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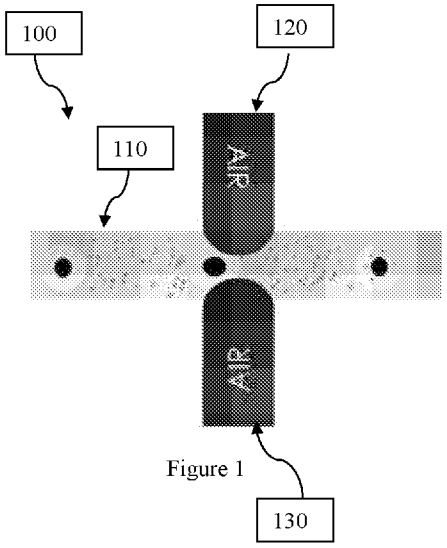

Figure 1

(57) Abstract: The present disclosure relates to a method for inducing enhanced poration in biological cells comprising: flowing a biological cell suspended in a medium through a microfluidic channel; introducing air through channels disposed in relation to the microfluidic channel in a cross-sectional manner, the air being introduced is to the extent to cause the biological cell to compress and thereby inducing enhanced poration in biological cell. The present invention in particular relates to a method for intracellular delivery of molecules or particulate matter by inducing enhanced poration in biological cell. The present disclosure further relates to a device for inducing enhanced poration in biological cell, more particularly for intracellular delivery of molecules or particles into the cell especially > 100 nm in size.

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A DEVICE AND METHOD FOR ENHANCED PORATION OF BIOLOGICAL CELLS

TECHNICALFIELD

[0001] The present disclosure relates generally to the field of poration in biological cells. In particular, the present disclosure relates to a device and method for inducing enhanced poration of biological cells without significant distortion of the biological cells.

BACKGROUND

[0002] Background description includes information that may be useful in understanding the present invention. It is not an admission that any of the information provided herein is prior art or relevant to the presently claimed invention, or that any publication specifically or implicitly referenced is prior art.

[0003] Cell membranes are natural barriers to free transport of molecules and particulate matter from an extracellular space (outside) to the intracellular space (inside) a cell. A key challenge in drug-delivery is overcoming this barrier, while maintaining the viability of a cell. A variety of methods exist for intracellular delivery and for forming pores on the cell membrane [M. P. Stewart, A. Sharei, X. Ding, G. Sahay, R. Langer, and K. F. Jensen, "In vitro and ex vivo strategies for intracellular delivery", Nature, vol. 538, no. 7624, pp. 183-192, Oct. 2016]. Cell-poration may be achieved using an electric field [P. E. Boukany et al., "Nanochannel electroporation delivers precise amounts of biomolecules into living cells", Nat. Nanotechnol., vol. 6, no. 11, pp. 747-54, Oct. 2011], deformability cytometry[A. Kollmannsperger et al., "Live-cell protein labelling with nanometre precision by cell squeezing", Nat. Commun., vol. 7, p. 10372, Jan. 2016], [A. Sharei et al., "Ex vivo cytosolic delivery of functional macromolecules to immune cells" PLoS One, vol. 10, no. 4, pp. 1-12, 2015]; lasers [Q. Fan, W. Hu, and A. T. Ohta, "Laser-induced microbubble poration of localized single cells", Lab Chip, vol. 14, no. 9, pp. 1572-1578, 2014]; acoustic waves[Z. Fan et al., ""Acoustic tweezing cytometry for live-cell subcellular modulation of intracellular cytoskeleton contractility", Sci. Rep., vol. 3, no. 1, p. 2176, Dec. 2013]; [Z. Fan, H. Liu, M. Mayer, and C. X. Deng, "Spatiotemporally controlled single cell sonoporation", Proc. Natl. Acad. Sci. U. S. A., vol. 109, no. 41, pp. 16486-91, Oct. 2012]; plasmonics [S. Courvoisier et al., "Plasmonic Tipless Pyramid Arrays for Cell Poration",

Nano Lett., vol. 15, no. 7, pp. 4461, 6, Jul. 2015]; molecular motors [V. García-López et al., "Molecular machines open cell membranes", Nature, vol. 548, no. 7669, pp. 567-572, Aug. 2017]; etc. Of these techniques, deformability cytometry has a significant advantage as it is capable of porating cells at high throughput without any external fields. However, existing techniques limit the maximum pore size of around ~50 nm with high efficiency and uptake[L. Shang, K. Nienhaus, and G. U. Nienhaus, "Engineered nanoparticles interacting with cells: size matters", J. Nanobiotechnology, vol. 12, p. 5, Feb. 2014]. Santos et al.[T. dos Santos, J. Varela, I. Lynch, A. Salvati, and K. A. Dawson, "Quantitative Assessment of the Comparative Nanoparticle-Uptake Efficiency of a Range of Cell Lines", Small, vol. 7, no. 23, pp. 3341-3349, Dec. 2011]have shown to porate cells beyond 50 nm but the efficiency reduces drastically. Existing techniques are thus limited by pore size, throughput, or efficiency of theparticle-intake.

[0004] There is therefore a requirement in the art for an efficient approach for inducing enhanced poration in biological cells, yet devoid of shortcoming associated with the existing techniques.

OBJECTS

[0005] A general object of the present disclosure is to provide a device for inducing enhanced poration in biological cells.

[0006] Another object of the present disclosure to provide a device for intracellular delivery, for example of molecules and particulate matter by inducing enhanced poration in biological cells.

[0007] Another object of the present disclosure is to provide a method for inducingenhanced poration in biological cells.

[0008] Another object of the present disclosure to provide a method for inducingenhanced poration in biological cells for intracellular delivery, for example of molecules and particulate matter.

SUMMARY

[0009] The present disclosure relates generally to the field of poration induction in biological cells. In particular, the present disclosure relates to a device for inducing enhanced poration of biological cells without significant distortion of the biological cells.

[0010] In an aspect, the present disclosure provides a device for inducing poration in biological cells, said device comprising: at least one first microfluidic channel configured for flow of a fluid medium through a lumen of the at least one first microfluidic channel, said fluid medium comprising biological cells suspended in the fluid medium; and at least one pair of second microfluidic channels configured for flow of air, disposed perpendicular to the at least one first microfluidic channel and facing one another, said at least one pair of second microfluidic channels pressure coupled to the lumen of the at least one first microfluidic channel, wherein flow of air through the at least one pair of second microfluidic channels causes compression of the biological cells flowing in the lumen of the at least one first microfluidic channel to induce poration in the biological cells.

[0011] In an embodiment, the fluid medium comprises any or a combination of molecules and particulate matter, wherein induction of poration in the biological cells enables intracellular delivery of said any or a combination of molecules and particulate matter into the biological cells.

[0012] In another embodiment, one or more first microfluidic channels are placed in the formation of a grid to enable higher throughput of porated biological cells.

[0013] In another embodiment, the microfluidic channels of the device are made of polydimethylsiloxane (PDMS).

[0014] In another embodiment, device is bonded to a rigid substrate such as glass and Silicon.

[0015] In another embodiment, the device is fabricated using soft lithography.

[0016] In an aspect, the present disclosure provides a method for using a device for inducing poration in biological cells, said method comprising the steps of: flowing a fluid medium through a lumen of at least one first microfluidic channel configured on the device, said fluid medium comprising biological cells suspended in the fluid medium; and flowing air through at least a pair of second microfluidic channels disposed perpendicular to the at least one first microfluidic channel and facing one another, said at least one pair of second microfluidic channels pressure coupled to the lumen of the at least one first microfluidic channel, wherein the biological cells flowing I the lumen of the at least one first microfluidic channel are compressed by the flow of air through the at least one pair of second microfluidic channels to induce poration in the biological cells.

[0017] In an embodiment, the fluid medium comprises any or a combination of molecules and particulate matter, wherein induction of poration in the biological cells enables intracellular delivery of said any or a combination of molecules and particulate matter into the biological cells.

[0018] In an aspect, the present disclosure provides a method for using the claimed device of the present disclosure for inducing poration in biological cells, said method comprising the steps of: flowing a fluid medium through a lumen of at least one first microfluidic channel configured on the device, said fluid medium comprising biological cells suspended in the fluid medium; and flowing air through at least a pair of second microfluidic channels disposed perpendicular to the at least one first microfluidic channel and facing one another, said at least one pair of second microfluidic channels pressure coupled to the lumen of the at least one first microfluidic channel, wherein the biological cells flowing I the lumen of the at least one first microfluidic channel are compressed by the flow of air through the at least one pair of second microfluidic channels to induce poration in the biological cells.

[0019] In an embodiment, the fluid medium comprises any or a combination of molecules and particulate matter, wherein induction of poration in the biological cells enables intracellular delivery of said any or a combination of molecules and particulate matter into the biological cells.

BRIEF DESCRIPTION OF DRAWINGS

[0020] The accompanying drawings are included to provide a further understanding of the present disclosure and are incorporated in and constitute a part of this specification. The drawings illustrate exemplary embodiments of the present disclosure and, together with the description, serve to explain the principles of the present disclosure.

[0021] FIG. 1 illustrates a schematic representation of a device forinducing enhancedporation in biological cells, in accordance with an exemplary embodiment of the present disclosure.

[0022] FIG. 2 illustrates a schematic representation of an enlarged portion of the device for intracellular delivery of molecules or particles into a biological cell by inducingenhanced poration in biological cell, showing the cellbeing compressedresulting in enhanced poration in cell and delivery of particles into the cell.

[0023] FIG. 3 illustrates a representation of a cell wall of a biological cell before introducing cells into the proposed device of the present disclosure.

[0024] FIG. 4 illustrates a representation of the cell wall of the biological cell after the biological cell is allowed to flow through the proposed device of the present disclosure.

[0025] FIG. 5 illustrates an exemplary fluorescence image showing a cell wall of an RBC along with 30nm beads at the inlet of the proposed device, where the beads are outside the cells.

[0026] FIG. 6 illustrates an exemplaryfluorescence imageshowingthe cell wall of the RBCalong with 30nm beads at the outlet of the proposed device, wherethe beads have entered the cell volume of the RBC.

[0027] FIG. 7 illustrates an exemplary fluorescence image showing a cell wall of an RBC along with 100nm beads at the inlet of the proposed device, where the beads are outside the cells.

[0028] FIG. 8 illustrates an exemplary fluorescence image showing the cell wall of the RBC along with 100nm beads at the outlet of the proposed device, where the beads have entered the cell volume of the RBC.

DETAILED DESCRIPTION

[0029] The following is a detailed description of embodiments of the disclosure depicted in the accompanying drawings. The embodiments are in such detail as to clearly communicate the disclosure. However, the amount of detail offered is not intended to limit the anticipated variations of embodiments; on the contrary, the intention is to cover all modifications, equivalents, and alternatives falling within the spirit and scope of the present disclosure.

[0030] Depending on the context, all references below to the "invention" or "disclosure" may in some cases refer to certain specific embodiments only. In other cases, it will be recognized that references to the "invention" or "disclosure" will refer to subject matter recited in one or more, but not necessarily all, of the claims.

[0031] As used in the description herein and throughout the claims that follow, the meaning of "a," "an," and "the" includes plural reference unless the context clearly dictates otherwise. Also, as used in the description herein, the meaning of "in" includes "in" and "on" unless the context clearly dictates otherwise.

[0032] All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all

examples, or exemplary language (e.g. "such as") provided with respect to certain embodiments herein is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention otherwise claimed. No language in the specification should be construed as indicating any non-claimed element essential to the practice of the invention.

[0033] The present disclosure generally relates to a method for inducingenhanced poration in biological cells.

[0034] In an embodiment, the present disclosure provides a method for inducing enhanced poration in biological cells, the methodcomprising the steps of: flowing a biological cell suspended in a medium through a microfluidic channel; introducing air through channels disposed in relation to the microfluidic channel in a cross-sectional manner, the air being introduced is to the extent to cause the biological cell to compress and thereby inducingenhanced poration in biological cell.

[0035] In an embodiment, the present disclosure provides a method for intracellular delivery of molecules or particulate matterby inducingenhanced poration in biological cells.

[0036] In another embodiment the present disclosure provides a method for intracellular delivery of molecules or particulate matter, the methodcomprising the steps of causing a cell to compress under the influence of the air to the extent it inducesenhanced poration in the biological cell; and allowing the delivery of molecules or particulate matter into cells.

[0037] In oneembodimentthe present disclosure provides a method for intracellular delivery of molecules or particulate matter, the methodcomprising the steps of: flowing a biological cell and molecules or particulate matterto be delivered suspended in a medium through a microfluidic channel; introducing air through channels disposed facing each other and in a cross-sectional manner in relation to the microfluidic channel, the air being introduced is to the extent to cause the biological cell to compress thereby inducingenhanced poration, and allowing the delivery of molecules or particulate matter into the biological cell.

[0038] In an embodiment the present disclosure provides a method for intracellular delivery of molecules or particles into the biological cell ranging from a few angstroms in size to > 100 nm in size into cells without cell lysis.

[0039] In another embodiment the present disclosure provides a device for inducing enhanced poration in biological cell.

[0040] In an embodimentthe present disclosure provides a device for inducing enhanced poration in biological cell, the device comprising: a microfluidic channel configured to allow the flow of the biological cell suspended in a medium through the lumen of said microfluidic channel; at least a set of microfluidic channels disposed facing each other and in a cross-sectional manner in relation to the microfluidic channel allowing flow of the biological cell; wherein the set of microfluidic channels are adapted to allow introducing air into the microfluidic channel to the extent causing the biological cell flowing inside the lumen of said microfluidic channel to compress and thereby inducingenhanced poration in the biological cell.

[0041] FIG. 1 illustrates a schematic representation of a device for inducing enhanced poration in biological cells, in accordance with an exemplary embodiment of the present disclosure. In an embodiment, the device (100) comprises: a microfluidic channel (110) configured to allow the flow of the biological cell suspended in a medium through the lumen of said microfluidic channel (110); at least a set of microfluidic channels (120) and (130) disposed facing each other and in a cross-sectional manner in relation to the microfluidic channel (110); wherein the set of microfluidic channels (120) and (130) are adapted to introduce air into the microfluidic channel (110) to the extent causing the biological cell (not shown in figure) flowing inside the lumen of microfluidic channel (110) to compress and thereby inducingenhanced poration in the biological cell.

[0042] In anotherembodimentthe present disclosure provides a devicefor intracellular delivery of molecules or particulate matter into the biological cellby inducing enhanced poration inthe biological cell.

[0043] In an embodimentthe present disclosure provides a device for intracellular delivery of molecules or particulate matter into the biological cell, the device comprising: a microfluidic channel configured to allow the flow of the biological cell suspended in a medium through the lumen of said microfluidic channel; at least a set of microfluidic channels disposed facing each other and in a cross-sectional manner in relation to the microfluidic channel allowing flow of the biological cell; wherein the set of microfluidic channels are adapted to introduce air into the microfluidic channel to the extent causing the biological cell flowing inside the lumen of said microfluidic channel to compress and thereby inducingenhanced poration in the biological cell; and allowing delivery of the molecules or particulate matter into the biological cell.

[0044] FIG. 2 illustrates a schematic representation of an enlarged portion of the device for intracellular delivery of molecules or particles into a biological cell by inducing enhanced poration in biological cell, showing the cell being compressed resulting in enhanced poration in cell and delivery of particles into the cell. In an embodimentthe device (100) for intracellular delivery of molecules or particulate matter into the biological cell (200), the device comprising: a microfluidic channel (110) configured to allow flowing a biological cell and molecules or particulate matterto be delivered suspended in a medium through the lumen of said microfluidic channel (110); at least a set of microfluidic channels (120) and (130) disposed facing each other and in a cross-sectional manner in relation to the microfluidic channel (110); wherein the set of microfluidic channels (120) and (130) are adapted to introduce air into the microfluidic channel (110) to the extent causing the biological cell (200) flowing inside the lumen of microfluidic channel (110) to compress thereby inducingenhanced poration (210) in the biological cell (200) and allow delivery of particle (300) into the cell.

[0045] FIG. 3 illustrates a representation of a cell wall of a biological cell before introducing cells into the proposed device of the present disclosure. The pores in the biological cell can be seen to allow only small sized particles to enter the cell volume.

[0046] FIG. 4 illustrates a representation of the cell wall of the biological cell after the biological cell is allowed to flow through the proposed device of the present disclosure.

[0047] The pressure on the flowing medium due to flow of air causes the cell to compress and thereby induces enhanced poration in biological cell allowing larger particles to enter the cell volume.

[0048] To demonstrate and validate the working of the device as per one of the embodiments of the present disclosure, an experiment was conducted by flowing through the device of the present disclosure, red blood cells and 30 nm latex beads suspended in a buffer.Further, confocal microscopy was carried out confirming the presence of 30 nm latex beads inside the cells.

[0049] FIG. 5 illustrates an exemplary fluorescence image showing a cell wall of an RBC along with 30nm beads at the inlet of the proposed device, where the beads are outside the cells. Here, RBCs and 30nm beads are suspended in the buffer at the inlet of the channels, where the beads are outside the cells.

[0050] FIG. 6 illustrates an exemplary fluorescence image showing the cell wall of the RBC along with 30nm beads at the outlet of the proposed device, where the beads have entered the cell volume of the RBC. As can be seen the 30 nm beads have occupied entire cell volume without shape of the RBC being distorted.

[0051] To further demonstrate and validate the working of the device of the present disclosure, another experiment was conducted by flowing through the device of the present disclosure, red blood cells and 100 nm polystyrene beads suspended in a buffer. Further, confocal microscope was carried out confirming the presence of 100 nm polystyrene beads inside the cells.

[0052] FIG. 7 illustrates an exemplary fluorescence image showing a cell wall of an RBC along with 100nm beads at the inlet of the proposed device, where the beads are outside the cells.

Here, RBCs and 100nm beads are suspended in the buffer at the inlet of the channels, where the beads are outside the cells.

[0053] FIG. 8 illustrates an exemplary fluorescence image showing the cell wall of the RBC along with 100nm beads at the outlet of the proposed device, where the beads have entered the cell volume of the RBC. As can be seen the 100 nm beads have occupied entire cell volume without shape of the RBC being distorted.

[0054] In embodiment the present disclosure provides a devicefor intracellular delivery of molecules or particles ranging from a few angstroms in size to > 100 nm in size into cells through inducing enhanced poration inthe biological cell, without significant distortion of the biological cell.

[0055] In an embodiment, the extent to which the air is introduced through the set of microfluidic channels can be varied to compress the biological cell to achieve poration of desired dimensions or allowing the delivery of molecules or particles > 100 nm in size into the biological cell without causing cell lysis. The extent of air introduced through the set of microfluidic channels can be controlled with the help of various means, tools, and/or techniques capable of achieving the same.

[0056] The device can be in the form of system comprising of grid of microfluidic channel configured to allow flow of the biological cell suspended in a suitable medium and sets of microfluidic channels facing each other and disclosed in cross-sectional manner in relation to the microfluidic channel allowing the flow of the biological cells and molecules or particles

suspended in a suitable medium to induce enhanced poration in biological cell, for example for intracellular delivery of molecules or particles of interest into the cell.

[0057] The disclosed device can be made of any material or combination of materials suitable for use in providing microfluidic channels that can allow biological cells to pass through the microfluidic channels. Preferably, the device can be made of a silicon, glass or polymeric material. More preferably, the microfluidic system can be made of polydimethylsiloxane (PDMS). The device including channels can be fabricated using micro-fabrication techniques such as, but not limited to, soft lithography. The fabricated device may be bonded onto a rigid supporting substrate like glass, silicon or the like. The microfluidic channels can have suitable dimensions so as to allow the flowing of the biological cell through the lumen of the microfluidic channel configured for said purpose and the allow introducing air through the set of microfluidic channels adapted for said purpose.

[0058] The method and device of the present disclosure is able to induceporation inthe biological cells at high throughput, higher efficiency and larger pore size (~ / >100 nm) than what is demonstrated until now.

[0059] While the foregoing describes various embodiments of the disclosure, other and further embodiments of the disclosure may be devised without departing from the basic scope thereof. The scope of the invention is determined by the claims that follow. The invention is not limited to the described embodiments, versions or examples, which are included to enable a person having ordinary skill in the art to make and use the invention when combined with information and knowledge available to the person having ordinary skill in the art.

ADVANTAGES

[0060] The present disclosure provides a device for inducing enhanced poration in biological cells.

[0061] The present disclosure provides a device for intracellular delivery, for example of molecules and particulate matter by inducing enhanced poration in biological cells.

[0062] The present disclosure provides a method for inducing enhanced poration in biological cells.

[0063] The present disclosure provides a method for inducing enhanced poration in biological cells for intracellular delivery, for example of molecules and particulate matter.

We Claim:

1. A device for inducing poration in biological cells, said device comprising:

at least one first microfluidic channel configured for flow of a fluid medium through a lumen of the at least one first microfluidic channel, said fluid medium comprising biological cells suspended in the fluid medium; and

at least one pair of second microfluidic channels configured for flow of air, disposed perpendicular to the at least one first microfluidic channel and facing one another, said at least one pair of second microfluidic channels pressure coupled to the lumen of the at least one first microfluidic channel,

wherein flow of air through the at least one pair of second microfluidic channels causes compression of the biological cells flowing in the lumen of the at least one first microfluidic channel to induce poration in the biological cells.

2. The device as claimed in claim 1, wherein the fluid medium comprises any or a combination of molecules and particulate matter, wherein induction of poration in the biological cells enables intracellular delivery of said any or a combination of molecules and particulate matter into the biological cells.

3. The device as claimed in claim 1, wherein one or more first microfluidic channels are placed in the formation of a grid to enable higher throughput of porated biological cells.

4. The device as claimed in claim 1, wherein the microfluidic channels of the device are made of polydimethylsiloxane (PDMS).

5. The device as claimed in claim 1, wherein said device is fabricated using soft lithography.

6. The device as claimed in claim 1, wherein said device is bonded to a rigid substrate such as glass and Silicon.

7. A method for using a device for inducing poration in biological cells, said method comprising the steps of:

flowing a fluid medium through a lumen of at least one first microfluidic channel configured on the device, said fluid medium comprising biological cells suspended in the fluid medium; and

flowing air through at least a pair of second microfluidic channels disposed perpendicular to the at least one first microfluidic channel and facing one another, said at least one pair of second microfluidic channels pressure coupled to the lumen of the at least one first microfluidic channel,

wherein the biological cells flowing I the lumen of the at least one first microfluidic channel are compressed by the flow of air through the at least one pair of second microfluidic channels to induce poration in the biological cells.

8. The method as claimed in claim 7, wherein the fluid medium comprises any or a combination of molecules and particulate matter, wherein induction of poration in the biological cells enables intracellular delivery of said any or a combination of molecules and particulate matter into the biological cells.

9. A method for using the device for inducing poration in biological cells of claim 1, said method comprising the steps of:

flowing a fluid medium through a lumen of at least one first microfluidic channel configured on the device, said fluid medium comprising biological cells suspended in the fluid medium; and

flowing air through at least a pair of second microfluidic channels disposed perpendicular to the at least one first microfluidic channel and facing one another, said at least one pair of second microfluidic channels pressure coupled to the lumen of the at least one first microfluidic channel,

wherein the biological cells flowing I the lumen of the at least one first microfluidic channel are compressed by the flow of air through the at least one pair of second microfluidic channels to induce poration in the biological cells.

10. The method as claimed in claim 9, wherein the fluid medium comprises any or a combination of molecules and particulate matter, wherein induction of poration in the biological cells enables intracellular delivery of said any or a combination of molecules and particulate matter into the biological cells.

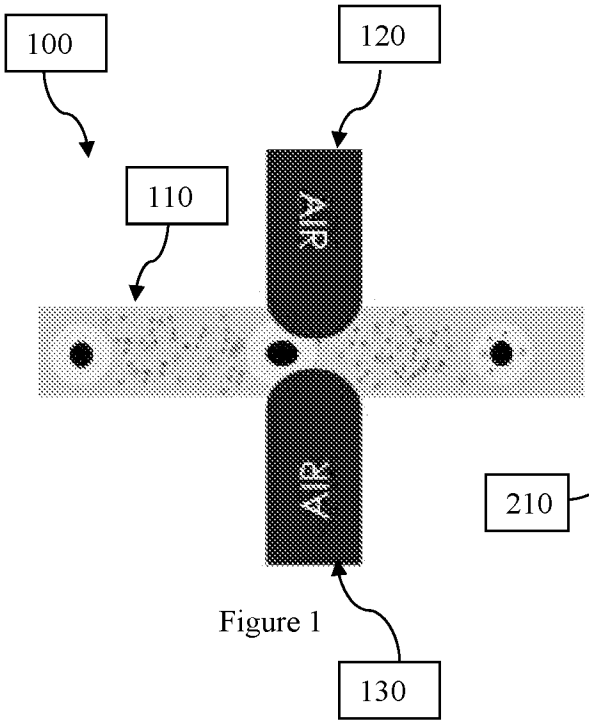

Figure 1

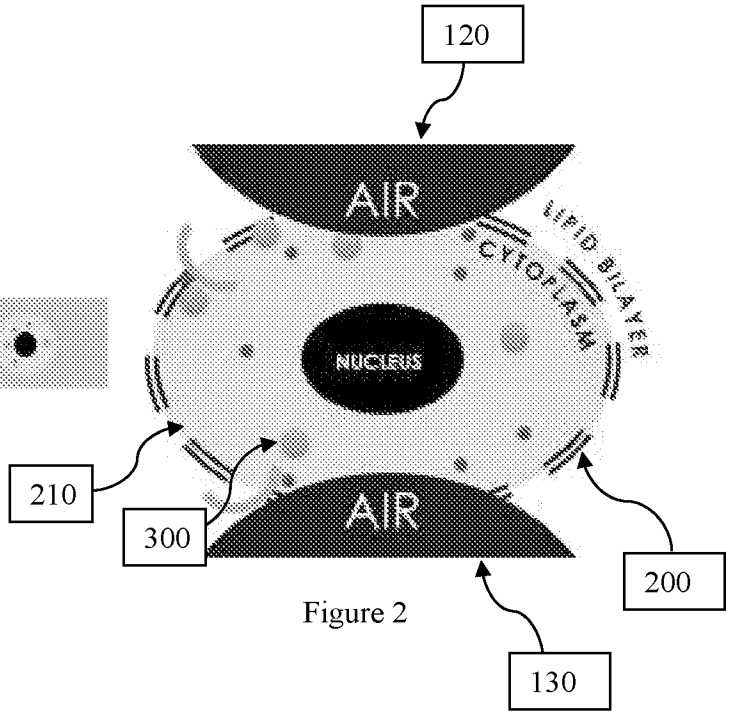

Figure 2

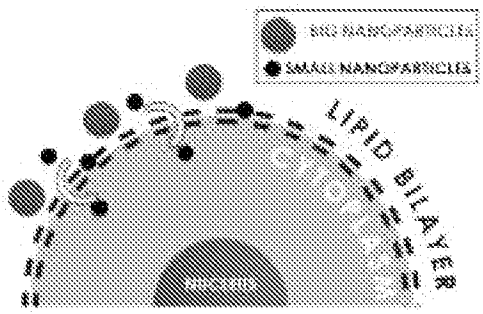

Figure 3

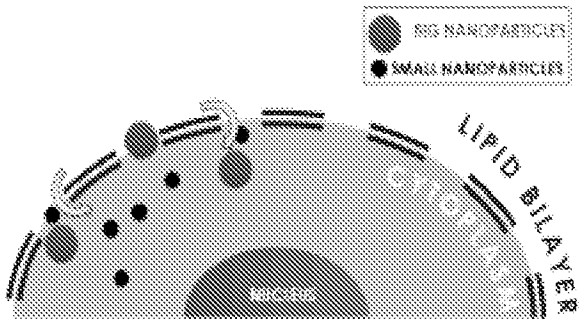

Figure 4

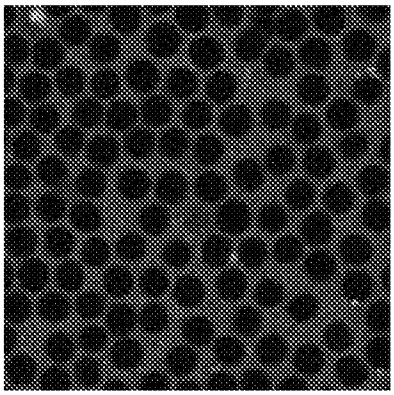

Figure 5

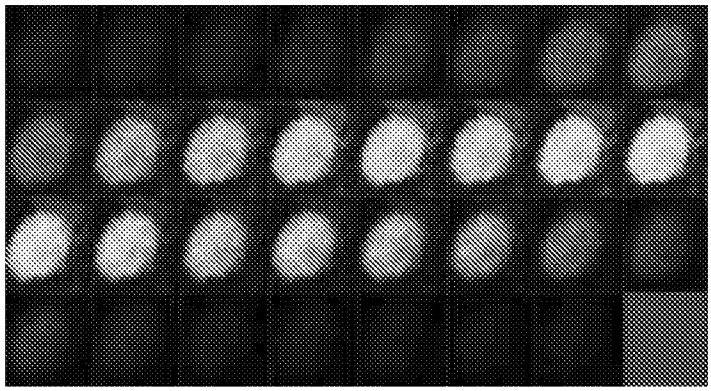

Figure 6

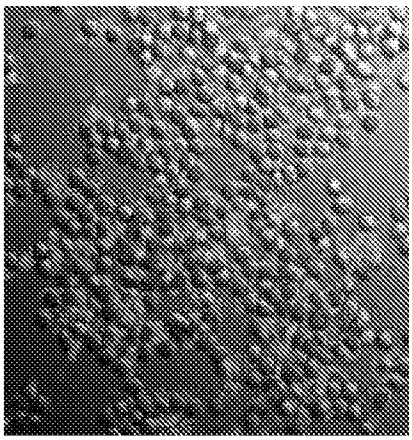

Figure 7

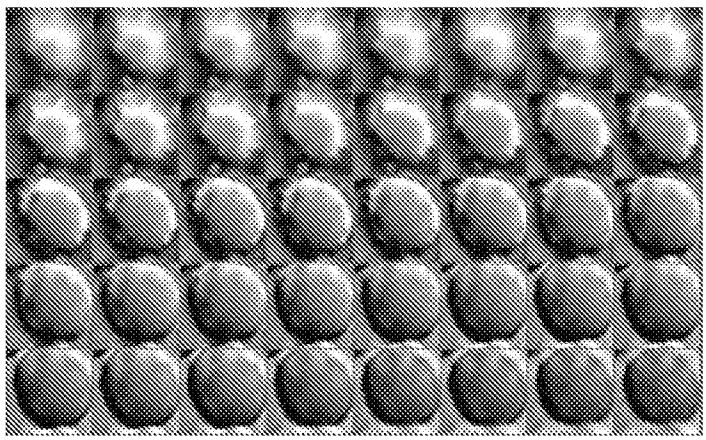

Figure 8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2019/055211

A. CLASSIFICATION OF SUBJECT MATTER
C12N15/00,C12M3/00,B01L3/00 Version=2019.01

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)

C12N, C12M, 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)

TotalPatent One, IPO Internal Database

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 20090280518 A1 (MASSACHUSETTS INSTITUTE OF TECHNOLOGY), 12 November 2009 (12-11-2009) Abstract, paragraphs [0005]& [0050]	1-3 & 5-10
Y	US 20110082056 A1 (ELECTRONICS AND TELECOMMUNICATIONS RESEARCH INSTITUTE), 7 April 2011 (07-04-2011) claims 1-3, paragraphs [0012] & [0029]	1-4 & 6-10
Y	US 8647861 B2 (CHILDREN'S MEDICAL CENTER CORPORATION), 11 February 2014 (11-02-2014) Abstract and claims 1, 2, 14 & 15	1-10
Y	Longsine-Parker et al. 2013. Microfluidic electro-sonoporation: a multi-modal cell poration methodology through simultaneous application of electric field and ultrasonic wave, Lab on a chip, 13(11):2144-52, 24 April 2013 Abstract	1-10
P,X	WO 2018207087 A1 (INDIAN INSTITUTE OF SCIENCE), 15 November 2018 (15-11-2018) Whole document	

☒ Further documents are listed in the continuation of Box C. ☒ 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
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"E" earlier application or patent but published on or after the international filing date
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"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
14-11-2019	14-11-2019
Name and mailing address of the ISA/ Indian Patent Office Plot No.32, Sector 14,Dwarka,New Delhi-110075 Facsimile No.	Authorized officer Raghavendra Madasetty Telephone No. +91-1125300200

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2019/055211

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	US 8857462 B2 (BIO-RAD LABORATORIES INC), 14 October 2014 (14-10-2014) Whole document	1-10
A	Wang et al. 2006. Electroporation of Mammalian Cells in a Microfluidic Channel with Geometric Variation, Analytical Chemistry, 78(14): 5158-5164, 15 July 2006 Whole document	1-10
A	LeGac, S and van den Berg, A. 2012. Single cell electroporation using microfluidic devices, Methods in Molecular Biology, 853:65-82 Whole document	1-10

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/IB2019/055211

Citation	Pub.Date	Family	Pub.Date
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		JP 2011528232 A	17-11-2011
		WO 2010009307 A2	21-01-2010
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		EP 3415235 A1	19-12-2018
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