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(54) **CELL DISPENSING SYSTEM AND METHOD**

VORRICHTUNG ZUR AUSGABE VON ZELLEN UND VERFAHREN

APPAREIL POUR DISTRIBUER DES CELLULES ET PROCÉDÉ

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Description

[0001] The present invention relates to the technical field of biological cell cultures and in vitro-tissues and provides means and methods for dispensing a suspension of biological cells for culture.

[0002] For the preparation of novel in vitro tissues from biological cells, in particular mammalian cells, cells are obtained, for example, from donor tissue, such as a biopsy, are then isolated and may be cultured for proliferation, conditioning and/or differentiation. The cells are brought into suspension in a liquid culture medium. The suspension of cells is then dispensed, on culture dishes where the isolated cells are allowed to grow into confluency to finally obtain a novel so called "in vitro" tissue. Particular examples are three-dimensional multi-layered tissues comprising one or more layers of cells. These in vitro tissues closely resemble tissue present in mammalian skin. For example, A model of the mammalian epidermis comprises layers of keratinocytes; a so called "3D skin equivalent" basically includes a first layer of keratinocytes and a second layer of fibroblast cells embedded in a collagenous biomatrix.

[0003] It is an object to accomplish a fully automated production process, from the isolation of cells to the seeding of isolated cells to grow and eventually to become a new tissue in a highly standardized, reproducible, and not least cost efficient fashion.

[0004] A first critical aspect in the preparation of such in vitro tissues is the process of initial dispending (or seeding) of isolated cells, in particular of isolated keratinocytes, onto culture dishes. For reliable and constant quality of the tissues produced it is required that the isolated cells be dispensed and seeded in substantially the same amount (aliquot) to each culture dish. It is also required that cells are seeded as a high-density suspension to ensure a high number of seeded cells in each culture dish. For that, high-density suspensions of cells shall be present in a feed vessel from which equal fraction or aliquots of the suspension are then transferred to a plurality of culture dishes or other receiving vessels such as multi titer plates (MTP), multi well plates (MWP) or culture inserts therein by repeated action of a transferring pipette comprising either (sterile) disposable pipette tips or a cleanable fixed hollow pipette needle. Aliquotation requires the dispension of virtually the same number of cells to each culture vessel. Known automated pipetting devices for transfer of liquid media are specifically designed to meter equal volumes. It is thus a pre-requisite in automated dispension and aliquotation by means of an automated pipette that each fraction of the suspension to be metered and dispensed is of the same "density", i.e. includes the same number of cells per fraction of volume. To the disadvantage, however, cell suspensions and in particular suspensions with high density have a tendency to settle within a feed vessel as well as, in a certain extent, within the transferring pipette or needle: With the cells following the gravity gradient and/or due to physical and/or biological processes of cell attraction, which results in a higher density of cells at the bottom and a lower density of cells towards the top of the vessel, i.e. an uneven distribution of cells and cell density occurs in a relatively short period of time. During common processes of transfer and aliquotation with an automated pipette the feed stock within a feed vessel thus soon begins to settle to the effect that the number of cells dispensed on a particular receiver vessel depends on the cell density of the suspension present at a particular place and at a particular moment in time where and when it is picked up by the tip of the pipette at the feed vessel.

[0005] A previously known fluid transfer mechanism is described in GB2068115 A. It is thus an object of the present invention to provide means for the automated transfer of cell suspensions from a feed vessel to a plurality of receiver vessels by repeated action over time which provide for a safe aliquotation of the cells, i.e. seeding in equal numbers in each receiver vessel, and avoids the deficiencies noted above.

[0006] This objective is obtained by the present invention as defined in the appended claims. To meet the objective, the present invention concerns a, in particular fully automatically operable, dispensing device for dispensing liquid suspensions of particles, in particular cell suspensions, more particular cell suspensions of high density. The device comprises at least one feed vessel containing the stock suspension and at least one, more particular a plurality of receiver vessels for receiving at least one fraction or aliquot of the stock suspension, and at least one transferring means, in particular a pipetting device including a needle, which is moveable relative to the position of the feed vessel to transfer the at least one fraction or aliquot of the stock suspension to the at least one of or to either one of the plurality of receiver vessels. The device of the present invention is characterized in that it further comprises a novel re-suspending means located at, and in particular fixed relative to the position of the feed vessel, and is specifically designed for ongoing constant re-suspending action on the stock suspension present in the feed vessel, that is a re-suspension of particles present in the liquid suspension.

[0007] The re-suspending means of the invention comprises a hollow needle and/or a tubing, the tip of which is submersed in the suspension of the feed vessel; the hollow needle and/or tubing is in fluid connection with a pump specifically designed for repeated sucking and expelling of a part of this suspension into and from the needle or tubing to effect re-suspension. While the preferably repeated transferring action of cells from the feed vessel to one or more of the receiver vessels can take place by the transferring means, the present invention pertains that at the same time the re-suspending means can constantly exert re-suspending action within the feed vessel coincidently and in parallel.

[0008] In the context of the present invention, the phrase "re-suspension" and similar as used herein define the process or state where a suspension of particles, and

in particular of biological cells, in a liquid medium present in a vessel is brought into or is maintained at a state where its density, i.e. the number of particles or cells at a fraction of the suspension's volume, is constant. A consistent density of the suspension is achieved or maintained for all or most part of the total volume of the suspension, but at least for that part of the volume where the suspension is taken up by the transferring means. The metes and bounds will become obvious for the skilled reader through the detailed description of the invention herein.

[0009] In a preferred embodiment, the transferring means, that is moveable to transfer aliquots of the suspension to receiver vessels is an automated manipulation with at least one axis, more preferred a robot arm, to which an automatic pipette, in particular comprised of at least one hollow needle in fluid connection with at least one pump or metering device for metering the volume of the suspension to be transferred in an embodiment known as such. The needle may be of stainless steel or other apt material known in the art and may be fitted to flexible or rigid tubing which establishes fluid connection to the metering device or pump. Transferring pipette and robot arm may be operated in a commonly known manner for sequential transfer of suspension from the feed vessel to one or more of the receiver vessel. More particular, the transferring means of the invention is specifically designed for automated dipping into the feed vessel, then automated uptake of a metered volume of suspension therefrom, then automated retracting of the pipette, and then automated moving of the pipette upon or into one of the receiver vessels, and then automated releasing of metered suspension (or a fraction thereof) onto or into the receiver vessel, and eventually automated returning of the pipette to the feed vessel for repeated performance of these steps.

[0010] The re-suspending means of the present invention more particular comprises a hollow needle which particularly is fitted to a flexible or rigid tubing in a two-pieced arrangement. The present invention also concerns an alternative single-pieced arrangement where a flexible or rigid or partially rigid tubing itself forms the tip part which, according to the invention, gets into contact with the suspension within the feed vessel without the need of a separate needle. It is thus to be understood that the term "needle" in the context of the present invention as used herein refers to both, a stiff hollow needle, preferably made of stainless steel or the like material, and also to a tubing, which is preferably more or less flexible and preferably comprises a chemically inert compound or plastic material, such as a poly-mere, preferably PE, PTFE, PVA, PET, ABC, and others.

[0011] In a preferred embodiment, the re-suspending needle comprises a tip which is without any or any significant burr and in particular is radiused and/or rounded to avoid such burrs. This may be accomplished in common manner by cutting the needle with a particular trimming process known as such, which avoids burrs in the first place. In a particular embodiment thereof, the tip is thoroughly machined and, alternatively or in addition, polished by an appropriate soft brush, for example, sisal or other soft material to remove burrs eventually present after cutting. The needle's tip of the re-suspending means is preferably free of any sharp or cutting edge such as indentation and serration. This avoids any turbulence that may impose mechanical stress to the cells repeatedly sucked in and expelled from it during the re-suspending action. This is in clear contrast to the design of common needles used in automated pipetting devices, such as the automated transferring means of the device described herein: where burrs or sharp geometrical elements are deliberately foreseen or even specifically designed at the tip in order to accomplish the full emptying of the needle for exact metering of volumes by avoidance of any droplets or remainder material adhering to the tip, which do not form in the presence of such sharp structures. Accordingly, it is held that a common steel needle for a transferring pipette cannot be directly used in or to form part of the re-suspending means to accomplish resuspension according to the present invention. The inventors have surprisingly found that a particularly defined and rounded tip of the needle of the re-suspending means is preferred for most of the biological cell types to be resuspended, albeit particular cell types may exist which are more robust and less prone to mechanical damage.

[0012] The transferring needle of the device of the invention is designed in a common fashion, which particularly includes a conical tip with a sharp tip and a radius increasing towards the shaft. In particular, the tip of the transferring needle exhibits an inner diameter of 0.5 mm increasing to an inner diameter of about 1.5 mm over the needle's length of several millimeters. Accordingly, the outer diameter of the transferring needle also increases given a constant wall thickness. The needle's shape at the tip region is typically conical or tapered. Alternatively or in addition, the tip may comprise a defined shoulder, where the diameter has a sudden rise from the initial approximately 0.5 mm to the final approximately 1.5 mm inner diameter.

[0013] On contrast, the geometry of the needle of the re-suspending means of the present invention resembles a cylindrical shape with a basically constant diameter at least at the tip part of the needle that is in contact with suspension, but alternatively over the full extent of the needle. Accordingly, the present invention preferably provides that at least the tip part the second needle or tubing, which is to be positioned in the suspension is of cylindrical shape. The inventors have surprisingly found that due to the provision of a cylindrical shape any turbulence disadvantageous to the cells is avoided during the re-suspending action. More particular, the re-suspending needle, at least at its cylindrical portion, has an inner diameter from about 1 mm to about 2 mm, more preferred of about 1.5 mm.

[0014] The needle of transferring pipette as well as of the re-suspending means may comprise a polished sur-

face on the outside as well as on the inside to facilitate cleaning of the needle, for example by action of ultrasonic waves and detergent to achieve sterility before and/or after getting into contact with the cell suspension.

[0015] The needle or tubing of the re-suspending means are located within the feed vessel at the position where a steric hindrance or mechanical interference with the pipette tip or needle of the transferring pipette may occur, such that the re-suspending means is able to rest in place within the feed vessel at least during the constant performance of the re-suspension action, while at the same time, the transferring pipette may repeatedly dip into or retract from the same feed vessel to accomplish the transfer.

[0016] In a particular embodiment, the tip of the needle of the re-suspending means is located in close vicinity of the place, where the tip of the transferring pipette takes in the suspension for transfer as described herein. This is meant to better provide for the uptake of an evenly dense suspension into the transferring needle for transfer, because the re-supending means of the invention is designed to confer and maintain a highly even density at least in vicinity to its tip. More particular, the tip of the re-suspending needle is around 3 mm or less apart from the tip of the movable transferring needle at the time the transferring needle is sucking in the suspension for transfer. Accordingly, it is preferred that tip of the first, i.e. transferring needle is designed to be positioned within a distance of from 1 mm to 3 mm of the tip of the second, i.e. re-suspending needle in the feed vessel. In order to achieve a rather intimate contact of the tip of the needle and the tip of the transferring needle within the feed vessel, the re-suspending needle may be introduced and positioned into the feed vessel at an angle relative to the moveable transferring needle, the latter being preferably introduced at or parallel to the long axis of the feed vessel in form of a tube.

[0017] It is also preferred that the tip of the needle of the re-suspending means rests in close vicinity of the bottom of the feed vessel in order to allow for close to complete emptying to minimize the remaining fraction of suspension within the feed vessel which else may have to be discharged.

[0018] While the first, i.e. transferring, needle is moveable, preferably by means of an automatic manipulation or robot arm, the second, i.e. re-suspending, needle is fixed relative to the feed vessel during the despensing process. But, of course, the needle may well be introduced into the feed vessel or retracted therefrom, preferably by means of a robot arm before and after the despensing process, for example to clean the needle and/ or to replace the feed vessel. Alternatively, the feed vessel is moveable relative to the re-suspending needle. Accordingly, the phrase "at the position of the feed vessel" or "fixed relative to the position of the feed vessel" in the context of the present invention means that the re-suspending means, in particular the re-suspending needle, is fixed during that period of time when suspension is transferred to one or more of the receiver vessel and when re-suspending action is ongoing. The phrase thus does not exclude any time period before it and thereafter when the first i.e. re-suspending needle is initially moveable relative to the feed vessel.

[0019] In a particular embodiment, the re-suspending means further includes a self-contained gas bubble which is present within the hollow needle or tubing and serves to separate an operation liquid, i.e. system fluid, present in the needle or tubing downstream towards the pump, from the suspension present upstream thereof at the tip. This gas bubble is specifically designed to dampen and/or separate pressure peaks within the operation fluid from the suspension. In common automated pipetting devices operated through a pump a small gas bubble may be present to seal the operation liquid of the device from the load volume to be taken in, transferred, and expelled from the pipette. There it is preferred that the volume of the gas bubble is as small as possible to allow for intimate coupling of the two liquids to insure the accuracy of volume taken in or dispended from the pipette. Accordingly, the volume of such separating gas bubbles in known automatic pipette devices is about 10 μl or less. On contrast, the present invention pertains to a separating gas bubble within the re-suspending means which is at least one order of magnitude larger than that and preferably has a gas volume from about 250 μl to about 500 μl, preferably of about 300 μl.

[0020] The gas bubble exhibits elastic characteristics due to the compressibility of gas. In connection with frictional forces exerted to the downstream suspension along the walls of the needle and frictions due to turbulence particularly on the tip of the needle and in connection with the mass inertness of the suspension this results in a mechanical low-pass system. Without wishing to be bound to the theory, this functions to dampen or cancel out peak movements, in particular pressure peaks, shock waves and the like, which may be present in the operation fluid, but are detrimental to cells present in the suspension. The dampening characteristics are dependent all of the above factors, among which the size or volume of the gas bubble can be varied in order to adapt the dampening characteristics to the particular set up.

[0021] According to preferred embodiments of the present invention, the receiver vessel or the plurality thereof may comprise a culture flask, one or more culture dishes or alternatively culture tubes. More particular, a plurality of receiver vessels is comprised on a common multi well or multi titer plate containing, for example, 6, 12, 24 identical wells regularly arranged on a common base. In a specific embodiment, the cells in suspension are seeded on the bottom of a well. In an alternative embodiment thereof, the cells are seeded on a membrane of an "insert" placed within a well in order to provide for a floating or air-lift culture of the cells.

[0022] The feed vessel may be a centrifuge tube, which is preferably directly obtained from a preceding centrifugation step for increase of density. The feed vessel pref-

erably comprises a conical or tapered bottom. While this embodiment is preferred, the present invention also concerns alternative embodiments of feed vessels, including common culture flasks or a plurality of feed vessels arranged in particular in a multi well or multi titer plate.

[0023] The present invention preferably avoids and any other known means for suspending or re-suspending of particles or cells in a liquid medium and excludes it from the device. These are particular means that confer re-suspension by thorough stirring or shaking of the vessel containing the suspension, for example, a so called "vortex" system or the like. It is considered that the presence and use of such systems in a device of the invention gives rather uncontrollable or unreliable re-suspending results, is detrimental to the action and efforts of the transferring means of the device to transfer the cells, and/or may cause mechanical damage to the cells in suspension.

[0024] The present invention also pertains to a method for automated dispensing of a suspension, in particular a cell suspension, from at least one feed vessel to a plurality of receiver vessels by a means for transferring at least one aliquot of the suspension utilizing the re-suspending means at the position of the feed vessel as described herein. The method comprises the steps of: (a) transferring one or more aliquot of the suspension from the feed vessel to one or more of the receiving vessels with a first needle, and (b) repeated sucking in and expelling from a second needle or tubing a partial volume of the suspension present in the feed vessel.

[0025] Steps (a) and (b) are performed simultaneously, that is in particular in parallel: preferably, re-suspending action of step (b) is ongoing while the transferring action of step (a) takes place. Step (b) preferably starts shortly before the action of step (a) takes place. Preferably, step (b) is still ongoing and is not stopped before the last aliquot is taken out of the feed vessel by the first needle in step (a).

[0026] Moreover, the method preferably avoids any re-suspending action by the first, i.e. transferring, needle itself by avoiding any single or repeated expulsion of suspension therefrom back to the feed vessel.

[0027] Re-suspension in step (b) is accomplished by repeated suction and expulsion of a partial volume of the suspension present in the feed vessel. Given the mechanical and fluidic characteristics of the suspending medium and of the cells suspended therein, the particular volume for repeated suction and expulsion, the frequency of the repetition and the flow rate of suction and/or expulsion are selected accordingly. According to a preferred embodiment this volume ranges from about 300 μl to about 600 μl and is more particular about 500 μl. Suction and expulsion preferably exhibits a peak flow rate of about 300 to about 500 μl/s and more particular of about 400 μl/s. Surprisingly, these values provide for a safe re-suspending action where the cells are thoroughly and efficiently held in suspension of constant density and, at the same time, are prevented from being damaged due to mechanical stress during the action. The above values are particularly selected for the suspension of keratinocytes and may be adapted to slightly different values for other cell types.

[0028] Preferably, any other known process for suspending or re-suspending of particles or cells in a liquid medium is excluded it from the method of the invention, more particular, thorough stirring or shaking of the feed vessel containing the suspension, in particular at least during action of the transferring needle in step (a).

[0029] The method of the invention preferably forms part of a whole automated process where isolated cells are automatically seeded onto culture vessels for growing tissue cultures. In an initial step of such an automated process cells are obtained, for example from biopsy, and then are isolated. Isolated cells are first cultured in a mono layer culture and are then presented in suspension. In order to achieve high numbers of cells to be seeded onto the culture dishes for tissue production, the process may include at least one amplification step where the density of the cells in suspension is increased by centrifugation. A high density cell suspension may be obtained in the bottom part of a centrifuge tube. The cell free supernatant obtained may be separated off and some fresh culture medium may be added. Then the re-suspending action according to step (b) may be started and kept ongoing while aliquots of the suspension are automatically transferred to a plurality of culture dishes according to step (a), simultaneously and in parallel.

[0030] The seeding of the cells onto the culture dishes by means of the first, i.e. transferring, needle may be performed as known as such, and its manner of metering may be adapted to the particular type of receiver vessel, i.e. either culture flask, culture dish, multi well plate or insert therein. In a particular embodiment, the tip of the transferring needle performs additional particular movements during metering of the suspension at the site of the receiver vessel which may include circular, spiral and/or meandering patterns in order to provide for a more even distribution of the cells seeded onto the culture surface. This is of particular importance in connection with the growing of multi-layered, three-dimensional tissues, where the layers shall be of constant thickness throughout most part of the tissue area and requires an even distribution of cell counts seeded per area.

[0031] In a particular embodiment step (a) includes the transfer of a single aliquot each time the transferring needle moves from the feed vessel to one receiver vessel. In an alternative yet preferred approach the method includes that in step (a) the transferring needle takes up a multiplicity of aliquots (n times a single aliquot) at the feed vessel at once and then transfers one single aliquot to each of a number n of receiver vessels - one after the other - before returning to the feed vessel. By that, the process of transfer and aliquotation of the stock suspension present in the feed vessel onto a plurality of receiver vessels may greatly increase in speed. Due to the repeated re-suspending action of step (b) a very even den-

sity suspension is obtained and constantly maintained at the feed vessel. This surprisingly allows for the uptake of a multiplicity of aliquots into the transferring needle at once. Any settling effect in the transferring needle is minimized and thus is ignorable to a considerable extend under practical conditions. This is in contrast to common multi-step pipetting techniques where suspensions of an already uneven density distribution are taken into the pipette.

[0032] The present invention also concerns the use of a re-suspending means as described herein for the purpose of providing, that is in particular establishing and maintaining, an even and constant density of cells in suspension present in a feed vessel.

[0033] More particular, the invention also pertains to a use, wherein the re-suspending second needle or tubing further includes a self-contained gas bubble located within the needle or tubing to dampen and separate pressure peaks present in an operation liquid from the suspension, in particular to avoid mechanical damage to biological cells present in the suspension.

[0034] In the following, illustrative examples of the present invention are provided, which are to be understood as working examples but not to limit the scope of the invention. Nevertheless, one or more of the technical details given within the examples not already disclosed as such in the general description of the invention are to be understood to form part, individually or in concert of particular preferred embodiments of the invention.

Figure 1 depicts a schematic side view of a particular embodiment of the device of the present invention including the re-suspending means comprising a cylindrical hollow needle 10 positioned within a centrifuge tube 50 with a conical bottom part 52 containing a stock of cell suspension 60. The needle 10 is shown here in operation in a schematic cross-sectional view: The tip or downstream part of the needle 10 contains a part of the suspension 62 which sucked into the needle 10 from the stock suspension and to be expelled therefrom. The needle 10 further contains an operation liquid 66 upstream thereof which is in fluid connection with a pump 70 (figures 3 and 4) for repeated sucking and expulsing action.

Located between the downstream suspension 62 and the upstream operation liquid 66 is gas bubble 64, particular comprised of inert gas or air to, firstly, separate or seal the suspension 62 from the operation liquid 66 and, secondly, to provide for mechanical dampening of rapid movement of the suspension 62 present in the operation fluid by means of the compressibility of the gas or air contained in the bubble 64.

Needle 10 rests firmly within arm 30. Downstream of the needle there is a fitting 16 for receiving a flexible tubing 18 (figure 2) to establish the fluid connection to the pump 70 (figures 3 and 4). In this particular embodiment needle 10 is introduced to the centrifuge tube 50 at an angle in order to give way to the transferring means for transfer comprising a transferring needle 20 seated in a robotic arm or system 40. Needle 20 comprises a tapered tip 22 which dives into the suspension 60 and ends in a fitting 26 for receiving a tubing 28 (figure 2). Due to action of arm 40, the transferring needle 20 may be retracted from the tube 50 for transfer to one or more of receiver vessels.

Figure 2 is a view on the setting of figure 1 with the tube 50 not visible. The re-suspending needle 10 has a tip 12 and a shaft part 14. The tip 12 is designed to be located in close vicinity to the tip 22 of the transferring needle 20.

Figure 3 depicts a top view of a complete apparatus for dispensing a stock suspension of cells 60 to a plurality of culture dishes presentation table 80. It includes arm 30 holding the re-suspending needle 10 which is shown here next to a centrifuge tube 50 which serves as the feed vessel to hold the stock suspension 60. In this particular embodiment, arm 30 is connected to a linear motor 32 for initial horizontal movement. A second tube 55 is provided for holding a cleaning solution for cleaning needles 10 and/or 20 before and/or after the dispensing procedure. Robot arm 40 holds the transferring needle 20 and includes slides for horizontal 42 and vertical 44 movement of needle 20. Further included is table 80 for receiving a multi well plate containing the plurality of culture dishes or inserts on which the cell suspension is seeded. In the particular embodiment depicted, table 80 is mounted on slides 82 and 84 for horizontal movement to retract and/or exchange table 80 and hence the multi well plate received thereon from the dispensing apparatus. Also contained are a group of pumps 70 which are in fluid connection with either the re-suspending needle 10 via tubing 18 or the transferring needle 20 via tubing 28. Figure 4 depicts a side view of the apparatus of figure 3.

Claims

1. Dispensing device for dispensing a suspension, comprising:

a feed vessel (50) containing the suspension,
at least one or a plurality of receiver vessel for receiving at least one aliquot of the suspension,
a first hollow needle (20) moveable relative to the position of the feed vessel for transferring aliquots of the suspension to the receiver vessels, and
a second hollow needle (10) or tubing at the position of the feed vessel for constant re-suspending of particles present in the suspension at the

feed vessel, the second hollow needle or tubing being in fluid connection with a pump (70) specifically designed for repeated sucking and expelling suspension into and from that needle.

2. Device according to claim 1, the first hollow needle being in fluid connection with a metering pump specifically designed for metering at least one or multiples of aliquots of the suspension.

3. Device according to any one of claims 1 or 2, the second needle or tubing comprising a self-contained gas bubble located within the needle or tubing to separate an operation liquid located downstream towards the pump from the suspension upstream thereof, wherein the gas bubble is specifically designed to dampen and separate pressure peaks present in the operation liquid from the suspension.

4. Device according to claim 3, wherein the gas bubble within the second needle or tubing has a volume of 250 μl to 500 μl.

5. Device according to any one of the preceding claims, wherein at least the tip part the second needle or tubing which is to be positioned in the suspension is of cylindrical shape and has an inner diameter of from 1 mm to 2 mm.

6. Device according to any one of the preceding claims, the second needle or tubing comprises a tip which is free of burr, indentation or serration.

7. Device according to any one of the preceding claims, wherein the tip of the first needle is designed to be positioned within a distance of 1 mm to 3 mm of the tip of the second needle.

8. Device according to any one of the preceding claims, wherein the receiver vessel is selected from culture flask, culture dish, well of a multi well plate, and a cell culture insert in a well.

9. Method for the transfer of a suspension from feed vessel to one or more of receiver vessels comprising the steps of:

(a) transferring one or more aliquots of the suspension from the feed vessel to one or more of the receiving vessels with a first hollow needle, movable relative to the position of the feed vessel for transferring aliquots of the suspension from the feed vessel to the receiver vessels, and
(b) repeatedly sucking in and expelling from a second hollow needle or tubing at the position of the feed vessel a partial volume of the suspension present in the feed vessel,

wherein step (a) and step (b) are performed simultaneously.

10. Method according to claim 9, wherein in step (a) the first needle is moved relative to the feed vessel while in step (b) a second needle or tubing is kept fixed relative to the feed vessel.

11. Method according to claim 9 or 10, wherein the first needle does not expel suspension at the feed vessel at any time.

12. Method according to any one of claims 9 to 11, wherein step (b) the partial volume comprises from 300 μl to 600 μl at a peak flow rate of the suspension of from 300 μl/s to 500 μl/s.

13. Use of a re-suspending second needle or tubing in the device of any one of claims 1 to 8, for preserving an even density of particles in suspension present in a feed vessel.

14. Use of claim 13, wherein the re-suspending second needle or tubing further includes a self-contained gas bubble located within the needle or tubing to dampen and separate pressure peaks present in an operation liquid from the suspension to avoid mechanical damage to biological cells present in the suspension.

Patentansprüche

1. Dosiervorrichtung zum Dosieren von Suspension, enthaltend:

ein Vorlagengefäß (50), enthaltend die Suspension,
mindestens eines oder mehrere Aufnahmegefäße zur Aufnahme mindestens eines Aliquots der Suspension,
eine erste Hohlnadel (20), welche in Bezug auf den Ort des Vorlagengefäßes bewegbar ist, um Aliquots der Suspension in die Aufnahmegefäße zu überführen, und
eine zweite Hohlnadel (10) oder Röhre am Ort des Vorlagengefäßes für andauerndes Resuspendieren von an dem Vorlagengefäß in der Suspension vorhandenen Partikeln, wobei die zweite Hohlnadel oder Röhre mit einer Pumpe (70), welche spezifisch ausgebildet ist zum wiederholten Einsaugen und Ausstoßen der Suspension in und aus dieser Nadel, in Fluidverbindung steht.

2. Vorrichtung nach Anspruch 1, wobei die erste Hohlnadel mit einer Dosierpumpe, welche spezifisch ausgebildet ist zum Dosieren mindestens eines oder mehrerer Aliquots der Suspension, in Fluidverbin-

dung steht.

3. Vorrichtung nach einem der Ansprüche 1 oder 2, wobei die zweite Nadel oder Röhre eine geschlossene Gasblase enthält, welche sich innerhalb der Nadel oder Röhre befindet, um eine Betriebsflüssigkeit, die stromabwärts, zu der Pumpe hin angeordnet ist, von der stromaufwärts liegenden Suspension trennt, wobei die Gasblase spezifisch ausgebildet ist, um in der Betriebsflüssigkeit vorhandene Druckspitzen zu dämpfen und von der Suspension fernzuhalten.

4. Vorrichtung nach Anspruch 3, wobei die Gasblase in der zweiten Nadel oder Röhre ein Volumen von 250 μl bis 500 μl besitzt.

5. Vorrichtung nach einem der vorstehenden Ansprüche, wobei zumindest der vordere Abschnitt der zweiten Nadel oder Röhre, welche in der Suspension zu positionieren ist, eine zylindrische Form aufweist und einen Innendurchmesser von 1 mm bis 2 mm besitzt.

6. Vorrichtung nach einem der vorstehenden Ansprüche, wobei die zweite Nadel oder Röhre eine Spitze aufweist, welche frei von Graten, Zacken oder Kerben ist.

7. Vorrichtung nach einem der vorstehenden Ansprüche, wobei die Spitze der ersten Nadel so ausgebildet ist, um innerhalb eines Abstands von 1 mm bis 3 mm zu der Spitze der zweiten Nadel positioniert zu sein.

8. Vorrichtung nach einem der vorstehenden Ansprüche, wobei das Aufnahmegefäß ausgewählt ist aus Kulturflasche, Petrischale, Vertiefung einer Multititerplatte und einem Zellkultureinsatz in einer Vertiefung.

9. Verfahren zum Übertragen einer Suspension aus einem Vorlagengefäß in eines oder mehrere Aufnahmegefäße, enthaltend die Schritte:

(a) Übertragen eines oder mehrerer Aliquots der Suspension aus dem Vorlagengefäß in eines oder mehrere der Aufnahmegefäße mit einer ersten Hohlnadel, welche in Bezug auf den Ort des Vorlagengefäßes bewegbar ist, um Aliquots der Suspension aus dem Vorlagengefäß in die Aufnahmegefäße zu übertragen, und
(b) wiederholtes Einsaugen und Ausstoßen einer Teilmenge der Suspension, welche in dem Vorlagengefäß vorhanden ist, in/aus der zweiten Hohlnadel oder Röhre am Ort des Vorlagengefäßes,

wobei Schritt (a) und Schritt (b) gleichzeitig durchgeführt werden.

10. Verfahren nach Anspruch 9, wobei in Schritt (a) die erste Nadel in Bezug auf das Vorlagengefäß bewegt wird, während in Schritt (b) eine zweite Nadel oder Röhre in Bezug auf das Vorlagengefäß ortsfest gehalten wird.

11. Verfahren nach Anspruch 9 oder 10, wobei die erste Nadel an dem Vorlagengefäß zu keinem Zeitpunkt Suspension ausstößt.

12. Verfahren nach einem der Ansprüche 9 bis 11, wobei in Schritt (b) die Teilmenge von 300 μl bis 600 μl bei einer maximalen Flussrate der Suspension von 300 μl/s bis 500 μl/s beträgt.

13. Verwendung einer resuspendierenden zweiten Nadel oder Röhre in der Vorrichtung nach einem der Ansprüche 1 bis 8, um eine gleichmäßige Dichte von Partikeln in einer Suspension, welche in einem Vorlagengefäß enthalten ist, aufrecht zu erhalten.

14. Verwendung nach Anspruch 13, wobei die resuspendierende zweite Nadel oder Röhre zusätzlich innerhalb der Nadel oder Röhre eine abgeschlossene Gasblase enthält, um in einer Betriebsflüssigkeit vorhandene Druckspritzen zu dämpfen und von der Suspension fernzuhalten, um mechanische Schäden von in der Suspension vorhandenen biologischen Zellen zu verhindern.

Revendications

1. Dispositif de dispension pour dispenser une suspension, le dispositif comprenant :

une cuve d'alimentation (50) contenant la suspension,
au moins un ou une pluralité des cuves réceptrices pour recevoir au moins une partie aliquote de la suspension,
une première aiguille creuse (20), mobile par rapport à la position de la cuve d'alimentation pour transférer des parties aliquotes de la suspension aux cuves réceptrices, et
une seconde aiguille creuse (10) ou tube à la position de la cuve d'alimentation pour constamment remettre en suspension des particules présentes dans la suspension au niveau de la cuve d'alimentation, la deuxième aiguille creuse ou tube étant en connexion fluidique avec une pompe (70) spécifiquement conçue pour aspirer et expulser de façon répétée la suspension dans et de cette aiguille.

2. Dispositif selon la revendication 1, dans lequel la pre-

mière aiguille creuse est en connexion fluidique avec une pompe de dosage spécifiquement conçue pour doser une ou plusieurs parties aliquotes de la suspension.

3. Dispositif selon l'une quelconque des revendications 1 ou 2, dans lequel la seconde aiguille ou tube comprend une bulle de gaz autonome située à l'intérieur de l'aiguille ou du tube pour séparer un liquide de travail, situé en aval vers la pompe, de la suspension en amont de celui-ci, la bulle de gaz étant spécifiquement conçue pour amortir et séparer les pics de pression présents dans le liquide de travail de la suspension.

4. Dispositif selon la revendication 3, dans lequel la bulle de gaz dans la seconde aiguille ou le tube a un volume de 250 µl à 500 µl.

5. Dispositif selon l'une quelconque des revendications précédentes, dans lequel au moins la partie de pointe de la seconde aiguille ou du tube qui doit être positionnée dans la suspension présente une forme cylindrique et a un diamètre intérieur de 1 mm à 2 mm.

6. Dispositif selon l'une quelconque des revendications précédentes, dans lequel la seconde aiguille comprend une pointe exempte de bavures, d'indentations ou de dentelures.

7. Dispositif selon l'une quelconque des revendications précédentes, dans lequel la pointe de la première aiguille est conçue pour être positionnée à une distance de 1 mm à 3 mm de la pointe de la seconde aiguille.

8. Dispositif selon l'une quelconque des revendications précédentes, dans lequel la cuve réceptrice est choisie parmi un flacon de culture, un plat de culture, un puits d'une plaque multi-puits et un insert de culture cellulaire dans un puits.

9. Procédé pour le transfert d'une suspension à partir d'une cuve d'alimentation à une ou plusieurs cuves réceptrices, le procédé comprenant les étapes consistant à :

(a) transférer une ou plusieurs parties aliquotes de la suspension à partir de la cuve d'alimentation à une ou plusieurs des cuves réceptrices avec une première aiguille, mobile par rapport à la position de la cuve d'alimentation pour transférer des parties aliquotes de la suspension à partir de la cuve d'alimentation aux cuves réceptrices, et
(b) aspirer et expulser de façon répétée un volume partiel de la suspension présente dans la cuve d'alimentation dans et d'une seconde aiguille ou tube à la position de la cuve d'alimentation,

dans lequel l'étape (a) et l'étape (b) sont effectuées simultanément.

10. Procédé selon la revendication 9, dans lequel la première aiguille est déplacée par rapport à la cuve d'alimentation dans l'étape (a), lorsqu'une seconde aiguille ou tube est maintenu(e) fixe par rapport à la cuve d'alimentation dans l'étape (b).

11. Procédé selon la revendication 9 ou 10, dans lequel la première aiguille n'expulse pas de suspension au niveau de la cuve d'alimentation à aucun moment.

12. Procédé selon l'une quelconque des revendications 9 à 11, dans lequel le volume partiel dans l'étape (b) comprend de 300 µl à 600 µl à un pic de débit de la suspension de 300 µl/s à 500 µl/s.

13. Utilisation d'une seconde aiguille ou tube de resuspension dans le dispositif selon l'une quelconque des revendications 1 à 8, pour maintenir une densité uniforme des particules en suspension présente dans une cuve d'alimentation.

14. Utilisation selon la revendication 13, dans lequel la seconde aiguille ou le tube de resuspension en outre comprend une bulle de gaz autonome située à l'intérieur de l'aiguille ou du tube pour amortir et séparer de la suspension les pics de pression présents dans un liquide de travail, pour éviter d'endommager mécaniquement les cellules biologiques présentes dans la suspension.

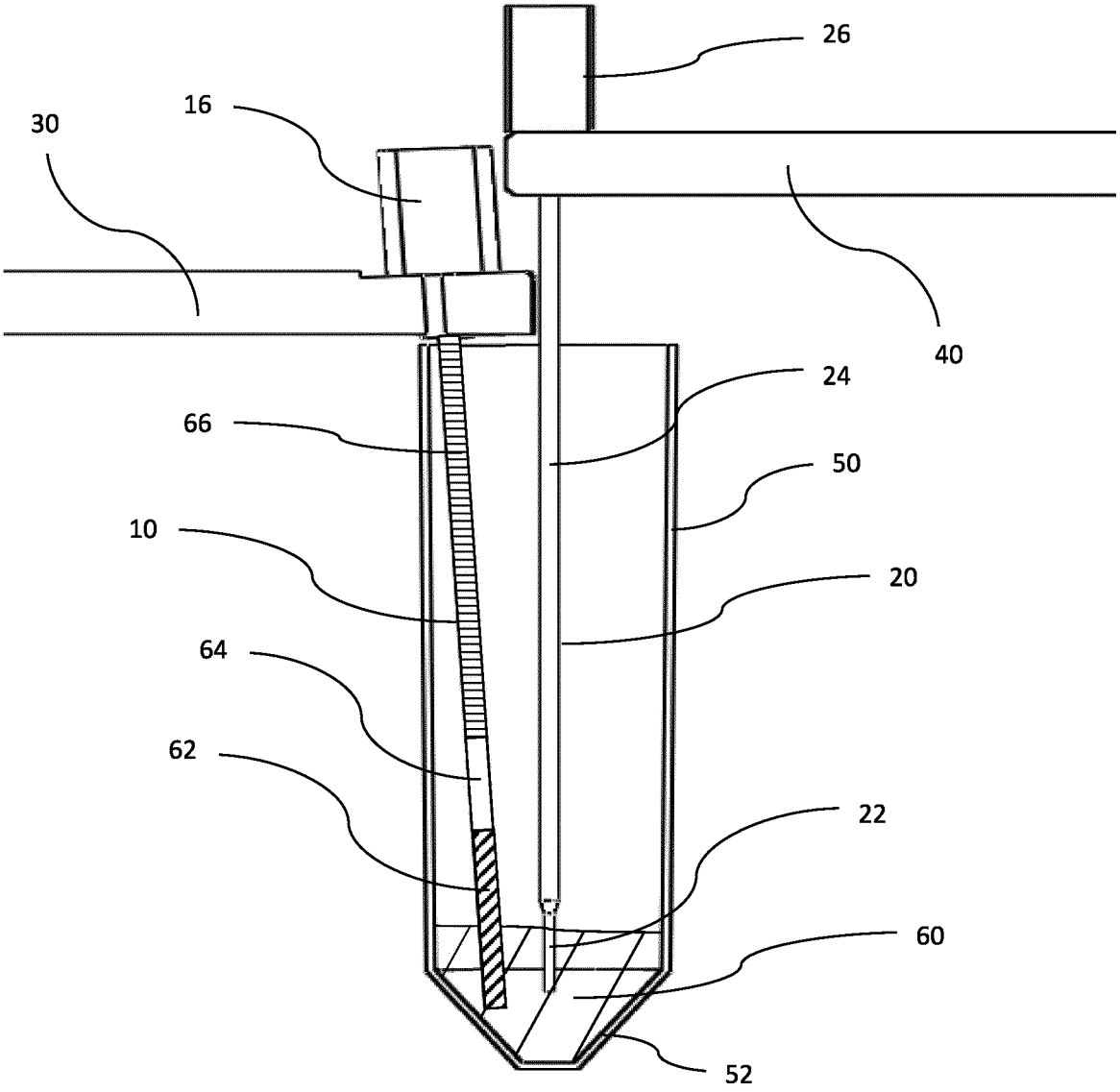

Fig. 1

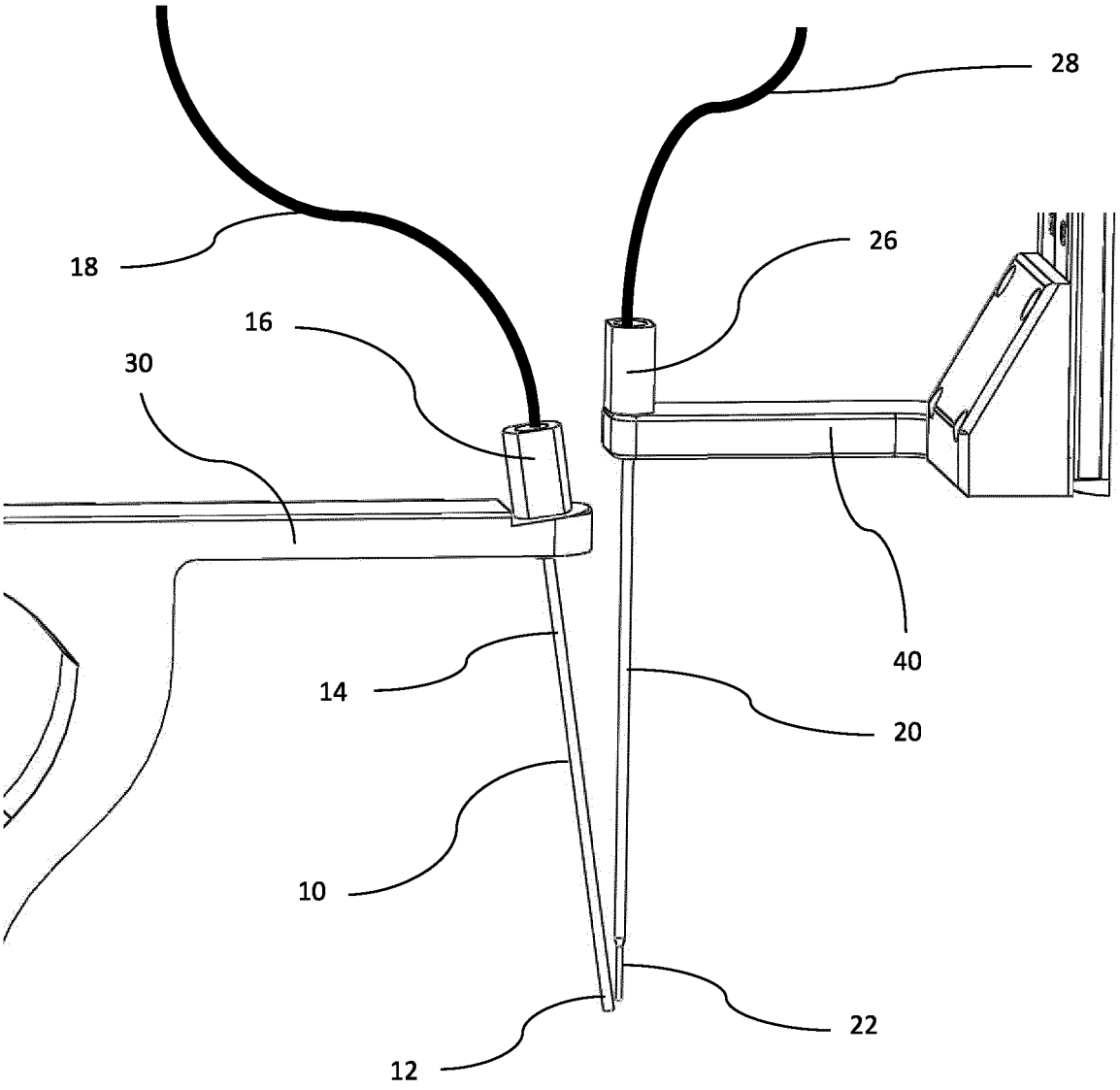

Fig. 2

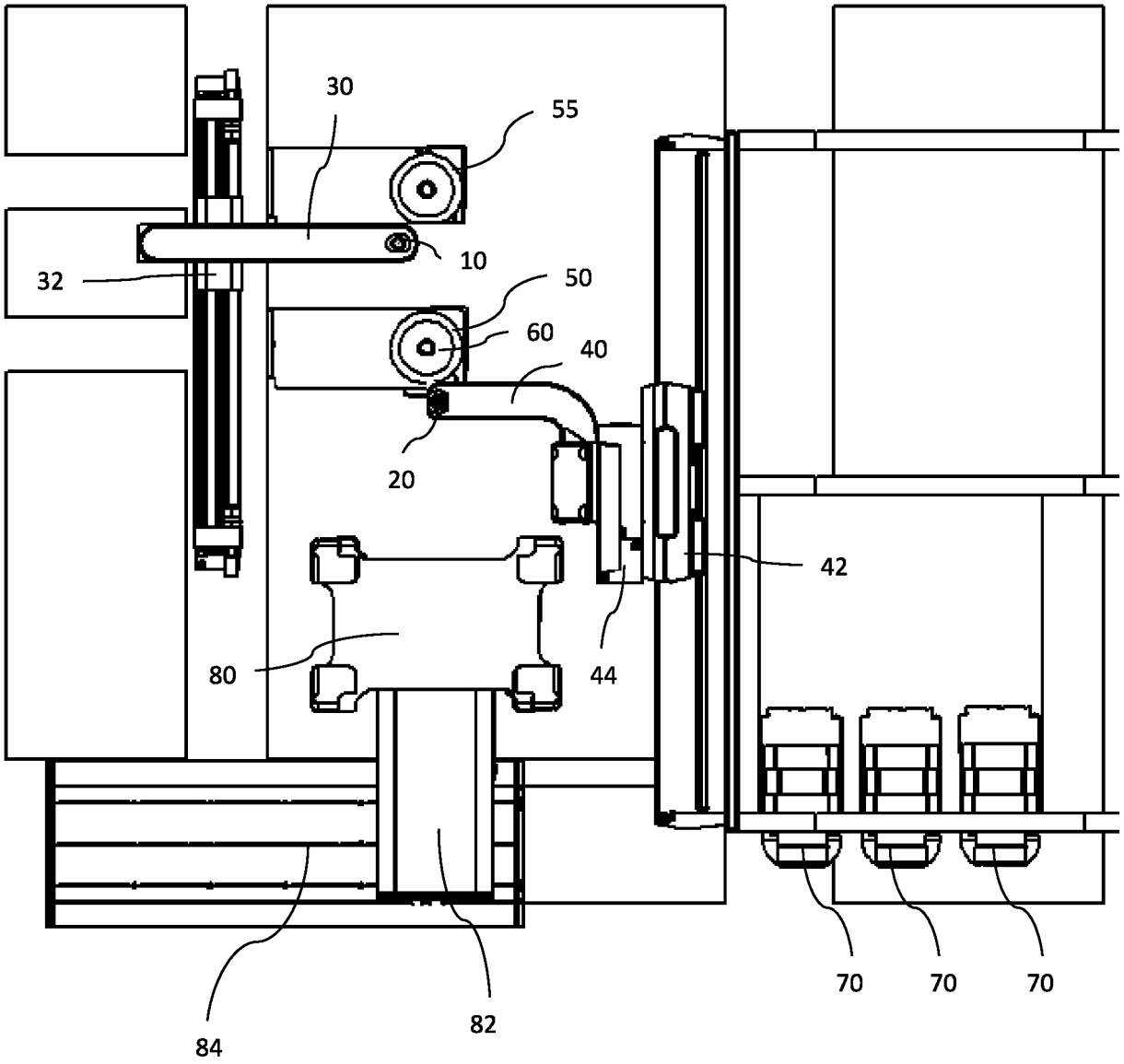

Fig. 3

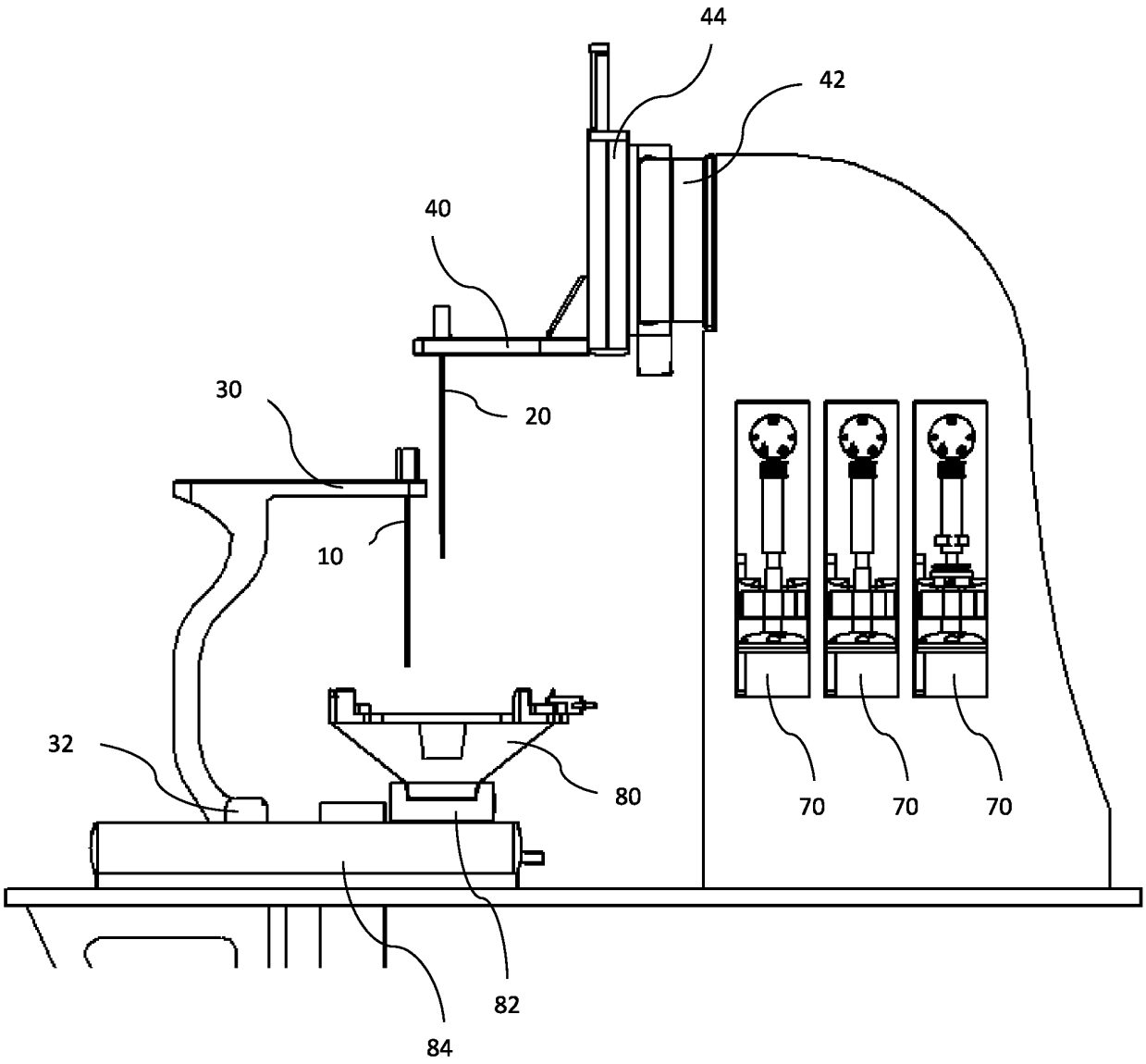

Fig. 4

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

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