

A MICROFLUIDIC DEVICE

Field of the Invention

5 The invention relates to microfluidic device design, manufacturing, and integration, for biomedical analysis applications.

Background

Microfluidic devices, especially biochips, have been studied world wide for biomedical,
10 chemical, food industries, and environmental diagnostic and analysis applications, and a number of microfluidic devices have been demonstrated and commercialized for various analytical and testing applications. It is expected more microfluidic devices will enter into the market with increasing speed in the near future. According to Nexus – an organization of European companies that specialize in MEMS – the BioMEMS industry
15 is worth US\$10 billion. Example applications of polymer biomedical devices are capillary electrophoresis chips and the so-called “lab on a chip”, where chemicals are delivered to specific reaction chambers at specific times and in predetermined order. Microstructures permit precise fluid handling of very low volumes (microlitres to picolitres) using minimum available space. Sophisticated designs enable such fluidic
20 functions as dosing, mixing, re-suspension of dried chemicals, triggering and valve functions. Microfluidics market has been expanding very rapidly in recent years. According to a report (by Yole Developpement, November 2004 Report Name: Emerging Markets for Microfluidics Applications, EMMA04. www.yole.fr) microfluidics market is expected to grow at 20% per year, reaching about Euro 4.5

billion in 2010. Biochips are becoming a big business, as healthcare gradually undergoes a major shift towards genomic-based medicine. The value of the biochip market in 2000 was \$US531 million and the market will generate \$US3.3 billion revenues in 2004, according to a report by the research firm Frost & Sullivan.

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Silicon has been the material most widely used for MEMS devices due to the advanced micro process technologies developed in the well-established microelectronics industry. However, for many BioMEMS device applications, silicon does not show advantages over other materials such as glass and polymers, for instance, the high cost associated with silicon and the batch process nature for silicon devices.

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For many laboratory and commercialized biochips, polymeric elastomer materials, such as Polydimethylsiloxane (PDMS), are used as the substrates for the devices. This is because microstructures of the biochips can be easily replicated on PDMS substrates through the casting process. However, for many applications the flexible substrate material is not preferred, as deformation can take place when samples are loaded on to the chip system, which will affect the functionality of the devices. On the other hand, the casting process is not an effective process for mass productions, and the curing process that takes place after the casting of the liquid components of the substrate material is also a time consuming process.

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Further, biochip components are manufactured as single components which need to go through further chip bonding, integration, and flow path control element installation processes before they can be used in a biochip system. Bonding of biochips to cover the

micro channels is still a challenging task in plastics biochip manufacturing. Adhesive bonding is used for many biochip systems, but it is not desired to use adhesives for many biomedical analyses, and the bonding strength is also limited for many applications at high pressure and temperature conditions. After the bonding process, connectors need to be installed onto polymer chips to form sample entry and exit paths for the biochip system. For many industrial biochip systems, control valves, filtering system etc also need to be installed into the system to manipulate sample flow paths to achieve the designed analysis task. All these assembly and integration processes are tedious, time consuming, and may result in considerable cost increase.

An important implication of such a cost increase is for applications requiring large scale diagnostic tests such as those conducted in commercial laboratories. For instance commercial and forensic DNA testing may involve a considerable volume of individual tests which essentially apply the same procedure repeatedly. A reduction in the cost of consumables for each test may have a significant effect on the economic viability of such a commercial laboratory or a saving of research funds for a research institution.

Further still, it will be appreciated that such biochips are well suited to the processing of samples in order to achieve relatively low costs diagnostic testing particularly when samples volumes are low. However, what has not been significantly developed is the cost involved in the preprocessing of said samples prior to introducing these to such biochips. It would therefore be further advantageous if the relatively expensive procedure of preprocessing samples was also reduced so as to better utilize the economic advantage provided by processing of samples using biochips.

Summary of the Invention

In a first aspect the invention provides a microfluidic device comprising a microfluidic core having a plurality of microfluidic channel blocks; wherein at least a portion of said blocks in selective fluid communication.

In a second aspect the invention provides a microfluidic device comprising a polymer microfluidic core having microfluidic channels on a first face and microfluidic features on a second face wherein said core is a single moulded unit.

In a third aspect, the invention provides a method of forming a microfluidic device the method comprising the steps of: moulding a microfluidic core having microfluidic channels on a first face and microfluidic features on an opposed second face and; sealing said microfluidic channels so as to permit the introduction of fluid to said microfluidic core.

In a fourth aspect, the invention provides a valve for controlling flow in a microfluidic device comprising a valve seat encompassing a microfluidic channel inlet and a microfluidic channel outlet; a rotatable disc coupled to said valve seat, said disc having a longitudinal recess wherein said disc is arranged to rotate between a first position whereby said recess aligns with the inlet and outlet providing fluid communication between said inlet and outlet and a second position sealing at least one of said inlet and outlet and so blocking fluid communication.

A microfluidic device according to the present invention may provide better fluidic flow manipulation and provide less complex flow entrance and exit connector moulding through moulding of such features directly to one or both faces of the core.

5 In a further aspect of the present invention, there may be provided a microfluidic device (biochip) having multiple microfluidic channel blocks located on the microfluidic device , with fluid flow between said blocks being selectively operable. Further, the plurality of blocks may also effect multi step preprocessing of a sample. Such a sample may then be further processed by another biochip which may also be a microfluidic
10 device designed for the processing of such a sample. In this context, a block may be defined as a discrete area on the device having a microfluidic channel with a long path within a confined space.

In this embodiment, there may be two, three, four or more microfluidic channel blocks
15 according to the number of steps required for the preprocessing of the sample.

In this case, a microfluidic device may be designed so as to provide a dedicated preprocessing procedure of multiple steps formed on a single biochip.

20 Said blocks may be arranged sequentially, from a first block to subsequent downstream blocks. Alternatively, blocks may form multiple branches of microfluidic channel blocks.

As an example, preprocessing of a blood sample to extract DNA may include a microfluidic device having three sequentially arranged microfluidic channel blocks. In this case the first block may be arranged to provide a concentration step, a second block to provide a purification step and a third block to provide an amplification step. The microfluidic device may further include features to extract the sample, such as DNA in this case. Such a feature may be an outlet for connection to another microfluidic device, an outlet to a diagnostic device either directly or indirectly or alternatively to a silicon chip for use with further biomedical analyses.

- 10 In a further aspect of the present invention, there may be located on the microfluidic device a valve for the control of the flow of fluid within the microfluidic device. Said control may include selectively permitting the pass or block of fluid. The valve may further allow the introduction of new materials to the microfluidic device. Further still the valve may permit the drainage of waste material from the microfluidic device.
- 15 In a further embodiment such valves may be located between adjacent microfluidic channel blocks so as to control the flow of fluid between said blocks. Said flow may be from an upstream block to a downstream block.

- It will be appreciated that the core may be planar, however other shapes of the core are possible, subject to design considerations. The invention may also enable direct injection moulding of multi-way flow control features on the microfluidic device during the moulding process, and facilitate installation of filtering devices into the flow paths.
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Accordingly, the present invention is directed to a microfluidic device having a moulded core form as a single unit. The core includes microfluidic channels on one face and features on an opposed second face. In one embodiment, the features on the second face may also include microfluidic channels. In a further embodiment, the microfluidic channels present on the first and second faces may be in fluid communication. The fluid communication may be provided by channels extending from the first face to the second face so as to provide a fluid pathway between the respective microfluidic channels. Other forms of fluid communication may be possible, including osmotic membranes or capillary structures.

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In a further embodiment, the first face may also include features other than the microfluidic channels. The features on the first and/or second faces may include valves for controlling the flow of fluid through the microfluidic channels for each face. Further, valves may be provided to control the flow of fluid between the first and second faces. Further still, said valves may be connectors, with said connectors arranged so as to not interfere with flow in the channels.

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Said features may further include entry points for the introduction of fluid to the microfluidic device.

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In a further embodiment, the features may include a mixing device moulded onto the first and/or second faces of the core. Said mixing device may include a plurality of entry points for the introduction of fluids to a bifurcator microfluidic arrangement. Said bifurcator may lead to a single microfluidic channel. Said microfluidic channels within

the mixing device may be arranged to provide sufficient residence time between the fluids introduced through the entry point so as to promote diffusion between the fluids prior to exiting the mixing device. The mixing device may exit onto a feature on the first and/or second faces. Said mixing device may exit into a further microfluidic channel.

Various embodiments of the present invention may provide certain advantages in isolation or in combination, subject to the designated application. For instance:

10 1) The polymer microfluidic devices may act as flow channel systems and so be connected and integrated in one moulded unit;

2) Fluid flow path may be controlled and re-directed through moulded channels in combination with a multi-way valve system in between flow channel systems;

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3) Fluid samples may be introduced into the flow path at the moulded entry points, or from any entry points designed and moulded downstream from an initial entry point

4) Fluid samples may also be conducted away from exit points moulded into the core,
20 subject to the designated application;

5) Fluid filters may be easily inserted into or removed from flow path

6) Other microfluidic devices may be incorporated into or removed from the moulded biochip system through pre-designed connection points to form hybrid microfluidic devices. Such multi-device, or multi-core devices, may be joined through connectors moulded into the core, so as to selectively couple and de-couple from another such core
5 of device.

7) As the flow channel systems may be designed so as to not interfere with the moulded sample entrance/exit points and the moulded valves on the device surfaces, the channel system may be sealed using elastomer sheets, or films, applied to the core surfaces
10 through designed chip holder. This may eliminate the need to seal the moulded chips using time consuming and costly bonding methods widely used in microfluidic devices.

Brief Description of Drawings

15 It will be convenient to further describe the present invention with respect to the accompanying drawings that illustrate possible arrangement of the invention. Other arrangements of the invention are possible and consequently the particularity of the accompanying drawings is not to be understood as superceding the generality of the preceding description of the invention.

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Figures 1A and 1B are isometric views of a microfluidic core according to one embodiment of the present invention;

Figure 2 is a plan view of a microfluidic core according to a further embodiment of the present invention;

5 Figure 3 is a plan view of an entry channel according to one embodiment of the present invention;

Figure 4 is a plan view of a sample channel according to a further embodiment of the present invention;

10 Figure 5 is a sectional view of a microfluidic device according to one embodiment of the present invention;

Figure 6 is an isometric view of a microfluidic device according to a further embodiment of the present invention;

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Figure 7 is a sectional view of a microfluidic valve according to one embodiment of the present invention.

Detailed Description

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Figures 1A, 1B and 2 show a core for a microfluidic device designed to preprocess a blood sample so as to extract DNA. The embodiment shown in Figures 1A, 1B and 2 provides an example of sample preprocessing which may be applied to a range of

different diagnostic procedures of which the example shown in Figures 1A, 1B and 2 for DNA detection and analysis is just one example.

The core 5 includes three microfluidic channel blocks 14, 15, 16 arranged on a first face 11 to conduct three sequential preprocessing steps. The core 5 further includes a mixing block 20, also on the first face 11, for introducing a sample to the core 5 to undergo preprocessing. Whilst each block is in fluid communication with the adjacent block, through channels in the second face 12, the fluid communication is controlled by respective valves 24, 25, 26. Said valves may control flow between adjacent blocks as well as provide a means for introducing further material to the fluid path for downstream preprocessing. The valves may permit further draining of fluid from an upstream process to a waste reservoir (not shown).

Further shown is a recess 35 for receiving for instance, a silicon chip (not shown). In the example provided in Figures 1 and 2, the core 5 is adapted for the preprocessing of DNA with the silicon chip arranged to receive the DNA sample for further diagnostic analysis. The recess 35 provides an inlet 28 and outlet 29 to achieve this purpose. The recess may also provide access to other diagnostic devices for instance, another bio chip which may receive a sample through inlet 28. The outlet 29 feeds into a further valve 30 which may prevent the flow of fluid out of the core 5 or may facilitate drainage of the fluid to a waste reservoir (not shown).

To discuss in further detail the anticipated preprocessing steps, one such application may include the introduction of the sample into the mixing block 20 through an inlet

port 55. Magnetic beads coated in a material to attract virus cells may be introduced to the inlet port 60 with the sample and beads mixing within the mixing block 65. Once fully mixed the fluid may exit 70 of the mixing block 20 so as to enter the concentration block 14. A magnetic field applying by a magnetic block (not shown) may be applied adjacent to the concentration block 14 such that the magnetic beads having mixed within the sample and attracted virus cells to the bead surface, may be halted from further progress within the concentration block 14. The excess fluid may then move further through a connecting channel 31 in the second face to be, for instance, drained through valve 24 to a waste reservoir.

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The purification block 15 having received the beads from the concentration block 14 via connecting channels 31, 32 will further receive a lysis solution which can be introduced through the first valve 24 such that the beads and lysis solution mix within the block 15.

15 The lysis solution acts to strip the virus cells leaving the resulting DNA. Coated silica beads can also be introduced to the purification chamber through valve 24 which acts to attract the DNA and so separating it from the remaining material. Figure 4 shows a filter 80 which is used to block the passage of the silica beads and so selectively traps the beads within the purification chamber.

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The fluid is then permitted to move into the amplification chamber 16 through connecting channels 33, 34 leading to and from a valve 25. It will be appreciated that a significant advantage of microfluidic channel devices is the ability to process very small

volume of samples. This does, however, lead to very low concentrations of DNA which is difficult to process.

However, polymerase chain reaction (PCR) can be implemented within the
5 amplification chamber 16 so as to increase the concentration of DNA removed from the initial sample. In this case the amplification block 16 may have a heat source applied (not shown) so as to promote replication of the DNA through the PCR process.

Once sufficient residence time within the amplification block 16 has been permitted, the
10 higher concentration of DNA may be allowed to flow into the recess 35, through connecting channel 37, to be received by a silicon chip (not shown) for subsequent DNA analysis. The remaining waste fluid may be drained through the valve 26 into a waste reservoir. The core 5 may then be discarded.

15 Thus the provision of a low cost core yields a low cost and efficient preprocessing procedure not previously available.

Additional benefits of the microfluidic core according to the present invention include a fully sealed procedure which may avoid contamination. In a commercial laboratory,
20 several DNA samples may be processed at one time. Having a fully sealed preprocessing procedure may therefore avoid cross contamination of samples.

It will be appreciated that with other preprocessing of diagnostic procedures applied to a core according to a present invention, multiple blocks corresponding to multiple steps

may be provided. In this instance if the preprocessing of a sample requires four, five or more steps, then a suitable number of microfluidic channel blocks may be provided to accommodate each step.

- 5 Further the core may provide provision to allow and “influence” to be applied to respective blocks as required. For instance, in the example provided in Figures 1A, 1B and 2 whereby a sample is preprocessed to extract DNA, the concentration block and amplification block both have external influences being a magnetic field and heat respectively. Accordingly, a microfluidic core designed for a different procedure may
10 allow for different external influences to be provided.

It will be appreciated that such external influences may be directly coupled to the core or provided in an external block adjacent to the respective block. Such external influences may be glued, clamped or screwed into place or may be inserted within an
15 external housing so as to hold said external influences adjacent to the respective microfluidic channel block.

It will be appreciated that the core 5 according to this aspect of the present invention may be glass, polymer, silicon or other acceptable material so as to provide the
20 microfluidic channels. Further, in this aspect the core may be a layer arrangement having two portions glued together in order to achieve the core as required.

In another aspect, Figures 1A, 1B and 2 may also show a microfluidic device 5 having a core 10 moulded as a single unit. The single unit may be injection moulded and

therefore using a polymer such as polycarbonate or polymethylmethacrylate (PMMA). Other appropriate rigid polymers may also be used, where rigid polymers are those having a glass transition above the operable temperature, such as ambient temperature for room temperature analyses.

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The core, formed as a single unit, is subject to an injection moulding process, whereby a suitable die is formed to provide the microfluidic channels and features. Apart from the benefits of dimensional stability, the injection moulding process also provides for repeatability and is particularly useful for providing microfluidic cores for application requiring repeated analysis.

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It will be appreciated that the various combinations of microfluidic channels, features, entry/exit point locations, valves and other ancillary devices such as mixing devices, will be subject to the designer of the analytical process. The formation of a device according to the present invention provides extended flexibility to the designer, together with the various advantages afforded by the simplified integrated process of a single unit moulding.

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As distinct from the first aspect, the second aspect having the microfluidic core 10 moulded as a single unit may or may not have multiple channel blocks. It will be appreciated that the combination of the two aspects of multiple block and single moulded unit may provide further economic and logistical benefits.

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In one embodiment of the second aspect shown in Figures 1A and 1B, the valves 24, 25, 26, 30 are moulded onto a first face 11 of the core 10. This provides for simple assembly of the valves to complete the microfluidic device 5. Said valves may be valve housings providing a base onto which a fully assembled valve may be placed.

5 Alternatively, the moulded valves may be connectors for other components to be added through an external housing into which the core may be placed.

Specifically the embodiment shown in Figures 1A and 1B show an entry arrangement, in this case also acting as a mixing device 20, for feeding into a series of three
10 microfluidic channel portions. Separating each microfluidic channel is a valve 25 allowing for cumulative or discreet use of the microfluidic channel portions. At the end of the microfluidic channel path are a further valve 30 and a recess 35 to receive a diagnostic chip (not shown).

15 Subsequent figures will show alternate embodiments of the present invention including more detailed analysis of the various features.

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Figures 5 and 7 show sectional views of a microfluidic device 5 according to a further embodiment of the present invention. Here the core 10 is shown in section having microfluidic channels 40, 100 on either face of said core. The microfluidic channels are connected by further channels or ducts 110 which allow for communication between the

faces of the core. Further shown is an entry point 95 for introduction of a material into the device 5.

In a still further aspect, moulded as part of the core is a valve 25 which separates a first microfluidic channel 100 from a second array of microfluidic channels 101. The valve may act as a point of introduction of a new material or for permitting flow between the two portions. Thus the valve provides for selective fluid communication between various portions of the microfluidic channels.

10 As a further aspect of the present invention, the valve disc 125 includes several features such as an orifice 126 and a longitudinal recess 138. Whilst in position, the disc 125 can be rotated so as to permit fluid communication between the first channel 100 and the outside of the device. By rotating the disc to this position, material may be added or extracted from the device such as the silica beads described in the previous aspect of the present invention. Similarly the longitudinal groove 138 is positioned on an underside of the disc 125. Rotating to a further position allows the first and second channels 100, 101 to be in fluid communication, thus allowing fluid to pass through the valve unfettered. By rotating the disc further to a position opposed to that of the orifice 126, the channels 100, 101 are sealed and so preventing any fluid communication. The upper surface of the disc 125 further provides a slot 139 for receiving a screw driver or similar implement and so permit the operator to selectively rotate the device and thus activate the various features of the valve 25.

Figure 6 shows the microfluidic device 135 having the core 155 placed within a reusable housing. The housing includes two portions 140, 145 which encompass the core 155. Valve portions 150 are also positioned to engage the valves 26 located on the core 155. Thus in the reusable housing the valves are free to operate as required. As shown in Figure 7, the external portion of the valve 150 includes a central core which permits access to the slot 139 for rotating the disc as well as providing a conduit through which material can be added to the orifice 136. The orifice 136 may also be used for draining waste material in the embodiment. As shown in Figure 7, the valve disc 125 also includes a positioning orifice 137. A positioning pin (not shown here) can be inserted to the orifice 137 through the positioning hole 146 within the reusable housing 145 to fix the position of the valve disc 125 once the rotation adjustment is finished. The use of the positioning orifice has also the advantage of precisely locating the position of disc 125 when doing rotating adjustment as it can be easily felt when the pin is pushed into the orifice position.

Returning to the core cross-section shown in Figure 5, the moulding process requires that the channels are formed as recesses on the core 10 and so in this embodiment the open channels formed by the moulding process are sealed by film 105A, B being applied to each side and so forming the device 5 from the core 10. Turning to the valve 25, the valve housing 115 is also part of the moulding process and formed in the single processing step of forming the core 10. To assemble a valve in this embodiment, a filter disc 120 is placed within the valve seat of the valve housing 115. A rotatable disc 125 is then added and a valve ring or disc 130 seals the peripheral edges of the disc such that selective flow between the microfluidic channels is provided through an orifice 126 of

the rotatable disc 125. Thus for a relatively simple assembly of the valve components into the pre-formed moulded valve housing 115, the microfluidic 5 becomes functional at a markedly reduced cost. It will be appreciated that the film 105A, B may become redundant when the core is used with the reusable housing shown in Figures 6 and 7, as
5 the housing may provide for sealing of the channels of the core.

Figure 6 shows the core 155 placed within a housing 135 with said housing including reusable components 140, 145 which are hingedly mounted. On completion of the preprocessing, the core 155 can be withdrawn and discarded once the preprocessed
10 sample has been extracted.

Claims:

1. A microfluidic device comprising;
a polymer microfluidic core having microfluidic channels on a first face and
5 microfluidic features on a second face wherein said core is a single moulded
unit.
2. The microfluidic device according to claim 1 wherein said features on the
second face include microfluidic channels in fluid communication with the
10 microfluidic channels on said first face.
3. The microfluidic device according to claim 2 wherein said fluid communication
is provided by channels connecting the microfluidic channels of the first and
second face.
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4. The microfluidic device according to any one of the preceding claims wherein
the first face further includes microfluidic features.
5. The microfluidic device according to any one of the preceding claims wherein
20 said features include: valves, connectors, entry points, exit points and mixing
devices.
6. The microfluidic device according to claim 5 wherein said mixing devices
include;

a plurality of entry points to introduce a plurality of fluids to be mixed;
said entry points in fluid communication with a microfluidic channel with said
mixing device;

said microfluidic channel arranged such that the fluids combine and move along
5 said microfluidic channel within the mixing device for a predetermined period
so as to provide sufficient residence time for diffusion of said fluids prior to
exiting said mixing device.

7. The microfluidic device according to claim 5 or 6 wherein said valves moulded
10 into said first or second face include a moulded housing for receiving
components, said components arranged to be assembled with said valve housing
to form an operable valve.

8. The microfluidic device according to any one of the preceding claims further
15 including a film placed on said first and/or second face to seal the microfluidic
channels.

9. The microfluidic device according to any one of the preceding claims wherein
said microfluidic device includes a reusable housing for receiving said core;
20 said housing including sealing faces on an inside surface such that on placement
of said core within said housing, the housing is arranged to close so as to seal
said microfluidic channels.

10. The microfluidic device according to claim 9 when said housing further includes valves for the control of fluid entering and exiting the core, said existing valves arranged to engage connectors moulded onto said first and/or second face.

5 11. A method of forming a microfluidic device the method comprising the steps of:
moulding a microfluidic core having microfluidic channels on a first face and microfluidic features on an opposed second face and;
sealing said microfluidic channels so as to permit the introduction of fluid to said microfluidic core.

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12. A microfluidic device comprising:
a microfluidic core having a plurality of microfluidic channel blocks;
wherein at least a portion of said blocks in selective fluid communication.

15 13. The microfluidic device according to claim 12 wherein each of said microfluidic channel blocks are arranged to correspond to steps of a pre-determined process, said plurality of blocks cumulatively corresponding to said process.

14. The microfluidic device according to claim 12 or 13 wherein said device is
20 arranged to permit the application of an external media so as to impose an influence on at least one of said blocks.

15. The microfluidic device according to claim 14 wherein said influence includes the application of heat and/or a magnetic field.

16. The microfluidic device according to any one of claims 12 to 15 further including a mixing block having a plurality of inlets for receiving materials to be combined within a common microfluidic channel mixing block in fluid communication with said inlets, said microfluidic channel within the mixing block having an outlet for introducing the mixed materials to the plurality of microfluidic channel blocks.
17. The microfluidic device according to any one of claims 12 to 16 wherein said selective fluid communication provided by an arrangement to selectively permit or block flow of fluid between adjacent microfluidic channel blocks.
18. The microfluidic device according to claim 17 wherein said arrangement includes a valve.
19. The microfluidic device according to claim 18 wherein said valve includes a valve inlet to permit the introduction of material to said blocks.
20. The microfluidic device according to claim 18 or 19 wherein said valve includes an outlet for draining waste material from said microfluidic channel blocks.
21. The microfluidic device according to any one of claims 12 to 20 further including a recess for receiving a diagnostic device.
22. A valve for controlling flow in a microfluidic device comprising;
a valve seat encompassing a microfluidic channel inlet and a microfluidic channel outlet;

a rotatable disc coupled to said valve seat, said disc having a longitudinal recess wherein said disc is arranged to rotate between a first position whereby said recess aligns with the inlet and outlet providing fluid communication between said inlet and outlet and a second position sealing at least one of said inlet and outlet and so blocking fluid communication.

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23. The valve according to claim 22 wherein said rotatable disc further includes an orifice such that rotation to a third position aligns said orifice with said outlet so as to permit the introduction of material through said valve into the microfluidic device.

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24. The valve according to claim 22 or 23 further including a vent such that rotation of the disc to a fourth position aligns the vent with the inlet so as to permit the drainage of waste material from said microfluidic device.

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