ELECTROPORATION DEVICE

Technical Field

The present invention relates to an electroporation device, in particular to a device for delivery of drugs, for instance into vertebrates, such as mammals using electroporation.

Background

Drug delivery, such as delivery of vaccines, into mammals using electroporation is known per se.

Now, an example of infectious disease will be described.
The human immunodeficiency virus (HIV) is a catastrophe for humanity. Prevention has proven difficult and not sufficiently effective. Treatment is expensive and viral resistance mutations are appearing. Antiretroviral treatment can prolong life, but here is still no cure.

The best way to permanently reduce new infections and stop the disease spreading would be by vaccination. Vaccination may also have a role in the treatment of HIV, by preventing continuous virus changes, or by slowing the course of the disease. One of the larger problems in the development of effective HIV vaccines is the difficulty in obtaining a sufficiently broad and long-lasting immune response.

This is described for instance in: "Enhancement of Cellular Immune Response to a Prostate Cancer DNA Vaccine by Intradermal Electroporation", by Roos et al, Molecular Therapy, Vol. 13, No. 2, February 2006, pages 320-327.

Roos et al disclose the use of electrical waves to deliver a polynucleotide vaccine into mammalian skin cells. It is also disclosed by Roos et al that successful genetic expression of the polynucleotide vaccine is demonstrated by detection of a genetic marker which expresses luciferase protein. In addition, Roos et al disclose that with the use of “slow” electrical waves to deliver a polynucleotide vaccine into mammalian skin cells, there is improved T-cell response involving improved secretion of good protein resulting from successful genetic expression of the polynucleotide vaccine.

There are several undesirable features common for present-day electroporation machines. Typically, they are connected to large, bulky devices, and they are powered by mains power.

The total delivery of the polynucleotide drug including deposit of the polynucleotide drug and electric pulses normally takes over 5 minutes for each shot and also causes pain, often due to several contractions of the underlying muscle.

In addition to what has been mentioned above, further disadvantages with present-day electroporation machines for vaccination are that they are also too large and fragile for easy transportation and dependent on a mains supply of electricity

In view of the above, it would be desirable to provide an electroporation device for the delivery of genetic material into superficial cells, where the whole procedure takes less than one minute. Using present state of the art electroporation machines, the manipulations of such a procedure typically takes more than 5 minutes.

Administration of a polynucleotide drug, to be successful, must typically also give evidence of successful genetic expression of the administered polynucleotide drug. Moreover, to be successful, the genetic expression of the administered polynucleotide must give evidence of providing a desired protein which results from the successful genetic expression of the polynucleotide sequence.

While the foregoing prior art indicates it to be well known to use electroporation machines per se, the prior art described above does not teach or suggest a device for the delivery of polynucleotide vaccines into mammalian skin cells which has the following combination of desirable features: (I); provides a device for the delivery of polynucleotide drug into mammalian skin cells which takes less than 1 minute (II) gives evidence of successful genetic expression of the administered polynucleotide drug; and (III) gives evidence of providing a desired protein which results from the successful genetic expression of the polynucleotide drug, and (IV) leads to an immune response, long-term secretion of an endogenous substance or a foreign antibody, which ever is the case.

An object of the present invention is to provide a new and improved electroporation device for the delivery of polynucleotide drug into mammalian skin cells which takes less than 1 minute both to administer the drug and to perform electroporation.

Another object of the present invention is to provide an electroporation device for the delivery of vaccines into mammalian skin cells that only causes one muscle contraction for the plural applied electrical waveforms.

Summary of the invention

According to an aspect of the present invention, there is provided a handheld drug delivery device for electroporation-mediated drug delivery to vertebrates, such as mammals, which is small (compared to prior art devices) and battery powered. The handheld drug delivery device comprises needles that are inserted and mounted such that they penetrate axially or essentially in parallel to a tissue (of the vertebrata) to deliver drugs which enters small fissures created in a skin surface of a vertebrata. The needles operate as electrodes for the electroporation. The moment the needles are inserted furthest into the skin, the polynucleotide drug is delivered, and typically seconds after that a small, almost painless (to the vertebrata), electric discharge will occur automatically. The drug can be delivered by the same needles as the electrodes or by means of a separate needle, or by one needle electrode only, or in some other suitable way depending on application.

This means that the procedure provided by the inventive device can be repeated several times for one individual with the same instrument (but different needles and same or different drug) which is usually the case desired for gene therapy applications. For vaccines usually one application is enough.

In this way, the inventive device (I); provides a device for the delivery of polynucleotide drug into mammalian skin cells which takes less than 1 minute (II) gives evidence of successful genetic expression of the administered polynucleotide drug; and (III) gives evidence of providing a desired protein which results from the successful genetic expression of the polynucleotide drug, and (IV) leads to an immune response, long-term secretion of an endogenous substance or a foreign antibody, which ever is the case.

Typically, skin cells are the most suitable for polynucleotide drugs introduction and protein production.

According to an aspect of the present invention, the use of a high power battery as a power source is of importance. Typically, the battery of lithium iron phosphate type. The device could

be used up to 500 - 1000 times without recharging. This is due to the use of lithium iron phosphate type battery. The lithium iron phosphate type battery also provides rapid upstart of the drug delivery device (seconds compared to minutes for prior art devices). The device is environment friendly due to the rechargeable battery type and provides a robust construction suitable also for difficult transport, for instance at field hospitals.

Herein, a the term “high power” means more than 10 W. Another definition is that high power means that the complete procedure takes less than 60 seconds, such that the delivery of polynucleotide drug into a vertebrata takes less than 1 minute both to administer the drug and to perform electroporation.

The present invention provides a device for the delivery of drugs containing large molecules such as DNA and other genetic material into surface tissues (skin of vertebrates such as mammals, fowl, and cells of plants) by means of using electroporation, for instance for new types of polynucleotide drugs (vaccines) that have been developed by the inventors, built on genes which express their proteins directly in the cells of the person to be immunized. This vaccine has no vector that may cause undesirable immune responses to the gene carrier.

Each small electrical charge opens up pores in the cell membranes, making it possible for drugs such as polynucleotide drug molecules to enter cells. The uptake/transfer over the membrane is essential for the efficacy of DNA vaccination. The strength of the electric field is presumed to pull the charged DNA molecules into the cells. Other effects, thermal and mechanical, may also be important for the observed beneficial effect.

According to an embodiment of the present invention, there is provided a handheld and portable electropoartion device for delivery of drugs into vertebrates such as mammals. The portable electropoartion device comprises:
means for electroporation arranged to be connected to an electric field generator powered by a high power battery power source; and a shaft, wherein a distal segment of the shaft comprises a needle means having at least one needle electrode arranged to be electrically coupled to the electric field generator and carried by the shaft, wherein the needle electrode is arranged to provide an electric field to provide electroporation.

According to an embodiment of the present invention, there is provided a handheld and portable

drug delivery device for delivery of drugs into vertebrates including mammals, the drug delivery device comprising: means for electroporation including an electric field generator powered by a high power battery power source; a shaft being configured for drug delivery, wherein a distal segment of the shaft comprises a needle means, being configured to cause drug to enter a skin surface of a vertebrata; and at least one set of electrodes electrically coupled to the electric field generator and carried by the shaft. At least one electrode is configured to enter a skin surface of a vertebrata.

The inventive device is small (the size of a large pen and typically battery powered in contrast to already existing devices that are large and require connection to mains power of 110V/220V.

Typically, the set of electrodes and/or the needles are arranged in an exchangeable part on the tip of the device.

Typically, the needles of the needle means are configured as hooks (typically hollow) or bifurcated forks (typically solid) or needles (hollow or solid) with an obliquely grounded surface.

Typically, the needles are configured as one bipolar electrode pair having a first electrode and a second electrode, the bipolar electrode pair coupled to the electric field generator.

Typically, the electric field generator comprises an electrical waveform generator, which is capable of applying a sequence of operator-controlled, independently programmed, electrical waveforms, which have programmable pulse intervals. An advantage with the present inventive device is that the whole procedure takes less than 60 seconds.

Typically, the handheld drug delivery device comprises a build-in injection mechanism arranged to deliver the drug. Alternatively, the drug can be loaded onto the needle by means of surface tension. This can be accomplished by using the types of bifurcated needles. According to another embodiment of the device of the invention, the drug is delivered from a reservoir, inside the device, to the hooked needle or the needle having an obliquely ground surface and is applied to the skin just after the needle is contacted with the skin and emptied.

Typically, a penetrating mechanism is arranged to thrust the needles into the skin at a rapid pace.

Typically delivered by a hollow needle formed as a hook that rapidly inserts in parallel to the skin or mucosal surface and delivers the drug,

Typically, the drug delivery is followed by a short electric pulse train

Typically, the full procedure, including drug delivery and electroporation, takes less than one minute.

The inventive electroporation device is suitable for and provides antibody-based therapy by delivering polynucleotide drug. The approach to deliver gene-encoded antibodies of varying reactivity is entirely new. The classical approach to deliver antibodies against a disease is passive immunization. During therapy based on passive immunization the antibodies are rapidly cleared from the body and the treatment must be frequently repeated. The suggested method provided by the inventive device would allow for a long-lived secretion of the antibody, which is produced inside the body of the patient as the inventive device delivers the gene encoding the antibody. In order to reach clinical efficiency the amount of plasmid delivered needs to be high and this will be achieved by the inventive drug delivery device, in which the procedure can be repeated easily.

New types of polynucleotide drugs have been developed by us, built on genes which express their proteins directly in the cells of the person to be immunized. This vaccine has no vector that may cause undesirable immune responses to the HIV gene carrier.

Skin cells are the most suitable for DNA vaccine introduction and protein production.

The foregoing desired characteristics are provided by the unique device for the delivery of drugs, such as polynucleotide vaccines into mammalian skin cells of the present invention as will be made apparent from the following description thereof.

The inventive device can be used to deliver genes that produce endogenous substances; similar to what otherwise is done by viral vectors, in order to obtain production of an endogenous substance to substitute for endocrinal defects as insulin in diabetes or collagen in skin diseases. The inventive device for delivering the polynucleotide drug encoding deficient production of

substances is specifically suitable for delivery to diseases areas of the skin, where the inventive device could deliver the lacking substance repeatedly, due to its rapidity and short time for each injection. Herein, the term “short” means typically less than 1 minute.

The inventive device is suitable for and provides antibody-based therapy by delivering polynucleotide drug. The approach to deliver gene-encoded antibodies of varying reactivity is entirely new. The classical approach to deliver antibodies against a disease is passive immunization. During therapy based on passive immunization the antibodies are rapidly cleared from the body and the treatment must be frequently repeated. The suggested method provided by the inventive device would allow for a long-lived secretion of the antibody, which is produced inside the body of the patient as the inventive device delivers the gene encoding the antibody. In order to reach clinical efficiency the amount of plasmid delivered needs to be high and this will be achieved by the inventive drug delivery device. in which the procedure can be repeated easily.

BRIEF DESCRIPTION OF THE DRAWINGS

Several embodiments of the present invention will be apparent upon consideration of the following detailed description, taken in conjunction with the accompanying drawings, in which like reference characters refer to like parts throughout, and in which:

FIG. 1a is a schematic view illustrating a handheld drug delivery device according to an embodiment of the present invention embodied as an electroporetic pen, partially in section, without outer casing. Different types of needles (3 basic types) are illustrated in FIG. 1b, c, and d.

FIG. 2 illustrates results from polynucleotide drug HIV vaccine delivered in animal experiments.

DETAILED DESCRIPTION

Now is referred to FIG. 1a, which illustrates an embodiment of a handheld drug delivery device 10 for delivery of drugs into vertebrates such as mammals according to the present invention. The drug delivery device as illustrated in FIG. 1a is embodied as a miniaturized drug delivery “pen” using electroporation to mediate drug delivery. The drug delivery device 10, comprises means 20 for electroporation including an electric field generator 22 powered by a high power battery power source 25, a shaft 32, which is configured for drug 40 delivery. A distal segment

34 of the shaft 32 comprises a needle means 24, herein a combined drug delivery means and electrode needle means 24, which needle means 24 is arranged to provide extended intradermal injection, and configured to cause one or more needles for drugs 40 to enter a skin surface 100 of a mammal. The needle means 24 comprises at least one set of needle electrodes 24´, 24´´ electrically coupled to the electric field generator 22 and carried by or being part of the shaft 32. The set of electrodes 24´, 24´´ are arranged to provide an electric field (F) to provide electroporation mediating drug delivery into a skin surface 100 of the mammal (schematically shown). In FIG. 1a, a needle means 24 having one or more needles 24´, 24´´ embodied as a hollow hook, and/or a forked needle means 24 or needles having obliquely grounded surface are shown. In an alternative embodiment (not shown), only one needle 24´is configured as a needle or hook, the other one 24´´ being configured as a non-skin penetrating electrode, such as a plate electrode.

Typically, the needles of the needle means are configured as hooks (typically hollow) or bifurcated forks (typically solid) or needles (hollow or solid) with an obliquely grounded surface. This is illustrated in FIG. 1b-d, of which FIG. 1b illustrates a hollow hook, c) a bifurcated fork (solid), where a cross-section taken along line A-A is shown, showing elliptical spaces between “the forks”,; and d) illustrates a (straight) needle having obliquely grounded surface.

The electrodes 24´ and 24´´ are electrically connected to the pulsed electric field generator 22 located inside the drug delivery device 10. The electrodes 24´ and 24´´, which form a bipolar electrode pair, optionally may be insulated at all regions, except their distal ends (not shown). It should be understood that several examples of electrodes 24´ and 24´´ described are electrically connected to the generator 22 even though the generator 22 is not explicitly shown or described with each embodiment. Typically, the first electrode 24´comprises an active electrode and the second electrode 24´´ comprises a return electrode.

Typically, the needle means 24 comprising needles/electrodes 24´, 24´´ are held in place in the device 10 by mechanical mounting means. Of course, also some other mounting providing electrodes 24´, 24´´ combined with needles having suitable configurations that ensure electrodes 24´, 24´´ that are fine, adjusted to the depth of the human epidermis or the epidermis of other animals, or to plants and easily exchangeable could be provided. Typically, they could be designed for the delivery of plasmids.

As illustrated in FIG. 1a, in this embodiment of the present invention, a pair of shoulders 37 prevent the needle(s)/electrodes 24´, 24´´ from being inserted too deeply (typically 1-3 mm is optimal for human skin) into the mammal. The needle means 24 can comprise needles 24´, 24´´ configured as hooks or two-, pronged needles or as needles having obliquely grounded surfaces 36. Because the needle means 24 is exchangeable, the risk for contamination could be reduced to almost zero for instance between vaccinated subjects or when repeating the procedure in the same individual. Between each polynucleotide drug delivery followed by electroporation, used needle means 24 are changed and discarded.

The driving mechanism 38 is typically a simple electromagnetic push-pull arrangement. The electric field generator 22 is typically a voltage generator designed as a blocking oscillator, capable of operating efficiently at voltages as low as 1.5 V, but also delivering more than 400 V if necessary. Short pulses of some 10 mA can be generated by the inventive electric field generator 22, which could be implemented using today's technology as a circuit comprising microprocessor for generation of high voltage, having a size smaller than a lump of sugar. The electrical characteristics can be varied according to operator preference and/or automatically according to default settings provided at factory, for instance.

Pulsed electric field parameters, typically controlled by the means 20 for electroporation including an electric field generator 22 can be altered and combined in any combination, as desired, typically according to an operator's preference. Such parameters can include, but are not limited to, voltage, field strength, pulse width, pulse duration, the shape of the pulse, the number of pulses and/or the interval between pulses (e.g., duty cycle), etc. For example, suitable field strengths can be up to about 10,000 V/cm and suitable pulse widths can be from nanoseconds up to about 1 second. Suitable shapes of the pulse waveform include, for example, AC waveforms, sinusoidal waves, cosine waves, combinations of sine and cosine waves, DC waveforms, DC-shifted AC waveforms, RF waveforms, square waves, trapezoidal waves, exponentially-decaying waves, or combinations. The field includes at least one pulse; but typically the field includes a plurality of pulses. Suitable pulse intervals include, for example, intervals less than about 10 seconds. These parameters are provided as suitable examples and in no way should be considered limiting. Suitable parameters will be further described as follows linked to typical applications.

Typically, the pulse generator 20 is directly digitally controlled.

Typically, suitable trains of pulses are used for the electroporation intradermally which cause no or very little pain: one starting with low pulses for plasmid transport followed by high pulses for membrane poration. Another, well established pulse train uses high pulses only and a third uses high pulses followed by a number of low voltage pulses. These three concepts can be selected for the antibody-based plasmid. Moreover, the drug, such as a DNA vaccine can be introduced by a separate needle, a hook, or by an ampoule and electroporation needle inserted in the exchangeable needle means 24, or by two closed bifurcated needles.

Typically, the power source 25 comprises a battery of lithium iron phosphate type. The device could be used up to 500- 1000 times without recharging. This is due to the use of lithium iron phosphate type battery. The lithium iron phosphate type battery also provides rapid upstart of the drug delivery device (seconds compared to minutes for prior art devices) and repeated use up to 1000 times without recharging batteries. The device is environment friendly due to the rechargeable battery type and provides a robust construction suitable also for difficult transport, for instance at field hospitals.

As illustrated in FIG. 1a, in the embodiment of the present invention, illustrated and described herein, a small, light-weight, battery-driven reciprocating mechanism 38 will thrust the thin hooked or forked needles 24´, 24´´ into the mammalian's skin 100. Typically, the penetrating mechanism 38 is driven by the same power source 25 as the electric field generator 22. Typically, the drug 40 flows by pressure via one or more of the needles 24´ and 24´´, which are also electrodes at the same time. When the needles 24´ and 24´´ are deepest (typically 1-2mm, corresponding to tattoo) in the skin 100, a small electric discharge will be given automatically to facilitate entrance of the drug into the skin cells and nuclei of the mammal. The movement is operator controlled started and can be stopped with a switch 26 on the device 10.

The inventive drug delivery device, including all electronics, is handheld and therefore easily portable and useful in all larger or smaller medical settings, also in hostile environments. The high voltage of the inventive device permits electroporation in the skin, typically by means of intradermal application, which is more efficient and more tolerable than prior art equipment, since the whole procedure, takes less than one minute.

The present invention can be used for gene vaccination, gene therapy and antibody gene delivery; as well as for human and veterinary applications.

The inventive device is provided for delivering genes for a polycucleotide drug that represents a polynucleotide vaccine of any specificity, a polynucleotide endogenous substance used for gene therapy such as skin healing substances or vaccine-empowering cytokines polynucleotide sequence of an antibody, directed against foreign infectious antigens or cancer substances.

Other advantages with the present invention are the following: exchangeable drug gene entities, safety due to exchangeable electrodes/needles and voltage lock, user friendly due to small size and handheld device.

The present invention could be used for antibody-based therapies for treatment of infectious diseases for instance. Of course, there are a great number of further applications for the present invention. Thus, antibody-based therapies for treatment of infectious diseases are only examples of application.

Electroporation-mediated delivery of polynucleotide drugs, very efficiently enhances the uptake of macromolecules by cells. As an electric current is applied at the site of injection of DNA, the cell membrane is perforated and allows transportation of the large DNA/RNA molecules into the cell. In the cell the transported DNA produces RNA and then protein of the same kind as the genetic structure permits. This protein is made by somatic cells in the body, which permits both humoral and cellular immunity as well as long-term synthesis of an antigenically tolerated substance such as antibodies or substances useful for gene therapy, for instance insulin or other hormones and cytokines.

A typical example of application of the inventive device for vaccination involves wetting the pair of forked needles 24´, 24´´ with vaccine 40, which will cling between the needle's 24´, 24´´ prongs. A constant volume of liquid polynucleotide drugs can be adsorbed between the forked needles 24´, 24´´, permitting up to 300 µg (50 µl at a concentration of 3 mg DNA/ml) of DNA plasmid at each vaccine point. The needles 24´, 24´´ are pressed against the skin 100 and the start button 26 for electroporation is engaged.

Alternatively, the handheld drug delivery device comprises a build-in injection mechanism arranged to deliver the drug. Alternatively, the drug can be loaded onto the needle by means of surface tension. This can be accomplished by using the types of bifurcated needles. According to another embodiment of the device of the invention, the drug is delivered from a reservoir, inside the device, to the hooked needle or needle having obliquely grounded surface and is applied to the skin just after the needle is contacted with the skin and emptied.

The inventive drug delivery device can be used to deliver various genetic materials (plasmid DNA, naked genes, microRNA, siRNA), and is in particular suitable to deliver plasmid-encoded antibodies against HIV. This later application is an entirely new use of antibody genes. Normally antibodies are delivered passively as proteins. Such protein antibodies are readily cleared from the body, resulting, in the case of chronic infection/disease, in a need to repeat the treatment at a weekly - 3 weekly basis. By instead introducing genes that encode the antibody, it will be possible to achieve a long-lasting treatment effect derived from the antibody synthesis in the body for months, since the plasmid is retained and antibody constantly produced intracellular from the transfected somatic cells. The duration of the production is, among other variables, dependent on the immunological tolerance of the antigen. When one chooses human antibodies, we expect the tolerance in humans to be as good as or better than passively given antibody. Since chronic diseases required frequent antibody injections, we will exploit a system with genetically constructed antibodies given by the efficient method of electroporation. Other areas of application include, but are not limited to passive vaccination against other serious diseases such as hepatitis C, influenza, other infections, and antibodies against proteins or cancer cells.

For gene antibody therapy purposes it is possible that the injections will have to be repeated within a time period, typically monthly.

We believe that the combination of a plasmid encoding a broadly neutralizing antibody against HIV and the highly efficient delivery that the inventive drug delivery device offers can create a new type of strategy for combating chronic diseases. The inventive device can also serve as a platform for delivering other DNA-encoded products, both for vaccine purposes as well as for gene therapy purposes.

The porosity of a cell membrane may be increased by inducing a sufficient voltage across the cell membrane through, e.g., short, high-voltage pulses. The duration of effect is a function of

multiple variables, such as pulse interval, field strength, pulse width, duty cycle, electric field orientation, (cell type) or size and other parameters. Herein, the term "pulse interval" means the time from the beginning of one pulse to the beginning of the next pulse.

By aligning an electric field so that the field preferentially aligns with the lengthwise aspect of the cell rather than the diametric or radial aspect of the cell, lower field strengths may be used to affect target neural cells, e.g. to alter gene expression, and/or to induce other suitable processes. This is expected to reduce total energy delivered to the system and to mitigate effects on non-target cells in the electric field.

The needle(s) may, for example, be fabricated from a flexible, pre-formed elastic material (e.g., thin-walled stainless steel) and typically easy to mount and/or dismount such that they could be easily changed.

The first electrode 24´ preferably comprises the active electrode and the second electrode 24´´ preferably comprises the return electrode. However, it should be understood that the electrode polarities optionally may be reversed.

Moreover, others utilize multi-electrode contact pieces, which are far too expensive for mass vaccination or field use because they have to be sterilized after use or discarded.

In summary, according to an aspect, the inventive drug delivery device provides:
Long-lived intracellular secretion of polynucleotide drugs including sequences for foreign antibody. The antibody gene delivery method can be applied to any antibody specificity or to molecules within the immological system such as cytokines, chemokines, complement, hormones. or other normally endogenous substances.

The technological alternatives applied by other manufacturers of electroporation devices result in large and inconvenient products that need main power to operate. The inventive drug delivery device embodied as a vaccine pen is a small portable device that can be used on up to 1000 individuals without any need to connect to mains power.

Large and inconvenient electroporation devices are available to be used experimentally to perform intramuscular injections, a much more difficult alternative due to exactness of antigen dose and to pain.

FIG. 2 shows results from polynucleotide drug HIV vaccine delivered in animal experiments.

ELISpot- (upper panel) and ELISA-results after 1 immunization with either DeviceX or Vaccipen. Animals were immunized at one occasion with either 1 or 3 injections (with same total amount of DNA).

The bifurcated needles were exchanged for conventional needles.
Antigen: pKCMVp37B 20ug/animal either at one or three locations (20ul/site – 10 or 20 ug/site, DNA conc 1 or 0,5ug/ul)
Mice: Male BALB/c, 5 animals/group

Group 1. Device X – 1 injection
Group 2 Device X – 3 injections
Group 3 Vaccipen – 1 injection
Group 4 Vaccipen – 3 injections

Device X is a device from one of the leading manufacturer of electroporation devices. Animals were immunized at one occasion and sacrificed 14 days later. Spleen and serum were collected from each mouse and analyzed by IFN-g ELISpot and ELISA, respectively (fig 2).

The phraseology and terminology employed herein are intended for the purpose of description and should not be regarded as limiting.