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(54) **Title**: ON-DEMAND SINGLE CELL ENCAPSULATION USING A LOCALISED ACOUSTIC FIELD

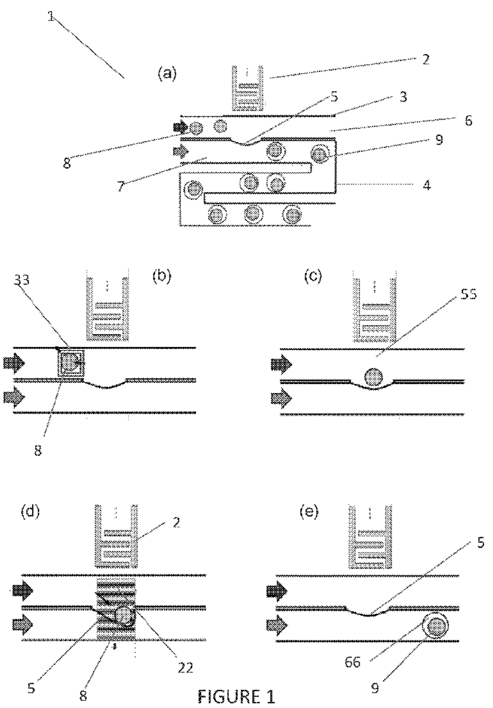

FIGURE 1

(57) **Abstract**: A microfluidic device for and method of isolating a single cell on-demand, the device including: a substrate; a first microfluidic channel (3) provided on the substrate adapted to have a solution containing cells flow within; a second microfluidic channel (4) provided on the substrate adapted to have a carrier liquid flow within; an interface zone (55) having an interface (5) where the solution containing cells flowing in the first microfluidic channel and the carrier liquid flowing in the second microfluidic channel meet; and an acoustic signal source (2); wherein: the solution containing cells and the carrier liquid are immiscible; and application of an acoustic signal from the acoustic signal source when the single cell passes into the interface zone causes deformation of the interface and thereby directs the single cell through the interface into the carrier liquid, such that the single cell is encapsulated in a droplet of the solution the cell was originally flowing within.

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ON-DEMAND SINGLE CELL ENCAPSULATION USING A LOCALISED ACOUSTIC FIELD

FIELD OF THE INVENTION

[0001] The present invention is directed to a device, system and method for single cell encapsulation using a localised acoustic field.

BACKGROUND TO THE INVENTION

[0002] Microfluidics is a research area which is fuelled by its ability to perform unique experiments. By reducing the size of a fluidic system it becomes possible to control the fluid environment, cell location and flow regimes highly accurately. One developing role of microfluidics is for encapsulated single cell analysis, where a single cell is fully contained in an aqueous droplet that is suspended in a mutually immiscible second fluid phase.

[0003] There are several benefits to encapsulating cells in droplets suspended within an immiscible carrier fluid: (1) the fluid interface acts as a physical barrier, eliminating undesired diffusion gradients, (2) undesired cell-cell interactions and cell-environment reactions, with the benefit of also (3) all but eliminating cell-cell or cell-wall adherence so that (4) mass quantities of encapsulated cells can be sorted, stored and handled independently. Because of these benefits, single cell analysis can be performed with significantly greater power, especially with regard to a cell's influence on its neighbouring environment. Only by limiting the volume in which cellular products can diffuse can individual cell information be obtained in many instances. This ability has wide-ranging application in cell studies and drug discovery.

[0004] The bulk of prior work in cell encapsulation relies on the mixing of the two phases in a droplet-producing geometry, where the aqueous phase contains a dilution of cells. However, these techniques are limited in that, due to the dilute initial population of cells required to make sure more than one cell is not captured in a single droplet, the vast majority of droplets contain no cells.

[0005] One prior method is cell encapsulation in a continuous flow device. This is explained in 'Droplet microfluidic technology for single-cell high-throughput screening' Proceedings of the National Academy of Sciences, 106(34):14195-14200, 2009 Brouzes et. al. While the encapsulation of cells in droplets has thus been recognized as an important precursor step to a variety of processes, implementation of high-efficiency cell encapsulation has proven difficult. Predominantly, efforts have focussed on integrating conventional droplet generation, where cells are suspended in the flowing aqueous phase and then are passively encapsulated at the droplet forming region of a t-junction or flow focussing device. However, it is impossible to guarantee that only single cells will be encapsulated in a droplet, with the random distribution of cells in the incoming flow leading to a cell content in droplets following the poisson distribution $P = \lambda^k e^{-\lambda}/k!$, where λ is the average number of cells per droplet and k is the number of cells. Here, tuning the concentration of cells is used to control the number of cells per droplet. However, to minimize the instances where multiple cells are captured in a given droplet, the cell concentration must be so low that the vast majority of vesicles contain no cells at all. This, however, limits the encapsulated cell production rate, and necessitates a downstream process to sort droplets containing cells from those that do not.

[0006] Another method used is inertial focussing to increase the seeding ratio. To address the issue of poor cell seeding with the method in the previous paragraph, some work has been undertaken to combine the ability to order particles using inertial focussing with passive droplet generation. Here, the concept is that an ordered line of cells arrives at the droplet generating region in sequence with the generation of droplets themselves, resulting in a single-cell emulsion that is mostly (but not completely) composed of single cells in droplets. Some droplets may not contain any cells and some droplets may contain multiple cells. This combination of inertial focussing and droplet generation has the potential to overcome the barrier imposed by the poisson distribution. However, in practice this system is difficult to implement, where the particle ordering prior to encapsulation is highly dependent on initial particle concentration and flow rate, where a minimum (high) flow rate is required to induce the inertial ordering effect, and the flow rates of water and oil must be specifically tuned.

[0007] Another method is active control of droplet production to encapsulate cells. This method is presented in WO 2013/120016 (The Regents of the University of California) and makes use of pulsed laser-induced cavitation to pinch off individual droplets containing cells. While theoretically viable, the method as presented has the disadvantages of having a relatively unfocussed pressure source; the cavitation bubble expands radially rather than directionally. Given cavitation bubbles potential to lyse cells, the viability of encapsulated cells is questionable without confirmatory experiments. Additionally, the optical method associated with integrating and focussing a pulsed laser system is quite complex and not able to be produced on a microchip. Another disadvantage of this system is that use of a laser creates heat which can damage the cell.

[0008] There is a need in encapsulating single cells to combine the detection of cells in a constant flow with the ability to produce droplets on-demand. It is an object of the present invention to overcome or ameliorate problems and difficulties of the prior art.

[0009] Discussion or mention of any piece of prior art in this specification is not to be taken as an admission that the prior art is part of the common general knowledge of the skilled addressee of the specification in Australia or any other country.

SUMMARY OF THE INVENTION

[0010] According to an aspect of the invention, there is provided a microfluidic device for isolating a single cell on-demand, the device including: a substrate; a first microfluidic channel provided on the substrate adapted to have a solution containing cells flow within; a second microfluidic channel provided on the substrate adapted to have a carrier liquid flow within; an interface zone having an interface where the solution containing cells flowing in the first microfluidic channel and the carrier liquid flowing in the second microfluidic channel meet; and an acoustic signal source; wherein: the solution containing cells and the carrier liquid are immiscible; and application of an acoustic signal from the acoustic signal source when the single cell passes into the interface zone causes deformation of the interface and thereby directs the single cell through the interface into the carrier liquid, such that the single cell is encapsulated in a droplet of the solution the cell was originally flowing within.

[0011] The device preferably further includes an interrogation area upstream of the interface zone for detecting the cell prior to encapsulation.

[0012] The interface zone may further include in the first microfluidic channel any one or more of: (a) obstacles; (b) electrically generated forces; or (c) acoustically generated forces, to encourage or direct the cell to the interface and/or to hold the single cell at the interface zone.

[0013] An acoustic mismatch may occur at the interface and the movement of the cell into the carrier liquid occurs because the interface is displaced.

[0014] The substrate is preferably a piezoelectric substrate with patterned electrodes for generating surface acoustic waves.

[0015] The carrier liquid is preferably a form of oil and the solution containing cells is preferably water, cell nutrient, biological fluid or buffer solution, such as phosphate buffer solution or phosphate buffer saline (PBS).

[0016] According to another aspect of the present invention there is provided a method of isolating a single cell on-demand using a device having: a substrate;

a first microfluidic channel adapted to have a solution containing cells flow within; a second microfluidic channel adapted to have a carrier liquid flow within; an interface zone having an interface where the solution containing cells flowing in the first microfluidic channel and the carrier liquid flowing in the second microfluidic channel meet; and an acoustic signal source, the method including: introducing into the first microfluidic channel a solution including cells; introducing into the second microfluidic channel a carrier liquid, wherein the solution including cells and the carrier liquid are immiscible fluids; and applying an acoustic signal from the acoustic signal source which produces a force at the interface zone, causing deformation of the interface and thereby directing the single cell through the interface into the carrier liquid in the second microfluidic channel, such that the single cell is encapsulated in a droplet of the solution the cell was originally flowing within.

[0017] Preferably the acoustic signal used in the method or device described above is a surface acoustic wave signal. The signal may be a travelling surface acoustic wave signal or a standing surface acoustic wave signal.

[0018] The method may further include the step of detecting the cell upstream of the interface zone prior to encapsulation.

[0019] In the method an acoustic mismatch may occurs at the interface and the movement of the cell preferably occurs because the interface is displaced.

[0020] The method may further include the step of holding the single cell at the interface zone through the use of any one or more of: (a) obstacles; (b) electrically generated forces; or (c) acoustically generated forces, in the interface zone of the first microfluidic channel.

[0021] Preferably the method further includes the step of directing one encapsulated cell into the second microfluidic channel and directing another encapsulated cell into a third microfluidic channel.

[0022] In the method, the substrate used is preferably a piezoelectric substrate with patterned electrodes for generating surface acoustic waves.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] It will be convenient to further describe the invention with respect to the accompanying drawings. Other embodiments of the invention are possible, and consequently, the particularity of the accompanying drawings is not to be understood as superseding the generality of the preceding description of the invention. In the drawings:

[0024] Figures 1(a) to 1(e) show the steps of a method according to an embodiment of the present invention. These figures also show a cross-sectional schematic of a device according to another embodiment of the present invention.

[0025] Figures 2(a) and 2(b) show a cross-sectional schematic of a device according to yet another embodiment of the present invention.

DESCRIPTION OF PREFERRED EMBODIMENTS

[0026] Figure 1(a) shows a part of a device or system for encapsulating single cells using surface acoustic waves (SAW). The device and method of the present invention will now be described with respect to Figures 1 and 2. The same reference numerals will be used throughout the drawings to refer to the same or like parts or method steps.

[0027] The device of the present invention produces droplets to encapsulate cells on-demand, that is, a single droplet is formed when required, and in this case as a result of a pulsed actuation of the acoustic source. As shown in Figures 1 and 2, the device 1 includes at least two channels, a cell solution channel 3 through which a solution 6 containing cells flows, and a carrier liquid channel 4, through which a carrier liquid 7 flows. The device 1 also includes an acoustic signal source 2 for generating an acoustic signal. In this embodiment, the acoustic signal source is a surface acoustic wave (SAW) device for generating SAWs. The device 1 also has a cell solution-carrier liquid interface 5. This interface 5 occurs at an area where there is a gap in a wall which is common to each channel 3, 4 , such that an interface 5 forms when the cell solution fluid 6 in one channel 3 meets the carrier liquid 7 in the other channel 4.

[0028] The channels are arranged on a substrate (not shown). The channels may be made using a material which is bonded to the substrate. The channel material is preferably polydimethylsiloxane (PDMS) but may be any other suitable polymer or other material. The channels may be made in the material which is then bonded to the substrate. Alternatively, the channels may be formed by a combination of the material and the substrate. For example, the material may form the side walls and top of the channel and the substrate forms the base of the channel. Alternatively, the channels may be etched into the substrate. The substrate may be a piezoelectric substrate with patterned electrodes for generating acoustic signals. As such, the acoustic signal source may be formed as part of the substrate or is provided on the substrate.

[0029] Figures 1(a) to 1(e) show the different stages in a method for producing encapsulated single cells using the device 1. Figure 1(a) shows cells 8 in the cell solution 6 flowing through the cell solution channel 3. The figure also shows single cells 9 that have been encapsulated in droplets of the cell solution 6 flowing along the carrier liquid 7 in the carrier liquid channel 4.

[0030] As the cells 8 in the cell solution 6 flow through the cell solution channel 3 they are detected in an upstream interrogation area 33 located in the cell solution channel 3. In Figure 1(b) the box 33 around cell 8 has been used to illustrate the interrogation area 33. It will be appreciated that in the embodiment shown the interrogation area does not have physical barriers such as a box or square, however, other embodiments may have physical obstacles in or which form the interrogation area.

[0031] An individual cell 8 is detected as it flows through the channel 3 when it approaches or is within the interrogation area 33 as shown in Figure 1(b). The individual cell may be detected by any conventional method including an electrical, optical or acoustic method. When the detected cell reaches the interfacial region 55 (as shown in Figure 1(c)), a focussed SAW 22 is applied (as shown in Figure 1(d)) which forces the cell 8 toward the interface 5. Applying the SAW also deforms the interface 5 as shown in Figure 1(d). By deforming the interface 5, the single cell 8 is encapsulated by a small amount of cell solution 66 (see Figure 1(e)). The encapsulated single cell 9 is forced into the carrier liquid channel 4 and flows within the carrier liquid 7 along the channel 4 down stream for further processing, sorting or other diagnostics.

[0032] As explained, when a cell 8 is detected, a burst of SAWs 22 pushes the cell to the interface 5 of the cell solution 6 and the carrier liquid 7, for example, a water-oil interface (that is, the solution 6 containing the cells is water, and the carrier liquid 7 is oil). The solution containing the cells could also be cell nutrient, biological fluid or buffer solution, such as phosphate buffer solution or phosphate buffer saline (PBS).

[0033] At the same time that the SAW 22 pushes the cell 8 towards the interface 5, the SAW 22 also deforms the interface 5 so that it extends into the carrier liquid 7 phase (See Figure 1(d)). If the SAW 22 is of sufficiently high amplitude, pressure conditions on either side of the distended interface 5 will result in droplet pinch-off, where the droplet of cell solution 66, for example water, contains the now encapsulated cell 9. The required energy which results in pinch-off may depend on a number of factors including: the frequency of excitation of the ultrasonic surface acoustic wave; the size of electrodes in the SAW generating device; the viscosity of the carrier liquid and the viscosity of the cell solution; the length of the electrical pulse applied to the electrodes; the size of the cell to be encapsulated; the flow rates of each of the carrier liquid and the cell solution; and the surface tension at the interface of the carrier liquid and cell solution.

[0034] The cell can be encouraged, directed or brought to the interface zone through various means, including obstacles, electrically generated forces or acoustically generated forces.

[0035] In another embodiment, as shown in Figure 2, the cells 8 travel in the channel 3 with the cell solution 6. As the cells near the interface 5, the device 1 encourages and forces the cells 8 to move towards the interface 5 of the cell solution 6 and the carrier liquid 7. Whilst similar to the previous embodiment described, in this embodiment, the cells are then held at the interface zone 55. This may be achieved by using obstacles in the channel or using electrically or acoustically generated forces (of various methods). As shown in Figure 2(b), once held at the interface, a burst of SAWs are applied from the SAW generating device 2. The SAW generating device 2 may have curved electrodes as shown in Figure 2. The SAWs push the cell 8 into the interface 5 and as a result cause part of the cell solution in which the cell is flowing to be pinched off from the rest of the cell solution 6. This results in a droplet 66 containing a cell 9 being pushed and directed into the carrier liquid 7.

[0036] The pulse of acoustic waves pushes the cell (with a small amount of solution 66) into the carrier liquid (or secondary) stream 7, for example, an oil stream, such that a droplet 66 is formed which encapsulates the cell 9.

[0037] This method is completely different to the prior art. In fact, the prior art teaches away from the present invention. The present invention allows a cell to be encapsulated on-demand as the cell passes a fixed point. The present invention detects the cell as it approaches the acoustic wave signal source and times the droplet formation by the acoustic signal around the timing or flow of the cell, rather than the other way around as taught by the prior art. The prior art tries to order the cells so that they arrive at an interface in time with a natural droplet formation rate, effectively controlling the cells to arrive at the interface of the cell solution and carrier liquid at the same periodicity as the natural production of a droplet. Instead, the present invention waits for a cell to arrive in a liquid stream, which does not necessarily arrive at a uniform spacing or periodicity. The present invention then forms a droplet around the cell while it travels. The present invention advantageously uses actively-controlled acoustic forces to control droplet formation, as and when it is needed, rather than controlling the cells to coincide with droplets that are naturally formed.

[0038] This device and method of the present invention has application in drug delivery and dosage control as well as for the analysis and diagnostics of cells in isolation allowing interrogation of physical properties without the influence of other cell, for example using flow cytometry. The device and method of the present invention could also be used for drug testing, for example, the droplets containing the single cell could be merged with droplets containing a drug solution and a response detected. The device may contain droplets of different drug dosages to ascertain the response. This device and method could be used to identify knowledge of the cell behaviour could be used to establish deviations from the standard response indicative of disease.

[0039] SAWs are optimal for this acoustic microfluidics application due to their planar nature, and SAWs are easily integrated directly into microfluidic systems with efficient energy transfer from the substrate to the fluid.

[0040] The active nature of the system of the present invention using an acoustic field improves cell seeding and substantially increases the cell seeding ratio. Further, the present invention controls when a droplet is formed, rather than controlling when the cell arrives to align with the produced droplet, as many prior art devices and methods do. The system of the present invention is able to be integrated onto a microchip unlike many prior art systems. The present invention significantly improves the efficiency of encapsulating single cells with no instances of multiple cells contained in a droplet, or no cells contained in a droplet.

[0041] As the present invention may be embodied in several forms without departing from the essential characteristics of the invention, it should be understood that the above described embodiment should not be considered to limit the present invention but rather should be construed broadly. Various modifications and equivalent arrangements are intended to be included within the spirit and scope of the invention. Modifications and variations as would be deemed obvious to the person skilled in the art are included within the ambit of the present invention as claimed in the appended claims.

CLAIMS:

1. A microfluidic device for isolating a single cell on-demand, the device including:

a substrate;

a first microfluidic channel provided on the substrate adapted to have a solution containing cells flow within;

a second microfluidic channel provided on the substrate adapted to have a carrier liquid flow within;

an interface zone having an interface where the solution containing cells flowing in the first microfluidic channel and the carrier liquid flowing in the second microfluidic channel meet; and

an acoustic signal source;

wherein:

the solution containing cells and the carrier liquid are immiscible; and

application of an acoustic signal from the acoustic signal source when the single cell passes into the interface zone causes deformation of the interface and thereby directs the single cell through the interface into the carrier liquid, such that the single cell is encapsulated in a droplet of the solution the cell was originally flowing within.

2. A microfluidic device according to claim 1, wherein the acoustic signal is a surface acoustic wave signal.

3. A microfluidic device according to claim 2, wherein the acoustic signal is a travelling surface acoustic wave signal.

4. A microfluidic device according to claim 2, wherein the acoustic signal is a standing surface acoustic wave signal.

5. A microfluidic device according to any one of the preceding claims, further including an interrogation area upstream of the interface zone for detecting the cell prior to encapsulation.

6. A microfluidic device according to any one of the preceding claims, wherein the interface zone includes in the first microfluidic channel any one or more of:

(a) obstacles;

(b) electrically generated forces; or

(c) acoustically generated forces,

to hold the single cell at the interface zone.

7. A microfluidic device according to any one of the preceding claims, wherein an acoustic mismatch occurs at the interface; and wherein the movement of the cell occurs because the interface is displaced.

8. A microfluidic device according to any one of the preceding claims, wherein the substrate is a piezoelectric substrate with patterned electrodes for generating surface acoustic waves.

9. A microfluidic device according to any one of the preceding claims, wherein the carrier liquid is a form of oil and the solution containing cells is water, cell nutrient, biological fluid or buffer solution.

10. A method of isolating a single cell on-demand using a device having: a substrate; a first microfluidic channel adapted to have a solution containing cells flow within; a second microfluidic channel adapted to have a carrier liquid flow within; an interface zone having an interface where the solution containing cells flowing in the first microfluidic channel and the carrier liquid flowing in the second microfluidic channel meet; and an acoustic signal source,

the method including:

introducing into the first microfluidic channel a solution including cells;

introducing into the second microfluidic channel a carrier liquid, wherein the solution including cells and the carrier liquid are immiscible fluids; and

applying an acoustic signal from the acoustic signal source which produces a force at the interface zone, causing deformation of the interface and thereby directing the single cell through the interface into the carrier liquid in the second microfluidic channel, such that the single cell is encapsulated in a droplet of the solution the cell was originally flowing within.

11. A method according to claim 10, wherein the acoustic signal is a surface acoustic wave signal.

12. A method according to claim 11, wherein the acoustic signal is a travelling surface acoustic wave signal.

13. A method according to claim 11, wherein the acoustic signal is a standing surface acoustic wave signal.

14. A method according to any one of claims 10 to 13, further including the step of detecting the cell upstream of the interface zone prior to encapsulation.

15. A method according any one of claims 10 to 14, wherein an acoustic mismatch occurs at the interface; and wherein the movement of the cell occurs because the interface is displaced.

16. A method according to any one of claims 10 to 15 further including the step of holding the single cell at the interface zone through the use of any one or more of: (a) obstacles; (b) electrically generated forces; or (c) acoustically generated forces, in the interface zone of the first microfluidic channel.

17. A method according to any one of claims 10 to 16 further including the step of directing one encapsulated cell into the second microfluidic channel and directing another encapsulated cell into a third microfluidic channel.

18. A method according to any one of claims 10 to 17, wherein the substrate is a piezoelectric substrate with patterned electrodes for generating surface acoustic waves.

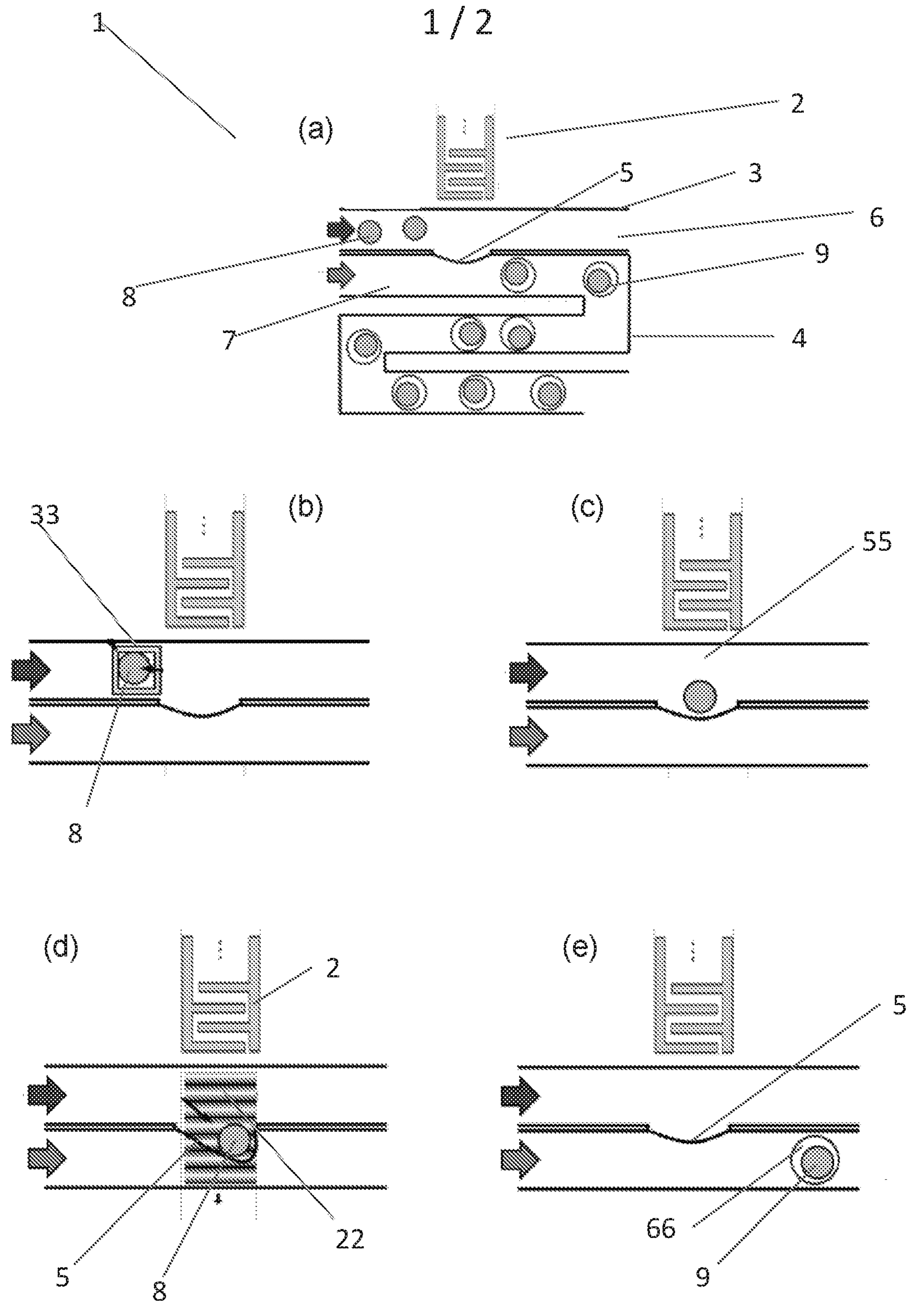

FIGURE 1

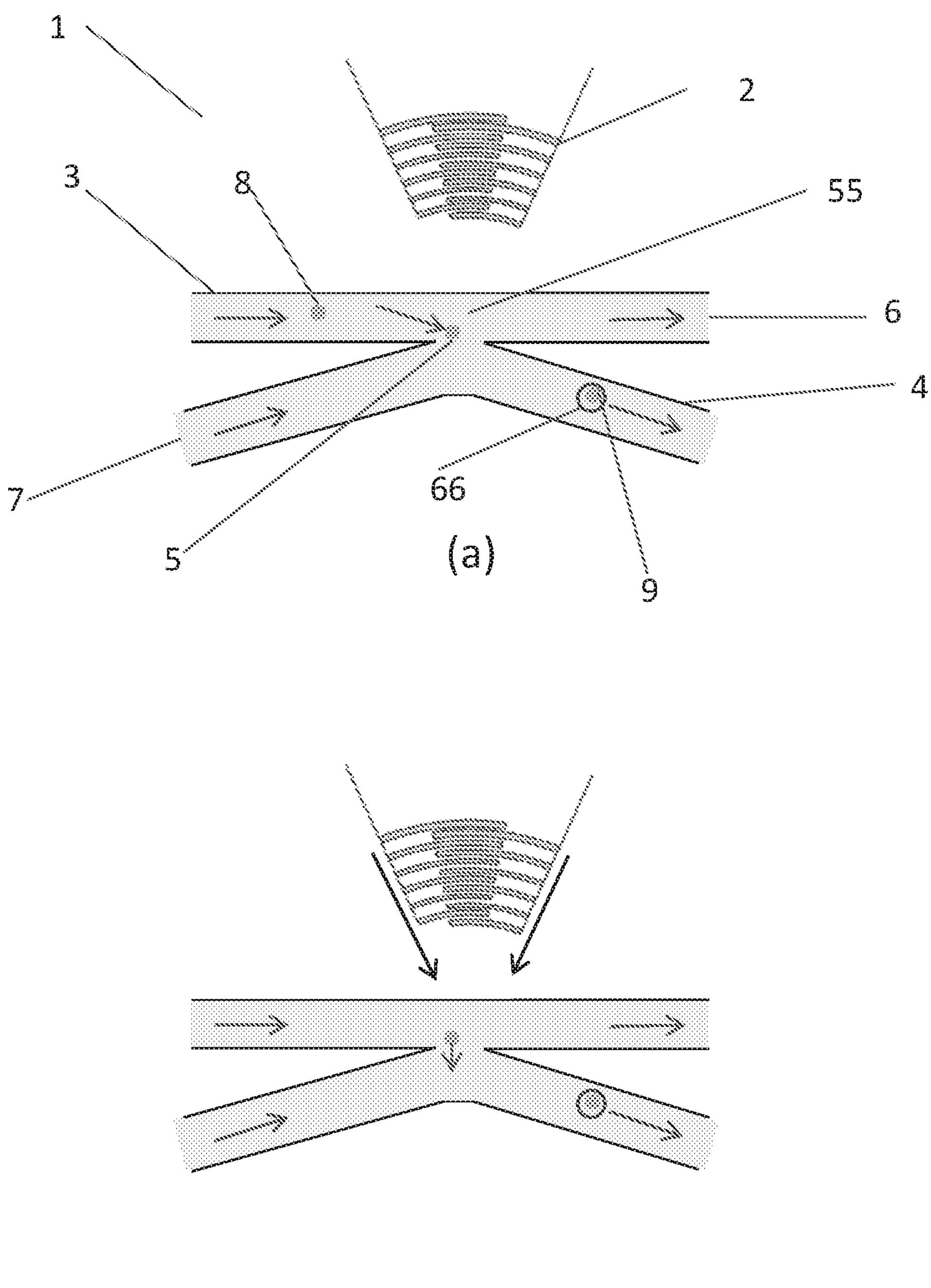

FIGURE 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2016/050590

A. CLASSIFICATION OF SUBJECT MATTER

C12M 1/42 (2006.01) B06B 1/00 (2006.01)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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)

WPIAP, EPODOC: IPC & CPC: C12M 1/42, B06B 1/00, B06B 1/10, B06B 1/18, B06B 1/20; keywords: acoustic, encapsulation, cell (and like terms); **EPODOC**: CPC: B01L 2200/0652, B01L 2400/0436, B01L 3/502715, B01L 2200/0647, C12N 5/0012, C12M 23/16, B01L 3/502761; **EPODOC, TXTE**: CPC: C12M 23/16, B01L 3/502761, C12N 5/0012, C12N 11/04; keywords: encapsulation, acoustic, immiscible (and like terms).

Google Patents and **Google Scholar**: keywords: microfluidic, single cell, encapsulation, droplet, acoustic wave (and like terms). Applicant and inventors name search in internal databases provided by IP Australia and **Espacenet**.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier application or patent but published on or after the international filing date
- "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	Date of mailing of the international search report
25 October 2016	25 October 2016

Name and mailing address of the ISA/AU

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(ISO 9001 Quality Certified Service)
Telephone No. 0262832138

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	COLLINS, D. J. et al., 'Surface acoustic waves for on-demand production of picoliter droplets and particle encapsulation', Lab on a Chip, 2013, vol. 13, pages 3225-3231 Abstract; page 3226, col. 1, lines 14-16; page 3226, col. 2, line 8-page 3227, col. 1, line 54; page 3227, col. 2, line 49-page 3228, col. 1, line 3; page 3230, col. 1, lines 10-47; figs. 1, 2 and 4	1-18
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INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/AU2016/050590**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

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