(54) **Title:** PRESSURE SYSTEM OF AN INJECTION DEVICE

(57) **Abstract:** A pressure system, by means of which it is possible to produce various pressure levels to be used advantageously in an injection device of living cells, which injection device comprises a pressure source for pressurizing a medium and a capillary (7). The pressure system comprises an input (IN) of pressurized medium, an output (OUT) for connecting to a capillary (7), and means (2, 8, 9) for directing positive pressure to the output (OUT). In addition, the system comprises means (3, 13) for forming negative pressure by using positive pressure and means (2, 4) for directing the negative pressure to the output (OUT).
PRESSURE SYSTEM OF AN INJECTION DEVICE

Field of the invention

The invention relates to a pressure system according to the preamble of the appended claim 1. The invention further relates to a method according to the preamble of the appended claim 6 for pressurizing a medium into different pressures.

Background of the invention

Precise control of both positive and negative pressure in a processing capillary is required for intensifying the versatile manipulation of living cells. Positive pressure is required in injecting cells and negative pressure is required in the aspiration processing of cells, as well as in forming a so-called giga connection in electrophysiological measurements.

The publication WO2005/016535 discloses a solution for injecting and aspirating small doses. The solution comprises inherent pressure sources for both positive and negative pressures. In addition, the solution comprises a regulator, by means of which the pressures in the air chamber are controlled. The gas and liquid sides of the system are separated by a membrane placed in the chamber.

The publication US 4,532,205 discloses a solution for injecting small doses into living cells. The solution discloses a structure enabling various pressure levels. The publication discloses a solution for how different positive pressure levels can be created with one pressure source. The different pressure levels are used, inter alia, for injecting and cleaning the system.

The solutions used for controlling pressure in known solutions are complex. Furthermore, it is difficult to set the dosage sizes provided by them.
Summary of the invention

The main purpose of the present invention is to disclose a new solution for producing different pressure levels of a cell processing device.

To attain this purpose, the system according to the invention is primarily characterized in what will be presented in the characterizing part of the independent claim 1. The method according to the invention, in turn, is primarily characterized in what will be presented in the characterizing part of the independent claim 6. The other, dependent claims will present some preferred embodiments of the invention.

Now a pressure system has been invented, by means of which it is possible to form both positive and negative pressure, and which can advantageously be controlled by means of a computer, which can in an advantageous embodiment be integrated into a manipulation system, and which can also be expanded to multimicromanipulator applications.

The system advantageously comprises the following functions: (i) positive pressure pulses for microinjection, (ii) low aspiration pressure, (iii) adjustable background pressure level for eliminating inflow and outflow, (iv) high cleaning pressure for cleaning a clogged capillary, and (v) holding pressure for holding cells if necessary.

By means of the pressure system according to the invention it is possible to produce various pressure levels, which can in an advantageous embodiment of the invention be in a positive and negative pressure. The pressure system is intended to be used advantageously in an injection device of living cells, which is suitable for injecting small doses into living cells, and which device, in addition to a pressure system, comprises a pressure source and a capillary (micropipette or microcapillary). The pressure system according to the invention, in turn, comprises connections to a pressure source and a capillary, as well as means for directing positive pressure to the capillary connection. In addition the system comprises means for creating negative
pressure by using positive pressure and means for directing the negative pressure to the capillary connection.

In an advantageous embodiment of the invention the system comprises means for forming a second positive pressure from the first positive pressure received from the pressure source by decreasing pressure.

In another advantageous embodiment of the invention the system comprises a valve, which is arranged for the capillary connection for controlling the effective time of the effective pressure. By placing the valve on the gas side, the speed of the operating times is affected advantageously.

In an advantageous embodiment of the invention the means forming the negative pressure is an ejector (which is also called an ejector pump and vacuum ejector pump), but it can also be some other suitable means, such as, for example, a blower or a pump, which uses positive pressure for forming negative pressure.

In an advantageous embodiment of the invention the pressure system is arranged to produce pressure to a gas, by means of which it is possible to convey pressure to liquid via an interface located in a capillary pipe. Thus, the solution does not require chamber and membrane structures for separating the gas and the liquid sides from each other, because this is taken care of by a glass capillary, which directly comprises a gas/liquid interface without a separating membrane.

The solution according to the invention utilizes only one pressure source, while known solutions require separate pressure sources for creating positive pressure and negative pressure. This will simplify the structure of the system.

The use of the valve according to the invention also enables fast operation, which is not possible with known solutions. Thanks to the fast operation the volume of the injected or aspirated dose can be controlled more precisely. Also, the repeatability of operation is good due to the precise operation of the apparatus, which is very significant in different studies.
Description of the drawings

In the following, the invention will be described in more detail with reference to the appended principle drawings, in which

Fig. 1 shows a first embodiment of the invention,

Fig. 2 shows a second embodiment of invention

Fig. 3 shows a third embodiment of the invention,

Fig. 4 shows a fourth embodiment of the invention.

For the sake of clarity, the figures only show the details necessary for understanding the invention. The structures and details that are not necessary for understanding the invention, but are obvious for anyone skilled in the art, have been omitted from the figures in order to emphasize the characteristics of the invention.

Detailed description of the invention

The system advantageously comprises the following functions: (i) positive pressure pulses for microinjection, (ii) low aspiration pressure, (iii) adjustable background pressure level for eliminating inflow and outflow, (iv) high cleaning pressure for cleaning a clogged capillary, and (v) holding pressure for holding cells, if necessary. Next, some typical values for the above-mentioned functions will be presented.

(i) The positive pressure pulses used for microinjection are advantageously within the pressure range 5 to 30 kPa, sometimes even 200 kPa. The setting precision must be as good as possible, advantageously below 3 kPa. The range of fluctuation of the repeatability and resolution of pressure is below 0.5 kPa. The length of the pulse is typically 5 ms to 2 s, repeatability and resolution below 5 ms.
(ii) For aspiration the low aspiration pressure (negative pressure) is below 50 kPa, typically below 5 kPa, and precision and repeatability below 100 Pa, and resolution below 10 kPa.

(iii) The pressure range of the adjustable background pressure level, which prevents unwanted inflow and outflow, is advantageously 0 to 20 kPa and the repeatability and resolution of pressure is below 0.5 kPa. Pressure can advantageously be formed continuously, in which case a pulse generator is not required.

(iv) The high cleaning pressure for cleaning a clogged capillary is typically 200 to 700 kPa. For example, particles or air bubbles are removed from the capillary with the pressure pulse. The duration of the pulse is not very significant.

(v) The holding pressure (negative pressure) for holding cells when necessary is advantageously 0 to 5 kPa, and the resolution and repeatability are below 1 kPa. The duration of the pulse is not substantial.

The above-described functions are thus located over at least three substantially different pressure ranges. The first pressure range is 200 to 700 kPa for function iv. The second pressure range is 0 to 30 kPa for functions i and iii. The third pressure range is 0 to 50 kPa negative pressure for functions ii and v. Application-specifically there may be more pressure range. In addition, the limit values of the pressure ranges may differ from the ones presented above.

The pressure system according to the invention can be implemented in a variety of ways. A basic idea is to form several pressures of different sizes (pressure levels, pressure ranges) in a manner most suitable for the pressures in question. Each pressure range is produced advantageously with a pressure formation unit suitable for the pressure range. The pressure pulse required by the function is formed by directing a pressure of a certain magnitude from the pressure formation unit to a pulsing unit (for example a quick valve), which releases pressure to the capillary (or the like) for a desired period of time. In an advantageous embodiment, there is only one
pulsing unit for each outlet. The precision of the component generally depends on its planned pressure range. By using different components on different pressure ranges, the precision is made good on each range.

Next, some advantageous ways for producing three pressure ranges are disclosed, the first pressure range of which is high positive pressure (200 to 700 kPa), the second pressure range is low positive pressure (0 to 30 kPa), and the third pressure range is negative pressure (0 to 50 kPa).

Fig. 1 shows a principle structure of a multichannel injection/aspiration system. It is composed of three pressure lines A, B, C (pressure formation units), which are connected to an injection line D. In the description, the term injection line D is used, irrespective of whether the line in question is affected by negative or positive pressure, i.e. the injection line can also be used for other purposes than injection, such as, for example, for aspiration. The injection line is connected to the output OUT of the system, to which, for example, a capillary 7 can be connected. The capillary can be used, for example, in injecting small doses into living cells.

The first pressure line A, i.e. the topmost one in the figure, is used for cleaning (function iv) and it comprises a first control means 8, such as a valve, by means of which the pressure is directed to the injection line D. The magnitude of its pressure is substantially the same as the input pressure of the system, which is connected to the input IN of the system. The input pressure is produced with some suitable technique, such as, for example, positive pressure pump and the magnitude of the positive pressure is advantageously within the range of 200 to 700 kPa.

The second pressure line B, i.e. the middle one in the figure, is used for injecting and for forming a background pressure (functions i, iii). It is composed of a pressure converter 1, such as an electro-pneumatic converter, which creates positive pressure and the constant pressure required in injection. In addition the pressure line B comprises a second control means 2, such as a valve, which directs the pressure to the injection line D.
The third pressure line C, i.e. the bottom one in the figure, is used for aspiration processing of cells and for forming a holding pressure (functions ii and v). The pressure line C comprises means for forming negative pressure, such as, for example an ejector 3, which converts input positive pressure into negative pressure, as well as a third control means 4, which directs the negative pressure to the injection line D.

For detecting different pressure levels, the device advantageously comprises suitable detectors, such as, for example, a positive pressure sensor 10 and a negative pressure sensor 11. There may be a different number of sensors 10, 11 and detectors than in the example, and they may be located at different points.

In addition, the system comprises a filter 5, which is located in connection with the injection line D in the example. The filter 5 prevents the clogging of the capillary 7. In the example, the injection line D branches into three channels, each one of which may comprise a capillary or other pipette 7. In addition, there is a quick-operated control means 6 in connection with each channel. The control means 6, such as a valve, is used for forming a pressure pulse, which is why it is also called a pulsing valve. In addition to the formation of the pressure pulse, the control means 6 is used for selecting the capillary 7 used in the injection/aspiration. Naturally, there may be a different number of branches than in the example, i.e. one or more.

The pressure pulse affecting the output OUT of the system and further the capillary 7 or a similar means is formed in such a manner that the pressure of the desired pressure level is released with the control means 2, 4, 8 of the pressure line A, B, C to the injection line D. The fast control means 6 in the injection line D, in turn, directs the pressure to the output OUT and further to the capillary 7 for the desired amount of time. With this arrangement, in the pressure lines A, B, C it is possible to use control means 2, 4, 8 possessing a slower operation time, and a fast control means 6 is used in the injection line D. Advantageously, the control means 6 used in the injection line D must be capable of operation times of below 5 ms.
In an advantageous embodiment the pressure converter 1, the control means 2, 4, 6, 8, such as, for example, solenoid valves, as well as the ejector 3 are remote controllable, for example by means of a computer. Thus, setting and/or using the values of the components is easily performed via a user interface. Thus, components do not need to be adjusted manually. This is advantageous, \textit{inter alia}, when pressure levels are desired to be changed often.

Figure 2 shows a principle structure of a second embodiment of a multichannel injection/aspiration system. It is composed of two pressure lines A, B, which are connected to an injection line D. The upper pressure line A is used for cleaning (function iv) and it comprises a control means, such as a valve 8, by means of which the pressure is directed to the injection line D.

The middle pressure line B is used for injecting and for forming a background pressure (functions i, iii), as well as for aspiration processing of cells and for forming holding pressure (functions ii and v). It is composed of a pressure converter 13, which creates positive pressure and the constant pressure required in injecting. In addition, the pressure converter 13 can form negative pressure from positive pressure. The pressure converter 13 can in a case be a combination of an electro-pneumatic converter and an ejector. In addition the pressure line B comprises a control means 2, such as a valve, which directs the pressure to the injection line D.

In addition, the system according to figure 2 comprises a filter 5, which is located in connection with the injection line D in the example. In this example as well, the injection line D branches into three channels, each one of which may comprise a capillary or other pipette 7. In connection with each channel there is a fast operating control means 6, which, in addition to the formation of the pressure pulse, is used for selecting the capillary 7 used in the injection/aspiration.

Figure 3, in turn, shows a principle structure of a third embodiment of a multichannel injection/aspiration system. It is composed of three pressure lines A, B, C, which are connected to an injection line D. The upper pressure line A is used for cleaning (function iv) and it comprises a control means,
such as a valve 8, by means of which the pressure is directed to the injection line D.

The middle pressure line B is used for injecting and for forming a background pressure (functions i, iii). It is composed of a pressure converter 1, such as an electro-pneumatic converter, which creates positive pressure and the constant pressure required in injecting. In addition, the pressure line B comprises a control means 2, such as a valve, which directs the pressure to the injection line D.

The bottom pressure line C is used for aspiration processing of cells and for forming a holding pressure (functions ii and v). The pressure line C comprises means for forming negative pressure, such as, for example an ejector 3, which converts input positive pressure into negative pressure, and a control means 4, which directs the negative pressure to the injection line. In the embodiment according to figure 3 the input positive pressure of the ejector 3 is directed with the control means 2 from the pressure converter. Thus, the pressure converter 1 can affect the level of the input pressure of the ejector 3, and at the same time affect the negative pressure formed by the ejector.

This system also comprises a filter 5, which is located in connection with the injection line D in the example. In this example as well, the injection line D branches into three channels, each one of which may comprise a capillary or other pipette 7. In addition, there is a fast operated control means 6 in connection with each channel. In addition to the formation of the pressure pulse, the control means 6, such as a valve, is used for selecting the capillary 7 used in the injection/aspiration.

Fig. 4 shows an advantageous structure of an injection/aspiration system. It is composed of three pressure lines A, B, C, which are connected to an injection line D. The first, i.e. the upper pressure line A is used for cleaning (function iv) and it comprises two solenoid valves 8, 9. There are advantageously at least two valves 8, 9, because one valve is generally not tight enough to be used alone as a shutter valve for cleaning pressure. The magnitude of the pressure produced into the pressure line A is substantially
the same as the input pressure of the system, which is connected to the input IN of the system.

The second, i.e. the middle pressure line B is used for injecting and for forming a background pressure (functions i, iii). The pressure line B is composed of an electro-pneumatic converter 1, which creates positive pressure and the balance pressure required in injection from the solenoid valve 2, which directs pressure to the injection line D and from the solenoid valve 6, by opening and closing which injection pulses are created. The electro-pneumatic converter 1 creates positive pressure, which is substantially linearly (in an application 0.6% in full scale) proportional to input pressure.

The third, i.e. the lower pressure line C is used for aspiration processing of cells and for forming a holding pressure (functions ii and v). In addition to an electro-pneumatic converter 1 and a solenoid valve 2 directing pressure to the injection line D (in this case rather an aspiration line), it comprises an ejector 3. The ejector 3 converts the input positive pressure formed by the electro-pneumatic converter 1 into negative pressure. The pressure line C also comprises another solenoid valve 4, which prevents air flow through the ejector 3 when the positive pressure is directed to another pressure line B. In addition, the system comprises a filter 5, which is located in front of the pressure lines A, B, C and prevents the capillary 7 from clogging.

The solution according to figure 4 has several advantages. The same pressure converter 1, i.e. the electro-pneumatic converter is used for forming pressure and for injecting cells, as well as for aspiration processing. The ejector 3 is used for converting the positive pressure of the electro-pneumatic converter 1 into negative pressure. In addition, a fast solenoid valve 6 is used for forming pressure pulses from the pressure formed by the electro-pneumatic converter 1 by opening and closing the valve. These arrangements enable an efficient injection and aspiration in the same device by means of only a few inexpensive components. The same valve 6 used for pulse formation can also be used for selecting a suitable micromanipulator in the multimanipulator system. All the valves 2, 4, 6, 8, 9 of the system are advantageously the same type of 3/2 solenoid valves (when a 2/2 valve is
required, one connection is closed). Because the components have several functions, and the same components can be used in different functions, the solution is very simple and cost-effective.

By means of the solution according to figure 4, the above-mentioned different pressure ranges are reached by selecting suitable components. For example, the following kinds of components have been used:

- an electro-pneumatic converter 1; positive pressure range of output 0 to 30 kPa, repeatability and resolution 0.5 kPa, linearity of the component advantageous
- a positive pressure sensor; measurement range 0 to 30 kPa (pressure difference), precision and resolution 300 Pa or better
- a solenoid valve 2; operation range 0 to 30 kPa, operation time not critical
- a negative pressure former 3; repeatability below 100 Pa, linearity of the component advantageous, adjustable negative pressure of 100 Pa and preferably lower
- a negative pressure sensor; measurement range 0 to 5 kPa (pressure difference), precision 30 and resolution 15 Pa or better
- a solenoid valve 8, 9; operation range 0 to 700 kPa, operation time not critical
- a solenoid valve 6 (bulging valve); operation range 0 to 700 kPa, operation time 5 ms
- a filter 5; 0.01 μm particle filter

By combining, in various ways, the modes and structures disclosed in connection with the different embodiments of the invention presented above, it is possible to produce various embodiments of the invention in accordance with the spirit of the invention. Therefore, the above-presented examples must not be interpreted as restrictive to the invention, but the embodiments of the invention may be freely varied within the scope of the inventive features presented in the claims hereinbelow.
Claims:

1. A pressure system of an injection device of living cells, by means of which system it is possible to produce different pressure levels, which injection device comprises a pressure source for pressurizing a medium and a capillary (7), and the pressure system comprises
   - input (IN) of pressurized medium,
   - output (OUT) for connecting to the capillary (7),
   - means (2, 8, 9) for directing positive pressure to the output (OUT), characterized in that the system in addition comprises
     - means (3, 13) for forming negative pressure by using positive pressure and
     - means (2, 4) for directing negative pressure to the output (OUT).

2. The pressure system according to claim 1, characterized in that in addition the system comprises means (1) for forming a second positive pressure from the first positive pressure by reducing pressure, which first positive pressure is received from the input (IN).

3. The pressure system according to claim 1 or 2, characterized in that the system in addition comprises a pulsing valve (6), which is arranged to control the effective time of the pressure affecting the output (OUT).

4. The pressure system according to any of the preceding claims, characterized in that the means (3, 13) forming the negative pressure is at least one of the following: an ejector, a blower, a pump.

5. The pressure system according to any of the preceding claims, characterized in that the pressure system is arranged to produce pressure to a gas, by means of which it is possible to convey pressure to liquid via an interface located in a capillary pipe (7).

6. A method for pressurizing a medium into different pressures in an injection device of living cells, which comprises a pressure source for pressurizing the medium and a capillary,
in which method:
- pressurized medium is conveyed to the input (IN)
- the pressure of the medium is changed
- the medium is conveyed further to the output (OUT)

characterized in that
a negative pressure medium is formed with the positive pressure medium, and either positive pressure or negative pressure is conveyed to the output (OUT).

7. The method according to claim 6, characterized in that a second positive pressure is formed from the first positive pressure by reducing the pressure of the medium, which first positive pressure is received from the input (IN).

8. The method according to claim 7, characterized in that the negative pressure medium is formed with a medium in the second positive pressure.

9. The method according to any of the preceding claims 6 to 8, characterized in that the duration of the positive pressure or negative pressure conveyed to the output (OUT) is adjusted with a pulsing valve (6).

10. The method according to any of the preceding claims 6 to 9, characterized in that the negative pressure is formed with a means (3, 13) that is one of the following: an ejector, a blower, a pump.
Fig. 3

Fig. 4
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

See extra sheet
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)

IPC8: C12M, C12N, C12Q, G01N, B01L, A61D

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

FI, SE, DK, NO

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

EPO-Internal, WPI, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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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
  "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

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Form PCT/ISA/210 (second sheet) (April 2005)
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CLASSIFICATION OF SUBJECT MATTER

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