal bovine serum was added to arrest trypsinisation and the cells were collected by centrifugation (900 rpm for 3 minutes) for resuspension in media and filtered. The cell density was corrected to 1×10^6 cells/mL for arraying in the microfluidic device. A 1 μL volume was added to the neuron input microwell with capillary flow acting to array the neurons (~50) in both compartments in <2 minutes (the time required to fill the volume metering channel). The device can be vertically orientated to reduce the upstream capture of neurons on the PDL coating and thereby increase the efficiency of neuron arraying.

The arrayed neurons were incubated at 37°C in a 5% CO₂ atmosphere for 1-2 hours to adhere and media was subsequently added to all 4 ports before returning the microfluidic neuron culture devices to the incubator.

Perfusion, Selective Treatments and Analytical End-Points: A column height difference between the flanking microwell ports and the neuron input microwell ports was used for media perfusion. This was sustained for >3 hours before height equilibration. In some experiments, a volume from the inlet microwell was added to these flanking channels to replace factors produced locally by the neurons as the original factors are lost during perfusion. Either compartment can be selectively treated with a test substance by adding this to the relevant microwell port for the gravity-driven treatment of the compartment. The opposite microwell port is filled with culture medium to the same height as the agent to ensure both cultures experience similar fluidic stresses, while also reducing the spread of the test substance to the opposing culture compartment. There are numerous means to analyse the co-culture, with fluorescent methods being especially suitable. Immunohistochemical staining can be used to determine the localisation and levels of biochemical and morphological structures (*e.g.* nucleus, vesicles, pre- and post-synapse structures (synaptophysin/PSD-95), somatodendritic mitogen activated protein 2 (MAP2) and the location and abundance of certain molecules that may also include RNA species). The transparent glass base of the microfluidic platform is suitable for imaging without labels, by standard bright field microscopy and also more advanced techniques such as Raman, coherent anti-Stokes Raman scattering (CARS) and second harmonic generation (SHG) imaging to assess, for example, microtubule integrity. In addition to material localisation data, the electrophysiological capacity of the neurons and the neuronal network can be measured indirectly by standard calcium imaging protocols. In addition to spontaneous firing, cells modified to express channelrhodopsin or other optogenetic systems, can be used for light-based interfacing with neuronal cultures. Direct electrophysiology measurements are also feasible, with these demanding electrode arrays to be patterned on the glass substrate.

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CLAIMS

1. A microfluidic device suitable for generating a capillary action during loading comprising:
 - one or more input channels;
 - one or more compartments connected to the one or more input channels, and comprising one or more traps or analysis sites;
 - a volume metering element connected to the traps or analysis sites of the one or more compartments via one or more microchannels;
 - wherein the volume metering element has a larger volume, or substantially equal volume, than the one or more compartments, and is arranged to control capillary action through the one or more microchannels.
2. The microfluidic device according to claim 1, wherein the volume metering element is arranged to control the volume of liquid flowing through the microfluidic device during the loading of the microfluidic device.
3. The microfluidic device according to claim 1 or claim 2, wherein the volume metering element is arranged to limit liquid flow via capillary action to a specific volume.
4. The microfluidic device according to any preceding claim, wherein the volume metering element comprises a capillary flow interruption point, at which the liquid flow is arranged to stop.
5. The microfluidic device according to claim 4, wherein the capillary flow interruption point is an enlargement in the hydraulic diameter or circumference of the volume metering element; or a change in surface properties of the volume metering element; or the end of the volume metering channel, for example as it emerges into an outlet well; or wherein the capillary flow interruption point is a significant reduction in the hydraulic diameter or circumference of the volume metering element to produce a flow rate of effectively zero; or combinations thereof.
6. The microfluidic device according to claim 5, wherein the change in surface properties is provided by a hydrophobic surface having a contact angle of greater than 60°.

7. The microfluidic device according to any preceding claim, wherein the microfluidic device comprises multiple compartments and each of these is connected, via microchannel(s) to a separate volume metering element.
8. The microfluidic device according to any preceding claim, wherein the volume metering element has a larger volume than the compartment or in the case of multiple compartments connected to a single volume metering channel, larger than the combined volume of the cell culture compartments.
9. The microfluidic device according to any preceding claim, wherein the volume metering element has a substantially equal volume to the combined volume of the microchannels and the compartment(s).
10. The microfluidic device according to any preceding claim, wherein the volume metering element is tapered; or splits into multiple channels.
11. The microfluidic device according to any preceding claim, wherein the volume metering element is arranged in a serpentine path.
12. The microfluidic device according to any preceding claim, wherein the microchannels comprise an array of channels extending from the compartment(s).
13. The microfluidic device according to any preceding claim, wherein two or more compartments are connected to each other by the one or more microchannels.
14. The microfluidic device according to any preceding claim, wherein the microchannel(s) are too small in hydraulic diameter or circumference for the passage of a cell, such as a eukaryote cell or bacterial cell; or too small in hydraulic diameter or circumference for the passage of a particle, such as an exosome, microvesicle, virus particle, or platelet.
15. The microfluidic device according to any preceding claim, wherein the microchannel(s) form, at least in part, the cell or particle trap(s).

16. The microfluidic device according to any preceding claim, wherein the microchannels comprise a hydraulic diameter or circumference sized to allow cell outgrowths.
17. The microfluidic device according to any preceding claim, wherein the traps of one compartment are positioned close to opposing traps of another compartment, whereby the microchannels are short enough to allow for cell-body to cell-body contact.
18. The microfluidic device according to any preceding claim, wherein the microchannel is in the form of an aperture, such as a sub-cell or sub-particle sized aperture, formed between a compartment and a volume metering channel.
19. The microfluidic device according to any preceding claim, wherein the traps comprise regions of affinity on a surface of the compartment.
20. The microfluidic device according to any preceding claim, wherein the agents, such as test substances or assay reagents are deliverable directly to the microchannels by an additional inlet channel or by the sample loading channel.
21. The microfluidic device according to any preceding claim, wherein the microfluidic device further comprises one or more input wells connected to the input channel or channels.
22. The microfluidic device according to claim 21, wherein the input well(s) are positioned substantially equal to an arrangement of wells for a standard 6-well, 24-well, 48-well, 96-well or 384-well plate format.
23. The microfluidic device according to any preceding claim, further comprising an additional input channel connecting directly to the microchannel(s).
24. The microfluidic device according to any preceding claim, wherein the microfluidic device comprises two or more compartments.

25. The microfluidic device according to any preceding claim, wherein the traps are in the form of an inlet to the microchannels; and

wherein the inlets to the microchannels from the compartment are enlarged to form the traps; or the traps comprise a recessed surface in the compartment; and/or the traps comprise pillars in the compartment, which flank or partially surround the inlets to the microchannels.

26. The microfluidic device according to any preceding claim, wherein the input channel, compartment, microchannel and volume metering element each comprise a hydrophilic surface.

27. The microfluidic device according to any preceding claim, wherein the volume metering element comprises a hydrophilic surface.

28. The microfluidic device according to any preceding claim, further comprising one or more sensors or detection sites.

29. The microfluidic device according to any preceding claim, wherein at least one surface of the microfluidic device is coated with poly-lysine, poly-ornithine, an alternative polyamine, or an amino silane such as, but not limited to, 3-aminopropyl triethoxysilane (APTES), diethylenetriaminosilane (DETA), bis(trimethoxy silylpropyl)amine (BTMSPA) or 3-aminopropyldiisopropyl-ethoxysilane (APDIPES); or combinations thereof.

30. The microfluidic device according to any preceding claim, wherein a surface of the microfluidic device is coated with cell adhesion-promoting molecules.

31. The microfluidic device according to any preceding claim, wherein a surface of the microfluidic device is coated with protein and/or cell rejecting materials, such as the co-polymer poly-lysine grafted to poly(ethylene glycol) or serum albumin.

32. The microfluidic device according to any preceding claim, wherein the microfluidic device is pre-treated, the pre-treatment comprising one or more of sterilisation, autoclaving, incubation of the device in media, and application of a solvent.

33. An array of microfluidic devices according to any preceding claim.
34. Use of the microfluidic device according to any of claims 1 to 32 for study and/or culture of cells, optionally neuronal cells, tissues, organs or whole organisms; or for biomarker detection or quantification; analyte analysis; cell selection; genome analysis; transcriptome analysis; proteome analysis; or *in vitro* tissue modelling.
35. A method of studying and/or culturing cells, such as neuronal cells, comprising the loading of the cells into the microfluidic device according to any of claims 1 to 32, occupying one or more cell traps with the cells, and incubating the microfluidic device.
36. A microfluidic device, or the use of a microfluidic device, or a method, substantially as described herein, with reference to the accompanying drawings.

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$$P_c = \gamma \left(\frac{\cos(\theta_{\text{substrate}}) + \cos(\theta_{\text{PDMS}})}{a} + \frac{2 \cos(\theta_{\text{PDMS}})}{b} \right)$$

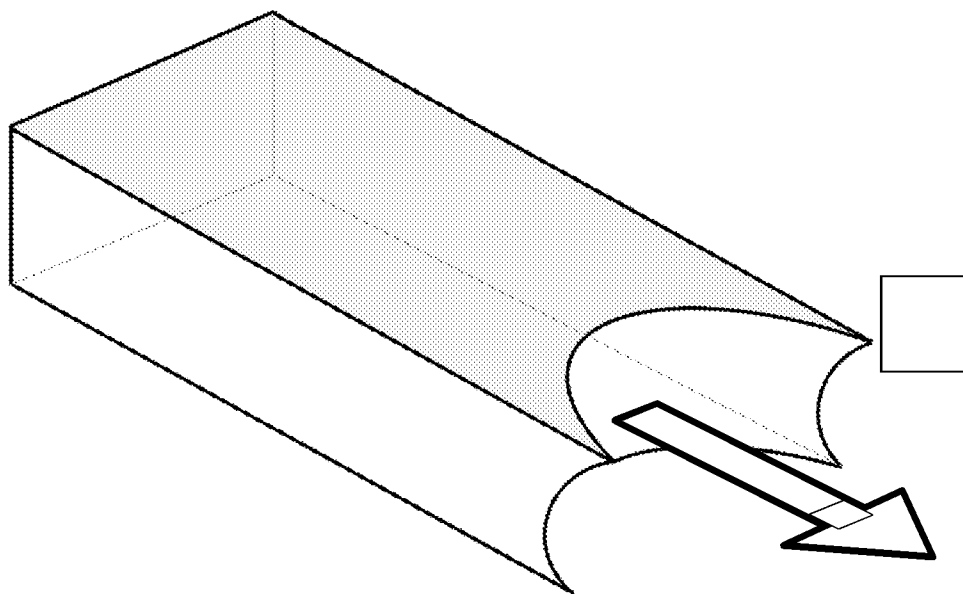


Figure 1

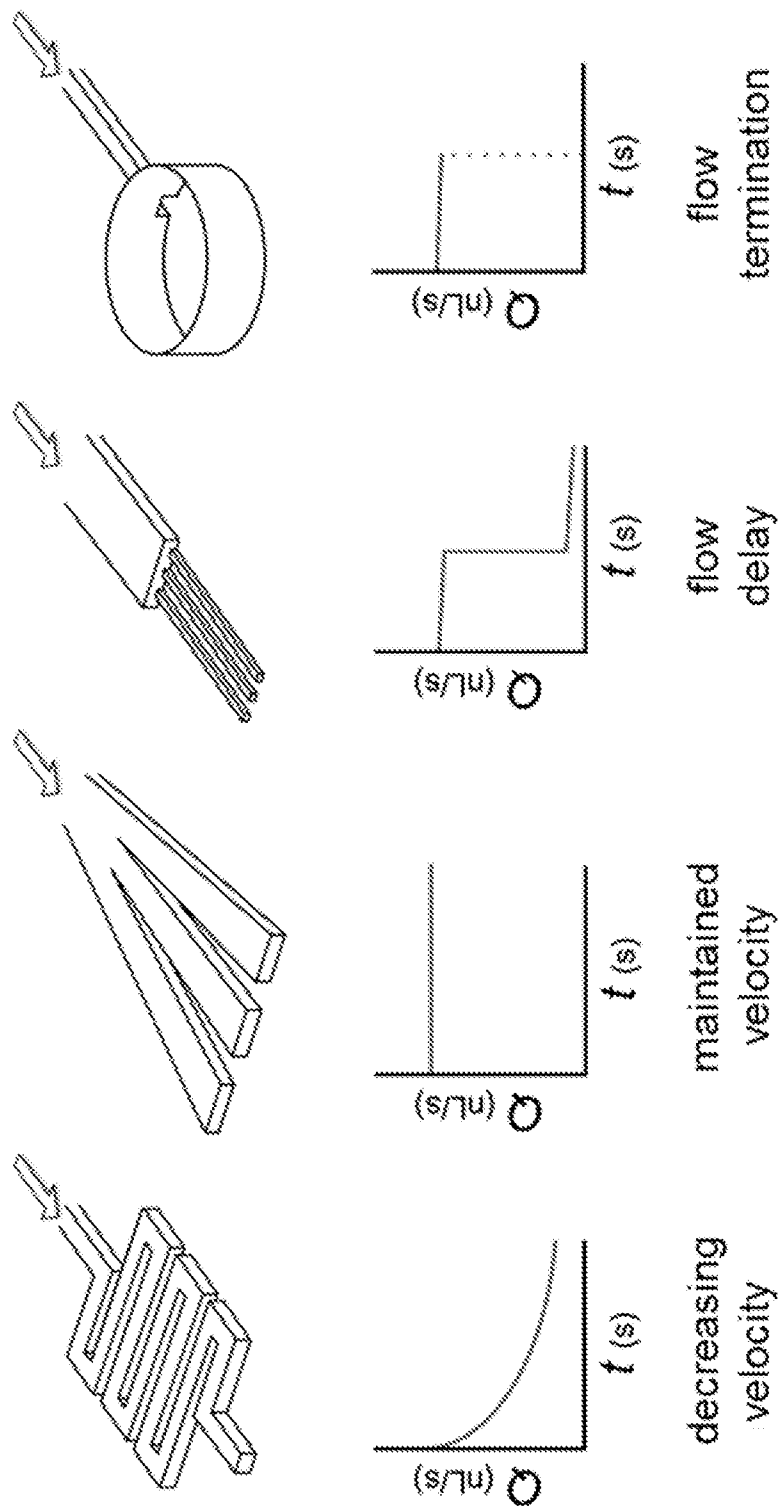


Figure 2

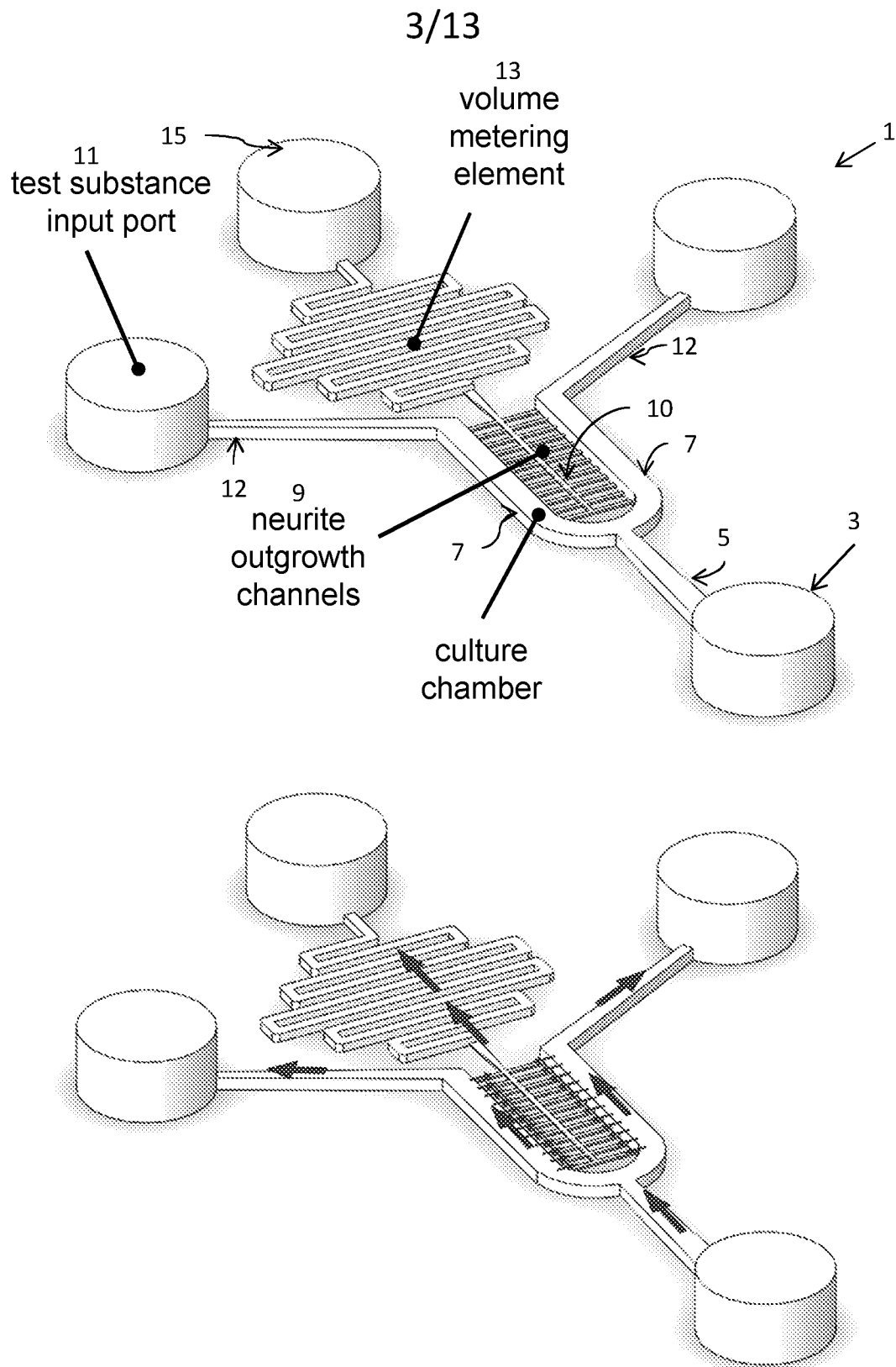
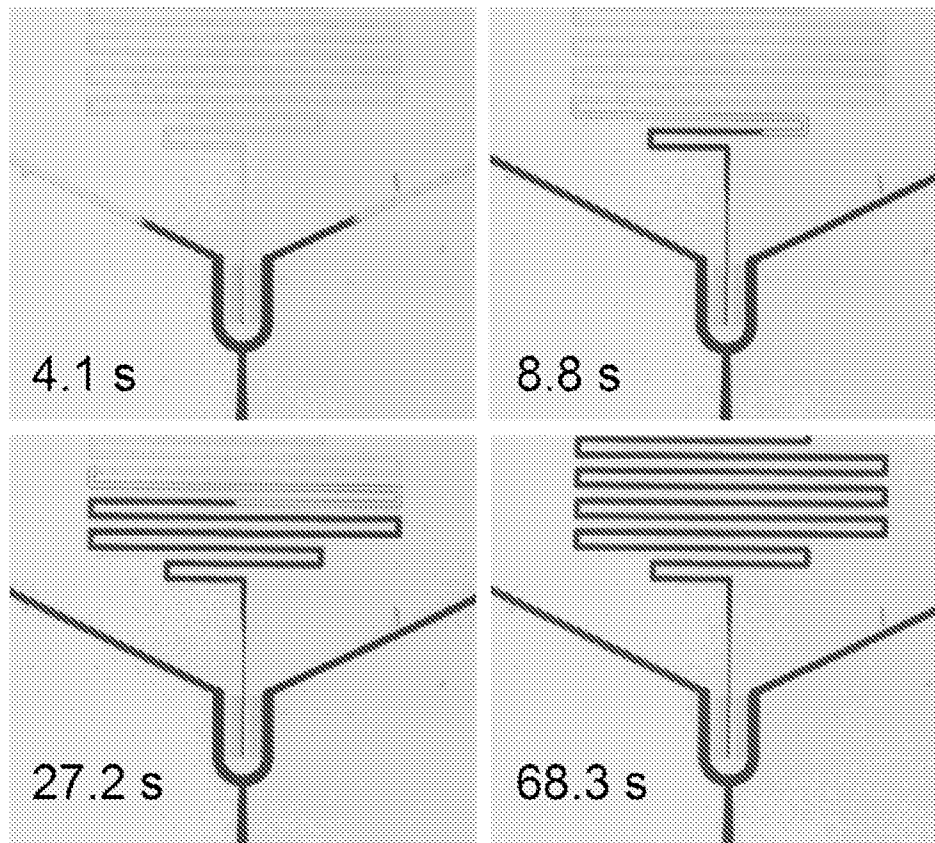


Figure 3

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flow rate characteristics

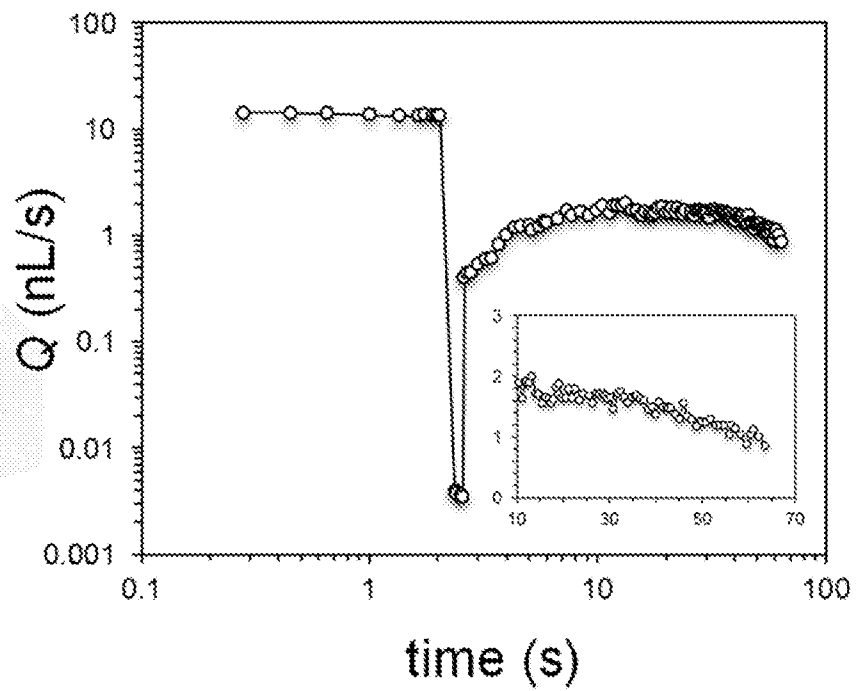


Figure 4

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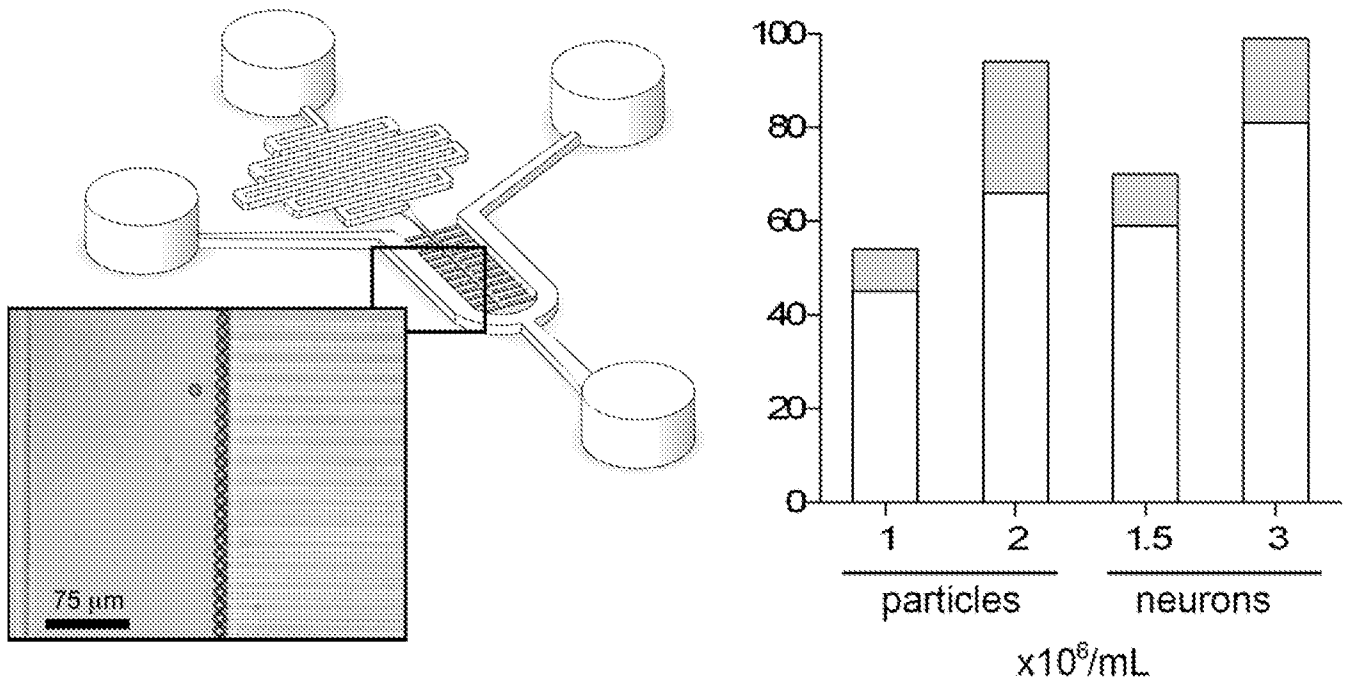


Figure 5

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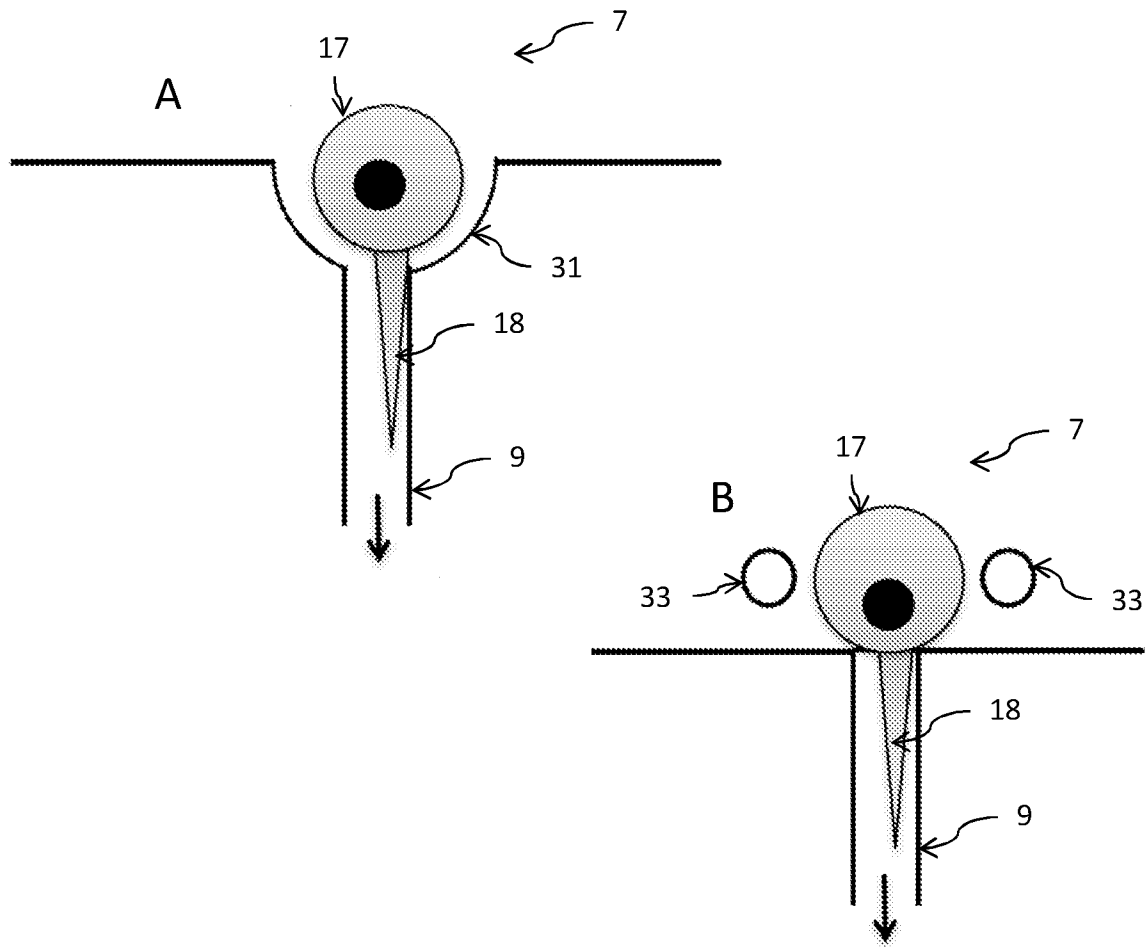


Figure 6

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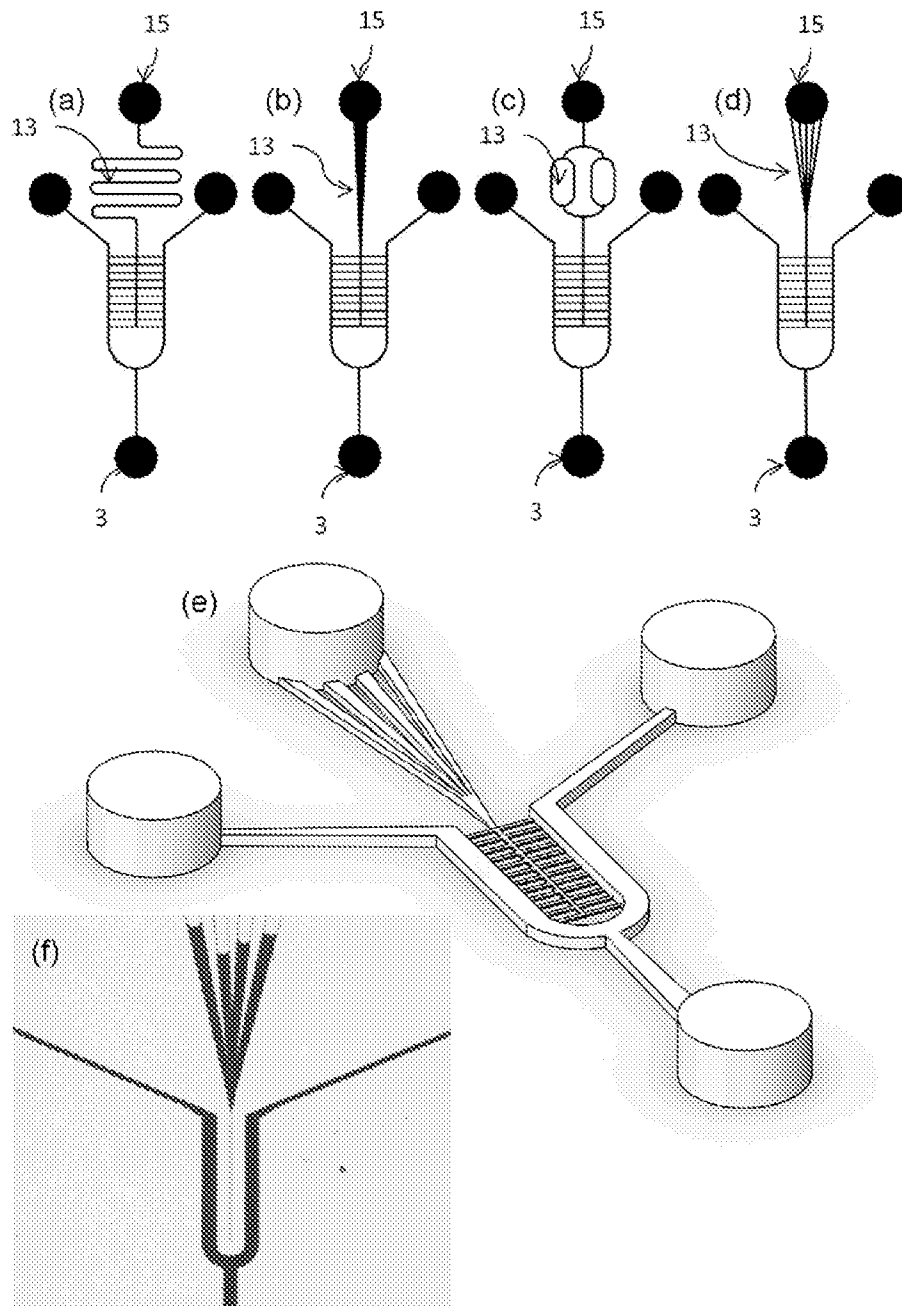


Figure 7

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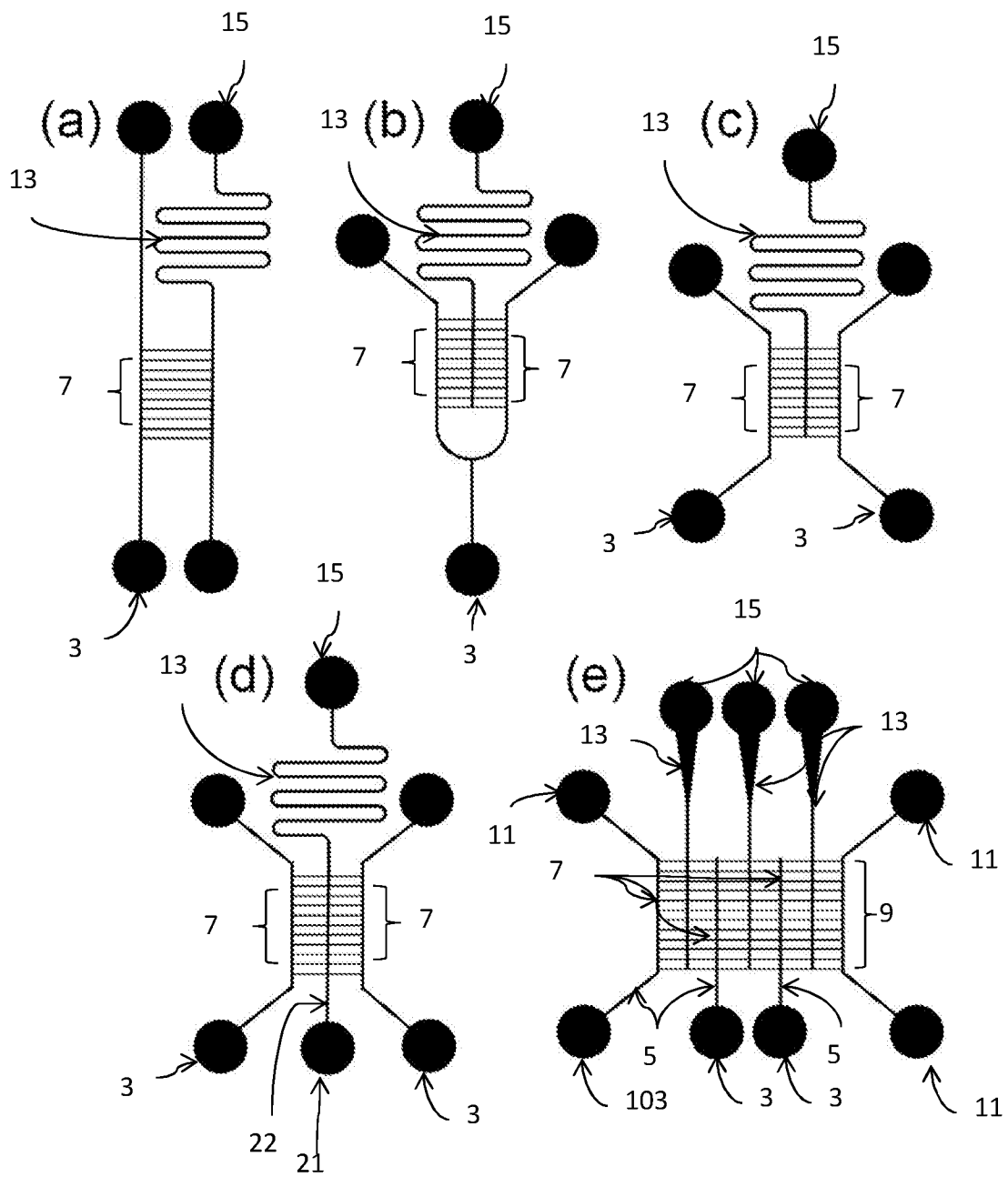


Figure 8

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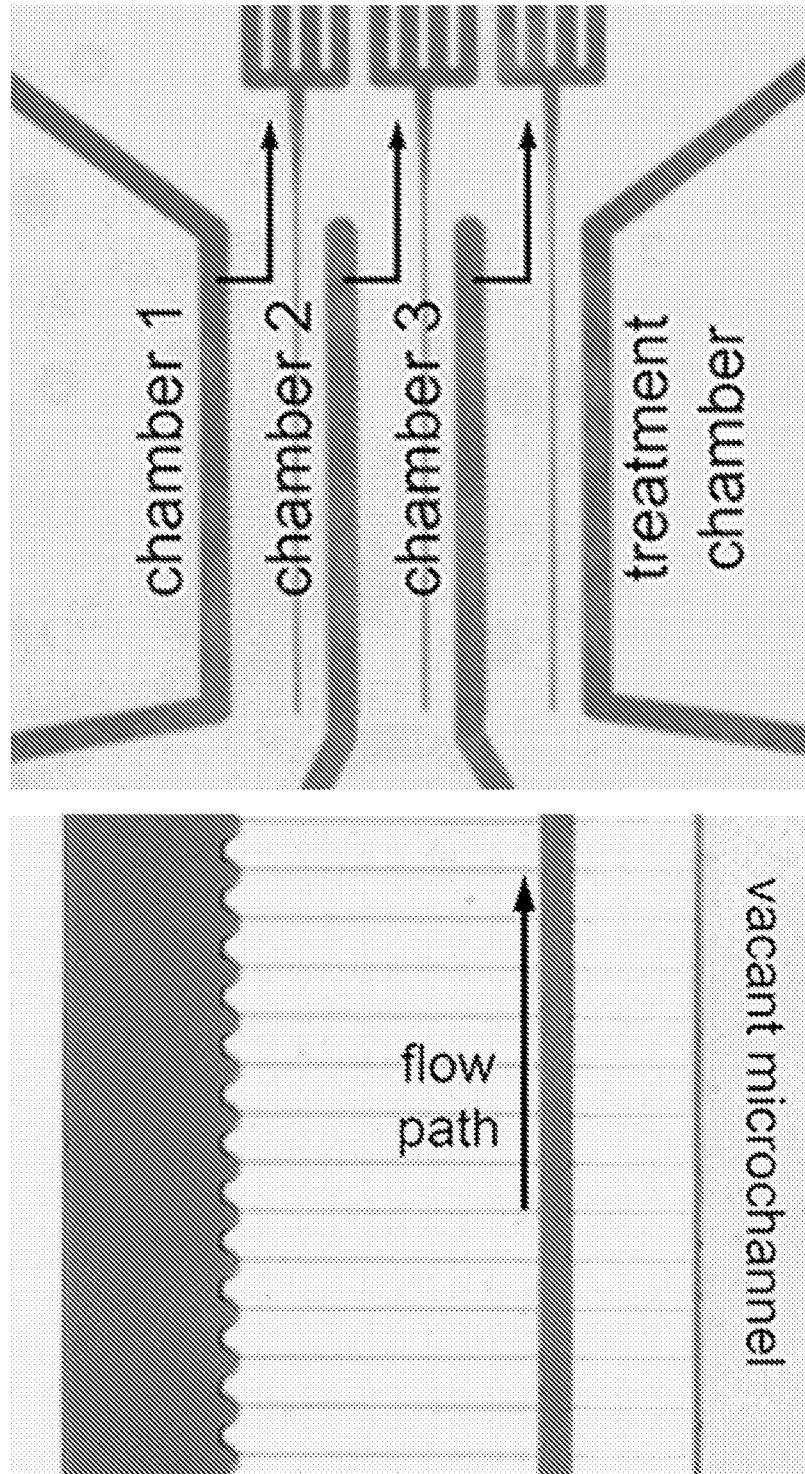
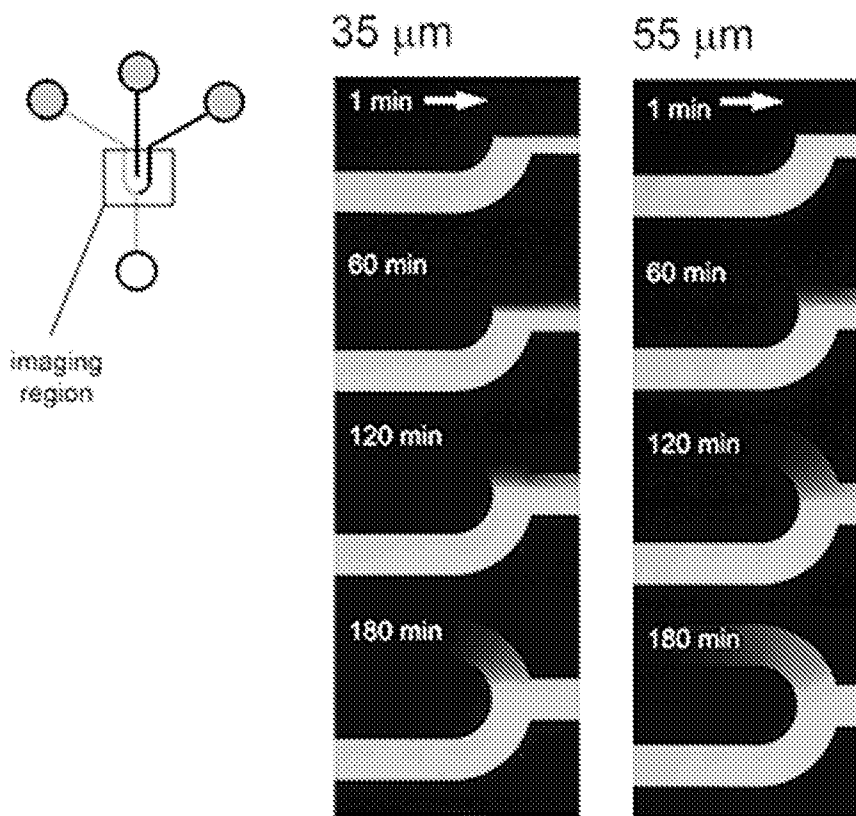


Figure 9

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treatment delivery rate

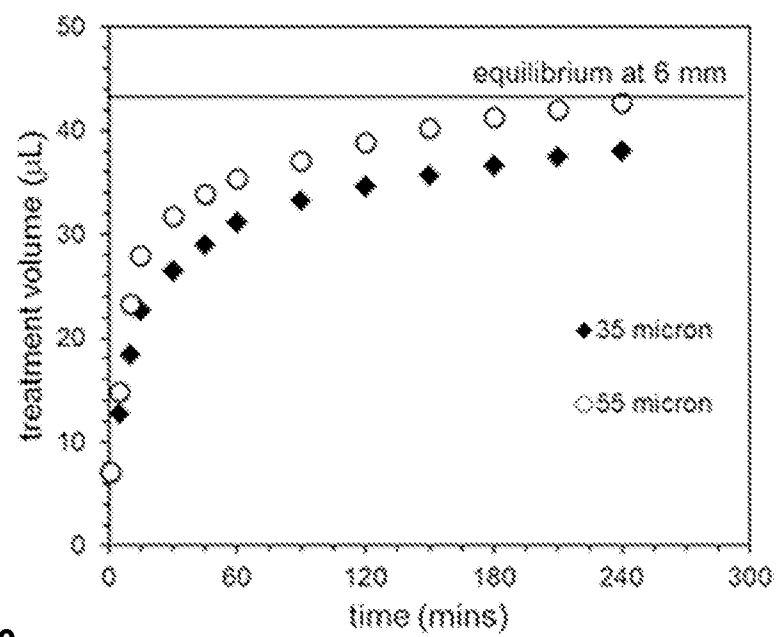


Figure 10

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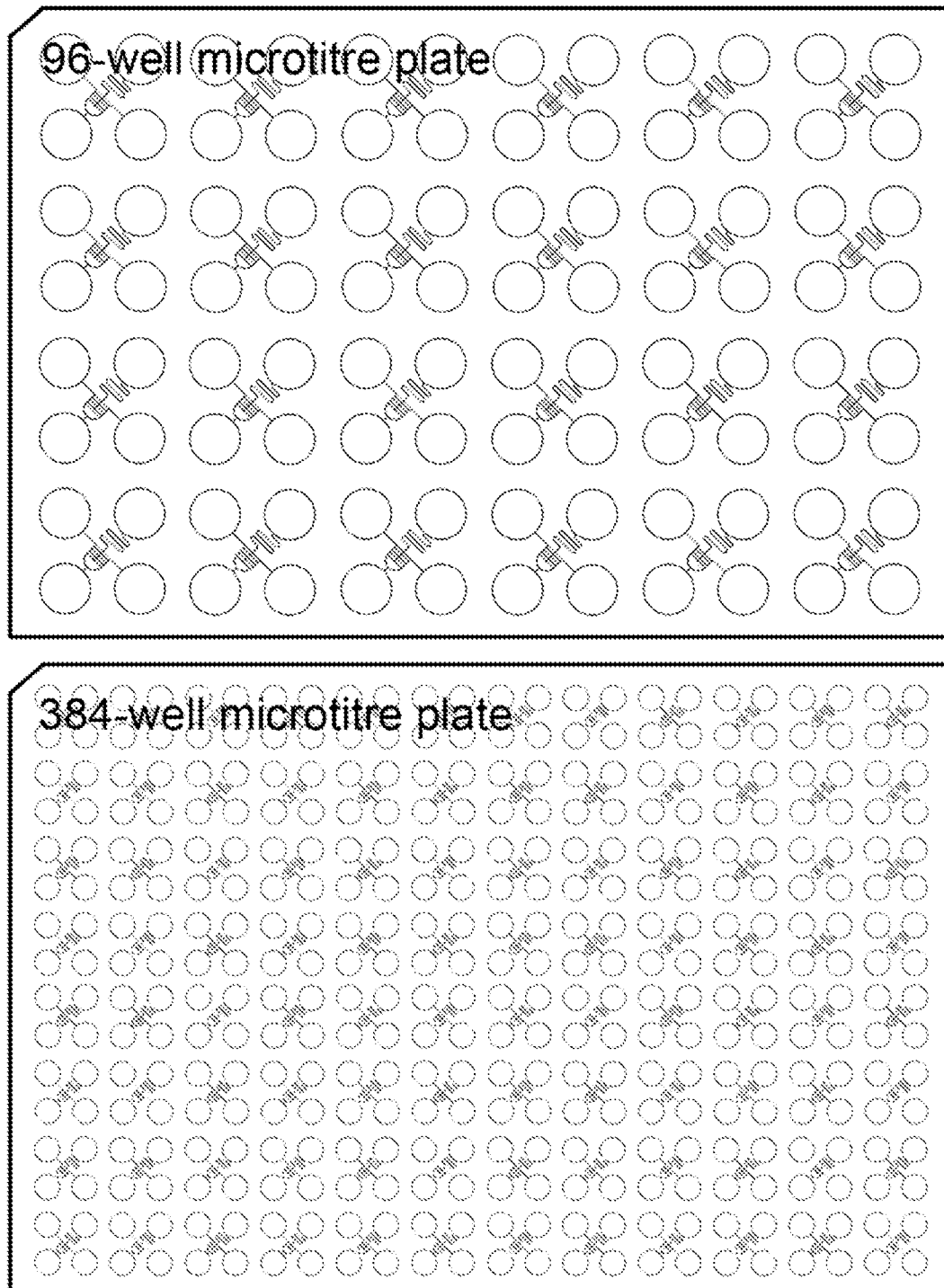


Figure 11

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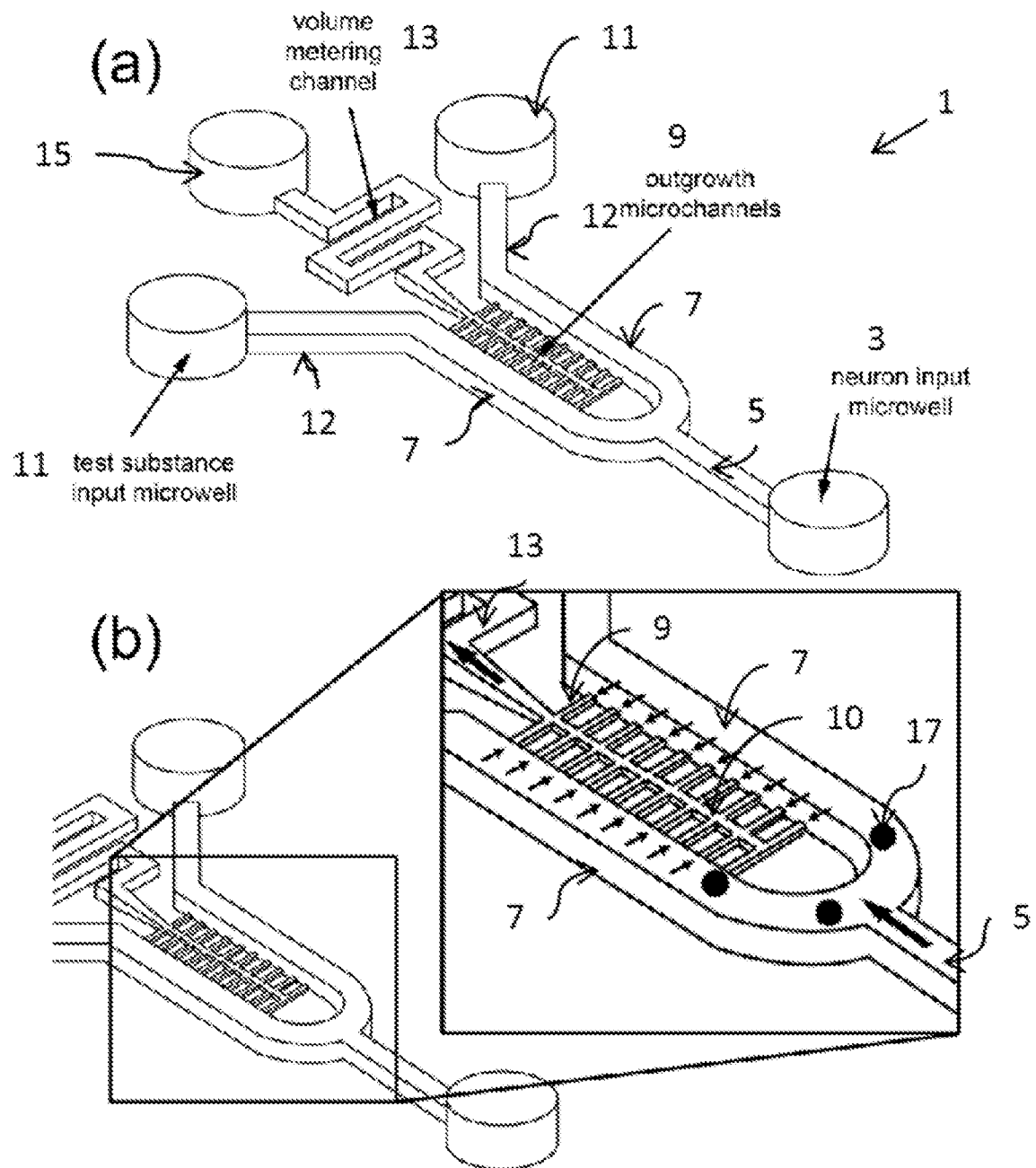


Figure 12

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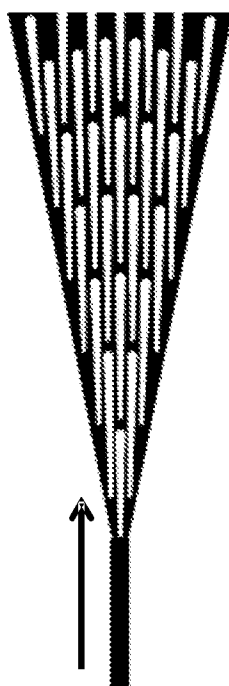


Figure 13

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2015/051971

A. CLASSIFICATION OF SUBJECT MATTER
INV. B01L3/00
ADD.

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)
B01L

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

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

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NGOC-DUY DINH ET AL: "Microfluidic construction of minimalistic neuronal co-cultures", LAB ON A CHIP, vol. 13, no. 7, 25 January 2013 (2013-01-25), pages 1402-1412, XP055213066, ISSN: 1473-0197, DOI: 10.1039/c3lc41224e abstract page 1403 - page 1407 page 1410 - page 1411 figure 1 ----- -/--	1-36



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

21 September 2015

Date of mailing of the international search report

28/09/2015

Name and mailing address of the ISA/

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Authorized officer

Sinn, Cornelia

INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2015/051971

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2012/164263 A1 (CARCLO TECHNICAL PLASTICS LTD [GB]; WILLIAMSON IAN [GB]; ALLEN GERALD) 6 December 2012 (2012-12-06) abstract page 1, lines 4-7 page 16, line 34 - page 20, line 19 figure 1 -----	1-36
A	WO 2013/003935 A1 (NAT RES COUNCIL OF CANADA NRC [CA]; CLIME LIVIU [CA]; MOUNIER MAXENCE) 10 January 2013 (2013-01-10) the whole document -----	1-36
A	US 2011/306041 A1 (VIOVY JEAN-LOUIS [FR] ET AL) 15 December 2011 (2011-12-15) the whole document -----	1-36

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/GB2015/051971

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