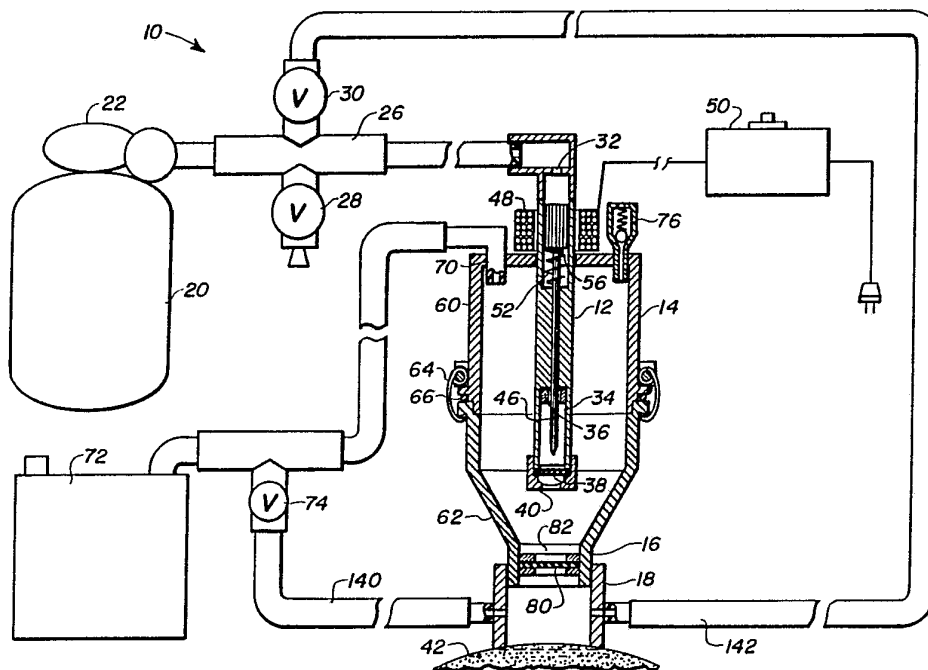




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(54) Title: IMPROVED METHOD AND APPARATUS FOR INTRODUCING BIOLOGICAL SUBSTANCES INTO LIVING CELLS



(57) Abstract

A process is described which uses a "cold" gas shock to accelerate microprojectiles, wherein particles are presented to the gas shock on a planar surface perpendicular to the plane of expansion of the gas shock wave. Several different apparatus capable of accomplishing this method are described.

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TITLEIMPROVED METHOD AND APPARATUS FOR INTRODUCING
BIOLOGICAL SUBSTANCES INTO LIVING CELLS5 Field of the Invention

This invention relates to an improved method and apparatus for introducing biological substances into living cells and/or tissues.

Background of the Invention

10 The transformation of living cells by bombardment with biological substances such as heterologous DNA or RNA is described in a patent application entitled Method For Transporting Substances Into Living Cells and Tissues and Apparatus Therefor, by Sanford et al., USSN
15 06/670,771, filed November 13, 1984. An improved apparatus for delivering substances into cells and tissue is described in a patent application filed by Sanford et al. entitled "Biolistic Apparatus for
20 Delivering Substances into Cells and Tissues in a Non-Lethal Manner," USSN 07/161,807 filed February 29, 1988, also by Sanford et al. The process described involves accelerating particles of an appropriate size to high velocities sufficient to penetrate cell walls and/or cell membranes thereby entering into the cell's
25 cytoplasm, nucleus, or organelles. If the particles are carrying biological substances and the velocity is sufficient to penetrate the cell wall without destroying the cell, the biological substances are introduced into the cell.

30 In this process, holes formed in the cell membrane need not be any larger than would be achieved using microinjection procedures and need only remain open for a fraction of a second. Biological substances such as RNA and DNA may be carried into the cells by a variety
35 of mechanisms such as precipitating the DNA onto the

surface of inert particles such as tungsten spheres, latex beads or ferrite crystals as is described by Sanford et al. DNA in a liquid carrier may be used as well or DNA in solid form may constitute the particles themselves. This process is particularly advantageous because large numbers of cells can be bombarded simultaneously and does not require the manipulation of individual cells.

This process is also described by Sanford et al. in J. Part. Sci. and Tech. 5:27-37 (1987) and is summarized in a recent review by Sanford in Trends in Biotechnology 6:229-302 (1988). The biolistic delivery of DNA into onion cells is described by Klein et al. in Nature 327:70-73 (1987). The biolistic delivery into corn cells is described by Klein et al. in Proc. Natl. Acad. Sci. 85:4305-4309 (1988) and Biotechnology 6:559-563 (1988). The transformations of microbes and organelles was first shown by Johnston et al. in Science 240:1538-1541 (1988) and Boynton et al. in Science 240:1534-1538 (1988) and Armeleo in Current Genetics 17:97-103 (1990). Organelle transformation was further elucidated by Fox et al. in PNAS 85:7288-7292 (1988), Blowers et al. The Plant Cell 1:123-132 (1989) and Daniell et al. PNAS (in press) (1989).

The utility of the process for higher plant transformation has been reviewed by Sanford Physiologia Plantarum (in press) (1989) and has been further demonstrated in numerous papers. Further the transformation of higher animal cells using this bombardment technique has been demonstrated in vitro in Zelenin et al. FEBS Letters 244: 65-67 (1989) Johnston et al. (in preparation) and Williams et al. Nature (submitted) (1990) and most recently in vivo in Johnston et al. (in preparation) Zelenin et al. (in preparation).

Based on these early teachings, various apparatus have been constructed to effect the delivery of particles carrying biological substances.

The apparatus described by Sanford et al. is
5 commercially available from E. I. du Pont de Nemours and Company, Wilmington, DE in the form of a gunpowder driven device in which the hot gasses generated by a gunpowder discharge form a hot gas shock wave which accelerate a macroprojectile carrying many tiny
10 microprojectiles. When the macroprojectile strikes a stopping plate having a hole therein, the microprojectiles continue their travel, striking the target cells. Morikawa et al. Appl. Microbiol. Biotechnol. 31:320-322 (1989) have constructed a device
15 based on the teachings of the Sanford et al. that uses pressurized nitrogen to drive the macroprojectile. Agracetus in European patent application 8731062.4 filed December 2, 1987 (publication number 0270356) describe a
20 macroprojectile accelerator. When the macroprojectile is a thin disk, it is accelerated by the use of a very high voltage discharge, which vaporizes a droplet of water, creating a hot gas which drives the projectile similar to the hot gases generated by the gunpowder discharge.

25 Dr. Laurie Mets has built a particle accelerator which is based on the same principle described in the Sanford et al. patent application, namely, gas flow entrainment of the particles. Finally Oard et al. (1990) achieved transient gene expression in maize, rice
30 and wheat cells using compressed air to accelerate a cylindrical macroprojectile. Plant Physiology 92:334-339 describes the use of such an airgun for propelling DNA coated microprojectiles. A polycarbonate vacuum chamber encloses the airgun muzzle and target material.

While these various apparatus represent various approaches to the problem of how best to accelerate microprojectiles for transferring biological materials into cells and tissues, they all suffer from one or more deficiencies. First of all, many of the apparatus constructed are not flexible as to mode of use. There are a wide array of applications for the ballistic process, each calling for different capabilities, settings, configurations, etc. The existing apparatus tend to be optimal for a single mode of use or for a single application but are not adaptable to plural needs. Secondly, the apparatus available generally does not provide the degree of repeatable results, target-to-target and day-by-day, that is desired. Existing accelerators have a high degree of variability in performance. Also the particle dispersion pattern is poor and not uniform over the target area. Existing apparatus often does not effectively break up particle aggregates and does not allow control over how large an area over which the particles are dispersed.

Most of the existing apparatus is bulky, generally immobile and generally cannot be handheld such as is needed for some veterinary or medical applications. In this connection, most of the apparatus that are available requires that the targets be placed inside a vacuum chamber and are not suited for use on targets larger than such chamber. Whatever the target used, there is a tendency for the accelerator apparatus to damage (or kill) a certain number of the cells, which impairs cell division or cell differentiation. This is especially true when the distance to the target needs to be short, as in medical applications. It is desirable therefor to have better velocity control, less gas blast, less acoustic shock, less high velocity debris, less heat, and less radiant energy.

Apparatus which employs gunpowder or high voltage discharges tend to generate high temperatures. Furthermore, high voltage discharges generate a blinding flash which may generate ultraviolet light and other forms of ionizing radiation. Such may be harmful to the cells being transformed, or the DNA being delivered.

SUMMARY OF THE INVENTION

Many of the deficiencies of the prior art acceleration methods for introducing particles carrying biological materials into a target of cells and/or tissue are overcome utilizing the method of this invention. Such method comprises the steps of accelerating the particles sufficiently to cause them to penetrate the surface of the cells and/or tissues and become incorporated into the interior of the cells and/or tissue without killing the cells and/or tissues and effecting the particle acceleration by subjecting the particles to the force of a gas shock wave generated from an ambient temperature gas (hereinafter referred to as a "cold" gas shock wave). The method for particle acceleration includes positioning the particles on the target side of a planar surface and subjecting the surface to the cold gas shock wave, thereby to accelerate the surface and hence the particles. If the planar surface is a mesh, the particles are optionally entrained into the shock wave. If the sheet is solid and is fixedly secured at its edges, the cold gas shock wave causes distention but not rupture of the membrane thereby launching particles while optionally protecting the target from the shock wave.

Alternatively, if the membrane is fixed and allowed to rupture, the particles are dispersed over a wider region of the target cells. Or, the biological materials may be dispersed on a screen in the path of

the gas shock wave. If a restraining screen is positioned between the target cells and a solid, unfixed membrane (a flying disk) flight of the disk will be restrained after a short distance, allowing the
5 particles to be launched and pass through the screen to the target.

Using the various methods set forth above provides a number of alternatives which may be utilized according to the need. For example, the use of a "flying disk"
10 provides protection of the target as well as very high particle speeds. When the particles are simply entrained in the shock wave, the damage to the cells and tissue can be more severe, but velocities may be highest. On the other hand with the use of a distended
15 membrane whose distention is limited by a screen, least cell damage occurs. The rupturable membrane achieves a better dispersion of the particles but tends to increase somewhat the cell damage.

An apparatus for performing the method of this
20 invention includes a closed housing capable of sustaining a vacuum, the housing having a first port for applying a vacuum thereto, a major axis, and a second port lying on the axis, and defining a throat portion opposite the second port encompassing the axis, a high
25 pressure chamber positioned in the second port and adapted to catastrophically release gas stored therein to provide a gas shock wave directed toward the throat portion, and means for positioning the particles in the throat portion for acceleration by the force of a shock
30 wave toward the target. An optional interface, secured to the throat portion, may be provided for coupling the throat portion to the target, when large targets are employed, or where target's exposure to vacuum and/or shock is to be limited. The invention provides
35 flexibility for positioning and launching the particles

in the throat portion, each configuration having various advantages and disadvantages.

In short, the apparatus can be seen to have five parts (a) a high pressure gas delivery system; (b) a
5 mechanism to generate an instantaneous gas shock out of the high pressure system; (c) an enclosure into which the gas shock is released, contained and vented; and (d) a throat region which allows for interchangeable inserts which translate the gas shock into microprojectile
10 acceleration, utilizing the force of the gas shock by several diverse mechanisms. Finally, an interchangeable interface mechanism may be used for different types of biological targets ranging from small animals or plants to large animals or plants, to cells contained in a
15 petri dish, etc.

Brief Description of the Drawings

The invention will be better understood with reference to the attached drawings in which:

20 Fig. 1 is a diagrammatic view of a system including an apparatus for introducing particles carrying biological materials into a target of cells and/or tissue constructed in accordance with this invention;

25 Fig. 2 is a cross section exploded view of the details of the apparatus of Fig. 1;

Fig. 3 is an alternative construction of the apparatus of Fig. 1 which is particularly useful as a handheld device;

30 Fig. 4 is an alternative construction for the throat portion of the particle accelerator of Fig. 1;

Figs. 5a and 5b are cross sectional representations of the throat portion of the particle accelerator depicting initial and operated conditions for one embodiment of the particle accelerator constructed to
35 operate in a gas entrainment mode;

5 Figs. 6a and 6b are cross sectional representations of the throat portion of the particle accelerator when constructed to operate as a fixed membrane;

10 Figs. 7a and 7b depict a cross sectional representation of the throat portion of the particle accelerator of this invention when constructed to operate as a captured membrane showing both the initial and operated conditions;

15 Figs. 8a, 8b, and 8c are cross sectional representations of the throat portion of the particle accelerator depicting the initial, intermediate and operated conditions of the particle accelerator when constructed to operate as in a ruptured membrane mode;

20 Figs. 9a, 9b, and 9c are cross sectional representations of the throat portion of the particle accelerator of this invention constructed to operate as a flying disk and showing the initial, intermediate and operated conditions;

25 Fig. 10 depicts three alternative arrangements of the nozzle portion of the particle accelerator of Fig. 1;

Fig. 11 is a cross sectional representation of an alternative embodiment for the nozzle portion of the particle accelerator of Fig. 1;

30 Fig. 12 is a cross sectional representation of a particle accelerator constructed in accordance with an alternative embodiment of this invention that is particularly adopted for use with large targets;

Fig. 13 is a cross sectional view of a baffle used in the accelerator of Fig. 12 taken along the section line 13-13; and

Fig. 14, 14a, and 14b are additional cross sectional views of an alternative embodiment of the invention.

Detailed Description of the Preferred Embodiment

With reference to Figs. 1 and 2, the apparatus of the invention may be seen to comprise five parts: (a) a high pressure gas delivery system 10; (b) a shock mechanism 12 to generate an instantaneous gas shock wave out of the high pressure system; (c) an enclosure or housing 14 into which the gas shock wave is released, contained and vented; (d) a throat region 16 of the device, which allows for interchangeable inserts, which translates the gas shock wave into microprojectile acceleration by diverse mechanisms; and (e) an optional interchangeable nozzle or interface mechanism 18 for targeting different types of biological targets.

The high pressure gas delivery system includes a source of gas 20 under high pressure. Typically the gas may be helium because of its light weight and characteristic of having a high speed of expansion. Other preferably inert gases may be used if desired such as nitrogen. Also air, hydrogen, etc. may be used. Gas source 20 is provided with a suitable regulator 22 and pressure indicator 24 and is coupled through suitable tubing 26 to the chamber 12 for generating the instantaneous gas shock wave. A bleed valve 28 and shutoff valve 30 are used. The valve 30 couples the source of gas 20 to the nozzle or interface 18 as will be described.

The shock generating system 12, the details of which are seen more clearly in Fig. 2, receives gas from the tubing 26 through a constriction 32 which serves to limit the flow rate of gas, thereby stabilizing the rupture-activating mechanism and preventing premature firing before the desired pressure is reached. It serves as a gas shock generating system. All of the fittings of the chamber 12, housing 14, throat inserts 18, interface 18, unless otherwise specified, may be

made of any suitable material capable of withstanding high pressures or vacuums, such as brass or stainless steel. The constriction 32 also serves to limit the flow of gas through the system after firing in the event
5 that other closure mechanisms fail. Because of the constriction, it takes some seconds to pressurize a high pressure chamber 34 contained in the lower portion of the shock generating system 12.

A valve which can reproducibly be cracked open
10 could replace the constriction, allowing adjustment of the flow rate through the constriction point. The dimensions of the pressurized gas chamber 34 within the shock generating system 12 should be sufficiently large to contain enough gas to allow generation of a powerful
15 gas shock wave but small enough to limit the amount of gas that needs to be vented and which will contribute to the gas impacting onto the biological target. Essentially this chamber area is denoted by the numeral 34. The volume of the chamber 34 may be adjusted by a
20 threaded sleeve insert 36 which may be sized as desired.

The gas shock wave generating mechanism 12 is designed to provide a very sharply defined pressure front which needs to expand freely into an area of relatively low pressure. Release mechanisms suitable
25 for generating the appropriate gas shock wave must open very rapidly. This can be accomplished either by the instantaneous rupture of a membrane, or by the use of a special very fast valve. Due to the lack of
30 availability of truly fast, high pressure valves the embodiment depicted in Figs. 1 and 2 is preferred which includes a rupturable membrane 38. The membrane is held by an endcap 40 (Fig. 2) threadingly engaged to the cylinder comprising shock generating system 12. The
35 endcap 40 is open at its central portion to allow for the gas to escape downwardly in the direction of a

target 42 as will be described. The membrane may be any suitable rupturable material such as Kapton® polyimide films or Mylar® polyester films. The characteristics of these type materials will be described below. The use
5 of five 2 mil thick Kapton® membranes has been used successfully (as an example four-five layers of 2 mil thick Kapton® membrane will contain 1200 psi of helium). The cap 40 has a frictional compression seal (not shown). Under this arrangement the pressurized
10 membranes are significantly deformed outwardly but do not rupture spontaneously. Alternatively, a weaker membrane or higher pressures can be used to achieve spontaneous rupture.

In order to assure a sudden rupture of the membrane
15 38, which must be catastrophic, an active rupture mechanism can be employed in the form of a rod 46 which passes actually through a bore in the center of the shock system 12. The rod 46 has a sharp point which ruptures the membrane from within the pressure chamber
20 36 such that there is no interference with the free expansion of the gas shock wave outwardly.

The high pressure tube of the shock system can be formed to have a bore or constriction in a central portion with a wider bore at both ends. The rod 46
25 extends through the constricted portion but may be either hollow or fluted (not shown) such that the gas can pass by it into the lower pressure chamber region 34. At the upper end (nearest the gas source) the rod 46 is wider (of larger diameter) such that upon firing
30 it is captured and cannot fly down through the constricted region. In this wider end region, the rod is made of a magnetically responsive metal and can be driven downwardly by the use of a solenoid 48 actuated by an electrical energizing mechanism 50. The high
35 pressure tube that forms the shock system 12 is made of

a magnetically non-responsive material such as brass as mentioned. A spring 54 between the upper enlarged end of the rod 46 and the constricted portion permits or facilitates the return of the rod to its up position at the end of each firing cycle.

When the rod 46 is actuated downwardly by the solenoid 48, its lower other end, which is sharpened, extends down into the pressure chamber 34 and pierces the membrane thereby generating a gas shock wave. At the base of the widened portion of the rod is a ring seal 56 such that, upon firing, the wider portion of the rod seals against the constriction, stopping any subsequent gas from escaping into the lower pressure chamber 34 (which has now been pressure released due to the rupture of the membrane). When the overlying gas pressure is then released, the small spring 52 returns the rod 46 to its original raised position whereby the lower pressure chamber 34 can again be closed off with a new membrane and repressurized.

The gas shock wave which is generated with this mechanism is important because it is unlike the gas shock wave obtained by Sanford et al. with a gunpowder cartridge producing an instantaneous release of heat and shock. In the case of the gas shock system of this invention, the gas shock wave is a gas shock wave generated from a pressurized gas at ambient temperature (hereinafter referred to as a "cold" gas shock wave) which has the advantages that it does not disturb the biological material in the target region either from an ultraviolet light standpoint or from a heat standpoint both of which are undesirable when dealing with biological materials. The gas shock wave itself causes a steep increase in pressure almost amounting to a step function that is propagated through the residual gas. Generally the width of the transmission region over

which the steep increase in pressure occurs is the molecular mean free path, i.e., the path distance a molecule moves before colliding with another molecule. It is the sudden catastrophic increase in pressure that is effective for providing the rapid acceleration of particles within a short distance that is necessary and desirable for this invention. The shock wave is cold. By "cold" it is meant that the gas is cool enough not to cause damage to biological materials of tissue.

10 The best bursts come from spontaneous membrane failures, since they fail at their tensile limit. Such membranes can be made uniformly to rupture spontaneously at a given pressure. In this case all that is needed is the gradual pressurization of the pressure chamber 34 until the critical pressure is reached which ruptures the membrane 38 and creates the gas shock wave which propagates in the direction of the target 42. The membranes may be layered. Each 2 mil Kapton® layer breaks at pressures of 275-300 psi, with the multiple layers being additive. Metal foils may also be used.

20 The gas shock wave which is generated is extremely loud and potentially hazardous to an operator's hearing. Also the gas shock wave tends to become somewhat attenuated in power unless it can expand freely into a partially evacuated space. Furthermore, the particles to be accelerated by the gas shock wave, being of light weight are soon velocity attenuated if they are required to go through a gas-filled region. For these reasons a housing 14 is provided to permit the use of a partial or full vacuum in the region of the gas shock release. The housing is cylindrical and formed of two parts, an upper and a lower part 60 and 62, respectively. The two parts, 60 and 62, are joined by a snap clip 64 and a suitable O-ring 66 to provide a seal between the parts.

30

35 The shock generating system 12 is threadingly engaged

along the major axis 11 of the cylindrical housing 14 and is threadingly engaged at the upper end of the housing so that the system's position may be adjusted vertically within the housing 14. More specifically,
5 the axis 11 of the chamber, as defined by the axis of the rod 46 coincide with the housing axis 11 which passes through the throat 16 and interface 18 to the target 42, all of which lie on or encompass the axis of the chamber to permit the shock wave to progress to the
10 target.

The throat 16 forms the lower portion of the lower half 62 of the housing 14 which is tapered to form a reduced diameter throat portion 16. It is in this region that the energy of the gas shock wave is
15 transferred to the microprojectiles for acceleration to the target. Because this energy transfer can occur by a variety of mechanisms in accordance with this invention, interchangeable inserts are placed in this region depending on the mode of use. The inserts can be
20 threaded and screwed or otherwise fixed in place individually or can be dropped into place in the throat region as preassembled unit as will be described. A typical insert used includes some type of particle carrier 80 held by two ring inserts 82 which are
25 threadingly engaged within the throat 16. The particle carrier 80 may be either a screen, a membrane or a combination thereof as will be described in connection with Figs. 5 through 9, inclusive. As will be
described, a nozzle/interface 18 (Fig. 1) or 18' (Fig.
30 2) may threadedly engage the end of the throat region. The lower end of the interface 18' is flanged inwardly and threaded to receive various attachments as is depicted in Figs. 10 and 11.

With reference to Fig. 4, there is depicted an
35 alternative throat section which includes a removeable

insert 100 that is adapted to slide within the throat section 16' and be supported by the inwardly extending flange 102. The interior of the insert 100 is threaded to accommodate a pair of rings 82'. A small handle 84
5 may be provided at the upper end of the insert for convenience of handling. The lower face of the insert 100 is provided with a O-ring 104 to aid in providing a seal to prevent air leakage around the insert 100. The exterior of the insert 100 and the interior of the
10 throat section 16 are unthreaded to facilitate the slideability of the insert. This removeable insert 100 may be used to facilitate handling of any of the alternative particle acceleration arrangements for the throat section depicted in Figs. 5 through 9, inclusive.

15 The particle acceleration embodiment illustrated in Fig. 6a and 6b is a fixed membrane arrangement for the throat section 16 and includes a strong but resilient material such as Kapton® polyimide films or Mylar® polyester films. The polyimide films are exceptionally
20 strong and thermally resistant. Polyester films have similar properties and maintain its tensile strength over a wide temperature range. Such films have sufficient strength to survive the impact of the gas shock wave. The term "resilient" is used to describe
25 material that is capable of withstanding the shock wave without rupture up to the limit of use required. The membrane 110 is held in place about its periphery by such mechanism as rings 82 which may have a matching groove and ridge (not shown) to facilitate locking the
30 membrane 110 in place. Locking can be accomplished by positioning the rings in the throat region 16 and tightening them down against each other with the membrane 110 between them.

35 Microprojectiles are adhered to the outer surface of the membrane 110 (lower surface in the drawing which

is the surface closest to the target). Thus, when the gas shock wave impacts the membrane 110, it is distended but does not rupture thereby launching the microprojectiles 112 at high speed towards the target 42 (Fig. 1). This embodiment is particularly suited where it is desired to prevent damage from the driving gas impact upon the target. The membrane forms a good seal above the target and isolates it from the shock wave source. The effect of the gas shock wave on the membrane and the subsequent propulsion of the microprojectiles is depicted in Fig. 6b wherein the particles 112 are seen flying to the target.

With reference to Figs. 7a and 7b, another insert embodiment known as the "captured membrane" configuration as contrasted to the "fixed membrane" of Figs. 6a and 6b is depicted. In this case, which is very similar to that illustrated in connection with Fig. 6, a thin spacer ring 122 and a rigid screen 120 preferably made of stainless steel or similar chemically inert, nonreactive, non-toxic material 120 is captured along with the anchored membrane 110 between the rings 82. Upon firing, i.e., creation of the gas shock wave, the fixed membrane 110 distends to nearly its fullest extent, at which point (as controlled by the thickness of the spacer ring 122), it impacts against the rigid screen 120. This sudden stop results in a better release from the surface of the membrane, higher velocities for the microprojectiles and better dispersion and deagglomeration of the microprojectiles 112 than occurs in the fixed membrane embodiment of Fig. 6. This mechanism is effective even if the membrane ruptures, and captures the membrane and any membrane debris, deflecting most of the gas shock away from the target thereby providing a safe, relatively gentle mode of bombardment. A smaller disk or membrane may be

attached to the back of the membrane 110, thereby reinforcing the center of the membrane and reducing its chance of rupture or if ruptures occurs ensuring that it occurs at the perimeter of the smaller membrane reducing any possible direct gas blowthrough onto the target.

The embodiment depicted in Figs. 8a, 8b and 8c, known as the "ruptured membrane" embodiment, utilizes a membrane 110 that is thinner polyimide or polyester films, or is made of weaker material than those heretofore described, (e.g., aluminum foil), such that it ruptures upon impact of the gas shock. At the moment of rupture there is a tremendous lateral shear force across the surface of the membrane, releasing the particles from the surface and deagglomerating them with very high efficiency and dispersing the particles over a relatively wide area. The initial launch of the particles is then enhanced by the flow of the gas shock wave through the region giving the particles still higher velocity. Much of the gas shock wave, however, is still reflected back into the evacuated enclosure (prior to rupture), thereby protecting the target from the worst of the gas shock. This configuration is desirable when a relatively severe shock is tolerable, where higher velocities are needed, and where excellent deagglomeration and dispersion of the microprojectiles over a wide area is needed. If the ruptured membrane generates any flying debris, a capture screen can be placed between the membrane 110 and the target 42.

An alternative embodiment of the invention depicted in Figs. 9a, 9b and 9c, and known as the "flying disk", is depicted. In this instance, the usual membrane 110, with particles 112, that is not rupturable of the type used in the embodiment of Fig. 7, is positioned between two rings 82 and adjusted in height as desired by positioning the rings. In accordance with this

invention, a relatively rigid membrane or disk 130 such as a 2 mil thick polyimide disk is positioned on the lower ring 82 but is not anchored with an overlying ring. It is simply held in place by a lip having a recess, or with a small amount of a suitable adhesive (i.e., a thin film of vacuum grease is sufficient to hold it in place). Other thickeners may be used as desired. A centering ring 132 positions a screen 134 along the axis 11 (Fig. 2) and both are supported by a lower ring 133 threaded in the throat 16. Upon firing, the disk 110 together with the particles 112 positioned on the lower side lifts off its platform and flies down the throat unimpeded until it impacts against a rigid screen 134 which is fixed in place at the bottom of the throat region by a ring support 132. Upon impact, particles are launched as in the case of the captured membrane of Fig. 7.

Potential velocities are higher than with the captured membrane of Fig. 7, although potential for the gas shock wave impacting against the target exists because gas can leak ahead of the disk while it's in flight. By using a flying disk fitted closely to the inside diameter of the region and a relatively short flight distance, the gas blowby can be small. By letting the disk, 130, upon impact, after the flight, seat in and seal against the support surface of the open screened area 134 significantly smaller than at the flying disk, the energy of the gas shock wave transmitted to the target region is kept minimal. Therefore relatively high velocities can be generated with reduced gas shock on the target area.

In another embodiment known as "gas entrainment", as seen in Figs. 5a and 5b, a screen 134 of suitable mesh and type is used, such as a suitable screen for this purpose is Swiss Polyester Monofilament screening

having 150 μm openings. Such screen is available as part no. 7-150/43 from Tetko, Inc., in Elmsford, NY. The screen 134 is captured between the rings 82 within the throat region 16. Microprojectiles are accelerated by the gas shock wave directly by placing them directly in the path of the gas shock wave. A drop of liquid containing the microprojectiles can be placed in the middle of the screen or mesh 134. Upon firing, the gas shock wave launches and atomizes the droplet and entrains the microprojectiles in it. The particles and gas shock wave then directly impact the target together. This configuration is effective when the target can tolerate the full impact of the gas shock wave.

5) Interchangeable interface for different biological targets - When the apparatus is used for intact animals, humans or other large organisms, an interface 18 is required which allows precise positioning, Fig. 10, control of flight distance to target surface, control of gas or vacuum over the target area, support of soft tissues against vacuum or shock, and the potential for dissipation of any gas shock wave which may be transmitted into this region. When smaller organisms, tissue, or cells are bombarded, a convenient mechanism for placing petri dishes or other cell containers at the proper distance within a chamber which can hold a vacuum is required. The following interface embodiments can be used.

The embodiments depicted in Fig. 10 are threefold all of which are adapted to fit on the throat portion 16. All have a tubular shaped nozzle 144 which is adapted to threadingly engage the nozzle interface 18 (Fig. 2) or 18' (Fig. 4). One version (lower right in the drawing) of the nozzle is provided with a vent tube 146. Both versions (lower left and right) have a

5 snap-in screen 148 (similar to screen 120 (Fig. 7)) that
engages a tapered recess 147 in the lower end of the
nozzle. The interface 18 may be provided with a
connection 140 to the vacuum pump 72 and alternatively
10 may be provided with a connection 142 to the pressurized
gas to alter the gas type within the system. An O-ring
145 is provided in a groove 149 in the upper face of the
nozzles 144 to provide a seal against the lower face of
the throat 18. The extended nozzle configuration is
15 especially suited to surgical applications, where the
interface is needed to be placed within a surgical
opening. It also facilitates precise targeting of an
area on a large surface (such as epidermis) to be
bombarded. The tip of the nozzle can be pressed
20 directly against the targeted area. At the end of the
nozzle, a screen or mesh 148 can be positioned as
described to support the tissue against shock and
prevent the tissue from being sucked upward into the
nozzle when vacuum is applied within the nozzle's
interior. Any one of the differently configured nozzles
can be screwed into place depending on the nature of the
application.

In another embodiment, as seen in Fig. 11, a clear
chamber, made of Lexan® polycarbonate or similar
25 shatter-resistant material, 150 can be attached to the
end of the throat 18 of the gun via a threaded fitting.
This embodiment, which can maintain a vacuum, has an O-
ring 152 fitted in a groove 153 to maintain a seal
between a side wall 154 and base plate 156. The chamber
30 which is formed of two parts, upper half 154 which may
be threadingly engaged and sealed by O-ring 160 against
the interface 18 and a lower plate 156 which may be
friction fitted and snapped into place and sealed by the
O-ring seal 152. In this manner the chamber can readily
35 be opened for insertion of a petri plate 158 or any

small sample. This simple chamber can be used in a bench-top laboratory mode.

As may be seen in Fig. 12 an alternative embodiment of the interface is provided which is particularly adapted to work with large animals or large surfaces. This embodiment allows maximum venting and deflection of gas blast from the target (42), while allowing minimum distance from particle launch position to target impact point. In this embodiment, the arrangement is substantially identical to that shown in Figs. 1 and 2, the only difference being that the lower half of the housing 14 does not have to be tapered and has a threaded nozzle insert 160 of constant diameter. It may, however, be used with the chamber 14' if desired.

An ante-chamber 164, in the form of an inverted housing, and cover 170 is adapted to fit on the outer portion of the housing 14' and is sealed about the periphery of the housing 14' by a grommet 162 captured between two inwardly flanged pieces of the ante-chamber 164. The lower or cover portion 170 of the ante-chamber 164 is formed to be a protuberant and relatively rigid which may as the ante-chamber be formed of Lexan®, polycarbonate or similar shatter-resistant material, as was the case for Fig. 11. The protuberant member 170 forms a orifice 172 in the center portion lying along the axis 11 of the housing 14' and is configured to accommodate a snap-in screen 174 to prevent the target tissue from being sucked up into the ante-chamber. The protuberant member 170 may be secured to the ante-chamber 164 by suitable screws (not shown). An O-ring 176 positioned a groove 177 to provide a seal maintains a vacuum.

A baffle plate 178 (Fig. 13) is placed on the protuberant member 170 and has sufficient openings 180 and closed regions to deflect most of the gas shock

waves, depicted by the arrows 182, away from the target 42. Fig. 13 is a sectional view taken along the section line 13-13 of Fig. 12. The grommet 162 permits the ante-chamber 164 to be slid along the axis of the housing and thereby one is able to adjust the distance of the target 42 from the gas shock wave. The baffle 178 facilitates the flow and venting of gases laterally away from the lower orifice region to prevent the receding gases from becoming trapped in the orifice region which could result in reflection of the gases back toward the oncoming particles or simple deacceleration of the particles due to the viscous drag. The baffle plate acts finally to divert gases and the shock wave away from the lower target region.

Operation

In operation, the target 42 whether it be cells or tissue is selected and prepared. No special preparation is required other than possible cell plating, removal of hair, surgical exposure to tissue, etc. The particular procedures for this do not form part of this invention. Based upon the target and the objective, the optimal microprojectile type, amount, and size is chosen and prepared and loaded into place and the desired throat assembly configuration is selected and properly positioned. Finally the desired gas pressure is set at 22. The interface assembly 18 best suited to the target and purpose has been selected and the high pressure membrane is sealed in place and the housing is closed and sealed. The high pressure chamber 12 is then pressurized, the target positioned in place relative to the interface assembly and the gas environmental or degree of vacuum in the enclosure in flight areas is set. The solenoid is activated causing the unit to generate the gas shock. The vacuum is then released and

the pressure to the high pressure component is vented and the process repeated as needed.

The particular manner of preparation of the biological particles, such as DNA coated particles or DNA in solution, are best loaded as a slurry in either water or ethanol used for gas entrainment, while for other configurations DNA coated particles are best loaded and spread onto the distal side of the membrane surface, suspended in ethanol and allowed to dry prior to bombardment. The particular manner of preparation of the DNA coated particles does not form part of this invention. As used herein, the term particle includes both the biological material itself, or coated on particles. Any of the well-known prior art techniques used today may be used. For example, those described in either the Agracetus patent application, or as described in Sanford et al. or Klein et al. may be used.

An alternative embodiment of the invention is illustrated in Fig. 3. In this figure, many of the essential parts of the gun described in connection with Figs. 1 and 2 are illustrated and bear the double prime reference symbol for parts which correspond to a part in Figs. 1 and 2. The primary difference between the embodiment of Fig. 3 is that it is reduced in size and has a pistol grip 206 to facilitate its being held for surgical applications. The hand-held pistol grip 206 is provided with switches 204 which control the several valves 28 double prime 30 double prime 200 along with the solenoid actuation 48 prime.

The nozzle portion 18 double prime, the throat portion 16 double prime, is similar to the throat portion 16 of Figs. 1 and 2 and is internally threaded so that it may receive the various discs, screens, and the like as is depicted in Figs. 5 through 9. No further infittings are provided for the apparatus

illustrated so that it may be used directly on animal tissue. Although not shown, a restraining screen of the type denoted by item 148 and Fig. 10 may be used. This device also includes a valve 200 for controlling the vacuum and a line 202 for supplying power to the unit is the adapter to receive high pressure from the gas source 20 and from the vacuum pump 72. Aside from this the operation is the same as that described in connection with Figs. 1 and 2 and need not be described further.

5
10 The cold gas shock wave generator of this invention may be retrofitted into an existing hot gas shock wave generator used in the PDS-1000 currently sold by E. I. du Pont de Nemours and Company, Wilmington, Delaware. The PDS-1000 is a cylindrical two chamber device shown
15 in cross section in Fig. 14 as housing 210. Connections are made in the upper and lower sections of the device to a source of vacuum 140. The target is positioned on a platform 220 that may be slid into the device upon opening a front door to permit access to the chambers.
20 At the top of the device is a central opening through which the cartridge used to generate the hot gas shock wave is directed. The cartridge device of the existing unit is removed and replaced with the gas shock wave generator 12 (Fig 2). The threads 56 on the shock wave
25 generator are threaded to engage flat disk like plates 214 and 216 which lock into place by the brackets 18 where the current barrel assembly of the PDS-1000 currently locks into place. Because this assembly will be connected with a high pressure gas source, it will be
30 restricted in how it can be manipulated. Accordingly, the plate may have multiple notches (not shown) around its parameter so that they can be locked into place at numerous points of rotation as is most convenient. A second set of plates 214' and 216 is secured by screws
35 218 to the lower plates 214 and 216. However, the

plates 214' and 216' are inverted in this manner. The gas shock wave generator may be removed and inverted when it is desired to replace the membrane 38 (Fig. 2) for the shock wave. This greatly facilitates changing the high pressure membranes 38. All other aspects of the high pressure gas shock wave generator remain essentially the same as previously described in connection with Figs. 1 and 2. The chamber 210 has an upper plate 220 which also is slideable in by opening the front door to the chamber 210. This plate 220 which normally would hold the stopping plate assembly in the PDS-1000 is replaced in accordance with this invention by a throat section assembly 16 which will typically include elements 82 and 80 (Fig 2.). More preferably, the throat assembly 16 will include the removable insert 100 illustrated in Fig. 4.. Thus, this simply can be dropped into the platform where the stopping plate assembly formerly was and the platform will retain its function as the partition is sealed between the upper and lower sections of the bombardment chamber 210. The lower section of the chamber serves as a target chamber as before, the target being depicted by the tray 212. Beyond this, the operation of the device is substantially the same as described previously.

The method and apparatus thus described may be used for enabling the transformation (or delivery of pharmaceuticals) of a variety of different living cells and tissues ranging from plants, microbes, and animals. The configurations described are suitable for use either as a handheld probe for surgical uses and large organism applications or as a non-mobile (bench mounted) device for in vitro cultures and other lab applications. A cold shock power source is used to derive the force for accelerating the particles carrying living organisms. The shock power source is enclosed within a housing

which is able to hold the vacuum. The vacuum in turn facilitates the device's use and facilitates maximal rates of expansion for the gas shock wave that is generated while at the same time protecting the operator's ears from severe noise generated by the gas shock. Finally the target itself is protected from potentially lethal shocks.

The device is capable of generating a catastrophic, essentially instantaneous gas shock, without extreme heating, through the rupture of a membrane, or by similar means, thereby venting a high pressure chamber of given volume and with adjustable pressure, such that the shock wave strength and speed is suitable for accelerating microprojectiles to speeds suitable for biolistic purposes of any particular nature. Such apparatus includes housing which simultaneously protects users from the gas shock, attenuates the impact of the gas shock on the target cell or tissue, and allows use of modified internal gas environments for better particle flight characteristics. Such housing also allows optionally for relatively small, hand-held "pistol" or "wand" configurations which allow a nozzle or antechamber to be pressed directly against epidermis, dermis, or surgically exposed tissues, for biolistic treatment of large intact organisms.

The throat area of the device accommodates a variety of interchangeable assemblies which allows the device to operate in different acceleration modes ranging from a fixed membrane to a captured membrane, ruptured membrane, flying disk and gas entrainment.

A target interface area through which the accelerated particles fly on their way to the target provides interchangeable interfaces to accommodate different uses ranging from large animals to small in vitro applications. This interface area provides

flexibility in what gas or vacuum the particles pass through, the distance of flight to the target, the dissipation of gas and shock prior to impact with the target and physical stabilization of the target.

5 In short, the apparatus and method provides a flexible transformation device that is relatively safe and provides a high degree versatility and repeatable results. The device is mobile and affords less target damage if the proper inserts are utilized. It also
10 provides better particle distribution and dispersal along with breaking up of the agglomerated particles.

Examples

Example 1

15 A prototype apparatus using a cold gas shock wave for introducing particles carrying biological materials into a target of cells and/or tissue constructed in accordance with this invention (Figs. 1 and 2) was used to transform yeast cells. The resulting number of
20 transformed colonies and the pattern of dispersion of the transformed colonies was compared to that achieved using the PDS-1000. This apparatus is now commercialized as the PDS-1000 by E. I. du Pont de Nemours and Company, Wilmington, DE. The gunpowder
25 driven apparatus uses a hot gas shock wave to drive a macroprojectile conveying microprojectiles coated with biological material. The macroprojectile strikes a stopping plate housing a central orifice, which stop the macroprojectile before it strikes the target and allows
30 the microprojectile to pass through the orifice striking the target.

 A growth medium (liquid YEP medium) was prepared by adding 5 g yeast extract, 10 g peptone, and 0.025 g adenine to 900 mL of distilled water, separately adding
35 20 g of glucose to 100 mL distilled water, autoclaving

both solutions, and then combining the two autoclaved solutions. A colony of yeast cells was taken from a stock culture and placed in a 250 mL flask containing 50 mL of liquid YEP medium. The yeast cells were grown to stationary phase by placing the 250 mL flask containing the yeast cells and liquid YEP medium on a rotary shaker rotating at 150 RPM for 72 hours and 37°C. The cells are then pelleted by centrifugation, the supernatant discarded, and the cells re-suspended in 10 mL water.

5

10 The concentration of the cells was determined by measuring the optical density of the suspension at 600 nm (1 unit equals approximately 2^{10} cells/mL). A growth medium (Uracil drop-out medium) was prepared by adding 3.35 g yeast nitrogen base without amino acids,

15 0.235 g Uracil dropout amino acid premix (prepared by grinding and mixing the D and L forms of the following: 0.4 g adenine, 0.4 g tryptophane, 0.4 g histidine, 0.4 g arginine, 0.4 g methionine, 0.6 g tyrosine, 1.2 g leucine, 0.6 g lysine, 1.0 g phenylalanine, and 4.0 g

20 threonine; the mixture is stored at room temperature in a dark tightly sealed bottle), 7.5 g agar, and sorbitol and mannitol to a final concentration of 0.75 M each, separately adding 10 g glucose to 50 mL distilled water, autoclaving the two solutions, and then combining the

25 two autoclaved solutions. 10^8 cells were spread out onto a petri dish containing the Uracil dropout medium.

M-10 tungsten particles obtained from GTE, Hawes St., Towanda, PA, were coated with the plasmid DNA YEP352, using the procedure described by S. A. Johnston,

30 R. Butow, K. Shark, and J. C. Sanford, Mitochondrial Transformation in Yeast by Bombardment with Microprojectiles, *Science* 240:1538-1541 (1988). 5 μ L of transforming DNA (1 μ g/ μ L transforming DNA in a buffer consisting of 10 mM Tris hydrochloride and 1 mM

35 ethylenediaminetetracetic acid) was mixed with 25 μ L of

a suspension of M-10 tungsten particles (60 mg/ μ L water), to which was added 25 μ L 2.5 M calcium chloride, and 5 μ L 0.1 M spermidine. This mixture was allowed to sit for 10 minutes at room temperature. The mixture was
5 then centrifuged and all of the the supernatant except for 10 μ L was discarded. In preparation for bombardment, the pelleted microparticles were re-suspended in the remaining 10 μ L of solution. For
10 bombardment, 3 μ L of the suspension was placed on the tip of a microprojectile.

Microparticles for use with the apparatus invention were prepared using the same procedure and were then further treated by re-suspending the particles in 50 μ L of a 70% ethanol solution, and pelleting the washed
15 microparticles by centrifugation. In preparation for introduction into the cells (bombardment), the pelleted coated microparticles were resuspended in 10 μ L of a 100% ethanol solution and 3 μ L of the suspension was placed on a membrane and spread to produce an even layer
20 about 5 mm in diameter. The ethanol was allowed to evaporate at room temperature, leaving a dry powder.

Cells were bombarded with the coated microparticles the same day that they were spread onto the petri dishes containing Uracil dropout medium. Cells were bombarded
25 with the PDS-1000. Both the top shelf and bottom shelf position were used for the target. Cells were also bombarded using the "captured membrane", "ruptured membrane", and "gas entrainment" embodiments of the present invention and the method described above in the
30 "Operation" section and shown in Figs. 5, 7, and 8. The desired gas (helium) pressure was selected using a high vacuum regulator at the tank source, and the presence of vacuum over the target was controlled with standard valves described. The target was placed in a Lexan®
35 chamber connected to a vacuum pump as disclosed in the

Detailed Description of the Preferred Embodiment and shown in Fig. 10.

Table 1

5	Colonies	Number of Transformed per Petri Dish				
		<u>Apparatus of the Present Invention</u>				
10	<u>Method</u>	<u>Petri Dish No.</u>			Average	Std. Dev.
		<u>1</u>	<u>2</u>	<u>3</u>		
15	Helium entrainment at 1000 psi	149	352	1492	664	723
	Ruptured membrane at 500 psi	1039	1623	---	1331	412
20	Ruptured membrane at 1000 psi	516	2115	712	1114	872
	Captured membrane at 600 psi	233	207	219	220	13
25	Captured membrane at 1000 psi	302	415	172	296	122
	<u>PDS-1000</u>					
30	Top shelf	261	231	604	365	207
	Bottom shelf	334	209	1215	586	548

Table 1 shows a comparison of the number of transformed colonies using the apparatus of the present invention and the the gunpowder driven apparatus. Results indicate that use of the "ruptured membrane" embodiment at both 500 and 1000 psi produced approximately two to three times as many colonies as the gunpowder driven apparatus. The apparatus of the present invention also produced a wider and more even dispersion of colonies over the surface of the petri dish. The gunpowder driven apparatus typically produced a dispersion in which there was a "dead zone" of approximately 1 cm in diameter in which no transformed colonies were apparent, and in which the agar was often blown out by the blast.

15 Example 2

A prototype apparatus as described in Example 1 was used to transform Bacillus megaterium bacteria. The resulting number of transformed colonies and the pattern of dispersion of the transformed colonies was compared to that achieved using the PDS-1000.

50 mL of Luria-Bertani (LB) broth medium (prepared by adding 10 g tryptone, 5 g yeast extract, and 5 g NaCl, to 900 mL distilled water, adjusting the pH to 7.5 with NaOH, adding additional distilled water to a total volume of 1000 mL, and autoclaving) was inoculated with a loop of Bacillus megaterium strain 7A17 cells, available from the Bacillus Genetic Stock Center, State University, Columbus, OH. The inoculated culture was incubated in a rotary shaker at 2500 RPM for 24 hours. The culture was centrifuged and 40 mL of supernatant was discarded and the cell pellet was re-suspended in the remaining supernatant. The concentration of the cells was determined by measuring the optical density of the suspension at 600 nm. A solid growth medium (solid LB medium plus methionine and osmoticum) was prepared by

adding 10 g tryptone, 5 g yeast extract, 5 g NaCl, and 15 g agar to 900 mL distilled water, adjusting the pH to pH 7.5 with NaOH, adding additional distilled water to a total volume of 1000 mL, adding 182.2 g D-sorbitol and 5 136.6 g D-mannitol, autoclaving, adding a sterile solution of methionine to a final concentration of 50 µg/mL, and adding 4 mL of a sterile solution of of D,L-methionine (12.5 mg/mL). 10⁸ cells were spread out onto a petri dish containing the solid LB medium plus 10 10 methionine and osmoticum. The cells were allowed to dry on the agar prior to bombardment. The medium described was optimized with respect to osmoticum concentration (sorbitol and mannitol) based on efficiency of transformation of B. megaterium. Increasing the 15 concentration of osmoticum can aid in the survival of cells immediately following bombardment.

M-5 tungsten particles obtained from GTE, Hawes St., Towanda, PA, were coated with the plasmid DNA DNA PUB110 obtained from the Bacillus Genetics Stock Center 20 in Columbus, OH using the procedure disclosed by Sanford et al. and as described in Example 1. The plasmid PUB110 is 4.5 kb in size and confers resistance to the antibiotics kanamycin (Km) and neomycin (Nm). The plasmid PUB110 was isolated from 24 hour old cultures of 25 Bacillus subtilis strain 1EG using the method described by T. Maniatis, E. F. Firtsch, and J. Sambrook, Molecular Cloning, A Laboratory Manual, 1982. The plasmid PUB110 was then purified using a cesium chloride-ethidium bromide gradient.

30 Microparticles for use with the apparatus of the present invention were also prepared using the method described by Sanford et al.

Cells were bombarded with the PDS-1000 described by Sanford et al. The "captured membrane", "ruptured 35 membrane", "flying disc", and "gas entrainment"

embodiments of the present invention were tested using the procedure described above in the "Operation" section and shown in Figs. 5, 7, and 8.

Stationary cells were plated on the medium
5 described above (solid LB medium plus methionine and osmoticum) and were bombarded with either the
gunpowderpowder driven apparatus of Sanford et al. or
the apparatus of the present invention and then covered
with 15 mL of an overlay medium (prepared by adding 4 mL
10 D,L-methionine and 2 mL kanamycin sulfate (25 mg/mL solution) to 1 liter of autoclaved LB broth medium to a
final concentration of 50 µg/mL each). After the
overlay medium hardened the petri dishes were maintained
at 37°C and the number of transformants were counted
15 after 72 hours. Transformed B. megaterium cells were
resistant to the antibiotics kanamycin and neomycin.
Transformants were thus selected by their ability to
grow in the presence of kanamycin.

Ten transformants were isolated and streaked on
20 solid LB medium with methionine and kanamycin
(10 µg/mL). This kanamycin concentration is
approximately the same as the concentration of kanamycin
in the petri dishes containing bombardment medium and
overlay medium from which the transformants were
25 isolated. Plasmid DNA was isolated from 10
transformants. The purified plasmid DNA was cut within
the restriction enzyme BamH1 and then visualized by
agarose gel electrophoresis. pUB110 is cut at one site
with BamH1. The digested plasmid DNA from each
30 transformant was compared to the known markers which
were lambda-phage digested with HindIII and lambda-phage
digested with HindII and ECoR1 and also compared to
pUB110 plasmid isolated from B. subtilis 1E6 digested
with BamH1. The digested plasmid DNA from the
35 transformants was identical in size (4.5 kb) to plasmid

pUB110 digested with BamH1. Thus the presence of transformation was confirmed in those cells selected as transformants.

5 The results of bombardment of B. megaterium with the gunpowder driven PDS-1000 apparatus and the apparatus of the present invention are shown in Table 2. Tables 3 and 4 indicate the results of bombardment experiments using various embodiments of the present invention.

Table 2

Comparison of Number of Transformants - Apparatus
of the Present Invention Compared to the PDS-1000

5 Colonies		Number of Transformed per Petri Dish				
		<u>Petri Dish No.</u>			Std.	
<u>Method</u>		<u>1</u>	<u>2</u>	<u>3</u>	<u>Average</u>	<u>Dev.</u>
10	Helium entrainment at 1000 psi	0	0	0	0	0
15	Ruptured membrane at 600 psi	14	77	93	61.3	41.8
	Ruptured membrane at 1000 psi	8	0	7	5.0	4.3
20	Captured membrane at 600 psi	37	4	15	18.7	16.8
25	Captured membrane at 1000 psi	16	28	8	17.3	10.1
=====						
30	PDS-1000	16 PETRI DISHES			NO COLONIES	

Table 3
 Comparison of Number of Transformed
 Colonies for Various Embodiments

5 Colonies	Method	Petri Dish No.			Number of Transformed per Petri Dish	
		1	2	3	Average	Std. Dev.
10	Flying disc at 800 psi	4	6	7	5.7	1.5
	Flying disc at 1200 psi	3	0	1	1.3	1.5
15	Ruptured membrane at 600 psi	3	1	0	1.3	1.5
	Ruptured membrane at 1000 psi	0	1	1	0.7	0.6
20	Controls run:					
25	1) Cells were mixed with DNA prior to being bombarded with naked particles using each of the embodiments above (18 petri dishes bombarded, no transformed colonies found).					
30	2) Cells were mixed with DNA coated particles with no subsequent bombardment, 18 petri dishes prepared, no transformed colonies found).					

Table 4
Comparison of Number of Transformed
Colonies for Various Embodiments

5	Colonies Method	Number of Transformed <u>per Petri Dish</u>			Average
		<u>Petri Dish No.</u>			
		<u>1</u>	<u>2</u>	<u>3</u>	
	Ruptured membrane				
10	at 800 psi (3 cm)	4	2	2	2.7
	Ruptured membrane				
	at 600 psi				
15	(3 cm)	1	0	1	0.7
	Ruptured membrane				
	at 400 psi				
	(3 cm)	0	0	0	0
20	Ruptured membrane				
	at 800 psi				
	(5 cm)	1	0	0	0.7
	Ruptured membrane				
25	at 1200 psi				
	(3 cm)	1	0	0	0.7
30	Controls run:				
	Cells untreated, cells mixed with DNA and bombarded with naked particles, cells bombarded with naked particles and then mixed with particles and DNA, cells mixed with DNA and then hit with a helium shock (no particles), and cells hit with a helium shock and then mixed with DNA. 20 petri dishes tested, no transformed colonies found.				
35					

Table 2 indicates that several transformants were observed with various embodiments of the present invention while no transformants were observed with the PDS-1000. Tables 2, 3 and 4 show that the apparatus of the present invention can be used successfully to produce transformants, sometimes at relatively high rates (colonies per petri dish), which were not possible with the PDS-1000. Furthermore, transformation rates up to several thousand or more are possible under optimal conditions. The preferred conditions for achieving such high transformation rates include use of 15 hour old Bacillus cultures, a cell density of 108 cells/petri dish, use of a flying disc embodiment at 900 psi, and a cell growth medium which includes 1.0 M sorbitol and 0.75 M mannitol as osmotic support for the cells. Under these conditions, the present invention provides approximately a 1000-fold increase in transformants per petri dish than the gunpowder driven apparatus of Sanford et al.

Example 3

A prototype apparatus for introducing particles carrying biological materials into a target of cells and/or tissue constructed in accordance with this invention was used to transform NT1 Nicotiana tabacum tobacco cells. The resulting number of transformed cells and the pattern of dispersion of the transformed cells was compared to that achieved using the PDS-1000.

NT1 Nicotiana tabacum cells were grown as suspension in a liquid growth medium (Daniell, et al. PNAS, 87:88-92, 1990) on a gyratory shaker. The NT1 cell line was obtained from G. An at the University of Washington (Daniell et al., PNAS, 87:88-92-1990). The NT1 cells have lost their ability to regenerate into plants but are a useful model system because of their

uniformity and rapid growth. The cells were generally found in clusters of three to four cells each. In preparation for bombardment, 1 to 5 mL of cell suspension was collected onto filter paper discs using a
5 Buchner funnel.

M-10 tungsten particles obtained from GTE, Hawes St., Towanda, PA, were coated with the plasmid DNA pIB1505 (obtained from Dr. Bill Crosby at the Molecular Genetics Plant Biotechnology, Institute, NRC Saskatoon,
10 Canada), as the transforming DNA using the procedure described by T. M. Klein, M. E. Fromm, A. Weissinger, D. Tomes, S. Schaaf, M. Sleeten, and J. C. Sanford, Proc. Natl. Acad. Sci. 85:4305-4309, 1988, T. M. Klein, E. Fromm, T. Gradziel, and J. C. Sanford, Biotechnology
15 6:559-563, 1988, T. M. Klein, E. C. Harper, Z. Svab, J. C. Sanford, M. E. Fromm, P. Maliga, Proc. Natl. Acad. Sci. 85: 8502-8503, 1988, and by Sanford et al.

20 Microparticles for use with the apparatus of the present invention were prepared using the same procedure as in Example 1.

The β -glucuronidase gene (GUS) gene was used as a reporter gene and used to assay rates of transformation in the plant cells. The GUS gene was cloned from the bacterium E. coli (R. A. Jefferson et al., EMBO, 6:3901-
25 3907, 1987). The GUS gene codes for the protein β -glucuronidase, which is not normally present in plant species. Plant cells which have been transformed with GUS will turn blue in the presence of the substrate x-gluc. The GUS assay was used to detect transient gene
30 expression in bombarded NT1 plant cultures. The cells are stained two days after bombardment. The staining procedure consisted of adding 1 mL of x-gluc solution to the cells using the method described by McCabe et al., Biotechnology 6:923 (1988). The solution consisted of
35 0.5 mg/mL x-gluc dissolved in DMSO, 10 mM EDTA, 100 mM

sodium phosphate, 0.5 mM potassium ferrocyanide, and 0.1% Triton X-100. The cells were incubated at 37°C for 24 hours and the number of blue spots then recorded.

Cells were bombarded with the PDS-1000 as disclosed
 5 by Sanford et al. and described by T. M. Klein,
 M. E. Fromm, A. Weissinger, D. Tomes, S. Schaaf,
 M. Sleeten, and J. C. Sanford, Proc. Natl. Acad. Sci.
85:4305-4309, 1988, T. M. Klein, E. Fromm, T. Gradziel,
 and J. C. Sanford, Biotechnology 6:559-563, 1988, and
 10 T. M. Klein, E. C. Harper, Z. Svab, J. C. Sanford,
 M. E. Fromm, P. Maliga, Proc. Natl. Acad. Sci. 85:8502-
 8503, 1988. The "flying disc" and "gas entrainment"
 embodiments of the present invention were tested using
 the procedure described above in the "Operation" section
 15 and shown in Figs. 5, 7, and 8.

Table 5 shows a comparison of transformation of NT1 cells using the PDS-1000 and the apparatus of the present invention.

Table 5

20 Comparison of Number of Transformants - Apparatus
 of the Present Invention Compared to the PDS-1000

Colonies Method	Number of Transformed per Petri Dish				
	Replicate No.			Average	Std. Dev.
	1	2	3		
Helium entrainment	406	440	475	440.3	34.5
25 Flying 30 Disc	773	610	603	662.0	96.2
PDS-1000	124	118	122	121.3	3.0

Table 5 shows the apparatus of the present invention provides superior transformation rates in comparison to the hot gas shock wave apparatus provided
5 by the PDS-1000.

The same procedures and plasmid described for transformation of NT1 tobacco cells were used to transform peach embryonic callus. The growth medium used for peach callus (DKW medium) consisted of 4.4 μ M
10 BAP, 0.05 μ M IBA, 2% sucrose, pH 5.8, and agar (0.6-0.7%) or Girlite (0.25%). Peach callus was provided by Dr. Ralph Scorza of the USDA. The peach callus was taken from a five year old culture derived from immature embryos, were autotrophic, and were growth-regulator
15 dependent, and could continue to produce somatic cells. In this case the peach callus was bombarded three consecutive times with the PDS-1000. The peach callus were bombarded only once using the apparatus of this invention. The results are shown in Table 6.

20

Table 6
 Comparison of Number of Transformants - Apparatus
 of the Present Invention Compared to the PDS-1000

5		Number of Blue Spots (Transformed Cells) <u>per Petri Dish</u>		
<u>Method</u>				
		<u>Lowest #</u>	<u>Highest #</u>	<u>Average</u>
10	Helium entrainment at 900 psi	62.0	554.0	329.7
15	Helium entrainment at 1200 psi	75.0	320.0	208.0
20	Flying Disc at 900 psi	111.0	345.0	172.7
25	Flying Disc at 1200 psi	89.0	172.7	230.0
	PPS-1000	86.0	340.0	215.4
25	(triple bombardment)			

Table 6 shows that the apparatus of the present invention can achieve rates of gene delivery in a single bombardment, that are greater than can be achieved by three consecutive bombardments with the PDS-1000.

5 Furthermore, the uniformity of dispersion and amount of area covered was observed to be qualitatively superior to that produced using PDS-1000.

Example 4

10 A prototype apparatus as described in Fig. 1 was used. The resulting number of transformed cells and the pattern of dispersion of the transformed cells was compared to that achieved using the PDS-1000.

M-10 tungsten particles obtained from GTE, Hawes St., Towanda, PA, were coated with the plasmid DNA 15 pHBluc obtained from R. S. Williams, Duke University, Durham, North Carolina using the procedure of Sanford et al. Plasmid pHBluc was used for the transformation of myotubes in vitro and for ear, skin, and liver in situ and contains the firefly luciferase gene fused to a 20 human beta-actin promoter in a puc19 based vector (ole Wet, J. R., et al., Mol. Cell Biol., 7:725-727 (1987), Leavitt, J. et al., Mol. Cell Biol. 4:1961-69 (1984).

25 Microparticles for use with the apparatus of the present invention were purchased from Alfa Johnson, Mathy, Danvers, MA) and were also prepared using the method described by Sanford et al.

Myotubes were prepared from chick embryos. Chick embryos were removed from the egg and the breast muscle was removed by dissection and placed in a drop of the 30 commercially available solution, Saline G. The muscle was minced and then diluted with 9 mL Saline G, 1 mL of a 10x solution of trypsin (2.5% solution in buffered saline). This mixture was rocked for 5 minutes and then agitated by pulling the mixture into and out of a pipet. 35 The mixture was then rocked for an additional 15 minutes

and the cells collected by filtration. The cells were re-suspended in 20 mL of CKI growth media (commercially prepared Dulbecco's Modified Eagles Medium consisting of 0.584 g/L L-glutamine, 1 g/L glucose, 3.7 g/L sodium bicarbonate, 15% horse serum, 5% embryo extract) and then counted (Embryo extract is prepared by removing 100 g of chick embryos are removed from the egg, decapitated, and then homogenized in 100 mL of a medium consisting of 121.12 g/L sodium chloride, 15.5 g/L potassium chloride, 12.72 g/L magnesium chloride, 7.8 g/L calcium chloride, 2 g/L dibasic sodium phosphate, and 5.19 g/L monobasic sodium phosphate; the homogenate was stirred in a cold room for one hour after addition of 10000 units of hyaluronidase. The mixture was centrifuged to remove debris; lipid was skimmed off the supernatant, which was then sterilized by filtration). The cells were then plated on 50 mm petri dishes at a density of 1×10^6 cells per mL. The myotubes were held for 5 days prior to bombardment.

Cells were bombarded with the PDS-1000 using standard protocols. This procedure was modified only in that the the liquid medium overlaying the myotubes was removed just prior to bombardment. The "captured membrane", embodiment of the present invention were tested using the procedure described above in the "Operation" section and shown in Fig. 5. The media was replaced immediately after bombardment. The cells were incubated for 24 hours at 37°C and then assayed for luciferase activity.

In order to assay for luciferase activity, the cells were scraped off the plate in 1 mL of extraction buffer (consisting of 100 mM potassium phosphate buffer, pH 7.8, 3 mM magnesium chloride, and 1 mM DTT), and then pelleted by centrifugation. The supernatant was removed and 100 μ L of extraction buffer and 50 μ L of lysis

buffer (consisting of 8.9 mL of 0.25 M Tris buffer, pH 7.8, 1.0 mL soybean trypsin inhibitor of 10 mg/mL concentration, 0.1 mL aprotinin) were added and the cells lysed by sonication for 6 seconds. The cell debris was pelleted by centrifugation and the supernatant assayed for luciferase activity.

The assay for luciferase activity measures the output of light (in photons) produced by the reaction catalyzed by the luciferase enzyme in the presence of its substrate luciferin. The quantity of light produced (number of photons) is proportional to the quantity of luciferase extracted from the tissue, which is determined by the number of transformed cells and the amount of luciferase produced by each transformed cell. The greater the luciferase activity the more efficient the transformation. The number of photons per shot area can be converted to the number of picograms of luciferase per shot area by developing a standard curve using purified commercially available luciferase. Table 7 indicates the results of a comparison of the transformation efficiency of the apparatus of the present invention and the PDS-1000

Table 7
 Peak Luciferase Expression: Comparison
 of PDS-1000 and the Wand One Day After
 Bombardment. Luciferase Is Expressed as Total Pico Grams
 from the Bombarded Area

5

Biolistic PDS-1000 (picograms luciferase per area bombarded)

	<u>Skin</u>	<u>Ears</u>	<u>Myotubes</u>
	(10.6 cm diameter)	(160 mm plate diameter)	
10 Mean	300	1312	2959
Std. Error of Mean	64	202	306
Sample Number	17	59	6

15 Present Invention
 (picograms luciferase per area bombarded)

	<u>Skin</u>	<u>Ears</u>	<u>Myotubes</u>
	(10.6 cm diameter)	(160 mm plate diameter)	
Mean	1543	5563	34775
20 Std. Error of Mean	448	1510	5114
Sample Number	4	18	5

25 Table 7 shows that the apparatus of the present invention for myotubes produces on the average about 11 times higher luciferase activity, and therefore transformation rate, than the gunpowder driven apparatus.

30 In another test, the coating of microparticles and the preparation for bombardment with DNA for transformation of living in situ tissues using the PDS-1000. Gold microparticles were used instead of tungsten. Gold particles used were spherical, and either of 1 to 3 μ diameter or 2 to 5 μ in diameter
 35 (available from Alpha products, product no. 00766). In

addition, the configuration of the apparatus was modified in that instead of a vacuum chamber, a nozzle (Fig. 10) was placed at the end of the device to direct the microparticles to a small patch of tissue. For skin and ear tissue the nozzle was connected to a vacuum pump so that the microparticles traveled through a reduced atmosphere. Liver tissue would have been damaged by a reduced atmosphere and was therefore not subjected to the reduced atmosphere.

10 Microparticles for use with the apparatus of the present invention were purchased from Alfa, Johnson, Mathey, Danvers, MA and also prepared using the method described by Sanford, et al.

15 To prepare the skin and ears for bombardment, the animal was anesthetized and its hair removed from the area to be bombarded with a depilatory. An area about 6 mm in diameter was then bombarded and the animal was then allowed to recover from the anesthetic. In 24 hours the animal was sacrificed and the bombarded area is cut out. The tissue was macerated in a mixture of 140 μ L of extraction buffer, 50 μ L of lysis buffer, and 10 μ L of 2% NP-40 detergent (commercially available). The cell debris was then pelleted by centrifugation, and the supernatant assayed for luciferase activity.

25 To transform liver tissue, a mouse is anesthetized and an incision made in its abdomen one expose of the lobe of liver. A 10 mm area was then bombarded (as the skin and ears except without drawing a vacuum). The incision was then closed and the animal allowed to recover. In 24 hours the animal is sacrificed and the bombarded area removed, macerated in 200 μ L extraction buffer, the cell debris was then pelleted by centrifugation and the supernatant assayed for luciferase activity.

Table 8 indicates the results obtained for livers bombarded with the apparatus of the present. It was not possible to transform liver with the gunpowder driven apparatus of Sanford et al.

5

Table 8

	Present invention (captured disk)		
	(picograms luciferase per area bombarded)		
10 Liver	1	3	5
Mean	293	243	5
Std. Error	122	133	2.3
of Mean			
Sample Number	8	4	3

15

WHAT IS CLAIMED IS:

1. A method of introducing particles carrying biological materials into a target of cells, and/or tissue, said method comprising the steps of:
 - 5 accelerating the particles sufficiently to cause them to penetrate the surface of the cells and/or tissue and become incorporated into the interior of the cells and/or tissue without killing the cells and/or tissue and
 - 10 effecting the particle acceleration by subjecting the particles to the force of an instantaneous cold gas shock wave.
2. A method according to claim 1 wherein the step of effecting particle acceleration includes:
 - 15 positioning the particles on the target side of a planar carrier sheet, and subjecting the sheet to the cold gas shock, thereby to accelerate the particles.
3. The method of claim 2 wherein the sheet is a resilient membrane fixedly secured at its edges against movement, and the cold gas shock wave causes distension but not rupture of the membrane, thereby protecting the target from the shock wave.
4. The method of claim 2 wherein the sheet is a resilient membrane fixedly secured at its edges against movement, and includes the step of positioning a restraining screen between the target cells and the membrane, such that the membrane is restrained against the screen prior to full distension or rupture, allowing the particles to be better launched, passing through the screen to the target.
 - 25
 - 30
5. The method of claim 2 wherein the sheet is a resilient membrane fixedly secured at its edges against movement, and permitting the shock wave to cause distension and rupture of the membrane, thereby

launching, deagglomerating and dispersing the particles over a broader region of the target cells.

6. The method of claim 5 wherein the sheet is formed of a non-resilient material.

5 7. The method of claim 6 wherein the sheet is aluminum foil.

8. The method of claim 2 wherein the sheet positioned to be subjected to the force of the gas shock wave is not tightly anchored in place, such that it will
10 be accelerated to move freely in the direction of the target cells until restrained by a barrier which permits the particles to move toward the target cells.

9. The method of claim 8 wherein the sheet is formed of polyimide or polyester film.

15 10. The method of claim 8 wherein the restraining of the sheet is done by positioning a screen in the path of movement of the sheet.

11. The method of claim 1 wherein the planar surface is a screen that permits the force of the shock
20 wave to pass through it to accelerate the particles by gas entrainment.

12. The method of claim 11 which includes the additional step of baffling the shock wave after it passes the screen, to deflect some of the force of the
25 shock wave away from the target cells.

13. The method of claim 1 wherein the cold gas shock wave is formed by providing a source of pressurized gas, providing a rupturable membrane seal for the gas, and rupturing the seal to catastrophically
30 release the gas in the direction of the cells.

14. A method according to claim 13 wherein the step of effecting particle acceleration includes:

positioning the particles on the target side of a planar surface, and subjecting the surface to the cold
35 gas shock, thereby to accelerate the particles.

15. The method of claim 14 wherein the sheet is a resilient membrane fixedly secured at its edges against movement, and the cold gas shock wave causes distension but not rupture of the membrane, hereby protecting the
5 target from the shock wave.

16. The method of claim 14 wherein the sheet is a resilient membrane fixedly secured at its edges against movement, and includes the step of positioning a
10 restraining screen between the target cells and the membrane, such that the membrane is restrained against the screen prior to full distension or rupture, allowing the particles to be launched, passing through the screen to the target.

17. The method of claim 14 wherein the sheet is a
15 resilient membrane fixedly secured at its edges against movement, and permitting the shock wave to cause distension and rupture of the membrane, thereby launching, deagglomerating and dispersing the particles over a broader region of the target cells.

20 18. The method of claim 17 wherein the sheet is formed of a non-resilient material.

19. The method of claim 18 wherein the sheet is aluminum foil.

20. The method of claim 14 wherein the sheet
25 positioned to be subjected to the force of the gas shock wave is not tightly anchored in place, such that it will be accelerated to move freely in the direction of the target cells until restrained by a barrier which permits the particles to move toward the target cells.

30 21. The method of claim 20 wherein the sheet is formed of polyimide or polyester film.

22. The method of claim 20 wherein the restraining of the sheet is done by positioning a screen in the path of movement of the sheet.

23. The method of claim 14 wherein the planar surface is a screen that permits the force of the shock wave to pass through it to accelerate the particles by gas entrainment.

5 24. The method of claim 23 which includes the additional step of baffling the shock wave after it passes the screen, to deflect some of the force of the shock wave away from the target cells.

10 25. The method of claim 1 wherein the cold gas shock wave is formed by providing a source of pressurized gas, rapidly releasing the gas in the direction of the cells.

26. A method according to claim 25 wherein the step of effecting particle acceleration includes:

15 positioning the particles on the target side of a planar carrier sheet, and subjecting the sheet to the cold gas shock, thereby to accelerate the particles.

20 27. The method of claim 26 wherein the sheet is a resilient membrane fixedly secured at its edges against movement, and the cold gas shock wave causes distension but not rupture of the membrane, hereby protecting the target from the shock wave.

25 28. The method of claim 26 wherein the sheet is a resilient membrane fixedly secured at its edges against movement, and includes the step of positioning a restraining screen between the target cells and the membrane, such that the membrane is restrained against the screen prior to full distension or rupture, allowing the particles to be launched, passing through the screen
30 to the target.

29. The method of claim 26 wherein the sheet is a resilient membrane fixedly secured at its edges against movement, and permitting the shock wave to cause distension and rupture of the membrane, thereby

launching, deagglomerating, and dispersing the particles over a broader region of the target cells.

30. The method of claim 26 wherein the sheet positioned to be subjected to the force of the gas shock wave is not tightly anchored in place, such that it will be accelerated to move freely in the direction of the target cells until restrained by a barrier which permits the particles to move toward the target cells.

31. The method of claim 26 wherein the planar surface is a screen that permits the force of the shock wave to pass through it to accelerate the particles by gas entrainment.

32. The method of claim 26 which includes the additional step of baffling the shock wave after it passes the screen to deflect some of the force of the shock wave away from the target cells.

33. Apparatus for introducing particles carrying biological materials into a target of cells and/or tissue comprising:

a closed housing capable of sustaining a vacuum, the housing having a first port for applying a vacuum thereto, a major axis, and two other ports lying on the axis, one defining a throat portion when particles are accelerated and the second port opposite the throat introducing pressurized gas into a high pressure chamber,

a high pressure chamber positioned in the second port and adapted to catastrophically release gas stored therein to provide a gas shock wave directed toward the throat portion,

means for positioning the particles in the throat portion for their acceleration by the force of a shock wave toward the target, and

an interface secured to the throat portion for coupling the throat portion to the target.

34. An apparatus as in claim 33 wherein the housing is a small, mobile, potentially hand-held cylinder

35. The apparatus of claim 34 wherein the chamber is hollow with the portion toward the throat sealed by a
5 rupturable membrane.

36. The apparatus of claim 35 wherein the chamber includes means to mechanically rupture the membrane.

37. The apparatus of claim 36 wherein the high pressure chamber has a restricted inlet to limit the
10 rate of flow of gas to the membrane.

38. The apparatus of claim 35 wherein the rupture means is electromagnetically actuated.

39. The apparatus of claim 35 wherein the membrane is constructed of polyimide film.

40. The apparatus of claim 33 which includes
15 disposable carrier sheets positioned in the throat portion, the particles adapted to being positioned on the target side of the carrier sheet, whereby the particles can be propelled by the force of the shock
20 wave to the target.

41. The apparatus of claim 40 wherein the carrier sheet is resilient and which includes means to secure it in the throat portion, by gripping the periphery of the carrier sheet, the anchoring means being positionable at
25 various locations along the axis of the throat.

42. The apparatus of claim 41 which includes a screen secured by its edges between the carrier sheet and nozzle, thereby to restrain the movement of the membrane, but allowing membrane to distend partially.

43. The apparatus of claim 40 wherein the carrier sheet is rupturable and which includes means to secure it in the throat portion by gripping the periphery of the carrier sheet, the anchoring means being
30 positionable along the height of the axis.

44. The apparatus of claim 40 which includes an optional rupturable membrane secured in the throat portion by means gripping the periphery of the membrane, a resilient carrier sheet which is not securely anchored, such that it can fly freely, and an anchored barrier such as a screen, capable of stopping the carrier sheet but allowing particles to continue.

45. The apparatus of claim 33 wherein the chamber includes means to mechanically rupture the membrane.

46. The apparatus of claim 45 which includes a carrier sheet positioned in the throat portion, the particles adapted to being positioned on the target side of the carrier sheet, whereby the particles can be propelled by the force of the shock wave to the target.

47. The apparatus of claim 46 wherein the carrier sheet is resilient and which includes means to secure it in the throat portion by gripping the periphery of the carrier sheet, the anchoring means being positionable at various locations along the axis of the throat.

48. The apparatus of claim 47 which includes a screen secured by its edges between the carrier sheet and nozzle, thereby to restrain the movement of the membrane, but allowing membrane to distend partially.

49. The apparatus of claim 46 wherein the carrier sheet is rupturable and which includes means to secure it in the throat portion by gripping the periphery of the carrier sheet, the anchoring means being positionable along the height of the axis.

50. The apparatus of claim 46 which includes an optional rupturable membrane secured in the throat portion by means gripping the periphery of the membrane, a resilient carrier sheet which is not securely anchored, such that it can fly freely, and an anchored barrier such as a screen, capable of stopping the carrier sheet but allowing particles to continue.

51. The apparatus of claim 40 which includes a nozzle attached to the throat for passing the particles to a limited region of the target.

5 52. The apparatus of claim 51 which includes a screen positioned across the open end of the interface to prevent the target from being drawn into the interface.

53. The apparatus of claim 51 which includes a port to a vacuum pump on the interface.

10 54. The apparatus of claim 40 which includes a closeable receptacle adopted to receive target cells, the receptacle encompassing the axis.

55. The apparatus of claim 54 which includes a port to a vacuum pump on the interface.

15 56. The apparatus of claim 40 which includes a removable sleeve insert adapted to be positioned in the throat portion, the disposable portions of which being positioned in the insert.

20 57. The apparatus of claim 56 which includes a carrier sheet positioned in the sleeve, the particles adapted to being disposed on the target side of the carrier sheet, whereby the particles can be propelled by the force of the shock wave to the target.

25 58. Apparatus for introducing particles carrying biological materials into a target of cells and/or tissue comprising:

30 a closed housing capable of sustaining a vacuum, the housing having a first port for applying a vacuum thereto, a major axis, and a second port lying on the axis, and a throat portion opposite the second port encompassing the axis,

35 a high pressure chamber positioned in the second port and adapted to catastrophically release gas stored therein to provide a gas shock wave directed toward the throat portion,

an antechamber lying on the axis and sealingly engaging the outside of the housing, the antechamber having a base which defines a protuberant portion adapted to be positioned contiguous the target, the
5 protuberant portion defining an opening encompassing the axis, the opening adapted to receive a screen to support target tissue.

59. The apparatus of claim 58 which includes a
10 baffle plate positioned orthogonally of the axis in the antechamber and defining an orifice for particles along the axis.

60. A method of introducing particles carrying biological materials into a target of cells, and/or tissue, said method comprising the steps of:
15 accelerating the particles sufficiently to cause them to penetrate the surface of the cells and become incorporated into the interior of the cells and/or tissue without killing the cells and/or tissue and effecting the particle acceleration by subjecting the
20 particles to the force of a gas shock wave, positioning the particles on the target side of a planar surface, and subjecting the surface to the gas shock, thereby to accelerate the particles.

61. The method of claim 60 wherein the planar
25 surface is a resilient membrane fixedly secured at its edges against movement, and includes the step of positioning a restraining screen between the target cells and the membrane, such that the membrane is restrained against the screen prior to full distension
30 or rupture, allowing the particles to be launched, passing through the screen to the target.

62. The method of claim 60 wherein the sheet is a resilient membrane fixedly secured at its edges against
35 movement, and permitting the shock wave to cause distension and rupture of the membrane, thereby

launching, deagglomerating and dispersing the particles over a broader region of the target cells.

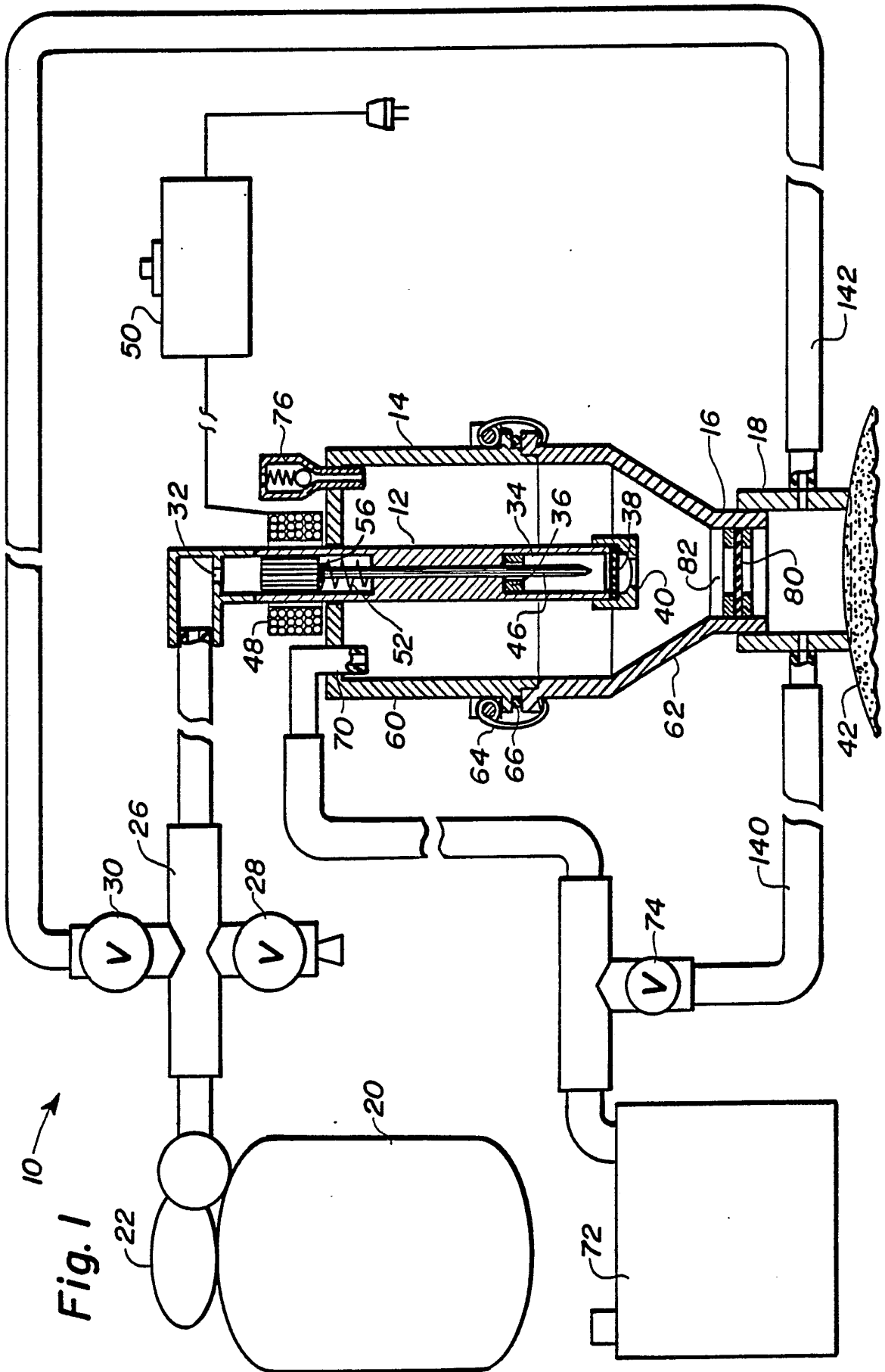
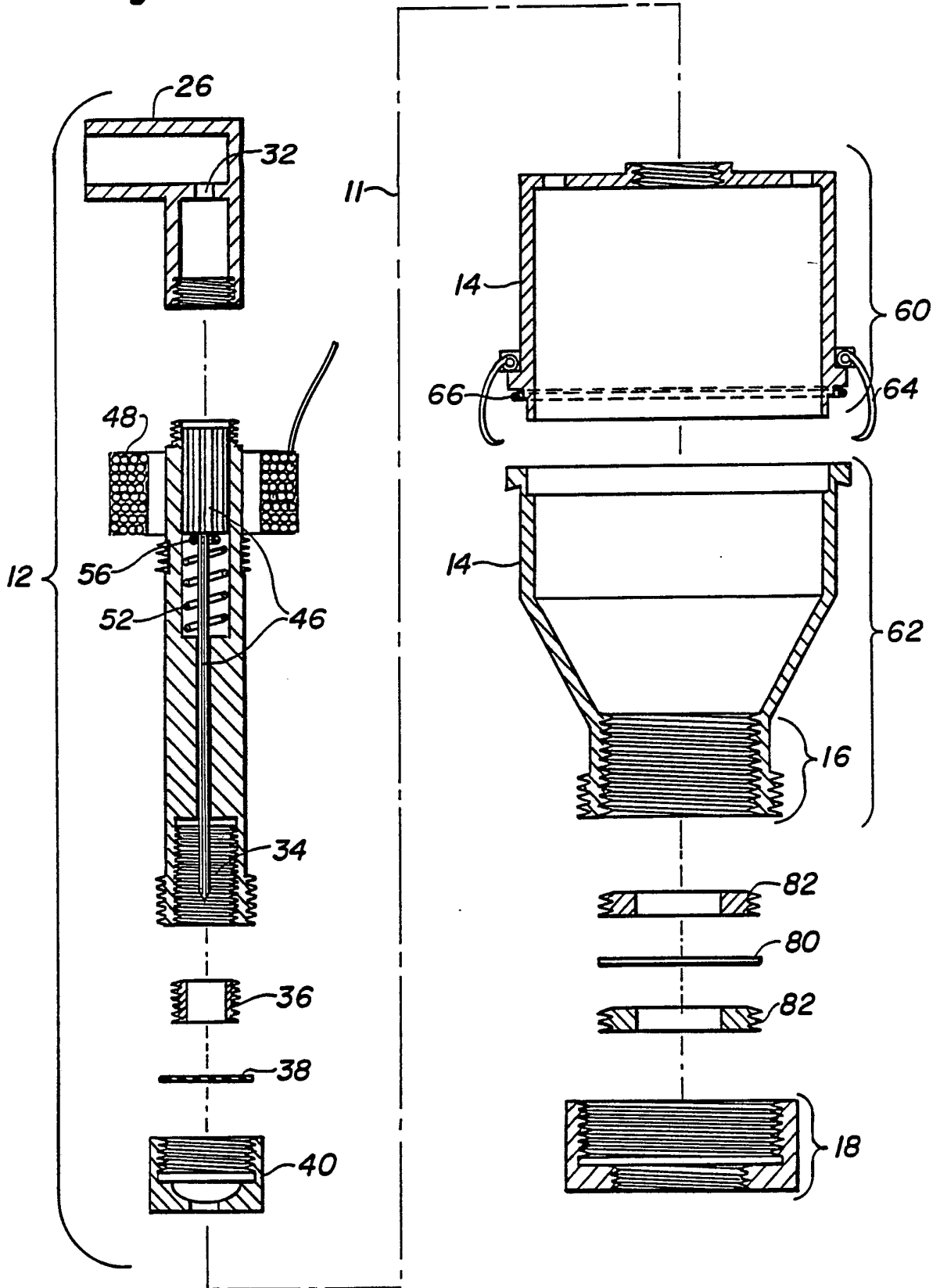


Fig. 2



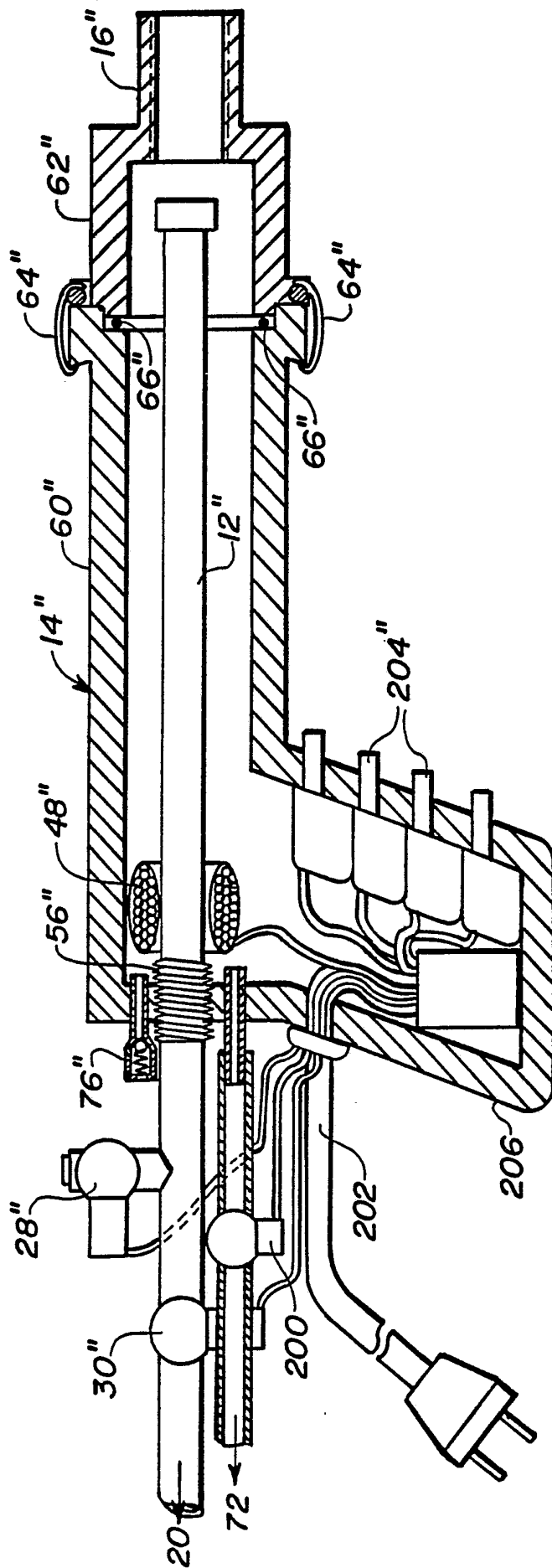


Fig. 3

Fig. 4

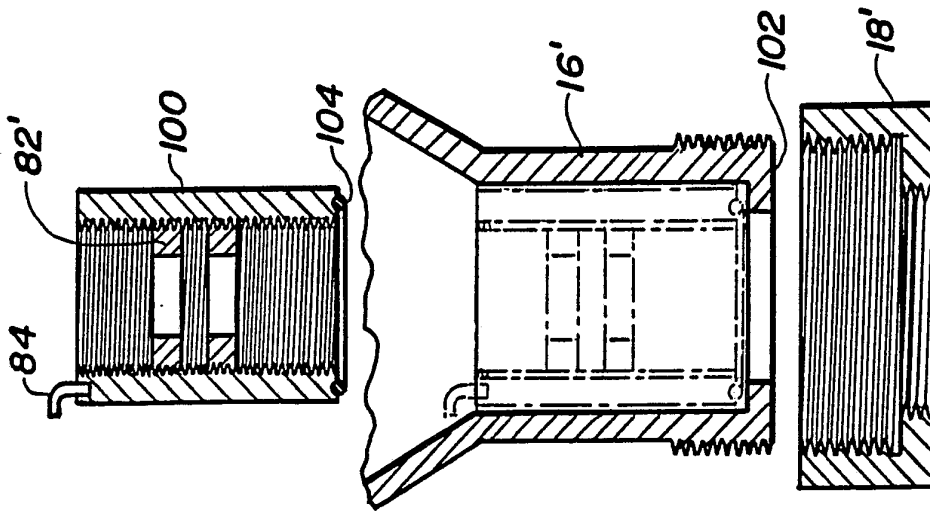


Fig. 5a

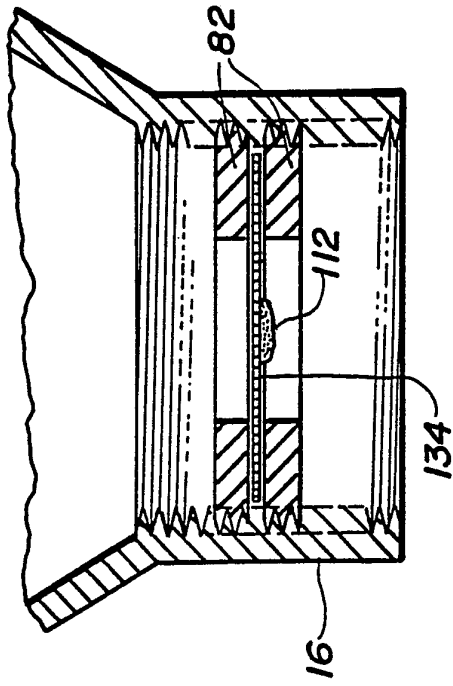
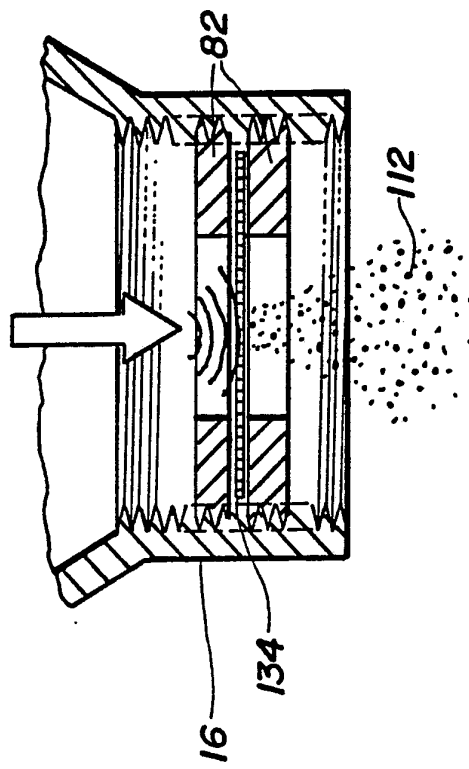


Fig. 5b



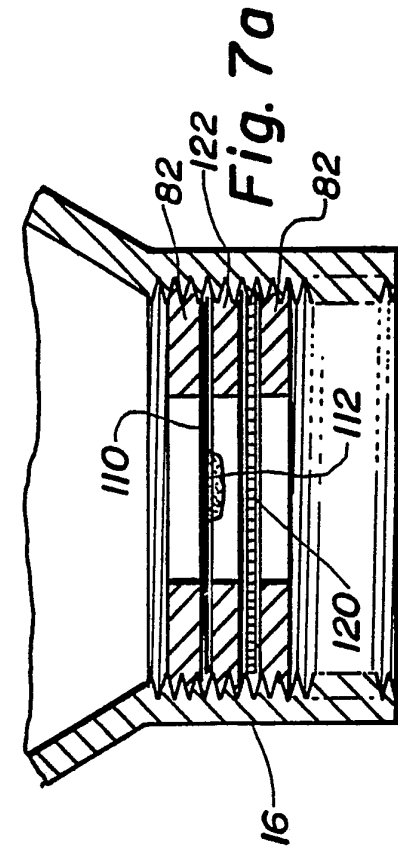


Fig. 6a

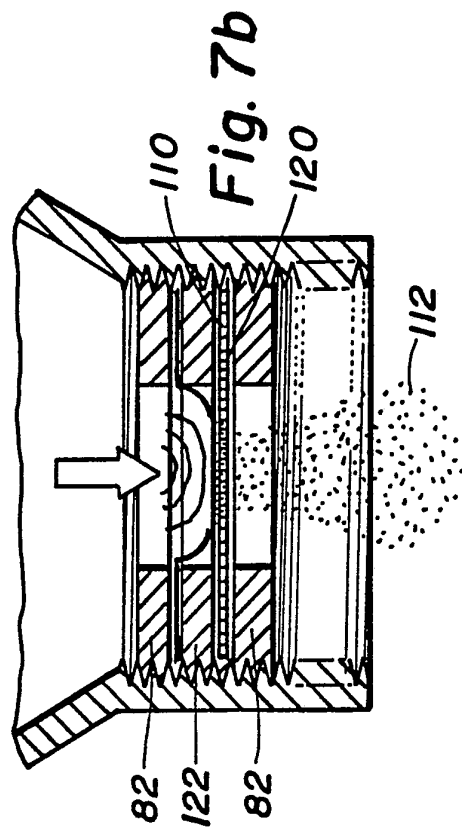


Fig. 6b

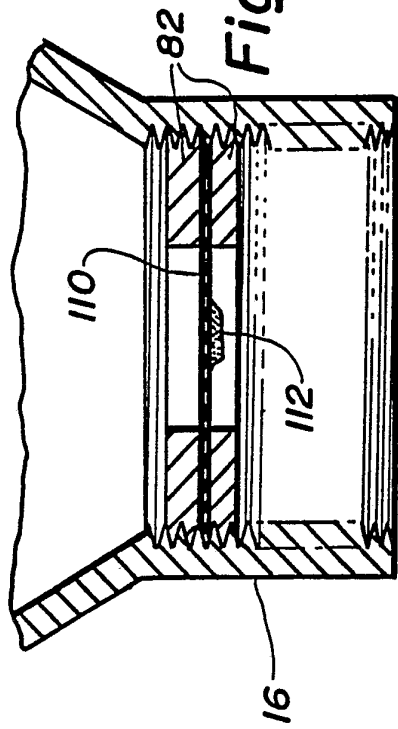


Fig. 7a

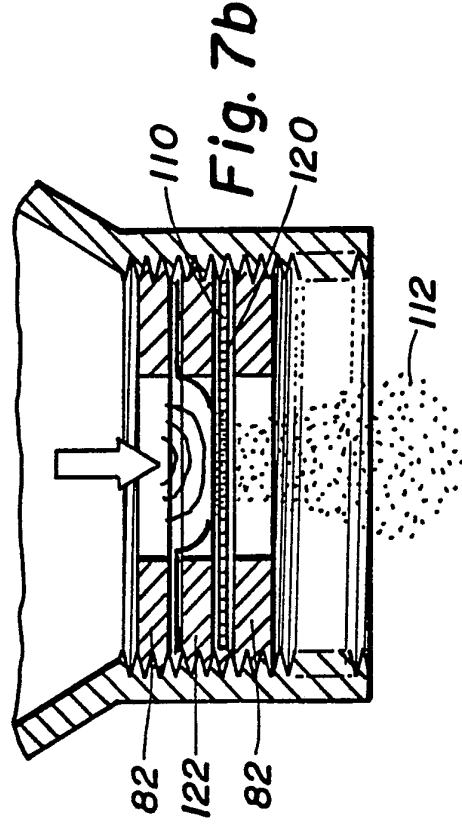


Fig. 7b

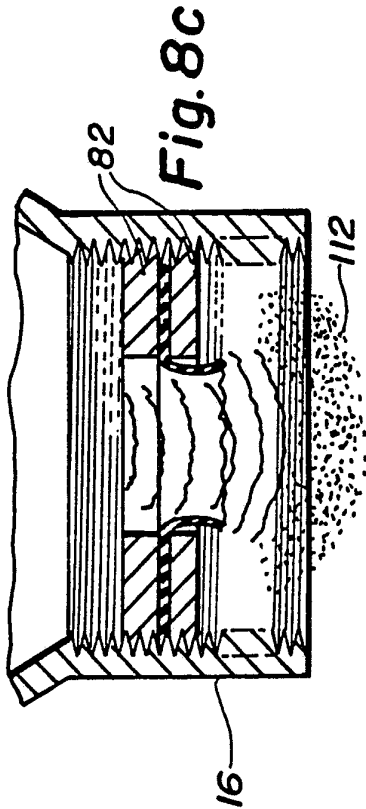
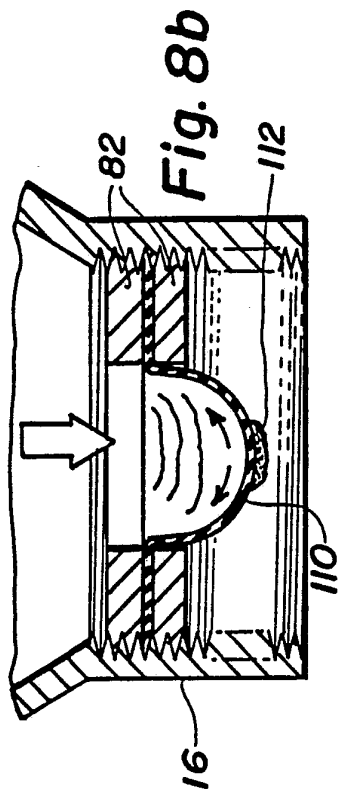
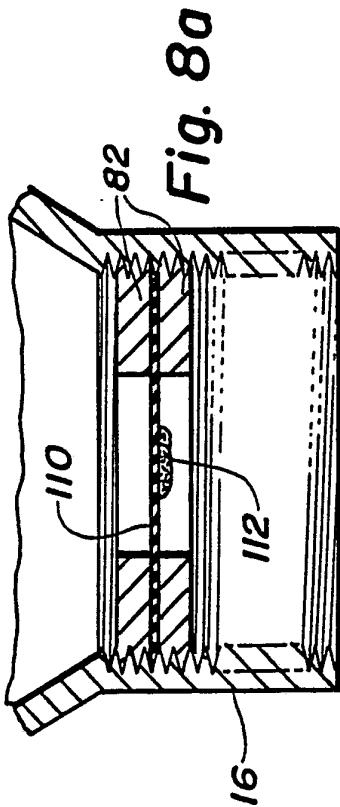
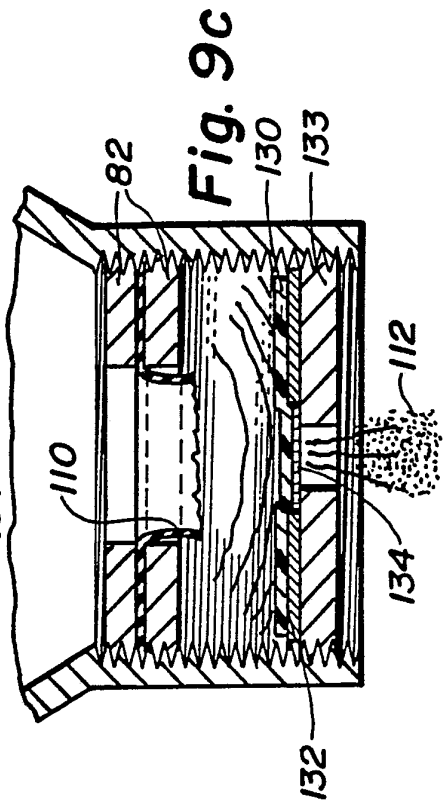
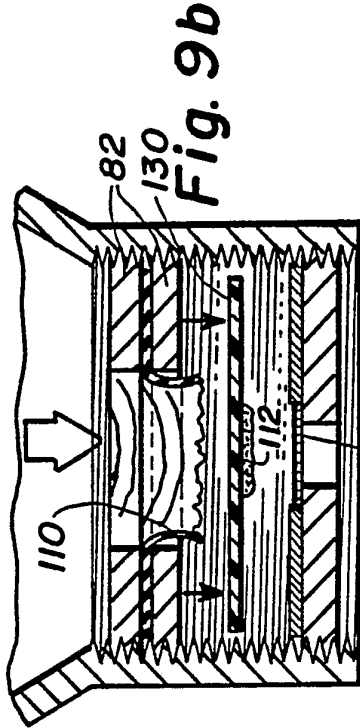
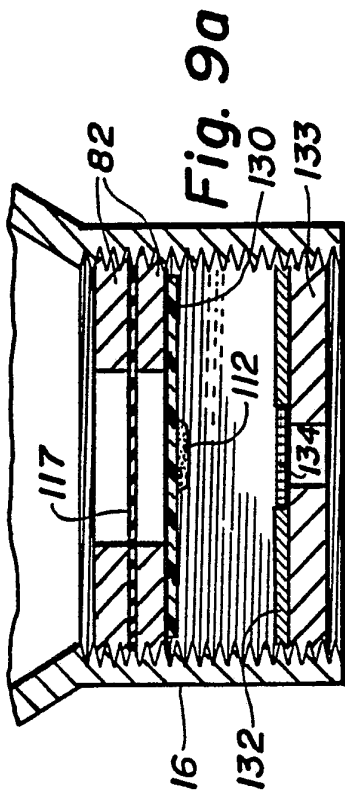


Fig. 10

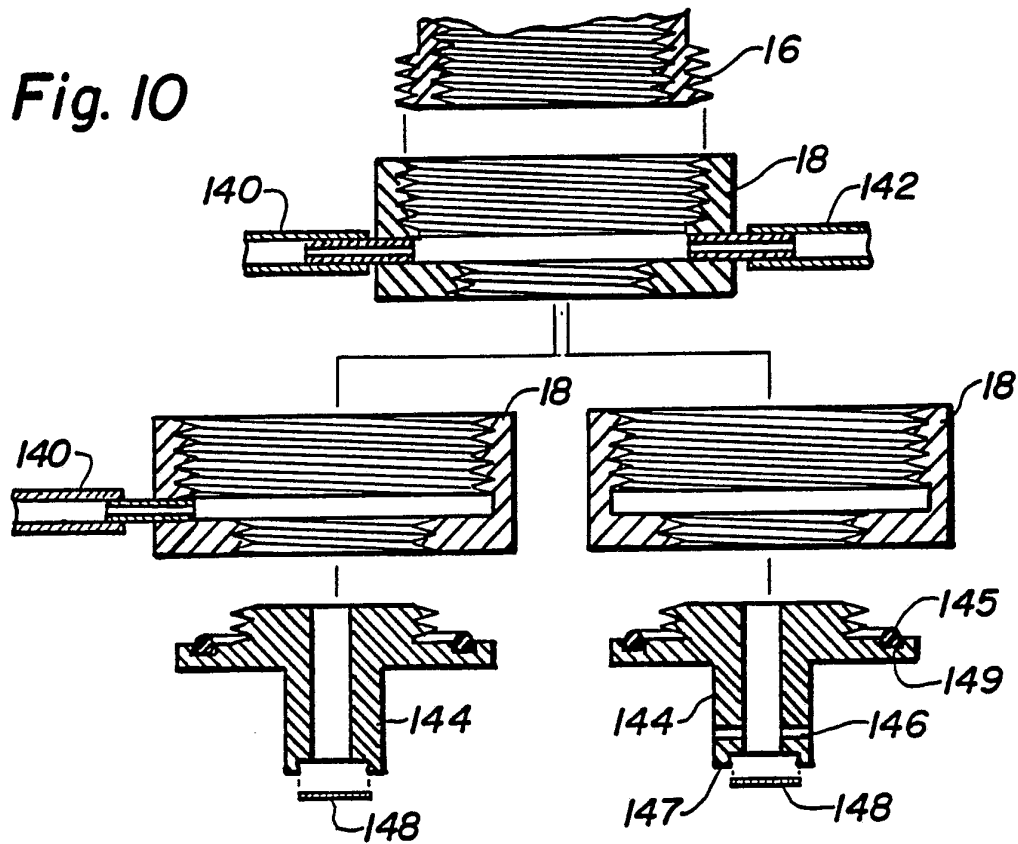


Fig. 11

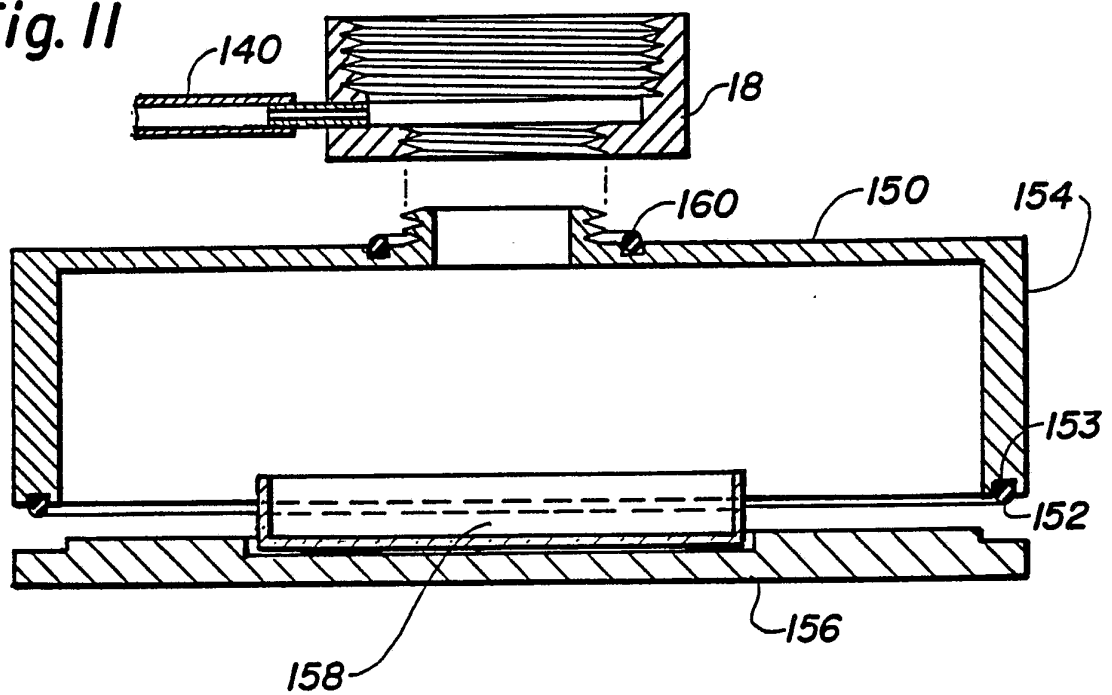


Fig. 12

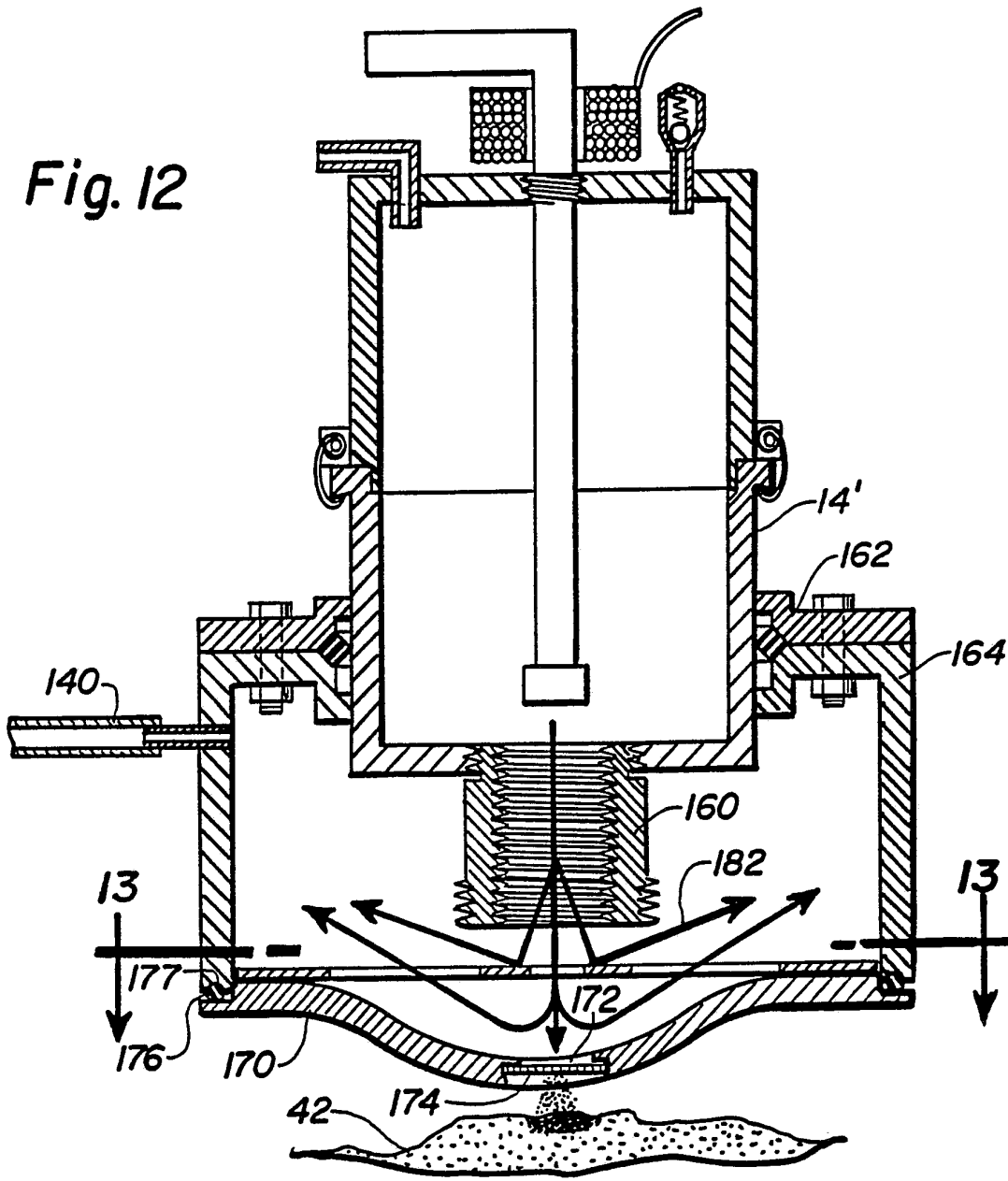


Fig. 13

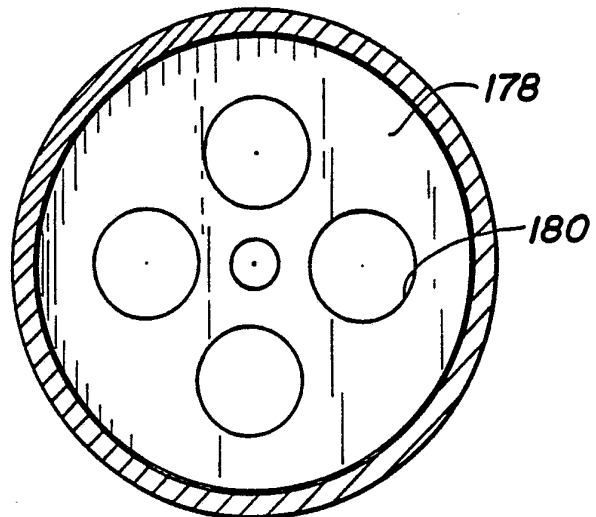


Fig. 14 a

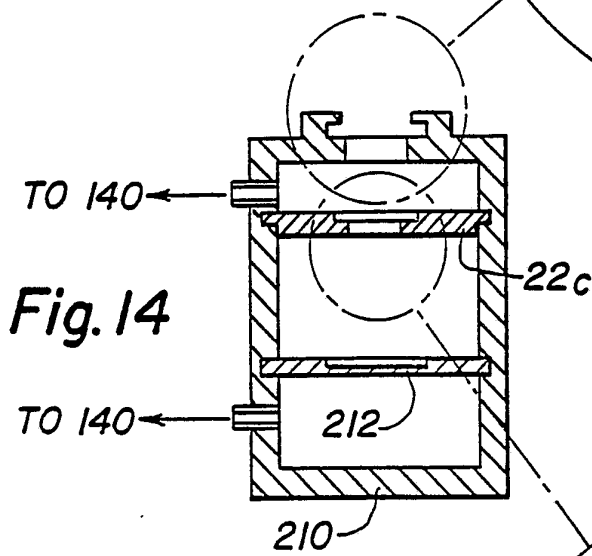
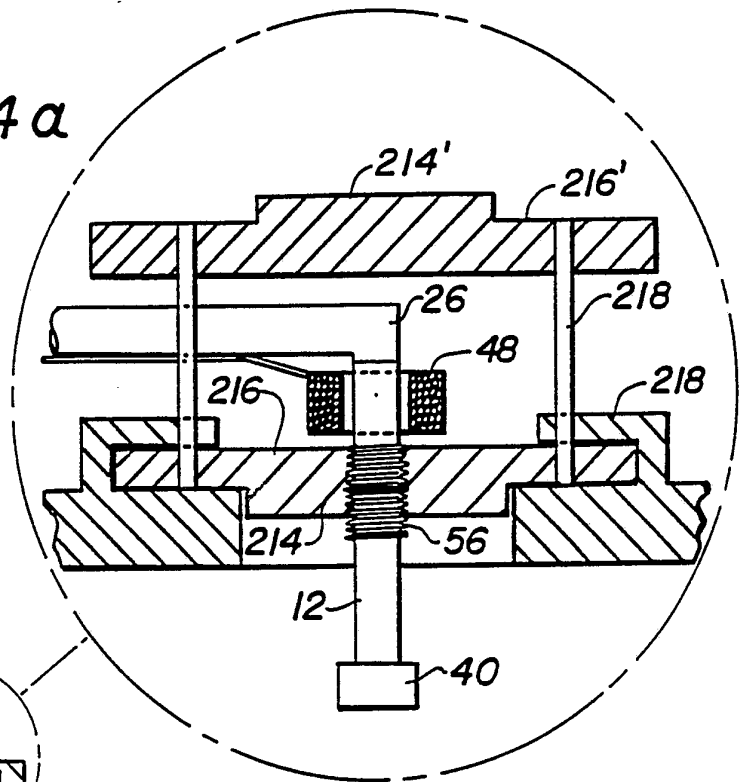
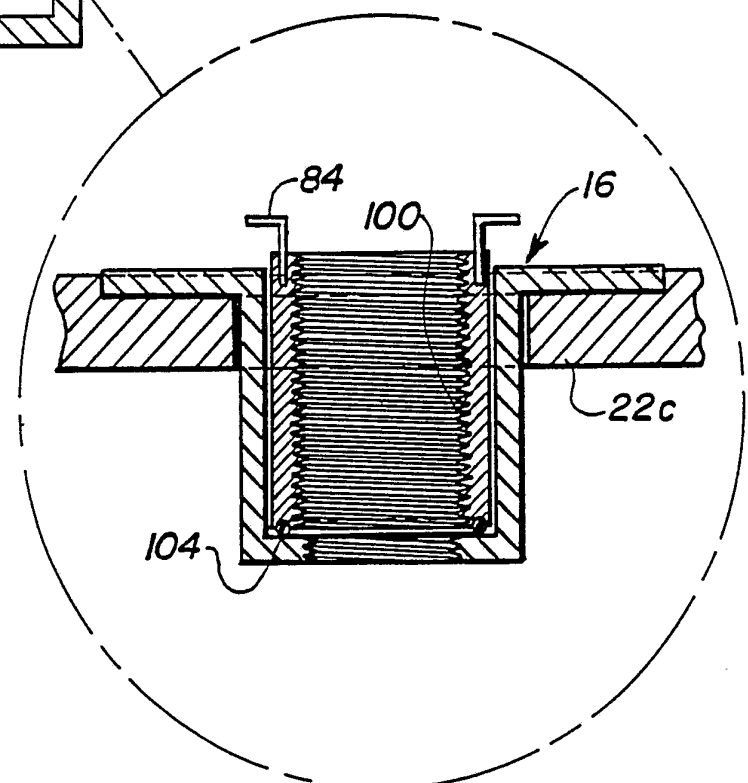


Fig. 14

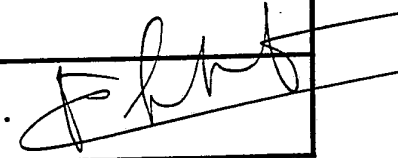
Fig. 14 b



INTERNATIONAL SEARCH REPORT

PCT/US 91/02816

International Application No

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC Int.Cl. 5 C12N15/87 ; C12M1/00 ; C12M3/00		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
Int.Cl. 5	C12N ; C12M	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹		
Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
P,X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. vol. 88, no. 7, April 1991, WASHINGTON US pages 2726 - 2730; WILLIAMS, R.S. ET AL.: 'In troduction of foreign genes into tissues of living mice by DNA-coated microprojectiles ' see the whole document	1,2, 8-10,13, 20,30, 33,60
X	JOURNAL OF PARTICULATE SCIENCE AND TECHNOLOGY vol. 5, no. 1, 1987, WASHINGTON, US pages 27 - 37; SANFORD, J.C. ET AL.: 'Delivery of substances into cells and tissues using a particle bombardment process ' cited in the application see figure 1	1
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<p>¹⁰ Special categories of cited documents : ¹⁰</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"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</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"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.</p> <p>"&" document member of the same patent family</p>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
14 AUGUST 1991	09.09.91	
International Searching Authority	Signature of Authorized Officer	
EUROPEAN PATENT OFFICE	CHAMBONNET F.J. 	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
Y	TRENDS IN BIOTECHNOLOGY. vol. 6, no. 12, December 1987, CAMBRIDGE GB pages 299 - 302; SANFORD, J.C.: 'The biolistic process ' cited in the application see the whole document ---	1,2,8, 14,30
Y	EP,A,270 356 (AGRACETUS) June 8, 1988 cited in the application see page 3, column 4, line 60 - page 5, column 7, line 5; claim 25; figure 1 ---	1,2,8, 14,30

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.**

US 9102816
SA 47598

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

14/08/91

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-270356	08-06-88	AU-B- 610534 AU-A- 8186387 JP-A- 63258525	23-05-91 09-06-88 26-10-88