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(54) **MICROSEEDING DEVICE FOR GENE DELIVERY BY MICRONEEDLE INJECTION**

(76) Inventors: **Elof Eriksson**, Wellesley, MA (US); **Christian Baker**, Weymouth, MA (US); **W. Robert Allison**, Sudbury, MA (US); **Tom Johnson**, Milford, NH (US); **Anthony Downing**, Ayer, MA (US); **David Porait**, Newton, MA (US); **Denis Labombard**, Georgetown, MA (US); **John Navickas**, Norfolk, MA (US); **Patrick Guiney**, Concord, MA (US)

Correspondence Address:
QUARLES & BRADY LLP
411 E. WISCONSIN AVENUE
SUITE 2040
MILWAUKEE, WI 53202-4497 (US)

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Publication Classification

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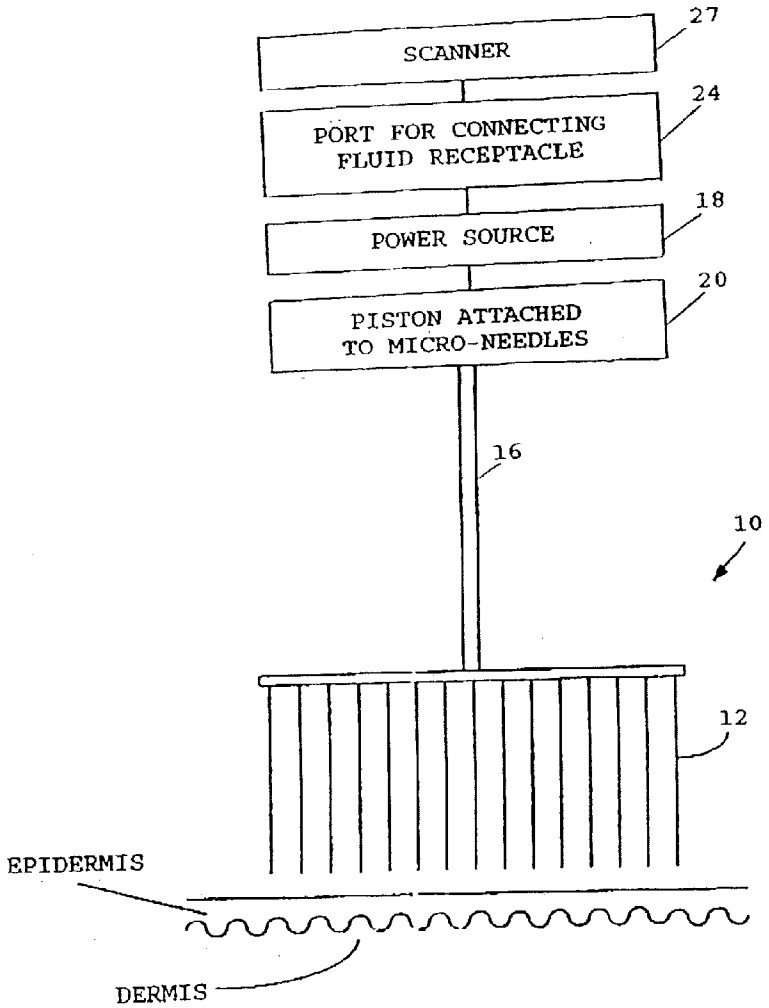
(52) **U.S. Cl.** **514/44**; 604/500

(57) **ABSTRACT**

An apparatus is provided to enable the direct gene transfer of genetic material into a target cell site ("microseeding"), as a means of obtaining long term expression of native or non-native polypeptides in a host. The apparatus includes a matrix of microneedles that oscillate at a predetermined frequency and receive the genetic material from a delivery system that is integrated with the apparatus.



Serendia, LLC
Exhibit 2142
Jeisys v. Serendia
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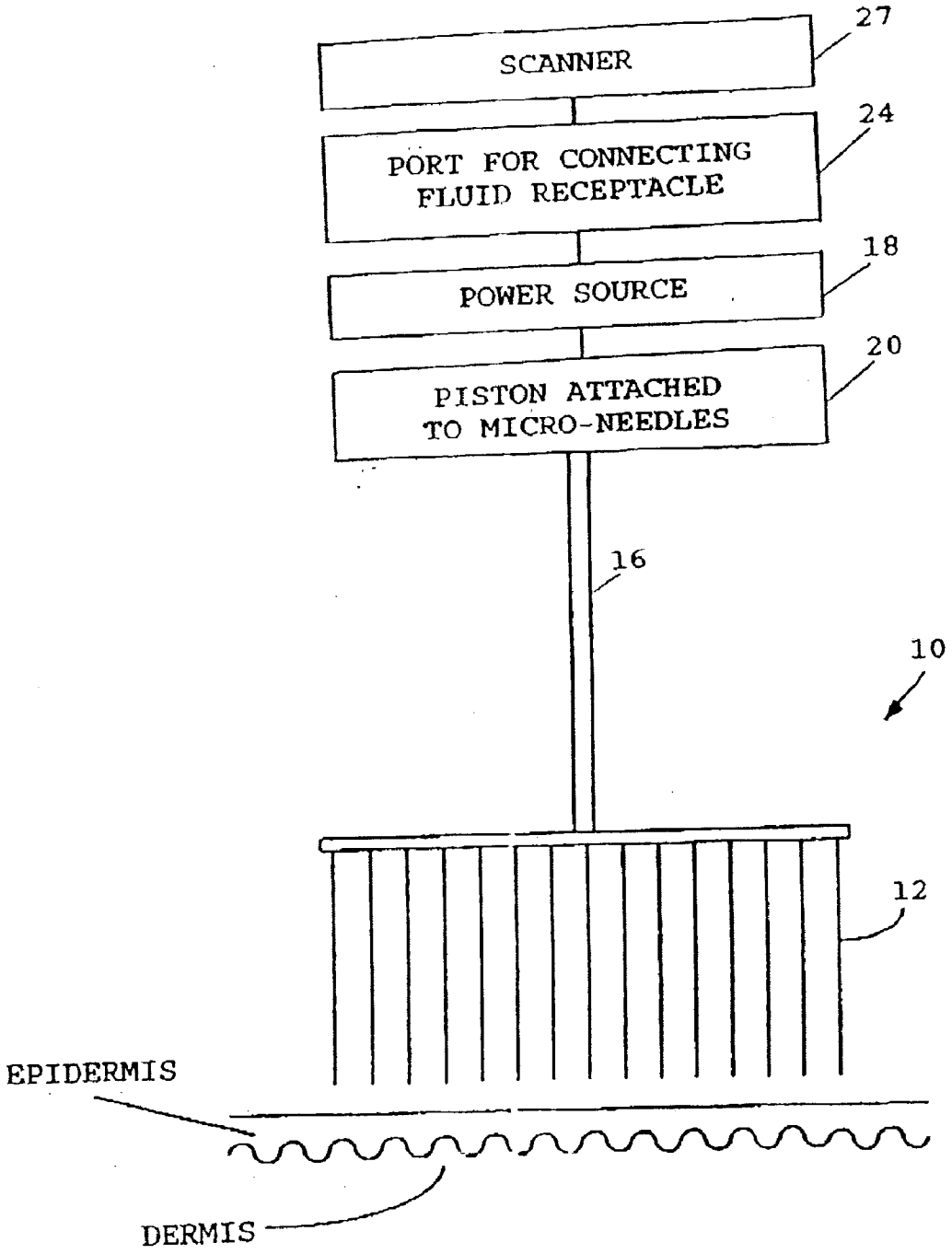
Ex. 2142-001


SCANNER
27
PORT FOR CONNECTING FLUID RECEPTACLE
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POWER SOURCE
18
PISTON ATTACHED TO MICRO-NEEDLES
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16
10
12
EPIDERMIS
DERMIS

FIG. 1

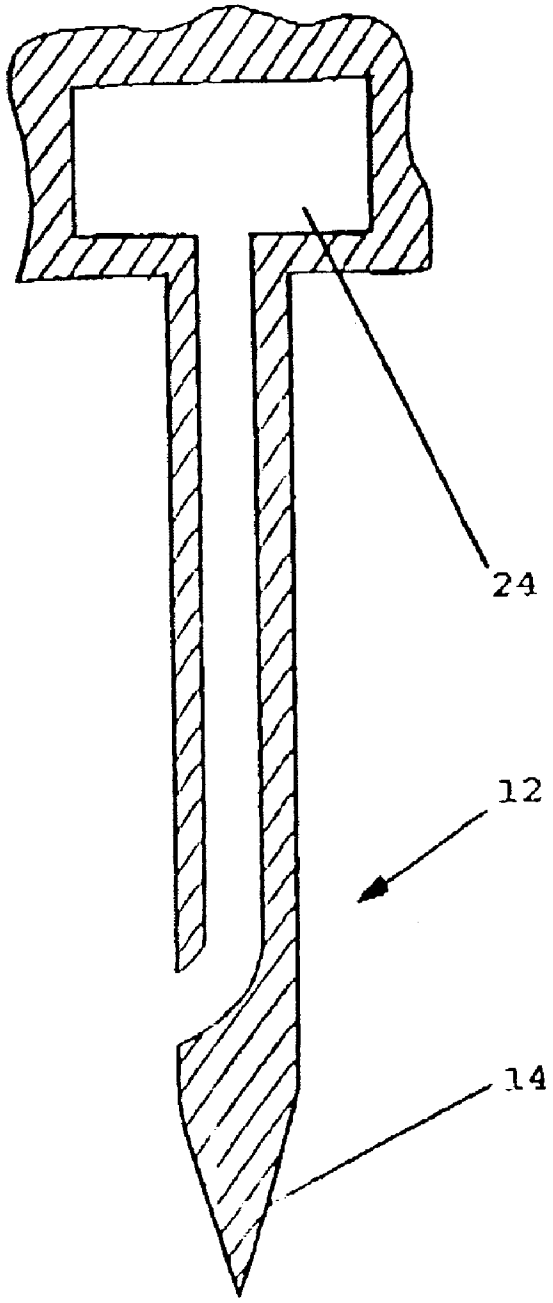


FIG. 2

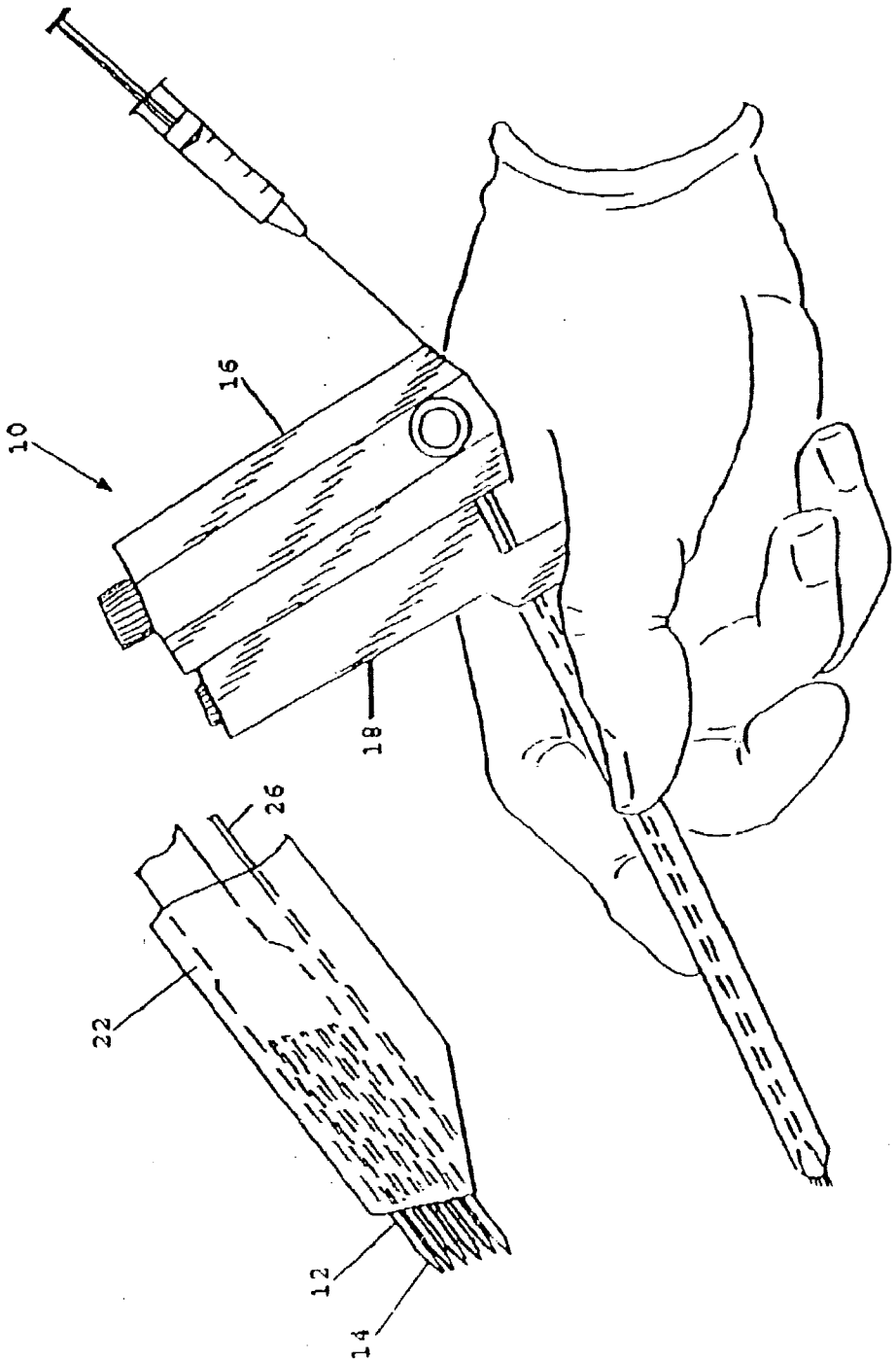


FIG. 3a

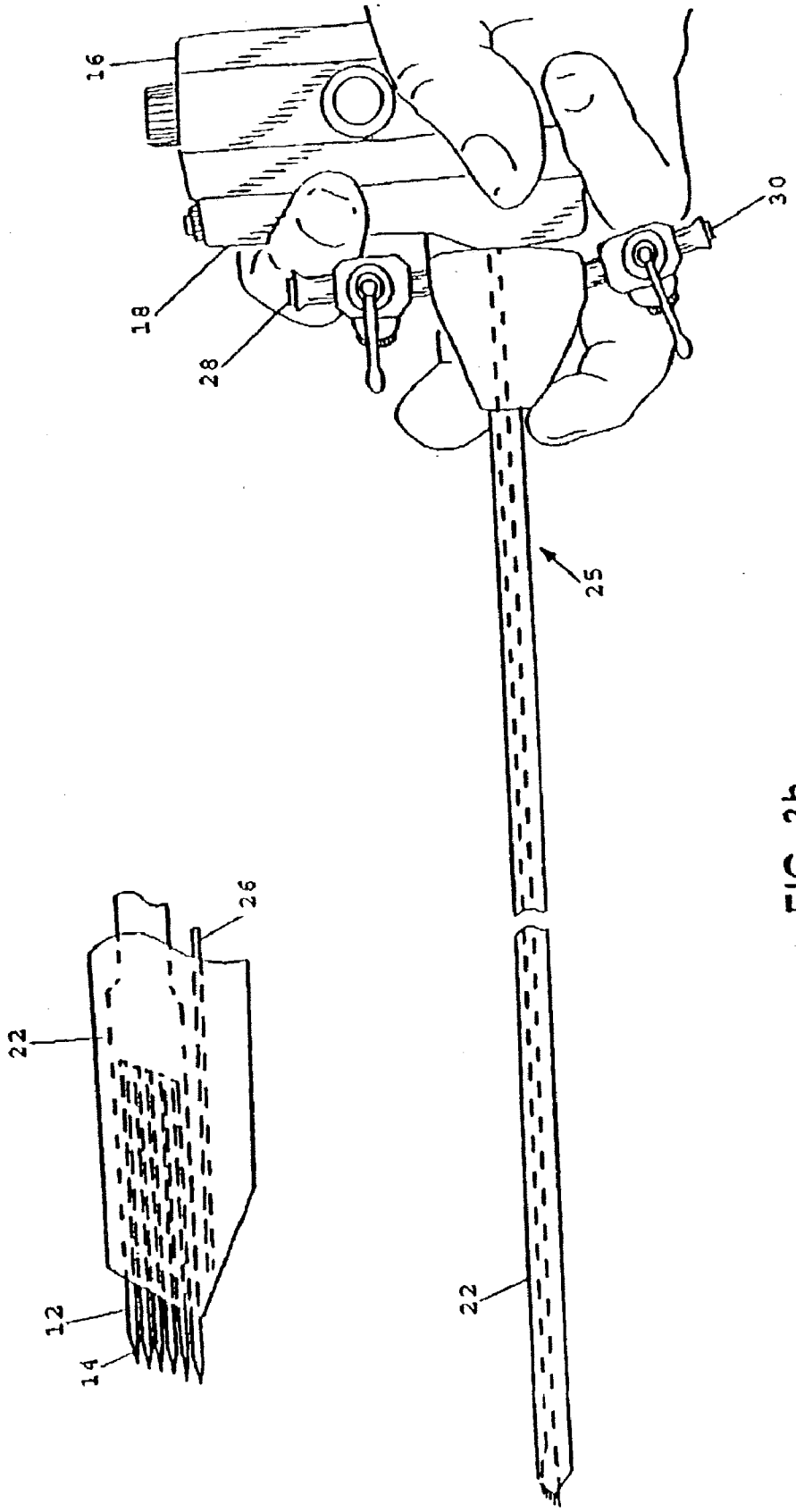
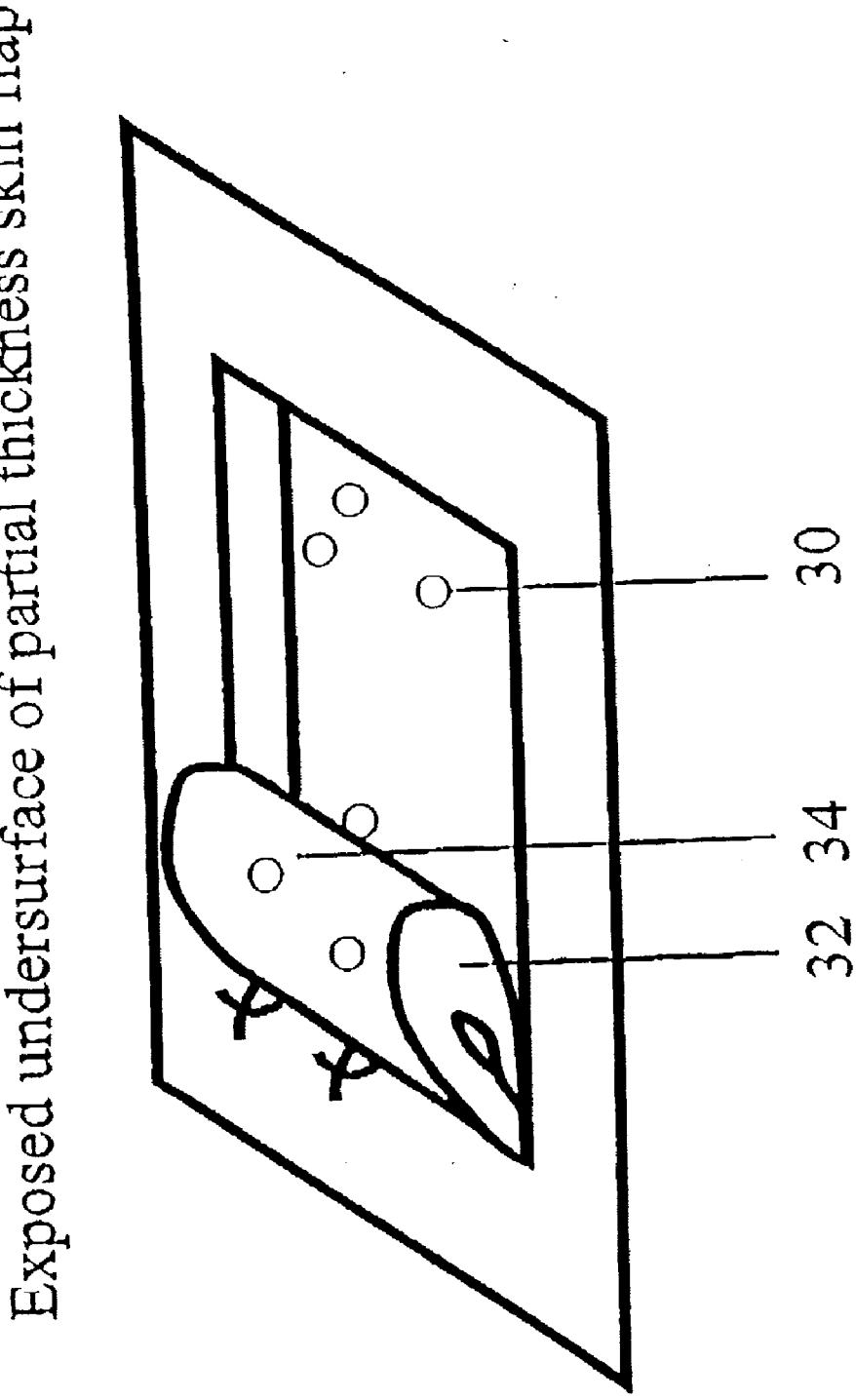


FIG. 3b


Exposed undersurface of partial thickness skin flap
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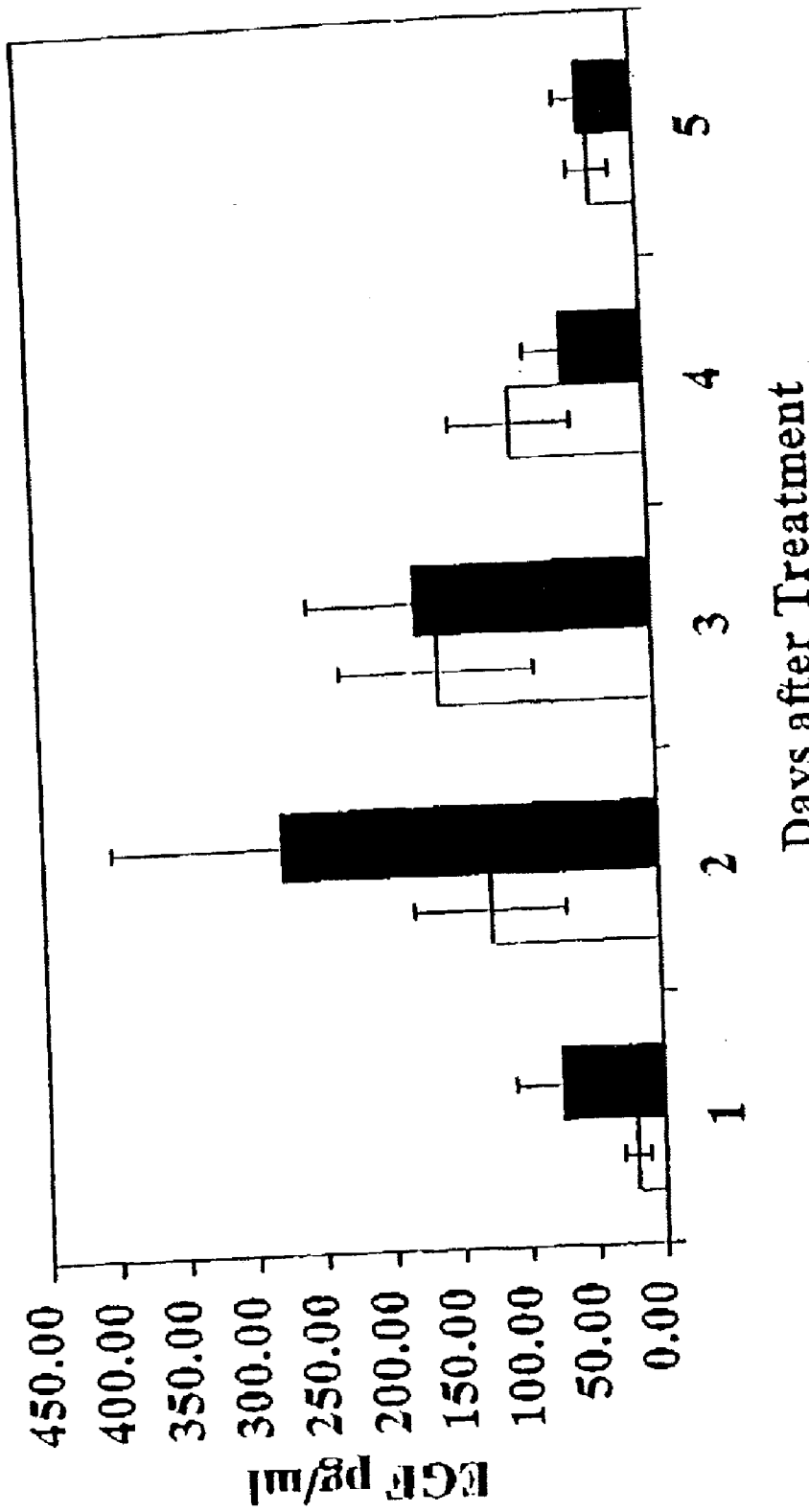
34
32

FIG 4


EGF pg/ml
450.00
400.00
350.00
300.00
250.00
200.00
150.00
100.00
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0.00
1
2
3
4
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Days after Treatment

FIG 5

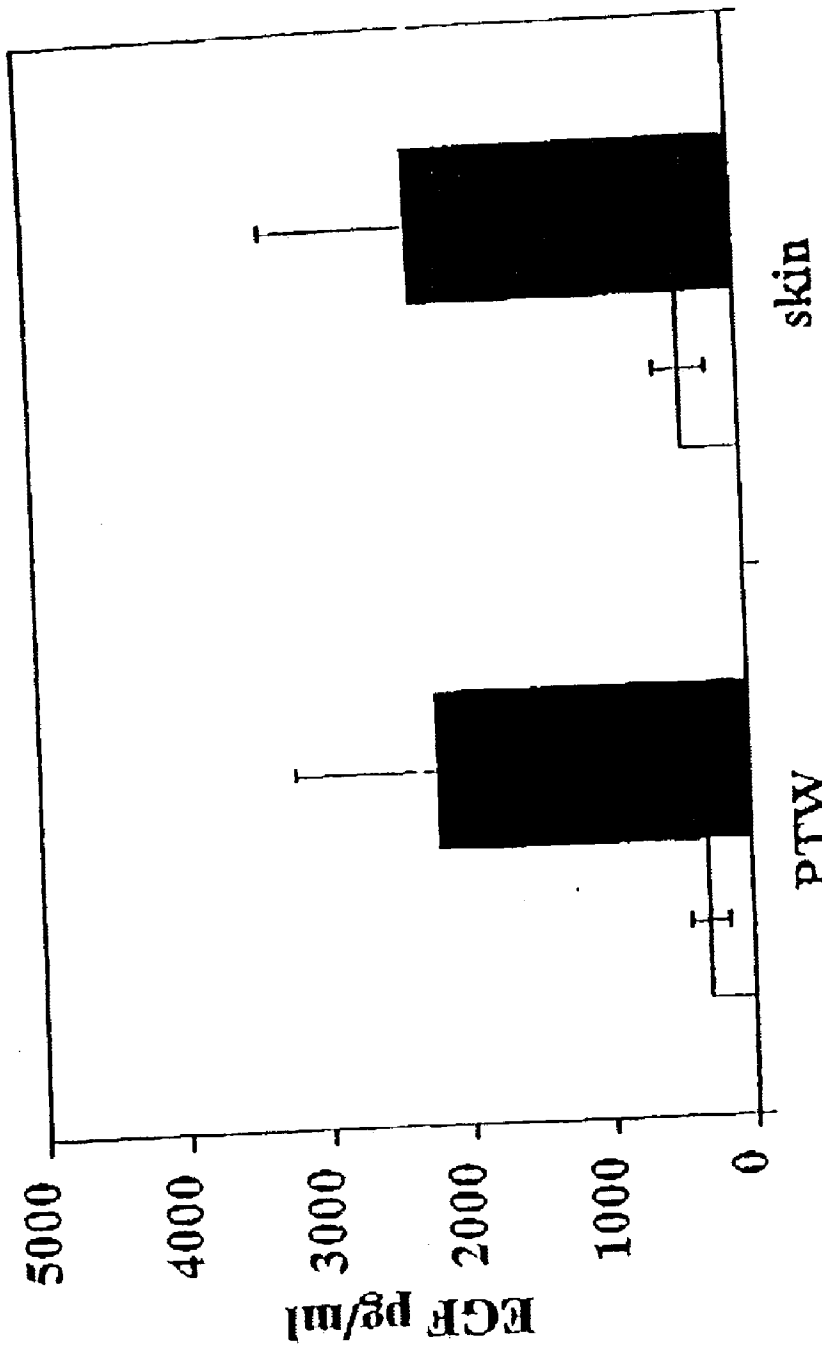

FIG 6

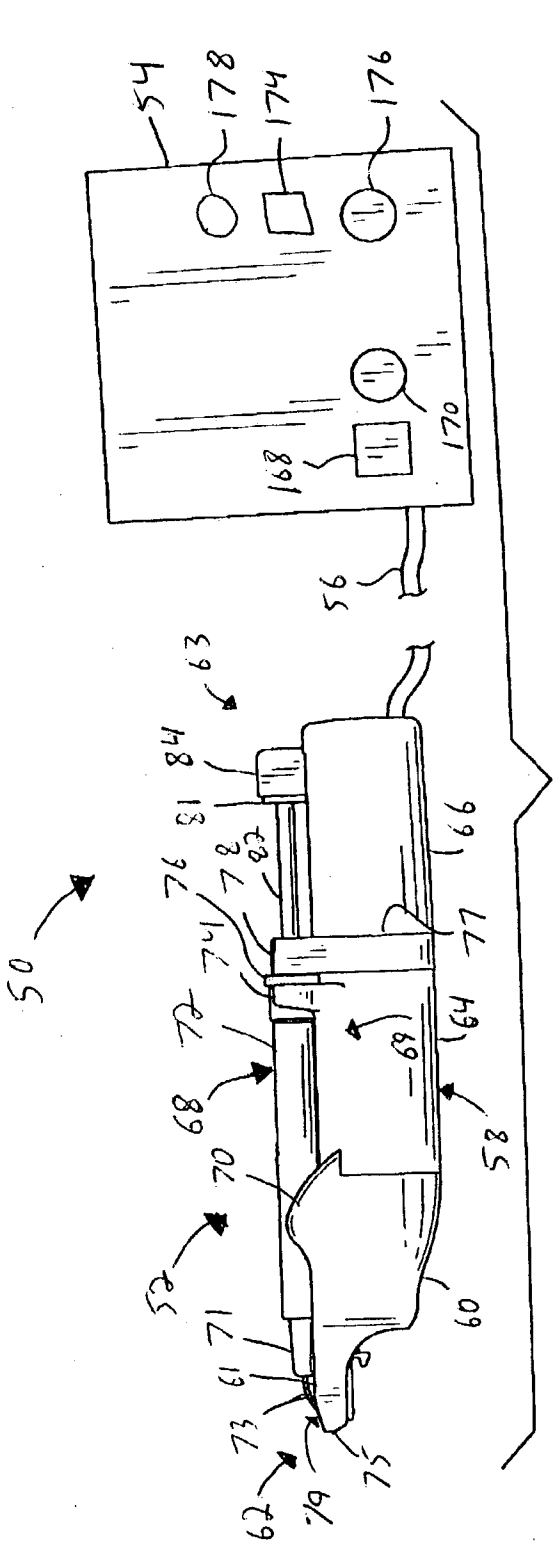

FIG. 7

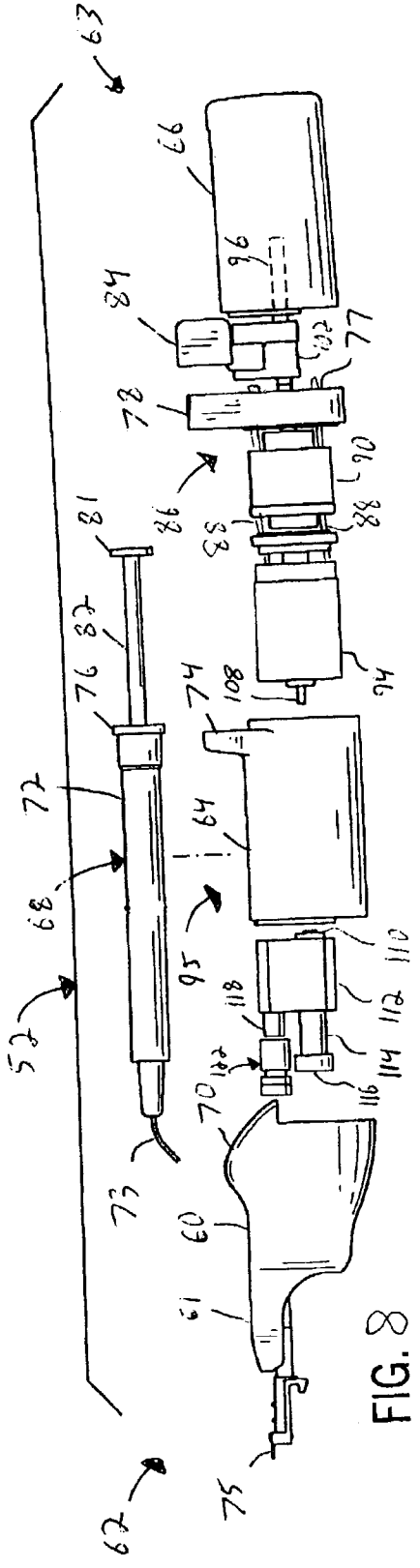

FIG. 8

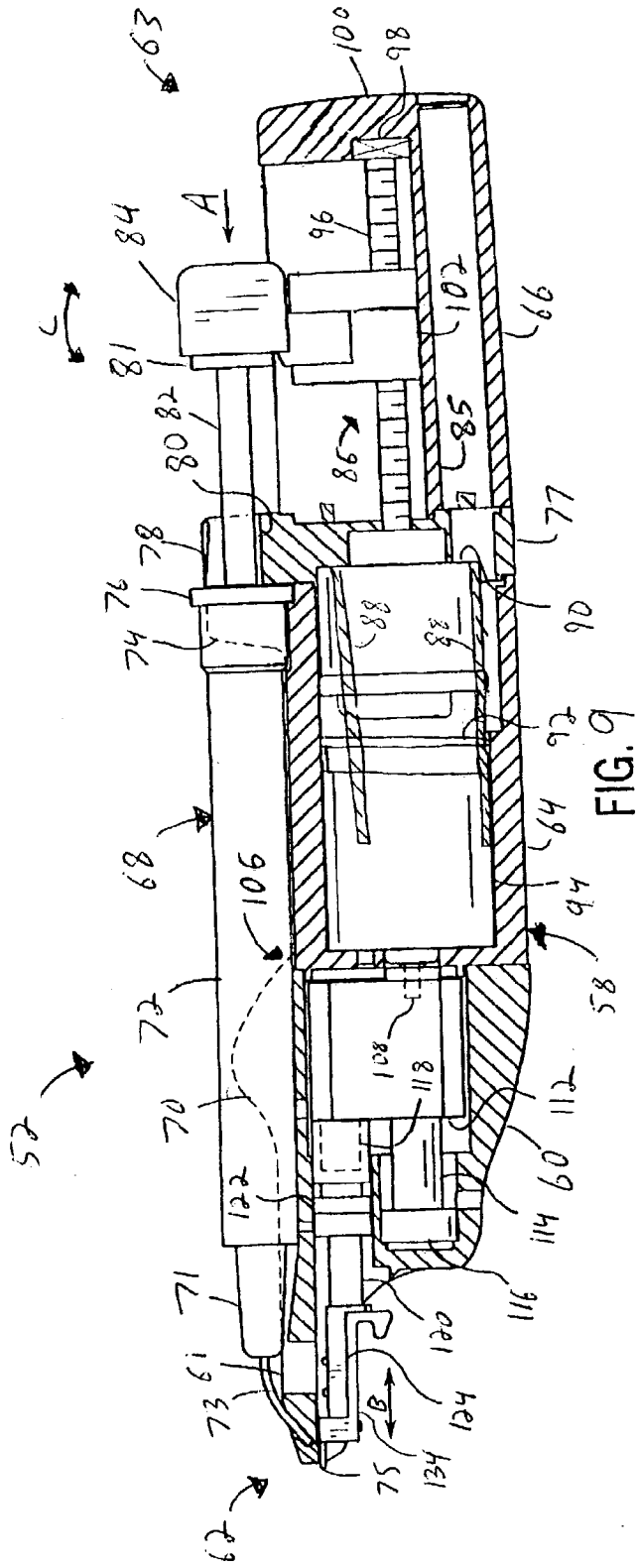
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FIG. 9

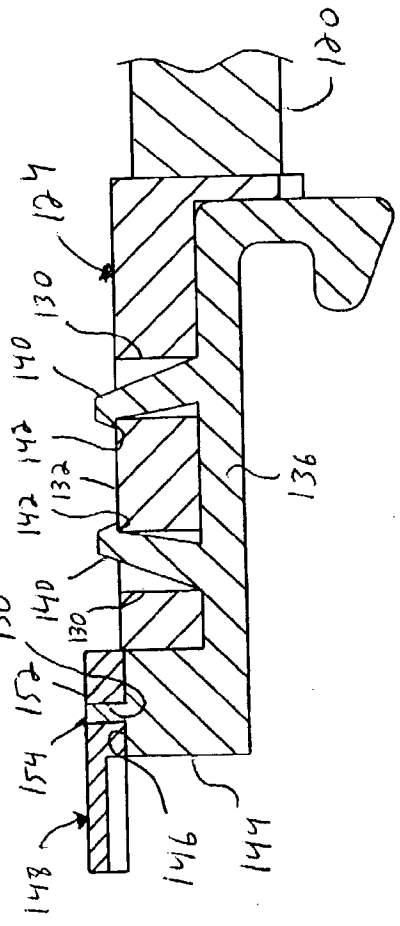
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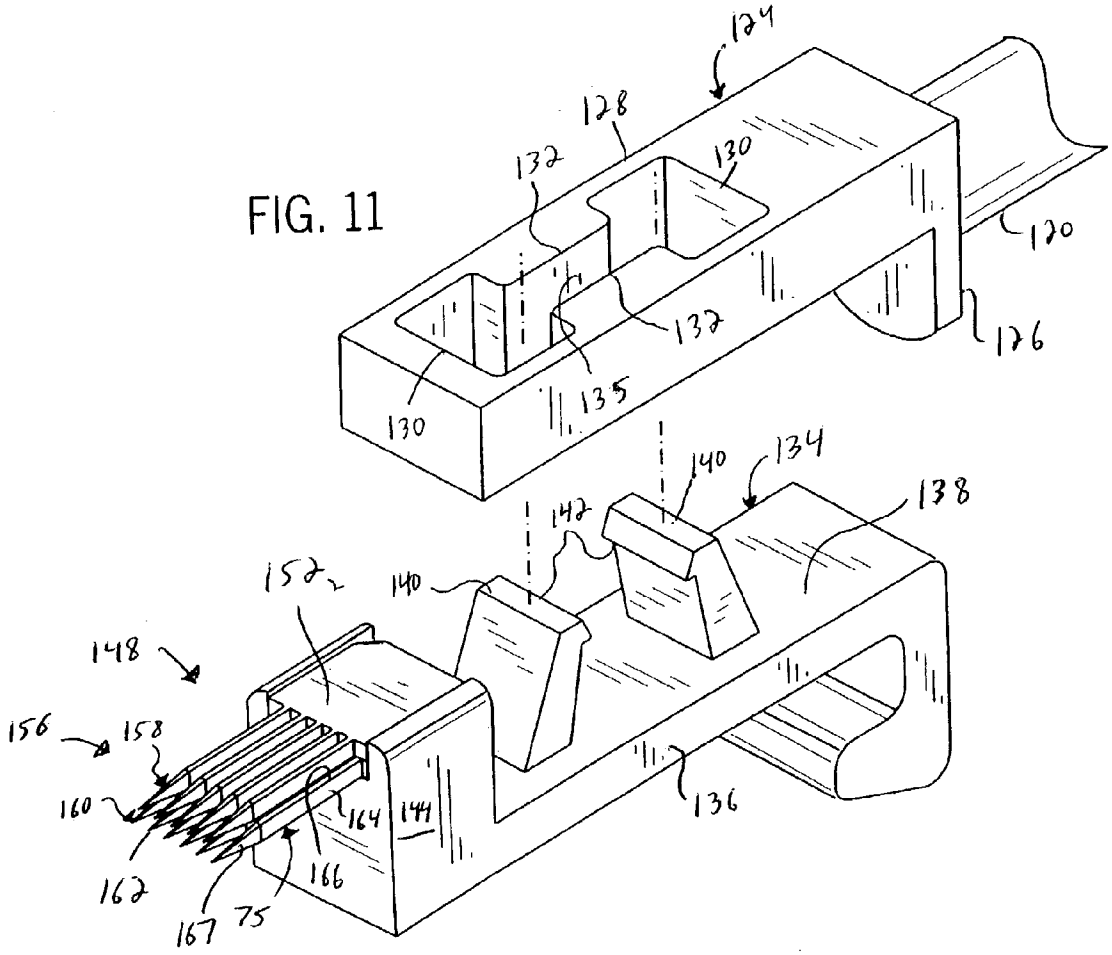
FIG. 10

FIG. 11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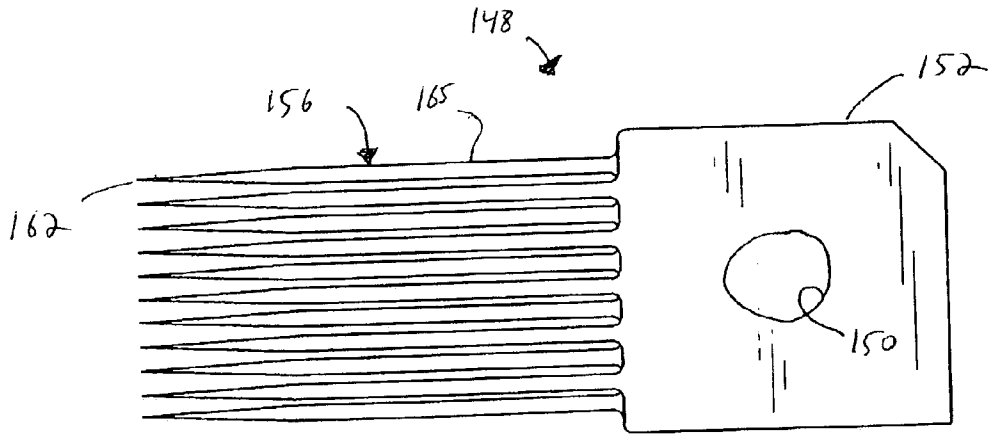
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FIG. 12

MICROSEEDING DEVICE FOR GENE DELIVERY BY MICRONEEDLE INJECTION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This is a continuation-in-part of U.S. Ser. No. 09/614,178, filed Jul. 11, 2000 which will issue on Feb. 25, 2003 as U.S. Pat. No. 6,525,030, and which is entitled "Gene Delivery by Microneedle Injection", which is a continuation-in-part of U.S. Ser. No. 08/445,265, filed May 19, 1995 which issued on Dec. 16, 1997 as U.S. Pat. No. 5,697,901 the disclosures of each which are hereby incorporated by reference as if set forth in their entirety herein.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] Not applicable.

BACKGROUND OF THE INVENTION

[0003] The invention relates to a system for delivering genetic material into cells in situ in a patient.

[0004] Various methods for introducing genetic material into an internal or external target site on an animal exist. Most prominent are methods of accelerated particle mediated gene transfer, such as are described in U.S. Pat. Nos. 4,945,050 (Sanford, et al.), 5,204,253 (Sanford, et al.) and 5,015,580 (Christou, et al.).

[0005] Other methods have focussed on introducing genetic material into skin cells, particularly keratinocytes. Keratinocytes are the principle cells which cover the surface of the body. They are capable of producing proteins, particularly keratin, which constitute the main surface barriers of the body. For several different reasons, keratinocytes are attractive potential targets for gene transfer. Since they are located on the surface of the body, they are easily accessed both for gene manipulation and monitoring. If complications from gene transfer would occur, for instance, the development of local tumors or local infections, these could more easily be treated in the skin than elsewhere.

[0006] To date, genetic manipulation of keratinocytes has been done in one principal way. Skin has been harvested, the keratinocytes have been separated from the fibroblasts, and then the keratinocytes individually isolated and brought into suspension. These suspensions of keratinocytes have then been cultured to confluence using tissue culture techniques, as reported by Rheinwald, J. G., Green, H. Serial Cultivation of Human Epidermal Keratinocytes: The Formation of Keratinizing Colonies From Cells. *Cell* G, 331-343, 1975.

[0007] The new genetic material has been introduced into the keratinocyte while being grown in vitro using either a viral vector or plasmid, as reported by Morgan, J. R., Barrandon, Y., Green, H., Mulligan, R. C. Expression of an Exogenous Growth Hormone Glue by Transplantable Human Epidermal Cells. *Science,* Vol. 237, 1476-1479 (1987) and Tenmer, J., Lindahl, A., Green, H. Human Growth Hormone in the Blood of Athymic Mice Grafted With Cultures of Hormone-Secreting Human Keratinocytes. *FASEB J.,* 4:3245-3250 (1990). The transfected cells are then usually resuspended and grown on selective media in order to increase the yield of transfection. Sheets of keratinocytes are then transplanted back to the mammal from which the keratinocytes were harvested.

[0008] Even though the in vitro yield has been acceptable, the in vivo yield has been unacceptably low, both short and long term. It has been very difficult to document any significant long term (more than thirty days) expression with these techniques, for example, as reported by Garlick, J. A., Katz, A. B., Fenvjes Esitaichman, L. B. Retrovirus Mediated Transduction of Cultured Epidermal Keratinocytes. *J. Invest. Dermatol.,* 97:824-829, 1991.

BRIEF SUMMARY OF THE INVENTION

[0009] Direct gene transfer of genetic material into internal or external target sites in optional combination with the use of an "in vivo" culture chamber is particularly effective for long term expression of polypeptides.

[0010] Direct delivery of genetic material is accomplished using a microseeding assembly for delivering genetic material from a dispenser to a target site. The assembly includes a housing and a matrix of microneedles disposed on a translatable shaft at one end of the housing. An integrated dispenser support assembly is connected to the housing for supporting a genetic material dispenser. The support assembly includes an actuator for initiating the delivery of genetic material from the dispenser to the microneedles. A drive assembly is connected to the housing and communicates with the actuator and the microneedles to initiate delivery of genetic material to the microneedles and to oscillate the microneedles at a predetermined frequency.

[0011] It is thus an object of the present invention to provide a microseeding apparatus having an integrated genetic material delivery system.

[0012] These and other aspects of the invention are not intended to define the scope of the invention for which purpose claims are provided. In the following description, reference is made to the accompanying drawings, which form a part hereof, and in which there is shown by way of illustration, and not limitation, a preferred embodiment of the invention. Such embodiment does not define the scope of the invention and reference must be made therefore to the claims for this purpose.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

[0013] The following figures are presented in which like reference numerals correspond to like elements throughout and in which:

[0014] **FIG. 1** is a schematic representation of a microseeding device for delivering genetic material into cells of a patient;

[0015] **FIG. 2** is a cross-sectional view of a microneedle **10** shown in **FIG. 1** constructed in accordance with one embodiment of the invention;

[0016] FIGS. **3**(*a* and *b*) are representations of microseeding devices for delivering genetic material into cells of a patient constructed in accordance with one embodiment of the invention;

[0017] **FIG. 4** is a schematic of the exposed undersurface of a partial thickness skin flap used to expose hair follicles to obtain epidermal stem cells;

[0018] **FIG. 5** shows the EGF concentration in target sites [intact skin (Stippled) and wound (Filled)] at various times after microseeding with an EGF-expressing genetic construct;

[0019] **FIG. 6** shows the levels of EGF in fluid and in tissue biopsies taken 3 days after delivery of pWRG1630 into intact skin and into partial thickness wounds;

[0020] **FIG. 7**.is a side elevation view of a microseeding device constructed in accordance with the preferred embodiment of the invention;

[0021] **FIG. 8** is an assembly view of the microseeding device illustrated in **FIG. 7**;

[0022] **FIG. 9** is a sectional side elevation view of the microseeding device illustrated in **FIG. 7**;

[0023] **FIG. 10** is a side elevation view of a yoke assembly of the microseeding device illustrated in **FIG. 7**;

[0024] **FIG. 11** is an assembly view of the yoke assembly illustrated in **FIG. 10**; and

[0025] **FIG. 12** is a side elevation view of a microneedle assembly constructed in accordance with the preferred embodiment of the invention.

DETAILED DESCRIPTION OF THE INVENTION

[0026] The present disclosure relates to a method and apparatus for delivering genetic material into cells at an internal or external target site on an human or non-human patient using a microneedle delivery apparatus. This process has been termed "microseeding" by the inventors. Examples of disorders that can be treated include wounds such as burns, pain, tumors, and infections.

[0027] Also described herein is a microneedle delivery apparatus for delivering DNA according to the method of the present invention.

[0028] Also described herein is use of a treatment system for wounds and other skin disorders that can optionally be employed in the method of the present invention when delivering DNA to an external target site. The treatment system has previously been described in U.S. Pat. No. 5,152,757, which is incorporated herein by reference.

[0029] Target Cells.

[0030] The method of the present invention delivers genetic material into any accessible target cell type in any human or non-human patient. Among non-human patients, most preferred are mammalian animals, especially domesticated animals such as dogs, cats, cattle, swine, goats, sheep and the like, in which the method is envisioned as a desired veterinary therapy.

[0031] The preferred target cells in all target animals are skin cells, which are most readily transduced with genetic material because of their proximity to the exterior of the patient. "Skin" is intended to encompass cells in all skin layers including epidermal, dermal, and subdermal layers. More specifically, skin includes superficial keratinocytes, stem cell keratinocytes and dermal fibroblasts. For purposes of this patent application, "skin" also encompasses the muscular tissue beneath the skin that can be accessed from the exterior by the microneedles described herein. The target skin can be intact or can be prepared for treatment by wounding. In addition, internal tissues and organs within a patient are also desirable target sites into which exogenous genetic material can be introduced in keeping with the method. Suitable internal sites include any soft tissue that can be pierced by microneedles as described herein. For purposes of this patent application, "internal sites" are those target sites which are not accessible from the exterior of the patient but which are accessible using the microneedle delivery device for internal use, as disclosed herein. Without intending to limit application of the method to particular internal sites, specifically envisioned as targets are smooth and striated muscle, connective and epithelial tissues, walls of abdominal passages, and internal organs including, but not limited to, liver, kidney, stomach, appendix, intestines, pancreas, lungs, heart, bladder, gall bladder, brain and other nervous system cells, as well as reproductive, endocrine, lymphatic and other glandular tissues. The region surrounding the bone, in particular the periosteum, is also well suited as a target tissue for in vivo gene transfer by microseeding in human and non-human animals.

[0032] In any particular target tissue, it is envisioned that a subpopulation of cells (e.g., keratinocytes in skin cells) may be most preferred target cells, insofar as they exhibit a superior ability to take up or express introduced genetic material. The particular preferred subpopulations can readily be determined empirically in an experimental system designed to measure a particular response to introduction of a particular genetic molecule. If a particular subpopulation of cells is found to be a preferred target, one of ordinary skill will understand how to direct the genetic material into that subpopulation either by orienting the microneedle delivery apparatus toward a portion of a target tissue, or by selecting a preferred delivery depth to which the genetic material is delivered, or both.

[0033] Genetic Material.

[0034] The genetic material that is introduced into the target cells using the method can be any native or non-native genetic molecule that can provide a desirable activity to a target cells. The genetic material can encode any native or non-native protein or polypeptide having such a desirable activity in a target cell. Alternatively, the introduction of the genetic material itself can alter the target in a desirable way, such as by interference with the transcription or translation of a gene normally present in the target. "Non-native" means that neither the genetic material, nor any protein or polypeptide product encoded by the genetic material is detectable in the untreated target cells or tissue. The nature of the introduced genetic material itself forms no part of the present invention.

[0035] The genetic material, can be RNA, but is preferably DNA and is most preferably supercoiled plasmid DNA. The genetic material is prepared according to any standard preparation or purification method among the many known to the art and is provided in an aqueous solution, buffered or unbuffered. The amount of genetic material delivered is not absolutely critical, though better results have been observed for DNA molecules tested when the amount of DNA is below 500 *g, and preferably below 200 *g. Suitable concentrations can readily be determined by routine experimentation. Delivery to the target site of approximately 100 *l of an aqueous solution containing genetic material in the

preferred concentration range is sufficient to function in the method. The genetic material can be attached to microparticles such as iron oxide particles in the range of 0.5 to 1 micron in size. Alternatively, unsupported genetic material in solution is also suitable for delivery according to the method.

[0036] The genetic material typically includes an expressible DNA sequence that encodes a native or non-native polypeptide. The DNA can be of any length and can include genomic DNA fragments, engineered DNA produced in a microbial host, or synthetic DNA produced according to known chemical synthetic methods, including, but not limited to, the Polymerase Chain Reaction. The art is cognizant of the various required and preferred elements (including promoters, terminators, transcription- and translation-regulating sequences, and the like) that one of ordinary skill would provide on an expressible genetic construct. One of ordinary skill is also able to select appropriate elements from the many known elements to facilitate or optimize expression in a particular target animal. The native or non-native polypeptide produced in the method can be maintained intracellularly or secreted to the extracellular space. One of ordinary skill in the art is familiar with the genetic elements necessary to direct a sequence to a particular cellular or extracellular microenvironment and with methods for constructing a genetic construct to facilitate such direction. For example, a polypeptide expressed after gene transfer can be directed to cross a cell membrane by adding an appropriate signal peptide to the gene that encodes the polypeptide.

[0037] Desirable proteins that can be expressed after gene transfer include, without limitation, growth factors, hormones, and other therapeutic proteins. These would speed the healing of wounds and correct certain deficiencies, such as parathyroid, growth hormone, and other hormone deficiencies, as well as deficiencies of certain clotting factors such as factor VIII. For instance, if the gene encoding the growth factor is introduced into the genome or cytoplasm of target skin cells in a wound, these cells can be made to produce a desirable growth factor. The expressed growth factor will then not only speed the healing of the wound, but may also help to heal wounds that would not heal otherwise.

[0038] Cells can also be engineered to not express a protein, such as a protein involved in an immune response, for example, a human leukocyte antigen (HLA). This can be accomplished in a variety of understood ways, the most common being the introduction of "antisense" genetic material that hybridizes with mRNA present in a cell to prevent translation of the mRNA.

[0039] This approach can be used to insert other genetic material of interest into target cells in order to eliminate, or restore, missing or defective functions of patients having any of a variety of skin diseases. Also, the mode in which cytokines act can be changed in that when a factor is expressed in a cell that normally does not produce that factor, a paracrine pathway can be changed into an autocrine pathway. In the same fashion, target cells can be genetically engineered to repair or compensate for inherent genetic defects, such as epidermolysis bullosa. The target cells in a superficial excisional wound can be microseeded with appropriate genetic material to generate a new epidermis with the desired features. Problematic wounds may require coverage of the defect and fast healing.

[0040] Microneedles

[0041] Genetic material can be delivered directly into target cells of a patient using a microneedle delivery apparatus to repeatedly puncture a target area, and to deliver, with each puncture, a small amount of the genetic material provided in an aqueous solution. A microneedle delivery device **10**, suitable for use in the method of the present invention, such as the devices shown in FIGS. **1-3**(*a* and *b*), include microneedles **12** having beveled tips **14**, mounted in a row on a support **16**, such as a handle. The microneedle diameter is about 300 microns. The beveled tip 14 tapers to a zero diameter along the 2 mm closest to the tip; at 100 microns from the tip, the diameter is about 60 microns, while at 50 microns from the tip, the diameter is about 35 microns. The beveled tip **14** facilitates puncturing of the selected target site by the microneedles **12** and opening of cells along the needle path, which is thought to facilitate high expression levels after delivery. The microneedles **12** can be solid (**FIG. 3**) or can have hollow centers (as in **FIG. 2**), although preferred microneedles are solid, since no genetic material is lost to the interior of the needle during delivery. If hollow needles are used, the hollow center should terminate to the side, rather than the bottom, of the tip **14**, as is shown in **FIG. 2**. Moreover, the solid interior applies significantly higher force than hollow needles to the solution containing the genetic material, to help direct the material into the target cells.

[0042] The microneedles **12** are reciprocally driven by a power source **18**, secured to the support, which can be an electric motor or any other suitable power source. The microneedles **12** have a proximal end and a distal end, relative to the power source **18**. If the motor is electric, as in the preferred devices, the motor is attached to a switch that controls whether electric power is applied to the motor. The switch can provide variable or constant power to the device. The motor is connected by a cam to a reciprocating piston **20**, which includes an attachment slot thereupon. The microneedles are joined to the piston attachment slot through a bar connected to the microneedles and inserted into the piston attachment slot. When energized, the piston can oscillate the microneedles at a wide range of speeds between 8 and 50 oscillations per second. The speed of the needles is not thought to be critical to their use in the invention. The amplitude of the oscillating microneedles can vary up to approximately 5 mm. The amplitude is also not believed to be critical, the effect of a greater amplitude simply being a deeper penetration depth. A 3 mm amplitude is suitable. Both the speed and amplitude can be predetermined by, for example, varying the power applied to the motor.

[0043] Surrounding the microneedles is a needle tube **22**, shaped to accommodate the microneedles **12**, which supports the microneedles **12** in all four directions and urges the microneedles **12** into a very small delivery area. The needle tube **22** itself is rigid or semi-rigid and is secured to the support **16** with a fastener, such as a wing nut, in a narrow opening.

[0044] If hollow microneedles **12** are used, as in **FIG. 2** (schematic), the hollow centers of the microneedles are in fluid communication with controllable port **24** for connecting a fluid receptacle that holds the solution comprising the genetic material. The fluid receptacle can be a syringe. The

solution can flow from the fluid receptacle through the hollow centers to the distal tips **14** of the microneedles **12**.

[**0045**] If solid microneedles are used, delivery of the solution comprising genetic material through the microneedles to the tips is not practical. No fluid delivery structure is needed if the device is to be used for delivery only to an external target site, such as an intact skin or wound target site because fluid delivery can be readily accomplished by manual delivery of the solution to the site before applying the microneedle device. However, tubing for delivering the genetic material, as described elsewhere herein, is preferably also used for delivery to an external target site because less DNA is consumed when delivery is performed through delivery tubing.

[**0046**] When the genetic material is to be injected into internal sites within the patient, the genetic material needs to be delivered to the distal tips **14** of the microneedles **12**. In an apparatus **25** that facilitates direct gene transfer to an internal or external target site, separate structures are provided as part of the device, to bring the genetic material in solution to the distal tips of the solid microneedles. As shown in both embodiments of **FIGS. 3***a* and **3***b*, the tube that surrounds the needle can be provided with one or more additional channels **26** on its inner or outer surface to serve as fluid conduits. The channels **26** can be separate from and attached to the needle tube, or can be formed directly thereto or therein. A first channel terminates at a first end at the motor end of the device at a controllable port **28** connectable to a fluid receptacle such as a syringe. A second end of the channel is at the distal end of the microneedles **12**. A second, optional, larger channel, connectable by a controllable port **30** at one end to an external suction device, also opens at the opposite end near the tips, and provides suction of fluid from the delivery site. The second channel can alternatively be provided with a second fluid receptacle and used as a second fluid delivery channel to deliver a second fluid, such as a sealant, a biological glue or a hemostatic agent, to the distal ends of the solid microneedle tips.

[**0047**] The overall size of the apparatus **25**, and, in particular the length of the microneedle **12**, is such that that power source remains user-operable and external to the patient when the distal end of the microneedle **12** is positioned at the internal target site. Thus, the length will depend upon the distance of the internal site from the incision through which the apparatus **25** is introduced into the patient. A suitable length of the device for internal use is 10-15 inches, preferably 13 inches, which allows adequate manipulation to numerous internal target sites.

[**0048**] The inventors have determined that optionally placing the microneedles **12** in a 35% (12.1 M) solution of hydrochloric acid for 12 hours before use in the method etches the needles slightly. Another way to modify the needles is to bombard the needles with aluminum oxide microbeads. It is not yet known whether needle surface modification provides additional surface area onto which the genetic material can adhere during delivery, or whether the modified needle surface advantageously affects the needle's ability to puncture the cells. Although not essential, dipping the microneedle tips **14** into hot paraffin can also prevent DNA from sticking irreversibly to the microneedles during microseeding.

[**0049**] Direct In Situ Introduction of the Genetic Material into Target Cells.

[**0050**] Microseeding can be used to insert genetic material directly into target cells in situ in a human or non-human patient. One or more microneedles are ideal for this purpose. In the method, a solution containing genetic material is placed on the target surface. The microneedles are then moved as a group across the target site to "inject" and thereby deliver the genetic material through the surface of the target to the underlying tissue along several parallel lines. The microneedle or microneedles repeatedly penetrate a site on a surface of the target tissue to a depth within the tissue at which a plurality of preferred or most preferred target cells are found. The penetrating and delivering steps are repeated in the vicinity of the target cells until sufficient genetic material has been delivered that a change in the patient attributable to the delivery of the genetic material is detectable, preferably within twenty-four hours. The change can be physiological, biochemical, histological, genetic, or immunological, or otherwise. Detection of an added or eliminated characteristic of the patient, genetic or phenotypic, is sufficient.

[**0051**] The method can be used to deliver unsupported genetic material in solution, or genetic material attached to carriers, such as iron oxide microparticles. This technique is useful for delivery of a suspension of genetic material alone, without delivery of infectious viral material or attachment to microparticles. This is desirable because of the minimal sample preparation time required. In addition to persistent expression, high transient expression levels are observed.

[**0052**] It is preferred that a large number of independent microneedle penetrations be performed to ensure delivery of an adequate amount of genetic material in the method. The number of penetrations should range from between about 500 to about 5,000 per cm^2 of surface at the target site. Preferably, between 3,000 and 4,000 independent penetrations should be performed at each 1 cm^2 site. To reduce the total delivery time, it is highly preferred that more than one oscillating microneedle, preferably six or more oscillating microneedles, be used to deliver the genetic material. Because of the large number of microinjections, it is also highly preferred that the oscillation be accomplished using an apparatus designed for repeated penetration of oscillating microneedles, such as either of the microneedle delivery devices described herein. Such a microneedle delivery device, placed under control of a scanner **27**, can systematically and accurately cover a predefined area of skin or other tissue, and can deposit the genetic material very evenly into a large number of target cells in the predefined area.

[**0053**] For delivery into a wound or intact skin, the microneedles are placed over a desired treatment site that has previously been prepared, as needed. No particular preparation is necessary for delivery into intact skin. The site for delivery of genetic material into a wound is prepared by removing infected or burned skin, if necessary, or by creating an appropriate wound for the purpose of in situ delivery of genetic material. An artificially created wound is shown in **FIG. 4**. Stem cells located in the hair follicles **30** deep to the epidermal dermal junction can be exposed by creating a flap **32** of epidermis where the deep portion of the flap contains the basal layer of the epidermis. The exposed wound surface **34** contains the portion of the hair follicles

with the stem cell keratinocytes. After delivery of the genetic material, the epidermal flap **32** of an intentional wound is sutured back in place and the wound is sealed with a dressing or in a chamber, as described below.

[**0054**] When internal delivery is desired, surgical access to a desired delivery site is provided for a microseeding apparatus such as that described herein. Surgical access to the interior of the patient can be provided through laparoscopic ports made in a manner known to the art. The laparoscopic ports can also accommodate a viewing scope and an assisting instrument for accurate placement of the microseeding apparatus. In such cases, no wound chamber is needed after treatment, since the treated cells are not exposed to the air and will not dry out.

[**0055**] Regardless of location of the delivery site, the beveled tips of a plurality of microneedles are surrounded with an aqueous solution comprising the genetic material. As noted above, the genetic material is provided to the microneedle tips manually or via a channel provided on the microneedle delivery device. Oscillation of the microneedles placed on the target site at a rate of between 8 and 50 oscillations per second is initiated by energizing the power source. The power source reciprocates the piston at a predetermined rate so that the microneedles repeatedly enter the treatment site to a desired depth. The desired depth, which can range from 0 to about 5 mm, is selected to deliver the solution containing genetic material to a depth at which a large number of cells competent to take up and express the genetic material are found. For instance, it has been determined by the inventors that, when the target is skin, the preferred target cells are keratinocytes, generally located at about 2 mm beneath the skin surface. The optimal penetration depth for other delivery sites can readily be determined empirically by looking under controlled experimental conditions for a desired change in an art-accepted model system, for example, a pig, using a physiological, biochemical, chemical, histological, genetic, immunological or like detection method.

[**0056**] After treatment, no particular method steps need to be followed. However, should one desire to either regulate the fluid in which the treatment site is bathed, or to analyze the products produced at the treatment site, isolation of the site in a vinyl adhesive chamber, or other such isolating chamber is recommended.

[**0057**] The technique can also be enhanced by practicing it in combination with other biological agents, such as liposomes.

[**0058**] This method of introducing genetic material into cells may be superior to gene transduction using plasmids or retroviral vectors, because the latter have an unacceptably low yield. Transfer of genetic material with accelerated particles, employing "gene guns," have a higher yield than plasmid or retroviral transduction, but can, in some embodiments, have other disadvantages, such as a very loud blast and a risk of accidental discharge. Moreover, this method does not leave particles inside patients after treatment. For internal delivery, the microneedles are advantageous because it is not necessary to fully expose internal organs before delivery; rather, only an few incisions large enough to receive a manipulable microneedle apparatus, assisting tool, and viewing scope are made.

[**0059**] Thus, microseeding of genetic material into cells with oscillating microneedles provides a practical, affordable, and a predictable method of insertion of genetic material for localized or systemic expression. The invention finds particular utility as a method for immunization against a non-native polypeptide, and as a method for introducing a foreign genetic material as a therapeutic agent.

[**0060**] The Chamber.

[**0061**] If a polypeptide is secreted from treated intact skin or wound cells, it may be desirable to localize the secreted polypeptide at or near the target site. Such is the case, for example, when the secreted polypeptide accelerates wound healing. In such cases, the target site can be isolated after treatment by enclosing the treated wound site in a sealed chamber, in the manner of US Pat. No. 5,152,757, which is incorporated herein by reference. The structure of the chamber is described in the referenced patent, and is not described in detail herein.

[**0062**] Isolation of the treatment site has an additional benefit of protecting the treatment site from external pathogens such as bacteria and viruses. Moreover, one has the ability to surround the treated target site with fluid having a desired composition, and the ability to analyze samples of the fluid to evaluate the concentrations of desired or undesired compounds in the fluid. However, if delivery is made into a non-wound skin site, use of the sealed chamber may not be necessary, as the skin provides sufficient protection to transduced cells.

[**0063**] In a preferred embodiment, treated skin and wound cells are protected by a system including a chamber, treatment fluid (which may be nutrient media, physiological saline, or some other compatible solution), treatment additives (such as antibiotics and buffering agents), means for controlling treatment variables and means for monitoring cell growth.

[**0064**] The treatment system includes a chamber securable about the periphery of a wound, having portal means for introduction into and removal of treatment fluids from the chamber, treatment fluid, at least one treatment additive, control means for treatment variables, and monitoring means for monitoring wound conditions. The chamber is secured about the periphery of a wound, a treatment fluid and at least one treatment additive are introduced into the chamber, and the treatment variables are controlled according to wound conditions. The wound chamber, which is made of vinyl or other flexible transparent material, such as polyurethane, has a bellows shape and an opening which corresponds to the size of the wound, either the chronic wound or a superficial wound created specifically for the purpose of gene transfer. The chamber contains a small amount of normal saline with antimicrobial agents. When the microneedle is used, the DNA is delivered into the cell and the chamber with normal saline and antibiotics is then attached to the perimeter of the wound. Wound fluid is sampled in order to assay expression of the secretable gene product (e.g., growth factor) at 24 and 48 hours. The wound is treated in the chamber until healed.

[**0065**] The chamber encloses a predetermined surface area about the treatment site. The chamber provides protection for the wound from the surrounding non-sterile environment, control of treatment variables, containment for continuous fluid treatment, an effective delivery system for additives, direct monitoring of cell growth. Monitoring can

be accomplished visually if the chamber is formed of a transparent material, or by extraction and analysis of fluid from the chamber. Fluid extracted from the system can be analyzed for factors which provide an indication of the status of healing, as well as the presence of undesirable components such as micro-organisms, low oxygen, high carbon dioxide and adverse pH. The fluid may be tested for the number and type of bacteria and other microorganisms, the number and type of cells, the amount and type of proteins secreted by the patient and the cells within the chamber, and other factors such as drug levels, oxygen, carbon dioxide and pH.

[0066] The treatment system provides control over variables including temperature, specific ion concentration, colloid osmotic pressure, glucose concentration, amino acid content, fat concentration, oxygen concentration and carbon dioxide concentration and pH.

[0067] Portal means provide access for the introduction of treatment fluids and treatment additives into the chamber and extraction of fluid from the chamber. In some embodiments, treatment fluid is introduced into the chamber by injection with a conventional hypodermic syringe through the wall of the chamber, preferably made of a flexible, self-repairing plastic. In other embodiments, the chamber has an inlet and outlet port or separate inlet and outlet ports. Valve mechanisms are necessary where the apparatus is not to be connected to a treatment fluid reservoir and a drain or connected to a continuous perfusion system. The seals of the ports would be broken at an appropriate time for connection to other apparatus, such as a continuous perfusion system, at a hospital for example.

[0068] A preferred embodiment of the treatment system incorporates continuous perfusion of treatment fluid through inlet and outlet ports. A pump or gravity may be used to move the treatment fluid. The treatment fluid may be recirculated after filtering and other appropriate action (eg. heating or cooling). Alternately, fresh treatment fluid may be introduced and contaminated fluid disposed of. In an embodiment described in U.S. Pat. No. 5,152,757, the chamber contains a reservoir or more than one chamber, with the additional chamber serving as a source of fresh culture media, oxygen, and treatment additives.

[0069] As those skilled in the art will readily recognize, a removable sheet for protecting the adhesive and maintaining the sterility of the interior of the chamber is desirable. The chambers may be stored in a sterile pack for years. This chamber can take many shapes in order to fit wounds from the size of one square centimeter up to the size of a whole extremity. It is important that the adhesive surface be sufficient to secure the bandage to the skin surface to ensure a leak-proof seal.

[0070] As previously mentioned, treatment fluid and treatment additive introduction and subsequent extraction may be accomplished directly through the chamber walls by a needle and syringe. A self-repairing material to construct chamber **10** is contemplated. An alternative method would be to use inlet and outlet ports allowing the introduction and extraction of various substances into the chamber.

[0071] If the chamber is to be used to cover a wound or intact skin site, the skin adjacent to the site is cleaned so that there will be good adhesion between the chamber and the skin. The open portion of the chamber is then placed over the site, with the adhesive edges securing the chamber to the skin, then an appropriate culture medium and cells are introduced into the sealed chamber. The treatment fluid may be introduced and then extracted in favor of fresh culture media in a continuous or batch process. Selected treatment additives may be introduced into the chamber continuously or at a predetermined time or at periodic intervals. Appropriate control of treatment variables is also effected. Monitoring is accomplished by examination of the patient and visual examination of the fluid within the chamber and the wound itself. In addition, samples of fluid are extracted from the chamber for analysis and diagnosis. The chamber is removed once sufficient healing of the wound has occurred.

[0072] For example, to determine whether or not the wound is healed, the protein content of the extracted fluid is analyzed. When the protein content of the extracted fluid decreases to the level present in chambers containing fluid that are placed over normal skin, the wound is healed. Methods for determining protein content are well known in the art and are inexpensive and fast. The types of protein and the relative amounts of the types of protein can also be determined to further evaluate healing and expression of exogenous genetic material.

[0073] Control of Treatment or Culture Variables Using Wound Chamber.

[0074] Treatment of each patient is specific for the conditions within the chamber. Control over treatment variables can include continuous cooling to 34*C. for the first 24 hours. Monitoring can include analyzing extracted fluid for protein and microorganisms, with samples extracted every 24 hours. For example, when the number of microorganisms is less than 10^4 per milliliter or per cc, infection has been resolved. Protein levels checked every day should be less than 24 mg/dl/cm^2.

[0075] As noted above, there are a number of treatment variables which may be controlled by the system. One such treatment variable which may be controlled is temperature. It has been found that heating the wound from a temperature of approximately 27*C. (a common temperature of a lower extremity wound) to 37*C. accelerates wound healing. Experimental data has shown that at a wound temperature of approximately 37*C., the rate of wound healing is more than twice as fast as at a temperature of 27*C. The temperature of the wound area can be achieved by heating the treatment fluid. Cooling has also been proven beneficial in the case of acute burn and other traumatic wounds. Cooling reduces pain, swelling and destruction of tissue. In general terms, acute wounds benefit from cooling during the first hours after occurrence of the wound and later, wounds benefit from a temperature of approximately 37*C. Cooling can similarly be effected by cooling the treatment fluid.

[0076] Other treatment variables may also be optimized. For example, ion concentrations should be kept close to extracellular ion levels. Glucose, amino acid and fat concentrations should be kept close to the concentrations present in plasma or corresponding to a skin tissue culture medium. Oxygen and carbon dioxide concentrations should also be maintained at their normal tissue levels. Oxygen is an important treatment additive, and is essential for cell growth.

[0077] Treatment Additives and Culture Media in Wound Chamber.

[0078] Normal or physiological buffered saline is the basic culture media. Buffering agents, anesthetics such as lidocaine, antibiotics such as penicillin or streptomycin, chemotherapeutic agents, and growth factors including epidermal growth factor (EGF), platelet-derived growth factor (PDGF), insulin-like growth factor (IGF), basic fibroblast growth factor (bFGF), and cholera toxin (CT) can be added to the culture/treatment media. Tissue culture mediums and fluids which increase osmotic pressure and oxygen accessibility may also be introduced to the chamber as treatment additives.

[0079] Selection of treatment additives is wound specific. For example, if an infection has been diagnosed, antibiotics are added in the amount of one single parenteral dose per 1,000 cc of fluid. Furthermore, a treatment additive of gentamicin, tobramycin or carbenicillin is appropriate for a wound infection with Pseudomonas, detected by analyzing extracted fluid. When hypoxia has been diagnosed, the liquid is passed through an oxygenating chamber before entering the chamber. If a tumor has been diagnosed, chemotherapy is given in an amount of one single parenteral dose per 1,000 cc of fluid. In situations involving a wound containing necrotic tissue and debris, proteolytic enzyme is added to the liquid. Immune modulators are added to the treatment fluid if an inflammatory reaction is exhibited. Epidermal growth factor is added in a concentration of 10 nanograms per cc when required.

[0080] The present invention will be further understood by reference to the following non-limiting examples.

EXAMPLES

Example 1

[0081] Introduction of DNA into Keratinocytes and Fibroblasts Using "Microseeding"

[0082] Iron oxide particles ranging in size from 0.05 microns to I micron in diameter were mixed with DNA plasmids in Tris-EDTA buffer. Drops of this material were placed on intact human skin. A microseeding was placed on the material on the skin to insert, or inject, the DNA into superficial keratinocytes as well as stem cell kera-tinocytes in the deep epidermis, or dermal fibroblasts.

Example 2

[0083] Microseeding of Expression Plasmids into Skin and Wounds, without Carrier Particles

[0084] The method of the present invention was tested in a laboratory model system for wound healing. Dorsal skin sites on domestic female Yorkshire pigs (3-4 months old, 40-45 kg) were outlined and were randomly assigned for partial-thickness wound or intact skin treatment. Wounds (15×15×1.2 mm) were created using a dermatome. Animals were maintained in accordance with the Harvard Medical Area Standing Committee on Animals. Surgical procedures were performed under Halothane (1-1.5%) anesthesia in a 3:5 mixture of oxygen and nitrous oxide.

[0085] Supercoiled plasmid DNA was introduced into the intact skin or into the wound bed using an oscillating microneedle apparatus driven by an electric motor and a piston. The oscillating microneedle apparatus for external (skin and wound) use is commercially available from Spaulding and Rogers Manufacturing, Inc. (Voorheesville, N.Y.). Controls included skin and wound sites untreated with DNA. 100 *1 of solution containing supercoiled plasmid DNA in water was introduced into the intact skin or into the wound bed. After delivery, the skin and wound site were covered with sealed vinyl adhesive chambers, of the type described in U.S. Pat. No. 5,152,757, containing 1.2 ml of isotonic saline with 100 units/mi penicillin and 100 mg/ml streptomycin.

[0086] a. Histochemical Determination of Transduced Cells

[0087] Plasmid pCMV*-gal, described by MacGregor, G. R. and C. T. Caskey, *Nuel. Acids Res.,* 17:2365 (1989), was delivered to wound sites. Plasmid pCMV*-gal encodes a *-galactosidase enzyme which is histochemically detectable upon the addition of 5-bromo-4-chloro-3-indolyl-*-D-galactoside (X-gal chromogen), which forms a blue precipitate in transduced cells. The treated tissues and control tissues were frozen and thin sections prepared from the frozen samples were histochemically stained for *-galactosidase activity. Positive staining was observed only in the epidermal keratinocytes and in the hair follicles of sites treated with pCMV*-gal DNA. No stained cells were observed in control sections.

[0088] Skin biopsies were flash-frozen, embedded in O.C.T. and cut into 8 micron cross-sections. The sections were then fixed in 1.5% glutaraldehyde and were stained for *-galactosidase activity. See MacGregor, G. R. et al., *Somat. Cell Molec. Genet.* 13:253 (1987). The tissues were counterstained with hematoxylin. Blue spots were observed in portions of the tissues having *-galactosidase activity.

[0089] b. Delivery of Epidermal Growth Factor-Encoding Plasmid DNA

[0090] Plasmid pWRG1630, an epidermal growth factor-encoding expression plasmid, contains an in-frame fusion of the hGH secretory signal peptide to the mature EGF polypeptide. Plasmid pWRG1630 includes, upstream of the chimeric hGH-EGF gene, the cytomegalovirus (CMV) immediate early transcriptional promoter. Downstream of the mature human EGF coding region is the 3' untranslated sequence and polyadenylation signal from the bovine growth hormone gene (obtained from pRc/CMV, commercially available from Invitrogen, Inc.).

[0091] The complete nucleotide sequence of plasmid pWRG1630 is attached hereto as SEQ ID NO: 1. The 93 amino acid long polypeptide, encoded in two separate exons by pWRG1630, is shown in SEQ ID NO: 2. Referring now to SEQ ID NO: 2, the hGH secretory signal peptide is the first **26** amino acids. These amino acids are cleaved during signal-peptide processing at a cleavage site between amino acids 26 and 27. The next 14 amino acids are encoded in part by the hGH DNA and in part by the plasmid polylinker. Following the 14 amino acid long portion, is a 53 amino acid long mature EGF portion that corresponds to the 53 amino acids of the naturally occurring mature EGF peptide. The ability of this plasmid to produce an EGF polypeptide has been demonstrated by showing that after transduction of the plasmid into cultured fibroblast KB-3-1 cells, a polypeptide

secreted into the culture medium reacted with hEGF monoclonal antibodies in ELISA and Western Blot assays. The culture medium containing the secreted polypeptide was biologically active in an [^{3}H]-thymidine incorporation assay using primary human foreskin fibroblasts and Madine-Darvy Canine Kidney (MDCK) cells.

[0092] c. Expression After Delivery

[0093] Initial experiments were performed to determine DNA transfer conditions to obtain high specific transfer (expression level per *g of input DNA). Parameters tested included the amount of DNA delivered per wound, the depth of needle penetration, and the density of penetrations. These parameters were adjusted to yield the greatest concentration of hEGF polypeptide in wound fluid after treatment. The preferred conditions for this plasmid were determined to be 20-200 *g of pWRG1630 DNA per wound delivered to a penetration depth of 2 mm at a density of 3,330 penetrations per cm^2 surface area. In the experiment, 7500 penetrations were performed using the oscillating microneedle apparatus in a 2.25 cm^2 surface area over a 25 second duration. Microneedles used for microseeding DNA were routinely pre-treated by dipping the tips of the microneedles into hot paraffin and then placing the microneedles into a 35% (12.5 M) solution of hydrochloric acid for 12 hours. This pre-treatment scarifies the needles and provides increased surface area for trapping DNA during delivery.

[0094] Plasmid pWRG1630 was delivered into wounds at 20 *g, 200 *g or 2,000 *g per wound. At 72 hours post delivery, the wound tissue and the chamber fluid bathing the wound were tested for EGF polypeptide. EGF gene expression was monitored daily after gene transfer by measuring EGF concentrations in wound tissue, intact skin, and wound fluid for at least 7 sites for each group. Wound fluid was withdrawn from the wound chambers every 24 hours. The fluid was immediately chilled on ice, filtered and centrifuged. Samples from each group were then pooled, flash frozen and stored at −70*C. Skin biopsies were homogenized and protein was extracted from the homogenate before analysis. A commercially available EGF ELISA assay (Quantikine, R & D Systems, Minneapolis, Minn.) was used to determine EGF concentrations in samples. The minimum detection limit was 0.2 pg EGF per ml.

[0095] When 20 *g of DNA were delivered, between about 25 and 75 (average about 50) pg/ml of EGF were observed.

[0096] When 10-fold more EGF-encoding DNA (200 *g) was delivered, both the chamber fluid and the skin extracts contained about 150-175 pg/ml on average. The range of concentrations in the chamber fluid samples was between about 75-250 pg/ml, while the range in the skin extract samples was between about 50-300 pg/ml and was, on average, slightly higher than in the chamber fluid samples.

[0097] When yet another 10 fold increase in EGF-encoding DNA (2000 *g) was delivered into wounds, only about a 2 fold increase in EGF concentration was observed over the previous level. In this case, the EGF concentration in the chamber fluid samples ranged from about 250 to about 400 with an average of about 325 pg/ml while the concentration in skin extract samples was, on average, about 400 pg/ml with a range of between about 250 and 575 pg/ml.

[0098] From these data, it was determined that a maximally efficient response was observed in the range of 20-200 *g of DNA per wound. In future examples, 20 *g of DNA were delivered per wound or skin site, unless otherwise indicated.

[0099] Temporal variation in EGF level after gene delivery was monitored in chamber fluid over 5 days. Maximal EGF concentrations in fluid from wound sites (276±149 pg/ml) were observed 48 hours after microseeding. In fluid from intact skin treatment sites, a maximal EGF level of 165±113 pg/ml was observed 72 hours after microseeding. Detectable EGF concentrations were maintained over the entire 5 day monitoring period, as is shown in **FIG. 5**. The figure shows the EGF concentration in intact skin sites (Stippled) and in wound sites (Filled). Controls from wound sites seeded with 20 *g of PCMV*-gal, and wound sites treated topically with 20 *g of EGF DNA are not shown. However, no EGF was detected in these controls using the ELISA assay. No evidence of abnormal cell growth, dysplasia tissue disorganization or the like has been observed in cells microseeded with pWRG1630.

[0100] d. EGF Yield in Fluid and Tissue After Delivery

[0101] Three days after delivery of the pWRGI630 EGF-encoding plasmid DNA into partial thickness wounds and into intact skin, analysis of the fluid surrounding the delivery site and of biopsies of the tissue itself were performed to determine the yield of EGF in such tissues. The results shown in **FIG. 6** demonstrate that EGF levels in biopsied tissue (filled bars) are much higher than EGF levels in the fluid (stippled bars), and can reach nanogram levels. In **FIG. 6**, EGF in both wound and intact skin was above 2000 pg (2 ng), on average, and in some samples was above 4000 pg (4 ng), three days after treatment.

[0102] e. Persistence of Transferred DNA

[0103] Wound biopsy specimens were evaluated using the polymerase chain reaction (PCR) on days 1 through 30 to analyze the persistence of the transgenes. DNA was prepared from wound biopsies on days 6, 9, 12, 15, and 30 after delivery using a Puregene kit, commercially available from Gentra. To monitor for the presence of the introduced DNA, 400 ng of DNA prepared from the wound biopsy was mixed in a PCR reaction with 0.2 *g of each primer and 2.5 units of AmpliTAQ* DNA polymerase (from Perkin Elmer) in 100 *l of 10 mM Tris-HCl, pH 9.0 (25*C.), 50 mM KCl, 0.1% Triton X-100, 1.5 mM $MgCl_2$, and 0.2 mM of each dNTP. The sequences of the primers used are attached hereto as SEQ ID NO: 3 and SEQ ID NO: 4. After 3 minutes at 96*C., the reactions were subjected to 30 cycles of 60*C. for 75 seconds, 72*C. for 60 seconds, 96*C. for 60 seconds. See Mullis, K. B. and F. A. Faloona, *Meth. Enzymol.* 155:335 (1987). A 10 *l aliquot of each reaction mix was analyzed by agarose gel electrophoresis. The PCR products were visualized by staining with an ethidium bromide and their identities were confirmed by Southern Blot (Southern, E. M., *J. Mol. Biol.* 98:503 (1975) using internal hybridization probes. The PCR results demonstrated that plasmid DNA persisted in the wound site for at least 30 days. It is as yet unclear whether the persistent DNA resides within cells or is present in the extracellular matrix. These results contrast with the observation that both EGF and *-galactosidase protein expression diminish after 5-6 days.

Example 3

[0104] Endoscopic DNA Delivery

[0105] An anesthetized Yorkshire pig was placed on its back. Laparoscopic ports were placed into the abdomen to accommodate a viewing scope, an assisting instrument, and a microseeding instrument adapted for use inside the patient as is shown in **FIG. 3**. A DNA solution containing the pCMV*-gal plasmid and a small amount of a black iron oxide pigment was delivered to four sites in the liver, two sites in the stomach, and two sites in the abdominal wall. The black iron oxide pigment was added to the DNA solution to facilitate visualization of the treated area after the experiment. The DNA in solution was not coated onto the iron oxide.

[0106] Three days later, the tissues were harvested from the pig for processing to visualize expression of the inserted lac-Z gene. Strong expression was observed in the stomach. No expression was found in the liver. Expression in the abdominal wall was questionable. A reference site on the skin that was microseeded as a control showed strong expression. It is possible that the rapid healing of the liver tissue may have obscured the target sites and that the sites were missed during harvesting. In any event, the utility of the microseeding instrument for internal use was demonstrated by the strong lac-Z expression levels in the stomach.

Example 4

[0107] In vivo gene transfer to the porcine and murine periosteum by microseeding pigs were anesthetized using 1.0-2.5% Halothane delivered in conjunction with a 30:50 mixture of oxygen and nitrous oxide via a facial mask. The heart rate and oxygen saturation of blood was monitored throughout the procedure. Rats were anesthetized using 3 mg of sodium pentobarbital per 100 g of body weight. Animal housing, feeding, and all performed animal procedures were reviewed and approved by the Harvard Medical Area Standing Committee on Animals.

[0108] Under sterile conditions the porcine femoral periosteum was exposed, elevated, and the target sites were marked with sutures. The target sites were microseeded with plasmid DNA at 35 *g of DNA per site, as described. Four sites were tested per animal. Likewise, the murine tibial periosteum was prepared and microseeded at 3 *g of DNA per site. The microseeded plasmid DNA was pWRG1630 that encodes a secretable, mature form of human epidermal growth factor. Control sites received sham microseeding treatments without plasmid.

[0109] Forty-eight hours after microseeding, the microseeded sites were harvested and were processed as described. Briefly, the biopsies were homogenized and protein was extracted from the homogenate before analysis. The protein was subjected to a commercially available EGF ELISA assay (Quantikine, R & D Systems, Minneapolis, Minn.) which was used to determine EGF concentrations in the samples. The minimum detection limit was 0.2 pg EGF per ml.

[0110] The levels of hEGF gene expression are shown below in Tables 1 and 2. No EGF expression was detected in the sham-microseeded controls.

TABLE 1

PERIOSTEUM, Pigs (35* g DNA/site), 48 h:				
periosteal site #	1	2	3	4
pg/ml of tissue extract	3976	1374	1225	1729.6

[0111]

TABLE 2

PERIOSTEUM, Rats (35 *g DNA/site), 48 h:				
periosteal site #	1	2	3	4
pg/ml of tissue extract	93.3	146.4	57.5	128.6

[0112] Collectively, these results demonstrate that the periosteal cells can be successfully made to express an exogenous gene by microseeding in an animal. Comparable results are anticipated in humans. The method described in Example 4 can bring about new gene transfer applications in bone healing. For example, by delivering the genes that encode one or more bone morphogenic proteins to the periosteum in an area near a bone defect, the cells that receive the gene by microseeding can express bone morphogenetic proteins which can enhance the healing of the bone defect. A preferred gene for delivery can include, but is not limited to, a gene that encodes a product that can modulate bone growth, which products can include, for example, cytokines or the products of a bone development regulatory gene, such as those listed in Table 3, and variants thereof. A plurality of genes may be delivered in combination. The BMP genes described by Wozney, J. M. et al., *Science* 242:1528-34 (1988), incorporated herein by reference, are well characterized and are, therefore, considered more preferred for delivery.

TABLE 3

CYTOKINES INVOLVED IN BONE OR CARTILAGE REGENERATION:

1) TGF-beta superfamily, particularly including BMP-1,2,7,12,13,14, and GDFs (growth differentiating factors)
2) LIF (Leukemia inhibitory factor)
3) OSM (oncostatin-M)
4) CT-1 (cardiotrophin-1)
5) IL-3,4,6,8,11 (Interleukins)
6) PDGF-AA,AB,BB
7) IGFs
8) FGFs
9) TNF-alpha
10) GM-CSF
11) EGF, HG-EGF
12) VEGF
13) IP-10
14) PF-4
15) MCP-1
16) HGF
17) RANTES
18) PGE (Prostaglandins)
19) Decorin

BONE DEVELOPMENT REGULATORY GENES:

1) Osf2/Cbfa1

[0113] This application is particularly enhanced by employing the described endoscopic modification of the

microseeding instrument **10** which further diminishes the trauma caused by the procedure.

[**0114**] Referring now to FIGS. **7-9**, a microseeding assembly **50** constructed in accordance with the preferred embodiment of the invention and suitable for use in the method of the present invention includes a microseeding device **52** connected to a control module **54** (illustrated schematically) via an electrical lead **56**. Device **52** includes an elongated housing **58** that is generally pen-shaped and may be operated by a single hand of a user gripping the device as a pen. While portions of housing **58** are cylindrical, the disclosure herein will refer to the housing as if it were entirely cylindrical. Accordingly, for the purposes of clarity and convenience, directional terms "radial" and "axial" will be referred to throughout the disclosure, it being appreciated that these terms may be interpreted and used synonymously and interchangeable with "lateral" and "transverse," and "longitudinal" respectively.

[**0115**] The device **52** defines a first front axial end **62** and a second rear end **63**. Accordingly, positional terms "forward" and "rearward" will be used throughout this disclosure relative to ends **62** and **63**. Housing **58** includes a nose body **60** that is disposed at front end **62** and is connected to a central oscillating drive body **64**, which is disposed rearwardly of nose body **60**. A nose **61** extends forwardly from nose body **60**, and defines the forward applicator end **62** of device **52**. Oscillating drive body **64** is, in turn, connected to a syringe drive housing **66** that is disposed rearwardly of body **64**. Electrical lead **56** extends outwardly from syringe drive housing **66** and is connected to control module **54** that is used to control the operation of microseeding device **52**, as will be described in more detail below.

[**0116**] A syringe **68** extends axially along the radially outer surface of housing **58**, and is preferably positioned above the housing and secured by a support assembly **69** when the device is orientated for use. In particular, a pair of symmetrical lips **70** extends radially outwardly (upwardly) from housing **58** at the rear end of nose body **60** and is elongated in the axial direction. Lips **70** are slightly radially offset with respect to each other to define an axially extending groove therebetween (not shown). Furthermore, lips **70** are curved to correspond to the contour of the syringe barrel **72**, such that the front end of the barrel snaps into place in the groove defined by curved lips **70**. The syringe tip **71** thus extends axially forwardly from barrel **72** and is disposed radially outwardly from (above) the rear end of nose **61**. A syringe dispenser tube **73** extends forwardly from syringe tip **71**, and provides a conduit to deliver genetic material from barrel **72** to a plurality of axially extending microneedles **75**.

[**0117**] A first support flange **74** extends radially outwardly (upwardly) from housing **58** at the rear end of oscillating drive body **64** and defines an upwardly facing groove (not shown) sized to receive syringe barrel **72** at a position immediately forward of the syringe flange **76**. The groove defines a radius of curvature corresponding to that of the syringe such that the syringe snaps into place in the groove defined by flange **74**. A drive system cap **77** is disposed at the forward end of syringe drive housing **66** and encloses the forward end of housing **66**. A second support flange **78** extends radially outwardly (upwardly) from cap **77**, and defines an upwardly facing groove **80** extending axially therethrough (See **FIG. 9**). Groove **80** is sized to receive the syringe plunger **82** which extends through hole **80**. Second flange **78** is positioned immediately rearwardly of syringe flange **76**, and spaced axially from flange **74** by a sufficient distance such that flange **76** is tightly sandwiched between the two flanges **74** and **78**.

[**0118**] A syringe pusher plate **84** extends radially outwardly (upwardly) from syringe drive housing **66**, and engages the rear surface of syringe plunger flange **81**. Plate **84** is slidable in the axial direction to enable the automatic delivery of genetic material during use, as will be described in more detail below. Members **70**, **74**, **78**, and **84** (and their corresponding grooves/apertures) are radially aligned so as to support syringe **68** in an axially extending position during operation.

[**0119**] Device **52** includes a syringe drive assembly **86** that translates pusher plate **84** in the axial direction during use. In particular, a stepper motor drive **90** is supported by a pair of screws **88** extending axially forwardly from drive system cap **77** and through the drive motor **90**. A radially extending mounting plate **92** is disposed in oscillating drive body **64** at a location axially forwardly of drive **90**, and supports screws **88** that extend through the plate **92**. The distal ends of screws **88** further extend through, and support, an oscillating drive motor **94**. A drive assembly **95** is thus provided that includes motors **90** and **94** to deliver genetic material to microneedles **75** and to oscillate microneedles **75** for the delivery of the genetic material into the target tissue, which will be described in more detail below.

[**0120**] A flat base plate **85** extends axially through syringe drive assembly **66**, and presents a horizontal surface (not shown) when device **52** is orientated such that syringe **68** is disposed above the housing **58**. The upper surface of base plate **85** supports a pusher assembly **102** whose upper end extends through housing **58**, and is connected to pusher plate **84**. A threaded shaft **96** extends rearwardly from drive motor **90** through cap **77**, and terminates at a bearing **98** that is mounted onto the inner surface of rear wall **100** of syringe drive housing **66** and is rotated during operation. Pusher assembly defines a nut (not shown) having a bore that extends axially through pusher assembly **102** having threads that mate with corresponding threads of shaft **96**. Accordingly, pusher assembly **102** travels along shaft **96** in the axial direction during rotation of the shaft **96**.

[**0121**] Pusher assembly **102** is hingedly connected to the internal nut to allow the assembly **102** to articulate slightly (a few degrees) forward and backward along the direction of double-arrow C. It has been found that the articulation prevents binding of the nut with respect to the screw **96** as the screw is rotated. Pusher plate **84** is thus correspondingly translated in the axial direction as indicated by Arrow A to depress the plunger **82** of syringe **68** and deliver genetic material to microneedles **75** via dispenser tube **73** it should be appreciated that the direction of shaft **96** rotation may be reversed to translate pusher plate **84** axially rearwardly, for example, when the contents of syringe **68** have been fully delivered.

[**0122**] Power is supplied to motor **90** via control module, which controls the speed of rotation of motor **90**, and thus the speed of axial translation of pusher plate **84**, as well as the direction of rotation of motor **90**. Motor **90** may be any suitable motor or drive element capable of rotating shaft **96** at a predetermined or variable speed as appreciated by one having ordinary skill in the art.

[0123] Device **52** further includes an oscillating drive assembly **106** that oscillates microneedles **75** to deliver genetic material to the target tissue. In particular, drive motor **94** is connected to mounting plate **92**, and extends axially forwardly therefrom. Motor **94** is further supported by screws **88** that extend axially partially through motor **94**. Motor **94** has a forwardly extending output drive shaft **108** that is received by the bushing **110** of a drive body **112**, such that operation of motor **94** rotates drive shaft **108** in a predetermined direction. Motor **94** may be any suitable motor or drive element capable of rotating shaft **108** at a predetermined or variable speed as appreciated by one having ordinary skill in the art. It should thus be appreciated that motors **94** and **90** may be constant or variable speed motors to control the oscillation frequency of microneedles and speed of the translation of pusher assembly **102**, respectively.

[0124] When energized, the piston **120** can oscillate the microneedles at a wide range of speeds between 4 and 67, and preferably between 8 and 50 oscillations per second. The speed of the needles is not thought to be critical to their use in the invention, but may be predetermined by, for example, varying the power applied to the motor using control module **54**. The amplitude of the oscillating microneedles **75** can vary between 0.1 and 10 mm, depending on the thickness of the target tissue and desired penetration path. A more preferred amplitude of oscillation is within the range of 0.5-2 mm. The amplitude may be configured by the internal components of drive body **112**, as will now be described.

[0125] Drive body **112** includes a driven input shaft **114** that is coupled to bushing **110** at one end, and extends forwardly from the bushing and terminates at a bearing **116** that is mounted inside nose body **60**. An output shaft **118** is linked to input shaft **114** via internal drive body **112** components (not shown) that translate rotational motion to linear oscillating motion, as is understood by one having ordinary skill in the art. In particular, the forward end of output shaft **118** is connected to the rear end of a piston **120** via a coupling **122**. Piston **120** extends forwardly from coupling **122** and terminates at a mounting plate **124** that is disposed in nose **61**.

[0126] Referring now also to FIGS. **10-12**, mounting plate **124** defines a radially extending rear-end **126** that is attached to the forward end of piston **120**, and an axially extending rectangular mounting section **128** that extends forwardly from rear end **126**. A pair of symmetrical rectangular apertures **130** extend through mounting section **128**, each aperture **130** being separated by a pair of opposing inwardly extending flanges **132**, such that a channel **135** extends between flanges **132** and connects apertures **130**.

[0127] A yoke **134** includes a substantially axially extending body **136** defining an upper horizontal surface **138**. A pair of opposing flexible clip members **140** extends upwardly from upper surface **138**. Clips **140** include corresponding inwardly facing notches **142** that are spaced apart from each other a distance slightly less than the length of channel **135**. Accordingly, yoke **134** may be attached to mounting plate **124** by inserting clips **140** upwardly into apertures **130** until notches **142** slide over flanges **132** and lock in place. It should be appreciated, however, that several variations from the design illustrated in **FIGS. 10 and 11** may be envisioned to facilitate a mechanical connection between mounting plate **124** and yoke **134**, and such variations are anticipated by the present invention.

[0128] Yoke **134** includes a forward section **144** that extends upwardly with respect to body **136**, and defines an upper horizontal surface **146** that supports a microneedle assembly **148**. In particular, a round peg **154** extends upwardly from horizontal surface **146**. Microneedle assembly **148** includes a base member **152** having a generally square shape corresponding to the shape of forward section **144**. A hole **150** extends through base **152** and is sized to snugly receive peg **154**. A user may place an adhesive, such as a glue, onto upper horizontal surface **146** to fasten the mounting assembly **148** to the yoke **134**. It should be appreciated that any suitable alternative removable mechanical fastener could be used to connect microneedle assembly **148** to yoke **134**.

[0129] Referring now to **FIGS. 11 and 12** in particular, a matrix **156** of solid microneedles **75** extends axially forwardly from base **152**. The term "solid" is used to denote that genetic material does not flow inside the microneedles **75**, but rather is delivered to the exterior of microneedles **75**, which in turn carry the genetic material into the target tissue. As illustrated, microneedles **75** are arranged in an upper row **158** and a lower row **160** such that the microneedles are vertically staggered with respect to microneedles in the opposite row. Microneedles **75** are positioned so as to be vertically aligned with the outlet of tubing **73** to enable genetic material to be delivered from syringe **68** onto the microneedles and subsequently delivered to the target tissue. The combination of oscillating microneedles **75** and genetic material flowing from tubing **73** provides a genetic material delivery system **79** that is advantageously integrated with device **52**.

[0130] Upper row **158** is comprised of five microneedles **75**, while lower row **160** is comprised of six microneedles. The outermost microneedles **75** of row **160** are thus disposed outwardly with respect to the outermost microneedles **75** of upper row **158**. Advantageously, this arrangement enables genetic material delivered downwardly from tubing **73** that spills past microneedles **75** of upper row **158** to be caught by microneedles **75** of lower row **160**, thereby reducing the amount of genetic material that is not delivered to the target tissue.

[0131] Microneedles **75** are preferably photoetched out of flat sheet metal and include a base **165** having a rectangular cross-section whose opposing vertical walls **164** are longer than the opposing horizontal walls **166**. In particular, the opposing horizontal walls **166** have a horizontal thickness between approximately 0.05-0.09 inches, and preferably 0.07 inches. The opposing vertical walls **164** have a height between approximately 0.002 and 0.018 inches, and preferably between 0.005 and 0.013 inches, and more preferably approximately 0.008 inches. Each base **165** terminates at its axially forward end at a tine **167** having a tip **162** at its forward end. In particular, opposing horizontal walls **166** and vertical walls **164** are beveled at their forward ends to a point that defines tip **162**. In some instances, it may be desirable for the upper horizontal wall **166** to be beveled at an angle less than the bevel of the lower horizontal wall **166** to assist in capturing the genetic material from dispenser tube **73**.

[0132] The base **165** of each microneedle **75** extends approximately 0.11 inches from base member **152**, though the length of base **165** is not thought to be critical to the use of the present invention. Each tine **167** extends forwardly from base **156** a distance greater than 0.02 inches, and approximately between 0.02 and 0.08 inches, and more preferably between 0.04 and 0.08 inches, from base **165**. Adjacent tips **162** of microneedles in a given row are spaced apart a distance between approximately 0.01 and 0.03 inches, and preferably 0.019 inches. Tips **162** of microneedles **165** of upper row **158** are preferably staggered a distance approximately between 0.005 and 0.015 inches, and preferably 0.01 inch with respect to adjacent tips of lower row **160**.

[0133] It should be appreciated that any suitable matrix of microneedles may be implemented in accordance with the present invention. For instance, the present invention contemplates a single row or more than two rows (e.g., 3, 4, 5, 6, etc . . .) of microneedles **75** having any number of microneedles between, for example, 1 and 20, with a preferred range being between 4 and 10, and more preferred range between 4 and 6. Preferably, the number of microneedles in a given row is less than the number of microneedles in a lower row to assist in capturing the genetic material, as discussed above. The number of needle penetrations may thus be determined as the product of the number of microneedles at the matrix **156** and the oscillation frequency. For instance, the illustrated matrix **156** has eleven needles which, when operating at a frequency of 50 oscillations per second, penetrates the target site 33,000 times per minute.

[0134] Advantageously, microneedle assembly **148** may be provided in individual sterilized packages (not shown). Accordingly, a used assembly **148** may be removed by applying upward pressure on the base **165** of needle matrix **156** to overcome the adhesive bond between base member **152** and yoke **124**. A new assembly **148** may be removed from the sterilized package and installed in yoke **124** in the manner described above. Furthermore, the base **165** of microneedles may advantageously have a rough exterior texture to assist in adhering the genetic material delivered from tubing **73** to the microneedles **75**. Additionally, small depressions or protrusions may be formed in microneedles **75** assist in capturing the genetic material.

[0135] During operation, a user fills barrel **72** of syringe **68** with genetic material to be delivered to a target tissue. Pusher plate **84** should be in its most rearward position to enable plunger to be installed into barrel such that plunger flange **81** abuts the forward edge of pusher plate. A "oscillator ON/OFF" button **168** on control module is depressed to energize motor **94**. When motor **94** is energized, piston **120**, and therefore microneedles **75**, oscillates axially along the direction indicated by double-arrow B. The oscillation frequency of motor **94** may be controlled using knob **170** on control module **54**. Once the device is properly positioned such that microneedles **75** are disposed proximal the target tissue, motor **90** may be energized using "syringe ON/OFF" button **174** on control module.

[0136] Activation of motor **90** begins the flow of genetic material through tubing **73** and onto oscillating microneedles **75** for delivery of the genetic material into the target tissue. The speed of motor **90**, and thus the flow rate of genetic material, may be controlled using knob **176** of control module. Once the contents of syringe **68** have been fully delivered, or if it is desired to replace syringe **68**, the direction of motor **90** revolution may be reversed using switch **178** on control module. Pusher plate **84** is then translated rearwardly to enable the removal and replacement of syringe **68**.

[0137] Control **54** may be further equipped with an output interface (not shown) that displays various information, such as oscillation frequency/amplitude of motor **90**, or speed/direction of plunger **82** translation. It should be appreciated that control may be hand or finger-operated as described above, or alternatively may include pedals that are foot-operated. Control is preferably sufficiently small to render assembly **50** fully portable. It should be appreciated that the method described above with reference to microseeding device **10** are equally applicable to microseeding assembly **50**, as would be appreciated by one having ordinary skill in the art.

[0138] Advantageously, device **52** provides a handheld unit having a genetic material delivery system integrated with the device for enhanced ease of operation and portability. It should be appreciated that drive assembly **95** may be simplified by integrating motors **90** and **94** into a single motor having a first output shaft in communication with threaded shaft **96**, and a second output shaft in communication with bushing **110**. The rotation of the first and second output shafts may have a predetermined ratio such that the oscillation frequency of microneedles **75** is correlated to the speed of axial translation of plunger **82**.

[0139] The invention has been described in connection with what are presently considered to be the most practical and preferred embodiments. However, the present invention has been presented by way of illustration and is not intended to be limited to the disclosed embodiments. Accordingly, those skilled in the art will realize that the invention is intended to encompass all modifications and alternative arrangements included within the spirit and scope of the invention, as set forth by the appended claims.

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We claim:

1. A microseeding assembly for delivering genetic material from a dispenser to a target site, the assembly comprising:

a housing;

a matrix of microneedles disposed on a translatable shaft at one end of the housing;

an integrated dispenser support assembly connected to the housing for supporting a genetic material dispenser, wherein the support assembly includes an actuator for initiating the delivery of genetic material from the dispenser to the microneedles; and

a drive assembly connected to the housing and communicating with the actuator and the microneedles to initiate delivery of genetic material to the microneedles and to oscillate the microneedles at a predetermined frequency.

2. The microseeding assembly as recited in claim 1, wherein the drive assembly comprises a first motor having an output shaft in mechanical communication with the microneedles.

3. The microseeding assembly as recited in claim 2, wherein the drive assembly further comprises a drive body disposed between the output shaft and the microneedles to convert rotational motion of the output shaft to linear oscillating motion of the microneedles.

4. The microseeding assembly as recited in claim 2, wherein the drive assembly further comprises a second motor having an output shaft in mechanical communication with the actuator.

5. The microseeding assembly as recited in claim 4, wherein the second motor is connected to a threaded output shaft, and wherein the actuator is connected to a follower receiving the threads of the output shaft and is translated in response to rotation of the output shaft.

6. The microseeding assembly as recited in claim 1, wherein the genetic material dispenser comprises a syringe;

7. The microseeding assembly as recited in claim 6, wherein the syringe comprises an output that delivers genetic material to the microneedles.

8. The microseeding assembly as recited in claim 2, wherein the microneedles are removably connected to the output shaft.

9. The microseeding assembly as recited in claim 8, further comprising a yoke connected to the output shaft.

10. The microseeding assembly as recited in claim 9, further comprising a microneedle assembly having a base and microneedles extending outwardly from the base, wherein the base is removably connectable to the yoke.

11. The microseeding assembly as recited in claim 4, further comprising a control module in electrical communication with the housing to control the first and second motors.

12. The microseeding assembly as recited in claim 11, wherein the control module controls activation of the first and second motors.

13. The microseeding assembly as recited in claim 11, wherein the control module controls a frequency of oscillation of the first motor.

14. The microseeding assembly as recited in claim 11, wherein the control module controls the rate of delivery of genetic material.

15. The microseeding assembly as recited in claim 1, wherein the microneedles are formed from photoetched flat sheet metal.

16. The microseeding assembly as recited in claim 1, wherein the microneedles are solid.

17. The microseeding assembly as recited in claim 16, wherein the microneedles are arranged into a multi-row matrix.

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