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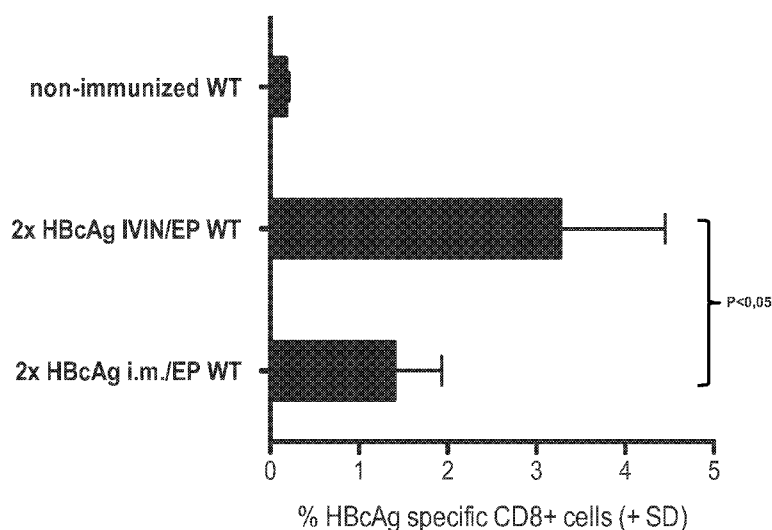
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Figure 69 **HBcAg-CTL precursor frequency**



(57) **Abstract:** Several needle assemblies and intracellular delivery devices that are used for the delivery of prophylactic and/or therapeutic material (i.e., delivered agents) into a tissue of a subject are disclosed. Preferably, the needle assemblies and/or the intracellular delivery devices comprise needles and/or needle electrodes, which are disposed in an array (e.g., a Y-type array having three outer needles and a center needle), wherein each needle in the array has a closed end and a plurality of apertures along each needle barrel, and the apertures on the needle barrels of the outer needles of the array are positioned to deliver the delivered agent toward the apertures of the center needle and/or an adjacent needle, but not outside of the active zone defined by the area within the needle array and the apertures on the needle barrel of the center needle are positioned to deliver the delivered agent toward the outer needles. The devices may be used to deliver immunogenic compositions such as those that illicit an immune response to the hepatitis c virus in naive or in chronically HCV infected individuals.

INJECTION NEEDLE, DEVICE, IMMUNOGENIC COMPOSITIONS AND METHOD OF USE

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims the benefit of priority to U.S. Application No. 61/710,634, filed October 5, 2012, which is hereby expressly incorporated by reference in its entirety, and the present application also claims the benefit of priority to U.S. Application No. 61/881,391, filed September 23, 2013, which is hereby expressly incorporated by reference in its entirety

REFERENCE TO SEQUENCE LISTING

[0002] The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled TRIPEP132WOSEQLISTING.TXT, created October 3, 2013, which is 177 kb in size. The information in the electronic format of the Sequence Listing is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0003] Aspects of the embodiments disclosed herein relate generally to devices and methods for the delivery and uptake of therapeutic material such as immunogenic compositions to illicit an immune response against HCV (e.g., chemicals, compounds, proteins and nucleic acids) by tissue of a subject (e.g. a human). Preferred embodiments concern devices and methods for the delivery of genetic material or nucleic acids including, but not limited to, DNA, RNA, and modified nucleic acids into a plurality of cells, preferably animal cells, such as human cells.

BACKGROUND OF THE INVENTION

[0004] The delivery of therapeutic material, such as genetic material, into tissue has a wide range of useful applications including vaccination, replacement of a defective gene, DNA immunization, introduction of an immunogen, anti-sense therapy, and miRNA, RNAi, aptamer, or siRNA therapy. For instance, nucleic acids, such as DNA, for example, can be injected into tissue, wherein the nucleic acids are taken up by the surrounding cells albeit inefficiently. DNA introduced in this manner will produce the protein that the DNA encodes. The successful delivery of nucleic acids into tissue and the uptake of the nucleic acids by the cells is difficult,

especially when significant amounts of protein expression are desired (e.g., as is desired for DNA-based vaccination). Conventional injection of genetic material into tissue generally results in poor uptake by the cells and low levels of protein expression, if any at all.

[0005] Gene therapy is an important tool in the future for treatment of human and animal disease. Some clinical progress has been made in recent years with one example of a patient with partial restoration of vision following gene therapy (Bainbridge New Engl J Med. 2008 358(21):2231). A major area for clinical application of gene therapy is genetic vaccination for infectious diseases. However, a major limitation to make gene therapy a reality is the difficulty to reproducibly deliver the genetic material. Genes can be delivered either by viral vectors or in the form of plasmid DNA. Viral vectors have limitations in that they generate anti-vector responses that limit their repeated use, and they are expensive to produce and to store. DNA has the advantage that it does not induce anti-vector responses and is relatively cheap to produce and to store. However, the major problem with DNA is the poor uptake into human cells *in vivo*. Thus, new robust and tolerable ways for DNA delivery to human cells *in vivo* can accelerate the whole field of gene therapy.

[0006] Intravascular administration approaches have also been developed to deliver therapeutic agents to animals (*see e.g.*, U.S. Pat. Nos. 6,379,966; 6,897,068; 7,015,040; 7,214,369; 7,473,419; and 7,589,059, all of which are hereby expressly incorporated by reference in their entireties). Intravascular administration can be very difficult to implement in practice; however, requiring skilled clinicians and, if performed incorrectly, the procedure can lead to punctured blood vessels, hematomas, and the development of internal blood clots, which could lead to an embolism. Furthermore, the intravascular administration approach can produce a wide dispersion of the introduced therapeutic agent (e.g., nucleic acid and protein), which is undesirable when trying to encourage the body to mount an immune response to the delivered agent. Accordingly, there remains a need for devices and methods that facilitate the delivery and uptake of therapeutic molecules such as nucleic acids and proteins.

SUMMARY OF THE INVENTION

[0007] Disclosed herein are devices and methods that deliver a prophylactic and/or therapeutic agent (e.g. a chemical, a compound, a chemotherapeutic agent, a protein, a specificity exchanger, a nucleic acid, such as DNA, RNA, other natural nucleic acid, a modified nucleic acid, or a DNA, or RNA or nucleic acid aptamer) into tissue of an animal (e.g., a human), whereby said agent (a “delivered agent”) can be taken up by cells in the tissue

surrounding the injection site and, the agent is expressed so as to provide a prophylactic, therapeutic or cosmetic benefit. Also disclosed herein are compositions, such as immunogenic compositions, and method of using compositions so as to provide a prophylactic, therapeutic or cosmetic benefit.

[0008] In some embodiments an immunogenic composition comprising (a) a polynucleotide encoding a hepatitis C virus NS3/4A polypeptide and (b) an IL-12 polypeptide or a polynucleotide encoding an IL-12 polypeptide is disclosed. Some of these immunogenic compositions further comprise (c) an HBcAg polypeptide or a polynucleotide encoding an HBcAg polypeptide. Some of these immunogenic compositions further comprise a polynucleotide encoding an IL-12 polypeptide. In some of these immunogenic compositions the polynucleotide encoding a hepatitis C virus NS3/4A polypeptide and the polynucleotide encoding an IL-12 polypeptide are present in the same vector. In some of these immunogenic compositions the polynucleotide encoding a hepatitis C virus NS3/4A polypeptide and the polynucleotide encoding an IL-12 polypeptide are present in different vectors. In some of these immunogenic compositions, the immunogenic composition comprises a polynucleotide encoding an HBcAg polypeptide. In some of these immunogenic compositions the polynucleotide encoding a hepatitis C virus NS3/4A polypeptide and the polynucleotide encoding an HBcAg polypeptide are present in the same vector. In some of these immunogenic compositions the polynucleotide encoding a hepatitis C virus NS3/4A polypeptide and the polynucleotide encoding an HBcAg polypeptide are present in different vectors. In some of these immunogenic compositions the immunogenic composition further comprises an attenuated modified *vaccinia Ankara*. In some of these immunogenic compositions the immunogenic composition further comprises a vesicular stomatitis virus (VSV). In some of these immunogenic compositions the immunogenic composition further comprises a polynucleotide encoding a rodent HBcAg. In some of these immunogenic compositions the immunogenic composition further comprises a replication defective Ad6 vector expressing a NS3/NS4A, NS3/4A/5A, NS3/4A/NS5B, or NS3/4A/5A/5B gene fusion. In some of these immunogenic compositions the immunogenic composition further comprises a replication defective Ad24 vector expressing a NS3/NS4A, NS3/4A/5A, NS3/4A/NS5B, or NS3/4A/5A/5B gene fusion. In some of these immunogenic compositions the immunogenic composition induces production of NS3-specific T cells. In some of these immunogenic compositions the immunogenic composition increases production of NS3-specific T cells relative to a comparative composition comprising the same components as said immunogenic composition except for both lacking said

IL-12 polypeptide and lacking said polynucleotide encoding an IL-12 polypeptide. In some of these immunogenic compositions the immunogenic composition induces production of NS3-specific T cells at twice the level relative to a comparative composition comprising the same components as said immunogenic composition except for both lacking said IL-12 polypeptide and lacking said polynucleotide encoding an IL-12 polypeptide. In some of these immunogenic compositions the immunogenic composition induces production of NS3-specific T cells at fourfold the level relative to a comparative composition comprising the same components as said immunogenic composition except for both lacking said IL-12 polypeptide and lacking said polynucleotide encoding an IL-12 polypeptide. In some of these immunogenic compositions the immunogenic composition is characterized as increasing by at least twentyfold the number of splenocytes expressing INF γ upon administration of said immunogenic composition relative to a control immunogenic composition lacking an HCV antigen. In some of these immunogenic compositions the immunogenic composition is characterized as producing at least 10^2 INF γ -expressing splenocytes per million cells upon administration of said immunogenic composition more than a control immunogenic composition lacking an HCV antigen. In some of these immunogenic compositions the immunogenic composition is characterized as producing at least 5×10^2 INF γ -expressing splenocytes per million cells upon administration of said immunogenic composition more than a control immunogenic composition lacking an HCV antigen. In some of these immunogenic compositions the immunogenic composition is characterized as producing at least 10^3 INF γ -expressing splenocytes per million cells upon administration of said immunogenic composition more than a control immunogenic composition lacking an HCV antigen. In some of these immunogenic compositions the immunogenic composition is characterized as producing at least 4×10^3 INF γ -expressing splenocytes per million cells upon administration of said immunogenic composition more than a control immunogenic composition lacking an HCV antigen. In some of these immunogenic compositions the immunogenic composition induces an NS3-specific T cell response in a patient that is chronically infected with HCV. In some of these immunogenic compositions the immunogenic composition is characterized as inducing an NS3-specific T cell response in a patient that is chronically infected with HCV. In some of these immunogenic compositions the immunogenic composition is present in an injection device or an injection device configured to be used in conjunction with an electroporation device.

[0009] In some embodiments a method of generating an immune response in a subject is disclosed. In some embodiments this method comprises delivering to an intracellular

space any of the immunogenic compositions provided herein. In some embodiments this method comprises delivering to an intracellular space, such as a plurality of muscle cells, of said subject a polynucleotide encoding an HCV NS3/NS4A, NS3/4A/5A, NS3/4A/NS5B, or NS3/4A/5A/5B polypeptide, and delivering to said animal an IL-12 polypeptide and/or a polynucleotide encoding an IL-12 polypeptide. In some embodiments this method comprises an IL-12 polypeptide that is encoded by a polynucleotide, and said polynucleotide is delivered to an intracellular space of said animal, such as a plurality of muscle cells or intradermally. In some embodiments this method comprises delivering to an intracellular space such as a plurality of muscle cells or intradermally of said subject an HBcAg polypeptide or a polynucleotide encoding an HBcAg. In some embodiments this method comprises HBcAg polypeptide encoded by a polynucleotide, and the polynucleotide is delivered to an intracellular space such as a plurality of muscle cells or intradermally of an animal and translated into an HBcAg polypeptide therein, thereby delivering said HBcAg polypeptide to said subject. In some embodiments the components of said immunogenic composition are delivered in a single injection. In some embodiments the components of said immunogenic composition are delivered in two or more injections. In some embodiments this method comprises providing ribavirin to said subject. In some embodiments this method comprises providing pegylated interferon to said subject. In some embodiments the pegylated interferon is pegylated interferon α 2a. In some embodiments this method comprises injecting said polynucleotide encoding an HCV NS3/4A polypeptide into said subject and subjecting a vicinity of said injected polynucleotide encoding an HCV NS3/4A polypeptide to a treatment comprising electroporation. In some embodiments the delivery comprises injecting said immunogenic composition into a subject. In some embodiments this method comprises subjecting the vicinity of said site of injection of said immunogenic composition to electroporation. In some embodiments the injection is effected using an IVIN injection device. In some embodiments this method comprises administering a boost vaccination to said subject. In some embodiments the boost vaccination comprises an attenuated modified *vaccinia Ankara*. In some embodiments the boost vaccination comprises a vesicular stomatitis virus. In some embodiments the boost vaccination comprises a rodent HBcAg polynucleotide. In some embodiments the boost vaccination comprises a replication defective Ad6 vector expressing a NS3-NS5B gene fusion. In some embodiments the boost vaccination comprises a replication defective Ad24 vector expressing a NS3-NS5B gene fusion. In some embodiments the boost vaccination is administered within 28 days of the administration of said polynucleotide encoding an HCV

NS3/4A polypeptide. In some embodiments the boost vaccination is administered within 14 days of the administration of said polynucleotide encoding an HCV NS3/4A polypeptide. In some embodiments the boost vaccination is administered within 7 days of the administration of said polynucleotide encoding an HCV NS3/4A polypeptide.

[0010] In some embodiments an apparatus for intracellular delivery in a living animal by overloading a tissue locally is disclosed. In some embodiments the apparatus comprises a delivery unit, which controls an injection parameter; a hub connected to the delivery unit and comprising at least one electrical connector; a plurality of needles connected to the hub, wherein each needle of the plurality of needles comprises: a closed end; a needle barrel; a plurality of apertures that are disposed on the needle barrel, wherein the apertures on each needle barrel oppose the apertures on at least one other needle barrel so as to generate an opposing direction of delivery of a delivered agent or a cross-spray pattern of delivery of a delivered agent; an electrode; an electrical power supply configured to generate an electric field at the electrode, wherein the electrical power supply is connected to the electrode through the at least one electrical connector; and further comprising one or more of the immunogenic compositions provided herein. In some embodiments at least one needle of the plurality of needles of the apparatus is a needle-electrode, which is electrically connected to the electrical power supply and is configured to generate an electric field. In some embodiments the intracellular delivery apparatus comprises a plurality of needle-electrodes, which are electrically connected to the electrical power supply, and configured to generate an electric field. In some embodiments the intracellular delivery apparatus comprises four needles arranged in a Y-type pattern with a center needle and three outer needles disposed around the center needle, wherein the center needle is a needle-electrode configured to apply a first polarity voltage, and the three outer needles are needle-electrodes, configured to apply a second polarity voltage. In some embodiments the center needle comprises apertures along the needle barrel, which are positioned to direct delivery of a delivered agent toward apertures that are present on each of the three outer needles and each of the three outer needles comprises apertures along the needle barrel, which are positioned to direct delivery of a delivered agent toward apertures that are present on the center needle, and wherein the outer needles do not have apertures positioned to deliver a delivered agent away from the center needle. In some embodiments the intracellular delivery apparatus comprises an array of needles comprising a center needle and a plurality of outer needles disposed around the center needle, wherein the center needle is a needle-electrode configured to apply a first polarity voltage, and the outer needles are needle-electrodes,

configured to apply a second polarity voltage, wherein the center needle comprises apertures along the needle barrel, which are positioned to direct delivery of a delivered agent toward the outer needles and the outer needles comprise apertures along the needle barrel, which are positioned to direct delivery of a delivered agent toward the center needle and/or an adjacent needle, and wherein the outer needles do not have apertures that allow delivery of a delivered agent away from an active zone defined by an area within the array of needles. In some embodiments the intracellular delivery apparatus further comprises seven needles that are arranged in a hexagonal pattern, wherein one needle, which is a needle-electrode, is located at the center of the hexagonal pattern. In some embodiments the apparatus further comprises: a syringe in fluid communication with the hub; a channel configured to receive the syringe; a collar operable to fasten the syringe within the channel; a handle, which operates to enclose the syringe and hub within the channel such that the closed ends of the needle barrels protrude from the channel and are available to engage a subject; a charging element configured to be charged by the operation of the handle, the charging element coupled to a trigger and the syringe such that operation of the trigger releases the charging element and the charging element acts on the syringe, thereby displacing prophylactic and/or therapeutic material out of the syringe; and an electrical port configured to mate with the electrical connector on the hub of the hypodermic needle device thereby establishing electrical contact between the electrical power supply and the electrodes and/or needle-electrodes. In some embodiments the hub is a pocket hub, comprising an individual reservoir of a delivered agent for each needle or needle electrode. In some embodiments the hub comprises a single reservoir of a delivered agent for each needle or needle-electrode. In some embodiments the apparatus further comprises an electrical power supply controller configured to control generation of the electric field. In some embodiments at least one needle of the plurality of needles is at least partially laminated with an electrically non-conductive material. In some embodiments the controlling generation of the electric field comprises controlling one or more of the pulse voltage, polarity of the needle-electrodes, the number of pulses, the pulse pattern, or the pulse duration. In some embodiments the charging element is a spring configured to be compressed by the operation of the handle and configured to decompress upon operation of the trigger or when activated by an electrically operated transducer. In some embodiments the electric field applied at the electrode and/or needle electrodes is commutating.

[0011] In some embodiments a method of introducing a delivered agent into a tissue of a subject is disclosed. In some embodiments the method comprises providing the intracellular

delivery apparatus above; providing a voltage source; inserting a plurality of needles and a plurality of electrodes or a plurality of needle-electrodes of said electroporation apparatus into a tissue of a subject; displacing the delivered agent through the lumen of the plurality of needles or the plurality of needle electrodes and into the tissue of the subject; and applying an electric field to the tissue of the subject. In some embodiments the displacing of the delivered agent is performed prior to applying the electric field to the tissue. In some embodiments the displacing of the delivered agent is performed subsequent to applying the electric field to the tissue. In some embodiments the displacing of the delivered agent is performed in a two stage process, wherein the first stage comprises displacing the delivered agent into the tissue into an active area and the second stage comprises energizing one or more electrodes or needle electrodes to electroporate the cells within the active area. In some embodiments the injection parameter of the apparatus above is for use in DNA vaccination. In some embodiments the delivered agent is any of the immunogenic compositions provided herein.

[0012] In some embodiments a product combination is disclosed. In some embodiments the product combination comprises an electroporation device that comprises a plurality of needles each having a closed end and a plurality of apertures along the barrel of said needles, wherein the apertures on separate needles oppose one other but said apertures do not face away from each other so as to create a zone of injection existing in a region of tissue between the opposing apertures of said plurality of needles, wherein at least two of said plurality of needles are capable of passing current so as to enhance delivery of a nucleic acid within said zone of injection, wherein said nucleic acid comprises at least one plasmid that comprises one or more polynucleotides encoding HCV NS3/4A and, optionally HCV NS5A, and HBcAg, optionally an HBcAg that infects a stork or heron, and IL-12, optionally a human IL-12, wherein said one or more polynucleotides are, optionally codon-optimized for human expression and wherein said product combination is used for treatment of HCV in an HCV infected or chronically HCV infected patient.

[0013] Disclosed herein are devices and methods that deliver a prophylactic and/or therapeutic agent (e.g. a chemical, a compound, a chemotherapeutic agent, a protein, a specificity exchanger, a nucleic acid, such as DNA, RNA, other natural nucleic acid, a modified nucleic acid, or a DNA or nucleic acid aptamer) into tissue of an animal (e.g., a human), whereby said agent (a “delivered agent”) can be taken up by cells in the tissue surrounding the injection site and, the agent is expressed so as to provide a therapeutic or cosmetic benefit. As used herein, the term “delivered agent” may refer a prophylactic and/or therapeutic agent

including any of those listed above, both prior to injection or delivery to a tissue or subject or after delivery to a tissue or subject.

[0014] In some embodiments, one or more of the needles and/or devices described herein are used to administer cell populations (e.g., regenerative cells, stem cells, progenitor cells, or a mixture thereof) to effectuate therapeutic and/or cosmetic benefit. In these embodiments, the cells are introduced into tissue (e.g., fatty tissue of the breast, heart, kidney, bone, skin, fat tissue, intervertebral discs) of a subject in need thereof to promote therapeutic or cosmetic benefit (e.g., to facilitate or effectuate breast reconstruction, ameliorate an ischemic region, repair degenerative discs, promote bone repair, promote wound healing, or to ameliorate wrinkles or pock marks on the skin).

[0015] Several embodiments disclosed herein include intracellular delivery devices that can be used with living animals, including humans. Some of the intracellular delivery devices operate by overloading a tissue of the animal locally (e.g., within an area defined by a needle array of the device) with the delivered agent and providing an electrical field to this area or injection region so as to promote greater delivery and/or uptake of the delivered agent. Some embodiments, for example, comprise: an intracellular delivery apparatus that comprises a delivery unit, which controls an injection parameter, a hub connected to the delivery unit comprising at least one electrical connector; a plurality of needles connected to the hub, wherein each needle of the plurality of needles comprises: a closed end; a needle barrel; a plurality of apertures that are disposed on the needle barrel, wherein the apertures on each needle barrel oppose the apertures on at least one other needle barrel so as to generate an opposing direction of delivery of a delivered agent or a cross-spray pattern of delivery of a delivered agent; an electrode; and an electrical power supply configured to generate an electric field at the electrode, wherein the electrical power supply is connected to the electrode through the at least one electrical connector.

[0016] In some embodiments, at least one needle of the plurality of needles of the intracellular delivery apparatus is a needle-electrode, which is electrically connected to the electrical power supply and is configured to generate an electric field.

[0017] In some embodiments, the intracellular delivery apparatus comprises a plurality of needle-electrodes, which are electrically connected to the electrical power supply, and configured to generate an electric field.

[0018] In some embodiments the intracellular delivery apparatus comprises four needles arranged in a Y-type pattern with a center needle and three outer needles disposed

around the center needle, wherein the center needle is a needle-electrode configured to apply a first polarity voltage, and the three outer needles are needle-electrodes, configured to apply a second polarity voltage.

[0019] In some embodiments of the intracellular delivery apparatus, the center needle comprises apertures along the needle barrel, which are positioned to direct delivery of a delivered agent toward apertures that are present on each of the three outer needles and each of the three outer needles comprises apertures along the needle barrel, which are positioned to direct delivery of a delivered agent toward apertures that are present on the center needle, and wherein the outer needles do not have apertures positioned to deliver a delivered agent away from the center needle.

[0020] In some embodiments, the intracellular delivery apparatus comprises an array of needles comprising a center needle and a plurality of outer needles disposed around the center needle, wherein the center needle is a needle-electrode configured to apply a first polarity voltage, and the outer needles are needle-electrodes, configured to apply a second polarity voltage, wherein the center needle comprises apertures along the needle barrel, which are positioned to direct delivery of a delivered agent toward the outer needles and the outer needles comprise apertures along the needle barrel, which are positioned to direct delivery of a delivered agent toward the center needle and/or an adjacent needle, and wherein the outer needles do not have apertures that allow delivery of a delivered agent away from an active zone defined by an area within the array of needles.

[0021] In some embodiments, the intracellular delivery apparatus further comprises seven needles that are arranged in a hexagonal pattern, wherein one needle, which is a needle-electrode, is located at the center of the hexagonal pattern.

[0022] Some embodiments disclosed herein describe an intracellular delivery apparatus as described above comprising a syringe in fluid communication with the hub; a channel configured to receive the syringe; a collar operable to fasten the syringe within the channel; a handle, which operates to enclose the syringe and hub within the channel such that the closed ends of the needle barrels protrude from the channel and are available to engage a subject; a charging element configured to be charged by the operation of the handle, the charging element coupled to a trigger and the syringe such that operation of the trigger releases the charging element and the charging element acts on the syringe, thereby displacing prophylactic and/or therapeutic material out of the syringe; and an electrical port configured to mate with the electrical connector on the hub of the hypodermic needle device thereby

establishing electrical contact between the electrical power supply and the electrodes and/or needle-electrodes.

[0023] In some embodiments, the hub is a pocket hub, comprising an individual reservoir of a delivered agent for each needle or needle-electrode.

[0024] In some embodiments, the hub comprises a single reservoir of a delivered agent for each needle or needle-electrode.

[0025] In some embodiments, at least one needle of the plurality of needles is at least partially laminated with an electrically non-conductive material.

[0026] In some embodiments, the intracellular delivery apparatus further comprises an electrical power supply controller configured to control generation of the electric field.

[0027] In some embodiments, controlling generation of the electric field comprises controlling one or more of the pulse voltage, polarity of the needle-electrodes, the number of pulses, the pulse pattern, or the pulse duration.

[0028] In some embodiments, the charging element is a spring configured to be compressed by the operation of the handle and configured to decompress upon operation of the trigger.

[0029] In some embodiments, the electric field applied at the electrode and/or needle electrodes is commutating.

[0030] Additional embodiments disclosed herein include methods of introducing a delivered agent into a tissue of a subject comprising: providing the intracellular delivery apparatus as described herein; providing a voltage source; inserting a plurality of needles and a plurality of electrodes or a plurality of needle-electrodes of said electroporation apparatus into a tissue of a subject; displacing the delivered agent through the lumen of the plurality of needles or the plurality of needle electrodes and into the tissue of the subject; and applying an electric field to the tissue of the subject.

[0031] In some embodiments, the displacing of the delivered agent is performed prior to applying the electric field to the tissue.

[0032] In some embodiments, the displacing of the delivered agent is performed subsequent to applying the electric field to the tissue.

[0033] In some embodiments, the displacing of the delivered agent is performed in a two stage process, wherein the first stage comprises displacing the delivered agent into the tissue into an active area and the second stage comprises energizing one or more electrodes or needle electrodes to electroporate the cells within the active area.

[0034] In some embodiments, the injection parameter is injection speed or injection pressure.

[0035] In some embodiments the electroporation apparatus described herein is for use in DNA vaccination.

[0036] In some embodiments the DNA vaccination comprises introduction of a polynucleotide encoding a hepatitis antigen.

[0037] In some embodiments the hepatitis antigen is a hepatitis C virus (HCV) antigen.

[0038] In some embodiments the HCV antigen comprises NS3.

[0039] In some embodiments an electroporation apparatus described herein is used to prime T cell responses in a subject specific for HCV by delivering an expression plasmid comprising a human codon-optimized HCV nucleotide sequence (e.g., a sequence encoding NS3/4A) fused to a human codon optimized HBcAg nucleotide sequence (e.g., a sequence encoding a stork or heron HBcAg), wherein the HCV nucleotide sequence and/or the HBcAg nucleotide sequence comprises one or more translation interruption or protease cleavage sites. Optionally, IL12 or a nucleic acid encoding IL12 (e.g., a nucleotide sequence encoding IL12 present on an expression vector) can be provided to said subject so as to improve the immune response. It has been demonstrated in the disclosure that follows that mice (e.g., wild-type C57BL6/J and NS3/4A transgenic mice), which have been administered (by IVIN and electroporation) an expression plasmid comprising a human codon optimized nucleotide sequence encoding NS3/4A joined to a human codon optimized nucleotide sequence encoding stork HBcAg, further comprising protease cleavage sites therein, exhibit a primed HCV-specific T cell response.

[0040] Surprisingly, it has also been discovered that the use of an IVIN device, as described herein, in combination with electroporation induces a robust immune response (e.g., IFN gamma production) when significantly lower amounts (e.g., as compared to conventional *i.m.* injection with electroporation or IVIN injection alone) of DNA immunogen (e.g., an expression plasmid comprising a human codon optimized nucleotide sequence encoding NS3/4A joined to a human codon optimized nucleotide sequence encoding stork HBcAg, further comprising protease cleavage sites therein) are delivered. Similar results are seen in mice and large animals, which have muscle and skin similar to that of humans (e.g., pigs). Additional experiments have shown that the combination of IVIN and electroporation can be used to induce potent immune responses to a DNA immunogen (e.g., an expression plasmid comprising a

human codon optimized nucleotide sequence encoding NS3/4A joined to a human codon optimized nucleotide sequence encoding stork HBcAg, further comprising protease cleavage sites therein) irrespective of whether the DNA immunogen is provided to the dermis (skin) or muscle (e.g., in pigs). In some experiments, potent immune responses to a DNA immunogen delivered by a combination of IVIN and electroporation (e.g., an expression plasmid comprising a human codon optimized nucleotide sequence encoding NS3/4A joined to a human codon optimized nucleotide sequence encoding stork HBcAg, further comprising translation interruption or protease cleavage sites therein) were observed when a prime and boost inoculations were provided to the skin of a pig (both by IVIN and electroporation) and when an intramuscular prime inoculation followed by a boost inoculation to the skin of a pig (both by IVIN and electroporation) were employed. Accordingly, the skilled artisan will readily appreciate in light of the present disclosure that the combination of IVIN and electroporation can induce significantly robust immune responses to DNA immunogens (e.g., an expression plasmid comprising a human codon optimized nucleotide sequence encoding NS3/4A joined to a human codon optimized nucleotide sequence encoding stork HBcAg, further comprising translation interruption or protease cleavage sites therein) in humans, as well; and IVIN in combination with electroporation allows one to deliver a lower amount of DNA-encoding antigen than conventional injection followed by electroporation.

[0041] The present studies show that IVIN technology is so efficacious that a far better immune response is achievable using the same or even much lower doses of DNA than when using a regular injection needle, irrespective of whether the injection is used in combination with *in vivo* electroporation or not. IVIN increases the number of vaccine specific T cells by more than 100% as compared to a regular needle when in combination with electroporation. Using IVIN strong immune responses can be achieved even when the dose is reduced 100-fold. The opportunity to reduce the amount of vaccine, or number of vaccinations, is one of the most important measures for an efficacious vaccine principle. Accordingly, aspects of the invention include use of an IVIN device with electroporation to increase the number of vaccine specific T cells by more than 100% as compared to a regular needle when in combination with electroporation and use of an IVIN device with electroporation to deliver a nucleic acid encoding an antigen in a protocol that requires or uses fewer administrations as compared to a regular needle when in combination with electroporation and, which provides an equivalent immune response as that obtained using a regular needle in combination with electroporation.

BRIEF DESCRIPTION OF THE DRAWINGS

[0042] FIG. 1A illustrates a side view of an embodiment of an intracellular delivery apparatus having two barrels, each barrel having five apertures for delivering a prophylactic and/or therapeutic agent to an area in between the barrels.

[0043] FIG. 1B illustrates an exploded perspective view of one embodiment of a hypodermic needle hub with four barrels for delivering a prophylactic and/or therapeutic agent to an area in between the barrels.

[0044] FIG. 2A illustrates a side view of an embodiment of an intracellular delivery apparatus with two barrels, each barrel having three apertures for delivering a prophylactic and/or therapeutic agent to an area in between the barrels.

[0045] FIG. 2B illustrates an embodiment of an intracellular delivery apparatus with five apertures on each needle that are equally spaced apart.

[0046] FIG. 2C illustrates an embodiment of a needle hub with three needles and depicts dimensions of the hypodermic needle hub.

[0047] FIG. 2D illustrates an embodiment of a needle hub with four needles in a staggered configuration.

[0048] FIG. 3 illustrates a side view of an embodiment of an intracellular delivery apparatus with two barrels, each barrel having ten apertures for delivering a therapeutic agent to an area in between the barrels.

[0049] FIG. 4 illustrates a side view of an embodiment of an intracellular delivery apparatus useful for delivering a prophylactic and/or therapeutic agent including DNA into a muscle cells.

[0050] FIG. 5A is a top view of an intracellular delivery apparatus having three needles.

[0051] FIG. 5B illustrates a side view of an embodiment of an intracellular delivery apparatus with three barrels.

[0052] FIG. 5C illustrates a perspective view of the intracellular delivery apparatus of FIG. 5B delivering a therapeutic agent to the tissue of a subject by providing a cross-spray pattern.

[0053] FIG. 6A illustrates a side view of an embodiment of an intracellular delivery apparatus having two barrels, wherein each barrel is disposed at an angle relative to the longitudinal axis of the device.

[0054] FIG. 6B illustrates a perspective view of an embodiment of an intracellular delivery apparatus with two barrels and a connector fitting.

[0055] FIG. 6C illustrates a top view of an intracellular delivery apparatus with a connector fitting.

[0056] FIG. 7A illustrates a perspective view of an embodiment of an intracellular delivery apparatus with six barrels, each barrel having a plurality of apertures for delivering a prophylactic and/or therapeutic agent to the tissue of a subject.

[0057] FIG. 7B is a top view of the intracellular delivery apparatus of FIG. 7A.

[0058] FIG. 8A illustrates a side view of an embodiment of an intracellular delivery apparatus with four barrels.

[0059] FIG. 8B illustrates a top view of an embodiment of the intracellular delivery apparatus of FIG. 8A having a “Y”-type pattern.

[0060] FIG. 8C illustrates another top view of an embodiment of the intracellular delivery apparatus of FIG. 8A having an “O”-type pattern.

[0061] FIG. 9 illustrates a top view of an embodiment of an intracellular delivery apparatus comprising four barrels.

[0062] FIG. 10A illustrates a top view of an embodiment of an intracellular delivery apparatus comprising seven barrels having a star-type pattern.

[0063] FIG. 10B illustrates a perspective view of a needle hub comprising seven barrels.

[0064] FIG. 11 illustrates a top view of an embodiment of an intracellular delivery apparatus including ten barrels.

[0065] FIG. 12 illustrates a top view of an embodiment of an intracellular delivery apparatus including three barrels.

[0066] FIG. 13 illustrates a top view of an embodiment of an intracellular delivery apparatus including three barrels.

[0067] FIG. 14 illustrates a top view of an embodiment of an intracellular delivery apparatus including four barrels.

[0068] FIG. 15 illustrates a top view of an embodiment of an intracellular delivery apparatus including four barrels.

[0069] FIG. 16 illustrates a top view of an embodiment of an intracellular delivery apparatus including a ring-shaped barrel.

[0070] FIG. 17 illustrates a top view of an embodiment of an intracellular delivery apparatus including a ring-shaped barrel.

[0071] FIG. 18 illustrates a top view of an embodiment of an intracellular delivery apparatus including a ring-shaped barrel.

[0072] FIG. 19 illustrates a cut-away view of an embodiment of a barrel including a single lumen.

[0073] FIG. 20 illustrates a cut-away view of an embodiment of a barrel including two lumens.

[0074] FIG. 21A-D are perspective and side views of one embodiment of a spring-actuated feature for use with the intracellular delivery devices described herein.

[0075] FIG. 22A-B are perspective and side view of one embodiment of a trigger feature for use with the intracellular delivery devices described herein.

[0076] FIG. 23A-C illustrate an embodiment of a needle hub having a top hub portion and a bottom hub portion.

[0077] FIG. 24 illustrates a perspective view of one embodiment of an adjustable delivery feature for use with the intracellular delivery devices described herein.

[0078] FIG. 25 illustrates a cross-sectional view of one embodiment of an adjustable delivery unit for use with an intracellular delivery device.

[0079] FIG. 26 illustrates an embodiment of an intracellular delivery apparatus having slit-type apertures and closed ends.

[0080] FIGS. 27A-C illustrate embodiments of an intracellular delivery apparatus having a micro-hub and closed ended needles.

[0081] FIGS. 28A-B illustrate perspective views of an adjustable delivery device being operated with an intracellular delivery apparatus having a micro-hub attached to a syringe.

[0082] FIGS. 29A-C illustrate the electrical connections to one or more needles of an intracellular delivery apparatus for use in electroporation.

[0083] FIGS. 30A-F illustrate embodiments of needle and electrode configurations of a microhub injection device showing polarity of needles or electrodes, electric fields, and direction of spray flow.

[0084] FIG. 31A illustrates an example of a distal end of an intracellular delivery apparatus having a non-pocket hub.

[0085] FIG. 31B illustrates another example of a distal end of an intracellular delivery apparatus having a pocket hub.

[0086] FIGS. 32A-B illustrate examples of an intracellular delivery apparatus having laminated needles, which are partially electrically insulated.

[0087] FIG. 33A-D illustrate examples of an intracellular delivery apparatus having dual syringes and delivery and electric field patterns of a hypodermic needle device having dual syringes.

[0088] FIG. 34A-C illustrate examples of an intracellular delivery apparatus having isolating valves and needle and electrode configurations of a hypodermic needle device having isolating valves.

[0089] FIG. 35A illustrates an example of an intracellular delivery apparatus during a first stage of a two stage delivery process.

[0090] FIG. 35B illustrate an example of a needle and electrode configuration during a first stage of a two stage delivery.

[0091] FIG. 35C illustrates an example of an intracellular delivery apparatus during a second stage of a two stage delivery.

[0092] FIG. 35D illustrates the needle and electrode configuration of the device of FIG. 35B during the second stage of a two stage delivery.

[0093] FIG. 36 illustrates perspective views of a reusable delivery device providing an electric power supply being operated with an intracellular delivery device having a micro-hub attached to a syringe.

[0094] FIG. 37 illustrates an internal view of reusable delivery device providing an electric power supply with an intracellular delivery apparatus disposed within.

[0095] FIGS. 38A-C illustrate embodiments of an intracellular delivery apparatus having a micro-hub configured for electroporation.

[0096] FIGS. 39A-C illustrate embodiments of an intracellular delivery apparatus with a Y-type configuration having a micro-hub configured for electroporation.

[0097] FIGS. 40A-B depict a small HIP (high injection pressure) injector and a large HIP injector.

[0098] FIG. 41 is a graphical depiction of a rabbit study five days post immunization with a nucleic acid encoding NS3/4A, the radioactivity of cells, as counts per minute, when incubated with various antigens at various concentrations to show radioactive thymidine uptake in a T cell proliferation assay is provided.

[0099] FIG. 42 is a chart illustrating HCV NS3-specific T cell proliferation as a result of immunization with the HIP injector. Proliferation is measured as radioactivity of cells incubated with antigen divided by the radioactivity of cells incubated with media alone.

[0100] FIGS. 43A-C are histological evaluations of tissue at the site of injection with a regular 27 gauge needle (FIG. 43A), a small HIP injector (FIG. 43B), and a large HIP injector (FIG. 43C).

[0101] FIGS. 44A-I depict various constructs containing the NS3/4A platform and the HBcAg with and without protease cleavage sites.

[0102] FIG. 45 depicts an embodiment of a setup for measuring the force requirements when injecting material using one of the injection needle devices disclosed herein.

[0103] FIG. 46A-F are top and cross-sectional views of Tests 7-9 showing dyed water injected into chicken breast using an intracellular delivery apparatus described herein.

[0104] FIG. 47A-F are top and cross-sectional views of Tests 25-27 showing dyed water injected into chicken breast using an intracellular delivery apparatus described herein.

[0105] FIG. 48A-F are top and cross-sectional views of Tests 16-18 showing dyed water injected into chicken breast using an intracellular delivery apparatus described herein.

[0106] FIG. 49A-F are top and cross-sectional views of Tests 34-36 showing dyed water injected into chicken breast using an intracellular delivery apparatus described herein.

[0107] FIG. 50A-F are top and cross-sectional views of chicken breast having dyed water injected by hand using an intracellular delivery apparatus described herein.

[0108] FIG. 51A-F are top and cross-sectional views of chicken breast having dyed water injected by hand using a single needle.

[0109] FIG. 52 shows a graph of the maximum force for injecting a placebo fluid with needle configurations "3Y01(72)", "3O01(96)", and "6X01(144)" using a 5 mL syringe.

[0110] FIG. 53 shows a graph of the maximum force for injecting water into air with needle configurations "3Y01(72)", "3O01(96)", and "6X01(144)" using a 10 mL syringe.

[0111] FIG. 54 shows a graph of the maximum force for injecting water into air with needle configurations "3Y05(72)", "3O05(72)", and "3X05(72)" using a 10 mL syringe.

[0112] FIG. 55 shows images of dyed water injected into chicken by hand delivery with a standard needle, or using a Lloyd force tensometer with the 3Y05(72)", "3O05(72)", "3X05(72)", 3Y01(72)", or "3O01(96)" needle configurations.

[0113] FIGS. 56A1-B2 show results of injection using a regular needle with and without electroporation and injection using an intracellular delivery apparatus described herein with and without with electroporation.

[0114] FIGS. 57A1-C2 show the results of two experiments evaluating injection using an intracellular delivery apparatus described herein at two injection force values with electroporation compared to a conventional needle with electroporation.

[0115] FIG. 58A illustrates immunization data in a mouse using an IVIN intracellular delivery apparatus, without electroporation, delivering a construct having a sequence encoding codon optimized NS3/4A-codon optimized stork HBcAg polynucleotide containing translation interruption sites introduced therein (C2.2 in a pVAX1 backbone).

[0116] FIG. 58B illustrates immunization data in a mouse using an a conventional injection needle, without electroporation, delivering a construct having a sequence encoding a codon optimized NS3/4Apolypeptide and a codon optimized stork HBcAg polynucleotide separated translation interruption sites introduced therein (C2.2 in a pVAX1 backbone).

[0117] FIG. 58C illustrates immunization data in a mouse using an IVIN intracellular delivery apparatus, with electroporation, delivering a construct having a sequence encoding a codon optimized NS3/4Apolypeptide and a codon optimized stork HBcAg polynucleotide separated by translation interruption sites introduced therein (C2.2 in a pVAX1 backbone).

[0118] FIG. 58D illustrates immunization data in a mouse using a conventional injection needle with electroporation, delivering a construct having a sequence encoding a codon optimized NS3/4Apolypeptide and a codon optimized stork HBcAg polynucleotide separated by translation interruption sites introduced therein (C2.2 in a pVAX1 backbone).

[0119] FIGS. 59A-B illustrate immunization data of non-immunized pigs.

[0120] FIGS. 59C illustrates immunization data of an immunized pig, which was injected with a construct having a sequence encoding a codon optimized NS3/4Apolypeptide and a codon optimized stork HBcAg polynucleotide separated by translation interruption sites introduced therein (C2.2 in a pVAX1 backbone).

[0121] FIG. 60 illustrates the percent of NS3-pentamer-specific CD8⁺ cells among untreated, injected, IVIN treated, injection/electroporated, and IVIN/electroporated subjects, wherein said subjects were immunized with construct having a sequence encoding a codon optimized NS3/4Apolypeptide and a codon optimized stork HBcAg polynucleotide separated by translation interruption sites introduced therein (C2.2 in a pVAX1 backbone).

[0122] FIG. 61 illustrates the proportion of IFN- γ SFCs per million cells induced upon administration of human codon optimized NS3/4A-human codon optimized stork HBcAg polynucleotide immunogenic compositions via injection, IVIN treatment, injection/electroporation, and IVIN/electroporation.

[0123] FIG. 62 illustrates the proportion of IFN- γ SFCs per million cells induced upon administration of human codon optimized NS3/4A-human codon optimized stork HBcAg polynucleotide immunogenic compositions further comprising murine (m) IL-12 (SEQ ID NO.: 36) polynucleotides in wild type and NS3/4A transgenic mice.

[0124] FIG. 63 shows detection of HCV NS3-protein expressing muscle fibers and inflammation (hematoxylin-eosin) in rabbit tibialis anterior muscles five days after immunization of 0.9mg in 0.3mL of human codon-optimized HCV NS3/4A DNA immunogen.

[0125] FIG. 64 A-J illustrate the proportion of IFN- γ SFCs per million cells induced upon IVIN and electroporation administration of nucleic acids encoding a construct having a sequence encoding a human codon optimized NS3/4Apolypeptide and a human codon optimized stork HBcAg polynucleotide separated translation interruption sites introduced therein (C2.2 in a pVAX1 backbone) further comprising mIL-12 (SEQ ID NO.: 36) polynucleotides in wild type mice.

[0126] FIG. 65 A-J illustrate the proportion of IFN- γ SFCs per million cells induced upon IVIN and electroporation administration of nucleic acids encoding a construct having a sequence encoding a human codon optimized NS3/4Apolypeptide and a human codon optimized stork HBcAg polynucleotide separated translation interruption sites introduced therein (C2.2 in a pVAX1 backbone) further comprising mIL-12 (SEQ ID NO.: 36) polynucleotides in NS3/4A transgenic mice.

[0127] FIG. 66 A-C illustrate the proportion of IFN- γ SFCs per million cells induced upon IVIN and electroporation administration of differing amounts of nucleic acids encoding a construct having a sequence encoding a human codon optimized NS3/4Apolypeptide and a human codon optimized stork HBcAg polynucleotide separated translation interruption sites introduced therein (C2.2 in a pVAX1 backbone) in mice demonstrating that IVIN and electroporation allows for the use of a lower dose of immunogen, as compared to conventional injection followed by electroporation.

[0128] FIG. 67 A-H illustrate the proportion of IFN- γ SFCs per million cells induced upon single or double (prime and prime/boost) IVIN and electroporation administrations into the dermis (Skin) or muscle (IM) of differing amounts of nucleic acids encoding a construct having

a sequence encoding a human codon optimized NS3/4A polypeptide and a human codon optimized stork HBcAg polynucleotide separated translation interruption sites introduced therein (C2.2 in a pVAX1 backbone) in pigs demonstrating that a prime and boost IVIN and electroporation administration allows for a more robust response.

[0129] FIG. 68 A-B illustrate the proportion of IFN- γ SFCs per million cells induced upon intramuscular and electroporation or IVIN and electroporation administration of differing amounts of polynucleic acids encoding a human codon optimized human HBcAg polynucleotide immunogenic compositions further comprising mL-12 (SEQ ID NO.: 36) polynucleotides demonstrating that IVIN and electroporation improves the immune response to HBcAg as compared to conventional intramuscular injection in combination with electroporation.

[0130] FIG. 69 illustrates the percent of HBcAg-pentamer-specific CD8⁺ T cells among untreated, conventional needle/electroporated or IVIN/electroporated subjects, wherein said subjects were immunized with a construct having human codon optimized HBcAg polynucleotide immunogenic compositions further comprising mL-12. The data demonstrate that IVIN significantly improve the number of HBcAg specific CD8⁺ T cells as compared to conventional needle injection.

DETAILED DESCRIPTION

[0131] Aspects of this invention described herein concern devices and methods for the delivery of agents (e.g., nucleic acids) into a tissue. Some embodiments concern an intracellular delivery apparatus configured to introduce agents, such as nucleic acids, especially DNA, into a target tissue, wherein the molecules are taken up by the cells in a region localized to a site near or proximal to the site of injection (e.g., within a region defined by the area within a needle array of an intracellular delivery apparatus described herein).

[0132] The needle(s) of an intracellular delivery apparatus may comprise a fitting connector or a needle hub, which may comprise a sleeve with an internal thread. The fitting connector or needle hub is configured to attach the needle to the syringe or vessel containing the agent to be introduced. In some embodiments, the sleeve forms the attachment means or fastener and it can be screwed onto an outer thread on an attachment part of a syringe. The fitting connectors or needle hubs can also comprise a press-on assembly, a snap-on assembly, or a Luer Taper connection, such as a Luer Lok or Luer Slip connection or a butterfly connector.

[0133] The needle(s) of an intracellular delivery apparatus described herein may be attached to one or more syringe barrels (e.g., permanently affixed or removably attached) and

said syringe barrels or the device may contain the prophylactic and/or therapeutic agent that is to be delivered. For example, in some embodiments, the needle(s) and attached syringe may be pre-loaded with a prophylactic and/or therapeutic agent, such as a nucleic acid, protein, modified nucleic acid, aptamer, or cell population for a single-use application. In some embodiments, the agent may be drawn into the syringe through apertures disposed on the needles(s). The syringe barrels can be of a variety of sizes (e.g., 0.3cc-100cc or more). That is, the syringe barrels can be greater than or equal to or any number or range in between 0.1, 0.3, 0.4, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 cc size. The syringe barrels can be constructed from a variety of materials (e.g., metal, plastic, nylon, polyethylene, glass).

[0134] The needle(s) of an intracellular delivery apparatus described herein may be attached to one or more devices that facilitate delivery of prophylactic and/or therapeutic molecules or agents to tissue, including but not limited to gene guns, electroporation systems, and microneedle devices. The injection needle(s) described herein can be modified for use with existing technologies, including gene gun delivery systems (*see e.g.*, U.S. Patent Nos. 5,036,006; 5,240,855; and 5,702,384, the disclosures of which are hereby expressly incorporated by reference in their entireties), delivery systems using electroporation (*see e.g.*, U.S. Patent Nos. 6,610,044 and 5,273,525, 6,132,419, and 6,527,216, the disclosures of which are hereby expressly incorporated by reference in their entireties) and microneedle delivery systems (*see e.g.*, U.S. Patent Nos. 6,960,193; 6,623,457; 6,334,856; 5,457,041; 5,527,288; 5,697,901; 6,440,096; 6,743,211; and 7,226,439, the disclosures of which are hereby expressly incorporated by reference in their entireties).

[0135] As described herein, the intracellular delivery devices comprising the needle(s) may also contain a variety of prophylactic and/or therapeutic agents (e.g. a cell population, such as a cell population comprising stem cells, a chemical, a compound, a chemotherapeutic agent, a protein, a specificity exchanger, a nucleic acid such as DNA, RNA, other natural nucleic acids, a modified nucleic acid, or a DNA or nucleic acid aptamer). In some embodiments, the intracellular delivery devices comprising one or more of the needle(s) described herein comprise a DNA that encodes an immunogen (preferably a viral antigen, such as hepatitis C virus (HCV), hepatitis B virus (HBV), human immunodeficiency virus (HIV), influenza, Japanese encephalitis virus (JEV), human papilloma virus (HPV), or a parasite

antigen, such as a malaria antigen, or a plant antigen, such as birch antigen, or a bacterial antigen, such as a staphylococcal or anthrax antigen, or a tumor antigen). In some embodiments, the intracellular delivery devices comprising one or more of the needles described herein comprise one or more of the aforementioned nucleic acids pre-loaded (e.g., a pre-loaded, single use syringe with coupled needle(s) containing a measured dose of delivered agent).

[0136] In some embodiments, the prophylactic and/or therapeutic agent that is delivered or contained in a syringe, needle, or injection device, as described herein, comprises a natural nucleic acid and in other embodiments, the prophylactic and/or therapeutic agent that is delivered or contained in a syringe, needle, or an intracellular delivery device, as described herein, comprises an unnatural nucleic acid (e.g., containing an artificial nucleotide, universal base, or spacer). Natural nucleic acids that can be used as the therapeutic agent that is delivered or contained in a syringe or injection device, as described herein, comprise a deoxyribose- or ribose-phosphate backbone. An artificial or synthetic polynucleotide that can be used as the prophylactic and/or therapeutic agent that is delivered or contained in a syringe, needle, or intracellular delivery device, as described herein, can comprise any polynucleotide that is polymerized *in vitro* or in a cell free system and contains the same or similar bases but may contain a backbone of a type other than the natural ribose-phosphate backbone. These backbones include: PNAs (peptide nucleic acids), phosphorothioates, phosphorodiamidates, morpholinos, and other variants of the phosphate backbone of native nucleic acids. Bases that may be included in one or more embodiments described herein include purines and pyrimidines, which further include the natural compounds adenine, thymine, guanine, cytosine, uracil, inosine, and natural analogs. Synthetic derivatives of purines and pyrimidines that may be included in one or more embodiments described herein include, but are not limited to, modifications which place new reactive groups such as, but not limited to, amines, alcohols, thiols, carboxylates, and alkylhalides.

[0137] The term “base,” as used herein, encompasses any of the known base analogs of DNA and RNA including, but not limited to, 4-acetylcytosine, 8-hydroxy-N6-methyladenosine, aziridinylcytosine, pseudoisocytosine, 5-(carboxyhydroxymethyl)uracil, 5-fluorouracil, 5-bromouracil, 5-carboxymethylaminomethyl-2-thiouracil, 5-carboxymethylaminomethyluracil, dihydrouracil, inosine, N6-isopentenyladenine, 1-methyladenine, 1-methylpseudo-uracil, 1-methylguanine, 1-methylinosine, 2,2-dimethyl-guanine, 2-methyladenine, 2-methylguanine, 3-methyl-cytosine, 5-methylcytosine, N6-methyladenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxy-amino-methyl-2-thiouracil, beta-D-

mannosylqueosine, 5'-methoxycarbonylmethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, oxybutosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, N-uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, pseudouracil, queosine, 2-thiocytosine, and 2,6-diaminopurine. The term polynucleotide includes deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) and combinations on DNA, RNA and other natural and synthetic nucleotides.

[0138] The prophylactic and/or therapeutic agent that is delivered or contained in a syringe, needle, or an intracellular delivery device, as described herein, can comprise DNA, which may be in the form of cDNA, *in vitro* polymerized DNA, plasmid DNA, parts of a plasmid DNA, genetic material derived from a virus, linear DNA, vectors (P1, PAC, BAC, YAC, artificial chromosomes), expression cassettes, chimeric sequences, recombinant DNA, chromosomal DNA, an oligonucleotide, anti-sense DNA, or derivatives of these groups. RNA may be in the form of oligonucleotide RNA, tRNA (transfer RNA), snRNA (small nuclear RNA), rRNA (ribosomal RNA), mRNA (messenger RNA), *in vitro* polymerized RNA, recombinant RNA, chimeric sequences, anti-sense RNA, siRNA (small interfering RNA), miRNA (micro RNA), ribozymes, or derivatives of these groups. The prophylactic and/or therapeutic agent that is delivered or contained in a syringe, needle, or an intracellular delivery device, as described herein, can also comprise an anti-sense polynucleotide that is a polynucleotide that interferes with the function of DNA and/or RNA. Antisense polynucleotides include, but are not limited to: morpholinos, 2'-O-methyl polynucleotides, DNA, RNA and the like. SiRNA comprises a double stranded structure typically containing 15 to 50 base pairs and preferably 21 to 25 base pairs and having a nucleotide sequence identical or nearly identical to an expressed target gene or RNA within the cell. Interference may result in suppression of expression. The polynucleotide can be a sequence whose presence or expression in a cell alters the expression or function of cellular genes or RNA. In addition, DNA and RNA may be single, double, triple, or quadruple stranded. Double, triple, and quadruple stranded polynucleotides may contain both RNA and DNA or other combinations of natural and/or synthetic nucleic acids. These polynucleotides can be delivered to a cell to express an exogenous nucleotide sequence, to inhibit, eliminate, augment, or alter expression of an endogenous nucleotide sequence, or to express a specific physiological characteristic not naturally associated with the cell. Polynucleotides may be coded to express a whole or partial protein, or may be anti-sense. The delivered polynucleotide can stay within the cytoplasm or nucleus apart from the

endogenous genetic material. Alternatively, the polymer could recombine with or become a part of the endogenous genetic material. For example, the therapeutic agent that is delivered or contained in a syringe or an intracellular delivery device, as described herein, can comprise a DNA that can insert itself into chromosomal DNA by either homologous or non-homologous recombination.

[0139] The prophylactic and/or therapeutic agent that is delivered or contained in a syringe, needle, or an intracellular delivery device, as described herein, can also comprise an RNA inhibitor, which is any nucleic acid or nucleic acid analog containing a sequence whose presence or expression in a cell causes the degradation of or inhibits the function or translation of a specific cellular RNA, usually a mRNA, in a sequence-specific manner. An RNA inhibitor may also inhibit the transcription of a gene into RNA. Inhibition of RNA can effectively inhibit expression of a gene from which the RNA is transcribed. RNA inhibitors include, but are not limited to, siRNA, microRNAs (miRNAs), interfering RNA or RNAi, dsRNA, RNA Polymerase III transcribed DNAs, ribozymes, and antisense nucleic acid, which may be RNA, DNA, or an artificial nucleic acid. MicroRNAs (miRNAs) also typically have a length of between 15-50 nucleotides, preferably between 20-25 nucleotides in length, and miRNAs can be used as post-transcriptional regulators that bind to complementary sequences on target messenger RNA transcripts (mRNAs), usually resulting in translational repression and gene silencing. Antisense polynucleotides can include, but are not limited to: morpholinos, 2'-O-methyl polynucleotides, DNA, RNA and the like. RNA polymerase III transcribed DNAs can contain promoters, such as the U6 promoter. These DNAs can be transcribed to produce small hairpin RNAs in the cell that can function as siRNA or linear RNAs that can function as antisense RNA. The RNA inhibitor may be polymerized *in vitro*, recombinant RNA, contain chimeric sequences, or derivatives of these groups. The RNA inhibitor may contain ribonucleotides, deoxyribonucleotides, synthetic nucleotides, or any suitable combination such that the target RNA and/or gene are inhibited. In addition, these forms of nucleic acid may be single, double, triple, or quadruple stranded.

[0140] The prophylactic and/or therapeutic agent that is delivered or contained in a syringe, needle, or an intracellular delivery device, as described herein, can also include a nucleic acid that is incorporated into a vector (e.g., an expression vector). Vectors are polynucleic molecules originating from a virus, a plasmid, or the cell of a higher organism into which another nucleic fragment of appropriate size can be integrated; vectors typically introduce foreign DNA into host cells, where it can be reproduced. Examples are plasmids, cosmids, and

yeast artificial chromosomes; vectors are often recombinant molecules containing DNA sequences from several sources.

[0141] As used herein, term “vector” refers any DNA molecule that could include associate molecules to transfer DNA sequences into a cell for expression. Examples include naked DNA, non-viral DNA complexes (e.g. DNA plus polymers [cationic or anionic], DNA plus transfection enhancing compounds, and DNA plus amphipathic compounds) and viral particles. As used herein, vector may also include a viral vector: for example, adenovirus; DNA; adenoassociated viral vectors (AAV) which are derived from adenoassociated viruses and are smaller than adenoviruses; and retrovirus (any virus in the family *Retroviridae* that has RNA as its nucleic acid and uses the enzyme reverse transcriptase to copy its genome into the DNA of the host cell's chromosome; examples include VSV G and retroviruses that contain components of lentivirus including HIV type viruses).

[0142] The prophylactic and/or therapeutic agent that is delivered or contained in a syringe, needle, or an intracellular delivery device, as described herein, can also comprise one or more compounds that enhance the uptake of the therapeutic agent (e.g., a nucleic acid, as described herein). The therapeutic agent that is delivered or contained in a syringe, needle, or an intracellular delivery device, as described herein, can comprise a polymer, for example, which is a molecule built up by repetitive bonding together of smaller units called monomers. The term “polymer” can include both oligomers, which have two to 80 monomers and polymers having more than 80 monomers. The polymer can be linear, branched network, star, comb, or ladder types of polymer. The polymer can be a homopolymer in which a single monomer is used or can be copolymer in which two or more monomers are used. Types of copolymers include alternating, random, block and graft.

[0143] The prophylactic and/or therapeutic agent that is delivered or contained in a syringe, needle, or an intracellular delivery devices, as described herein, may also comprise a nucleic acid-polycation complex. Cationic proteins like histones and protamines or synthetic polymers like polylysine, polyarginine, polyornithine, DEAE dextran, polybrene, and polyethylenimine are effective intracellular delivery agents. A polycation is a polymer containing a net positive charge, for example poly-L-lysine hydrobromide. The polycation can contain monomer units that are charge positive, charge neutral, or charge negative, however, the net charge of the polymer is desirably positive. The term “polycation” also can refer to a non-polymeric molecule that contains two or more positive charges. A polyanion is a polymer containing a net negative charge, for example polyglutamic acid. The polyanion can contain

monomer units that are charge negative, charge neutral, or charge positive, however, the net charge on the polymer must be negative. The term “polyanion” can also refer to a non-polymeric molecule that contains two or more negative charges. The term “polyion” includes polycation, polyanion, zwitterionic polymers, and neutral polymers that contain equal amounts of anions and cations. The term “zwitterionic” refers to the product (salt) of the reaction between an acidic group and a basic group that are part of the same molecule. Salts are ionic compounds that dissociate into cations and anions when dissolved in solution. Salts increase the ionic strength of a solution, and consequently decrease interactions between nucleic acids with other cations.

[0144] The prophylactic and/or therapeutic agent that is delivered or contained in a syringe, needle, or an intracellular delivery device, as described herein, may also comprise a specificity exchanger. Several types of specificity exchangers are known and any one or more of these molecules can be delivered or contained in a syringe, needle, or injection device, as described herein. For example, U.S. Patent Nos. 7,318,926; 7,019,111; 6,933,366; 6,660,842; 6,469,143; 6,245,895; 6,040,137; 5,869,232; 7,943,149; 6,417,324 describe specificity exchangers, the disclosure of which are expressly incorporated by reference in their entireties. Preferably, specificity exchangers that comprise a ligand for a receptor on a pathogen joined to an oligosaccharide (e.g., the gal epitope or Gal α 1-3Gal- β 1-4GlcNAc-R (preferably synthetically assembled specificity exchangers or glycoconjugates are used, for example, specificity exchangers or glycoconjugates prepared by solid phase peptide synthesis).

[0145] Some embodiments relate to an intracellular delivery device that comprises a plurality of needles, which are arranged or configured to deliver a therapeutic agent to a targeted tissue. In some embodiments, the agent is delivered through the proximal end of the injection device by a syringe and the agent is delivered to the targeted tissue through a plurality of apertures disposed on or near the distal ends of the plurality of needle barrels. In other embodiments, the end of the apertures can be disposed on the proximal ends of the needles barrels. In some embodiments, a plurality of needles of any one or more of the design features described herein are provided on an injection device. Embodiments described herein also include a cannula that comprises a plurality of needles configured as described above. That is, in some embodiments the intracellular delivery device and/or cannula can comprise, consist, or consist essentially of 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more needles. The needles can be of the same size and length or can be of different sizes and lengths. Each needle in embodiments that have more than one needle can have a plurality of apertures, which can be in a first or second

zone, as described above, or both (e.g., along the length of the band). Preferably, the needles of the intracellular delivery devices have a closed end, as described above. Intracellular delivery devices and/or cannulas that comprise, consist, or consist essentially of 2, 3, 4, 5, 6, 7, 8, 9, or 10 needles can be configured such that at least two needles have a different amount of apertures and/or different sizes of apertures and/or different shapes of apertures and/or different positions of apertures and said needles preferably have a closed end. Preferably, the needles and apertures are oriented such that the apertures on each needle oppose the apertures on another needle so as to generate an opposing field of delivery of a delivered agent or a cross-spray pattern of delivery of a delivered agent (e.g., in a four needle array having a central needle and three outer needles, the outer needles can have apertures that direct delivered agent toward apertures present on the central needle and the central needle has apertures in three zones, wherein each zone opposes apertures present on the outer needles and delivered agent exiting the central needle is directed to apertures present on each of the outer needles). In some embodiments of an intracellular delivery device, such needle arrays are connected to or disposed on a hub that comprises at least one electrical connector. Again, preferably, the apertures on each needle in the array oppose the apertures on another needle so as to generate a cross-spray pattern or a region of opposing delivery of a delivered agent. In this way, a region within the needle array is overloaded locally with the delivered agent, thereby providing a high concentration of the delivered agent within the region, which is particularly useful when an electric field is applied thereto so as to improve uptake of the delivered agent. The injection devices, hypodermic needle assemblies, or intracellular delivery devices also preferably include an electrode (in some embodiments, one or more of the needles themselves are electrodes) and an electrical power supply configured to generate an electric field at the electrode, wherein the electrical power supply is connected to the electrode through the at least one electrical connector. In some embodiments, one needle or a plurality of needles has apertures in a first zone proximal to a closed end of the barrel and one needle or a plurality of needles that has apertures in a second zone that is distal to a closed end of the needle barrel. Additionally, some embodiments may have a first needle (e.g., a single needle on a single needle device, as described below, or the first needle in a device having a plurality of needles) or a first plurality of needles with apertures that are a size equal to, greater than or less than or any number or range of numbers in between 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5, 0.55, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85, 0.9, 0.95, 1.0, 1.05, 1.10, 1.15, 1.20, 1.25, 1.30, 1.35, 1.40, 1.45, 1.50, 1.55, 1.60, 1.65, 1.70, 1.75, 1.80, 1.85, 1.90, 1.95, 2.0, 2.05, 2.10, 2.15, 2.20, 2.25,

2.30, 2.35, 2.40, 2.45, 2.50, 2.55, 2.60, 2.65, 2.70, 2.75, 2.80, 2.85, 2.90, 2.95, 3.0, 3.05, 3.10, 3.15, 3.20, 3.25, 3.30, 3.35, 3.40, 3.45, 3.50, 3.55, 3.60, 3.65, 3.70, 3.75, 3.80, 3.85, 3.90, 3.95, or 4.0 mm in its widest portion). In some embodiments, one needle or a plurality of needles has apertures that less than 1 μm at its widest portion. In some embodiments, one needle or a plurality of needles has apertures that less than 900 nm at its widest portion. In some embodiments, one needle or a plurality of needles has apertures that less than 500 nm at its widest portion. In some embodiments, the first needle in an intracellular delivery device having a plurality of needles has apertures that are smaller or substantially smaller than a second needle or a second plurality of needles in the device.

[0146] In some embodiments, the intracellular delivery device includes only one needle. The needle can include any of the design features disclosed herein. For example the needle can have a closed or open end. The needle may include a plurality of apertures, for example, at least, greater than or equal to or any number or range in between 5, 10, 20, 30, 50, 70, 100, 120, 140, 160, 180, 200, 500 apertures (e.g., at least 5-100, 10-100, 20-100, 30-100, 40-100, 50-100 or 5-200, 10-200, 20-200, 30-200, 40-200, 50-200, 100-200, 100-500, 140-500, 150-500, 200-500 apertures). The apertures can be evenly spaced, or randomly distributed. The apertures may, in some embodiments, form a regular pattern on the needle. For example, the apertures may form a pattern having a rotational symmetry along the axis of the needle. The rotational symmetry may include 2-fold, 3-fold, 4-fold, 5-fold, six-fold, or a higher degree of rotational symmetry. As another example, the apertures may form a pattern having a screw axis symmetry. The screw axis symmetry can be include 2-fold, 3-fold, 4-fold, 5-fold, six-fold, or a higher degree of rotational symmetry. The translation vector for the screw axis can, for example, be at least, not more than, not more than 0.01 mm, 0.05 mm, 0.1 mm, 0.15 mm, 0.2 mm, 0.25 mm, 0.3 mm, 0.4 mm, 0.5 mm, 0.6 mm, 0.7 mm, 0.8 mm, 0.9 mm, 1 mm, 2 mm, 3 mm, 4 mm, 5mm, 6 mm, 7 mm, 8 mm, 9 mm, 1 cm, 2 cm or 3 cm. In some embodiments, the apertures are configured to produce a radial pattern when a prophylactic and/or therapeutic material is injected.

[0147] In some embodiments, the intracellular delivery device comprises one or more needles that are fluidly coupled to a syringe or a reservoir containing the prophylactic and/or therapeutic material so that the relative orientation of the one or more needles with the syringe or reservoir is fixed. The end(s) of the one or more needles may, in some embodiments, be both fluidly coupled and disposed near the syringe or a reservoir containing the prophylactic and/or therapeutic material. For example, an end of the one or more needles may be not more

than 1 mm, 5 mm, 1 cm, 2 cm, 3 cm, 4 cm, 5 cm, 6 cm, 7 cm, 8 cm, 9 cm, or 10 cm apart from the syringe or a reservoir containing the prophylactic and/or therapeutic material. In some embodiments, the end(s) of the one or more needles are no more than 10 cm apart from the syringe or a reservoir that is fluidly coupled with the needle(s) and contains the prophylactic and/or therapeutic material.

[0148] Some embodiments relate to injection devices, intracellular delivery devices, cannulas, and needles comprising a fluid containing an agent, as described herein (e.g., a medicinal compound, chemical, nucleic acid, peptide, specificity exchanger, and, in particular, a DNA). In some embodiments, the intracellular delivery devices, cannulas, and needles, described herein, are for single use. That is, some embodiments comprise one or more of the needle designs, described herein, joined to a receptacle (preferably a sterile container, such as a sterilized syringe) that comprises a single application or dose of delivered agent (e.g., medicinal compound, chemical, nucleic acid, peptide, specificity exchanger, and, in particular a DNA). Accordingly, a single application or device can be conveniently packaged and provided to medical practitioners or end-consumers, which can administer said agent at an appropriate site and, following administration, the used injection device, needle, or cannula comprising a plurality of needles can be appropriately discarded. Some embodiments also include methods of making and using the aforementioned devices and methods of inducing an immune response to a desired antigen.

[0149] In some embodiments, the intracellular delivery device is not configured to apply an electric field shortly after or simultaneous with the introduction of the prophylactic and/or therapeutic material (e.g., DNA) at the tissue around and/or through the site of the injection. For example, the intracellular delivery device may not include a voltage source coupled to the device and configured to apply an electric field to the tissue at or near the site of injection. In other embodiments, the intracellular delivery device is configured to apply an electric field shortly after or simultaneous with the introduction of the prophylactic and/or therapeutic material (e.g., DNA) at the tissue around and/or through the site of the injection. For example, the intracellular delivery device may include a voltage source coupled to the device and configured to apply an electric field to the tissue at or near the site of injection.

[0150] Some embodiments disclosed herein include a method of delivering a prophylactic and/or therapeutic material to a subject in need thereof, where the prophylactic and/or therapeutic material is administered using any of the intracellular delivery devices disclosed herein. The prophylactic and/or therapeutic material may be any of those materials

disclosed herein. In some embodiments, the method may also include maintaining the one or more needles inserted within the tissue for at least a predetermined time after injecting the prophylactic and/or therapeutic material but before withdrawing the one or more needles. The one or more needles may be maintained in the tissue, for example, at least, greater than or equal to 1 s, 2 s, 3 s, 4 s, 5 s, or more after injecting the therapeutic material but before withdrawing the one or more needles. In some embodiments, the entire dosage is delivered in a period of time less than 60 seconds, 40 seconds, 30 seconds, 20 seconds, 15 seconds, 10 seconds, 5 seconds, 4 seconds, 3 seconds, 2 seconds, 1.5 seconds, 1.0 second, 0.8 seconds, 0.5 seconds, 0.4 seconds, 0.3 seconds, or 0.2 seconds.

[0151] The period of time between inserting the one or more needles into the tissue and removing the one or more needles into the tissue can also vary. The period of time may, for example, be at least, not more than, not more than 0.5 seconds, 1 second, 2 seconds, 3 seconds, 4 seconds, 5 seconds, 6 seconds, 7 seconds, 8 seconds, 9 seconds, 10 seconds, 15 seconds, 30 seconds, 45 seconds, or 60 seconds.

[0152] In some embodiments, the needle(s) and any of the devices described herein can be affixed to the body of a subject for greater periods of time so as to allow for a long term delivery of a prophylactic and/or therapeutic agent (e.g., delivery for at least, greater than or equal to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days) and such needles and devices can be affixed to miniature pumps so as to administer small amounts of prophylactic and/or therapeutic material (e.g. a cell population, such as a cell population comprising stem cells, chemical, a compound, a chemotherapeutic agent, a protein, a nucleic acid, such as DNA, RNA, other natural nucleic acid, a modified nucleic acid, or a DNA or nucleic acid aptamer), to said subjects over an extended period of time.

[0153] The prophylactic and/or therapeutic material may be delivered to the body, in some embodiments, at high pressures through any of the intracellular delivery devices disclosed herein, using a pressure generation element. The pressure generation element may be a plunger on a syringe, and may comprise a spring or other element which depresses the plunger to generate a high force or pressure on the prophylactic and/or therapeutic material. The high force or pressure generated by the pressure generation element is applied to the prophylactic and/or therapeutic material to displace it through the needle (e.g., by applying force to a plunger on a syringe coupled to the needle(s)). The maximum force can, for example, be at least or not more than 25 N, 40 N, 50 N, 75 N, 100 N, 150 N, 200 N, or 500 N. The maximum pressure applied to the therapeutic material can, for example, be at least, or not more than, or any number or range

in between 50 kPa, 100 kPa, 200 kPa, 300, kPa, 400 kPa, 500 kPa, 600 kPa, 700 kPa, 800 kPa, 900 kPa, 1000 kPa, 1100 kPa, 1200 kPa, 1300 kPa, 1400 kPa, 1500 kPa, 1600 kPa, 1700 kPa, 1800 kPa, 1900 kPa, 2000 kPa, 2100 kPa, 2200 kPa, 2300 kPa, 2400 kPa, 2500 kPa, 2600 kPa, 2700 kPa, 2800 kPa, 2900 kPa, 3000 kPa, 3100 kPa, 3200 kPa, 3300 kPa, 3400 kPa, 3500 kPa, 3600 kPa, 3700 kPa, 3800 kPa, 3900 kPa, 4000 kPa, 4100 kPa, 4200 kPa, 4300 kPa, 4400 kPa, 4500 kPa, 4600 kPa, 4700 kPa, 4800 kPa, 4900 kPa, 5000 kPa, 6000 kPa, 7000 kPa, 8000 kPa, 9000 kPa, 10 MPa, 15 MPa, 20 MPa, 25 MPa, or 30 MPa.

[0154] In some embodiments, the prophylactic and/or therapeutic material can be delivered at high hole velocities (i.e., the average velocity of the fluid exiting the apertures). The hole velocity can be, for example, at least, or not more than 1 mm/s, 1.5 mm/s, 2 mm/s, 2.5 mm/s, 3 mm/s, 3.5 mm/s, 4 mm/s, 5 mm/s, 6 mm/s, 7 mm/s, 8 mm/s, 9 mm/s or 10 mm/s. In a preferred embodiment, the hole velocity can be at least 3 mm/s. The hole velocity may be determined, for example, by the volume rate of injecting the prophylactic and/or therapeutic material relative the average size of the apertures. The intracellular delivery devices disclosed herein may optionally be configured to delivery at any of the hole velocities disclosed above. For example, the intracellular delivery device can be coupled with a spring piston configured to inject prophylactic and/or therapeutic material at a volume rate sufficient to produce the desired hole velocity.

[0155] Some aspects of the invention concern a intracellular delivery device comprising a needle that comprises a lumen adapted for the passage of a prophylactic and/or therapeutic material and a needle barrel that comprises a plurality of apertures on the length of the barrel, wherein said needle barrel has a closed-end; and a connector configured to join said needle to a pressure generation element. In some embodiments, the intracellular delivery device above comprises a plurality of needles as described herein and in some embodiments, the intracellular delivery device comprises a circular, diamond, triangular, square, rectangle, trapezoidal, ovoid, or otherwise shaped array of needles. Preferably, the intracellular delivery device is designed such that the plurality of said needles is configured such that the apertures on the needle barrels face each other but in some embodiments, the hypodermic needle assembly has a plurality of said needles that is configured such that the apertures on the needle barrels face away from each other. In some embodiments, the intracellular delivery device further comprises a pressure generation element joined to said intracellular delivery devices and this pressure generation element can be a syringe. The intracellular delivery devices above of can have

apertures that have a diameter of 10nm-4mm, 0.01 mm-4mm, 0.1 mm-4mm, 1.0 mm-4mm, 1.5 mm-4mm, 2.0 mm-4mm, or 3.0 mm-4mm.

[0156] In some embodiments, the intracellular delivery device above comprises a single syringe joined to at least three of said needles. In some embodiments, the at least three of said needles are between 2 and 10 mm apart. In other embodiments, the intracellular delivery device above can comprise a single syringe joined to at least four hypodermic needles. In some embodiments, the intracellular delivery device has at least four hypodermic needles that are between 3 and 6 mm apart. A single use intracellular delivery device is also an embodiment and such devices preferably comprise a plurality of needles attached to at least one syringe, wherein the needles comprise a plurality of apertures distributed along the barrel of said needles and a closed end; and said at least one syringe comprises a single dose of a prophylactic and/or therapeutic agent. In some embodiments, the prophylactic and/or therapeutic agent in the intracellular delivery device is a nucleic acid. The prophylactic and/or therapeutic agent can be a DNA that encodes a protein. In some embodiments, the intracellular delivery device above comprises a single syringe joined to at least three hypodermic needles and in some embodiments, the at least three hypodermic needles are between 2 and 10 mm apart. In other embodiments, the intracellular delivery device above comprises a single syringe joined to at least four needles and in some embodiments, the at least four hypodermic needles are between 3 and 6 mm apart.

[0157] Aspects of the invention also include methods of making and using the aforementioned devices. By one approach, some of the devices described herein are used to deliver a prophylactic and/or therapeutic agent to a subject and said methods are practiced by providing one of the delivery devices described herein, inserting the needles of said device into a tissue of a subject; and displacing the therapeutic agent from the syringe through the needles and into the tissue. In some embodiments, the prophylactic and/or therapeutic agent is a nucleic acid, the nucleic acid can encode an antigen, such as a viral antigen, preferably, a hepatitis antigen such as an HCV or HBV antigen such that some of the delivery devices described herein can be used for the purposes of inducing an immune response in a subject to an antigen that is delivered by said device.

[0158] Additional embodiments include an intracellular delivery device for the delivery of prophylactic and/or therapeutic material into tissue, the device comprising a connection to a pressure generation element; a lumen adapted for the passage of a prophylactic and/or therapeutic material; and a needle barrel, wherein the needle barrel comprises a plurality

of apertures that extend along the length of the barrel. In some embodiments, the therapeutic material comprises a nucleic acid, a polypeptide, a carbohydrate, a specificity exchanger, a steroid, a cell population, a chemical or an immunogen. In some embodiments, the prophylactic and/or therapeutic agent induces the immune system to generate an immune response.

[0159] The needle barrel can be adapted to transmit an electric current and the device can further comprise an electrode adapted to transmit an electromagnetic field. In some embodiments, the prophylactic and/or therapeutic agent enters a cell and in others it remains extracellular. In some embodiments, the pressure is transmitted using a fluid medium or a gas medium. In some embodiments, the nucleic acid comprises a sequence from a hepatitis virus such as a hepatitis B antigen (HBV), such as HBcAg, or a hepatitis C virus (HCV) antigen, such as NS3/4A, or a combination thereof, such as HBcAg from an HBV virus that infects stork or heron joined to NS3/4A. In other embodiments, the nucleic acid comprises a sequence from a human simian virus antigen. Preferably, the nucleic acid comprises a sequence encoding an antigen capable of generating a proliferative T-cell response and, in some embodiments, the nucleic acid comprises a sequence from a human immunodeficiency virus.

[0160] Additional embodiments include, a intracellular delivery device for the delivery of prophylactic and/or therapeutic material into tissue comprising a prophylactic and/or therapeutic material pressure generation element; an array of needle barrels coupled to the pressure generation element; wherein at least one of the needle barrels in the array comprises a plurality of apertures adapted to deliver a pressure transmitted from the pressure generation element into a tissue to cause an increase in the permeability of a cell membrane, and at least one of the needle barrels in the array is adapted for the passage of the prophylactic and/or therapeutic material as described elsewhere herein.

[0161] More embodiments, include an intracellular delivery device having a longitudinal axis, the device comprising a connector configured to engage a source of pressurized fluid; and a needle assembly, the needle assembly comprising a stem extending from the connector in a direction substantially parallel to the longitudinal axis of the device, the stem comprising a first lumen that is fluidly coupled with the connector, a first needle barrel extending from the stem in a direction substantially parallel to the longitudinal axis of the device, the first needle barrel comprising a second lumen that is fluidly coupled with the stem and at least one aperture that is fluidly coupled with the second lumen, and a second needle barrel extending from the stem in a direction substantially parallel to the longitudinal axis of the

device, the second needle barrel comprising a third lumen that is fluidly coupled with the stem and at least one aperture that is fluidly coupled with the third lumen.

[0162] In some embodiments, the first needle barrel and the second needle barrel form an injection cavity space there between. In some embodiments, the injection cavity space is configured to receive at least a portion of a subject, such as a tissue. In some embodiments, the first needle barrel and second needle barrel each comprise the same number of apertures. In some embodiments, each aperture on the first needle barrel faces an aperture on the second needle barrel. In some embodiments, the first needle barrel and the second needle barrel comprise a pointed distal tip disposed opposite the stem. In some embodiments, the apertures are generally curvilinear. In some embodiments, the apertures are generally polygonal. In some embodiments, the apertures are evenly disposed along a line segment that is substantially parallel to the longitudinal axis of the device. In some embodiments, a third needle barrel extending from the stem in a direction substantially parallel to the longitudinal axis of the device, the third needle barrel comprising a fourth lumen that is fluidly coupled with the stem and at least one aperture that is fluidly coupled with the fourth lumen. In some embodiments, at least one aperture is configured to apply negative pressure to the injection cavity space.

[0163] Still more embodiments concern an intracellular delivery device for delivering a prophylactic and/or therapeutic agent to subject, the device having a longitudinal axis and comprising a plurality of syringes disposed generally parallel to the longitudinal axis of the device, each syringe comprising a needle with a plurality of apertures disposed along a length of the needle, wherein the apertures face the longitudinal axis of the device. In these embodiments, the at least one syringe comprises a prophylactic and/or therapeutic agent comprising a gene. In some embodiments, each needle comprises a tip and the tips of the plurality of needles are disposed on a plane that lies substantially normal to the longitudinal axis of the device. Additional embodiments include a hypodermic needle comprising a plurality of apertures distributed along the barrel of said needle, wherein the end of said needle is closed. In some embodiments, said closed end is blunt.

[0164] In some embodiments, the assembly further comprises a syringe attached to the needle. In some embodiments, said syringe comprises a prophylactic and/or therapeutic agent, which can be a nucleic acid such as a DNA that encodes a protein. Still more aspects of the invention concern an intracellular delivery device comprising a plurality of hypodermic needles that comprise a plurality of apertures distributed along the barrel of said needles joined to one or more syringes. Preferably, the end of said needles are closed. In some embodiments,

the end of said needles are blunt. In some embodiments, said syringe comprises a prophylactic and/or therapeutic agent such as a DNA that encodes a protein. In some embodiments, the injection device above comprises a single syringe joined to at least three hypodermic needles. Some embodiments concern a single use intracellular delivery device comprising a plurality of needles attached to at least one syringe, wherein the needles comprise a plurality of apertures distributed along the barrel of said needles and said at least one syringe comprises a single dose of a prophylactic and/or therapeutic agent. In some embodiments, the end of said needles are closed. In some embodiments, the end of said needles are blunt. In some embodiments, the prophylactic and/or therapeutic agent is a nucleic acid. In some embodiments, the nucleic acid is a DNA that encodes a protein.

[0165] In some embodiments, the intracellular delivery device comprises a needle hub having a plurality of needles partially disposed therein; the plurality of needles each comprising a lumen adapted for the passage of a therapeutic material and a needle barrel, the needle barrel comprising a plurality of apertures along the length of the barrel, wherein said barrel has a closed-end and wherein the apertures are evenly spaced apart on each of the needle barrels and have an average diameter in the range of 0.01 mm to 6 mm; a syringe comprising a plunger; a connector configured to fluidly couple the syringe and needle hub, the connector configured to form a gap between the connector and needle hub. In some embodiments, the hypodermic needle comprises zones on the needle barrel, wherein each zone is configured to pass therapeutic material in a different direction. In some embodiments, the hypodermic needle comprises a slit having a first dimension corresponding to the axis of the needle barrel and a second dimension perpendicular to the axis of the needle barrel wherein the first dimension is longer than the second dimension.

[0166] Methods of using any one or more of the aforementioned devices are also embodiments, including a method of delivering a nucleic acid into a cell comprising using the injection devices as described herein. In some embodiments, the method of delivering to a subject a prophylactic and/or therapeutic agent comprises using electroporation by providing a voltage source, inserting one or more needles of an intracellular delivery device into a tissue of a subject, wherein the one or more needles each comprises a barrel having a lumen and a plurality of apertures, the barrel made from an electrically conductive material, and an electrical connection to the voltage source; displacing the prophylactic and/or therapeutic agent through the lumen of the needle and into the tissue through the plurality of apertures, wherein the one or more needles are joined to a syringe containing said prophylactic and/or therapeutic agent such

that the syringe and one or more needles form a rigid or non-flexible structure; and applying an electric field to the tissue of the subject using the one or more needles as electrodes. In some embodiments, displacing the prophylactic and/or therapeutic agent is performed prior to applying the electric field to the tissue. In some embodiments, displacing the prophylactic and/or therapeutic agent is performed subsequent to applying the electric field to the tissue. In some embodiments, displacing the prophylactic and/or therapeutic agent is performed prior to applying the electric field to the tissue. In some embodiments, displacing the prophylactic and/or therapeutic agent is performed at the same time as applying the electric field to the tissue (e.g., a prophylactic and/or therapeutic agent, such as a nucleic acid encoding an antigen, can be administered by passing the prophylactic and/or therapeutic agent through the apertures on the needle barrel before, during, or after applying an electric field to the recipient tissue at or near the site of injection of the prophylactic and/or therapeutic agent. In some embodiments, the nucleic acid is a DNA that encodes a protein, such as an antigen.

[0167] Some embodiments relate to an intracellular delivery device comprising a plurality of pairs of needles, each needle of the plurality of pairs of needles comprising a closed end, a barrel, and a plurality of apertures along the barrel, wherein the apertures on at least one of the needles of the plurality of pairs of needles is aligned in relation to at least one other needle to generate a cross-spray pattern; wherein at least one of the plurality of pairs of needles comprises a pair of electrodes having opposite polarity, the electrodes configured to apply an electric field to a subject.

[0168] In some embodiments, said DNA encodes a viral antigen. In some embodiments, said viral antigen is an HCV or HBV antigen. Furthermore, in some embodiments a use of a HBcAg or a fragment thereof or a nucleic acid encoding HBcAg or a fragment thereof as an adjuvant. By some approaches, said HBcAg or a fragment thereof or a nucleic acid encoding HBcAg or a fragment thereof is a sequence selected from the group consisting of SEQ. ID NOs. 1-32. Methods of enhancing an immune response to an antigen are also embodiments and said methods can comprise providing said antigen or a nucleic acid encoding said antigen to a subject in mixture with or shortly after providing said subject with HBcAg or a fragment thereof or a nucleic acid encoding HBcAg or a fragment thereof. In some methods, said HBcAg or a fragment thereof or a nucleic acid encoding HBcAg or a fragment thereof is a sequence selected from the group consisting of SEQ. ID NOs. 1-32. In some methods, the DNA encodes NS3/4A and/or HBcAg (e.g., an HBcAg derived from a virus that infects stork and heron).

[0169] In some embodiments, the intracellular delivery devices described herein are used to deliver an immunogenic composition to a subject in need thereof. Preferably, the immunogenic composition is a nucleic acid encoding an antigen (e.g., an HCV antigen, such as NS3/4A or NS5, and/or an HBV antigen, such as HBcAg, preferably from an HBV that infects stork or heron). In some of these approaches, the immune response to the immunogenic composition (e.g., the antigen encoded by the nucleic acid, such as NS3/4A, NS5, or HBcAg), is evaluated, measured, analyzed, or observed. A preferred device for delivery of such immunogenic compositions is one that comprises a syringe capable of connecting to one or more of the needles described herein such that the syringe and needle assembly is non-flexible or rigid (i.e., not a catheter). In some embodiments, at least 0.3 mL of prophylactic and/or therapeutic material (e.g., an immunogenic composition suspended in 0.2, 0.3, 0.4, or 0.5 ml) is delivered to said subject in less than 60, 45, 30, 15, 10, 5, or 1 second and the pressure of delivery is at least 200 kPa. In some embodiments, the amount of prophylactic and/or therapeutic material may be at least 1 mL.

[0170] Accordingly, aspects of the invention concern an intracellular delivery device that is configured for delivery of a prophylactic and/or therapeutic agent (e.g. a cell population, such as a cell population comprising stem cells, chemical, a compound, a chemotherapeutic agent, a protein, a specificity exchanger, a nucleic acid, such as DNA, RNA, other natural nucleic acid, a modified nucleic acid, or a DNA or nucleic acid aptamer), wherein said intracellular delivery device comprises a plurality of needles that comprise a closed or open end and a plurality of apertures that extend along the length of each needle. In some embodiments the intracellular delivery device comprises one needle as described herein. In some embodiments, the intracellular delivery device comprises a plurality of needles as described herein. The needle(s) can be blunt-ended (e.g., having a closed end) or can have a beveled, pointed, or a sharp end, each of which may also have a closed end. The needle can be made to a variety of gauges (e.g., at least, equal to or greater than or any number or range in between 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, or 34 gauge). Preferably, the needle(s) is of a gauge that is greater than or equal to 20 (e.g., greater than or equal to or a range of numbers in between 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, or 34 gauge) and more preferably, the needle(s) is of a gauge that is greater than or equal to 23 (e.g., 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, or 34 gauge) and most preferably, the needle(s) is of a gauge that is greater than or equal to 25 (e.g., 25, 26, 27, 28, 29, 30, 31, 32, 33, or 34 gauge). In some embodiments, the apertures are not located at or near the tip of the one or

more needles. For example, the apertures can be located at least 1 mm, 2 mm, 3 mm, 4 mm, 5 mm, 6 mm, 7 mm, 8 mm, 9 mm, 10 mm, 11 mm, 12 mm, 13 mm, 14 mm, 15 mm, 16 mm, 17 mm, 18 mm, 19 mm, 2 cm, 3 cm, 4 cm, or more from the tip of the needle.

[0171] The length of the needle(s) can vary according to the type of delivery desired. In order to target specific cells in the skin or particular tissues, for example, the preferred target depth depends on the particular cell or tissue being targeted and the thickness of the skin of the particular subject (e.g., to target the Langerhan's cells in the dermal space of human skin, it is desired that the delivery encompass, at least, in part, the epidermal tissue depth typically ranging from 0.025 mm to 0.2 mm in humans). Accordingly, in embodiments, wherein delivery to Langerhan's cells is desired, needle lengths can be between 0.025mm to 0.2mm. In some embodiments, it is desired that the therapeutic agents are delivered at a targeted depth just under the stratum corneum and encompassing the epidermis and upper dermis (e.g., in these embodiments preferred needle lengths include between 0.025 mm to 2.5 mm). In other embodiments, the therapeutic agents are delivered into the muscle tissue or adipose tissue (e.g., in these embodiments, it is desired that the preferred needle lengths include between 0.5 cm to 15cm). Accordingly, aspects of the invention concern intracellular delivery devices that comprise one or more needles and uses thereof, wherein the length of the needle(s) is greater than, equal to, less than or any number or range of numbers in between 0.025mm, 0.05mm, 0.075mm, 0.1mm, 0.2mm, 0.3mm, 0.4mm, 0.5mm, 0.6mm, 0.7mm, 0.8mm, 0.9mm, 1mm, 5mm, 10mm, 15mm, 20mm, 25mm, 30mm, 35mm, 40mm, 45mm, 50mm, 55mm, 60mm, 65mm, 70mm, 75mm, 80mm, 85mm, 90mm, 95mm, 100mm, 125mm, 150mm, 175mm, 200mm, 225mm, 250mm, 275mm, 300mm, 325mm, 350mm, 375mm, 400mm, 425mm, 450mm, 475mm, 500mm, 525mm, 550mm, 575mm, 600mm, 625mm, 650mm, 675mm, 700mm, 725mm, 750mm, 775mm, 800mm, 825mm, 850mm, 875mm, 900mm, 925mm, 950mm, 975mm, 1cm, 1.25cm, 1.5cm, 2.0cm, 2.25cm, 2.5cm, 2.75cm, 3.0cm, 3.25cm, 3.5cm, 3.75cm, 4.0cm, 4.25cm, 4.5cm, 4.75cm, 5.0cm, 5.25cm, 5.5cm, 5.75cm, 6.0cm, 6.25cm, 6.5cm, 6.75cm, 7.0cm, 7.25cm, 7.5cm, 7.75cm, 8.0cm, 8.25cm, 8.5cm, 8.75cm, 9.0cm, 9.25cm, 9.5cm, 9.75cm, 10.0cm, 10.25cm, 10.5cm, 10.75cm, 11.0cm, 11.25cm, 11.5cm, 11.75cm, 12.0cm, 12.25cm, 12.5cm, 12.75cm, 13.0cm, 13.25cm, 13.5cm, 13.75cm, 14.0cm, 15.25cm, 14.5cm, 14.75cm, or 15 cm. In some embodiments, the length of the needle(s) is greater than 10 mm. In some embodiments, the length of the needle(s) is greater than 15 mm. In some embodiments, the length of the needle(s) is greater than 20 mm. In some embodiments, the length of the needle(s) is greater than 30 mm.

[0172] Any of the needle(s) described herein (e.g., any one or more of the needles of the shapes and dimensions described above) can include a plurality of apertures of a variety of sizes and shapes (e.g., oval, circular, slit, or ovoid shape), which can be produced by machine cutting or laser. The one or more needles can each comprise, for example, greater than, equal to, less than, or any number or range of numbers between 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 300, 400, or 500 apertures. In preferred embodiments, the total number of apertures in all of the one or more needles in the device can be 72, 96, or 144. In some embodiments, the apertures can be evenly spaced along the length of the needle. In some embodiments, the apertures can be grouped in one area (e.g., spaced in a first or a second zone of the needle, (such as, wherein the two zones are demarcated by the two sides opposing the middle point of the length of the needle) or said apertures can be along the length of the needle), or unevenly spaced along the length of the needle. The needle(s) can have a closed or open end but a closed end is preferred, as such a design is configured to increase the pressure of delivery when small diameter apertures (e.g., a size equal to or less than 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5, 0.55, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85, 0.9, 0.95, 1.0 mm in its widest portion) are employed. The needle(s) can be composed of surgical steel, stainless steel, or a metal alloy (e.g., consisting essentially of at least 52% Ni and at least 48% Ti). In some embodiments, the needle may comprise an electrically conductive material. In some embodiments, when the needle is attached or coupled to a pressurized fluid reservoir, such as a syringe, for example by a luer lock mechanism, the syringe and needle form a rigid, non-flexible body such that the syringe and needle assembly is not a catheter. Similarly, many of the embodiments described herein comprise a syringe joined to one or more of the needles described herein such that the syringe and needle assembly lack an exterior outer sheath covering the needle, as is required for catheters. While many of the devices described herein have features similar to that found in a catheter, many of the embodiments of the present invention are not catheters and many of the devices described herein are rigid and non-flexible when the syringe is connected to the needle (e.g., when a syringe is directly coupled to a hub comprising one or more of the needles described herein).

[0173] Embodiments disclosed herein are not limited to any particular manufacturing process to create the barrels or apertures disclosed. The needle barrels can be manufactured using any of the standard needle manufacturing techniques including, by way of example only,

die-casting, injection molding, blow molding, machine tooling, laser fabrication and others. Similarly, the material for the needle can be chosen from any number of well-known needle materials such as stainless steel, carbon steel, and various metal alloys. The apertures on the barrels can be created as a part of the barrel manufacturing process, or can be added later by drilling or laser etching. These various manufacturing methods are all well-known in the art.

[0174] Aspects of the present invention also relate generally to methods of transmembrane delivery of drugs, nucleic acids, or other bioactive molecules and compounds using the intracellular delivery devices described above. The active ingredients (e.g. DNA, RNA, nucleic acids, protein, or compounds) can be formulated in a number of solutions for delivery through the needles described herein. In some embodiments, the active ingredients (e.g. DNA, RNA, nucleic acids, protein, or compounds) may be mixed in with a carrier solution such water, a buffer, saline, an oil emulsion, oil, or glycerin. The liquid can then be passed through a needle as described herein. In some embodiments the active ingredients (e.g. DNA, RNA, nucleic acids, protein, or compounds) can be attached to a support (e.g. a nanoparticle, protein, sugar, or pellet) and mixed with one or more of the aforementioned carrier solutions (e.g. water, a buffer, saline, an oil emulsion, oil, or glycerin) and the support bound agent is passed through the needles described herein. It will be understood that there exists a variety of carrier mediums and supports, and using carrier mediums or supports not specifically mentioned herein will not depart from the spirit of the invention. For instance, the carrier medium may be a cationic oil.

[0175] The nucleic acid contemplated for use with the intracellular delivery devices described herein can be nucleic acids from human, non-human primates, mice, bacteria, viruses, mold, protozoa, bird, reptiles, birds – such as stork, and heron, mice, hamsters, rats, rabbits, guinea pigs, woodchucks, pigs, micro-pigs, goats, dogs, cats, humans and non-human primates, e.g., baboons, monkeys, and chimpanzees, as mentioned above. In certain embodiments, the intracellular delivery devices described herein can be used for the delivery of nucleic acids encoding proteins found in the hepatitis C virus (HCV). The HCV gene products can be viruses known to infect animals of any species, including, but not limited to, amphibians, reptiles, birds such as, stork, and heron, mice, hamsters, rats, rabbits, guinea pigs, woodchucks, pigs, micro-pigs, goats, dogs, cats, humans and non-human primates, e.g., baboons, monkeys, and chimpanzees. In certain embodiments, the intracellular delivery devices described herein can be used for the delivery of nucleic acids encoding proteins found in the hepatitis B virus (HBV). The HBV gene products can be viruses known to infect animals of any species, including, but

not limited to, amphibians, reptiles, birds such as, stork, and heron, mice, hamsters, rats, rabbits, guinea pigs, woodchucks, pigs, micro-pigs, goats, dogs, cats, humans and non-human primates, e.g., baboons, monkeys, and chimpanzees.

[0176] In certain embodiments an adjuvant is used in addition to the active ingredient. For instance, a pharmacologic agent can be added to a drug being delivered by a device described herein as needed to increase or aid its effect. In another example, an immunological agent that increases the antigenic response can be utilized with a device described herein. For instance, US Patent Number 6,680,059, which is hereby incorporated in its entirety by reference, describes the use of vaccines containing ribavirin as an adjuvant to the vaccine. However, an adjuvant may refer to any material that has the ability to enhance or facilitate an immune response or to increase or aid the effect of a prophylactic and/or therapeutic agent. In some embodiments, an adjuvant administered in conjunction with one or more of the antigens or nucleic acids encoding an antigen (e.g., NS3/4A) disclosed herein is IL-12 or a nucleic acid encoding IL-12, which may or may not be present on the same construct as the nucleic acid encoding the antigen of interest (e.g., NS3/4A).

[0177] In certain embodiments, any nucleic acid can be used with the device and methods presented, for example, plasmid DNA, linear DNA, antisense DNA and RNA. For instance, the nucleic acid can be a DNA expression vector of the type well known in the art. In some embodiments, the invention is used for the purpose of DNA or RNA vaccination. That is, the invention includes a method of enhancing the transmembrane flux rate of an injected DNA or RNA nucleic acid into the intracellular space.

[0178] In certain embodiments, the intracellular delivery device can be used for high pressure injection into various tissues of organisms, wherein it is desirable to deliver a prophylactic and/or therapeutic material. For instance, the tissue could be skeletal muscle, adipose tissue, an internal organ, bone, connective tissue, nervous tissue, dermal tissue, and others. For instance, DNA vaccines may delivered by intramuscular injection into skeletal muscle or by intradermal injection into the dermis of an animal. In other embodiments, a therapeutic material may be delivered via parenteral delivery into subcutaneous or intraperitoneal tissues. Depending on the target tissue and prophylactic and/or therapeutic agent or agents being delivered, parameters of the needles may be appropriately modified to accommodate the desired physical properties necessary to achieve generation of the pressure sufficient to enhance agent delivery.

[0179] In some embodiments, the intracellular delivery device may be configured to deliver a prophylactic and/or therapeutic material at a predetermined delivery rate. For example, the syringe may be controlled by a spring-actuated device that produces a desired stroke speed for pressing the syringe plunger to produce a desired delivery rate. U.S. Patent No. 6,019,747 discloses one example of such a device and is hereby incorporated by reference in its entirety. In some embodiments, the syringe may be controlled by a gas spring, an electromechanical device, compressed air, or other similar actuation mechanism. The delivery rate may, for example, be at least or any number or range in between 0.1 mL/s, 0.3 mL/s, 0.5 mL/s, 0.8 mL/s, 0.9 mL/s, 1.0 mL/s, 1.1 mL/s, 1.2 mL/s, 1.3 mL/s, 1.4 mL/s, 1.5 mL/s, 2.0 mL/s, or 3.0 mL/s. The delivery rate may, for example, be no more than 20.0 mL/s, 10.0 mL/s, 7 mL/s, 6 mL/s, 5 mL/s, 4 mL/s, 3 mL/s, or 2 mL/s. As discussed further below, the present application includes methods of using the injection device. Accordingly, the method may include delivering a therapeutic material at a predetermined rate, such as any of the rates disclosed above.

[0180] In some embodiments, an intracellular delivery device contains a prophylactic and/or therapeutic agent. The device can comprise, for example, a nucleic acid that is formulated for intra muscular delivery. Desirably, DNA encoding an immunogen or a DNA-containing immunogenic composition (e.g., a DNA vaccine) is provided in a device comprising one or more of the needles described herein. However, a wide variety of nucleic acids can be delivered by an embodiment described herein. That is, one or more of the embodiments described herein can comprise one or more of a nucleic acid selected from the group consisting of: mRNA, tRNA, rRNA, cDNA, miRNA (microRNA), siRNA, (small interfering RNA), RNAi (interfering RNA), piRNA (Piwi-interacting RNA), aRNA (Antisense RNA), snRNA (Small nuclear RNA), snoRNA (Small nucleolar RNA), gRNA (Guide RNA), shRNA (Small hairpin RNA), stRNA (Small Temporal RNA), ta-siRNA (Trans-acting small interfering RNA), cpDNA, (Chloroplast DNA), gDNA (Genomic DNA), msDNA (Multicopy single-stranded DNA), mtDNA (Mitochondrial DNA), GNA (Glycol nucleic acid), LNA (Locked nucleic acid), PNA (Peptide nucleic acid), TNA (Threose nucleic acid), Morpholino containing nucleic acids, sulfur-containing nucleic acids, 2-O-methyl nucleic acids, and nucleic acids containing one or more modified bases or spacers.

[0181] The concentration of the nucleic acid contained in or delivered by a device described herein can vary from 0.1 ng/ml to 50 mg/ml. In some aspects, the nucleic acid concentration that is contained in or delivered by a device described herein (e.g., a suitable dose of nucleic acid for delivery by a device described herein) is between 10 ng/ml to 25 mg/ml. In

still other aspects, the nucleic acid concentration is between 100 ng/ml to 10 mg/ml. In some aspects, the nucleic acid concentration contained in or delivered by a device described herein (e.g., a suitable dose of nucleic acid for delivery by a device described herein) is greater than or equal to or less than 100 ng/ml, 150 ng/ml, 200 ng/ml, 250 ng/ml, 300 ng/ml, 350 ng/ml, 400 ng/ml, 450 ng/ml, 500 ng/ml, 550 ng/ml, 600 ng/ml, 650 ng/ml, 700 ng/ml, 750 ng/ml, 800 ng/ml, 850 ng/ml, 900 ng/ml, 950 ng/ml, 1 µg/ml, 2 µg/ml, 3 µg/ml, 4 µg/ml, 5 µg/ml, 6 µg/ml, 7 µg/ml, 8 µg/ml, 9 µg/ml, 10 µg/ml, 11 µg/ml, 12 µg/ml, 13 µg/ml, 14 µg/ml, 15 µg/ml, 16 µg/ml, 17 µg/ml, 18 µg/ml, 19 µg/ml, 20 µg/ml, 21 µg/ml, 22 µg/ml, 23 µg/ml, 24 µg/ml, 25 µg/ml, 26 µg/ml, 27 µg/ml, 28 µg/ml, 29 µg/ml, 30 µg/ml, 31 µg/ml, 32 µg/ml, 33 µg/ml, 34 µg/ml, 35 µg/ml, 36 µg/ml, 37 µg/ml, 38 µg/ml, 39 µg/ml, 40 µg/ml, 41 µg/ml, 42 µg/ml, 43 µg/ml, 44 µg/ml, 45 µg/ml, 46 µg/ml, 47 µg/ml, 48 µg/ml, 49 µg/ml, 50 µg/ml, 55 µg/ml, 60 µg/ml, 65 µg/ml, 70 µg/ml, 75 µg/ml, 80 µg/ml, 85 µg/ml, 90 µg/ml, 95 µg/ml, 100 µg/ml, 150 µg/ml, 200 µg/ml, 250 µg/ml, 300 µg/ml, 350 µg/ml, 400 µg/ml, 450 µg/ml, 500 µg/ml, 550 µg/ml, 600 µg/ml, 650 µg/ml, 700 µg/ml, 750 µg/ml, 800 µg/ml, 850 µg/ml, 900 µg/ml, 950 µg/ml, 1.0 mg/ml, 1.1 mg/ml, 1.2 mg/ml, 1.3 mg/ml, 1.4 mg/ml, 1.5 mg/ml, 1.6 mg/ml, 1.7 mg/ml, 1.8 mg/ml, 1.9 mg/ml, 2.0 mg/ml, 2.1 mg/ml, 2.2 mg/ml, 2.3 mg/ml, 2.4 mg/ml, 2.5 mg/ml, 2.6 mg/ml, 2.7 mg/ml, 2.8 mg/ml, 2.9 mg/ml, 3.0 mg/ml, 3.1 mg/ml, 3.2 mg/ml, 3.3 mg/ml, 3.4 mg/ml, 3.5 mg/ml, 3.6 mg/ml, 3.7 mg/ml, 3.8 mg/ml, 3.9 mg/ml, 4.0 mg/ml, 4.1 mg/ml, 4.2 mg/ml, 4.3 mg/ml, 4.4 mg/ml, 4.5 mg/ml, 4.6 mg/ml, 4.7 mg/ml, 4.8 mg/ml, 4.9 mg/ml, 5.0 mg/ml, 5.1 mg/ml, 5.2 mg/ml, 5.3 mg/ml, 5.4 mg/ml, 5.5 mg/ml, 5.6 mg/ml, 5.7 mg/ml, 5.8 mg/ml, 5.9 mg/ml, 6.0 mg/ml, 6.1 mg/ml, 6.2 mg/ml, 6.3 mg/ml, 6.4 mg/ml, 6.5 mg/ml, 6.6 mg/ml, 6.7 mg/ml, 6.8 mg/ml, 6.9 mg/ml, 7.0 mg/ml, 7.1 mg/ml, 7.2 mg/ml, 7.3 mg/ml, 7.4 mg/ml, 7.5 mg/ml, 7.6 mg/ml, 7.7 mg/ml, 7.8 mg/ml, 7.9 mg/ml, 8.0 mg/ml, 8.1 mg/ml, 8.2 mg/ml, 8.3 mg/ml, 8.4 mg/ml, 8.5 mg/ml, 8.6 mg/ml, 8.7 mg/ml, 8.8 mg/ml, 8.9 mg/ml, 9.0 mg/ml, 9.1 mg/ml, 9.2 mg/ml, 9.3 mg/ml, 9.4 mg/ml, 9.5 mg/ml, 9.6 mg/ml, 9.7 mg/ml, 9.8 mg/ml, 9.9 mg/ml, 10.0 mg/ml, 11 mg/ml, 12 mg/ml, 13 mg/ml, 14 mg/ml, 15 mg/ml, 16 mg/ml, 17 mg/ml, 18 mg/ml, 19 mg/ml, 20 mg/ml, 21 mg/ml, 22 mg/ml, 23 mg/ml, 24 mg/ml, 25 mg/ml, 26 mg/ml, 27 mg/ml, 28 mg/ml, 29 mg/ml, 30 mg/ml, 31 mg/ml, 32 mg/ml, 33 mg/ml, 34 mg/ml, 35 mg/ml, 36 mg/ml, 37 mg/ml, 38 mg/ml, 39 mg/ml, 40 mg/ml, 41 mg/ml, 42 mg/ml, 43 mg/ml, 44 mg/ml, 45 mg/ml, 46 mg/ml, 47 mg/ml, 48 mg/ml, 49 mg/ml, or 50 mg/ml, or within a range defined by, and including, any two of these values.

[0182] The amount of nucleic acid provided by an intracellular delivery device described herein can vary from 1 ng to 10g. In some aspects, the amount of nucleic acid

contained in the intracellular delivery device or provided by the intracellular delivery device is less than greater than or equal to 1 ng, 5 ng, 10 ng, 20 ng, 30 ng, 40 ng, 50 ng, 60 ng, 70 ng, 80 ng, 90 ng, 100 ng, 150 ng, 200 ng, 250 ng, 300 ng, 350 ng, 400 ng, 500 ng, 600 ng, 700 ng, 800 ng, 900 ng, 1 µg, 2 µg, 3 µg, 4 µg, 5 µg, 6 µg, 7 µg, 8 µg, 9 µg, 10 µg, 11 µg, 12 µg, 13 µg, 14 µg, 15 µg, 16 µg, 17 µg, 18 µg, 19 µg, 20 µg, 21 µg, 22 µg, 23 µg, 24 µg, 25 µg, 26 µg, 27 µg, 28 µg, 29 µg, 30 µg, 31 µg, 32 µg, 33 µg, 34 µg, 35 µg, 36 µg, 37 µg, 38 µg, 39 µg, 40 µg, 41 µg, 42 µg, 43 µg, 44 µg, 45 µg, 46 µg, 47 µg, 48 µg, 49 µg, 50 µg, 55 µg, 60 µg, 65 µg, 70 µg, 75 µg, 80 µg, 85 µg, 90 µg, 95 µg, 100 µg, 105 µg, 110 µg, 115 µg, 120 µg, 125 µg, 130 µg, 135 µg, 140 µg, 145 µg, 150 µg, 155 µg, 160 µg, 165 µg, 170 µg, 175 µg, 180 µg, 185 µg, 190 µg, 195 µg, 200 µg, 205 µg, 210 µg, 215 µg, 220 µg, 225 µg, 230 µg, 235 µg, 240 µg, 245 µg, 250 µg, 255 µg, 260 µg, 265 µg, 270 µg, 275 µg, 280 µg, 285 µg, 290 µg, 295 µg, 300 µg, 305 µg, 310 µg, 315 µg, 320 µg, 325 µg, 330 µg, 335 µg, 340 µg, 345 µg, 350 µg, 355 µg, 360 µg, 365 µg, 370 µg, 375 µg, 380 µg, 385 µg, 390 µg, 395 µg, 400 µg, 405 µg, 410 µg, 415 µg, 420 µg, 425 µg, 430 µg, 435 µg, 440 µg, 445 µg, 450 µg, 455 µg, 460 µg, 465 µg, 470 µg, 475 µg, 480 µg, 485 µg, 490 µg, 495 µg, 500 µg, 505 µg, 510 µg, 515 µg, 520 µg, 525 µg, 530 µg, 535 µg, 540 µg, 545 µg, 550 µg, 555 µg, 560 µg, 565 µg, 570 µg, 575 µg, 580 µg, 585 µg, 590 µg, 595 µg, 600 µg, 605 µg, 610 µg, 615 µg, 620 µg, 625 µg, 630 µg, 635 µg, 640 µg, 645 µg, 650 µg, 655 µg, 660 µg, 665 µg, 670 µg, 675 µg, 680 µg, 685 µg, 690 µg, 695 µg, 700 µg, 705 µg, 710 µg, 715 µg, 720 µg, 725 µg, 730 µg, 735 µg, 740 µg, 745 µg, 750 µg, 755 µg, 760 µg, 765 µg, 770 µg, 775 µg, 780 µg, 785 µg, 790 µg, 795 µg, 800 µg, 805 µg, 810 µg, 815 µg, 820 µg, 825 µg, 830 µg, 835 µg, 840 µg, 845 µg, 850 µg, 855 µg, 860 µg, 865 µg, 870 µg, 875 µg, 880 µg, 885 µg, 890 µg, 895 µg, 900 µg, 905 µg, 910 µg, 915 µg, 920 µg, 925 µg, 930 µg, 935 µg, 940 µg, 945 µg, 950 µg, 955 µg, 960 µg, 965 µg, 970 µg, 975 µg, 980 µg, 985 µg, 990 µg, 995 µg, 1.0 mg, 1.1 mg, 1.2 mg, 1.3 mg, 1.4 mg, 1.5 mg, 1.6 mg, 1.7 mg, 1.8 mg, 1.9 mg, 2.0 mg, 2.1 mg, 2.2 mg, 2.3 mg, 2.4 mg, 2.5 mg, 2.6 mg, 2.7 mg, 2.8 mg, 2.9 mg, 3.0 mg, 3.1 mg, 3.2 mg, 3.3 mg, 3.4 mg, 3.5 mg, 3.6 mg, 3.7 mg, 3.8 mg, 3.9 mg, 4.0 mg, 4.1 mg, 4.2 mg, 4.3 mg, 4.4 mg, 4.5 mg, 4.6 mg, 4.7 mg, 4.8 mg, 4.9 mg, 5.0 mg, 5.1 mg, 5.2 mg, 5.3 mg, 5.4 mg, 5.5 mg, 5.6 mg, 5.7 mg, 5.8 mg, 5.9 mg, 6.0 mg, 6.1 mg, 6.2 mg, 6.3 mg, 6.4 mg, 6.5 mg, 6.6 mg, 6.7 mg, 6.8 mg, 6.9 mg, 7.0 mg, 7.1 mg, 7.2 mg, 7.3 mg, 7.4 mg, 7.5 mg, 7.6 mg, 7.7 mg, 7.8 mg, 7.9 mg, 8.0 mg, 8.1 mg, 8.2 mg, 8.3 mg, 8.4 mg, 8.5 mg, 8.6 mg, 8.7 mg, 8.8 mg, 8.9 mg, 9.0 mg, 9.1 mg, 9.2 mg, 9.3 mg, 9.4 mg, 9.5 mg, 9.6 mg, 9.7 mg, 9.8 mg, 9.9 mg, 10.0 mg, 11 mg, 12 mg, 13 mg, 14 mg, 15 mg, 16 mg, 17 mg, 18 mg, 19 mg, 20 mg, 21 mg, 22 mg, 23 mg, 24 mg, 25 mg, 26 mg, 27 mg, 28 mg, 29 mg, 30 mg, 31 mg, 32 mg, 33 mg, 34 mg, 35 mg, 36 mg, 37 mg, 38 mg, 39

mg, 40 mg, 41 mg, 42 mg, 43 mg, 44 mg, 45 mg, 46 mg, 47 mg, 48 mg, 49 mg, 50 mg, 55 mg, 60 mg, 65 mg, 70 mg, 75 mg, 80 mg, 85 mg, 90 mg, 95 mg, 100 mg, 150 mg, 200 mg, 250 mg, 300 mg, 350 mg, 400 mg, 450 mg, 500 mg, 550 mg, 600 mg, 650 mg, 700 mg, 750 mg, 800 mg, 850 mg, 900 mg, 950 mg, 1g, 2g, 3g, 4g, 5g, 6g, 7g, 8g, 9g, or 10g or within a range defined by, and including, any two of these values.

[0183] The volume of prophylactic and/or therapeutic material provided by an intracellular delivery device described herein can vary, for example, from 100 μ L to 100 mL. In some aspects, the volume of prophylactic and/or therapeutic material contained in the hypodermic injection pressure device or provided by the hypodermic injection pressure device is less than greater than or equal to 100 μ L, 150 μ L, 200 μ L, 250 μ L, 300 μ L, 350 μ L, 400 μ L, 500 μ L, 600 μ L, 700 μ L, 800 μ L, 900 μ L, 1 mL, 2 mL, 3 mL, 4 mL, 5 mL, 6 mL, 7 mL, 8 mL, 9 mL, 10 mL, 20 mL, 30 mL, 40 mL, 50 mL, 60 mL, 70 mL, 80 mL, 90 mL, or 100 mL or within a range defined by, and including, any two of these values.

[0184] Aspects of the invention also concern methods of making one or more of the aforementioned devices. By one approach, one or a plurality of the needles described herein are provided and said needle(s) are attached to a syringe that contains a therapeutic agent (e.g., a nucleic acid such as DNA, RNA, protein, or a compound). The attachment of the needle(s) and the syringe can be made such that the needle cannot be removed from the syringe (e.g., the needle and syringe are molded together) or the attachment can be made such that the needle and the syringe are detachable. Preferably, the attachment of the needle(s) and the syringe is done prior to loading the syringe with the therapeutic agent. The needle and syringe can be sterilized prior to or after adding the therapeutic agent. Preferably, the needle and syringe assembly is sterilized prior to addition of the therapeutic agent and shortly after sterilization, sterilized therapeutic agent is added in a sterile fashion. Desirable manufacturing processes are used to produce a single use device comprising one or more of the sterilized needles described herein, which are attached to one or more sterilized syringes that contain a single dose of one or more sterilized therapeutic agents. These single use devices can be separately sterile packaged such that a user merely needs to tear open a package and inject the therapeutic agent into a suitable tissue (e.g., single use DNA vaccination by injection into muscle).

[0185] Aspects of the invention also concern methods of using one or more of the devices described herein. By one approach, methods of intracellular delivery of a compound are provided, wherein a compound contained in a device described herein is administered to a subject. In some embodiments, a compound (e.g., a nucleic acid, such as DNA or protein) is

provided in a device described herein (e.g., a syringe comprising one or more of the needles described herein). The compound is then delivered to the subject by inserting the needles into tissue of the subject, deploying the plunger to provide pressure on the solution in the syringe thereby pressing the compound out the apertures of the needles at a desired pressure. The increased pressure in the tissue promotes the uptake of the compound by the cells thereby allowing for the intracellular delivery of the compound. Indeed, any prophylactic and/or therapeutic material for which it is desirable for the material to be injected into under a high-injection pressure can be used in conjunction with the invention, including, but not limited to, polypeptides, carbohydrates, microparticles, steroids, or low-molecular weight molecules. For instance, nucleic acid and proteins can be simultaneously or serially introduced into a tissue undergoing high injection pressure.

[0186] Some embodiments concern methods of expressing a protein from DNA, wherein a device as described herein is provided (e.g., a syringe comprising one or more of the needles described herein and a DNA), the needles are inserted into a tissue of a subject (e.g., muscle), the DNA is introduced into the tissue by exiting the apertures under pressure (e.g., pressure exerted by deploying the plunger and pressing it toward the DNA solution in the syringe), and the DNA is taken up by the muscle cells. Optionally, the device containing the DNA is introduced or deployed in a manner that promotes an inflammatory response (e.g., mobilization of or activation of cells associated with an inflammatory response). Optionally, the needle design (e.g., plurality of apertures) or configuration of the device produces an inflammatory response (e.g., mobilization of or activation of cells associated with an inflammatory response). Optionally, the amount of protein expression and/or mobilization of cells associated with an inflammatory response is measured. Such measurements can be made using immunology and/or histochemistry.

[0187] Accordingly, some aspects of the invention concern methods of inducing an immune response to a desired antigen, whereby, a device as described herein is provided (e.g., a syringe comprising one or more of the needles described herein and a DNA), the needles are inserted into a tissue of a subject (e.g., muscle), the DNA is introduced into the tissue by exiting the apertures under pressure (e.g., pressure exerted by deploying the plunger and pressing it toward the DNA solution in the syringe), and the DNA is taken up by the muscle cells. Subsequently, protein encoded by the DNA is made in the cells, and the immune system responds to the protein. Optionally, an immune response to the antigen produced from the introduced DNA is measured (e.g., presence of antibody, specific T cells, reduction or clearance

of infection, or a reduction or disappearance of disease symptoms). Using certain embodiments of the invention, gene constructs may be administered directly into a skeletal muscle tissue for the uptake of the gene by a cell for the subsequent synthesis of the encoded product. In some methods, a high-pressure injection needle may be used to propel a liquid that contains DNA or RNA molecules into a subject's tissue. The liquid is propelled at a sufficient velocity such that upon impact with the tissue the liquid exerts a high pressure onto the tissue, increasing cell permeability, and causing the DNA or RNA molecule to permeate the cells in the area. In some embodiments, an intracellular delivery device as described herein may be used to deliver genetic material to tissue of other organs in order to introduce a nucleic acid molecule to cells of that organ. Indeed, it will be readily recognized that other gene delivery mechanisms well known in the art can be adapted to be used with embodiments of the present invention, including liposome-derived systems, artificial viral envelopes, and other systems known in the art (Rossi, J. J. (1995) *Br. Med. Bull.* 51:217-225; Boado, R. J. et al. (1998) *J. Pharm. Sci.* 87:1308-1315; Morris, M. C. et al. (1997) *Nucleic Acids Res.* 25:2730-2736, all of which are hereby included in their entirety by reference). Additionally, one may use a variety of adjuvants (e.g., ribavirin or IL-12 or a nucleic acid encoding IL-12), to either enhance immunogenicity and/or cell permeability.

[0188] For instance, by way of example only and not by way of any limitation, certain embodiments of the invention can be used in conjunction with the constructs described in U.S. Publication Number 2005-0277192 and U.S. Publication Number 2005-0124573, the entireties of which are hereby expressly incorporated by reference. These references describe the use of a nucleic acid encoding hepatitis C virus (HCV) nonstructural protein 3/4A (NS3/4A) to promote an immune response in humans. For example, it was observed that when HCV NS3/4A gene was transfected into mammalian cells, via a eukaryotic expression vector, appreciable levels of expression of NS3 were observed. Further, mice immunized with the NS3/4A gene were found to have primed high levels of NS3-specific antibodies and antigen specific T cells. Recently, similar constructs have been found to produce a potent immune response in clinical trials with patients that are infected with HCV.

[0189] Accordingly, some embodiments concern methods of treating and preventing HCV infection, wherein one or more of the devices described herein, which contain one or more of the HCV DNA constructs that have been shown to produce a potent immune response in humans, is provided to a patient that is infected with or who is at risk of infection by HCV. Optionally, an individual in need of a medicament that prevents and/or treats HCV infection is

identified and said individual is then provided a medicament comprising one or more of the HCV constructs that have been found to produce a potent immune response in humans (e.g., an expression construct encoding NS3/4A) using a high-pressure injection needle device, as described herein. Optionally, an immune response to NS3/4A, a reduction in viral titer, or production anti-HCV antibodies is measured in the inoculated individual after treatment or during the course of treatment. However, the current invention is not limited to antigens of HCV for DNA immunization. Indeed, the invention can be used any time in which expression of any antigenic peptide within cell is desirable. For instance, some non-limiting examples of known antigenic peptides in relation to specific disease states include the following:

HBV: PreS1, PreS2 and Surface env proteins, core and pol

HIV: gp120, gp40, gp160, p24, gag, pol, env, vif, vpr, vpu, tat, rev, nef

Papilloma: E1, E2, E3, E4, E5, E6, E7, E8, L1, L2

HSV: gL, gH, gM, gB, gC, gK, gE, gD, ICP47, ICP36, ICP4,

as taught in U.S. Patent Number 7,074,770 to Charo, et al., entitled "Method of DNA vaccination," and which is hereby incorporated by reference in its entirety. Some of the embodiments described herein also include and/or administer one or more of the nucleic acids selected from the group consisting of: mRNA, tRNA, rRNA, cDNA, miRNA (microRNA), siRNA, (small interfering RNA), piRNA (Piwi-interacting RNA), aRNA (Antisense RNA), snRNA (Small nuclear RNA), snoRNA (Small nucleolar RNA), gRNA (Guide RNA), shRNA (Small hairpin RNA), stRNA (Small Temporal RNA), ta-siRNA (Trans-acting small interfering RNA), cpDNA, (Chloroplast DNA), gDNA (Genomic DNA), msDNA (Multicopy single-stranded DNA), mtDNA (Mitochondrial DNA), GNA (Glycol nucleic acid), LNA (Locked nucleic acid), PNA (Peptide nucleic acid), TNA (Threose nucleic acid), Morpholino containing nucleic acids, sulfur-containing nucleic acids, and 2-O-methyl nucleic acids, and nucleic acids containing one or more modified bases or spacers.

[0190] Referring to the Figures, FIG. 1A depicts a side view of an embodiment of a hypodermic needle device also referred to as an intracellular delivery device having two barrels, each barrel having five apertures for delivering a prophylactic and/or therapeutic agent to an area in between the barrels. Referring to FIG. 1A, the embodiment of a hypodermic needle device includes a proximal end 103, a distal end 101 opposite the proximal end, and a longitudinal axis running from the distal end 101 to the proximal end 103. In some embodiments, an intracellular delivery device can contain one or a plurality of needles. In some embodiments, the an intracellular delivery device comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19,

20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 needles. The intracellular delivery device comprises a standard connector 100 and a needle body 102 extending from the connector 100. The standard connector 100 and needle body 102 are disposed on an axis that is substantially parallel to the longitudinal axis. In some embodiments, the standard connector 100 is a luer lock or similar mechanism configured to connect the device to a pressure delivery device (not shown), for example, a syringe or pump.

[0191] The distal tip of the needle can be blunt, beveled, tapered, sharpened, or pointed to permit an operator to pierce the skin of a subject (e.g., a human, domestic animal, such as a cat or dog, or farm animal, such as a horse, cow, pig, or chicken) in order to reach the underlying desired target tissue. For example, the tips 105a, 105b can comprise a regular medical point (e.g., a “lancet point”). In some embodiments, the tips 105a, 105b can be blunted. In some embodiments, the distal tip of the needle is closed such that the tip does not establish fluid communication between the lumens of the needle barrel and the distal end of the needle body. In other embodiments, the distal tip is open such that the tip establishes fluid communication between the needle barrel and the distal end of the needle.

[0192] In some embodiments, the hypodermic needle device or an intracellular delivery device can be configured to be a one-time disposable device, wherein the prophylactic and/or therapeutic agent is contained within the device and no additional connection is required. The needle body 102 may comprise one or more needle delivery barrels 120a and 120b that extend from a stem or cannula 115. The stem 115 can include a central lumen or channel. Each needle barrel 120a, 120b also includes at least one lumen that is fluidly connected to the stem 115 and standard connector 100. In the illustrated embodiment, the needle body 102 includes two needle delivery barrels 120a, 120b with each needle barrel 120 including a distal tip 105a, 105b. The lengths of the needle barrels 120a, 120b can vary. In some embodiments, the needle barrels 120a, 120b are each the same length and in some embodiments, the needle barrels are different lengths. The needle barrels 120a, 120b can range from 2 mm to 100 mm. The gauges of the needles barrels 120a and 120b can vary from device to device or from barrel 120a to barrel 120b on a single device.

[0193] In some embodiments, the opening created by the space between the needle barrels 120a, 120b at the distal end of the device is sufficiently large in size to enable the needle barrels 120a, 120b to surround one or more cells.

[0194] The needle barrels 120a, 120b can each comprise apertures 110a, 110b disposed along a length of the barrels. In some embodiments, each needle barrel 120a, 120b

comprises at least one aperture 110a, 110b. In other embodiments, at least one needle barrel 120a, 120b does not comprise an aperture 110a, 110b. In some embodiments, the size and shape of each aperture 110a, 110b can vary from barrel to barrel. In some embodiments, the length of the needle can vary from barrel to barrel.

[0195] Each needle barrel 120a, 120b may comprise 0 to 100 apertures. In some embodiments, the needle has 1 or 2 apertures along the length of the needle (e.g., a closed ended needle having at least two apertures along the length of the needle). In other embodiments, the needle has a number of apertures that is exactly, less than, or greater than 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 or within a range defined by, and including, any two of these values. The apertures can be located near the distal end of a barrel or anywhere along the length of the barrel. The apertures on each barrel may each be disposed in a plane that is substantially parallel to the longitudinal axis. The apertures can also be disposed along a line segment that is substantially parallel to, and facing, the longitudinal axis of the device. In other embodiments, the apertures may be disposed on one or more planes that are not substantially parallel to the longitudinal axis of the device. Each aperture can face a common point, for example, a point on an axis that is substantially parallel to the longitudinal axis or each aperture can face a different point or direction.

[0196] The apertures can vary in size and shape. For example, apertures can be circular, round, generally curvilinear, square, rectangular, triangular, generally polygonal, generally symmetrical, generally asymmetrical, or irregularly shaped. Additionally, the apertures can vary in size and shape within each barrel. For example, in some embodiments, a first aperture on a barrel can be generally curvilinear and have a diameter of 1 mm and a second aperture on the barrel can have the same shape as the first aperture and have a diameter of 1.50 mm. In some embodiments, each aperture can have generally the same shape and same size. The apertures can vary in size and shape. For example, apertures can be circular, round, generally curvilinear, square, rectangular, triangular, generally polygonal, generally symmetrical, generally asymmetrical, or irregularly shaped. Additionally, the apertures can vary in size and shape within each barrel. For example, in one embodiment, a first aperture on barrel can be generally curvilinear and have a diameter of 1 mm and a second aperture on barrel can

have the same shape as the first aperture and have a diameter of 1.50 mm. In other embodiments, each aperture can have generally the same shape and same size.

[0197] FIG. 1B illustrates an exploded perspective view of one embodiment of a needle hub with four barrels for delivering a prophylactic and/or therapeutic agent to an area in between the barrels. Threaded luer adaptor 130 is configured to engage a syringe (not shown) containing a prophylactic and/or therapeutic material. Hub insert 140 comprises a plurality of needles 150 at the distal side of hub insert 140. Needles 150 may be of varying configuration, including any configuration described herein. Collar 160 is configured to engage threaded luer adaptor 130 and secure hub insert 140. Gasket 170 may optionally be disposed on hub insert 140 to maintain a sealed channel from a syringe to plurality of needles 150. The needles may optionally include a plurality of apertures (e.g., as depicted in FIG. 1A), as discussed above.

[0198] The size, shape, and quantity of apertures can be selected in order to maximize the efficient delivery of injected fluid or genetic material, to create the optimal pressure within the injection cavity space to enhance cell membrane permeability, or both. Referring to FIG. 2A, an intracellular delivery device includes two needle barrels 220a, 220b each including three apertures 210a, 210b and a pointed distal tip 205a, 205b. The distal tips 205a, 205b are separated from one another by a distance 203. Moving in the proximal direction from the distal tips 205a, 205b the opening 203 forms an injection cavity space 204 formed between the needle barrels 220a, 220b. In some embodiments, the opening 203 created by the space between needle barrels 220a, 220b between the tips 205a, 205b is sufficiently large in size to enable the needle barrels 220a, 220b to surround one more or cells in the injection cavity space 204.

[0199] In some embodiments, in order to create an intracellular delivery device for the delivery of a fluid containing a desired agent to targeted tissue, a needle 220a and/or 220b may comprise a plurality of generally curvilinear apertures 210a, 210b with widths or diameters ranging from 0.01 to 4.0 mm. In some embodiments, the width of the apertures 210a, 210b at their widest portion is greater than, less than or equal to 0.01 μ m, 0.02 μ m, 0.03 μ m, 0.04 μ m, 0.05 μ m, 0.06 μ m, 0.07 μ m, 0.08 μ m, 0.09 μ m, 0.1 μ m, 0.15 μ m, 0.2 μ m, 0.25 μ m, 0.3 μ m, 0.35 μ m, 0.4 μ m, 0.45 μ m, 0.5 μ m, 0.55 μ m, 0.6 μ m, 0.65 μ m, 0.7 μ m, 0.75 μ m, 0.8 μ m, 0.85 μ m, 0.9 μ m, 0.95 μ m, 1.0 μ m, 1.5 μ m, 2.0 μ m, 2.5 μ m, 3.0 μ m, 3.5 μ m, 4.0 μ m, 4.5 μ m, 5.0 μ m, 5.5 μ m, 6.0 μ m, 6.5 μ m, 7.0 μ m, 7.5 μ m, 8.0 μ m, 8.5 μ m, 9.0 μ m, 9.5 μ m, 0.01mm, 0.02mm, 0.03mm, 0.04mm, 0.05mm, 0.06mm, 0.07mm, 0.08mm, 0.09mm, 0.1mm, 0.15mm, 0.2mm, 0.25mm, 0.3mm, 0.35mm, 0.4mm, 0.45mm, 0.5mm, 0.55mm, 0.6mm, 0.65mm, 0.7mm,

0.75mm, 0.8mm, 0.85mm, 0.9mm, 0.95mm, 1.0mm, 1.05mm, 1.10mm, 1.15mm, 1.20mm, 1.25mm, 1.30mm, 1.35mm, 1.40mm, 1.45mm, 1.50mm, 1.55mm, 1.60mm, 1.65mm, 1.70mm, 1.75mm, 1.80mm, 1.85mm, 1.90mm, 1.95mm, 2.0mm, 2.05mm, 2.10mm, 2.15mm, 2.20mm, 2.25mm, 2.30mm, 2.35mm, 2.40mm, 2.45mm, 2.50mm, 2.55mm, 2.60mm, 2.65mm, 2.70mm, 2.75mm, 2.80mm, 2.85mm, 2.90mm, 2.95mm, 3.0mm, 3.05mm, 3.10mm, 3.15mm, 3.20mm, 3.25mm, 3.30mm, 3.35mm, 3.40mm, 3.45mm, 3.50mm, 3.55mm, 3.60mm, 3.65mm, 3.70mm, 3.75mm, 3.80mm, 3.85mm, 3.90mm, 3.95 mm, or within a range defined by, and including, any two of these values. In other embodiments, one can select a plurality (e.g., ten) generally curvilinear apertures 210a, 210b with diameters ranging from 10nm to 2.0 mm.

[0200] By adjusting the size, shape, and quantity of apertures 210a, 210b and taking into account the physical properties of the pressure transmitting medium into which needle 220a and 220b are inserted, the intracellular delivery device can deliver a local pressure in the range of 1 to 5000 kilopascals. The needles described herein may be configured to deliver a fluid at a pressure in the range of greater than, less than, equal to, 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 300, 400, 500, 1000, 2000, 3000, 4000, or 5000 kilopascals or within a range defined by, and including, any two of these values. An increased local pressure in the tissue contained within the injection cavity space 204 alters the cell membrane permeability characteristics of cells within the tissue and promotes entry of an agent (e.g., DNA) into the cells.

[0201] The length of the needles 220a and 220b can vary from 0.5 cm to 15 cm. In certain embodiments, the needle is at least or not more than 0.5, 0.75, 1.0, 1.25, 1.5, 2.0, 2.25, 2.5, 2.75, 3.0, 3.25, 3.5, 3.75, 4.0, 4.25, 4.5, 4.75, 5.0, 5.25, 5.5, 5.75, 6.0, 6.25, 6.5, 6.75, 7.0, 7.25, 7.5, 7.75, 8.0, 8.25, 8.5, 8.75, 9.0, 9.25, 9.5, 9.75, 10.0, 10.25, 10.5, 10.75, 11.0, 11.25, 11.5, 11.75, 12.0, 12.25, 12.5, 12.75, 13.0, 13.25, 13.5, 13.75, 14.0, 15.25, 14.5, 14.75, or 15 cm, or within a range defined by, and including, any two of these values. In some embodiments, the needle(s) have a length more than 1 cm. In some embodiments, the needle(s) have a length more than 15 mm. In some embodiments, the needle(s) have a length more than 20 mm.

[0202] Although the tips 205a, 205b are shown with the beveling angling towards the longitudinal axis of the device, the bevels may be angled in the opposite direction, or different directions (see FIG. 4), in order to spread tissue and deliver at least some targeted tissue through an area disposed between the needle barrels 220a, 220b and into an injection cavity space 204 disposed there-between. In some embodiments, each tip can include multiple beveled edges,

such two, three, four, five, six, or more beveled edges. This can result in a tip having generally a rotational symmetry about its axis and may provide for uniform insertion of each needle. In some embodiments, tips 205a and 205b may comprise a trocar tip, or a “quadcar” tip which has four beveled edges which may be used on one or more needles in the injection devices disclose herein. In some embodiments, needles 220a and 220b may similar or different tips 205a and 205b. In some embodiments, at least one beveled edge on the needle tip faces generally the same direction as one or more apertures on the same needle. In some embodiments, none of the beveled edges on the needle tip face in generally the same direction as any of the apertures on the same needle.

[0203] The delivery pressure of an agent may affect the efficacy and safety of a treatment. For example, applying too much pressure may result in undesirable damage to the cell, while applying too little pressure may not yield a sufficient permeability shift so as to allow for uptake of the agent. The laws of fluid dynamics and associated equations can be used to generate a profile of acceptable pressures in the injection cavity space 204. For example, the needle barrel 220a, 220b geometry and the fluid characteristics of the agent, for example, viscosity and density, will affect the local pressure in the injection cavity space 204. In some embodiments, the size and shape of the apertures 210a, 210b, the fluid and delivered agent, as well as, the driving pressure may be selected to produce a desired local pressure in the injection cavity space 204. The Darcy-Weisbach equation, for example, may be used to define the pressure drop with regards to the velocity of flow, the viscosity of the fluid, and the ratio of the diameter of the barrel lumen to the pipe length. The equation is useful, among other things, in determining the appropriate aperture 210a, 210b size when using different carrier medium fluids (e.g. phosphate buffered saline, glycerin, ethanol, deionized water, filtered water, various oils, emulsions, etc.), as each type of fluid has its own viscosity properties. Standard computational fluid dynamics software can be utilized in determining the optimal physical parameters of the needle barrels and apertures to achieve a desired pressure drop. However, embodiments are not limited to the use of fluid for the creation of the pressure drop, and can utilize other types of pressure transmitting mediums. For instance, in some embodiments, air or other gas, such as CO₂ or N₂, may be used to transmit pressure onto tissue.

[0204] FIG. 2B illustrates an embodiment of a hypodermic needle device or an intracellular delivery device having five apertures on each needle, the apertures spaced equally apart. Needles 230 each comprise five apertures 235 having a distance 240 between each aperture, measured from the center of each aperture, or from a corresponding edge of each

aperture. The spacing between the apertures may, in some embodiments, be the same for all the apertures in the needles, or in some embodiments, distance 240 may vary. The spacing may be at least or not more than 0.01 mm, 0.05 mm, 0.1 mm, 0.15 mm, 0.2 mm, 0.25 mm, 0.3 mm, 0.4 mm, 0.5 mm, 0.6 mm, 0.7 mm, 0.8 mm, 0.9 mm, 1 mm, 2 mm, 3 mm, 4 mm, 5 mm, 6 mm, 7 mm, 8 mm, 9 mm, 1 cm, 2 cm or 3 cm or within a range defined by, and including, any two of these values. Apertures 235 are configured such that each aperture on a first needle faces a corresponding second aperture on a second needle. This arrangement results in opposing fluid flow or a cross-spray of the therapeutic material between apertures that face each other. In some embodiments, all of the apertures are configured to face (or oppose) another aperture on a different needle. In some embodiments, at least 2, 4, 6, 8, 10, 16, 20, 30, 40, 50, or 60 of the apertures or within a range defined by, and including, any two of these values are configured to face (or oppose) another aperture on a different needle.

[0205] FIG. 2C illustrates an embodiment of a needle hub with three needles and depicts dimensions of the hypodermic needle hub. Hub 245 includes three needles 250 fluidly coupled to a distal end of hub 245. Needles 250 each have a needle length 255 from the distal end of hub 245 to needle tip 257. Needle length 255 may vary depending upon the target tissue for delivering a prophylactic and/or therapeutic material. Distance 265 between needle point 257 and the aperture 235 on the needle furthest from needle point 257 can vary. For example, distance 265 may be between 0.1 mm and 5 cm, such as 1 mm, 2 mm, 3 mm, 4 mm, 5 mm, 6 mm, 7 mm, 8 mm, 9 mm, 10 mm, 11 mm, 12 mm, 13 mm, 14 mm, 15 mm, 16 mm, 17 mm, 18 mm, 19 mm, 20 mm, 21 mm, 22 mm, 23 mm, 24 mm, 25 mm, 26 mm, 27 mm, 28 mm, 29 mm, 3 cm, 3.5 cm, 4 cm or more, or within a range defined by, and including, any two of these values. In some embodiments, distance 265 may be greater than 10 mm. In some embodiments, distance 265 may be greater than 11 mm. In some embodiments, distance 265 may be greater than 15 mm. In some embodiments, distance 265 may be greater than 20 mm. Similarly, distance 270 between the aperture 235 closest to needle point 257 and the aperture furthest from needle point 257 may also vary. In some embodiments, distance 257 may be between 0.5 mm and 10 cm, such as 1 mm, 2 mm, 3 mm, 4 mm, 5 mm, 6 mm, 7 mm, 8 mm, 9 mm, 1 cm, 2 cm, 3 cm, 4 cm, 5 cm, 6 cm, 7 cm, 8 cm, 9 cm, or within a range defined by, and including, any two of these values or more.

[0206] FIG. 2D illustrates an embodiment of a needle hub with four needles in a staggered configuration. Hub 275 includes four needles 280, 285, 287, and 290 fluidly coupled to the distal end of hub 245. Needle 287 is longer than needle 290 by distance 295. Needle 280

is longer than needles 285, 290 but shorter than needle 287. Numerous other variations of the staggered arrangement may also be used. In some embodiments, the injection device includes a plurality of needles, where at least one or more needles have a first length and one or more needles have second length that is longer than the first length. In some embodiments, the each needle may have a different length. In some embodiments, distance 295 may be, for example, at least 0.1 mm, 0.15 mm, 0.2 mm, 0.25 mm, 0.3 mm, 0.4 mm, 0.5 mm, 0.6 mm, 0.7 mm, 0.8 mm, 0.9 mm, 1 mm, 2 mm, 3 mm, 4 mm, or 5 mm or within a range defined by, and including, any two of these values. The difference in length between the needles may, for example, be no more than 5 cm, 2 cm, 1 cm, 5 mm, 4 mm, 3 mm, 2 mm, or 1 mm.

[0207] FIG. 3 illustrates a side view an embodiment of a hypodermic needle device or an intracellular delivery device having two barrels, each barrel having ten apertures for delivering a therapeutic agent to an area in between the barrels. The needle barrels 320a, 320b include lumens that are in fluid communication with a central lumen 315. A pressurized prophylactic and/or therapeutic agent can be directed through the central lumen 315 to the needle barrels 320a, 320b and can exit the needle barrels 320a, 320b via apertures 310a, 310b. Needle barrels 320a, 320b each comprise ten curvilinear apertures evenly distributed along a distal length of the barrels. The apertures 310a, 310b are configured to direct the pressurized agent towards the longitudinal axis of the device and thus, the apertures 310a on needle barrel 320a face the apertures 310b on needle barrel 320b. In one embodiment, the apertures can be disposed proximally from the tips of the barrels 320a, 320b between 1 and 3 mm towards the proximal ends of the barrels.

[0208] FIG. 4 illustrates a side view of an embodiment of a hypodermic needle device or an intracellular delivery device delivering a prophylactic and/or therapeutic agent including DNA into a muscle cell. The prophylactic and/or therapeutic agent 430 may comprise a gene, a nucleic acid, protein, or other large molecule for delivery into part of a cell 450, or into multiple cells, as described herein. As depicted in FIG. 4, the intracellular delivery device has been introduced into the muscle tissue such that the injection cavity space 404 surrounds at least part of one muscle cell 450. A high pressure source of fluid (not shown) is directed into the central lumen 415 of the device and through the lumens of each of the needles barrels 420a, 420b before it is expelled through the apertures 410a, 410b into the injection cavity space 404. The high pressure that exists at each aperture 410a, 410b results from pressure applied to the fluid as it is expelled into the tissue located in the injection cavity space 404. The resulting increase in local pressure alters the permeability properties of the membrane in order to enhance

uptake of the injected element. The resulting permeability change allows pharmaceutical drugs, nucleic acids and other compounds to gain access to the interior of the cell.

[0209] As described herein, the number of needle barrels may vary depending on the intended application for the injection device, the manufacturing process used to create the injection device, the amount of local pressure desired, and/or other factors. In some embodiments, the number of barrels can be equal or greater than 1, 2, 3, 4, 5, 6, 7, 8, 8, 10, or within a range defined by, and including, any two of these values.

[0210] FIG. 5A illustrates a top view of an intracellular delivery device. Needle barrels 520a, 520b, 520c are disposed around the center of the connector or central lumen housing 500. The needle barrels 520a, 520b, 520c can form an equilateral, isosceles, scalene, or right triangle. The diameter D_1 of connector 500 can vary as can the length L_1 between the needle barrels 520. In some embodiment, the diameter D_1 of the connector 500 ranges from 3 to 25 mm and the length L_1 between the needle barrels ranges from 1 to 8 mm, or more. L_1 may be measured from the center of a barrel to the center of an adjacent barrel, or from the edge of one barrel to the corresponding edge of an adjacent barrel

[0211] FIG. 5B illustrates a side view of an embodiment of a hypodermic needle device or an intracellular delivery device having three barrels. Barrels 520a-c are connected to three separate syringes 501a, 501b, 501c, respectively. The syringes can be configured to contain similar or different volumes of a therapeutic agent for delivery to a patient. In one embodiment, each syringe is configured to contain 1 mL of a prophylactic and/or therapeutic agent. Each syringe 501a, 501b, 501c includes a needle barrel 520a, 520b, 520c extending longitudinally therefrom. Each needle barrel 520 includes a plurality of apertures 510a, 510b, 510c facing the longitudinal axis of the device. The number of apertures 510a, 510b, 510c on each needle barrel 520a, 520b, 520c can range from one to twenty. In one embodiment, the apertures 510 on a barrel 520 are evenly distributed with one aperture disposed over 0.2mm. The volume range per length of needle barrel 520 can vary depending on the distance between apertures 510. In one embodiment, each millimeter of length of needle barrel 520 corresponds to 75 μ l of prophylactic and/or therapeutic agent. The three syringes 501 can be arranged in an equilateral triangle shape centered around the longitudinal axis of the device with each needle barrel 520 being equal distance from each of the other two needle barrels.

[0212] The distance between the needle barrels 520 can vary depending on the number of apertures 510. In one embodiment, each needle barrel 520 comprises ten apertures 510 and the needles are disposed 3.0 mm apart from one another. In another embodiment, each

needle barrel 520 comprises 8 apertures 510 and the needles are disposed 2.2 mm apart from one another. In another exemplary embodiment, each needle barrel 520 comprises six apertures and the needles are disposed 1.5 mm apart from one another. In yet another embodiment, each needle barrel 520 comprises apertures 510a-c and the needles are disposed 1.0 mm apart from one another. Apertures 510a-c may be oriented in a variety of orientations. In some embodiments, apertures 510a-c may face an adjacent needle. In some embodiments, apertures 510a-c may face an opposite needle, and produce a cross-spray pattern as illustrated in FIG. 5C.

[0213] FIG. 5C illustrates a perspective view of the hypodermic needle device or the intracellular delivery device of FIG. 5B delivering a therapeutic agent to the tissue of a subject 590.

[0214] Referring now to FIG. 6A, a side view of an embodiment of a hypodermic needle device or an intracellular delivery device having two barrels, each barrel being disposed at an angle relative to the longitudinal axis of the device is illustrated. The injection device in FIG. 6A comprises two syringes 620a, 620b each disposed at an angle relative to the longitudinal axis of the device. A support 670 holds the syringes 620 in position relative to one another and is generally aligned with the longitudinal axis of the device.

[0215] FIG. 6B illustrates a perspective view of an embodiment of a hypodermic needle device or an intracellular delivery device having two barrels and a connector fitting. Needle barrels 620a, 620b are fluidly connected to a common lumen 615 that is housed within a housing or connector 600. Needle barrels 620a, 620b are generally parallel to one another and distribute a prophylactic and/or therapeutic agent to a subject that is directed to the barrels by the common lumen 615.

[0216] FIG. 6C illustrates a top view of the hypodermic needle device or the intracellular delivery device with a connector fitting. Needles barrels 620a, 620b can be separated one another by a length L_2 and the connector 600 can have a diameter or width D_2 . The diameter D_2 of the connector 600 can vary as can the length L_2 between the needle barrels 620. In one embodiment, the diameter D_2 of the connector 600 ranges from 3 to 25 mm and the length L_2 between the needle barrels ranges from 1 to 6 mm.

[0217] FIG. 7A illustrates a perspective view of an embodiment of a hypodermic needle device or an intracellular delivery device having six barrels, each barrel having a plurality of apertures for delivering a prophylactic and/or therapeutic agent to the tissue of a subject. Six needle barrels 720a-f extend generally parallel to one another from a connector 700. The

connector 700 houses a common lumen 715 that distributes a pressurized prophylactic and/or therapeutic agent to the needle barrels 720.

[0218] FIG. 7B illustrates a top view of the injection device of FIG. 7A. As shown in FIG. 7B, five of the needles barrels 720 form a pentagram or five-sided polygon centered around the center of the connector 700. Each of these five needle barrels 720 can be separated from a left and right needle barrel 720 by a length L_3 . The sixth needle barrel 720 is disposed in the center of the five-sided polygon and separated from the other five needle barrels by a length L_4 . The connector 700 can also have a diameter of maximum width D_3 . In some embodiments, the diameter D_3 can be between 3 and 25 mm. The lengths L_4 and L_3 can be equal to one another or different. In some embodiments, length L_4 ranges from 1 to 6 mm and length L_3 ranges from 1 to 6 mm.

[0219] FIG. 8A illustrates a side view of an embodiment of a hypodermic needle device or an intracellular delivery device having four barrels. Needle barrels 820 are fluidly connected with a common lumen 815 housed within a connector 800. Each needle barrel 820 can include any number of inner facing apertures 810a-d, for example, six or ten. In some embodiments, a needle barrel 820a-d is disposed along the longitudinal axis of the device and includes no apertures 810a-d, or includes apertures 810a-d that face away from the center or longitudinal axis of the device. For example, needle barrel 820b may comprise three zones of apertures, where each zone includes apertures that face one needle selected from needle 820a, needle 820c or needle 820d. The zones may include apertures which each face a separate needle 820a-d as compared with the other zones. The needle barrels 820a-d can extend from the connector 800 for a length L_5 between 3 and 100 mm.

[0220] FIG. 8B illustrates a top view of the injection device of FIG. 8A including the connector 800 and the needle barrels 820a-d. Three of the needle barrels 820a, 820c, and 820d can be disposed in a triangle, centered around the longitudinal axis of the device and sharing a common center with the connector 800. Needle barrel 820b is located equidistant from the surrounding needle barrels 820a, 820c, and 820d. This needle arrangement may be termed a “Y-type configuration.” Needle barrels 820a-d can be separated from one another by a length L_6 . This length L_6 can vary between 2 and 12 mm. For example, L_6 can be 3 mm or 6mm. The connector 800 can have a diameter or maximum width D_4 dimension ranging from 3 to 20 mm.

[0221] The intracellular delivery device may comprise four needles as depicted in FIG. 8B. In some embodiments, L_6 is 6 mm. In some embodiments, L_6 may be from 0.1 mm to 6 mm. The total number of apertures in the four needles is 72. The center needle includes 36

apertures distributed in three rows along the axis of the needle, each row facing an outer needle. The three outer needles each include a single row of 12 apertures facing the center needle to produce a cross-flow between needles. The apertures may have a diameter of 0.1 mm and a spacing of 0.2 mm. In a Y-type configuration, the central needle may have apertures positioned to provide flow in 3 directions, out from the barrel toward each of the outer needles. Similarly, the outer needles may have apertures disposed along the length of the barrel such that therapeutic agent flows from each needle barrel toward the center needle.

[0222] In some embodiments, the an intracellular delivery device includes four needles as depicted in FIG. 8B, where L_6 is 3 mm. The total number of apertures in the four needles is 72. The center needle includes 36 apertures distributed in three rows along the axis of the needle, each row facing an outer needle. The three outer needles each include a single row of 12 apertures facing the center needle to produce a cross-flow between needles. The apertures may have a diameter of 0.05 mm and a spacing of 0.2 mm.

[0223] FIG. 8C illustrates a top view of an embodiment of an intracellular delivery device of FIG. 8A. Needles 830a-d are arranged as the points of a square, or any other quadrilateral, such as a trapezoid, isosceles trapezoid, parallelogram, kite, rhombus, or rectangle, having a length L_7 between needle 830d and needle 830b. This length L_7 can vary between 2 and 12 mm, such as 3 mm or 6 mm. In some embodiments, L_7 may be from 0.1 mm to 6 mm or any distance there between.

[0224] In some embodiments, each needle may be configured with a first zone of apertures that face a first adjacent needle. For example, needle 830b may include a first zone of apertures that face needle 830a. In some embodiments, each needle may be configured with a second zone of apertures that oppose a second adjacent needle. For example, needle 830b may include a first zone of apertures that face needle 830a and a second zone of apertures that face needle 830c. In some embodiments, each needle may be configured with a third zone of apertures that oppose a third adjacent needle. For example, needle 830b may include: a first zone of apertures that face needle 830a, a second zone of apertures that face needle 830c, and a third zone of apertures that face needle 830d. In some embodiments, each needle is configured with the same number of zones. In some embodiments, each zone includes the same number of apertures. In some embodiments, each needle may comprise any one of, or a combination of the three zones. Needles 830 may optionally be configured to form a diamond-shape, such as a parallelogram or rhombus. This needle arrangement may be called an "O-type or X-type pattern," depending on the specific composition of aperture zones. In an O-type pattern, the

zones on a needle 830a-d are configured to face only adjacent needles, resulting in a spray pattern that resembles an “O”. In an X-type pattern, the zones on a needle 830a-d are configured to face adjacent and opposite needles, resulting in a spray pattern that, in part, resembles an “X.”

[0225] In some embodiments, the injection device includes needles as depicted in FIG. 8C, where L_7 is 3 mm and each needle includes 18 apertures (4 times 18 is 72 total apertures) distributed in three rows along the axis of the needle. Each row faces one of the three other needles to produce a cross-flow between the needles and converging flows at the center equidistant from the four needles. The apertures may have a diameter of 0.05 mm and a spacing of 0.2 mm.

[0226] In some embodiments, the intracellular delivery device includes needles as depicted in FIG. 8C, where L_7 is 3 mm and each needle includes 24 apertures (4 times 24 is 96 total apertures) distributed in two rows along the axis of the needle. Each row faces an adjacent needle to produce a cross-flow between needles. The apertures may have a diameter of 0.1 mm and a spacing of 0.2 mm.

[0227] In some embodiments, the intracellular delivery device comprises needles as depicted in FIG. 8C, where L_7 is 6 mm and each needle includes 36 apertures (4 times 36 is 144 total apertures) distributed in three rows along the axis of the needle. Each row faces one of the three other needles to produce a cross-flow between the needles and converging flows at the center equidistant from the four needles. The apertures may have a diameter of 0.1 mm and a spacing of 0.2 mm.

[0228] In some embodiments, the intracellular delivery device comprises needles as depicted in FIG. 8C, where L_7 is 6 mm and each needle includes 18 apertures (4 times 18 is 72 total apertures) distributed in two rows along the axis of the needle. Each row faces an adjacent needle to produce a cross-flow between needles. The apertures may have a diameter of 0.05 mm and a spacing of 0.2 mm.

[0229] FIGS. 9-15 illustrate top views of various other embodiments of injection devices. Each of these injection devices includes a plurality of needle barrels and can include apertures disposed on the needle barrels. The apertures can be configured to deliver a pressurized prophylactic and/or therapeutic agent to a subject and/or apply a negative pressure to a subject.

[0230] FIG. 9 illustrates a top view of an embodiment of a hypodermic needle device or an intracellular delivery device comprising four needle barrels. Each needle barrel 920a-d

comprises at least one inward or center facing aperture 910 configured to deliver a pressurized prophylactic and/or therapeutic material into an injection space 904.

[0231] FIG. 10A illustrates a top view of an embodiment of a hypodermic needle device or an intracellular delivery device comprising seven barrels. Six of the needle barrels 1020 form a hexagon with the seventh needle barrel disposed near the center of the hexagon.

[0232] FIG. 10B illustrates a perspective view of a hypodermic needle hub comprising seven barrels. Needle barrels 1020a-g are disposed at least partially within a hub 1045. Referring to FIGS. 10A and 10B, six needle barrels 1020a-c and 1020e-g are arranged around a seventh center needle barrel 1020d. The apertures 1010 may be arranged in zones such that the apertures are directed to adjacent needle barrels and the center needle barrel. For example, on one needle barrel 1020a in a first zone face a first adjacent needle barrel 1020g, the apertures in a second zone face the second adjacent needle barrel 1020b, and the apertures in a third zone face the center needle barrel 1020d, as depicted by the arrows on FIG. 10A. Length D_{10} may, in some embodiments, be any distance from 0.5 mm to 12 mm or any distance there between. In some embodiments, D_{10} may be 3 mm. In some embodiments D_{10} may be 6 mm.

[0233] FIG. 11 illustrates an embodiment of an intracellular delivery device having ten needle barrels 1120a-j with each needle barrel comprising at least one inward or center facing aperture 1110a-j configured to deliver a pressurized prophylactic and/or therapeutic material into an injection space 1104.

[0234] FIGS. 12-14 illustrate embodiments of intracellular delivery devices wherein a pressurized therapeutic agent is delivered asymmetrically about an injection cavity space. This may be desirable in some circumstances, for instance, to deliver more focused positive pressure on only a portion or region of the tissue, rather than on all sides.

[0235] FIG. 12 illustrates an embodiment of an intracellular delivery device having three needle barrels 1220a-c with two of the three needle barrels comprising at least one inward or center facing aperture 1210b-c configured to deliver a pressurized prophylactic and/or therapeutic material into an injection space 1204. The third needle barrel 1220a does not comprise any apertures configured to deliver pressurized fluid to the injection space 1204.

[0236] In some embodiments, a hypodermic needle device or an intracellular delivery device is configured to apply negative pressure via one or more apertures to an injection cavity space. Negative or counter-pressure can be used to deliver an optimal amount of pressure onto a cell membrane. In these embodiments, negative pressure is represented by arrows

directed toward one or more of the needle barrels. Negative pressure can be applied by connecting certain apertures to a different lumen than other apertures.

[0237] FIG. 13 illustrates an embodiment of an intracellular delivery device having three needle barrels 1320a-c with two of the three needle barrels 1320b-c comprising at least one inward or center facing aperture 1310b, 1310c configured to deliver a pressurized prophylactic and/or therapeutic material into an injection space 1304. The third needle barrel 1320a comprises at least two inward or center facing apertures 1310a configured to apply a negative pressure to the injection space 1304.

[0238] FIG. 14 illustrates an embodiment of an intracellular delivery device having four needle barrels 1420a-d with two of the four needle barrels 1420c-d comprising at least one inward or center facing aperture 1410c-d configured to deliver a pressurized prophylactic and/or therapeutic material to an injection space 1404. The third and fourth needle barrels 1420a-b do not comprise any apertures configured to deliver pressurized fluid into the injection space 1404.

[0239] FIG. 15 illustrates an embodiment of an intracellular delivery device having four needle barrels 1520a-d with two of the four needle barrels 1520b, 1520d comprising at least one inward or center facing aperture 1510b, 1510d configured to deliver a pressurized prophylactic and/or therapeutic material into an injection space 1504. The third and fourth needle barrels 1520a, 1520c comprise apertures 1510a, 1510c configured to apply a negative pressure to the injection space 1504.

[0240] As described herein, the shape of each needle barrel can vary. FIGS. 16-18 illustrate embodiments of ring shaped needle barrels that include inward or center facing apertures. FIG. 16 illustrates a needle barrel 1620 that is ring shaped and includes three inward or center facing apertures 1610. Two of the three apertures 1610 are configured to deliver a pressurized prophylactic and/or therapeutic material into an injection space 1604 and the third aperture 1610 is configured to apply a negative pressure to the injection space. The apertures 1610 can form a triangle, for example, an equilateral triangle. FIG. 17 illustrates a needle barrel 1720 that is ring shaped and includes two inward or center facing apertures 1710 that face one another. One of the two apertures 1710 is configured to deliver a pressurized prophylactic and/or therapeutic material into an injection space 1704 and the other aperture 1710 is configured to apply a negative pressure to the injection space. FIG. 18 illustrates a needle barrel 1820 that is ring shaped and includes two inward or center facing apertures 1810 that face one another. Both of the apertures 1820 are configured to deliver a pressurized prophylactic and/or

therapeutic material into an injection space 1804. The apertures 1810 can comprise any suitable shape, for example, a generally polygonal shape.

[0241] In some embodiments, a needle barrel can comprise one lumen that is fluidly connected to a plurality of apertures or more than one lumen. FIG. 19 illustrates a cut-away view of an embodiment of a barrel including a single lumen. Needle barrel 1920 comprises a single lumen 1935 and three apertures 1910 that are each fluidly connected with the single lumen 1935. The lumen 1935 is used for both the transmission of pressure and the delivery of the prophylactic and/or therapeutic agent.

[0242] FIG. 20 illustrates a cut-away view of an embodiment of a barrel including two lumens. Needle barrel 2020 comprises a first lumen 2035 that is fluidly connected with two apertures 2010. The needle barrel 2035 also includes a second lumen 2037 that is fluidly connected with a third aperture 2012. This embodiment may be employed, for example, if it becomes desirable to use a first lumen for the delivery of a pressurized prophylactic and/or therapeutic agent and a second lumen for the delivery of another fluid, prophylactic and/or therapeutic agent, and/or the application of negative pressure, or vice versa.

[0243] In some embodiments, an adjustable delivery unit for use with an adjustable intracellular delivery device comprises a channel configured to receive the hypodermic needle, a handle which operates to enclose the syringe and needle hub within the channel such that the closed ends of the needle barrels protrude from the channel and are available to engage a biological specimen, a charging element configured to be charged by the operation of the handle, the charging element coupled to a trigger and the syringe such that operation of the trigger releases the charging element, wherein the charging element acts on the syringe, thereby displacing prophylactic and/or therapeutic material out of the syringe. In some embodiments, the adjustable delivery unit comprises a spring configured to be compressed by the operation of the handle and configured to decompress upon operation of the trigger.

[0244] FIG. 21A is one example of spring-actuated device that can be used with the needles devices described herein. Spring-actuated device 2100 includes loading ring grip 2110 on one side and depth adjusting member 2120 on an opposite side. Depth adjustment member 2120 may rotatably engage spring-actuated device 2100 and be configured to adjust the depth that needles penetrate tissue when administering to a subject. Trigger button 2130 can be pressed to trigger the device to compress the needle plunger and inject prophylactic and/or therapeutic material.

[0245] Fig. 21B shows intracellular delivery device 2140 being inserted into spring actuated device 2100. Loading ring grip 2110 is withdrawn so that the needles can be inserted into a cavity of spring actuated device 2100. Fig. 21C shows an intracellular delivery device 2140 loaded within spring actuated device 2100. Fig. 21D shows a side view of spring-actuated device 2100 where springs 2150 are disposed along the lumen of spring-actuated device 2120 extending along a length of intracellular delivery device 2140. Springs 2150 are configured to extend when loading ring grip 2110 is withdrawn and compress the plunger of syringe 2140 upon pressing trigger button 2130.

[0246] Fig. 22A is a perspective view of one embodiment of a spring-actuated delivery device for using with the injection needle devices of the present application. Spring – actuated trigger device 2200 includes plunger aperture 2210 configured to receive the plunger portion of the syringe, and barrel aperture 2220 configured to receive the barrel portion of the syringe. Trigger 2230 is configured so that squeezing trigger 2230 depresses the plunger of a syringe. Fig. 22A depicts needle device 2240 being inserted into trigger device 2200. Needle device 2240 may be any needle device or needle hub as described herein.

[0247] Fig. 22D is a side view of trigger device 2200 where trigger 2230 is coupled to plunger aperture 2210 (e.g., coupled by a lever or gear) so that squeezing trigger 2230 compressed the plunger of the needle device and injects the prophylactic and/or therapeutic material.

[0248] FIG. 23A illustrate an embodiment of a needle hub. Bottom-hub component 2300 is configured to receive a plurality of needles, each needle having needle barrel 2310 and hub-engaging member 2320 disposed at one end of the needle. Bottom-hub component 2300 includes apertures 2330 that receive the needle barrel 2310 and engage hub-engaging member 2320 to maintain the needle within the hub. FIG. 23B illustrates the needles after being inserted into apertures 2330. The depth of apertures 2330 may vary so that the needles are staggered relative to each other (e.g., as depicted in FIG. 2D). FIG. 23C shows top-hub component 2340 having aperture-engaging members 2350 that are configured to engage apertures 2330 when top-hub component 2340 is disposed on bottom-hub component 2300. Aperture-engaging members 2350 can secure the hub-engaging member 2320 within the hub. Bottom-hub component 2300 and top-hub component 2340 may be secured together by, for example, welding the two components together.

[0249] Fig. 24 is perspective view of one embodiment of an adjustable delivery device or adjustable delivery unit for use with an intracellular delivery device. Adjustable

delivery unit (ADU) 2400 includes spring piston 2410 coupled to spring trigger 2420. Spring piston 2410 is configured to depress the plunger in syringe 2430 when spring trigger 2420 is actuated (e.g., by depressing). Syringe 2430 is secured within lumen 2435 of ADU 2400 by ball-bearing 2440 and collar 2450. Syringe 2430 is coupled to hub 2460 including one or more needles for injecting a prophylactic and/or therapeutic material. Hub 2460 can have any of the designs disclosed herein. ADU 2400 may optionally include slot 2470 through the body of ADU 2400 and spring trigger 2420. Slot 2400 can be configured to receive a priming member that compresses spring piston 2410 before delivering the prophylactic and/or therapeutic material. ADU 2400 may also optionally include threaded adjuster 2480 that is rotatably coupled to spring piston 2410 and configured to adjust the force (or pressure) applied to the syringe when spring trigger 2420 is actuated.

[0250] Fig. 25 illustrates a cross-sectional view of one embodiment an adjustable delivery unit with a priming jig. ADU 2400 may be configured to be operated with priming member which couples to an alignment aid 2510 and a priming jig 2520. The ADU 2400 may be seated in alignment aid 2510 and engaged with priming jig 2520, thereby raising spring trigger 2420, and allowing access to lumen 2435. Syringe 2430 is inserted into lumen 2435, and syringe 2430 is fastened to secure the syringe within the lumen of ADU 2400. The primed ADU 2400 can now be removed from alignment aid 2510 for use in a subject. To dispense the prophylactic and/or therapeutic material, spring trigger 2420 is depressed, spring 2530 is released, thereby operating spring piston 2410 which, in turn, operates the plunger of syringe 2435.

[0251] In some embodiments, the trigger device and spring can be configured to perform any of the methods of delivering the prophylactic and/or therapeutic material using the needles devices disclosed herein. For example, the trigger device can configured to deliver the prophylactic and/or therapeutic material within a time period (e.g., 1 second or less), apply a maximum force or pressure to the prophylactic and/or therapeutic material (e.g., 50-150 N or 50 to 5000 kPa), or deliver the therapeutic material at a maximum rate (e.g., at least 2.5 mL/s).

[0252] FIG. 26 illustrates an embodiment of an intracellular delivery device having slit-type apertures and closed ends. Four needle barrels 2620 arranged in a Y-type pattern. The four needle barrels 9120 are disposed partially within hub 2645 with a center needle and three outer needles positioned equidistant from the center needle and from each other. The aperture 2610 may comprise at least one elongate slit having a first dimension along the axis of the needle barrel and a second dimension perpendicular to the axis of the needle barrel.

[0253] The first dimension of aperture 2610 may, for example, be greater than, equal to, or less than 0.01 μ m, 0.02 μ m, 0.03 μ m, 0.04 μ m, 0.05 μ m, 0.06 μ m, 0.07 μ m, 0.08 μ m, 0.09 μ m, 0.1 μ m, 0.15 μ m, 0.2 μ m, 0.25 μ m, 0.3 μ m, 0.35 μ m, 0.4 μ m, 0.45 μ m, 0.5 μ m, 0.55 μ m, 0.6 μ m, 0.65 μ m, 0.7 μ m, 0.75 μ m, 0.8 μ m, 0.85 μ m, 0.9 μ m, 0.95 μ m, 1.0 μ m, 1.5 μ m, 2.0 μ m, 2.5 μ m, 3.0 μ m, 3.5 μ m, 4.0 μ m, 4.5 μ m, 5.0 μ m, 5.5 μ m, 6.0 μ m, 6.5 μ m, 7.0 μ m, 7.5 μ m, 8.0 μ m, 8.5 μ m, 9.0 μ m, 9.5 μ m, 10 μ m, 15 μ m, 20 μ m, 25 μ m, 30 μ m, 35 μ m, 40 μ m, 45 μ m, 50 μ m, 55 μ m, 60 μ m, 65 μ m, 70 μ m, 75 μ m, 80 μ m, 85 μ m, 90 μ m, 95 μ m, 0.1mm, 0.2mm, 0.3mm, 0.4mm, 0.5mm, 0.6mm, 0.7mm, 0.8mm, 0.9mm, 1mm, 1.05mm, 1.10mm, 1.15mm, 1.20mm, 1.25mm, 1.30mm, 1.35mm, 1.40mm, 1.45mm, 1.50mm, 1.55mm, 1.60mm, 1.65mm, 1.70mm, 1.75mm, 1.80mm, 1.85mm, 1.90mm, 1.95mm, or 2.0mm or within a range defined by, and including, any two of these values.

[0254] The second dimension of aperture 2610 may, for example, be greater than, equal to, or less than 0.01 μ m, 0.02 μ m, 0.03 μ m, 0.04 μ m, 0.05 μ m, 0.06 μ m, 0.07 μ m, 0.08 μ m, 0.09 μ m, 0.1 μ m, 0.15 μ m, 0.2 μ m, 0.25 μ m, 0.3 μ m, 0.35 μ m, 0.4 μ m, 0.45 μ m, 0.5 μ m, 0.55 μ m, 0.6 μ m, 0.65 μ m, 0.7 μ m, 0.75 μ m, 0.8 μ m, 0.85 μ m, 0.9 μ m, 0.95 μ m, 1.0 μ m, 1.5 μ m, 2.0 μ m, 2.5 μ m, 3.0 μ m, 3.5 μ m, 4.0 μ m, 4.5 μ m, 5.0 μ m, 5.5 μ m, 6.0 μ m, 6.5 μ m, 7.0 μ m, 7.5 μ m, 8.0 μ m, 8.5 μ m, 9.0 μ m, 9.5 μ m, 10 μ m, 15 μ m, 20 μ m, 25 μ m, 30 μ m, 35 μ m, 40 μ m, 45 μ m, 50 μ m, 55 μ m, 60 μ m, 65 μ m, 70 μ m, 75 μ m, 80 μ m, 85 μ m, 90 μ m, 95 μ m, 0.1mm, 0.2mm, 0.3mm, 0.4mm, 0.5mm, 0.6mm, 0.7mm, 0.8mm, 0.9mm, 1mm, 1.05mm, 1.10mm, 1.15mm, 1.20mm, 1.25mm, 1.30mm, 1.35mm, 1.40mm, 1.45mm, 1.50mm, 1.55mm, 1.60mm, 1.65mm, 1.70mm, 1.75mm, 1.80mm, 1.85mm, 1.90mm, 1.95mm, 2.0mm, 2.05mm, 2.10mm, 2.15mm, 2.20mm, 2.25mm, 2.30mm, 2.35mm, 2.40mm, 2.45mm, 2.50mm, 2.55mm, 2.60mm, 2.65mm, 2.70mm, 2.75mm, 2.80mm, 2.85mm, 2.90mm, 2.95mm, 3.0mm, 3.1mm, 3.2mm, 3.3mm, 3.4mm, 3.5mm, 3.6mm, 3.7mm, 3.8mm, 3.9mm, or 4.0mm or within a range defined by, and including, any two of these values.

[0255] In some embodiments, the at least one slit may have a first dimension of 2.15mm and a second dimension of 0.05mm. The elongate slits may have any aperture dimensions as described herein, provided that the first dimension is greater than the second dimension. The apertures 2610 on the outer needles 2620 may be configured such that they face the center needle, and the apertures on the center needle may be configured to face each outer needle respectively.

[0256] FIGS. 27A-C illustrate an embodiment of an intracellular delivery device having a plurality of needles partially disposed within a micro-hub. The hub of this device is a

micro-hub because of its smaller size relative to the injection devices described elsewhere herein.

[0257] Referring to FIG. 27A, an exploded perspective view of a micro-hub injection device 2700 is depicted. Referring to FIG. 27B, side cutaway view of a micro-hub injection device is depicted. Micro-hub injection device 2700 comprises a plurality of needle barrels 2720 disposed within micro-hub 2745 such that the portion of the needle barrel comprising apertures is not disposed within the micro-hub. Needle barrels 2720 comprise a plurality of apertures 2710 as described elsewhere herein. The apertures are disposed along the length of the needle barrels and are disposed on the portion of needle barrel 2720 not disposed within micro-hub 2745. Micro-hub 2745 may be generally of cylindrical shape.

[0258] The micro-hub intracellular delivery device also comprises a luer connector 2730. Luer connector 2730 is configured to mate with a luer fitting on a syringe on a first end 2731 and micro-hub 2745 on a second end 2732. Luer connector forms a conduit 2735 passing through the center of luer connector 2730. The conduit 2735 provides a flow path for fluid through luer connector 2735. The first end 2731 of luer connector 2730 may mate with a luer fitting on a syringe using a threaded connection, a friction fit, or other suitable attaching method. The second end 2732 of luer connector 2730 which mates with micro-hub 2745 has an internal hollow diameter sized to receive a first cylindrical diameter of hub 2745.

[0259] Micro-hub 2745 has a first cylindrical diameter 2746, which is sized to provide a surface which is in direct contact with a surface of luer connector 2730. Micro-hub 2745 has a second cylindrical diameter 2747, which is sized to fit within second end 2732 of a portion of luer connector 2730. The second cylindrical diameter 2747 of micro-hub 2745 which corresponds to the portion of micro-hub 2745 which is disposed within second end 2732 of luer connector 2730 is sized such that a gap L_{92} is created when needle holder 2745 is fully engaged within luer connector 2730. In some embodiments, L_{92} may be approximately 0.5 mm. The gap is sized to create a chamber 2740 between an end of micro-hub 2745 and the second end 2732 of luer connector 2730 which allows for fluid which flows through luer connector 2730 to access the open end 2715 of each needle barrel 2720. Chamber 2740 provides fluid communication between the open end 2715 of each needle barrel 2720 and chamber 2735 such that fluid from an attached syringe may ultimately flow through all apertures 2710 on all needle barrels 2720.

[0260] FIG. 27C illustrates a view of a micro-hub intracellular delivery device having trocar-tip needles. Trocar-tip needles have three beveled surfaces defined by planes intersecting at an angle relative the long axis of the needle barrel and culminating in a point.

Referring to FIG. 27C, L_{92A} may, in some embodiments, be from 0.01 mm to 3 mm. In some embodiments, L_{92A} is 1 mm. In some embodiments, L_{92B} may be from 0.01 mm to 4 mm. In some embodiments, L_{92B} is 1 mm. In some embodiments, L_{92C} may be from 1 to 6 mm. In some embodiments, L_{92C} is 4 mm. Needle barrels 2720 may, for example, have 6 apertures 2710 per needle barrel, each aperture having a diameter of 0.5 mm and spaced 0.2 mm apart. In some embodiments, the apertures may have a diameter of 0.05 mm.

[0261] FIGS. 28A-B illustrate perspective views of an adjustable delivery unit being operated with an intracellular delivery device having a micro-hub attached to a syringe. Referring to FIGS. 28A and 28B, the adjustable delivery unit comprises a cavity 2850 configured to receive a syringe and micro-hub intracellular delivery device, a locking handle 2860, and a button 2870. The syringe and micro-hub injection device are positioned within cavity 2850 such that the needle barrels are protruding from a first end of the adjustable delivery device. Once positioned within the cavity, locking handle 2850 is lowered. Lowering handle 2860 primes a charging element within adjustable delivery device, which is coupled to button 2870, which is disposed near a second end of the adjustable delivery device. The charging element may be a spring, which is compressed by the action of lowering handle 2860. Once the handle is completely lowered, the syringe and micro-hub device are within cavity 2850, and the charging element is primed, the adjustable delivery device may be inserted in to a subject or tissue, as desired. User may press button 2870 which releases the charging element, causing the plunger of the syringe to be operated. Operating the plunger of the syringe expels fluid or therapeutic agent out of the syringe, through the luer connector, through the needle barrels, out the apertures, and ultimately into the tissue or subject, as described elsewhere herein.

[0262] The needle barrels and intracellular delivery device embodiments described herein may be used in conjunction with other known methods and systems for enhancing gene delivery. Accordingly, some embodiments of the present invention utilize control circuitry to generate an electric current or an electromagnetic field to alter cell permeabilities. In some embodiments, it may be desired to utilize one or more of the needle barrels themselves to conduct or transmit the generated current or field into the tissue. The needle barrels may be used in conjunction with any number of known alternative microporation methods using optionally one or more of sonic, electromagnetic, mechanical and thermal energy or a chemical enhancer, such as that disclosed in U.S. Patent Number 6,527,716 to Eppstein, which is expressly incorporated by reference in its entirety herein.

[0263] Aspects of the invention also concern use of the aforementioned devices as intracellular delivery devices or in conjunction with electroporation. Electroporation is a known therapeutic treatment, which uses electrodes to generate electric fields in a tissue or sample to increase permeability of a cell's outer membrane, thus allowing greater cell uptake of therapeutic agents. (See U.S. Pat. No. 6,241,701, U.S. Pat. No. 6,516,223, U.S. Pat. No. 6,678,556, U.S. Pat. No. 6,610,044, and U.S. Pat. No. 6,110,161, all of which are hereby expressly incorporated by reference in their entireties.)

[0264] The skilled artisan, guided by the teaching of the present application, can readily configure the intracellular delivery devices and adjustable delivery units of the present application to comprise electrodes for use in electroporation. In some embodiments, the needles of an intracellular delivery device described herein may comprise electrodes (also referred to as needle-electrodes or combined needle and electrode) configured to apply an electric field to a tissue or sample. The needles may be configured as electrodes for use in electroporation. In some embodiments, the needles of the aforementioned devices may be inserted into a sample or a tissue. The needles, configured as electrodes, may generate an electric field in the tissue or sample in order to permeabilize the cell membranes of cells in the vicinity of the needles. The therapeutic agent may then be delivered via the intracellular delivery devices described herein, and cell uptake may be improved. In some embodiments, the needles of the aforementioned devices may be inserted into a sample or a tissue and deliver a therapeutic agent, as described herein. The needles, configured as electrodes, may also be used to generate an electric field in the sample or tissue, which permeabilizes the cell membranes of the cells in the vicinity of the needles. Upon permeabilization of the cell membranes, cell uptake of the therapeutic agent can be improved.

[0265] In some embodiments, injection speed and pressure may be varied by using various delivery units. The delivery unit may have a pressure generation element actuated by spring, gas spring, electromechanical mechanism, compressed air, or other similar device. By varying the force with which the pressure generation element is actuated, the injection speed, and/or injection pressure may be varied. In some embodiments the intracellular delivery apparatus is configured to rapidly deliver an agent to an area defined by the needle array. The rapid injection of prophylactic and/or therapeutic along with increased intracellular pressure overloads a tissue locally, thereby increasing permeability of the cell membrane. By varying injection pressure and/or speed, the local tissue overload can be varied, resulting in varying uptake of the prophylactic and/or therapeutic agent.

[0266] FIGS. 29A-C illustrate the electrical connections to one or more needles of an intracellular delivery device for use in electroporation. Referring to FIG. 29A, needles 2920a-d, are constructed of an electrically conductive material, and are individually electrically connected via electrical connectors 2925a-d, to a voltage source, current source, or other power source (not depicted). In some embodiments, the voltage source can be battery powered. The voltage source may be configured to apply signals of varying polarity to individual needles to achieve a desired electric field. In some embodiments, a positive voltage may be applied to needles 2920a, 2920b, and 2920c, and a negative voltage may be applied to needle 2920d. In some embodiments, a negative voltage may be applied to needles 2920a, 2920b, and 2920c, and a positive voltage may be applied to needle 2920d. In some embodiments, a constant voltage may be applied to needles 2920a-d. In some embodiments, the voltage may be pulsed or varying. In some embodiments, the same voltage may be applied to each needle 2920a-d. In some embodiments, different voltages or voltage patterns may be applied to needles 2920a-d. A person of skill in the art would understand that needles 2920a-d may have any combination of polarities or voltages.

[0267] Referring to FIG. 29B, needles 2930a-d are constructed of an electrically conductive material, and are individually electrically connected via electrical connectors 2935a-d to a voltage source (not depicted). In some embodiments, a positive voltage may be applied to needles 2930a and 2930b, and a negative voltage may be applied to needles 2930c and 2930d. In some embodiments, a positive voltage may be applied to needles 2930a and 2930c, and a negative voltage may be applied to needles 2930b and 2930d.

[0268] Referring to FIG. 29C, needles 2940a-f are constructed of an electrically conductive material, and are individually electrically connected via electrical connectors 2945a-f to a voltage source (not depicted). The voltage source may be configured to apply signals of varying polarity to individual needles. In some embodiments, a positive charge may be applied to needles 2940a-e and a negative voltage may be applied to needle 2940f. In some embodiments, a negative charge may be applied to needles 2940a-e and a positive voltage may be applied to needle 2940f. In some embodiments, a positive charge may be applied to needles 2940a, 2940c, and 2940f, and a negative voltage may be applied to needles 2940b, 2940d, and 2940e. Although specific embodiments of voltage polarity patterns are disclosed, the skilled artisan will appreciate that the polarity of the voltage applied to the needles in the present application can be varied, and, without limitation, any combination of polarities may be applied to the individual needles in any pattern.

[0269] FIGS. 30A-F illustrate embodiments of needle and electrode configurations of a microhub injection device. The microhub injection device 3001 can include needles 3010, electrodes 3020 and/or combined needle-electrodes 3030. Combined needle-electrodes 3030 and/or separate needles 3010 can be utilized for delivery of a prophylactic or therapeutic agent as described elsewhere herein. The direction of flow of prophylactic or therapeutic agent out of the needles of the microhub injection device is depicted by arrows extending from needle-electrodes 3030. Combined needle-electrodes 3030 and/or separate electrodes 3020 can be utilized to produce one or more electrode fields 3040. A plurality of needles 3010, electrodes 3020, and/or combined needle-electrodes 3030 may be configured to produce a desired delivery type and a desired field pattern of the electrode fields. Delivery types can include, but are not limited to 'star' type, 'X' type and 'Y' type. Field patterns can include, but are not limited to parallel field pattern, 'O' field pattern and 'Δ' field pattern. Field patterns can be commutating. Referring to FIG. 30A, combined needle-electrodes 3030 are configured for 'star' type delivery and field pattern. Referring to FIG. 30B, combined needle-electrodes 3030 are configured for 'Y' type delivery and field pattern. Referring to FIG. 30C, separate needles 3010 are configured for 'Y' type delivery and separate electrodes 3020 are configured for parallel field pattern. Referring to FIG. 30D, combined needle-electrodes 3030 and a central needle 3010 are configured for 'star' type delivery and commutating 'O' field pattern. Referring to FIG. 30E, separate needles 3010 are configured for 'Y' type delivery and commutating electrodes 3020 are configured for 'Δ' field pattern. Referring to FIG. 30F, combined needle-electrodes 3030 and separate needles 3010 are configured for 'Y' type delivery and separate electrodes 3020 are configured for parallel field pattern. Polarities of needles 3010 shown in FIGS. 30A-F are for illustrative purposes only. The polarities may be reversed or commutated. Needle polarity pairings and field patterns may be different from that shown. For example, the needle polarity pairings and field patterns may be altered to enhance electroporation. FIGS. 30A-F also depict the direction of flow of a prophylactic and/or therapeutic material out of the needles and/or the apertures located thereon. The direction is indicated by arrows extending from each needle and/or needle electrode. For example, FIGS. 30A and B depict two types of arrays of needles having one needle at the center and outer needles disposed in a hexagonal or triangular shape, respectively, around the center needle. The arrays of needles enclose an active zone or area into which flow of prophylactic and/or therapeutic material is directed. As depicted, the center needle/needle-electrode has apertures positioned along the needle barrel to direct the flow of prophylactic and/or therapeutic material toward each outer needle. The outer needles have

apertures positioned along the needle barrels which direct flow toward the center needle. In some embodiments (for example, FIG. 33D, described later), the outer needles may also have apertures positioned along the needle barrels which direct flow of a prophylactic and/or therapeutic agent toward an adjacent needle, or to one of the outer needles, as well as, apertures directing flow toward the center needles. In some embodiments, the outer needles do not have apertures which direct flow out of the active zone defined by the area within the needle array.

[0270] FIG. 31A-31B illustrate examples of a distal end of an intracellular delivery device having needle hub configurations. FIG. 31A, illustrates a non-pocket hub, while FIG. 31B, illustrates a pocket hub having a distance between electrodes 3110, a sleeve layer 3120 and an isolated area of the electrode 3130. The sleeve layer 3120 may be insulated to prevent electroporation of patients. Non-pocket hub assumes acceptable field losses at distal end of the needles 3010/electrodes 3020. The isolated area of the electrode 3130 can create a localized field. Pocket hub assumes the increased distance between distal ends of the needles 3010 and/or electrodes 3020 results in acceptable system losses at distal end of the needles 3010 and/electrode 3020. In some embodiments, the pocket hub reduces electrical losses at the distal end of the needles 3010.

[0271] FIGS. 32A-B illustrate examples of intracellular delivery devices having laminated needles 3010. Laminated needles 3010 may be used to focus charge and minimize losses. For example, the flow of electrical current through the drug within the hub can be minimized to minimize the electrical power losses. Laminated needles 3010 can include a conductive coating 3250 positioned between two insulated layers 3210. In some embodiments, the conductive coating 3250 can act as the electrode, while the needle and drug do not have a direct coupling to the electrical signal. In other embodiments, the laminated needle 3010 can act as the electrode and the inside of the needle can be coated with a non-conductive coating 3215. Non-conductive coating 3215 prevents the prophylactic and/or therapeutic agent from being in direct electrical contact with the electrodes while in the needle barrel. This arrangement may prevent break-down or degradation of the prophylactic or therapeutic material due to the electrical field inside the needle barrel. Laminated needles 3010 may be any needle described herein. For example, as depicted, needles 3010 have apertures along the length of each barrel oriented to direct flow of therapeutic material toward an aperture on the barrel of an adjacent or opposite needle.

[0272] FIG. 33A illustrates an example of an intracellular delivery device having dual syringes. Dual syringes 3380 can include two or more syringes. The dual syringes 3380

can be used to control needle-electrode polarity. Dual drug chambers of the dual syringes 3380 can prevent electrical current from flowing through the drug within the hub. The hub can include electrical connections 3310 to the needle-electrodes 3330. The hub may comprise one or more electrodes, which are not coupled to a reservoir containing a prophylactic and/or therapeutic material, but act only as an electrode.

[0273] FIGS. 33B-D illustrate examples of delivery and field patterns of an intracellular delivery device having dual syringes. Each syringe 3380 of the dual syringes may be coupled to one or more needle electrodes 3330. The number of needle-electrodes 3330 is not particularly limited, and can be chosen as desired to accomplish a desired configuration or pattern of injection and electroporation. As discussed herein, combination needle-electrodes 3330 can be configured to produce any desired delivery type and/or field pattern. FIG. 33B illustrates a hexagonal shaped delivery and field pattern, the arrows indicating the direction of flow of prophylactic and/or therapeutic material from the apertures on the barrel of the needle-electrodes 3330. FIG. 33C illustrates 'Row' type delivery and field pattern. FIG. 33D illustrates 'O & Y' type delivery and 'O & Y' field pattern, where the arrows indicate the direction of flow of prophylactic and/or therapeutic material from the apertures on the barrel of the needle-electrodes 3330. In a 'row' type delivery and field pattern, six needles are needle electrodes, configured in pairs, wherein one of each pair has a positive polarity and the other in each pair has a negative polarity. The pairs may be aligned across from one another as depicted in FIG. 33C, or they may be arranged in another pattern of pairs, as depicted in FIG. 33D. In some embodiments, there may be one, two, three, four, or five or more pairs of needle electrodes, arranged to provide electroporation. In some embodiments, the electric field can be commutated, or, the polarity of individual needle-electrodes or needle-electrode pairs may have alternating polarity as the electrical signal is applied.

[0274] FIG. 34A illustrates an example of an intracellular delivery device having isolating valves. Hub 3405 can include isolation valves 3410. The isolation valves 3410 can help to eliminate losses at the distal end of the needle-electrode. For example, the isolation valves can help to prevent electrical current from flowing through the drug within the hub. One or more of the needle-electrodes 3010 can include an isolation valve 3410. Isolation valves can be in the form of non-conductive mechanical check-valves. Isolation valves can include, but are not limited to, membrane, ball valve, or other. The hypodermic needle device can include electrical connections to the needle-electrodes 3430. The electrical connections to needle-electrodes 3430 can be configured to provide a desired polarity or pattern

[0275] FIGS. 34B-C illustrate examples of needle and electrode configurations of an intracellular delivery device having isolating valves. Isolation valves allow any configuration of combined needle-electrodes 3430, such as those described herein. FIG. 34B illustrates a commutating field in a 'star' type pattern, wherein individual needles are commutated in pairs in a clockwise or counter clockwise direction, leading to a commutating and varying electric field produced in an area bounded by the array of needles within a subject tissue.

[0276] FIG. 35A illustrates an example of an intracellular delivery device during a first stage of a two stage delivery. In some embodiments, the methods described herein can include a two stage delivery including a first stage and a second stage. The needles can be separate from the electrodes and may be of any type described herein. During the first stage, retractable needles 3510 are inserted into a subject. The prophylactic and/or therapeutic material is delivered substantially within an active area 3525 of the electrodes 3520.

[0277] FIG. 35B illustrates an example of a needle and electrode configuration during the first stage of a two stage delivery. As discussed herein, needles 3530 and electrodes 3520 can be configured to produce any desired delivery type and/or field pattern. For example, in the illustrated configuration, the needles 3530 are configured for 'Y' delivery and the electrodes 3520 are configured to provide parallel electrical field.

[0278] FIG. 35C illustrates an example of an intracellular delivery device in a second stage of a two stage delivery. During the second stage, the retractable needles 3530 are removed or retracted following delivery of the therapeutic and/or prophylactic material, and the electrodes 3520 are energized. Energized electrodes 3520 deliver an electrical field during electroporation. The retractable needles 3530 can include metal parts. It will be appreciated that retracting the needles 3530 removes metal parts of the needles 3530 from the electrical field during the electroporation.

[0279] FIG. 35D illustrates the example needle and electrode configuration of FIG. 35B during the second stage of a two stage delivery.

[0280] FIG. 36 illustrates a perspective view of a reusable delivery device configured for use with an intracellular delivery device comprising a micro-hub attached to a syringe and electrical connections for electroporation. Reusable delivery device 3600 comprises priming lever 3610, an electrical supply cable 3620, a syringe receiving portion 3630, and electrical port 3640. Reusable delivery device 3600 is configured to receive a syringe 3680. Syringe 3680 comprises a hub 3685 and electrical connector 3690. Syringe 3680 is inserted into syringe receiving portion 3630 such that electrical connector 3690 mates with electrical port 3640,

thereby creating a conductive path from an electrical power supply (not shown) through electrical supply cable 3620, and to the needle electrodes in hub 3685. Priming lever 3610 is configured to operate to retain syringe 3680 within syringe receiving portion 3630, and to actuate a compression spring within reusable delivery device, which upon actuation is primed to operate syringe 3680 when delivery is desired.

[0281] FIG. 37 depicts reusable delivery device 3600 with syringe 3680 disposed within following actuation of priming lever 3610. As depicted, priming lever 3610 actuates compression spring 3730 for release upon operation of trigger device 3750. Upon operation of trigger device 3750, compression spring 3730 expands and operates either directly, or indirectly through one or more components, syringe 3680, forcing therapeutic and/or prophylactic material out of syringe 3680, through needles 3695 disposed within hub 3685, and into a subject. Needles 3695 may be electrode-needles or needles as described elsewhere herein. Reusable delivery device 3600 is electrically connected to a power supply via electrical supply cable 3620. Reusable delivery device 3600 may also be connected to an electric signal generator (not shown) controllable by an operator, to deliver a desired electroporation pattern or signal to a subject via electrodes or needle-electrodes disposed within hub 3685. It will be appreciated by those skilled in the art that the trigger device could be operated and/or released by an electrical or electromechanical mechanism, e.g., solenoid, motor, shape memory alloy muscle wire, and other similar mechanisms.

[0282] FIGS. 38A-C illustrate embodiments of an intracellular delivery device having a micro-hub configured for electroporation. Intracellular delivery device 3800 comprises a micro-hub 3810 and syringe attachment portion 3820. Micro-hub 3810 comprises needles 3830. In some embodiments, needles 3830 may comprise electrode-needles or non-electrode needles in any combination. Electrodes and needle electrodes are in electrical contact with electrical conduits 3840. As depicted in FIG. 38B, two needles 3830 are in electrical contact with connectors 3845 which are in electrical contact with electrical conduits 3840. In some embodiments, one or more needles 3830 may be in electrical contact with one or more electrical connectors 3845 and arranged in any pattern or shape to provide a desirable electrical field. Needles 3830 may be similar to any of the needles described elsewhere herein, for example, needles having apertures along the barrel where the apertures are zoned for a particular spray pattern, or any other suitable design. For example, one or more of needles 3830 may have a positive polarity when charged, or one or more of needles 3830 may have a negative polarity when charged, as described elsewhere herein.

[0283] FIG. 38C illustrates a micro-hub 3810 comprising needles 3830 and electrodes 3835. As described elsewhere herein, one of skill in the art would understand that needles 3830 and electrodes 3835 could be electrically connected in various patterns in order to provide various electrical fields in a subject or tissue.

[0284] FIGS. 39A-C illustrate embodiments of an intracellular delivery device with a Y-type configuration having a micro-hub configured for electroporation. As depicted, needles 3930 are arranged in a Y-type pattern, and have apertures along the barrel to provide a Y-type spray pattern as described elsewhere herein. Needles 3930 are all in electrical contact with electrical connectors 3945 and thus in electrical contact with electrical conduits 3940. Micro-hub 3910 and syringe connecting portion 3920 may be configured to provide either a pocket design or non-pocket design. FIG. 38C depicts a pocket design, FIG. 39C depicts a non-pocket design.

[0285] In some embodiments, the electric field applied to a subject may vary based on the voltage applied to the needle electrodes. As cell types vary, so does the electrical field strength required to electroporate the cells. The voltage required across a cell is inversely proportional to the radius of the cell. For example, muscle cells require a lower voltage, and liver cells require a higher voltage. Therefore, the electric field created by the hypodermic needle devices as described herein may be variable according to the target cell type. The electric field strength delivered by the hypodermic needle devices described herein may range from a few Volts/cm, 100 Volts/cm, 200 Volts/cm, 300 Volts/cm, 400 Volts/cm, 500 Volts/cm, 600 Volts/cm, 800 Volts/cm, 900 Volts/cm, 1000 Volts/cm, 1500 Volts/cm, 2000 Volts/cm or greater, or within a range defined by, and including, any two of these values. If the electric field strength gets too high, for example, 3000 Volts/cm, cell death becomes a possibility.

[0286] The injection systems described herein may be termed *in vivo* intracellular injection (IVIN), to achieve a highly localized injection and uptake of the DNA. During the injection the therapeutic and/or prophylactic agent will overload the volume between the needles in a subject or tissue, thereby the increase local pressure forces the prophylactic and/or therapeutic agent into the cells. Thus, IVIN, as described herein, achieves both a highly localized deposit of a therapeutic and/or prophylactic agent, such as DNA, and an improved local uptake into target cells when compared to injection with a standard hypodermic needle. Electroporation (EP) devices can be combined with the technology, as has been described. For example, IVIN may be used to locally overload a volume of muscle cells with DNA that will be treated with *in*

vivo EP. Without EP, standard intra-muscular IVIN greatly improves the efficiency of DNA uptake and expression.

[0287] The IVIN technology may overload muscle tissue with a DNA-containing solution such that the cells become permeable and the DNA is taken up. With the IVIN technology this can be achieved at a highly localized site in a muscle with needles placed along a circle surround the tissue and injects the DNA tissue. Thus, the tissue is locally overloaded resulting in both a local inflammation and an improved uptake of DNA and antigen expression.

[0288] More importantly, in a larger muscle, here represented by the rabbit tibialis anterior, the IVIN shows a similar efficiency in transfecting muscle fibres *in vivo* as a regular needle injection combined with *in vivo* EP.

[0289] In some embodiments, when IVIN is combined with *in vivo* EP the uptake is further improved without causing additional tissue damage. In some embodiments, the *in vivo* EP pulse pattern has one short high-voltage pulse to make cells permeable, and a second longer low-voltage pulse to promote cellular uptake of DNA. The pulse pattern has a minimal destructive effect on the tissue and is more tolerable than pulse patterns also intended to cause tissue destruction and inflammation. Thus, the combination of IVIN and *in vivo* EP may result in a highly effective and tolerable mode of DNA delivery. Furthermore, the combination of IVIN and *in vivo* EP results in a synergistic effect, as is shown below in Example 12.

[0290] The *in vivo* EP pulse pattern may have one short high-voltage pulse to make cells permeable, and a second longer low-voltage pulse to promote cellular uptake of DNA. The pulse pattern may have a minimal destructive effect on the tissue and may be more tolerable than pulse patterns also intended to cause tissue destruction and inflammation. Thus, the combination of IVIN and *in vivo* EP results in a highly effective and tolerable mode of DNA delivery.

[0291] In some embodiments sequences from the highly immunogenic hepatitis B core antigen (HBcAg) have been added. By doing this the immunogenicity is not much improved as determined in a wild-type animal. However, when the combined NS3/4A-HBcAg vaccine is used in a host with dysfunctional T cells to HCV, as in the chronically infected human, responses are greatly improved.

[0292] Accordingly, an embodiment may include HBcAg of a non-human origin since around 2 billion humans already have been in contact with HBV, for example, avian (stork) HBcAg (sHBcAg), which no human has been in contact with. In addition, there is no cross reactivity between human and non-human HBcAg. Ideally, four final genotype-1 and -2 plasmids will be tested in the wild-type and NS3/4A-Tg models to identify the most

immunogenic version, i.e. selection of the best version of sHBcAg added to NS3/4A. The genotype 3 and 4 NS3/4A-sHBcAg vaccines are built as copies of these since they are expected to behave similarly. Optimized vaccine sequences for all four NS3/4A genotype have been generated. Thus, the complete vaccine-mix contains HCV NS3/4A(gt1)-sHBcAg, NS3/4A(gt2)-sHBcAg, NS3/4A(gt3)-sHBcAg, and NS3/4A(gt4)-sHBcAg. This is then combined with the correct dose of IL-12 plasmid and be formulated in to a final optimal mix, which constitutes the final vaccine.

[0293] Also disclosed herein is a polynucleotide immunogenic composition construct and method of use of said construct as an immunogenic composition, wherein the construct has a polynucleotide encoding hepatitis B core antigen (HBcAg). The HBcAg of the polynucleotide construct can be derived from a number of HBV sources, for example, the HBcAg may be isolated from human (SEQ ID NO.: 1), or stork (SEQ ID NO.: 43), heron (SEQ ID NO.: 45), or other avian HBV genome, or it may be isolated from HBV isolated from a rodent such as ground squirrel or woodchuck, or it may be isolated from any mammal or any vertebrate.

[0294] Any HBcAg core antigens known in the art as having useful immunogenic properties can be used herein. Dimer clustering of HBcAg subunits produces spikes on the surface of the core shell, which consist of radial bundles of four long α -helices. The immunodominant B cell epitope on HBcAg is localized around amino acids 76-82 (Salfeld et al., J Virol, 63:798-808, 1989; and Schodel et al., J Virol, 66:106-114, 1992), apparently forming a loop connecting adjacent helices (See, FIG. 1). The spacing of the spikes on the core shell is optimal for B cell mIg receptor cross-linking. See United States Patent No. 7,811,576, to Milich, et al., issued October 12, 2010, which is hereby incorporated by reference in its entirety.

[0295] Any core antigens from other hepatitis viruses isolated from other organisms known in the art as having useful immunogenic properties also can be used herein. For example, Woodchuck Hepatitis Virus WHcAg is only 67% identical at the amino acid level to the HBcAg, and the comparative studies revealed that, the HBcAg and WHcAg proteins do not significantly cross-react at the antibody level and only partially at the CD4⁺ T cell level. However, these two particulate antigens share a number of characteristics including: enhanced immunogenicity of T cell-independent, as well as T cell-dependent antibody production; the absence of nonresponder MHC-haplotypes; efficient antigen-specific B cell activation; the ability of naive WHcAg or HBcAg-specific B cells to act as primary APC for naive core-specific Th cells; and the ability to act as a carrier moiety for foreign epitopes. See, e.g., United

States Patent No. 7,811,576, to Milich, et al., issued October 12, 2010, which is hereby incorporated by reference in its entirety.

[0296] Similarly, ground squirrel hepadna virus (GHV) core protein (GSHcAg) and the arctic ground squirrel hepadna virus (ArGHV) core protein (ArGHcAg) can be used as antigens. The GSHcAg is 91% identical at the amino acid level to the WHcAg. Primate hepatitis B virus core antigens for woolly monkey, orangutan, gibbon, and chimpanzee can also be used, both as native proteins and through known immunogenically active modifications. Similarly antigens that can be used with the embodiments provided herein include avian core antigens from duck avihepadnavirus core antigen sequence, Ross' goose avihepadnavirus core antigen sequence, heron avihepadnavirus core antigen sequence, Sheldgoose avihepadnavirus core antigen sequence, and stork avihepadnavirus core antigen sequence. See, e.g., United States Patent No. 7,883,843 to Milich, et al. issued February 8, 2011, which is hereby incorporated by reference in its entirety.

[0297] The HCV genome contains a single open reading frame that is transcribed into a polyprotein. This HCV polyprotein can be cleaved in order to produce at least ten distinct parts, in the order NH₂-Core-E1-E2-p7-NS2-NS3-NS4a-NS4b-NS5a-NS5b-COOH.

[0298] The protein NS3 is a protein of 630 amino acids, which appears approximately from amino acid 1027 to amino acid 1657 of the polyprotein. The protein NS4, a protein of 314 amino acids, appears approximately from amino acid 1658 to amino acid 1972, as is known in the art (numbering with respect to HCV-1) (Choo et al., 1991, Proc. Natl. Acad. Sci., vol 88: 2451-2455). The polyprotein NS3/NS4 therefore appears approximately from amino acid 1027 to amino acid 1972, as is known in the art.

[0299] The polypeptide NS5b also contained in the composition of the invention is constituted by 590 amino acids and appears approximately from amino acid 2421 to amino acid 3011 of the polyprotein, as is known in the art (Choo et al., 1991).

[0300] The protein NS3 comprises two distinct structural domains, namely an N-terminal domain endowed with an active serine protease activity that is involved in the maturation of the viral polyprotein, and a C-terminal domain comprising a helicase activity associated with an NTPase activity that plays a role in the replication of the viral genome.

[0301] Compositions containing a polyprotein corresponding to the two collinear proteins NS3 and a polypeptide constituted by NS5b, the vectors, such as adenovirus or poxvirus, capable of expressing this composition and their use as vaccine have been reported.

See, e.g., United States Patent No. 8,057,162, to Fournillier, et al., issued November 15, 2011, which is hereby incorporated by reference in its entirety.

[0302] The polynucleotide immunogenic composition may comprise a construct having any polynucleotide encoding an HCV NS3/4A protein (SEQ ID NO.: 48), and the polynucleotide immunogenic composition may also comprise an additional HCV antigen, such as NS5A. Polyprotein antigens compositions corresponding to the two collinear proteins NS3 and NS4 (hereinafter referred to as HCV NS3/NS4A protein) and a polypeptide constituted by NS5b, have been reported in immunogenic compositions by, for example, United States Patent 8,057,162, to Fournillier, et al., issued November 15, 2011, which is hereby incorporated by reference in its entirety.

[0303] Protective immunity induced by vaccination is dependent on the capacity of the vaccine to elicit the appropriate immune response to resist or eliminate the pathogen. Depending on the pathogen, this may involve a cell-mediated and/or humeral immune response. The current paradigm for the role of the helper T cells in the immune response is that T cells can be separated into subsets on the basis of the cytokines they produce, and that the distinct cytokine profile observed in these cells determines their function. This T cell model includes two major subsets: Th1 cells that produce IL-2 and interferon-gamma (IFN- γ .) which augment both cellular and humoral immune responses, and Th2 cells that produce IL-4, IL-5 and IL-10 which augment humoral immune responses (Mosmann et al., J. Immunol. 126:2348 (1986)). It can be desirable to enhance the immunogenic potency of an antigen in order to obtain a stronger immune response in the organism being immunized, orient the immune response toward a particular type of response, and strengthen host resistance to the antigen-bearing agent. A substance that enhances the immunogenicity of an antigen with which it is administered is known as an adjuvant. See, e.g., United States Patent No. 7,374,751, to Hancock, issued May 20, 2008, which is hereby incorporated by reference in its entirety.

[0304] As provided herein, IL-12 can act as an adjuvant for prophylactic or therapeutic vaccines against infectious and metastatic diseases. See also Rodolfo and Colombo, Methods 19:114-120 (1999); and Scott and Trinchieri, Semin. Immunol. 146:431-427 (1991). IL-12 is a heterodimeric cytokine that is primarily produced by dendritic cells, macrophages and neutrophils and plays a role in the generation of type 1 immune responses and cell-mediated immunity (Brunda, J. Leukocyte Biol. 55:280-288 (1994); Scott and Trinchieri, Semin. Immunol. 9:285-291 (1997)). As contemplated herein, IL-12 has the potential to serve as an

adjuvant for HCV antigenic compounds such that administration of IL-12 to a subject immunized with HCV antigenic compounds elicits a superior anti-HCV immune response.

[0305] The polynucleotide immunogenic composition of the present disclosure may also comprise a polynucleotide (e.g., SEQ ID NO.: 34) encoding an IL-12 polypeptide (e.g., SEQ ID NO.: 35). A polynucleotide encoding IL-12 from any metazoan having IL-12 may be used, preferably an IL-12 from a vertebrate, even more preferably an IL-12 from a mammal, and even more preferably mouse (SEQ ID NO.: 38) or human IL-12. In some embodiments an IL-12 is selected so that its origin matches the genus and species of the subject to be vaccinated.

[0306] The polynucleotide immunogenic composition may also comprise a polynucleotide encoding a galactosyl transferase protein. A polynucleotide encoding galactosyl transferase from any metazoan having galactosyl transferase may be used, preferably an galactosyl transferase from a vertebrate, even more preferably an galactosyl transferase from a mammal, and even more preferably mouse or non-human primate galactosyl transferase (e.g., SEQ ID NO.: 40, encoding SEQ ID NO.: 39).

[0307] Anti- α Gal, a naturally occurring antibody present in all humans, specifically interacts with the carbohydrate epitope Gal α (1-3)Gal β (1,4)GlcNAc-R (α Gal epitope). This antibody does not interact with any other known carbohydrate epitope produced by mammalian cells (Galili, 1993, Springer Seminar Immunopathology 15:153). anti- α Gal constitutes approximately 1% of circulating IgG (Galili et al., 1984, J. Exp. Med. 160:1519) and is also found in the form of IgA and IgM (Davine et al., 1987, Kidney Int. 31:1132; Sandrin et al., 1993, Proc. Natl. Acad. Sci. USA 90:11391). It is produced by 1% of circulating B lymphocytes (Galili et al., 1993, Blood 82:2485). Production of this natural anti- α Gal Ab in humans is constantly stimulated by the presence of α Gal carbohydrate residues present in intestinal and pulmonary bacterial flora. In humans anti- α Gal reacts to the presence of this epitope in hyperacute xenograft rejection and complement is swift and certain resulting in destruction of foreign tissues in minutes to hours. Galactyl transferases are effective at eliciting an immune response because of their ability to catalyze the synthesis of an α Gal epitope, on cell surface glycoproteins present on cells.

[0308] For example, a pharmaceutical composition is generated by introducing to smooth muscle cells, for example using the IVIN-EP methods and devices disclosed herein, a polynucleotide sequence, which encodes upon expression an α Galactosyl Transferase enzyme. The sequence is introduced through any nucleotide transfer vehicle, which can comprise a viral or non-viral vector, or can comprise naked polynucleotide galactosyl transferase. See, e.g.,

United States Patent No. 7,763,461, to Link, Jr., et al., issued July 27, 2010, which is hereby incorporated by reference in its entirety.

[0309] Other adjuvants may be used in embodiments of the present disclosure. For example, the enzymatically-active region of cholera toxin (CTA1) may be used as an adjuvant to facilitate or to potentiate an immune response to an antigen such as the antigens contemplated herein.

[0310] In some preferred embodiments, more than one of the above-mentioned polynucleotides are present on a single polynucleotide construct. In some preferred embodiments, at least two of the polynucleotides mentioned above are present on distinct polynucleotide molecules in an immunogenic composition. The polynucleotides encoding the HBcAg, HCV antigens such as NS3/4A, and IL-12 can be on the same construct or can be on separate constructs. The polynucleotides encoding the HBcAg, HCV antigens such as NS3/4A, and galactosyl transferase can be on the same construct or can be on separate constructs.

[0311] The IL-12 encoding polynucleotide can be a modified IL-12, as well. For example, the IL-12 encoding polynucleotide may be codon-optimized for improved expression in human (e.g., SEQ ID NO.: 36). The codon optimization may not affect the sequence of amino acid residues in the encoded protein. The codon optimization may change the codon which specifies a given amino acid residue in the encoded protein.

[0312] The IL-12 polypeptide may be modified otherwise as well. IL-12 variant polypeptides having less than 100% identity to IL-12 are known in the art, and these variant or variants with similar degrees of difference but retaining IL-12 activity may be used. For example, human or mouse mutant IL-12p40 subunits containing mutant Asn-222 (human) or Asn-220 (mouse) which is essential for the secretion of IL-12p40 can be used for DNA vaccine immunization. See, e.g., United States Patent No. 7,910,564, to Sung, et al., issued March 22, 2011, which is hereby incorporated by reference in its entirety. In some embodiments the IL-12 polypeptide of the immunogenic compositions and methods disclosed herein is modified from the sequence disclosed herein so as to improve or facilitate immunogenicity.

[0313] The galactosyl transferase encoding polynucleotide can be a modified galactosyl transferase, as well. For example, the galactosyl transferase encoding polynucleotide may be codon-optimized for improved expression in humans (e.g., SEQ ID NO.: 41). The codon optimization may not affect the sequence of amino acid residues in the encoded protein. The codon optimization may change the codon which specifies a given amino acid residue in the encoded protein. In some embodiments the galactosyl transferase polypeptide of the

immunogenic compositions and methods disclosed herein is modified from the sequence disclosed herein so as to improve or facilitate immunogenicity.

[0314] In some embodiments, one or more of the HBcAg, NS3/4A, NS5A, IL-12 and galactosyl transferase polynucleotide sequences may be codon optimized for expression in humans. In some embodiments said codon optimization may not affect the sequence of the encoded polypeptide.

[0315] The IL-12, HBcAg, and HCV can each optionally be present in an immunogenic composition in protein form. Similarly, the galactosyl transferase, HBcAg, and HCV can each optionally be present in an immunogenic composition in protein form. For example, an immunogenic composition may comprise a codon optimized DNA construct encoding HBcAg and NS3/4A (with or without NS5A), and the construct can also include codon-optimized IL-12 polynucleotide sequence, or a separate construct encoding codon-optimized IL-12 polynucleotide sequence, or IL-12 protein can be provided as a constituent of said immunogenic composition. Additionally or alternately, the construct may also include codon-optimized galactosyl transferase polynucleotide sequence, or a separate construct encoding codon-optimized galactosyl transferase polynucleotide sequence, or galactosyl transferase protein can be provided as a constituent of said immunogenic composition such as an immune composition comprising NS3/4A and HBcAg or an immunogenic composition comprising polynucleotides encoding NS3/4A, and HBcAg.

[0316] Additionally, ribavirin can be given before, during or after introduction of the DNA immunogenic composition with the IL-12. Ribavirin is a small molecule inhibitor of HCV maturation. It is one of a number of small molecules given to treat HCV. Such therapeutic agents active against HCV also include, but are not limited to, levovirin, viramidine, thymosin alpha-1, interferon-beta, interferon-alpha, pegylated interferon-alpha (peginterferon-alpha), a combination of interferon-alpha and ribavirin, a combination of peginterferon-alpha and ribavirin, a combination of interferon-alpha and levovirin, and a combination of peginterferon-alpha and levovirin. The compounds of the present invention may also be administered in combination with the antiviral agent amantadine (1-aminoadamantane). See, e.g., United States Patent No. 8,278,322, to Holloway, et al., issued October 2, 2012, which is hereby incorporated by reference in its entirety. The ribavirin can be administered with pegylated interferon after vaccination, before or concurrently therewith. Small molecule inhibitors may be administered individually or in combination. Small molecule inhibitors may be administered with, up to 7 days before, up to 7 days after, up to 14 days before, up to 14 days after, up to 21 days before,

up to 21 days after, up to 28 days before, or up to 28 days after administration of an immunogenic composition such as an immune composition comprising NS3/4A, HBcAg and IL-12 or an immunogenic composition comprising polynucleotides encoding NS3/4A, HBcAg and IL-12.

[0317] The immunogenic composition can be administered in coordination with a prime boost strategy. That is, the prime can be with the DNA immunogenic composition with IL-12 and the boost can be with an MVA (attenuated modified *vaccinia Ankara*) or VSV (vesticular stomatis virus) vector having a polynucleotide encoding NS3/4A (with or without HBcAg, NS5A, and/or IL-12).

[0318] Prime/boost strategies also can be used with the compositions and methods provided herein for improving immunity to viral pathogens. In particular, heterologous prime/boost strategies, wherein a DNA priming event is combined with a virus boost can enhance response levels in clinical studies against pathogens such as HIV or malaria (WO98/56919; Hanke et al., 1999, *Vaccine* 17, 589-596; Hill et al., 2000, *Dev. Biol.* 104, 171-179; Estcourt et al., 2002, *Int. Immunol.* 14, 31-37; McConkey et al., 2003, *Nat. Med.* 9, 729-35; McMichael and Hanke, 2003, *Nat. Med.* 9, 874-80; Robinson, 2003, *Nat. Med.* 9, 642-643; Moorthy et al., 2004, *PLOS Med.* 1, 128-136; Winstone, 2009, *Eur. J. Immunol.* 39, 975-85). Various prime boost strategies were investigated in chimpanzees with the goal of increasing HCV-specific immunity following challenge with infectious HCV viruses. Potent HCV specific peripheral and intrahepatic CD8⁺ T cell responses were induced by employing a complex immunization regimen involving administrations of two replication-defective Ad6 and Ad24 vectors expressing NS3-NS5B gene fusion followed by 3 intramuscular injections of a DNA plasmid encoding the same antigens and the clearance of HCV was observed in 4 of the 5 challenged chimpanzees (Fattori et al., 2006, *Gen. Ther.* 13, 1088-96). This tripartite vaccine is now being tested in healthy volunteers (Barnes et al., 2009, *Hepatology* 50:A198). In a separate study, chimpanzees were vaccinated with a DNA plasmid encoding HCV-core-E1-E2 and NS3 followed by booster immunizations with a recombinant MVA encoding the same antigens. All animals elicited Th1 and Th2 cytokine responses together with HCV-specific CD8⁺ T cell responses. Upon HCV challenge the DNA prime/MVA boost was associated with the control of HCV viremia in the acute phase but unfortunately did not protect against chronic infection (Rollier et al. 2007, *Hepatology* 45, 602-13).

[0319] In some embodiments the prime boost strategy comprises rodent HBcAg. In some embodiments the prime boost strategies include MVA vectors or VSV and IL-12.

Additionally, a prime boost strategy may comprise addition of a plasmid encoding a galactosyl transferase gene as an adjuvant. Prime boost strategies may comprise an immunization regimen involving administrations of two replication-defective Ad6 and Ad24 vectors expressing NS3-NS5B gene fusion followed by 3 intramuscular injections of a DNA plasmid encoding the same antigens (Fattori et al., 2006, Gen. Ther. 13, 1088-96). Prime boost strategies may comprise a DNA plasmid encoding HCV-core-E1-E2 and NS3 followed by booster immunizations with a recombinant MVA encoding the same antigens (Rollier et al. 2007, Hepatology 45, 602-13). Prime boost strategies may comprise subcutaneous injections (e.g. three) of a construct expressing HCV NS3, NS4 and NS5B genes at one week interval followed by a fourth SC injection 11 weeks after the last priming injection.

[0320] For example, an MVA prime boost strategy may comprise MVA administration at a dose of 10^7 pfu per mouse per injection by subcutaneous (SC) injection at the base of the tail. For example, Seven to 11 weeks-old HLA-A2.1 mice (2 to 4 mice per group) may be used in each experiment. The immunization regimen includes priming with 2 electroporated intramuscular (IM) injections of a codon optimized HCV NS3/4A gene sequence at 4 weeks interval and boosting with 3 sub-cutaneous (SC) injections of an attenuated modified *vaccinia Ankara* (MVA) expressing HCV NS3, NS4 and NS5B genes at one week interval given 5 or 12 weeks after the prime. Various controls are performed in parallel involving either vaccine alone, negative control including priming and boosting with the empty counterparts p-VAX1 and MVA N33, or an homologous prime boost involving 3 SC injections of an attenuated modified *vaccinia Ankara* (MVA) expressing HCV NS3, NS4 and NS5B genes at one week interval followed by a fourth SC injection of an attenuated modified *vaccinia Ankara* (MVA) expressing HCV NS3, NS4 and NS5B genes 11 weeks after the last priming injection. Blood samples are obtained by retro-orbital puncture. For detection of specific cellular immune responses, spleen and lymph nodes are collected from mice that are sacrificed 14 days after the last immunization.

[0321] Other prime boost strategies may be used, for example strategies that differ in the timing and mode of delivery rather than or in addition to differing in the immunogenic composition delivered. In some embodiments a strategy may be used involving a prime with DNA/IVIN/EP expressing HCV antigen(s) introduced intra muscularly (im) then boost with subcutaneous (sc) delivery. In some embodiments a strategy may be used involving a prime with DNA/IVIN/EP expressing HCV antigen(s) introduced subcutaneous (sc) then boost with intra muscularly (im) delivery. In some embodiments a strategy may be used involving a prime

with DNA/IVIN/EP expressing HCV antigen(s) im and then boost with a viral vector expressing HCV. In some embodiments a strategy may be used involving a prime with DNA/IVIN/EP expressing HCV antigen(s) sc and then boost with a viral vector expressing HCV. In some embodiments a strategy may be used involving a prime with a viral vector expressing HCV and then boost with DNA/IVIN/EP expressing HCV antigen(s) im. In some embodiments a strategy may be used involving a prime with a viral vector expressing HCV and then boost with DNA/IVIN/EP expressing HCV antigen(s) sc.

[0322] Interferon gamma (IFN γ) production is associated with the stimulation of the immune system to generate an immune response. Assays for IFN γ are well known proxies for *in vivo* immune response stimulation. See, e.g., United States Patent No. 7,122,633, to Trinchieri, et al., issued October 17, 2006, which is hereby incorporated by reference in its entirety.

[0323] In experiments described in further detail below, it was discovered that IVIN injection of a plasmid comprising a nucleic acid sequence encoding a human codon optimized NS3/4A gene into a rabbit tibialis anterior muscle improved *in vivo* transfection of muscle fibers that express the NS3-protein and generated an appreciable localized inflammatory response. An expression plasmid comprising a nucleotide sequence encoding human codon-optimized HCV NS3/4A was injected into rabbit tibialis anterior muscle. Five days after immunization, embedded sections of the muscle were generated and stained with labeled antibodies to NS3 and hematoxylin-eosin staining was used to detect inflammation. As shown in FIG. 63, it was found that conventional *i.m.* injection showed no or limited detectable NS3 expression and no or limited localized inflammation; whereas, IVIN injection showed appreciable NS3 expression and localized inflammation around the injection site. Accordingly, these experiments provided evidence that IVIN injection more efficiently delivers a DNA immunogen to muscle and more efficiently generates an inflammatory response at the site of injection than conventional *i.m.* injection of a DNA immunogen.

[0324] In more experiments described in further detail below, it was discovered that the use of IVIN in combination with electroporation efficiently primes T cell responses in wild-type mice. Differing amounts of an expression plasmid containing a nucleotide sequence encoding a human codon optimized NS3/4A gene fused to a human codon optimized stork HBcAg gene, having protease cleavage sites introduced therein, were administered wild-type mice using IVIN in combination with electroporation. Additionally, a plasmid encoding IL12 was administered to the mice by IVIN and electroporation. The proportion of IFN- γ SFCs per

million cells induced upon IVIN and electroporation administration was then determined using methods described in greater detail below. As shown in FIG. 64 A-J, appreciable T cell responses were observed.

[0325] Similar experiments were then conducted in NS3/4A transgenic mice. NS3/4A-Tg mice express NS3/4A and are immunotolerant to the NS3/4A epitopes making them useful as models of the immune systems of chronically infected HCV individuals. In these experiments it was discovered that the use of IVIN in combination with electroporation efficiently primes T cell responses in the NS3/4A transgenic mice. Differing amounts of an expression plasmid containing a nucleotide sequence encoding a human codon optimized NS3/4A gene fused to a human codon optimized stork HBcAg gene, having translation interruption sites or protease cleavage sites introduced therein, were administered to NS3/4A transgenic mice using IVIN in combination with electroporation. Additionally, a plasmid encoding IL12 was administered to the mice by IVIN and electroporation. The proportion of IFN- γ SFCs per million cells induced upon IVIN and electroporation administration was then determined. As shown in FIG. 65 A-J, appreciable T cell responses were observed providing evidence that IVIN in combination with electroporation can improve an immune response in an individual that is immunotolerant to the HCV NS3/4A (e.g. mimics an individual chronically infected with HCV).

[0326] In more experiments, it was discovered that by using IVIN in combination with electroporation an appreciable immune response to a DNA immunogen can be generated with amounts of a DNA immunogen that are significantly lower than that used in conventional injection followed by electroporation. 10-fold differing amounts of an expression plasmid containing a nucleotide sequence encoding a human codon optimized NS3/4A gene fused to a human codon optimized stork HBcAg gene, having protease cleavage sites introduced therein, were administered to mice and the IFN- γ SFCs per million cells induced upon IVIN and electroporation administration was then analyzed. As shown in FIG. 66 A-C, an appreciable immune response was generated when IVIN in combination with electroporation was used to deliver vanishingly small amounts of the expression plasmid, (levels of the expression plasmid recognized to be below the amount of expression plasmid required to generate an immune response using conventional injection in combination with electroporation). Accordingly, these results provide evidence that IVIN used with electroporation allows for the use of a lower dose of DNA immunogen, as compared to conventional injection followed by electroporation.

[0327] Pigs provide a model system that closely resembles humans and further experiments were conducted in this model system. A porcine IFN gamma ELISpot assay was used to evaluate the prime-boost immune activation resulting from administration of a DNA construct using IVIN in combination with electroporation. A human codon optimized NS3/4A-human codon optimized stork HBcAg polynucleotide immunogenic composition containing translation interruption sites introduced therein (2A sites) was administered to pigs using IVIN in combination with electroporation. The proportion of IFN- γ SFCs per million cells induced upon single or double (prime and prime/boost) IVIN and electroporation administrations into the dermis (Skin) or muscle (IM) of in pigs was then analyzed. As shown in FIG. 67 A-H, IVIN in combination with electroporation efficiently delivers a DNA immunogen to the pigs irrespective of whether the immunogen is delivered to the skin or muscle and thereby promotes the generation of a potent immune response to the immunogen. Additionally, it is shown that prime-boost administrations to either the skin or to the skin and muscle (prime to the muscle and boost to the skin) generated a significant immune response to the immunogen.

[0328] In more experiments, it was found that intramuscular administration of a DNA immunogen using the IVIN device, in combination with electroporation, induced a more robust immune response as compared to conventional intramuscular injection in combination with electroporation. In these experiments, an expression plasmid comprising a nucleotide sequence having a human codon optimized HBcAg gene was administered to animals using conventional intramuscular injection in combination with electroporation or IVIN in combination with electroporation. An expression plasmid comprising a nucleic acid encoding IL12 was also provided to the same animals using conventional intramuscular injection in combination with electroporation or IVIN in combination with electroporation. The proportion of IFN- γ SFCs per million cells induced was then monitored and recorded. As shown in FIG. 68 A-B, the combination of IVIN and electroporation was vastly superior at inducing an immune response in the animals, as compared to using conventional intramuscular injection in combination with electroporation. These results provide further evidence of the surprising improvement of using IVIN and electroporation to deliver a DNA immunogen. In more experiments, using a conventional needle followed by electroporation or IVIN followed by electroporation, subjects were immunized with a construct comprising a human codon optimized HBcAg polynucleotide immunogenic composition and said subjects were provided mIL-12. FIG. 69 illustrates the percent of HBcAg-pentamer-specific CD8⁺ T cells among untreated, conventional needle/electroporated or IVIN/electroporated subjects. The data demonstrate that

IVIN significantly improve the number of HBcAg specific CD8⁺ T cells as compared to conventional needle injection.

[0329] Delivering or transfecting pharmaceutical drugs, proteins, and DNA into skeletal muscle and other cells residing within the skeletal muscle is a desired outcome of the injection of immunogenic compositions. Electroporation works on the principle that a cell acts as an electrical capacitor and is generally unable to pass current. Subjecting cells to a high-voltage electric field, therefore, creates transient permeable structures or micropores in the cell membrane. These pores are large enough to allow pharmaceutical drugs, DNA, and other polar compounds to gain access to the interior of the cell. With time, the pores in the cell membrane close and the cell once again becomes impermeable. The increase in DNA transfection efficiency is observed only if the muscle is electrically stimulated immediately, or shortly after the DNA injection. Thus, the semipermeable quality of the tissue induced by the stimulation is reversible. Moreover, it is dependent on current through the muscle; activity induced through the nerve does not affect transfection efficiency.

[0330] Once transfected, the muscle cells are able to express the proteins coded by the nucleic acid. Therefore, the transfection method of the present invention can be used, for example, to transfect expression vectors for genetic immunization (i.e., DNA vaccines). See, e.g., United States Patent No. 7,931,640, to Mathiesen, et al., issued April 26, 2011, which is hereby incorporated by reference in its entirety.

[0331] In some embodiments transfection is aided by the inclusion of one or more polynucleotide stabilizer molecules, such as molecules that innervate with polynucleotide helices to improve delivery into target tissue. In some embodiments the stabilizer is bipuvicane.

[0332] Several studies now show that the control of an infection caused by HCV either naturally (spontaneous resolution), or after treatment (therapeutic resolution) is associated with the induction or potentialization of cell-mediated immune responses involving the T-CD4⁺ and T-CD8⁺ lymphocytes (as described for example in LECHNER, F. et al., Eur. J. Immunol., 30: 2479-2487 (2000) and in Thimme R. et al., 2001, J. Exp. Med., 194 (10): 1395-1406).

[0333] Some embodiments relate to product combinations for use in improving an immune response comprising an electroporation device that comprises a plurality of needles each having a closed end and a plurality of apertures along the barrel of said needles, wherein the apertures on separate needles oppose one other but said apertures do not face away from each other, so as to create a zone of injection existing in a region of tissue between the opposing apertures of said plurality of needles, wherein at least two of said plurality of needles are capable

of passing current so as to enhance delivery of a nucleic acid within said zone of injection; and a nucleic acid encoding an antigen; and wherein said product combination improves an immune response in a subject as compared to an immune response resulting from delivery of said nucleic acid using a conventional injection device in combination with electroporation. In some aspects the nucleic acid comprises at least one polynucleotide encoding a first polypeptide. In some aspects the nucleic acid comprises at least one polynucleotide encoding a second polypeptide. In some aspects the nucleic acid comprises at least one polynucleotide encoding IL-12. In some aspects the IL-12 is human IL-12. In some aspects the nucleic acid comprises at least one polynucleotide encoding HCV NS3/4A. In some aspects the nucleic acid comprises at least one polynucleotide encoding HCV NS5A. In some aspects the nucleic acid comprises at least one polynucleotide encoding and HBcAg. In some aspects the HBcAg is isolated from an HBV that infects stork or heron. In some aspects the nucleic acid comprising said first polynucleotide encoding said first polypeptide and comprising said second polynucleotide encoding said second polypeptide are separated by a polynucleotide encoding a translation interruption sequence. In some aspects the polynucleotide encoding a translation interruption sequence comprises a T2A sequence. In some aspects the at least one polynucleic acid is codon optimized for expression in humans. In some aspects the immune response is a mammalian immune response. In some aspects the immune response is a human immune response. In some aspects the immune response is an HCV immune response. In some aspects the immune response is in an individual chronically infected with HCV. In some aspects the nucleic acid is provided in an amount insufficient to elicit an immune response when delivered intramuscularly in the absence of electroporation, and wherein said amount is sufficient to elicit an immune response. In some aspects the immune response is 2x stronger than an immune response observed in the absence of electroporation. In some aspects the immune response is comparable to an immune response observed upon administration of a greater amount of said nucleic acid to said zone of injection in the absence of electroporation. In some aspects the immune response is comparable to an immune response observed upon administration of twice as much of said nucleic acid to said zone of injection in the absence of electroporation.

[0334] Some embodiments comprise an immunogenic composition comprising (a) a polynucleotide encoding a hepatitis C virus NS3/4A polypeptide and (b) an IL-12 polypeptide or a polynucleotide encoding an IL-12 polypeptide. Some embodiments further comprise (c) an HBcAg polypeptide or a polynucleotide encoding an HBcAg polypeptide. In some embodiments the immunogenic composition comprises a polynucleotide encoding an IL-12

polypeptide. In some embodiments the polynucleotide encoding a hepatitis C virus NS3/4A polypeptide and the polynucleotide encoding an IL-12 polypeptide are present in the same vector. In some embodiments the polynucleotide encoding a hepatitis C virus NS3/4A polypeptide and the polynucleotide encoding an IL-12 polypeptide are present in different vectors. In some embodiments the immunogenic composition comprises a polynucleotide encoding an HBcAg polypeptide. In some embodiments the polynucleotide encoding a hepatitis C virus NS3/4A polypeptide and the polynucleotide encoding an HBcAg polypeptide are present in the same vector. In some embodiments the polynucleotide encoding a hepatitis C virus NS3/4A polypeptide and the polynucleotide encoding an HBcAg polypeptide are present in different vectors. Some embodiments further comprise an attenuated modified *vaccinia Ankara*. Some embodiments further comprise a vesicular stomatitis virus (VSV). Some embodiments further comprise a polynucleotide encoding a rodent HBcAg. Some embodiments further comprise a replication defective Ad6 vector expressing a NS3/NS4A, NS3/4A/5A, NS3/4A/NS5B, or NS3/4A/5A/5B gene fusion. Some embodiments further comprise a replication defective Ad24 vector expressing a NS3/NS4A, NS3/4A/5A, NS3/4A/NS5B, or NS3/4A/5A/5B gene fusion. In some embodiments the immunogenic composition induces production of NS3-specific T cells. In some embodiments the immunogenic composition increases production of NS3-specific T cells relative to a comparative composition comprising the same components as said immunogenic composition except for both lacking said IL-12 polypeptide and lacking said polynucleotide encoding an IL-12 polypeptide. In some embodiments the immunogenic composition induces production of NS3-specific T cells at twice the level relative to a comparative composition comprising the same components as said immunogenic composition except for both lacking said IL-12 polypeptide and lacking said polynucleotide encoding an IL-12 polypeptide. In some embodiments the immunogenic composition induces production of NS3-specific T cells at fourfold the level relative to a comparative composition comprising the same components as said immunogenic composition except for both lacking said IL-12 polypeptide and lacking said polynucleotide encoding an IL-12 polypeptide. In some embodiments the immunogenic composition is characterized as increasing by at least twentyfold the number of splenocytes expressing INF γ upon administration of said immunogenic composition relative to a control immunogenic composition lacking an HCV antigen. In some embodiments the immunogenic composition is characterized as producing at least 10^2 INF γ -expressing splenocytes per million cells upon administration of said immunogenic composition more than a control immunogenic composition lacking an HCV

antigen. In some embodiments the immunogenic composition is characterized as producing at least 5×10^2 INF γ -expressing splenocytes per million cells upon administration of said immunogenic composition more than a control immunogenic composition lacking an HCV antigen. In some embodiments the immunogenic composition is characterized as producing at least 10^3 INF γ -expressing splenocytes per million cells upon administration of said immunogenic composition more than a control immunogenic composition lacking an HCV antigen. In some embodiments the immunogenic composition is characterized as producing at least 4×10^3 INF γ -expressing splenocytes per million cells upon administration of said immunogenic composition more than a control immunogenic composition lacking an HCV antigen. In some embodiments the immunogenic composition induces an NS3-specific T cell response in a patient that is chronically infected with HCV. In some embodiments the immunogenic composition is characterized as inducing an NS3-specific T cell response in a patient that is chronically infected with HCV. In some embodiments the immunogenic composition is present in an injection device or an injection device configured to be used in conjunction with an electroporation device. Some embodiments comprise a method of generating an immune response in a subject, comprising delivering to an intracellular space, such as a muscle cell, of said subject the immunogenic composition of any of the above-mentioned embodiments.

[0335] Some embodiments comprise a method of generating an immune response in a subject, comprising delivering to an intracellular space, such as a plurality of muscle cells, of said subject a polynucleotide encoding an HCV NS3/NS4A, NS3/4A/5A, NS3/4A/NS5B, or NS3/4A/5A/5B polypeptide, and delivering to said animal an IL-12 polypeptide and/or a polynucleotide encoding an IL-12 polypeptide. In some embodiments the IL-12 polypeptide is encoded by a polynucleotide, and wherein said polynucleotide is delivered to an intracellular space of said animal, such as a plurality of muscle cells or intradermally. Some embodiments further comprise delivering to an intracellular space such as a plurality of muscle cells or intradermally of said subject an HBcAg polypeptide or a polynucleotide encoding an HBcAg. In some embodiments the HBcAg polypeptide is encoded by a polynucleotide, and wherein said polynucleotide is delivered to an intracellular space such as a plurality of muscle cells or intradermally of said animal and translated into an HBcAg polypeptide therein, thereby delivering said HBcAg polypeptide to said subject. In some embodiments the components of said immunogenic composition are delivered in a single injection. In some embodiments the components of said immunogenic composition are delivered in two or more injections. Some

embodiments further comprise providing ribavirin to said subject. Some embodiments further comprise providing pegylated interferon to said subject. In some embodiments the pegylated interferon is pegylated interferon $\alpha 2a$. Some embodiments further comprise the steps of injecting said polynucleotide encoding an HCV NS3/4A polypeptide into said subject and subjecting a vicinity of said injected polynucleotide encoding an HCV NS3/4A polypeptide to a treatment comprising electroporation. In some embodiments the delivery comprises injecting said immunogenic composition into a subject. Some embodiments further comprise subjecting the vicinity of said site of injection of said immunogenic composition to electroporation. In some embodiments the injection is effected using an IVIN injection device. Some embodiments further comprise administering a boost vaccination to said subject. In some embodiments the boost vaccination comprises an attenuated modified *vaccinia Ankara*. In some embodiments the boost vaccination comprises a vesicular stomatitis virus. In some embodiments the boost vaccination comprises a rodent HBcAg polynucleotide. In some embodiments the boost vaccination comprises a replication defective Ad6 vector expressing a NS3-NS5B gene fusion. In some embodiments the boost vaccination comprises a replication defective Ad24 vector expressing a NS3-NS5B gene fusion. In some embodiments the boost vaccination is administered within 28 days of the administration of said polynucleotide encoding an HCV NS3/4A polypeptide. In some embodiments the boost vaccination is administered within 14 days of the administration of said polynucleotide encoding an HCV NS3/4A polypeptide. In some embodiments the boost vaccination is administered within 7 days of the administration of said polynucleotide encoding an HCV NS3/4A polypeptide.

[0336] Some embodiments comprise an apparatus for intracellular delivery in a living animal by overloading a tissue locally comprising: a delivery unit, which controls an injection parameter; a hub connected to the delivery unit and comprising at least one electrical connector; a plurality of needles connected to the hub, wherein each needle of the plurality of needles comprises: a closed end; a needle barrel; a plurality of apertures that are disposed on the needle barrel, wherein the apertures on each needle barrel oppose the apertures on at least one other needle barrel so as to generate an opposing direction of delivery of a delivered agent or a cross-spray pattern of delivery of a delivered agent; an electrode; an electrical power supply configured to generate an electric field at the electrode, wherein the electrical power supply is connected to the electrode through the at least one electrical connector; and further comprising one or more of the immunogenic compositions above. In some embodiments at least one needle of the plurality of needles is a needle-electrode, which is electrically connected to the electrical

power supply and is configured to generate an electric field. In some embodiments the intracellular delivery apparatus comprises a plurality of needle-electrodes, which are electrically connected to the electrical power supply, and configured to generate an electric field. In some embodiments the intracellular delivery apparatus comprises four needles arranged in a Y-type pattern with a center needle and three outer needles disposed around the center needle, wherein the center needle is a needle-electrode configured to apply a first polarity voltage, and the three outer needles are needle-electrodes, configured to apply a second polarity voltage. In some embodiments the center needle comprises apertures along the needle barrel, which are positioned to direct delivery of a delivered agent toward apertures that are present on each of the three outer needles and each of the three outer needles comprises apertures along the needle barrel, which are positioned to direct delivery of a delivered agent toward apertures that are present on the center needle, and wherein the outer needles do not have apertures positioned to deliver a delivered agent away from the center needle. In some embodiments the intracellular delivery apparatus comprises an array of needles comprising a center needle and a plurality of outer needles disposed around the center needle, wherein the center needle is a needle-electrode configured to apply a first polarity voltage, and the outer needles are needle-electrodes, configured to apply a second polarity voltage, wherein the center needle comprises apertures along the needle barrel, which are positioned to direct delivery of a delivered agent toward the outer needles and the outer needles comprise apertures along the needle barrel, which are positioned to direct delivery of a delivered agent toward the center needle and/or an adjacent needle, and wherein the outer needles do not have apertures that allow delivery of a delivered agent away from an active zone defined by an area within the array of needles. In some embodiments the intracellular delivery apparatus further comprises seven needles that are arranged in a hexagonal pattern, wherein one needle, which is a needle-electrode, located at the center of the hexagonal pattern.

[0337] Some embodiments comprise a syringe in fluid communication with the hub; a channel configured to receive the syringe; a collar operable to fasten the syringe within the channel; a handle, which operates to enclose the syringe and hub within the channel such that the closed ends of the needle barrels protrude from the channel and are available to engage a subject; a charging element configured to be charged by the operation of the handle, the charging element coupled to a trigger and the syringe such that operation of the trigger releases the charging element and the charging element acts on the syringe, thereby displacing prophylactic and/or therapeutic material out of the syringe; and an electrical port configured to

mate with the electrical connector on the hub of the hypodermic needle device thereby establishing electrical contact between the electrical power supply and the electrodes and/or needle-electrodes. In some embodiments the hub is a pocket hub, comprising an individual reservoir of a delivered agent for each needle or needle electrode. In some embodiments the hub comprises a single reservoir of a delivered agent for each needle or needle-electrode. In some embodiments at least one needle of the plurality of needles is at least partially laminated with an electrically non-conductive material. Some embodiments further comprise an electrical power supply controller configured to control generation of the electric field. In some embodiments controlling generation of the electric field comprises controlling one or more of the pulse voltage, polarity of the needle-electrodes, the number of pulses, the pulse pattern, or the pulse duration. In some embodiments the charging element is a spring configured to be compressed by the operation of the handle and configured to decompress upon operation of the trigger or when activated by an electrically operated transducer. In some embodiments the electric field applied at the electrode and/or needle electrodes is commutating.

[0338] Some embodiments comprise A method of introducing a delivered agent into a tissue of a subject comprising: providing the intracellular delivery apparatus of any one of Claims 49 to 63; providing a voltage source; inserting a plurality of needles and a plurality of electrodes or a plurality of needle-electrodes of said electroporation apparatus into a tissue of a subject; displacing the delivered agent through the lumen of the plurality of needles or the plurality of needle electrodes and into the tissue of the subject; and applying an electric field to the tissue of the subject; wherein the delivered agent comprises one or more of the immunogenic compositions above. In some embodiments the displacing of the delivered agent is performed prior to applying the electric field to the tissue. In some embodiments the displacing of the delivered agent is performed subsequent to applying the electric field to the tissue. In some embodiments the displacing of the delivered agent is performed in a two stage process, wherein the first stage comprises displacing the delivered agent into the tissue into an active area and the second stage comprises energizing one or more electrodes or needle electrodes to electroporate the cells within the active area. In some embodiments the injection parameter is injection speed. In some embodiments the injection parameter is injection pressure. In some embodiments the delivery apparatus is for use in DNA vaccination.

[0339] Some embodiments comprise a product combination comprising an electroporation device that comprises a plurality of needles each having a closed end and a plurality of apertures along the barrel of said needles, wherein the apertures on separate needles

oppose one other but said apertures do not face away from each other so as to create a zone of injection existing in a region of tissue between the opposing apertures of said plurality of needles, wherein at least two of said plurality of needles are capable of passing current so as to enhance delivery of a nucleic acid within said zone of injection, wherein said nucleic acid comprises at least one plasmid that comprises one or more polynucleotides encoding HCV NS3/4A and, optionally HCV NS5A, and HBcAg, optionally an HBcAg that infects a stork or heron, and IL-12, optionally a human IL-12, wherein said one or more polynucleotides are, optionally codon-optimized for human expression and wherein said product combination is used for treatment of HCV in an HCV infected or chronically HCV infected patient.

[0340] Some embodiments comprise a method of reducing an amount of an immunogenic composition necessary to elicit an immune response in a mammalian subject comprising the steps of: injecting an immunogenic composition into said subject at an injection site, wherein said immunogenic composition is injected in an amount insufficient to elicit an immune response in the absence of further treatment; and electroporating said subject at said injection site; thereby eliciting an immunogenic response. In some embodiments the immunogenic composition comprises a polynucleic acid. In some embodiments the polynucleic acid encodes an HCV polypeptide. In some embodiments the polynucleotide encodes an HCV NS3/NS4A, NS3/4A/5A, NS3/4A/NS5B, or NS3/4A/5A/5B polypeptide. In some embodiments the polynucleotide encodes an HBcAg polypeptide. In some embodiments the polynucleotide encodes a polypeptide cleavage site. In some embodiments the cleavage site is a protease cleavage site. In some embodiments the cleavage site is a translation interruption site. In some embodiments the translation interruption site is a 2A ribosomal polypeptide translation interruption site.

[0341] Some embodiments comprise a method of generating an immune response to an antigen in a subject comprising: administering to a subject by the apparatus disclosed herein, a nucleic acid encoding an antigen, wherein the amount of nucleic acid encoding the antigen that is administered to said subject by said apparatus is lower than the amount of nucleic acid encoding the antigen that is required to obtain an equivalent immune response in a subject when said nucleic acid encoding said antigen is administered by injection with a needle having a single aperture, such as a conventional hypodermic needle, and electroporation. In some embodiments the polynucleic acid encodes an HCV polypeptide. In some embodiments the polynucleotide encodes an HCV NS3/NS4A, NS3/4A/5A, NS3/4A/NS5B, or NS3/4A/5A/5B polypeptide. In some embodiments the polynucleotide encodes an HBcAg polypeptide. In

some embodiments the polynucleotide encodes a polypeptide cleavage site. In some embodiments the cleavage site is a protease cleavage site. In some embodiments the cleavage site is a translation interruption site. In some embodiments the translation interruption site is a 2A ribosomal polypeptide translation interruption site.

[0342] Some embodiments comprise a method of generating an immune response to an antigen in a subject comprising: administering to a subject by the apparatus disclosed herein, an amount of a nucleic acid encoding an antigen that is insufficient to generate an immune response in a subject by injection with a needle having a single aperture, such as a conventional hypodermic needle, and electroporation. In some embodiments the polynucleic acid encodes an HCV polypeptide. In some embodiments the polynucleotide encodes an HCV NS3/NS4A, NS3/4A/5A, NS3/4A/NS5B, or NS3/4A/5A/5B polypeptide. In some embodiments the polynucleotide encodes an HBcAg polypeptide. In some embodiments the polynucleotide encodes a polypeptide cleavage site. In some embodiments the cleavage site is a protease cleavage site. In some embodiments the cleavage site is a translation interruption site. In some embodiments the translation interruption site is a 2A ribosomal polypeptide translation interruption site. In some embodiments the apparatus of any one of the above-mentioned embodiments is for use in administering an amount of a nucleic acid encoding an antigen that is lower than the amount of nucleic acid encoding the antigen that is required to obtain an equivalent immune response in a subject when said nucleic acid encoding said antigen is administered by injection with a needle having a single aperture, such as a conventional hypodermic needle, and electroporation. In some embodiments the polynucleic acid encodes an HCV polypeptide. In some embodiments the polynucleotide encodes an HCV NS3/NS4A, NS3/4A/5A, NS3/4A/NS5B, or NS3/4A/5A/5B polypeptide. In some embodiments the polynucleotide encodes an HBcAg polypeptide. In some embodiments the polynucleotide encodes a polypeptide cleavage site. In some embodiments the cleavage site is a protease cleavage site. In some embodiments the cleavage site is a translation interruption site. In some embodiments the translation interruption site is a 2A ribosomal polypeptide translation interruption site.

[0343] In some embodiments the apparatus of any one of the above-mentioned embodiments is for use in administering an amount of a nucleic acid encoding an antigen that is insufficient to generate an immune response in a subject by injection with a needle having a single aperture, such as a conventional hypodermic needle, and electroporation. In some embodiments the polynucleic acid encodes an HCV polypeptide. In some embodiments the

polynucleotide encodes an HCV NS3/NS4A, NS3/4A/5A, NS3/4A/NS5B, or NS3/4A/5A/5B polypeptide. In some embodiments the polynucleotide encodes an HBcAg polypeptide. In some embodiments the polynucleotide encodes a polypeptide cleavage site. In some embodiments the cleavage site is a protease cleavage site. In some embodiments the cleavage site is a translation interruption site. In some embodiments the translation interruption site is a 2A ribosomal polypeptide translation interruption site.

[0344] In some embodiments any of the apparatus embodiments above is used to deliver an amount of a nucleic acid encoding an antigen that is lower than the amount of nucleic acid encoding the antigen that is required to obtain an equivalent immune response in a subject when said nucleic acid encoding said antigen is administered by injection with a needle having a single aperture, such as a conventional hypodermic needle, and electroporation. In some embodiments the polynucleic acid encodes an HCV polypeptide. In some embodiments the polynucleotide encodes an HCV NS3/NS4A, NS3/4A/5A, NS3/4A/NS5B, or NS3/4A/5A/5B polypeptide. In some embodiments the polynucleotide encodes an HBcAg polypeptide. In some embodiments the polynucleotide encodes a polypeptide cleavage site. In some embodiments the cleavage site is a protease cleavage site. In some embodiments the cleavage site is a translation interruption site. In some embodiments the translation interruption site is a 2A ribosomal polypeptide translation interruption site.

[0345] In some embodiments any of the apparatus embodiments above is used to deliver an amount of a nucleic acid encoding an antigen that is insufficient to generate an immune response in a subject by injection with a needle having a single aperture, such as a conventional hypodermic needle, and electroporation. In some embodiments the polynucleic acid encodes an HCV polypeptide. In some embodiments the polynucleotide encodes an HCV NS3/NS4A, NS3/4A/5A, NS3/4A/NS5B, or NS3/4A/5A/5B polypeptide. In some embodiments the polynucleotide encodes an HBcAg polypeptide. In some embodiments the polynucleotide encodes a polypeptide cleavage site. In some embodiments the cleavage site is a protease cleavage site. In some embodiments the cleavage site is a translation interruption site. In some embodiments the translation interruption site is a 2A ribosomal polypeptide translation interruption site.

[0346] Some embodiments comprise an improved method of delivering an immunogenic composition comprising a nucleic acid encoding an antigen, wherein the improvement comprises delivering an amount of a nucleic acid encoding an antigen that is lower than the amount of nucleic acid encoding the antigen that is required to obtain an equivalent

immune response in a subject when said nucleic acid encoding said antigen is administered by injection with a needle having a single aperture, such as a conventional hypodermic needle, and electroporation. In some embodiments the polynucleic acid encodes an HCV polypeptide. In some embodiments the polynucleotide encodes an HCV NS3/NS4A, NS3/4A/5A, NS3/4A/NS5B, or NS3/4A/5A/5B polypeptide. In some embodiments the polynucleotide encodes an HBcAg polypeptide. In some embodiments the polynucleotide encodes a polypeptide cleavage site. In some embodiments the cleavage site is a protease cleavage site. In some embodiments the cleavage site is a translation interruption site. In some embodiments the translation interruption site is a 2A ribosomal polypeptide translation interruption site.

[0347] Some embodiments comprise an improved method of delivering an immunogenic composition comprising a nucleic acid encoding an antigen, wherein the improvement comprises delivering an amount of a nucleic acid encoding an antigen that is insufficient to generate an immune response in a subject by injection with a needle having a single aperture, such as a conventional hypodermic needle, and electroporation by the electroporation apparatus, wherein the improvement comprises elicitation of an immune response. In some embodiments the polynucleic acid encodes an HCV polypeptide. In some embodiments the polynucleotide encodes an HCV NS3/NS4A, NS3/4A/5A, NS3/4A/NS5B, or NS3/4A/5A/5B polypeptide. In some embodiments the polynucleotide encodes an HBcAg polypeptide. In some embodiments the polynucleotide encodes a polypeptide cleavage site. In some embodiments the cleavage site is a protease cleavage site. In some embodiments the cleavage site is a translation interruption site. In some embodiments the translation interruption site is a 2A ribosomal polypeptide translation interruption site.

[0348] The following examples are given to illustrate various embodiments of the present invention in the field of DNA immunization, which can be delivered to a subject in need of an immune response to the antigen contained therein. It is to be understood that the following examples are not comprehensive or exhaustive of the many types of embodiments which can be prepared in accordance with the present invention.

EXAMPLE 1

[0349] New Zealand white rabbits weighing 3.5 Kg were injected with a solution containing 0.3 ml 0.9% NaCl containing 0.9 mg of either ChronVac-C (coNS3/4A DNA) or coHBcAg in the tibialis anterior using either a large high injection pressure (HIP) injector (also

referred to herein as “IVIN”), a small HIP injector, or a regular 27 gauge needle. Rabbits were injected either in the right tibialis (TA) anterior, left tibialis anterior (TA), or both.

[0350] As depicted in FIG. 40A, the small HIP injector has needles 4-5 mm in length. The small HIP injector has 4 needles. As depicted in the figure, the three outer needles are oriented in a triangular formation, equally spaced with approximately 3 mm between each needle to form an equilateral triangle. The center needle is placed in the middle of the triangle formed by the three outer needles. Each needle has 6 apertures. The outer needles all have apertures opening to the center and the center needle has apertures opening at four directions at 90 degree angles. The large HIP injector, as depicted in FIG. 40B has needles 8-9 mm in length. The large HIP injector has 4 needles oriented in a triangular formation, equally spaced with 6 mm between each needle. The center needle is placed in the middle of the equilateral triangle formed by the three outer needles. Each needle of the large HIP injector has 10 apertures. The outer needles all have apertures opening to the center and the center needle has apertures opening at four directions at 90 degree angles. The injection scheme is shown in table 1 below.

TABLE 1

Rabbit #	Needle Type	Injection Site	Plasmid	Dose	Sacrificed
115	HIP-large	Right TA	coNS3/4A	0.9 mg/0.3ml	Day 5
	Regular Needle	Left TA	coNS3/4A	0.9 mg/0.3ml	
116	HIP-large	Right TA	coNS3/4A	0.9 mg/0.3ml	Day 5
	Regular Needle	Left TA	coNS3/4A	0.9 mg/0.3ml	
117	HIP-small	Right TA	coNS3/4A	0.9 mg/0.3ml	Day 5
	None	--	--	--	
118	HIP-small	Right TA	coNS3/4A	0.9 mg/0.3 ml	Day 5
	None	--	--	--	
119	HIP-large	Right TA	coNS3/4A	0.9 mg/0.3 ml	Day 10
	HIP-large	Left TA	coHBcAg	0.9 mg/0.3 ml	
120	HIP-large	Right TA	coNS3/4A	0.9 mg/0.3 ml	Day 10
	HIP-large	Left TA	coHBcAg	0.9 mg/0.3 ml	
121	Regular	Right TA	coNS3/4A	0.9 mg/0.3 ml	Day 10
	Regular	Left TA	coHBcAg	0.9 mg/0.3 ml	
122	None	--	--	--	Day 10

Rabbit #	Needle Type	Injection Site	Plasmid	Dose	Sacrificed
	none	--	--	--	

[0351] At day 5, rabbits 115-118 were sacrificed and peripheral blood mononuclear cells (PBMCs) were analyzed for T cell proliferation. The PBMCs were assayed for *in-vitro* proliferative recall responses using a standard 96h proliferation assay. (See Lazinda et al., J. Gen. Virol. 82:1299-1308 (2001), herein expressly incorporated by reference in its entirety.) In brief, microtiter plates were seeded with approximately 200,000 cells/well and the cells were incubated with media alone, recombinant NS3 or HBcAg. PBMCs were also incubated with Concanavalin A (ConA) as a positive control. After 72 hours, radioactive thymidine was added and 16-24 hours later the cells were harvested. The radioactivity of the cells as counts per minute are depicted in FIG. 41 and listed in TABLE 2. The proliferation was determined as radioactivity of the cells as the counts per minute (cpm) of cells incubated with the antigen divided by the CPM of the cells incubated with the media alone (sample to negative ration; S/N). The results are shown in FIG. 42.

TABLE 2

Rabbit	5 µg Con-A	media	1 µg NS3	0.1 µg NS3	0.01 µg NS3	1 µg HBcAg
115	14792	958	8570	14141	6816	Not tested
116	172935	406	21595	22360	Not tested	Not tested
117	71133	3632	7465	8625	10658	Not tested
118	32152	7632	3705	11152	7724	Not tested
119/120	67470	191	717	Not tested	Not tested	6838

[0352] The results show that rabbits immunized with the large HIP injector show a more robust immune response displayed through greater T cell proliferation than rabbits immunized with the small HIP injector. The data also provide strong evidence that the DNA that was introduced into the muscle tissue by the HIP injectors was effectively transferred into the cell, wherein it was transcribed, translated, and was used by the immune system of the animal to generate a potent immune response. Both the DNA encoding the HCV antigen NS3/4A and the DNA encoding the HBV antigen HBcAg effectively generated a potent immune response in mammals demonstrating that a variety of DNAs that encode immunogens can be

effectively introduced into mammals using a delivery device described herein to induce an immune response in the inoculated animal.

[0353] The injection site for each rabbit was also collected for histological evaluation (as described in Ahlen et al., *In Vivo* Electroporation Enhances the Immunogenicity of Hepatitis C Virus Nonstructural 3/4A DNA by Increased Local DNA Uptake, Protein Expression, Inflammation and Infiltration of CD3⁺ T Cells. *J. Immunol.* 2007 179(7):4741-53, herein incorporated by reference in its entirety). Briefly, the tissue was fixed in a buffered 4% formaldehyde solution, dehydrated, and embedded in paraffin. The embedded tissues were sectioned in 4-6 μ m sections. The sections were mounted onto glass slides and stained with hematoxylin and eosin stain (H&E), or polyclonal mouse sera from a coNS3/4A DNA-immunized mouse, which was detected by a biotinylated goat anti-mouse secondary antibody and peroxidase labeled streptavidin using an insoluble peroxidase substrate.

[0354] The results are shown in FIG. 43A-C. The injection of 0.9 mg of coNS3/4A with both HIP injectors produced significant amounts of local inflammation, regeneration, and fibrosis, as indicated by the high concentration of stained immune cells that localized to the injection site, in particular, between the needles. The data show that the large injector produced a better inflammatory response than the small injector in the rabbits. The injection of 0.9 mg of coNS3/4A with the conventional 27 gauge needle caused very little local inflammation, regeneration, and fibrosis, as indicated by the almost absent stained immune cells localized to the injection site. Additionally, both the HIP injectors induced the cells surrounding the injection site to produce significant amounts of NS3 protein, as indicated by the antibody labeling; whereas, the conventional injection with the 27 gauge needle under these conditions produced no detectable NS3 protein. Accordingly, the data show that the HIP injectors effectively delivered DNA into the cells, wherein it was transcribed and translated in significant amounts, which could be detected by an antibody specific for NS3 but the conventional injection with the 27 gauge needle did not.

[0355] The results provided in this example demonstrate that the HIP injectors described herein effectively deliver an expression plasmid that encodes an antigen into a cell of a subject in quantities sufficient to allow for a level of protein expression that is detectable by an antibody directed to the antigen and in an amount that is sufficient to generate appreciable amounts of antigen-specific T cells. That is, the data show that the HIP injectors described herein effectively deliver nucleic acids to cells of the body in an amount sufficient to produce a

potent immune response in the subject. Thus, injecting a DNA vaccine using the HIP injector improves the immune response relative to standard methods of delivering vaccines.

EXAMPLE 2

[0356] The mechanisms by which a high injection pressure (HIP) needle (also referred to herein as “IVIN”) improves the potency of intramuscular DNA vaccination are characterized by using the hepatitis C virus nonstructural (NS) 3/4A gene. Sustained control and clearance of HCV infection is related to an effective immune response, in particular a T cell response targeted to the nonstructural NS3 protein. By activating T cells outside the liver via vaccination, one may allow for the complementing or reshaping of the existing T cell repertoire. The present NS3/4A plasmid-based vaccine example is tested in mice. *In vivo* HIP needle administered vaccine is contemplated to increase the permeability of myocyte cell members, wherein the plasmid is effectively taken up in the nucleus and expressed, thereby inducing a functional *in vivo* immune response. The use of an *in vivo* HIP needle enhances the immunogenicity of coNS3/4A by both increasing protein expression levels and the duration of expression and by enhancing the infiltration of CD3+ T cells and a local inflammatory response at the site of injection.

[0357] Male and female C57BL/6 mice are bred and caged at five mice per cage. The mice are fed a commercial diet (RM3 (p) PL IRR diet; Special Diet Service) with free access to food and water. All animals are at least 6 weeks of age before start of the experiment. The SV40-luciferase plasmid (pGL4.13-[Luc2-SV40]; Promega) is produced in-house by standard technologies. The coNS3/4A plasmid is produced under Good Manufacturing Practice regulations.

[0358] The coNS3/4A DNA vaccine is administered by a single intramuscular injection (0.05 ml in mice) with a two-barrel 27-gauge HIP needle into the right tibialis anterior (TA) muscle. Doses range from 0.5 to 50 µg of DNA in mice. One two-barrel needle is used per injection and per animal. The procedure is repeated up to three times in mice at monthly intervals.

[0359] Detection of mouse antibody to NS3 by enzyme immunoassay is performed using standard immunoassay techniques. Antibodies titers are determined as the last serum dilution giving an OD at 405 nm of three times the OD at the same dilution of a non-immunized animal serum. With respect to NS3 antibody levels, a dose-response relationship is seen after vaccination with different doses of coNS3/4A-DNA administered with or without using the HIP

needle. The boost effect is seen after immunization. The lower dose given with the HIP needle induces the same mean NS3-specific antibody levels as a greater dose delivered without the HIP needle. In conclusion, the HIP needle makes the coNS3/4A DNA-based immunization more effective with respect to antibody responses, supporting the benefits of the adjuvant effects mediated by using a HIP needle.

EXAMPLE 3

[0360] New Zealand White rabbits weighing 2.5–3.5 kg, are purchased from commercial vendors. The coNS3/4A DNA vaccine is administered by a single intramuscular injection with a four-barrel 27-gauge HIP needle into the right tibialis anterior (TA) muscle. Doses range from 70 to 700 µg of DNA. One four-barrel needle is used per injection and per animal. The procedure is repeated up to five times in rabbits at monthly intervals.

[0361] Detection of rabbit antibodies to NS3 by enzyme immunoassay is performed using standard immunoassay techniques. Antibodies titers are determined as the last serum dilution giving an OD at 405 nm of three times the OD at the same dilution of a non-immunized animal serum.

[0362] Proliferative responses to NS3 are determined in rabbit whole blood. A total of 4 ml of whole blood is obtained from the ear artery of each rabbit immediately before the first vaccination and 2 weeks after each vaccination and collected in heparin tubes. Plasma and peripheral mononuclear cells (PMBC) are isolated by gradient centrifugation. Plasma is stored at -80° C until the analysis of NS3-specific antibody by enzyme immunoassay. PBMCs are immediately assayed for *in vitro* proliferative recall responses using a standard 96 hour proliferation assay. In brief, microplates are seeded with 200,000 cells per well and the cells are incubated with medium alone, ConA, PHA, or rNS3. After 72 hours, radioactive thymidine is added and 16–24 hours later, the cells are harvested. Proliferation is determined from the radioactivity of the cells as the counts per minute (cpm) of cells incubated with an antigen divided by the cpm of the cells incubated with medium alone, sample to negative (S/N) ratio. Groups are compared by the mean S/N ratios at each time point.

[0363] Rabbits are injected in the right TA with 0.3 ml of saline containing the indicated amount of coNS3/4A DNA. Antibody levels are recorded as the mean end point titers. Peak antibody end point titers are reached after several injections.

[0364] Data is recorded showing the dose-response relation with respect to induction of NS3-specific proliferative responses in PBMC in rabbits immunized using a HIP needle.

Data is collected to indicate a proliferative result as the mean S/N of duplicate or triplicate determinations in the presence of rNS3 *in vitro*.

[0365] NS3-specific proliferation will be detectable. The mean NS3-recalled proliferation is consistently higher in the groups receiving higher doses of coNS3/4A DNA as compared with the control group. Thus, the vaccination primes *in vitro* detectable T cell responses in rabbits.

EXAMPLE 4

[0366] In a next series of experiments, the injection needle(s) described herein (IVIN devices) are modified for use with existing gene transfer technologies, including gene gun delivery systems, delivery systems using electroporation, and microneedle delivery systems. In these experiments, the NS3/4A-pVAX1 vector is administered to mice or rabbits via the modified gene gun delivery system, the modified electroporation device, or the modified microneedle delivery system. Purified NS3/4A-pVAX1 vector is used to immunize groups of mice or rabbits. The plasmid is injected directly into regenerating tibialis anterior (TA) muscle via either the modified gene gun delivery system, the modified electroporation device, or the modified microneedle delivery system. Immunization of is performed with approximately 0.25mg/kg of DNA of plasmid DNA. Immunizations are performed on weeks 0, 4, and 8.

[0367] Enzyme immunosorbent assays (EIAs) are used to detect the presence of murine NS3-specific antibodies. These assays are performed essentially as described (Chen et al., *Hepatology* 28(1): 219 (1998)). Briefly, rNS3 is passively adsorbed overnight at 4°C to 96-well microtiter plates (Nunc, Copenhagen, Denmark) at 1 µg/ml in 50 mM sodium carbonate buffer (pH 9.6). The plates are then blocked by incubation with dilution buffer containing PBS, 2% goat serum, and 1% bovine serum albumin for one hour at 37°C. Serial dilutions of mouse sera starting at 1:60 are then incubated on the plates for one hour. Bound murine and rabbit serum antibodies are detected by an alkaline phosphatase conjugated goat anti-mouse or goat anti-rabbit IgG (Sigma Cell Products, Saint Louis, MO) followed by addition of the substrate pNPP (1 tablet/5ml of 1M Diethanol amine buffer with 0.5 mM MgCl₂). The reaction is stopped by addition of 1M NaOH and absorbency is read at 405 nm.

[0368] After four and six weeks, all mice and rabbits immunized with NS3/4A-pVAX1 will develop NS3 antibodies. Similarly, all mice and rabbits immunized with NS3/4A-pVAX1 will develop potent T cell responses. All mice and rabbits immunized with NS3/4A-pVAX1 via either the modified gene gun delivery system, the modified electroporation device,

or the modified microneedle delivery system will develop a potent immune response to the desired antigen.

EXAMPLE 5

[0369] It is well known that the exogenous capsid protein (HBcAg) of the hepatitis B virus (HBV) is highly immunogenic on a CD4⁺ T cell level in all species tested. However, HBcAg has not been explored as an adjuvant for genetic vaccines, and in particular the non-human forms of HBcAg. A key feature of using non-human HBcAg is that HBV is a very common infection that affects almost a third of the world's population. Thus, HBcAg sequences from highly distant species should be used in order to be able to use these vaccines also in areas highly endemic for HBV. The use of HBcAg as a DNA vaccine adjuvant is explored here. HBcAg-sequences effectively improved the immunogenicity of hepatitis C virus derived genes supporting that HBcAg can act as an intracellular adjuvant (iac). Importantly, the major role of the addition of HBcAg-sequences was seen in models mimicking the human HCV infection. HBcAg-based vaccines could overcome the profound T cell tolerance in transgenic mice co-expressing the human leucocyte antigen (HLA)-A2 and the HCV non-structural (NS) 3/4A complex. Here the presence of "healthy" non-tolerized heterologous T cells aided in the activation of the dysfunctional HCV NS3/4A-specific T cells. Thus, HBcAg effectively acts as an intracellular adjuvant that can help restoring a dysfunctional T cell response in a host with persistent presence of a viral antigen, as generally seen in chronic viral infections.

[0370] Some embodiments include, for example, one or more of the HBcAg nucleic acid or protein sequences disclosed in International Patent Application Publication Number WO 2009/130588, which designated the United States and was published in English, the disclosure of which is hereby expressly incorporated by reference in its entirety. Some embodiments include the NS3/4A/HBcAg fusions or a nucleic acid encoding said fusion identified in FIGS. 44 A-I, or a nucleic acid or a nucleic acid or a nucleic acid encoding a protein described in SEQ. ID NOS 1-32. Additional nucleic acid sequences encoding antigenic peptides, such as those described in WO 2009/130588 (e.g., birch antigen) and WO 2010/086743, both of which designated the United States and published in English, the disclosure of which is hereby expressly incorporated by reference in their entirety can also be joined to an HBcAg encoding nucleic acid sequence and said fusions can be administered to a subject in need thereof using one or more of the injection devices described herein. Some embodiments also include additional adjuvants, including but not limited to ribavirin or a CPG nucleotide e.g., SEQ. ID NO. 33. Any

of the aforementioned embodiments can be incorporated into one or more of the injection devices described herein and can be administered to a subject in need thereof.

EXAMPLE 6

[0371] The force requirements for injecting material using an intracellular delivery device described herein were studied. Placebo liquid was injected into open space or chicken breast and the applied forces were measured using a Lloyd force tensometer.

[0372] FIG. 45 is an example of the setup for measuring the force requirements when injecting material using one of the injection needle devices (IVIN) disclosed herein. Lloyd Force Tester 4500 was used to compress syringe 4510 containing fluid 4520 at a predetermined velocity to measure the applied force while injecting 0.3 mL of fluid (e.g., air or water). Support jig 4530 secured syringe 4510 during compression and high-speed camera 4540 recorded the spray pattern from the needles barrels 4550. Two different syringes were tested: (i) a 3 mL syringe requiring a plunger depth of 5.09 mm to inject 0.3 mL, and (ii) a 5 mL syringe requiring a plunger depth of 2.63 mm to inject 0.3 mL. An initial test studied the force required for injecting air into an open area (i.e., not positioned within muscle tissue). Tests were also completed for injecting died water into an open area or into chicken breast.

[0373] The tested injection device comprises four needles configured with generally the same structure depicted in FIG. 8B. The length L_6 is 6 mm. Specifically, needle 820b includes three zones, each having 15 apertures that all face one of the adjacent needles 820a, 820c, and 820d. That is, needle 820b include a first zone having 15 apertures that all face needle 820a, a second zone having 15 apertures that all face needle 820c, and a third zone having 15 apertures that all face needle 820d. Meanwhile, needles 820a, 820c, and 820d each include one zone of 15 apertures that all face needle 820b. All of the apertures in a given zone were spaced vertically apart along the axis of the needle barrel. Each aperture was separated by distance of 0.2 mm between the centers of each apertures. Needles with 0.05 mm circular apertures or 0.1 mm circular apertures were tested.

[0374] The results are shown Table 3.

TABLE 3

Test	Aperture Size (mm)	Syringe Volume (mL)	Compression Speed (mm/s)	Flow Rate (mL/s)	Fluid	Target Material	Maximum Force (N)
1	0.1	3 mL	17	1.0	Air	None	2.9

Test	Aperture Size (mm)	Syringe Volume (mL)	Compression Speed (mm/s)	Flow Rate (mL/s)	Fluid	Target Material	Maximum Force (N)
2	0.1	3 mL	10.2	0.6	Air	None	2.6
3	0.1	3 mL	5.1	0.3	Air	None	2.1
4	0.1	3 mL	17	1.0	H ₂ O	None	16.0
5	0.1	3 mL	10.2	0.6	H ₂ O	None	8.5
6	0.1	3 mL	5.1	0.3	H ₂ O	None	4.0
7	0.1	3 mL	17	1.0	Died H ₂ O	Chicken	18.0
8	0.1	3 mL	10.2	0.6	Died H ₂ O	Chicken	9.8
9	0.1	3 mL	5.1	0.3	Died H ₂ O	Chicken	5.25
10	0.1	5 mL	17	1.9	Air	None	1.9
11	0.1	5 mL	10.2	1.2	Air	None	1.2
12	0.1	5 mL	5.1	0.6	Air	None	0.6
13	0.1	5 mL	17	1.9	H ₂ O	None	36.0
14	0.1	5 mL	10.2	1.2	H ₂ O	None	36.5
15	0.1	5 mL	5.1	0.6	H ₂ O	None	15.9
16	0.1	5 mL	17	1.9	Died H ₂ O	Chicken	46.0
17	0.1	5 mL	10.2	1.2	Died H ₂ O	Chicken	37.0
18	0.1	5 mL	5.1	0.6	Died H ₂ O	Chicken	16.9
19	0.05	3 mL	17	1.0	Air	None	2.8
20	0.05	3 mL	10.2	0.6	Air	None	2.7
21	0.05	3 mL	5.1	0.3	Air	None	2.25
22	0.05	3 mL	17	1.0	H ₂ O	None	18.25
23	0.05	3 mL	10.2	0.6	H ₂ O	None	10.1
24	0.05	3 mL	5.1	0.3	H ₂ O	None	5.0
25	0.05	3 mL	17	1.0	Died H ₂ O	Chicken	24.4
26	0.05	3 mL	10.2	0.6	Died	Chicken	12.9

Test	Aperture Size (mm)	Syringe Volume (mL)	Compression Speed (mm/s)	Flow Rate (mL/s)	Fluid	Target Material	Maximum Force (N)
					H ₂ O		
27	0.05	3 mL	5.1	0.3	Died H ₂ O	Chicken	7.6
28	0.05	5 mL	17	1.9	Air	None	1.9
29	0.05	5 mL	10.2	1.2	Air	None	1.2
30	0.05	5 mL	5.1	0.6	Air	None	0.6
31	0.05	5 mL	17	1.9	H ₂ O	None	47.0
32	0.05	5 mL	10.2	1.2	H ₂ O	None	41.0
33	0.05	5 mL	5.1	0.6	H ₂ O	None	18.2
34	0.05	5 mL	17	1.9	Died H ₂ O	Chicken	42.0
35	0.05	5 mL	10.2	1.2	Died H ₂ O	Chicken	47.0
36	0.05	5 mL	5.1	0.6	Died H ₂ O	Chicken	23.0

The spray patterns for water into an open area were studied using a high-speed camera. Generally, tests that produced a 1 mL/s flow rate or higher produced a well-defined, symmetric spray pattern that is expected to increase pressure and may be suitable for delivering therapeutic material. Figs. 46-49 show top and cross-sectional views of chicken breast after injection with dyed water.

EXAMPLE 7

[0375] This example describes using an intracellular delivery device disclosed herein to inject material into a tissue sample by hand to consider the practical pressure limits for manually delivering material. The needles were configured the same as Example 6 and included 0.05 mm apertures with a 3 mm spacing between needles. The 3 mL syringe was supported using a support jig and the plunger was manually depressed as quickly as possible. The plunger motion was recorded using a high-speed camera and used to calculate the time for injecting 0.3 mL of dyed water into the chicken breast.

[0376] The test was repeated three times and the time required for delivering the material was 0.48 s, 0.40 s, and 0.48 s. Therefore, the average hand delivery speed was 0.45

seconds. FIG. 50 shows top and cross-sectional views of chicken breast after manual injection with died water. FIG. 51 is a comparative example showing top and cross-sectional views of chicken breast after manual injection with died water using only a single needle.

EXAMPLE 8

[0377] The force requirements for injecting material using an intracellular delivery device described herein were studied using generally the same procedures described in Example 6.

[0378] Several different injection devices were tested. Injection device “3Y01(72)” included four needles as depicted in FIG. 8B, where L_6 was 3 mm. The total number of apertures in the four needles was 72. The center needle included 36 apertures distributed in three rows along the axis of the needle, each row facing an outer needle. The three outer needles each include a single row of 12 apertures facing the center needle to produce a cross-flow between needles. The apertures had a diameter of 0.1 mm and a spacing of 0.2 mm.

[0379] Injection device “3O01(96)” included needles as depicted in FIG. 8C, where L_7 was 3 mm and each needle includes 24 apertures (4 times 24 is 96 apertures) distributed in two rows along the axis of the needle. Each row faces an adjacent needle to produce a cross-flow between needles. The apertures had a diameter of 0.1 mm and a spacing of 0.2 mm.

[0380] Injection device “6X01(144)” included needles as depicted in FIG. 8C, where L_7 was 6 mm and each needle includes 36 apertures (4 times 36 is 144 apertures) distributed in three rows along the axis of the needle. Each row faced one of the three other needles to produce a cross-flow between needles and also converging at the center equidistant from the four needles. The apertures had a diameter of 0.1 mm and a spacing of 0.2 mm.

[0381] Injection device “3O05(72)” had generally the same configuration as “3O01(96)” except that each aperture was 0.05 mm, L_7 was 3 mm, and each needle included 18 apertures. Injection device “3X05(72)” had generally the same configuration as “6X01(144)” except that each aperture was 0.05 mm, L_7 was 3 mm, and each needle included 18 apertures. Injection device “3Y05(72)” had generally the same configuration as “3Y01(72)” except that each aperture was 0.05 mm and , L_6 was 3 mm,.

[0382] FIG. 52 shows the maximum force at varying jet velocities (in mm/sec.) for the water exiting the aperture using a 5 mL syringe.

[0383] FIG. 53 shows the maximum force at varying jet velocities (in mms/sec.) for the water exiting the aperture using a 10 mL syringe. The ‘X’, ‘O’, and ‘Y’ type holes are “6X01(144)”, “3O01(96)”, and “3Y01(72)”, respectively.

[0384] FIG. 54 shows the maximum force at varying jet velocities (in mm/sec.) for the water exiting the aperture using a 10 mL syringe. The ‘X’, ‘O’, and ‘Y’ type holes are “3X05(72)”, “3O05(72)”, and “3Y05(72)”, respectively. The maximum injection forces for the six needle devices described above were tested when (i) injecting air into open space; (ii) injecting water into open space; and (iii) injecting water into chicken. The maximum forces are shown in the Table 4 below.

[0385] The delivery of dyed water into the chicken breast was tested. The dyed water was injected using the Lloyd force tensometer with needles “3Y05(72)”, “3O05(72)”, “3X05(72)”, “3Y01(72)”, and “3O01(96)”. As a comparative example, dyed water was delivered into chicken bread using a single needle with one aperture at the end of the barrel. 0.3 mL was injected for each test and FIG. 55 shows the results. The results show improved, localized tissue delivery for the needles “3Y05(72)”, “3O05(72)”, “3X05(72)”, “3Y01(72)”, and “3O01(96)”.

TABLE 4

Hub	Number of Holes /Hub	Velocity / Hole (m/sec)	AIR: Load @ 0.3ml (N)	PLACEBO: Load @ 0.3ml (N)	CHICKEN: Load @ 0.3ml (N)
3Y01	72	5.0	38.0	44.3	53.6
3O01	96	3.7	33.4	42.5	59.5
6X01	144	2.5	36.9	42.1	N/A
3Y05	72	19.9	60.4	49.1	93.7
3O05	72	19.9	56.1	56.7	62.4
3X05	72	19.9	57.3	65.5	73.2

EXAMPLE 9

[0386] A combined acute and repeated toxicology study is performed using a total of 72 rabbits (36 females and 36 males). After two weeks of acclimatization all animals are injected intramuscularly with either buffer, the clinical dose (amount DNA)/kg or $10 \times$ the clinical dose (amount DNA)/kg intended for human use in combination with electroporation, using needles

and methods described herein. The health status of the animals is monitored daily after injection of DNA. At 7 days after the first vaccination 4 animals from each group of animals is subjected to blood sampling for haematology and clinical chemistry analyses. The animals are thereafter euthanized and relevant organs are be dissected for histopathological analyses. Of particular interest for histopathology is the site of injection.

[0387] To study toxicity after repeated administration the remaining 48 animals (24 males, 24 females) are vaccinated, totally 4 times with one month between vaccinations, with buffer, the clinical dose or $10 \times$ the clinical dose (amount DNA)/kg intended for human use. At 7 days after the last injection the animals are subjected to blood sampling for haematology and clinical chemistry analyses. The animals are thereafter euthanized and relevant organs are dissected for histopathological analyses. Of particular interest for histopathology is the site of injection. During the period of repeated administration, blood samples are taken to determine the development of antibodies in response to the vaccination and to validate the effectiveness of the vaccine.

EXAMPLE 10

[0388] In a first study, HCV infected individuals are injected with a solution containing approximately 0.5 ml 0.9% NaCl containing approximately 0.25mg/kg bodyweight of ChronVac-C (coNS3/4A DNA), an expression plasmid encoding codon-optimized HCV NS3/4A, in the thigh muscle using a large high injection pressure (HIP) (also referred to as "TVIN") injector. In a second study, HBV infected individuals are injected with a solution containing approximately 0.5 ml 0.9% NaCl containing approximately 0.25mg/kg bodyweight of coHBcAg (an expression plasmid encoding codon-optimized HBV core antigen) in the thigh muscle using a large HIP injector. The large HIP injector has 4 needles oriented in a triangular formation, equally spaced with 6 mm between each needle. The center needle is placed in the middle of the equilateral triangle formed by the three outer needles. Each needle of the large HIP injector has 10 apertures. The outer needles all have apertures opening to the center and the center needle has apertures opening at four directions at 90 degree angles.

[0389] At day 5 and 10 blood is drawn from the inoculated individuals, peripheral blood mononuclear cells (PBMCs) are isolated, and the PBMCs are analyzed for T cell proliferation. The PBMCs are assayed for *in-vitro* proliferative recall responses using a standard 96h proliferation assay. (See Lazinda et al., J. Gen. Virol. 82:1299-1308 (2001), herein expressly incorporated by reference in its entirety.) Microtiter plates are seeded with approximately 200,000 cells/well and the cells are incubated with media alone or recombinant

NS3 or HBcAg. PBMCs are also incubated with Concanavalin A (ConA) as a positive control. After 72 hours, radioactive thymidine is added and 16-24 hours later the cells are harvested. The radioactivity of the cells as counts per minute are measured. Additionally, the presence of antibodies specific for NS3/4A and or HBcAg can be determined using standard assays (e.g., ELISA). Optionally, a boost injection is provided at two or three week intervals. The results will show that humans immunized with the large HIP injector show appreciable immune response to NS3/4A and/or HBcAg.

EXAMPLE 11

[0390] Electrification of needle electrodes in various combinations were studied to determine the effect of electric field generation on a prophylactic and/or therapeutic material contained within an electrified needle electrode and to determine the effect on the electrical properties of electrode needles, e.g. current draw, voltage, etc. as the injection medium and subject changed. The combinations and the results are described in Table 5.

[0391] In Table 5, two embodiments of intracellular delivery devices as described herein configured with needle electrodes were tested. Probe IV is a seven needle injection device having a pocket hub, as described herein. Probe VII is a 4 needle, Y-type injection device having a pocket hub, as described herein. The configuration column depicts which needles, identified by number had a positive polarity, and which had a negative polarity. Needles were numbered in a clockwise progression with the center needle having the highest number. Voltage was adjusted to maintain a desired 600 V/cm electrical field created in the subject or EP medium. The electrical field strength at the tips of the needles changes depending on needle pitch.

TABLE 5

Test	Probe	Voltage	Configuration		EP Medium	Needle Contents	Max. Current
			+	-			
1	IV	240	1	7	Saline	Empty	4.32
2	IV	190	1	7	Saline	Empty	3.52
3	IV	190	2	7	Saline	Empty	3.68
4	IV	190	3	7	Saline	Empty	3.41
5	IV	190	4	7	Saline	Empty	3.41
6	IV	190	1	3	Saline	Empty	2.95
7	IV	220	1	3	Saline	Empty	3.45

8	VII	190	1	4	Saline	Empty	3.82
9	VII	180	1	4	Saline	Empty	3.59
10	VII	170	1	4	Saline	Empty	3.41
11	VII	170	2	4	Saline	Empty	3.41
12	VII	170	2	4	Saline	Saline	3.57
13	VII	170	1	4	Saline	DNA	3.59
14	VII	170	1	4	Banana	DNA	0.70
15	VII	240	1	4	Banana	DNA	1.27
16	VII	170	1	4	Chicken	Empty	1.27
17	VII	240	1	4	Chicken	Empty	1.43
18	VII	240	1	4	Chicken	DNA	1.73
19	VII	170	1	4	Chicken	DNA	1.07
20	VII	170	1	4	Chicken	DNA	1.16
21	VII	170	1	4	Chicken	DNA	1.18
22	VII	170	1	4	Chicken	DNA	0.59
23	VII	170	1	4	Chicken	DNA	1.27

[0392] The testing of the present example demonstrated that using needles as electrodes and as carriers of prophylactic and/or therapeutic material (delivered agents) can be accomplished without degrading the prophylactic and/or therapeutic agent, and without undesirably increasing the current draw. Needle electrodes may be manufactured from electrically conducting material. In some embodiments, the hub is constructed from electrically non-conductive material.

EXAMPLE 12

[0393] As will be appreciated from the example disclosed herein, it has been found that utilizing IVIN delivery followed by electroporation in a two stage delivery, achieves a synergistic response over either method alone or the additive effect of both methods. Delivery, uptake and expression results, in larger muscles, represented by the rabbit tibialis anterior, are illustrated in FIGS. 56A1-B2 and FIGS. 57A1-C2.

[0394] In the current experiment, a first group of animals was injected with NS3/4A using a regular hypodermic needle (may also be referred to as a regular needle). A second group of animals was injected with NS3/4A using a regular needle followed by *in vivo* EP. A third group of animals was injected with NS3/4A, using an IVIN device as described herein. A fourth

group of animals was injected with NS3/4A followed by *in vivo* EP, using the same IVIN device.

[0395] In the present example, the EC marked Cliniporator EP device provided by IGEA/Kiritec was used to deliver the *in vivo* EP. The pulse pattern of the *in vivo* EP had one short high-voltage pulse followed by a second longer low-voltage pulse. Without being limited by theory, it is believed the one short high-voltage pulse helped to make cells permeable, while the second longer low-voltage pulse helped to promote cellular uptake of DNA. As observed, the pulse pattern had a minimal destructive effect on the tissue and was more tolerable than pulse patterns also intended to cause tissue destruction and inflammation.

[0396] Following injection and *in vivo* EP (if any) tissue samples from each sample population were assayed for NS3/4A expression levels.

[0397] As shown in FIG. 56A1, moderate to no expression of NS3/4A was observed in the tissue sample injected with the regular needle and no *in vivo* EP. As shown in FIG. 56A2, appreciable expression levels of NS3/4A were detected in the tissue sample injected with the regular needle and receiving *in vivo* EP. Thus, as shown in FIG. 56A1 and FIG. 56A2, results from the use of a regular needle are greatly enhanced when coupled with *in vivo* EP (FIG. 96A1) than the use of a regular needle when not coupled with *in vivo* EP (FIG. 96A2).

[0398] Similarly, as shown in FIG. 56B1-B2, results from the use of an IVIN needle with a 125N injection force were enhanced when coupled with *in vivo* EP (FIG. 56B1) than the use of the IVIN needle with a 125N injection force when not coupled with *in vivo* EP (FIG. 56B2).

[0399] Comparing FIG. 56A2 with FIG. 56B1, the IVIN injection with a 125N injection force showed a similar efficiency in transfecting muscle fibres *in vivo* (FIG. 56B1) as a regular needle injection combined with *in vivo* EP (FIG. 56A2).

[0400] Without being limited in theory, it is believed injection with the IVIN technology achieved a highly localized site in the tissue since the configuration of the hub placed the needles along a circle fixing the tissue and injecting the NS3/4A centrally in the tissue. It is believed the tissue was locally overloaded resulting in both a local inflammation and an improved uptake of DNA and antigen expression.

[0401] Further, as can be seen by comparing FIG. 56A2 and FIG. 56B1 with FIG. 56B2, the combined IVIN needle with a 125N injection force and *in vivo* EP (FIG. 56B2), showed both an enhanced inflammation and transfection over either the *in vivo* EP (FIG. 56A2) or the IVIN needle (FIG. 56B1) alone. Furthermore, as can be seen in FIG. 56B2 the combined

IVIN needle with a 125N injection force and *in vivo* EP, producing a truly synergistic effect over the additive effect of *in vivo* EP (FIG. 56A2) and the IVIN needle (FIG. 56B1).

[0402] FIG. 57A1-C2 are example results of injection using the hypodermic needle device described above with two injection force values coupled with electroporation as compared to a regular needle with electroporation. As shown in FIG. 57A1-A2, results from the use of a regular needle are greatly enhanced when coupled with *in vivo* EP (FIG. 57A1) than the use of a regular needle when not coupled with *in vivo* EP (FIG. 57A2). Similarly, as shown in FIG. 57B1-B2, results from the use of an IVIN needle with a 75N injection force were enhanced when coupled with *in vivo* EP (FIG. 57B1) than the use of the IVIN needle with a 75N injection force when not coupled with *in vivo* EP (FIG. 57B2). Similarly, as shown in FIG. 57C1-C2, results from the use of an IVIN needle with a 125N injection force were enhanced when coupled with *in vivo* EP (FIG. 57C1) than the use of the IVIN needle with a 125N injection force when not coupled with *in vivo* EP (FIG. 57C2).

[0403] As seen in FIG. 57C2, the combination of IVIN with a 125N injection force and *in vivo* EP transfection was highly effective.

[0404] FIG. 58A illustrates the number of interferon gamma producing cells/million splenocytes from a C57BL/6 mouse injected with 5 µg NS3/4A DNA in using an IVIN intracellular delivery apparatus into the tibialis anterior muscle, with no electroporation.

[0405] FIG. 58B illustrates the same quantity using standard injection methods to deliver 5 µg NS3/4A DNA in a C57BL/6 mouse.

[0406] FIG. 58C illustrates number of interferon gamma producing cells/million splenocytes from a C57BL/6 mouse injected with 5 µg NS3/4A DNA in using an IVIN intracellular delivery apparatus into the tibialis anterior muscle together with electroporation.

[0407] FIG. 58D illustrates the number of interferon gamma producing cells/million splenocytes from a C57BL/6 mouse injected with 5 µg NS3/4A DNA in using a conventional needle into the tibialis anterior muscle combined with electroporation.

[0408] Thus, DNA delivery using IVIN results in a localized deposition and uptake and expression of the DNA, as well as a local inflammation. Also, when combined with *in vivo* EP the uptake and expression is further improved without causing additional tissue damage. Thus, the combination of IVIN and *in vivo* EP results in a highly effective and tolerable mode of DNA delivery, which can be particularly useful for delivering genetic vaccines (e.g., nucleic acid constructs encoding a desired antigen, such as NS3/4A with or without HBcAg).

EXAMPLE 13

[0409] Outbred pigs were injected using an intracellular delivery apparatus having an IVIN injection system to deliver NS3/4A DNA in the skin (6x 50 ug) or in the right thigh muscle (1x 500 ug). FIG. 59A and B illustrate the number of pig-interferon gamma producing cells/million peripheral blood mononuclear cells of pigs receiving no NS3/4A DNA IVIN injection (non-immunized pigs). FIGS. 59C and D illustrate the number of pig-interferon gamma producing cells/million peripheral blood mononuclear cells of pigs after injection with NS3/4A DNA (immunized pigs). As the data shows, an appreciable immune response was induced after using an intracellular delivery device delivering the NS3/4A construct.

EXAMPLE 14

[0410] Codon optimized NS3/4A-codon optimized stork HBcAg immunogenic compositions were introduced into WT C57BI/6 mice. NS3-specific CD8⁺ T cell expansion was determined by direct ex vivo pentamer staining two weeks after a single immunization. Elicitation of IFN γ in SFC quantification of the immunogenic compositions was compared among compositions introduced through regular injection, IVIN injection, regular injection followed by electroporation (EP), and IVIN-EP treatment. It was observed that the IVIN administration, regular injection-EP, and IVIN-EP co-administration increased the percent of NS3-pentamer specific CD8⁺ T cells by approximately 1.5 fold, four fold and five fold over regular injection alone. See FIG. 60.

EXAMPLE 15

[0411] Codon optimized NS3/4A-codon optimized stork HBcAg immunogenic compositions were introduced into WT C57BI/6 mice. IFN γ SFC quantification was performed as discussed in Example 14, above. NS3-specific CD8⁺ T cell expansion was determined by direct ex vivo pentamer staining two weeks after a single immunization. Elicitation of IFN γ in SFC quantification of the immunogenic compositions was compared among compositions introduced through regular injection, IVIN injection, regular injection followed by electroporation (EP), and IVIN-EP treatment. It was observed that the IVIN administration, regular injection-EP, and IVIN-EP co-administration increased the ratio of IFN γ SFCs per 10⁶ cells by 2-3 fold, 4 fold and 5 fold, respectively, compared to the number of SFC cells observed upon regular injection alone. See FIG. 61.

ELISPOT assay

[0412] Splenocytes of immunized mice are collected and red blood cells are lysed (Sigma, R7757). Cells (2×10^5 per well in triplicate) are cultured in Multiscreen plates (Millipore, MSRA S4510) coated with anti-mouse IFN γ monoclonal antibody (BD Biosciences; 10 μ g/ml, 551216) in α MEM culture medium (Gibco, 22571) supplemented with 10 % FCS (Sigma, F7524 lot 103K3398), 80 U/mL penicillin / 80 μ g/mL streptomycin (PAN, P06-07-100), 2 mM L-glutamin (Gibco, 25030), 1 X non-essential amino acids (Gibco, 11140), 10 mM Hepes (Gibco, 15630), 1 mM sodium pyruvate (Gibco, 11360) and 50 μ M β -mercaptoethanol (Gibco, 31350) and in presence of 10 units/ml of recombinant murine IL-2 (Peprotech, 212-12, referred to as mIL-12), alone as negative control ("medium only" condition), or with the indicated concentrations of:

- NS3 CTL peptide, a mouse cytotoxic T lymphocyte epitope from the NS3 protein
- rNS3, a recombinant NS3 polypeptide
- OVA protein, ovalbumin protein
- OVA CTL, a mouse cytotoxic T lymphocyte epitope from the ovalbumin protein
- OVA Th, a mouse T helper cell epitope from the ovalbumin protein
- E13K (SEQ ID NO: 49: EIPFYGKAIPIEAIK), a mouse T helper cell epitope (a class II epitope) 15 mer peptide present in NS3 of genotype 1b;
- Concanavalin A (Sigma, C5275) as positive control.

[0413] After a 40-hour incubation at 37°C, 5 % CO₂, wells are washed with PBS 1X (1 time) and with PBS 1X-0.05 % Tween (3 times) and incubated for 2 h at room temperature with biotinylated anti-murine IFN γ (BD Biosciences 0.5 mg/ml; 554410). Wells are washed in PBS1X-0.05 % Tween (3 times), and streptavidin-conjugated HRP (horseradish peroxidase) (Southern Biotechnology 7100-05; 1/1000 dilution) is added for 1 h at room temperature. After rewashing with PBSIX-0.05 % Tween (3 times) and with PBSIX (3 times), spots representing individual cytokine-producing cells are visualized by developing with 3-amino-9-ethylcarbazole.

[0414] Spots are counted using an ELISPOT microplate reader (ImmunoSpot® S5 MicroAnalyzer; ImmunoSpot® Professional Software Version 5.0, CTL). A response is considered positive if the number of spots is higher than the mean value of the "medium only" condition plus 2 times the standard deviation related to 10^6 cells (cut-off of the experiment) and above 50 spots / 10^6 cells (technical cut-off related to the reader).

EXAMPLE 16

[0415] Codon optimized NS3/4A immunogenic compositions were delivered intramuscularly in mice using IVIN technology with electroporation (EP). Vaccinations were

performed on WT C57BI/6 and NS3/4A-Tg mice lines. NS3/4A-Tg mice express NS3/4A and are thus immunotolerant to the NS3/4A epitopes. They are useful, for example, as models of the immune systems of chronically infected HCV individuals.

[0416] Mice were twice immunized with 50 μ g of immunogenic composition constructs in combination with 5 μ g of mL-12. ELISpot assay was used for quantification of HCV NS3-specific, IFN γ -producing spot forming cells (SFC) after a 36 hour in vitro stimulation of splenocytes with indicated antigens. A cut-off of 50 SFC/10⁶ cells indicates a positive response. Antigens separately vaccinated included the OVA protein, and nucleic acid constructs encoding NS3 CTL, E13K, rNS3, OVA CTL, and OVA Th. Nucleic acids encoding mL-12 were included as an adjuvant in each of the above-mentioned vaccinations. Nucleic acids for each antigen were included in its respective immunogenic composition at the concentration indicated. See FIG. 62.

[0417] It was observed that inclusion of an IL-12 polypeptide, mL-12 (SEQ ID NO.: 37), in immunogenic compositions applied to wild-type mice improved the number of SFC/10⁶ cells observed in an ELISpot assay by 2 fold relative to a similar treatment (e.g., codon optimized NS3/4A-codon optimized stork HBcAg, IVIN+EP administration) in the absence of IL-12. Compare with results in Example 15, above.

[0418] It was further observed that inclusion of an IL-12 polypeptide, mL-12, in immunogenic compositions applied to Tg mice improved the number of SFC/10⁶ cells observed in an ELISpot assay by up to 150 fold relative to a vehicle control treatment.

EXAMPLE 17

[0419] In this example, it is shown that IVIN injection of a plasmid comprising a nucleic acid sequence encoding a human codon optimized NS3/4A gene into a rabbit tibialis anterior muscle improves *in vivo* transfection of muscle fibers that express the NS3-protein and generates an appreciable localized inflammatory response. An expression plasmid comprising a nucleotide sequence encoding HCV NS3/4A (0.9mg in 0.3mL) was injected into rabbit tibialis anterior muscle. Five days after immunization, embedded sections of the muscle were generated and stained with labeled antibodies to NS3 and hematoxylin-eosin staining was used to detect inflammation. Detection of HCV NS3-protein expressing muscle fibers and localized inflammation in rabbit tibialis anterior muscle sections was then analyzed. As shown in FIG. 63, conventional *i.m.* injection showed no or limited detectable NS3 expression and no or limited localized inflammation; whereas, IVIN injection showed appreciable NS3 expression and localized inflammation around the injection site at the concentration of DNA immunogen

used. Accordingly, these experiments provide evidence that IVIN injection more efficiently delivers a DNA immunogen to muscle and more efficiently generates an inflammatory response at the site of injection than conventional *i.m.* injection of the DNA immunogen.

EXAMPLE 18

[0420] In this example, it is shown that the use of IVIN in combination with electroporation efficiently primes T cell responses in wild-type mice. 5 μ g or 50 μ g of an expression plasmid containing a nucleotide sequence encoding a human codon optimized NS3/4A gene fused to a human codon optimized stork HBcAg gene, having translation interruption sites introduced therein, were administered to C57BL6/J wild-type mice using IVIN in combination with electroporation. Additionally, 5 μ g of a plasmid encoding IL12 was administered to the mice by IVIN and electroporation. The proportion of IFN- γ SFCs per million cells induced upon IVIN and electroporation administration was then determined using methods described in the previous example. As shown in FIG. 64 A-J, appreciable T cell responses were observed.

EXAMPLE 19

[0421] The experiments in Example 18 were then repeated in NS3/4A transgenic mice. NS3/4A-Tg mice express NS3/4A and are immunotolerant to the NS3/4A epitopes. They are useful as models of the immune systems of chronically infected HCV individuals. In this example, it is shown that the use of IVIN in combination with electroporation efficiently primes T cell responses in the NS3/4A transgenic mice. 5 μ g or 50 μ g of an expression plasmid containing a nucleotide sequence encoding a human codon optimized NS3/4A gene fused to a human codon optimized stork HBcAg gene, having translation interruption sites introduced therein, were administered to NS3/4A transgenic mice using IVIN in combination with electroporation. Additionally, 5 μ g of a plasmid encoding IL12 was administered to the mice by IVIN and electroporation. The proportion of IFN- γ SFCs per million cells induced upon IVIN and electroporation administration was then determined using methods described in the previous example. As shown in FIG. 65 A-J, appreciable T cell responses were observed providing evidence that IVIN in combination with electroporation can improve an immune response in an individual that is immunotolerant to the HCV NS3/4A (e.g. mimics an individual chronically infected with HCV).

EXAMPLE 20

[0422] In this example, it is demonstrated that by using IVIN in combination with electroporation an appreciable immune response to a DNA immunogen can be generated with

amounts of a DNA immunogen that are significantly lower than that used in conventional injection followed by electroporation. 50 μ g, 5 μ g, or 0.5 μ g of an expression plasmid containing a nucleotide sequence encoding a human codon optimized NS3/4A gene fused to a human codon optimized stork HBcAg gene, having translation interruption sites introduced therein, were administered to mice and the IFN- γ SFCs per million cells induced upon IVIN and electroporation administration was analyzed using the approaches described in the previous examples. As shown in FIG. 66 A-C, an appreciable immune response was generated when IVIN in combination with electroporation was used to deliver 0.5 μ g of the expression plasmid, which is an amount readily appreciated to be below the amount of expression plasmid required to generate an immune response using conventional injection in combination with electroporation. Accordingly, these results provide evidence that IVIN used with electroporation allows for the use of a lower dose of DNA immunogen, as compared to conventional injection followed by electroporation.

EXAMPLE 21

[0423] Pigs provide a model system that closely resembles humans. Pigs are a large animal with muscle and skin very similar to that found in humans. A porcine IFN gamma ELISpot assay was used following the methodology described in previous examples so as to allow for the study of prime-boost immune activation in pigs. In this example, it is shown that IVIN in combination with electroporation can efficiently deliver a DNA immunogen thereby promoting the generation of an immune response to the immunogen when the DNA construct is administered both the dermis (skin) and to the muscle of pigs. Additionally, these experiments show that prime-boost administrations to either the skin (prime and boost to the dermis) or to the skin and muscle (prime to the muscle and boost to the skin) generated an appreciable immune response in the animals when IVIN in combination with electroporation was used. 300 μ g of human codon optimized NS3/4A-human codon optimized stork HBcAg polynucleotide immunogenic compositions containing translation interruption sites introduced therein (C2.2 in a pVAX1 backbone) was administered to pigs using IVIN in combination with electroporation. The proportion of IFN- γ SFCs per million cells induced upon single or double (prime and prime/boost) IVIN and electroporation administrations into the dermis (Skin) or muscle (IM) of in pigs was then analyzed. As shown in FIG. 67 A-H, IVIN in combination with electroporation efficiently delivers a DNA immunogen to the pigs irrespective of whether the immunogen is delivered to the skin or muscle and thereby promotes the generation of a potent immune response to the immunogen. Additionally, it is shown that prime-boost administrations to either

the skin or to the skin and muscle (prime to the muscle and boost to the skin) generated a significant immune response to the immunogen.

EXAMPLE 22

[0424] In this example it is shown that intramuscular use of the IVIN device, in combination with electroporation, induces a more robust immune response as compared to conventional intramuscular injection in combination with electroporation when a DNA immunogen is delivered. In these experiments, 5 μ g of an expression plasmid comprising a nucleotide sequence having a human codon optimized HBcAg gene was administered (e.g., B cell deficient C57BL/6 mice) to animals using conventional intramuscular injection in combination with electroporation or IVIN in combination with electroporation. 5 μ g of an expression plasmid comprising a nucleic acid encoding IL12 was also provided to the same animals using conventional intramuscular injection in combination with electroporation or IVIN in combination with electroporation. Using the techniques described in other examples, the proportion of IFN- γ SFCs per million cells induced was then monitored and recorded. As shown in FIG. 68 A-B, the combination of IVIN and electroporation was vastly superior at inducing an immune response in the animals, as compared to using conventional intramuscular injection in combination with electroporation. These results provide further evidence of the surprising improvement of using IVIN and electroporation to deliver a DNA immunogen.

EXAMPLE 23

[0425] In this example it is shown that intramuscular use of the IVIN device, in combination with electroporation, induces an immune response which is 100% or more than 100% greater than conventional intramuscular injection in combination with electroporation when a DNA immunogen is delivered. In these experiments, an expression plasmid comprising a nucleotide sequence having a human codon optimized HBcAg gene was twice administered to animals using conventional intramuscular injection in combination with electroporation or IVIN in combination with electroporation. Using the techniques described in other examples, the proportion of IFN- γ SFCs per million cells induced was then monitored and recorded. As shown in FIG. 69, the combination of IVIN and electroporation was greater than 100% more effective at inducing an immune response in the animals, as compared to using conventional intramuscular injection in combination with electroporation. These results provide further evidence of the surprising improvement of using IVIN and electroporation to deliver a DNA immunogen.

[0426] The term “comprising” as used herein is synonymous with “including,” “containing,” or “characterized by,” and is inclusive or open-ended and does not exclude additional, unrecited elements or method steps.

[0427] Accordingly, in some contexts, the numerical parameters set forth herein are approximations that may vary depending upon the desired properties sought to be obtained. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of any claims in any application claiming priority to the present application, each numerical parameter should be construed in light of the number of significant digits and ordinary rounding approaches.

[0428] The above description discloses several methods and materials of the present invention. This invention is susceptible to modifications in the methods and materials, as well as alterations in the fabrication methods and equipment. Such modifications will become apparent to those skilled in the art from a consideration of this disclosure or practice of the invention disclosed herein. Consequently, it is not intended that this invention be limited to the specific embodiments disclosed herein, but that it cover all modifications and alternatives coming within the true scope and spirit of the invention.

[0429] All references cited herein, including but not limited to published and unpublished applications, patents, and literature references, are incorporated herein by reference in their entirety and are hereby made a part of this specification. To the extent publications and patents or patent applications incorporated by reference contradict the disclosure contained in the specification, the specification is intended to supersede and/or take precedence over any such contradictory material.

WHAT IS CLAIMED IS:

1. A product combination for use in improving an immune response comprising
a) an electroporation device that comprises
a plurality of needles each having a closed end and a plurality of apertures along the barrel of said needles,
wherein the apertures on separate needles oppose one other but said apertures do not face away from each other,
so as to create a zone of injection existing in a region of tissue between the opposing apertures of said plurality of needles,
wherein at least two of said plurality of needles are capable of passing current so as to enhance delivery of a nucleic acid within said zone of injection; and
b) a nucleic acid encoding an antigen; and
wherein said product combination improves an immune response in a subject as compared to an immune response resulting from delivery of said nucleic acid using a conventional injection device in combination with electroporation.
2. The product combination for use in improving an immune response of Claim 1, wherein said nucleic acid comprises at least one polynucleotide encoding a first polypeptide.
3. The product combination for use in improving an immune response of Claim 2, wherein said nucleic acid comprises at least one polynucleotide encoding a second polypeptide.
4. The product combination for use in improving an immune response of Claim 1, wherein said nucleic acid comprises at least one polynucleotide encoding IL-12.
5. The product combination for use in improving an immune response of Claim 4, wherein said IL-12 is human IL-12.
6. The product combination for use in improving an immune response of any of Claims 1-5, wherein said nucleic acid comprises at least one polynucleotide encoding HCV NS3/4A
7. The product combination for use in improving an immune response of any of Claims 1-6, wherein said nucleic acid comprises at least one polynucleotide encoding HCV NS5A.
8. The product combination for use in improving an immune response of any of Claims 1-7, wherein said nucleic acid comprises at least one polynucleotide encoding and HBcAg.

9. The product combination for use in improving an immune response of Claim 8, wherein said HBcAg is isolated from an HBV that infects stork or heron.

10. The product combination of any of Claims 1-9 wherein said nucleic acid comprising said first polynucleotide encoding said first polypeptide and comprising said second polynucleotide encoding said second polypeptide are separated by a polynucleotide encoding a translation interruption sequence.

11. The product combination of Claim 10, wherein said polynucleotide encoding a translation interruption sequence comprises a T2A sequence.

12. The product combination for use in improving an immune response of any of Claims 1-11, wherein said at least one polynucleic acid is codon optimized for expression in humans.

13. The product combination for use in improving an immune response of any of Claims 1-12, wherein said immune response is a mammalian immune response.

14. The product combination for use in improving an immune response of Claim 13, wherein said immune response is a human immune response.

15. The product combination for use in improving an immune response of any of Claims 13-14, wherein said immune response is an HCV immune response.

16. The product combination for use in improving an immune response of Claim 15, wherein said immune response is in an individual chronically infected with HCV.

17. The product combination for use in improving an immune response of any of Claims 1-16, wherein said nucleic acid is provided in an amount insufficient to elicit an immune response when delivered intramuscularly in the absence of electroporation, and wherein said amount is sufficient to elicit an immune response.

18. The product combination for use in improving an immune response of any of Claims 1-16, wherein said immune response is 2x stronger than an immune response observed in the absence of electroporation.

19. The product combination for use in improving an immune response of any of Claims 1-16, wherein said immune response is comparable to an immune response observed upon administration of a greater amount of said nucleic acid to said zone of injection in the absence of electroporation.

20. The product combination for use in improving an immune response of any of Claims 1-16, wherein said immune response is comparable to an immune response observed

upon administration of twice as much of said nucleic acid to said zone of injection in the absence of electroporation.

21. An immunogenic composition comprising (a) a polynucleotide encoding a hepatitis C virus NS3/4A polypeptide and (b) an IL-12 polypeptide or a polynucleotide encoding an IL-12 polypeptide.

22. The immunogenic composition of Claim 21, further comprising (c) an HBcAg polypeptide or a polynucleotide encoding an HBcAg polypeptide.

23. The immunogenic composition of Claim 21 or Claim 22, wherein said immunogenic composition comprises a polynucleotide encoding an IL-12 polypeptide.

24. The immunogenic composition of Claim 23, wherein the polynucleotide encoding a hepatitis C virus NS3/4A polypeptide and the polynucleotide encoding an IL-12 polypeptide are present in the same vector.

25. The immunogenic composition of Claim 23, wherein the polynucleotide encoding a hepatitis C virus NS3/4A polypeptide and the polynucleotide encoding an IL-12 polypeptide are present in different vectors.

26. The immunogenic composition of any of Claims 21-25, wherein said immunogenic composition comprises a polynucleotide encoding an HBcAg polypeptide.

27. The immunogenic composition of Claim 26, wherein the polynucleotide encoding a hepatitis C virus NS3/4A polypeptide and the polynucleotide encoding an HBcAg polypeptide are present in the same vector.

28. The immunogenic composition of Claim 26, wherein the polynucleotide encoding a hepatitis C virus NS3/4A polypeptide and the polynucleotide encoding an HBcAg polypeptide are present in different vectors.

29. The immunogenic composition of any of Claims 21 to 28, further comprising an attenuated modified *vaccinia Ankara*.

30. The immunogenic composition of any of Claims 21 to 29, further comprising a vesicular stomatitis virus (VSV).

31. The immunogenic composition of any of Claims 21 to 30, further comprising a polynucleotide encoding a rodent HBcAg.

32. The immunogenic composition of any of Claims 21 to 31, further comprising a replication defective Ad6 vector expressing a NS3/NS4A, NS3/4A/5A, NS3/4A/NS5B, or NS3/4A/5A/5B gene fusion.

33. The immunogenic composition of any of Claims 21 to 32, further comprising a replication defective Ad24 vector expressing a NS3/NS4A, NS3/4A/5A, NS3/4A/NS5B, or NS3/4A/5A/5B gene fusion.

34. The immunogenic composition of any of Claims 21 to 33, wherein said immunogenic composition induces production of NS3-specific T cells.

35. The immunogenic composition of any of Claims 21 to 34, wherein said immunogenic composition increases production of NS3-specific T cells relative to a comparative composition comprising the same components as said immunogenic composition except for both lacking said IL-12 polypeptide and lacking said polynucleotide encoding an IL-12 polypeptide.

36. The immunogenic composition of any of Claims 21 to 35, wherein said immunogenic composition induces production of NS3-specific T cells at twice the level relative to a comparative composition comprising the same components as said immunogenic composition except for both lacking said IL-12 polypeptide and lacking said polynucleotide encoding an IL-12 polypeptide.

37. The immunogenic composition of any of Claims 21 to 36, wherein said immunogenic composition induces production of NS3-specific T cells at fourfold the level relative to a comparative composition comprising the same components as said immunogenic composition except for both lacking said IL-12 polypeptide and lacking said polynucleotide encoding an IL-12 polypeptide.

38. The immunogenic composition of any of Claims 21 to 37, wherein said immunogenic composition is characterized as increasing by at least twentyfold the number of splenocytes expressing INF γ upon administration of said immunogenic composition relative to a control immunogenic composition lacking an HCV antigen.

39. The immunogenic composition of any of Claims 21 to 38, wherein said immunogenic composition is characterized as producing at least 10^2 INF γ -expressing splenocytes per million cells upon administration of said immunogenic composition more than a control immunogenic composition lacking an HCV antigen.

40. The immunogenic composition of any of Claims 21 to 38, wherein said immunogenic composition is characterized as producing at least 5×10^2 INF γ -expressing splenocytes per million cells upon administration of said immunogenic composition more than a control immunogenic composition lacking an HCV antigen.

41. The immunogenic composition of any of Claims 21 to 38, wherein said immunogenic composition is characterized as producing at least 10^3 INF γ -expressing splenocytes per million cells upon administration of said immunogenic composition more than a control immunogenic composition lacking an HCV antigen.

42. The immunogenic composition of any of Claims 21 to 38, wherein said immunogenic composition is characterized as producing at least 4×10^3 INF γ -expressing splenocytes per million cells upon administration of said immunogenic composition more than a control immunogenic composition lacking an HCV antigen.

43. The immunogenic composition of any of Claims 21 to 42, wherein said immunogenic composition induces an NS3-specific T cell response in a patient that is chronically infected with HCV.

44. The immunogenic composition of any of Claims 21 to 43, wherein said immunogenic composition is characterized as inducing an NS3-specific T cell response in a patient that is chronically infected with HCV.

45. The immunogenic composition of any of Claims 21 to 44, wherein said immunogenic composition is present in an injection device or an injection device configured to be used in conjunction with a an electroporation device.

46. A method of generating an immune response in a subject, comprising delivering to an intracellular space, such as a muscle cell, of said subject the immunogenic composition of any of Claims 21-45.

47. A method of generating an immune response in a subject, comprising delivering to an intracellular space, such as a plurality of muscle cells, of said subject a polynucleotide encoding an HCV NS3/NS4A, NS3/4A/5A, NS3/4A/NS5B, or NS3/4A/5A/5B polypeptide, and delivering to said animal an IL-12 polypeptide and/or a polynucleotide encoding an IL-12 polypeptide.

48. The method of Claim 47, wherein said IL-12 polypeptide is encoded by a polynucleotide, and wherein said polynucleotide is delivered to an intracellular space of said animal, such as a plurality of muscle cells or intradermally.

49. The method of Claim 47 or Claim 48, further comprising delivering to an intracellular space such as a plurality of muscle cells or intradermally of said subject an HBcAg polypeptide or a polynucleotide encoding an HBcAg.

50. The method of Claim 49, wherein said HBcAg polypeptide is encoded by a polynucleotide, and wherein said polynucleotide is delivered to an intracellular space such as a

plurality of muscle cells or intradermally of said animal and translated into an HBcAg polypeptide therein, thereby delivering said HBcAg polypeptide to said subject.

51. The method of any of Claims 46 to 50, wherein the components of said immunogenic composition are delivered in a single injection.

52. The method of any of Claims 46 to 51, wherein the components of said immunogenic composition are delivered in two or more injections.

53. The method of any of Claims 46 to 52, further comprising providing ribavirin to said subject.

54. The method of any of Claims 46 to 53, further comprising providing pegylated interferon to said subject.

55. The method of Claim 54, wherein said pegylated interferon is pegylated interferon $\alpha 2a$.

56. The method of any of Claims 46 to 55, further comprising the steps of injecting said polynucleotide encoding an HCV NS3/4A polypeptide into said subject and subjecting a vicinity of said injected polynucleotide encoding an HCV NS3/4A polypeptide to a treatment comprising electroporation.

57. The method of any of Claims 46 to 56, wherein said delivery comprises injecting said immunogenic composition into a subject.

58. The method of Claim 57, further comprising subjecting the vicinity of said site of injection of said immunogenic composition to electroporation.

59. The method of any of Claims 46 to 58, wherein said injection is effected using an IVIN injection device.

60. The method of any of Claims 46 to 59, further comprising administering a boost vaccination to said subject.

61. The method of Claim 60, wherein said boost vaccination comprises an attenuated modified *vaccinia Ankara*.

62. The method of Claim 60 or Claim 61, wherein said boost vaccination comprises a vesicular stomatitis virus.

63. The method of any of Claims 60 to 62, wherein said boost vaccination comprises a rodent HBcAg polynucleotide.

64. The method of any of Claims 62 to 63, wherein said boost vaccination comprises a replication defective Ad6 vector expressing a NS3-NS5B gene fusion.

65. The method of any of Claims 62 to 64, wherein said boost vaccination comprises a replication defective Ad24 vector expressing a NS3-NS5B gene fusion

66. The method of any of Claims 62 to 65, wherein said boost vaccination is administered within 28 days of the administration of said polynucleotide encoding an HCV NS3/4A polypeptide.

67. The method of any of Claims 62 to 66, wherein said boost vaccination is administered within 14 days of the administration of said polynucleotide encoding an HCV NS3/4A polypeptide.

68. The method of any of Claims 62 to 67, wherein said boost vaccination is administered within 7 days of the administration of said polynucleotide encoding an HCV NS3/4A polypeptide.

69. An apparatus for intracellular delivery in a living animal by overloading a tissue locally comprising:

- a delivery unit, which controls an injection parameter;

- a hub connected to the delivery unit and comprising at least one electrical connector;

- a plurality of needles connected to the hub, wherein each needle of the plurality of needles comprises:

- a closed end;

- a needle barrel;

- a plurality of apertures that are disposed on the needle barrel, wherein the apertures on each needle barrel oppose the apertures on at least one other needle barrel so as to generate an opposing direction of delivery of a delivered agent or a cross-spray pattern of delivery of a delivered agent;

- an electrode;

- an electrical power supply configured to generate an electric field at the electrode, wherein the electrical power supply is connected to the electrode through the at least one electrical connector;

- and further comprising one or more of the immunogenic compositions of Claims 21-45.

70. The intracellular delivery apparatus of Claim 69, wherein at least one needle of the plurality of needles is a needle-electrode, which is electrically connected to the electrical power supply and is configured to generate an electric field.

71. The intracellular delivery apparatus of Claim 69 or 70, wherein the intracellular delivery apparatus comprises a plurality of needle-electrodes, which are electrically connected to the electrical power supply, and configured to generate an electric field.

72. The intracellular delivery apparatus of any one of Claims 69 to 71, wherein the intracellular delivery apparatus comprises four needles arranged in a Y-type pattern with a center needle and three outer needles disposed around the center needle, wherein the center needle is a needle-electrode configured to apply a first polarity voltage, and the three outer needles are needle-electrodes, configured to apply a second polarity voltage.

73. The intracellular delivery apparatus of Claim 72, wherein the center needle comprises apertures along the needle barrel, which are positioned to direct delivery of a delivered agent toward apertures that are present on each of the three outer needles and each of the three outer needles comprises apertures along the needle barrel, which are positioned to direct delivery of a delivered agent toward apertures that are present on the center needle, and wherein the outer needles do not have apertures positioned to deliver a delivered agent away from the center needle.

74. The intracellular delivery apparatus of any one of Claims 69 to 73, wherein the intracellular delivery apparatus comprises an array of needles comprising a center needle and a plurality of outer needles disposed around the center needle, wherein the center needle is a needle-electrode configured to apply a first polarity voltage, and the outer needles are needle-electrodes, configured to apply a second polarity voltage, wherein the center needle comprises apertures along the needle barrel, which are positioned to direct delivery of a delivered agent toward the outer needles and the outer needles comprise apertures along the needle barrel, which are positioned to direct delivery of a delivered agent toward the center needle and/or an adjacent needle, and wherein the outer needles do not have apertures that allow delivery of a delivered agent away from an active zone defined by an area within the array of needles.

75. The intracellular delivery apparatus of any one of Claims 69 to 74, wherein the intracellular delivery apparatus further comprises seven needles that are arranged in a hexagonal pattern, wherein one needle, which is a needle-electrode, located at the center of the hexagonal pattern.

76. The intracellular delivery apparatus of any one of Claims 69 to 75, further comprising:

- a syringe in fluid communication with the hub;
- a channel configured to receive the syringe;

a collar operable to fasten the syringe within the channel;

a handle, which operates to enclose the syringe and hub within the channel such that the closed ends of the needle barrels protrude from the channel and are available to engage a subject;

a charging element configured to be charged by the operation of the handle, the charging element coupled to a trigger and the syringe such that operation of the trigger releases the charging element and the charging element acts on the syringe, thereby displacing prophylactic and/or therapeutic material out of the syringe; and

an electrical port configured to mate with the electrical connector on the hub of the hypodermic needle device thereby establishing electrical contact between the electrical power supply and the electrodes and/or needle-electrodes.

77. The intracellular delivery apparatus of any one of Claims 69 to 76, wherein the hub is a pocket hub, comprising an individual reservoir of a delivered agent for each needle or needle electrode.

78. The intracellular delivery apparatus of any one of Claims 69 to 77, wherein the hub comprises a single reservoir of a delivered agent for each needle or needle-electrode.

79. The intracellular delivery apparatus of any one of Claims 69 to 78, wherein at least one needle of the plurality of needles is at least partially laminated with an electrically non-conductive material.

80. The intracellular delivery apparatus of any one of Claims 69 to 79, further comprising an electrical power supply controller configured to control generation of the electric field.

81. The intracellular delivery apparatus of Claim 80, wherein controlling generation of the electric field comprises controlling one or more of the pulse voltage, polarity of the needle-electrodes, the number of pulses, the pulse pattern, or the pulse duration.

82. The intracellular delivery apparatus of any one of Claims 76 to 81, wherein the charging element is a spring configured to be compressed by the operation of the handle and configured to decompress upon operation of the trigger or when activated by an electrically operated transducer.

83. The intracellular delivery apparatus of any one of Claims 69 to 82, wherein the electric field applied at the electrode and/or needle electrodes is commutating.

84. The intracellular delivery apparatus of any one of Claims 69 to 82, wherein the injection parameter is injection speed.

85. The intracellular delivery apparatus of any one of Claims 69 to 82, wherein the injection parameter is injection pressure.

86. The intracellular delivery apparatus of any one of Claims 69 to 82, for use in DNA vaccination.

87. The apparatus of any one of Claims 69-86 for use in administering an amount of a nucleic acid encoding an antigen that is lower than the amount of nucleic acid encoding the antigen that is required to obtain an equivalent immune response in a subject when said nucleic acid encoding said antigen is administered by injection with a needle having a single aperture, such as a conventional hypodermic needle, and electroporation.

88. The apparatus of Claim 87, wherein said polynucleic acid encodes an HCV polypeptide.

89. The apparatus of Claim 88, wherein said polynucleotide encodes an HCV NS3/NS4A, NS3/4A/5A, NS3/4A/NS5B, or NS3/4A/5A/5B polypeptide.

90. The apparatus of any of Claims 87-89, wherein said polynucleotide encodes an HBcAg polypeptide.

91. The apparatus of any of Claims 87-90, wherein said polynucleotide encodes a polypeptide cleavage site.

92. The apparatus of Claim 91, wherein said cleavage site is a protease cleavage site.

93. The apparatus of Claim 91, wherein said cleavage site is a translation interruption site.

94. The apparatus of Claim 93, wherein said translation interruption site is a 2A ribosomal polypeptide translation interruption site.

95. The apparatus of any one of Claims 69-86 for use in administering an amount of a nucleic acid encoding an antigen that is insufficient to generate an immune response in a subject by injection with a needle having a single aperture, such as a conventional hypodermic needle, and electroporation.

96. The apparatus of Claim 95, wherein said polynucleic acid encodes an HCV polypeptide.

97. The apparatus of Claim 96, wherein said polynucleotide encodes an HCV NS3/NS4A, NS3/4A/5A, NS3/4A/NS5B, or NS3/4A/5A/5B polypeptide.

98. The apparatus of any of Claims 95-97, wherein said polynucleotide encodes an HBcAg polypeptide.

99. The apparatus of any of Claims 95-98, wherein said polynucleotide encodes a polypeptide cleavage side.

100. The apparatus of Claim 99, wherein said cleavage site is a protease cleavage site.

101. The apparatus of Claim 99, wherein said cleavage site is a translation interruption site.

102. The apparatus of Claim 101, wherein said translation interruption site is a 2A ribosomal polypeptide translation interruption site.

103. Use of the apparatus of any one of Claims 69-86 to deliver an amount of a nucleic acid encoding an antigen that is lower than the amount of nucleic acid encoding the antigen that is required to obtain an equivalent immune response in a subject when said nucleic acid encoding said antigen is administered by injection with a needle having a single aperture, such as a conventional hypodermic needle, and electroporation.

104. The use of Claim 103, wherein said polynucleic acid encodes an HCV polypeptide.

105. The use of Claim 104, wherein said polynucleotide encodes an HCV NS3/NS4A, NS3/4A/5A, NS3/4A/NS5B, or NS3/4A/5A/5B polypeptide.

106. The use of any of Claims 103-105, wherein said polynucleotide encodes an HBcAg polypeptide.

107. The use of any of Claims 103-106, wherein said polynucleotide encodes a polypeptide cleavage side.

108. The use of Claim 107, wherein said cleavage site is a protease cleavage site.

109. The use of Claim 107, wherein said cleavage site is a translation interruption site.

110. The use of Claim 109, wherein said translation interruption site is a 2A ribosomal polypeptide translation interruption site.

111. Use of the apparatus of any one of Claims 69-86 to deliver an amount of a nucleic acid encoding an antigen that is insufficient to generate an immune response in a subject by injection with a needle having a single aperture, such as a conventional hypodermic needle, and electroporation.

112. The use of Claim 111, wherein said polynucleic acid encodes an HCV polypeptide.

113. The use of Claim 112, wherein said polynucleotide encodes an HCV NS3/NS4A, NS3/4A/5A, NS3/4A/NS5B, or NS3/4A/5A/5B polypeptide.

114. The use of any of Claims 111-113, wherein said polynucleotide encodes an HBcAg polypeptide.

115. The use of any of Claims 111-114, wherein said polynucleotide encodes a polypeptide cleavage site.

116. The use of Claim 115, wherein said cleavage site is a protease cleavage site.

117. The use of Claim 115, wherein said cleavage site is a translation interruption site.

118. The use of Claim 117, wherein said translation interruption site is a 2A ribosomal polypeptide translation interruption site.

119. A method of introducing a delivered agent into a tissue of a subject comprising:
providing the intracellular delivery apparatus of any one of Claims 69 to 82;

providing a voltage source;

inserting a plurality of needles and a plurality of electrodes or a plurality of needle-electrodes of said electroporation apparatus into a tissue of a subject;

displacing the delivered agent through the lumen of the plurality of needles or the plurality of needle electrodes and into the tissue of the subject; and

applying an electric field to the tissue of the subject;

wherein the delivered agent comprises one or more of the immunogenic compositions of Claims 21-45.

120. The method of Claim 119, wherein the displacing of the delivered agent is performed prior to applying the electric field to the tissue.

121. The method of Claim 119, wherein the displacing of the delivered agent is performed subsequent to applying the electric field to the tissue.

122. The method of Claim 119, wherein the displacing of the delivered agent is performed in a two stage process, wherein the first stage comprises displacing the delivered agent into the tissue into an active area and the second stage comprises energizing one or more electrodes or needle electrodes to electroporate the cells within the active area.

123. A method of generating an immune response to an antigen in a subject comprising: administering to a subject by the apparatus disclosed herein, a nucleic acid encoding an antigen, wherein the amount of nucleic acid encoding the antigen that is administered to said subject by said apparatus is lower than the amount of nucleic acid encoding the antigen that is required to obtain an equivalent immune response in a subject when said nucleic acid encoding said antigen is administered by injection with a needle having a single aperture, such as a conventional hypodermic needle, and electroporation.

124. The method of Claim 123, wherein said polynucleic acid encodes an HCV polypeptide.

125. The method of Claim 124, wherein said polynucleotide encodes an HCV NS3/NS4A, NS3/4A/5A, NS3/4A/NS5B, or NS3/4A/5A/5B polypeptide.

126. The method of any of Claims 123-125, wherein said polynucleotide encodes an HBcAg polypeptide.

127. The method of any of Claims 123-126, wherein said polynucleotide encodes a polypeptide cleavage site.

128. The method of Claim 127, wherein said cleavage site is a protease cleavage site.

129. The method of Claim 127, wherein said cleavage site is a translation interruption site.

130. The method of Claim 129, wherein said translation interruption site is a 2A ribosomal polypeptide translation interruption site.

131. A method of generating an immune response to an antigen in a subject comprising: administering to a subject by the apparatus of any one of Claims 69-86, an amount of a nucleic acid encoding an antigen that is insufficient to generate an immune response in a subject by injection with a needle having a single aperture, such as a conventional hypodermic needle, and electroporation.

132. The method of Claim 131, wherein said polynucleic acid encodes an HCV polypeptide.

133. The method of Claim 132, wherein said polynucleotide encodes an HCV NS3/NS4A, NS3/4A/5A, NS3/4A/NS5B, or NS3/4A/5A/5B polypeptide.

134. The method of any of Claims 131-133, wherein said polynucleotide encodes an HBcAg polypeptide.

135. The method of any of Claims 131-134, wherein said polynucleotide encodes a polypeptide cleavage site.

136. The method of Claim 135, wherein said cleavage site is a protease cleavage site.

137. The method of Claim 135, wherein said cleavage site is a translation interruption site.

138. The method of Claim 137, wherein said translation interruption site is a 2A ribosomal polypeptide translation interruption site.

139. An improved method of delivering an immunogenic composition comprising a nucleic acid encoding an antigen, wherein the improvement comprises elicitation of an immune

response by delivering an amount of a nucleic acid encoding an antigen that is lower than the amount of nucleic acid encoding the antigen that is required to obtain an equivalent immune response in a subject when said nucleic acid encoding said antigen is administered by injection with a needle having a single aperture, such as a conventional hypodermic needle, and electroporation.

140. The method of Claim 139, wherein said polynucleic acid encodes an HCV polypeptide.

141. The method of Claim 140, wherein said polynucleotide encodes an HCV NS3/NS4A, NS3/4A/5A, NS3/4A/NS5B, or NS3/4A/5A/5B polypeptide.

142. The method of any of Claims 139-141, wherein said polynucleotide encodes an HBcAg polypeptide.

143. The method of any of Claims 139-142, wherein said polynucleotide encodes a polypeptide cleavage site.

144. The method of Claim 143, wherein said cleavage site is a protease cleavage site.

145. The method of Claim 143, wherein said cleavage site is a translation interruption site.

146. The method of Claim 145, wherein said translation interruption site is a 2A ribosomal polypeptide translation interruption site.

147. An improved method of delivering an immunogenic composition comprising a nucleic acid encoding an antigen, wherein the improvement comprises elicitation of an immune response by delivering an amount of a nucleic acid encoding an antigen that is insufficient to generate an immune response in a subject by injection with a needle having a single aperture, such as a conventional hypodermic needle, and electroporation by the electroporation apparatus.

148. The method of Claim 147, wherein said polynucleic acid encodes an HCV polypeptide.

149. The method of Claim 148, wherein said polynucleotide encodes an HCV NS3/NS4A, NS3/4A/5A, NS3/4A/NS5B, or NS3/4A/5A/5B polypeptide.

150. The method of any of Claims 147-149, wherein said polynucleotide encodes an HBcAg polypeptide.

151. The method of any of Claims 147-150, wherein said polynucleotide encodes a polypeptide cleavage site.

152. The method of Claim 151, wherein said cleavage site is a protease cleavage site.

153. The method of Claim 151, wherein said cleavage site is a translation interruption site.

154. The method of Claim 153, wherein said translation interruption site is a 2A ribosomal polypeptide translation interruption site.

155. A product combination comprising an electroporation device that comprises a plurality of needles each having a closed end and a plurality of apertures along the barrel of said needles, wherein the apertures on separate needles oppose one other but said apertures do not face away from each other so as to create a zone of injection existing in a region of tissue between the opposing apertures of said plurality of needles, wherein at least two of said plurality of needles are capable of passing current so as to enhance delivery of a nucleic acid within said zone of injection, wherein said nucleic acid comprises at least one plasmid that comprises one or more polynucleotides encoding HCV NS3/4A and, optionally HCV NS5A, and HBcAg, optionally an HBcAg that infects a stork or heron, and IL-12, optionally a human IL-12, wherein said one or more polynucleotides are, optionally codon-optimized for human expression and wherein said product combination is used for treatment of HCV in an HCV infected or chronically HCV infected patient.

156. A method of reducing an amount of an immunogenic composition necessary to elicit an immune response in a mammalian subject comprising the steps of:

injecting an immunogenic composition into said subject at an injection site, wherein said immunogenic composition is injected in an amount insufficient to elicit an immune response in the absence of further treatment; and
electroporating said subject at said injection site;
thereby eliciting an immunogenic response.

157. The method of Claim 156, wherein said immunogenic composition comprises a polynucleic acid.

158. The method of Claim 157, wherein said polynucleic acid encodes an HCV polypeptide.

159. The method of Claim 158, wherein said polynucleotide encodes an HCV NS3/NS4A, NS3/4A/5A, NS3/4A/NS5B, or NS3/4A/5A/5B polypeptide.

160. The method of any of Claims 157-159, wherein said polynucleotide encodes an HBcAg polypeptide.

161. The method of any of Claims 157-160, wherein said polynucleotide encodes a polypeptide cleavage side.

162. The method of Claim 161, wherein said cleavage site is a protease cleavage site.
163. The method of Claim 161, wherein said cleavage site is a translation interruption site.
164. The method of Claim 163, wherein said translation interruption site is a 2A ribosomal polypeptide translation interruption site.

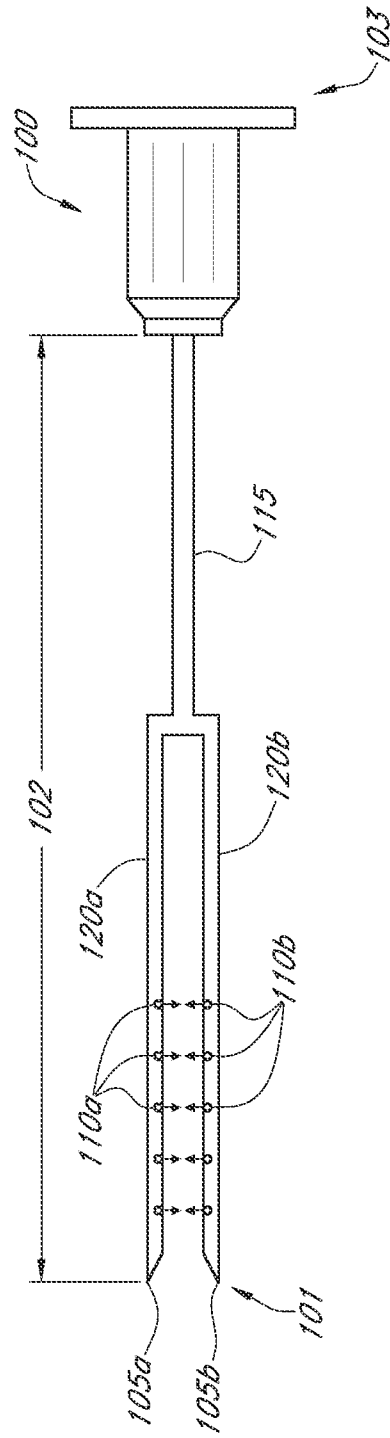


FIG. 1A

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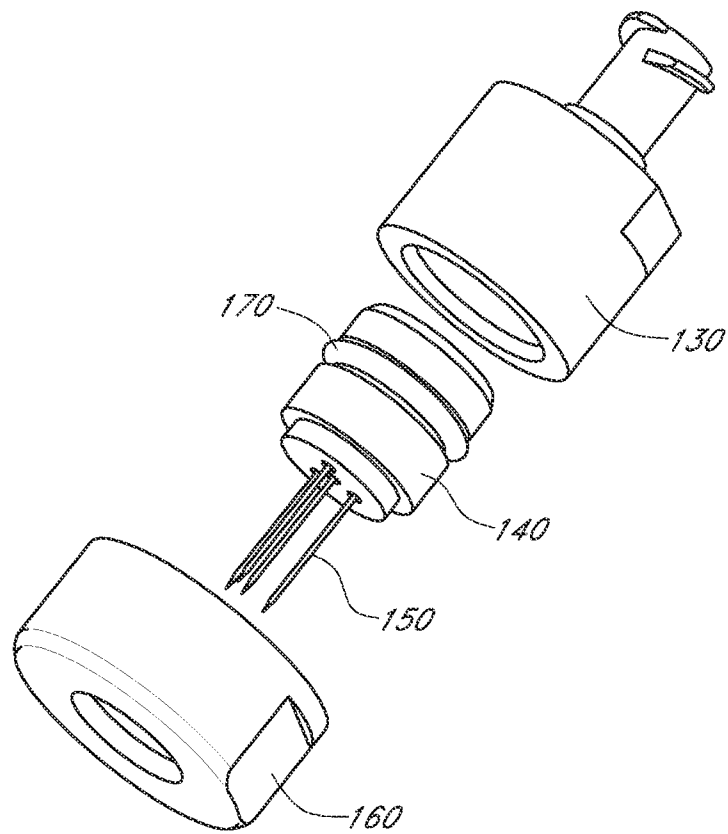


FIG. 1B

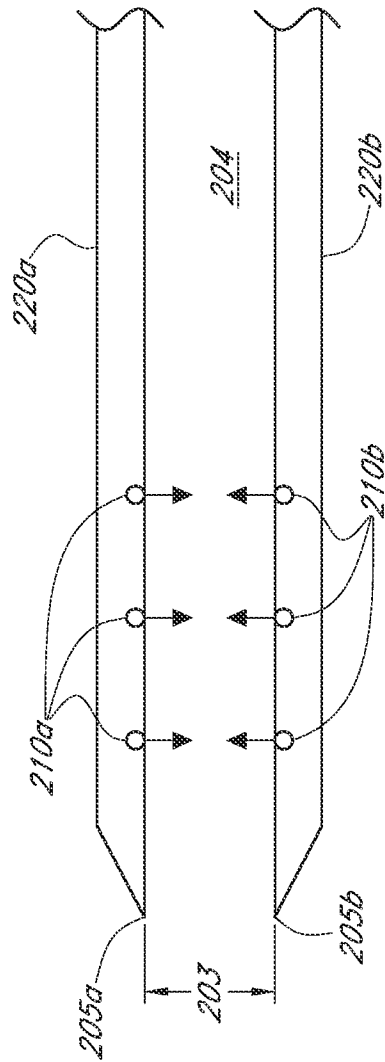


FIG. 2A

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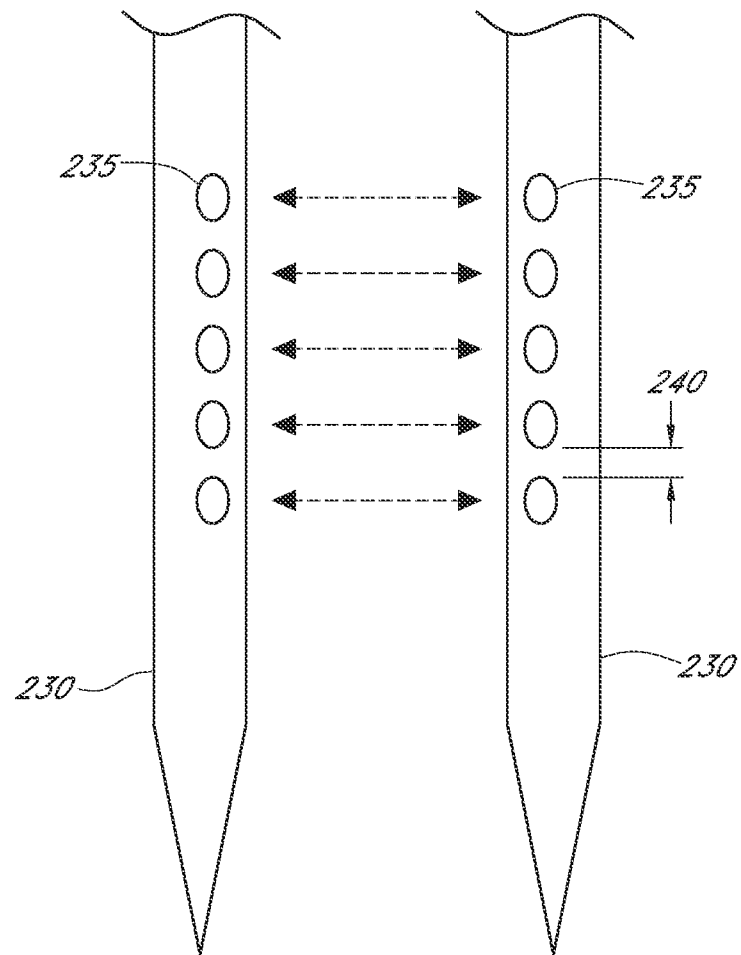


FIG. 2B

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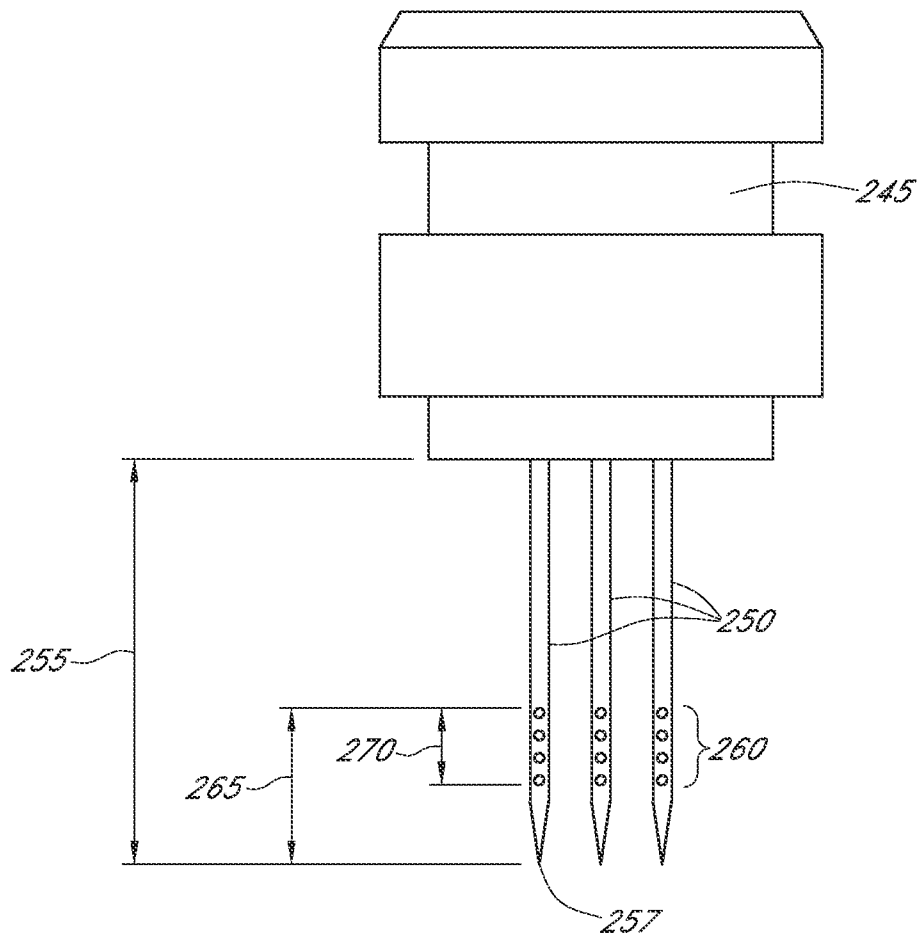


FIG. 2C

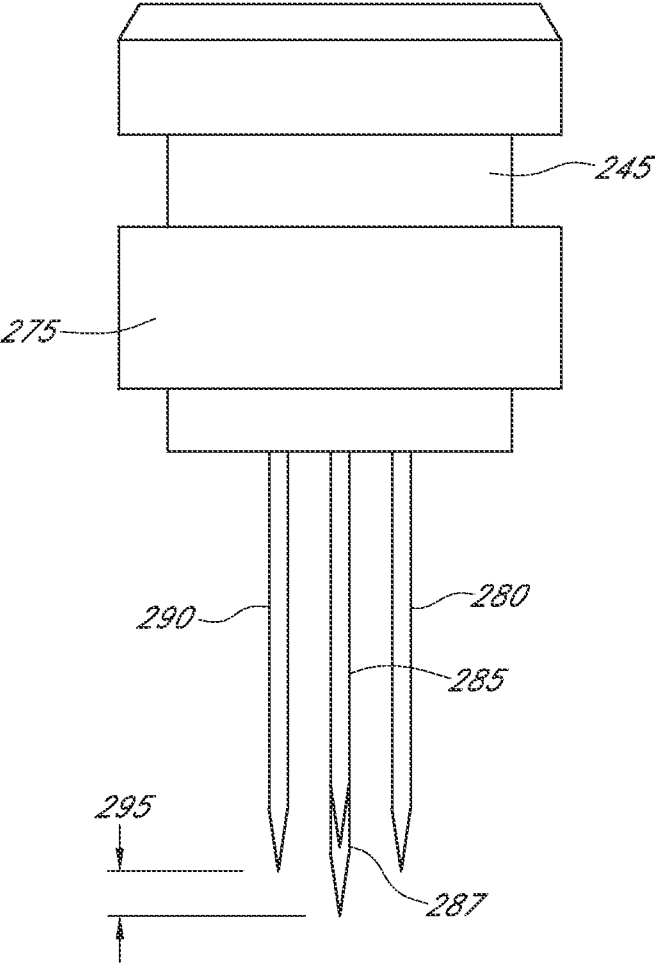


FIG. 2D

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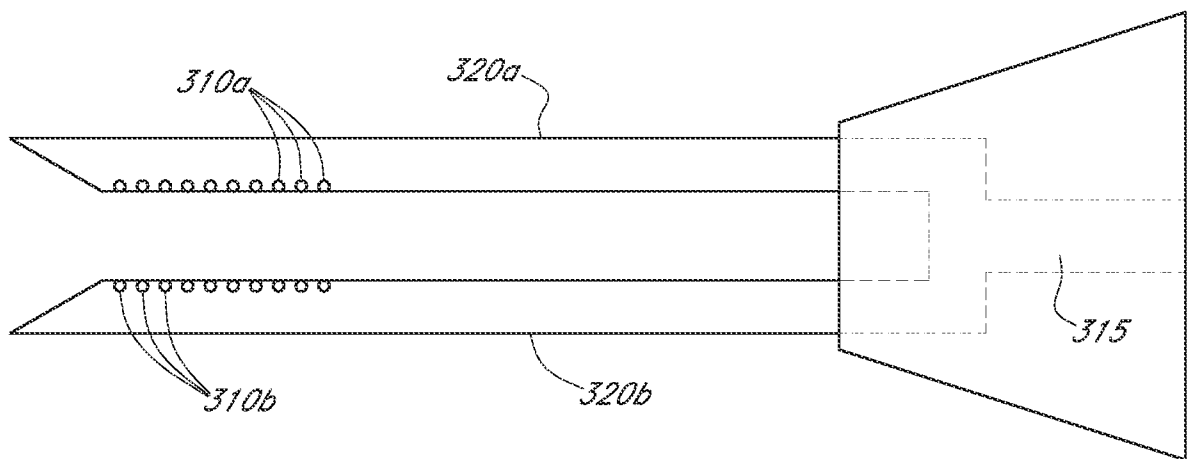


FIG. 3

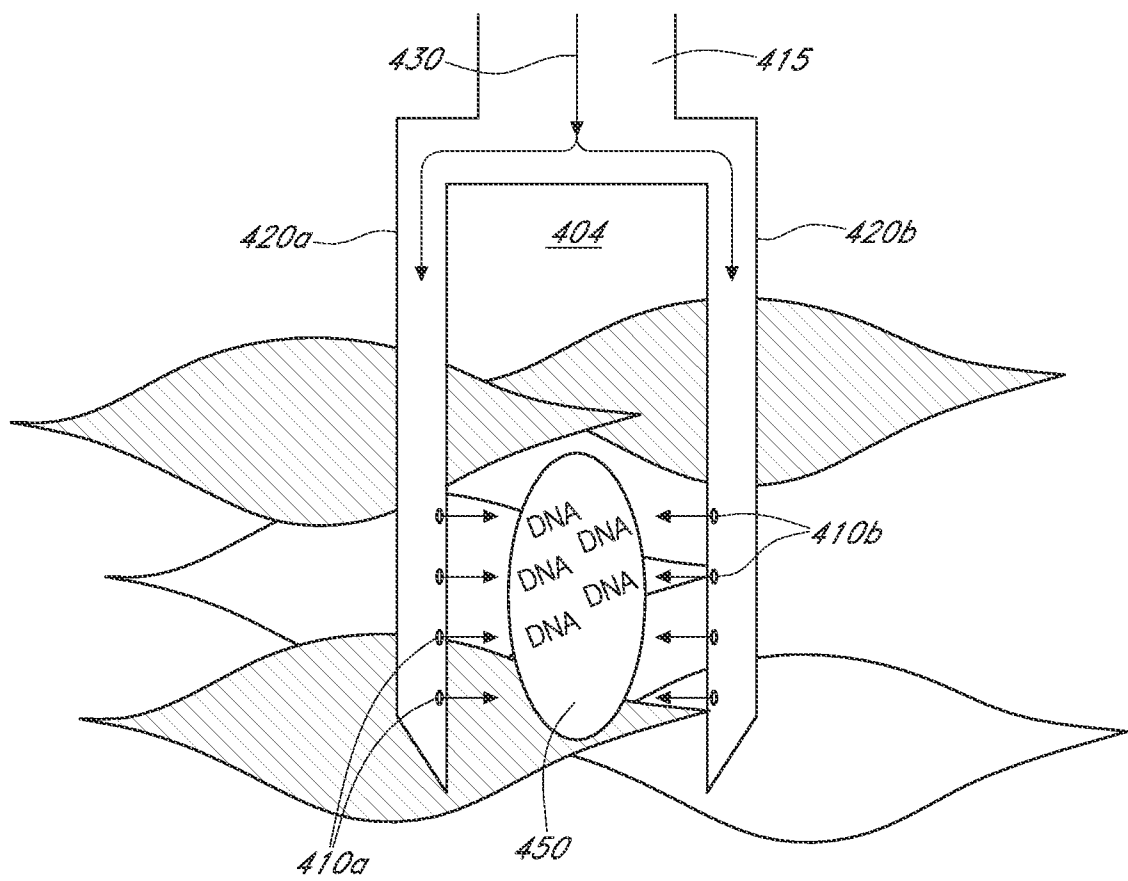


FIG. 4

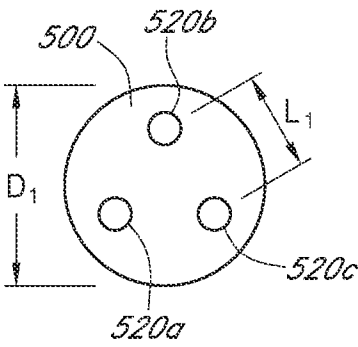


FIG. 5A

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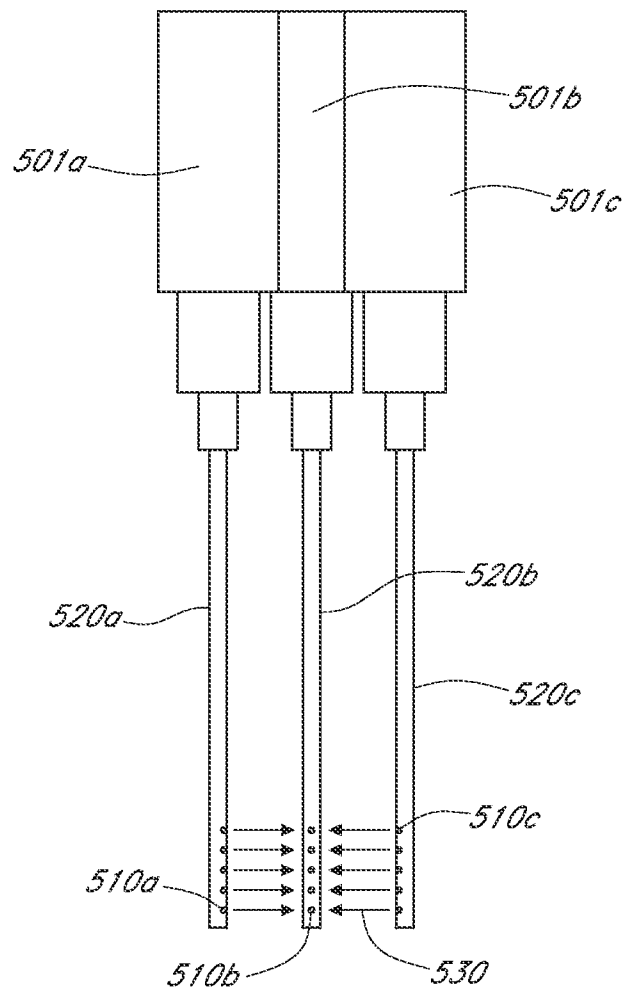


FIG. 5B

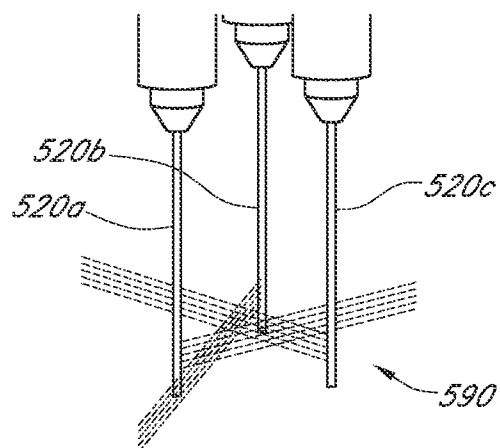


FIG. 5C

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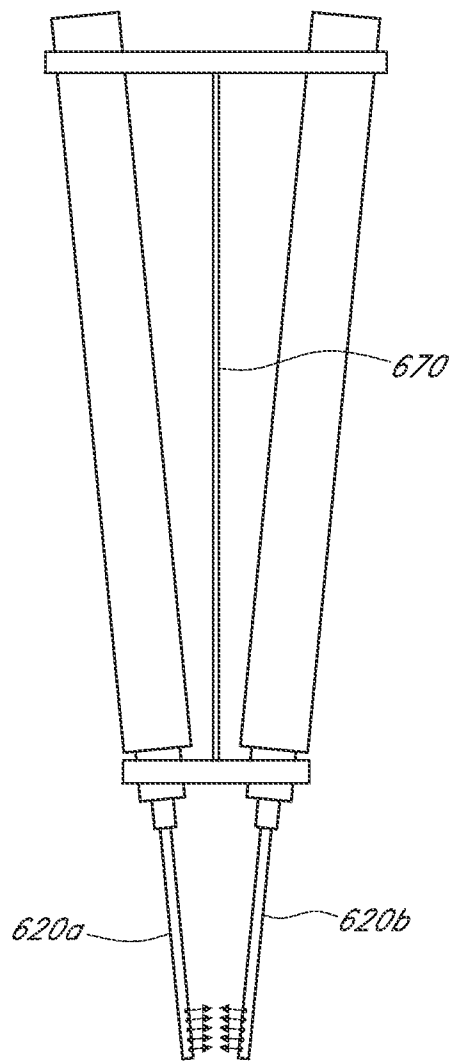


FIG. 6A

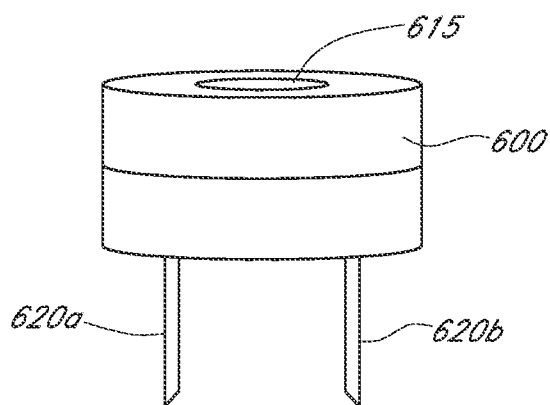


FIG. 6B

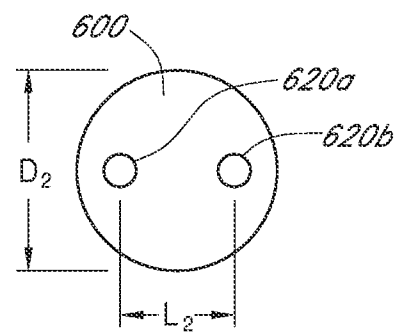


FIG. 6C

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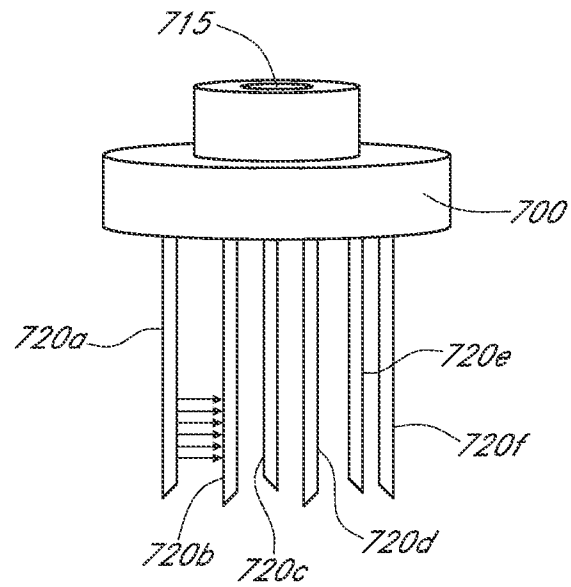


FIG. 7A

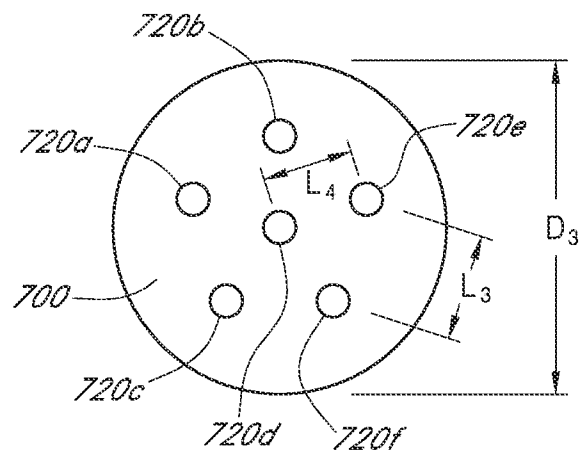


FIG. 7B

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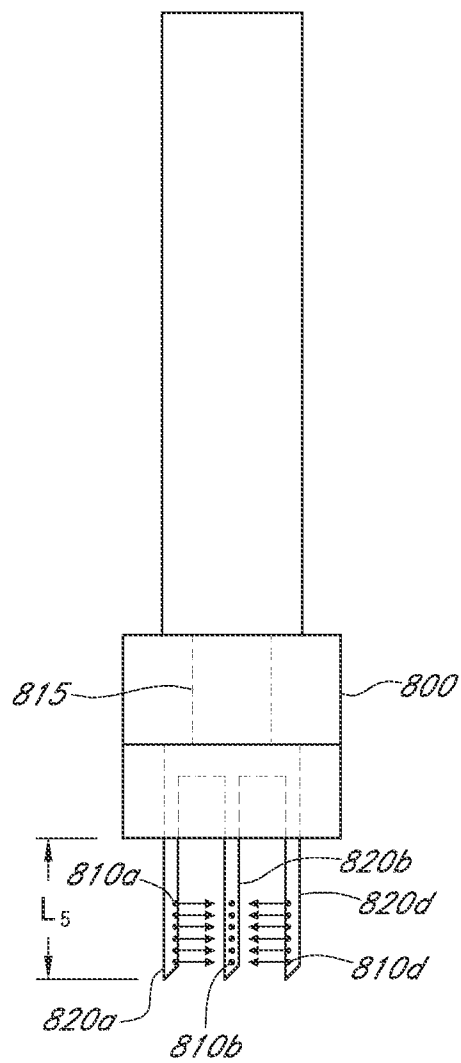


FIG. 8A

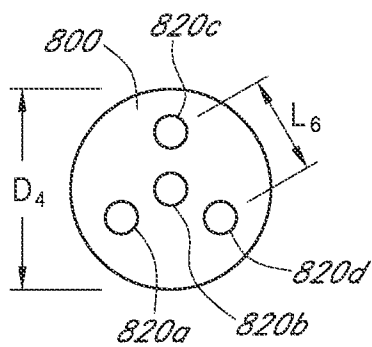


FIG. 8B

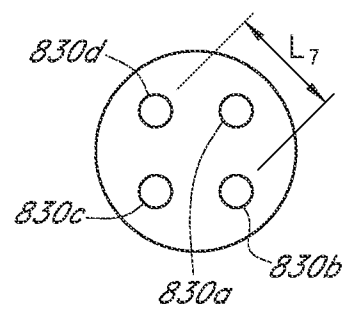


FIG. 8C

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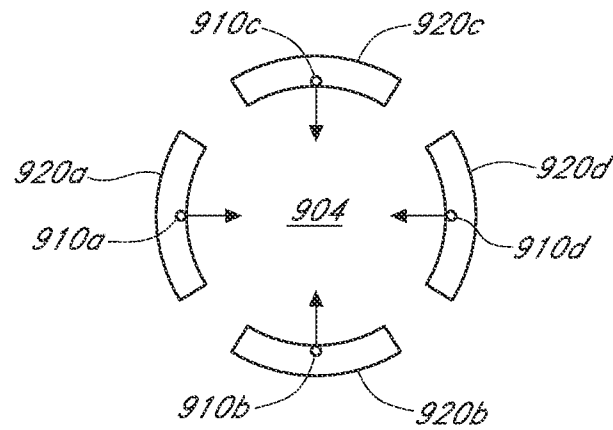


FIG. 9

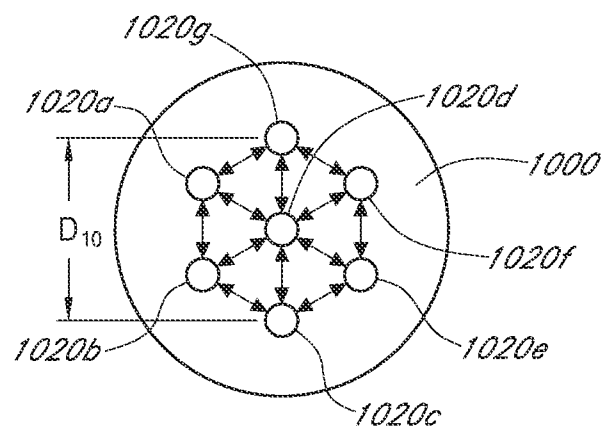


FIG. 10A

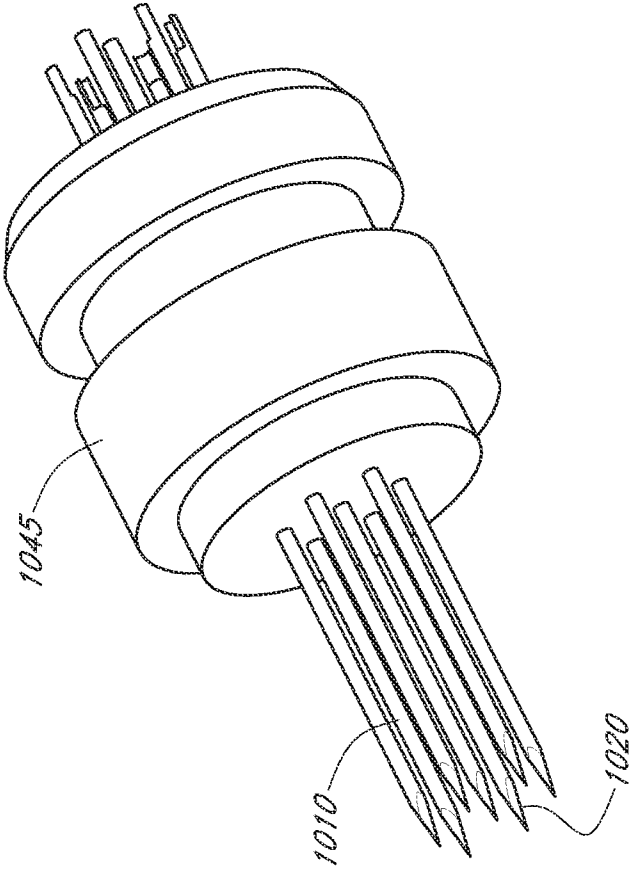


FIG. 10B

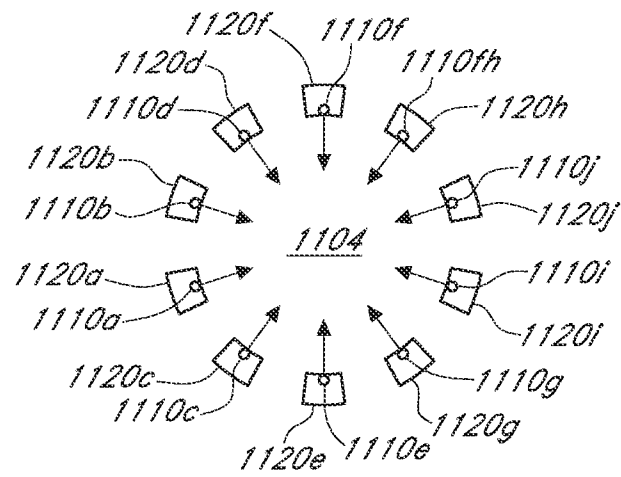


FIG. 11

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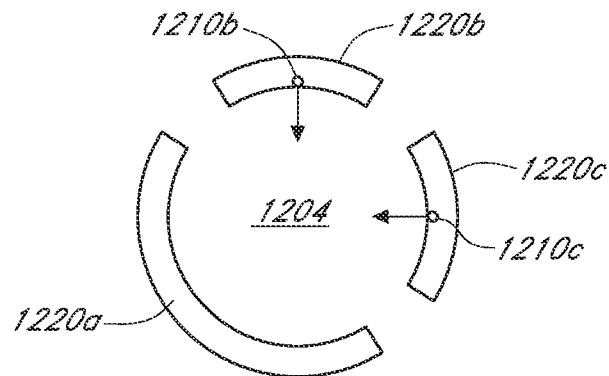


FIG. 12

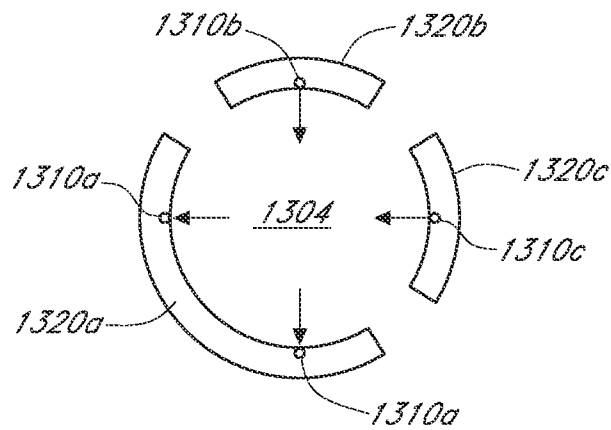


FIG. 13

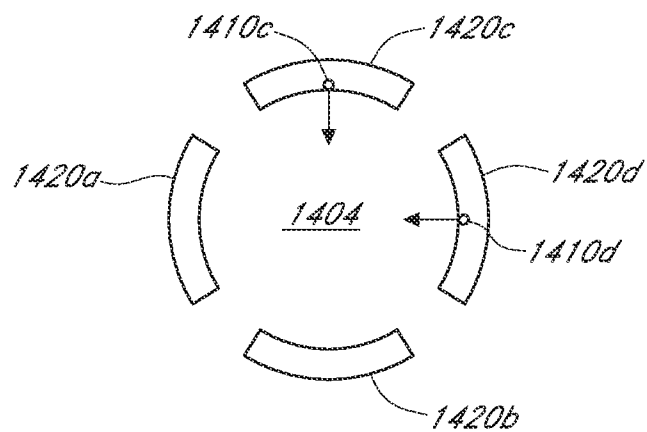


FIG. 14

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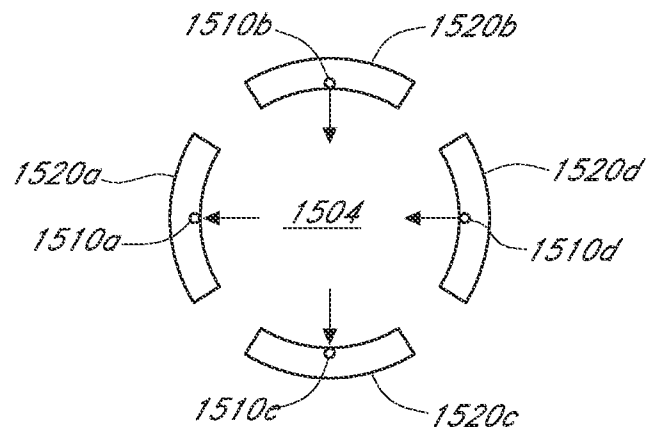


FIG. 15

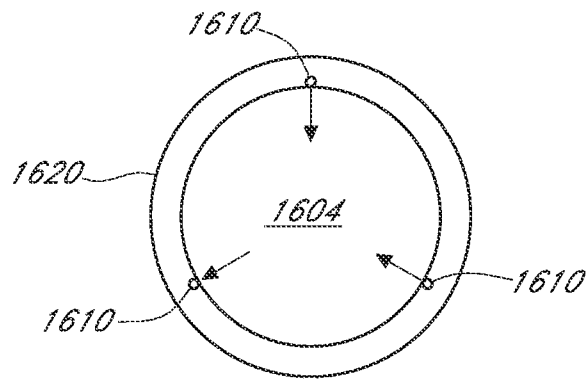


FIG. 16

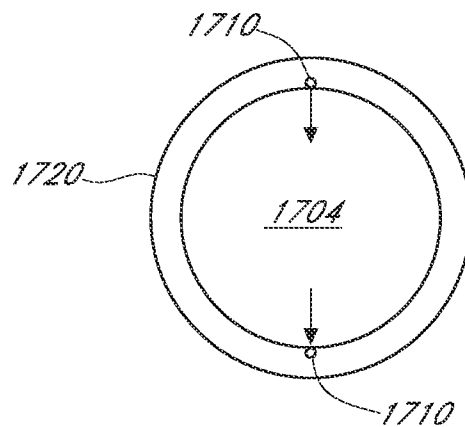


FIG. 17

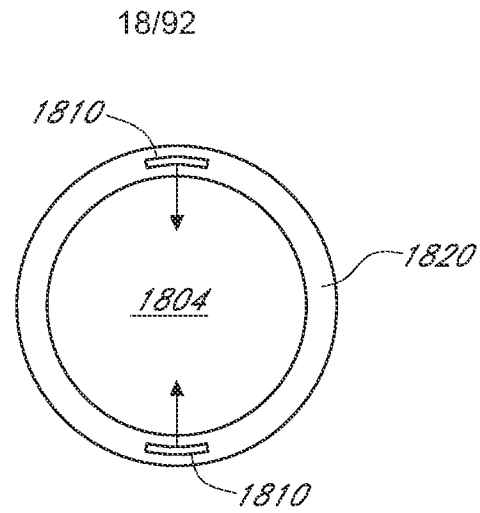


FIG. 18

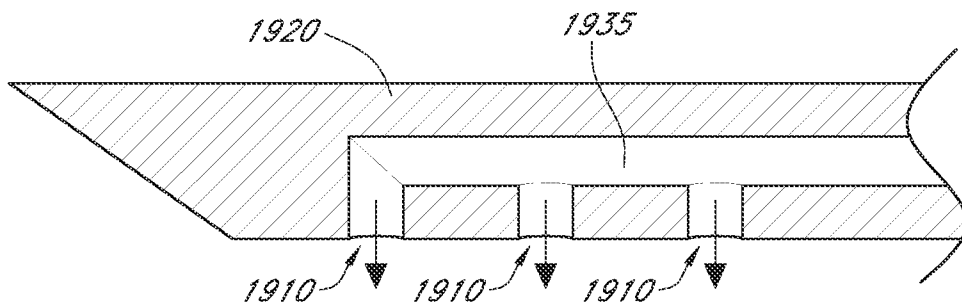


FIG. 19

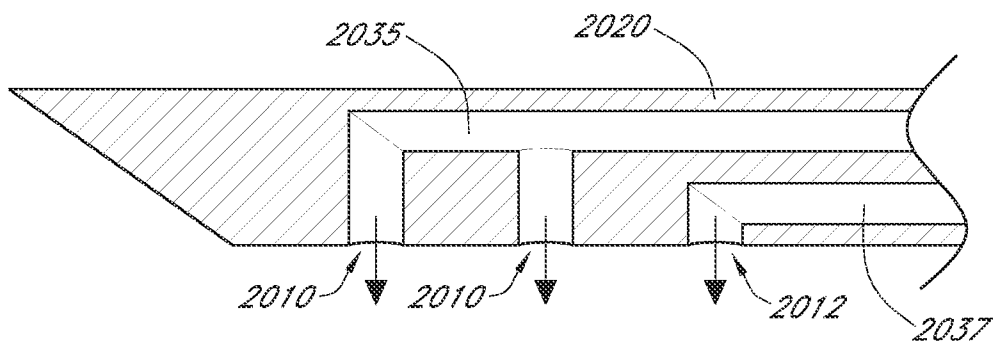
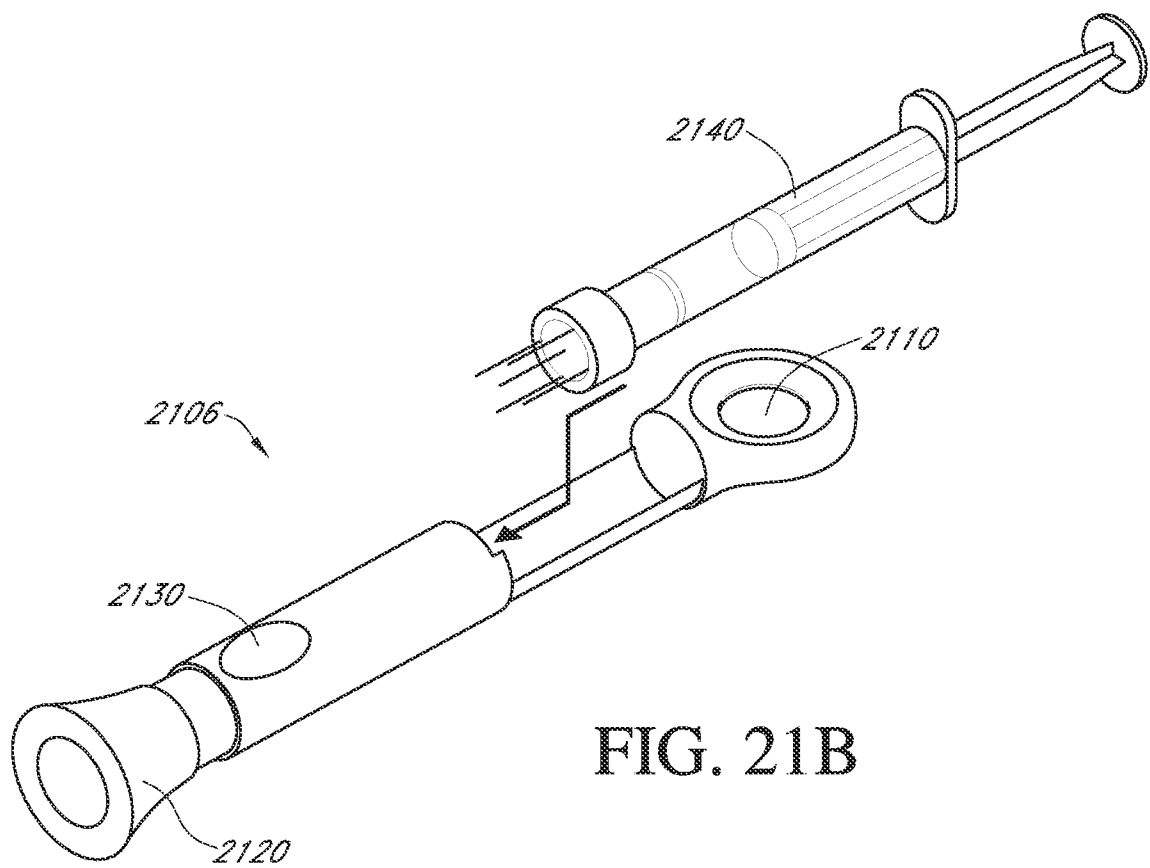
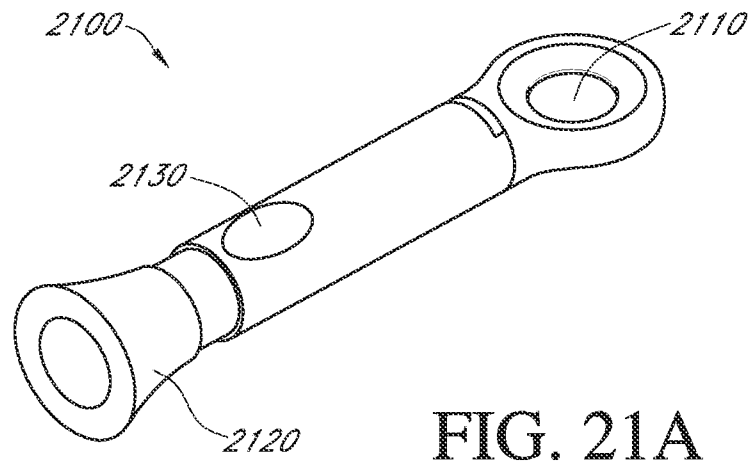
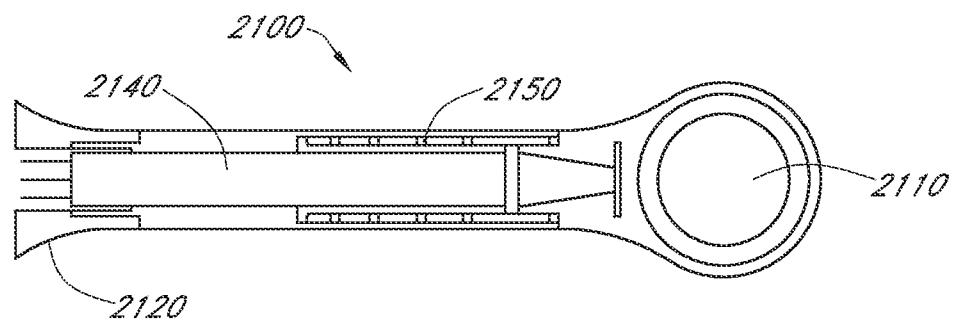
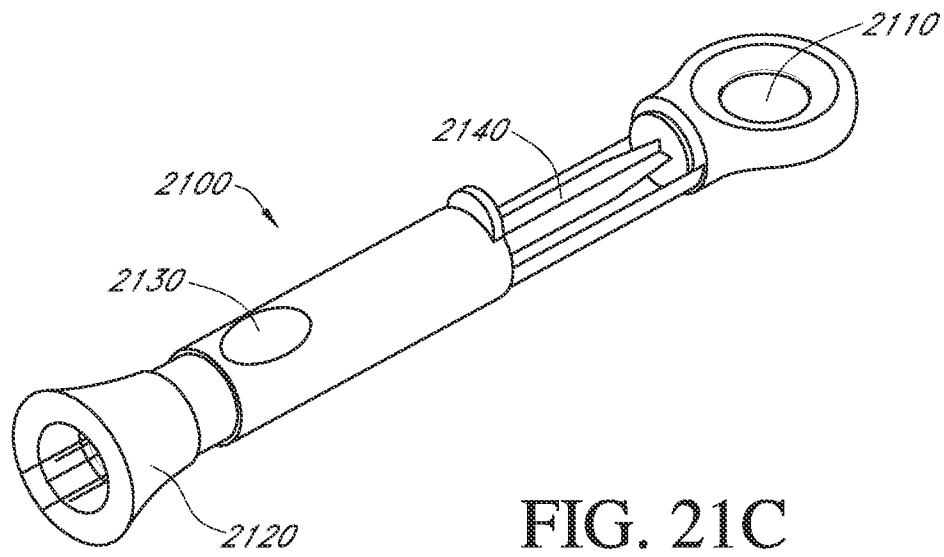


FIG. 20

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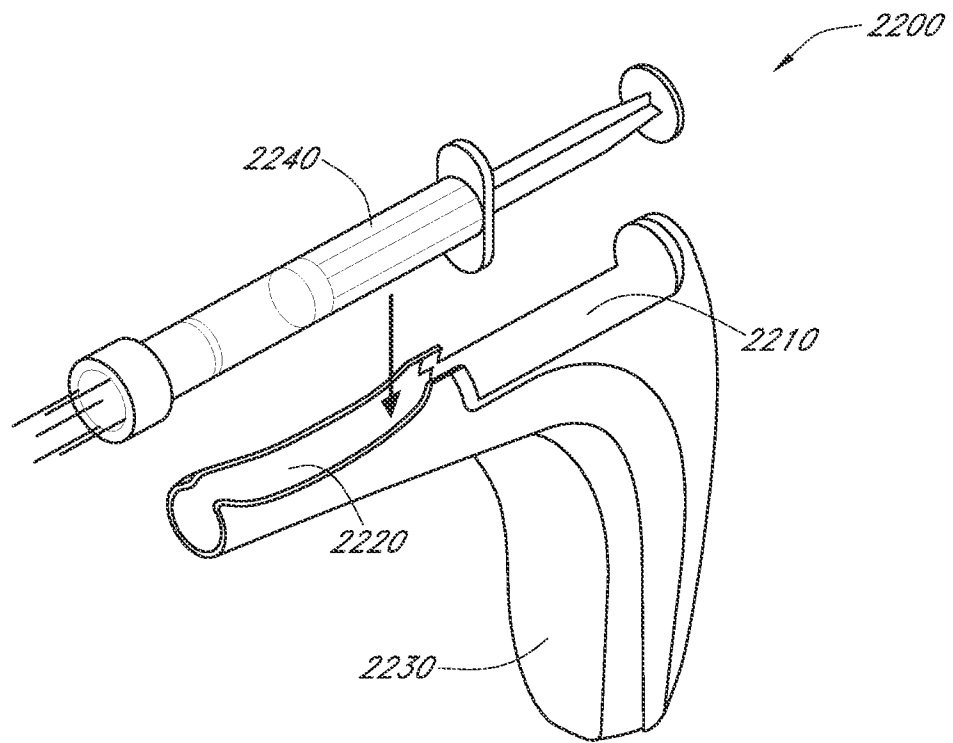


FIG. 22A

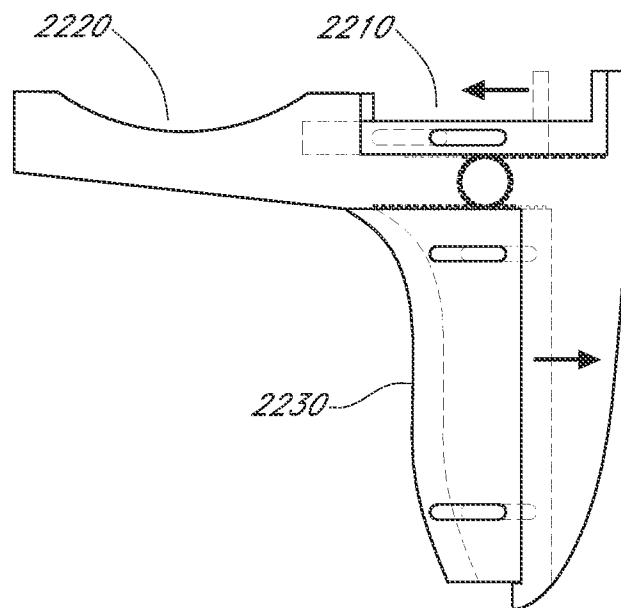


FIG. 22B

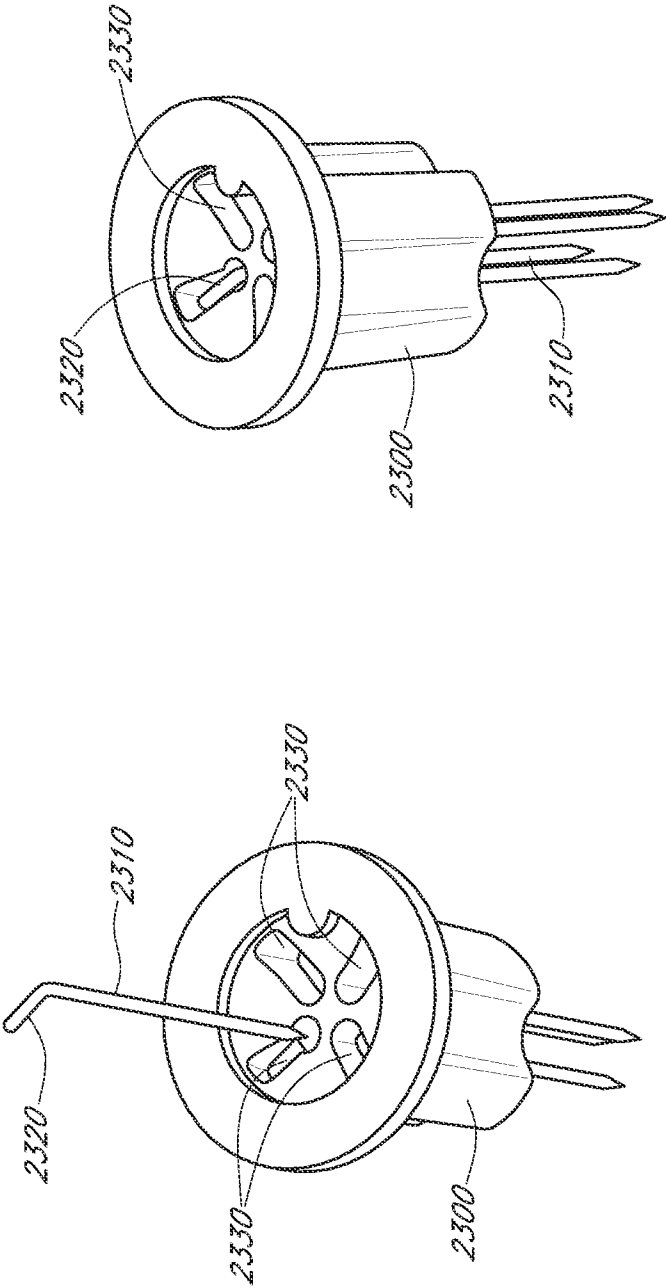


FIG. 23B

FIG. 23A

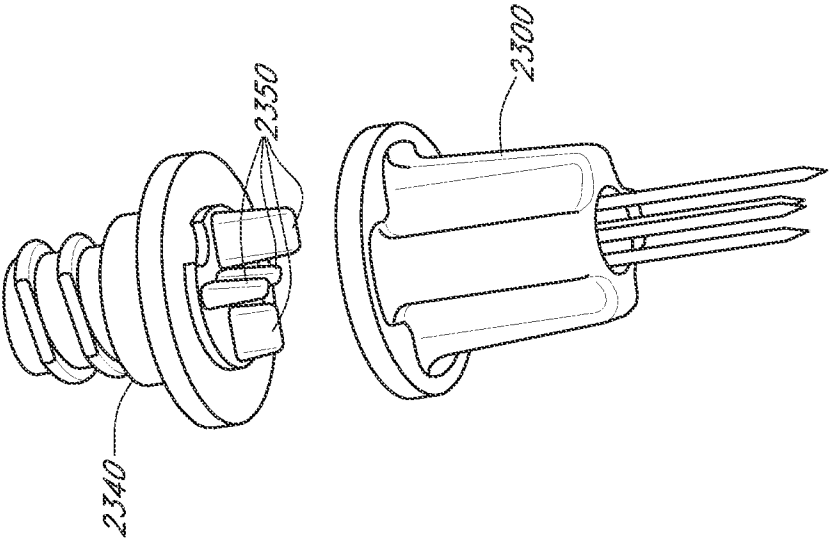


FIG. 23C

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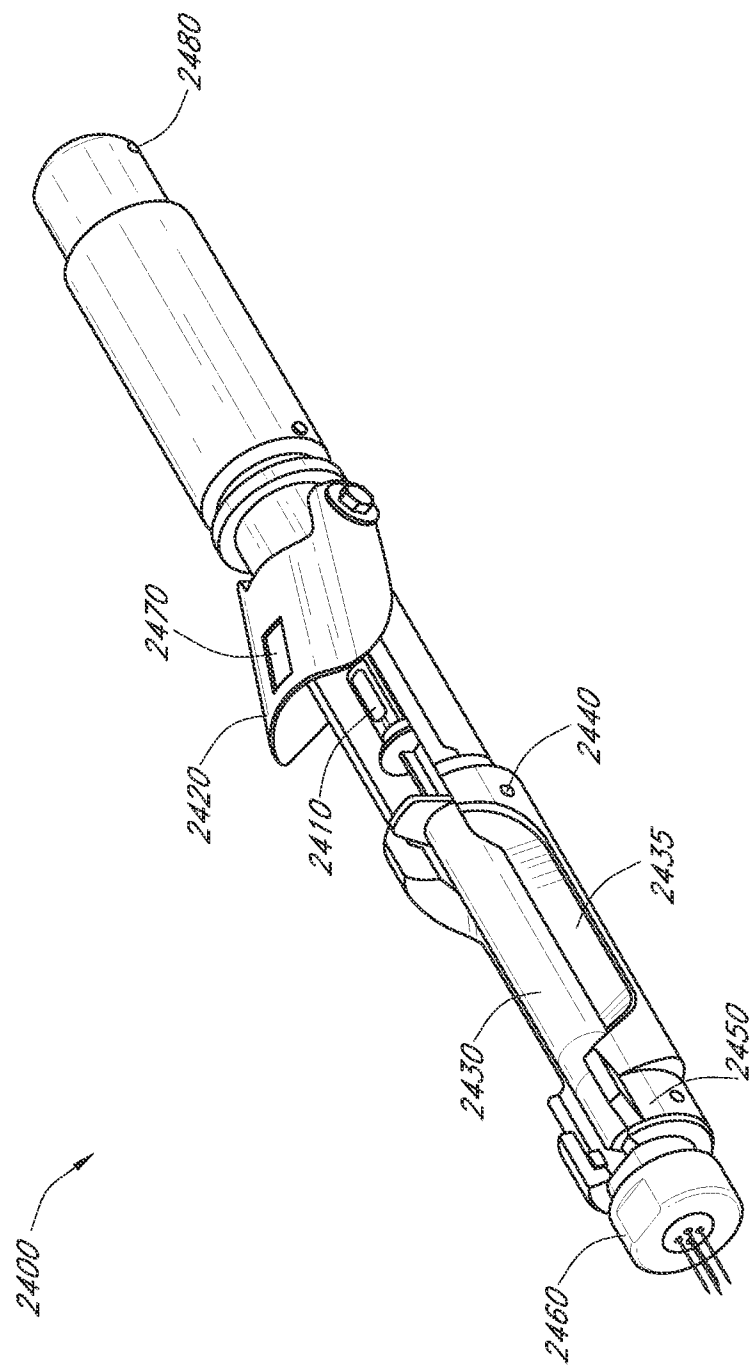


FIG. 24

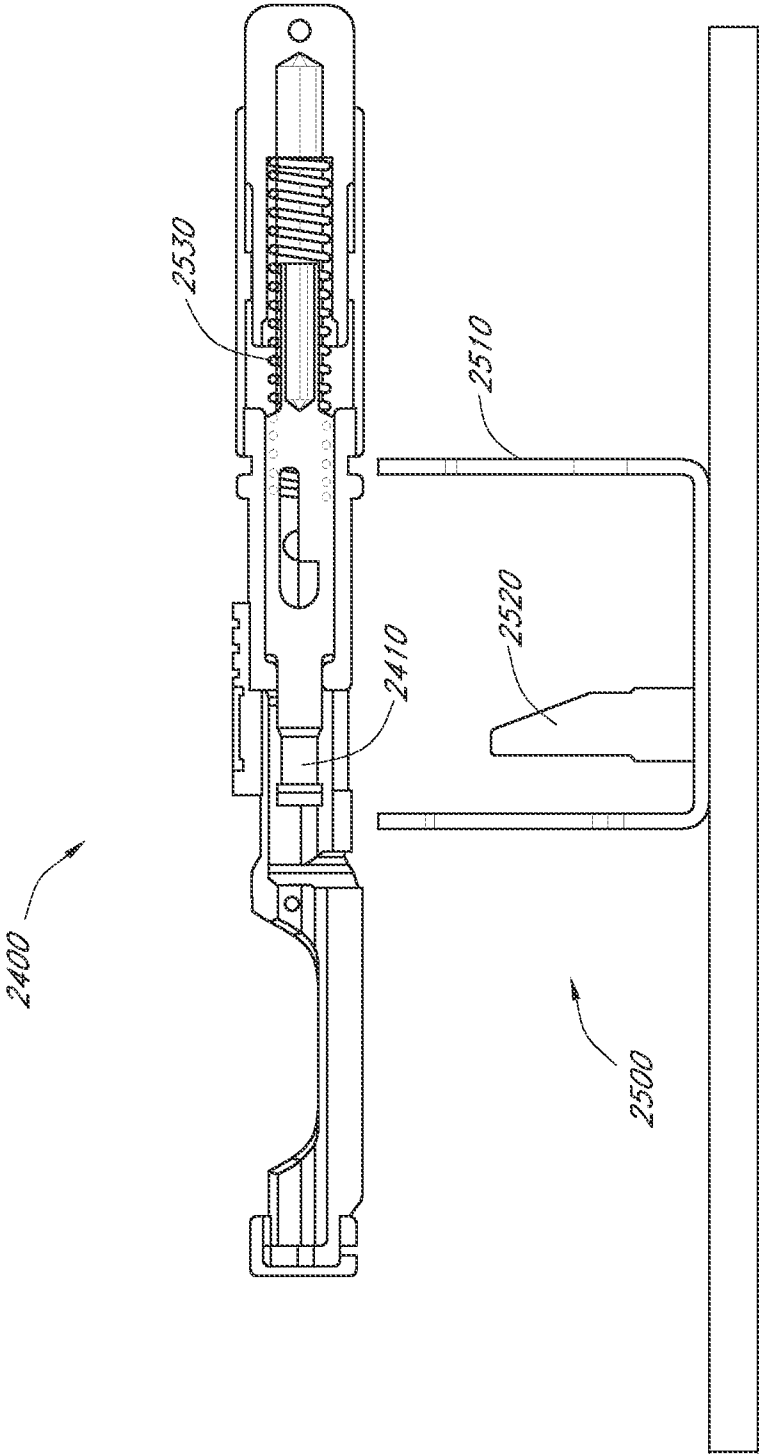


FIG. 25

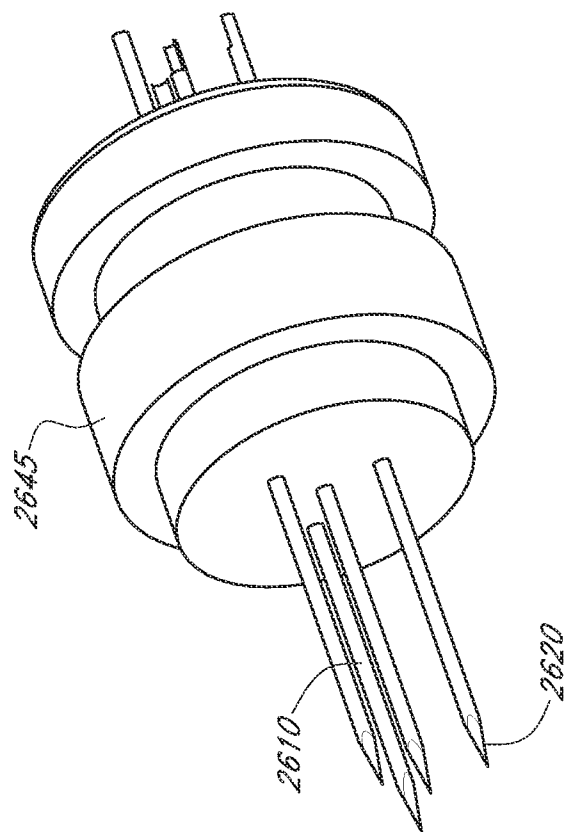


FIG. 26

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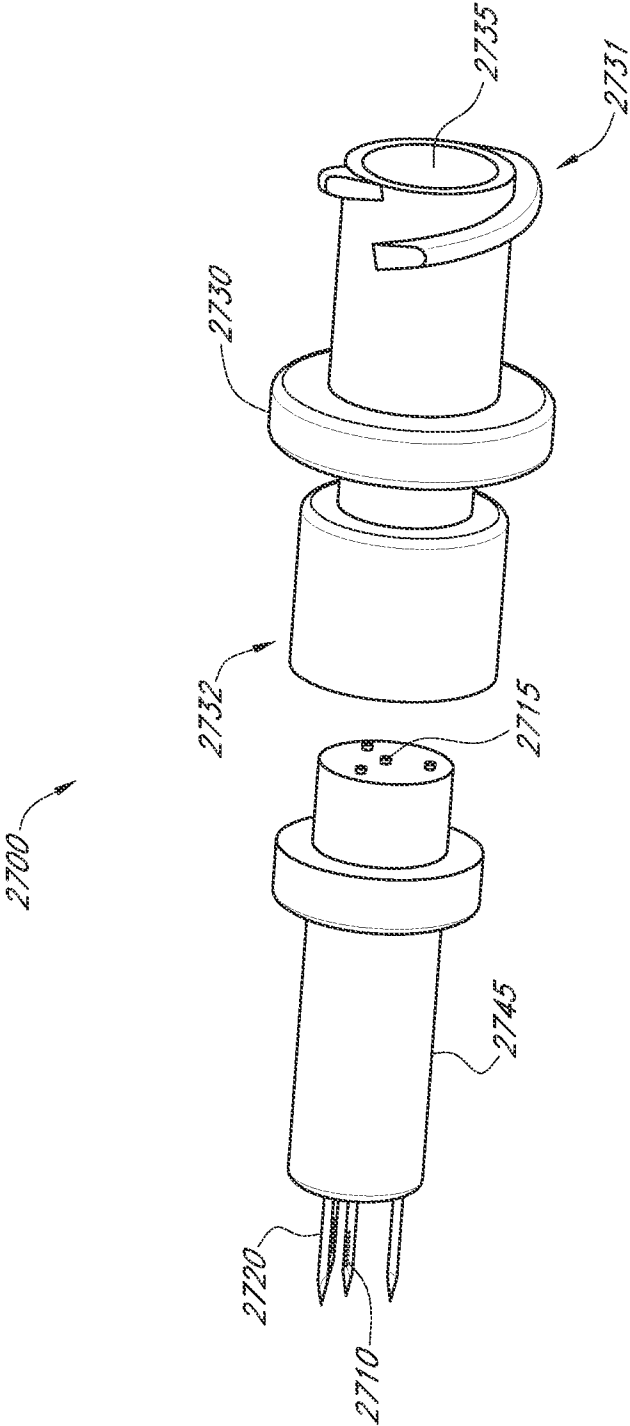


FIG. 27A

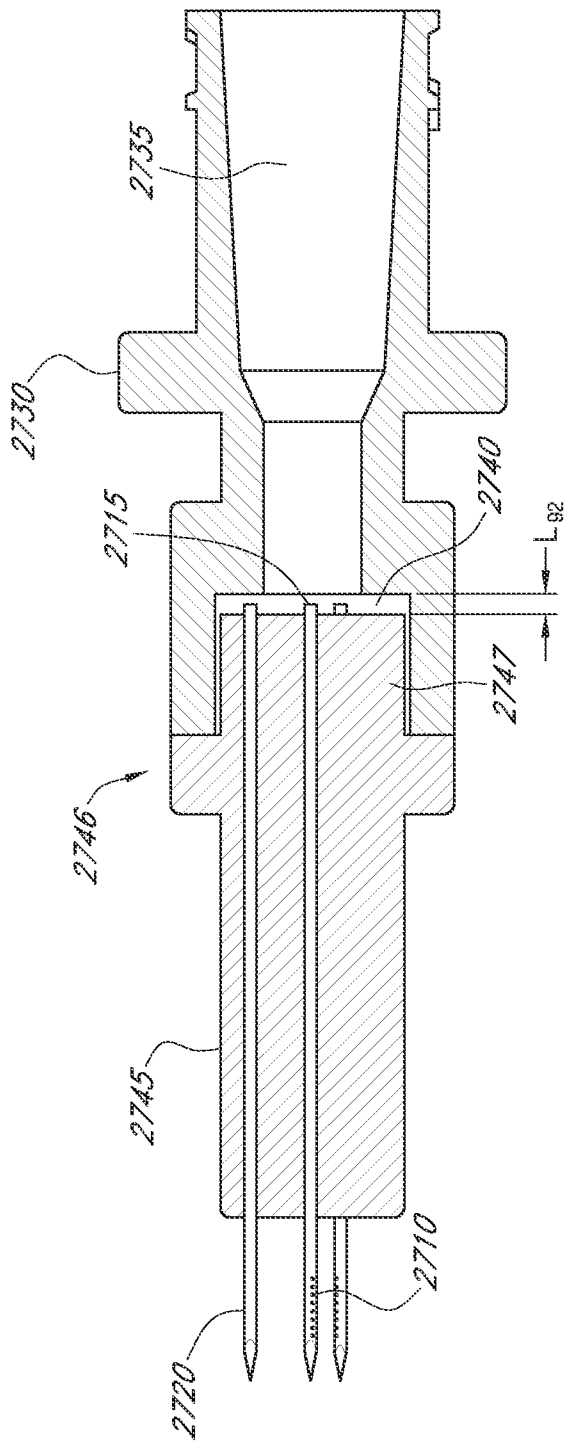


FIG. 27B

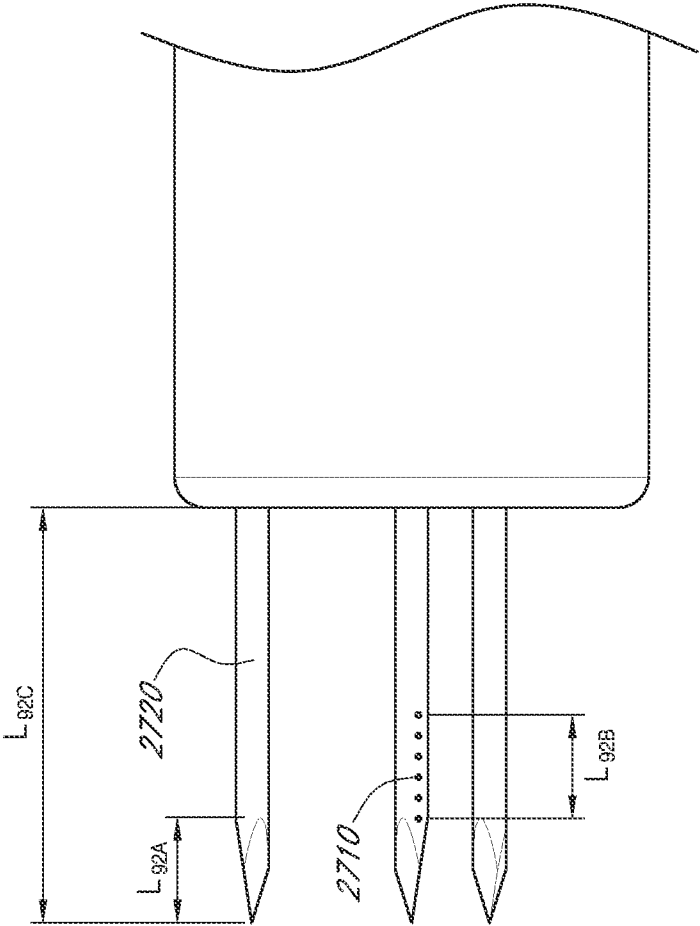


FIG. 27C

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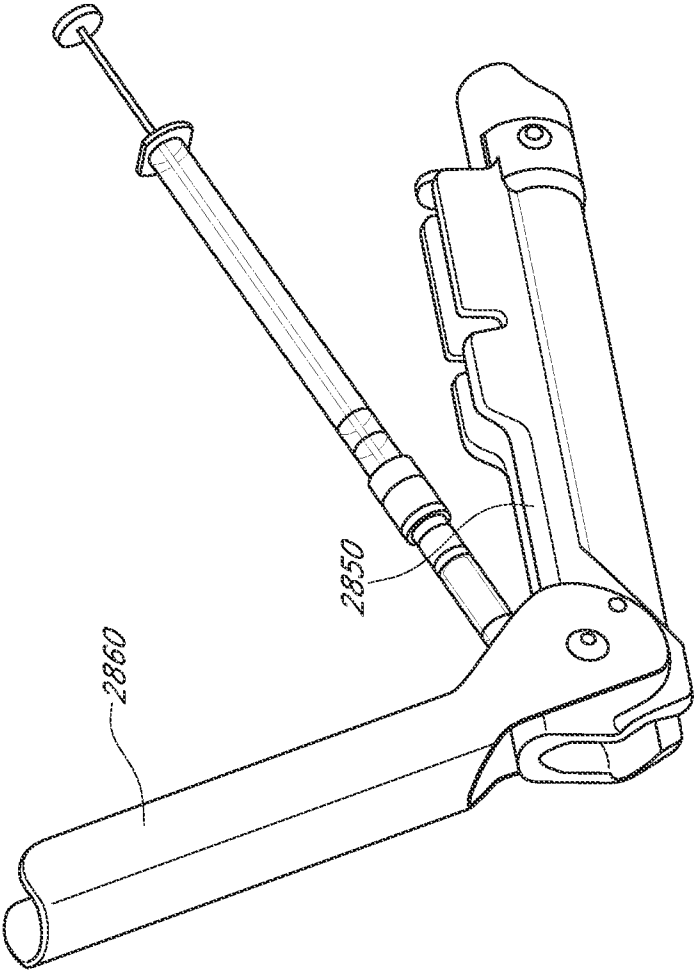


FIG. 28A

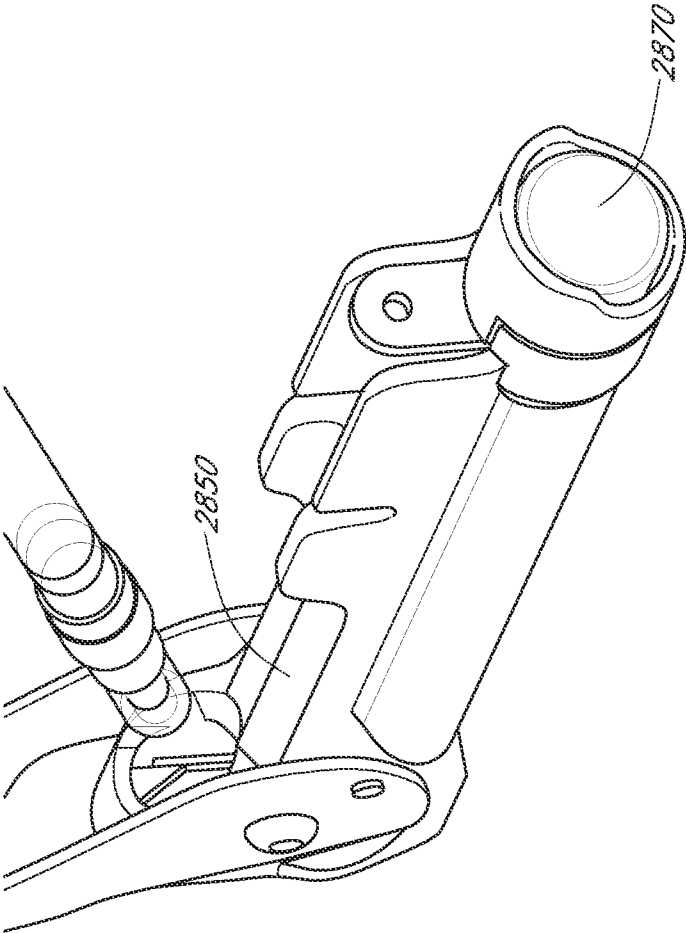


FIG. 28B

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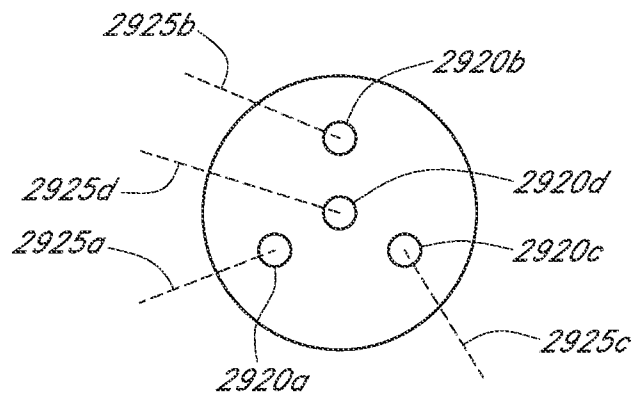


FIG. 29A

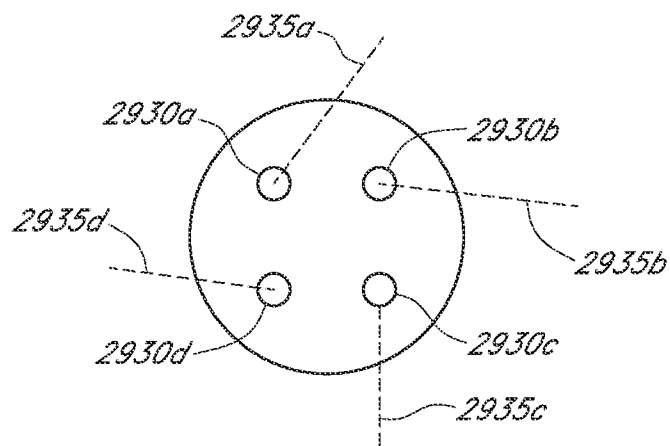


FIG. 29B

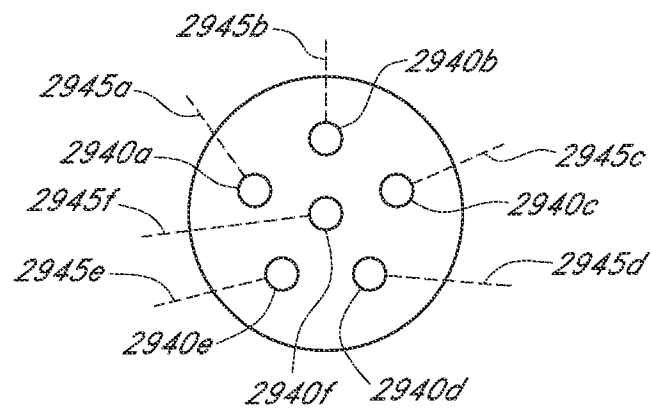


FIG. 29C

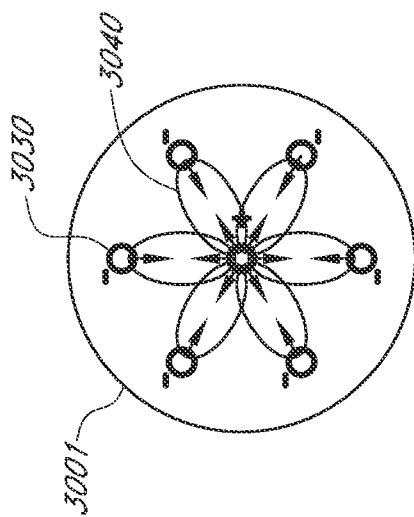


FIG. 30A

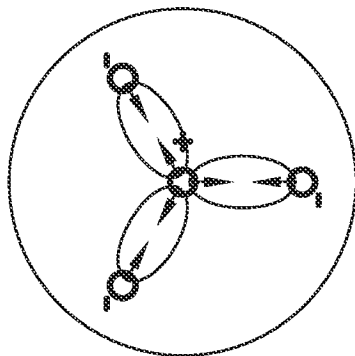


FIG. 30B

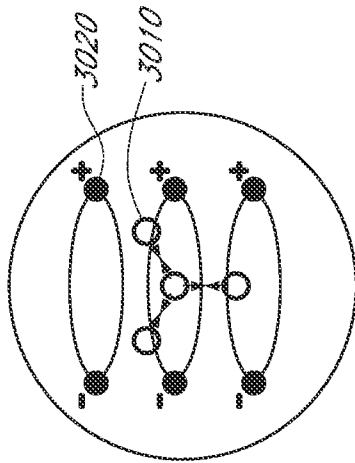


FIG. 30C

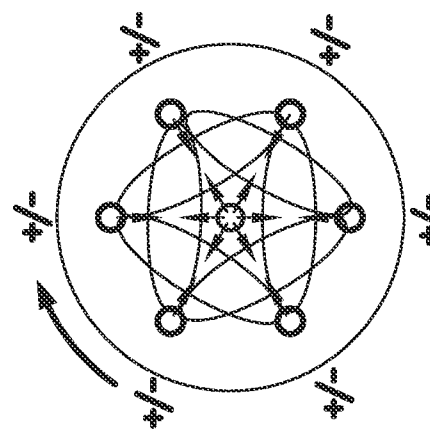


FIG. 30D

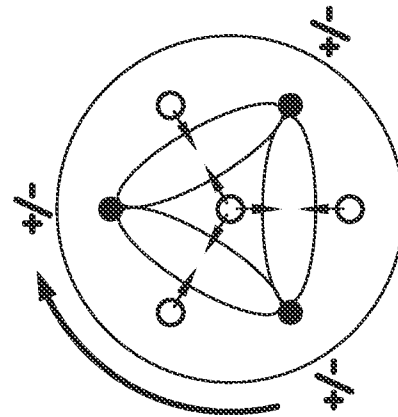


FIG. 30E

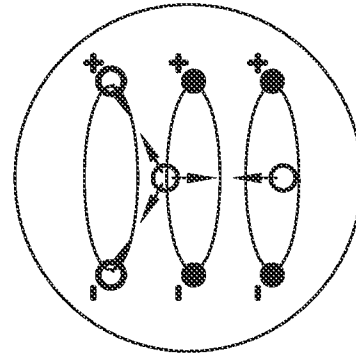


FIG. 30F

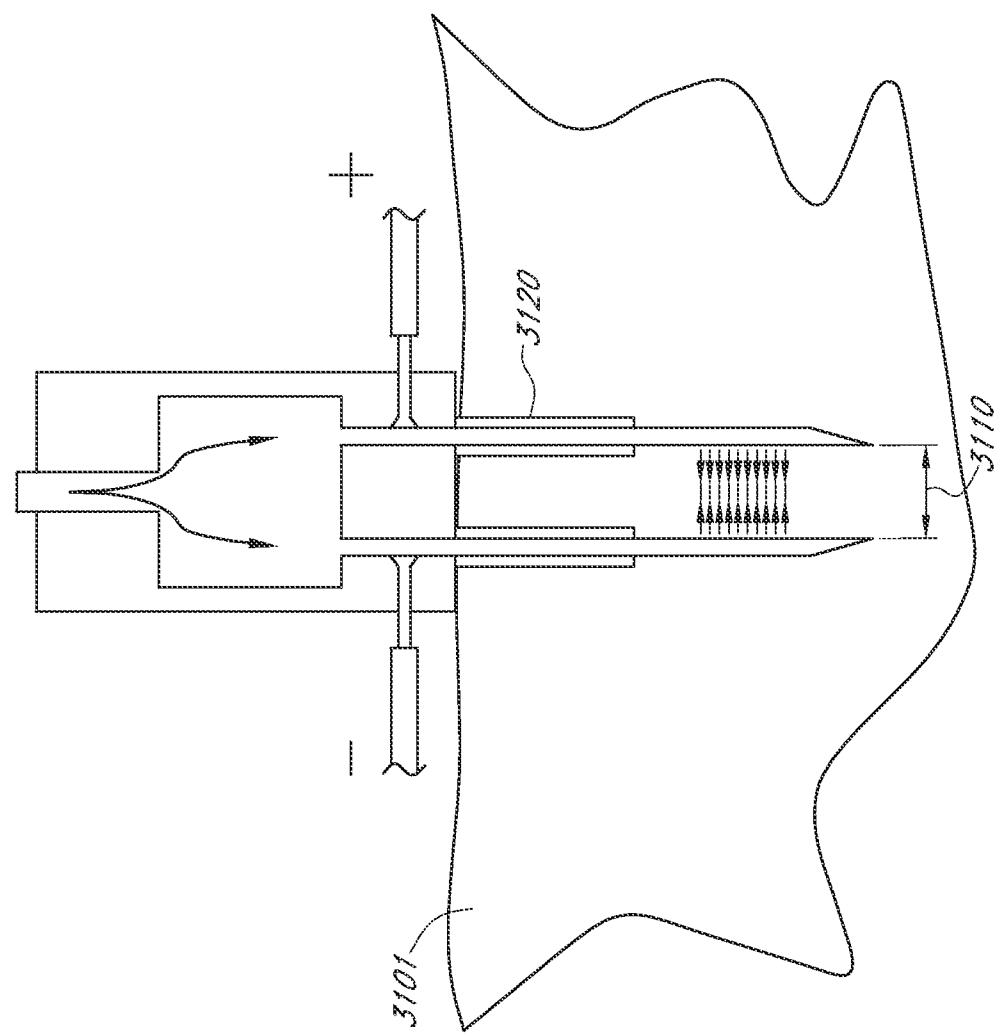


FIG. 31A

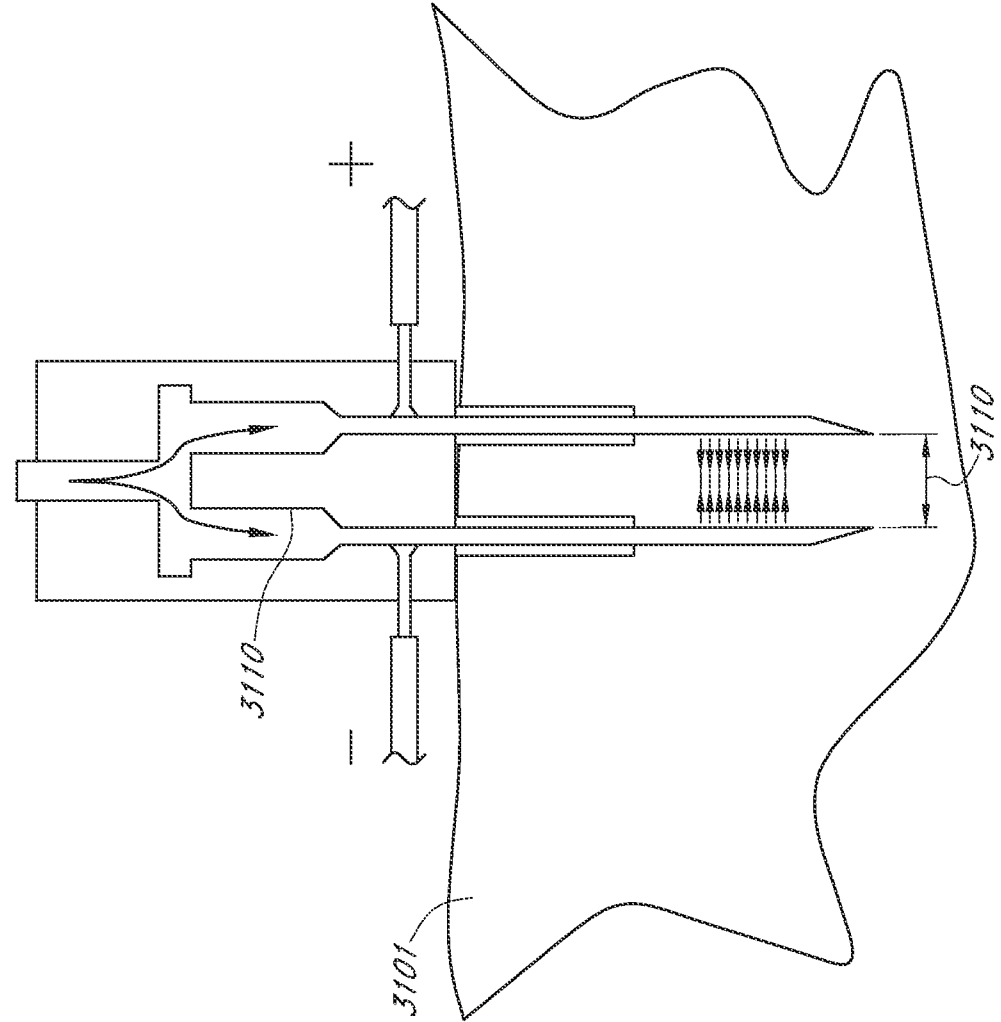


FIG. 31B

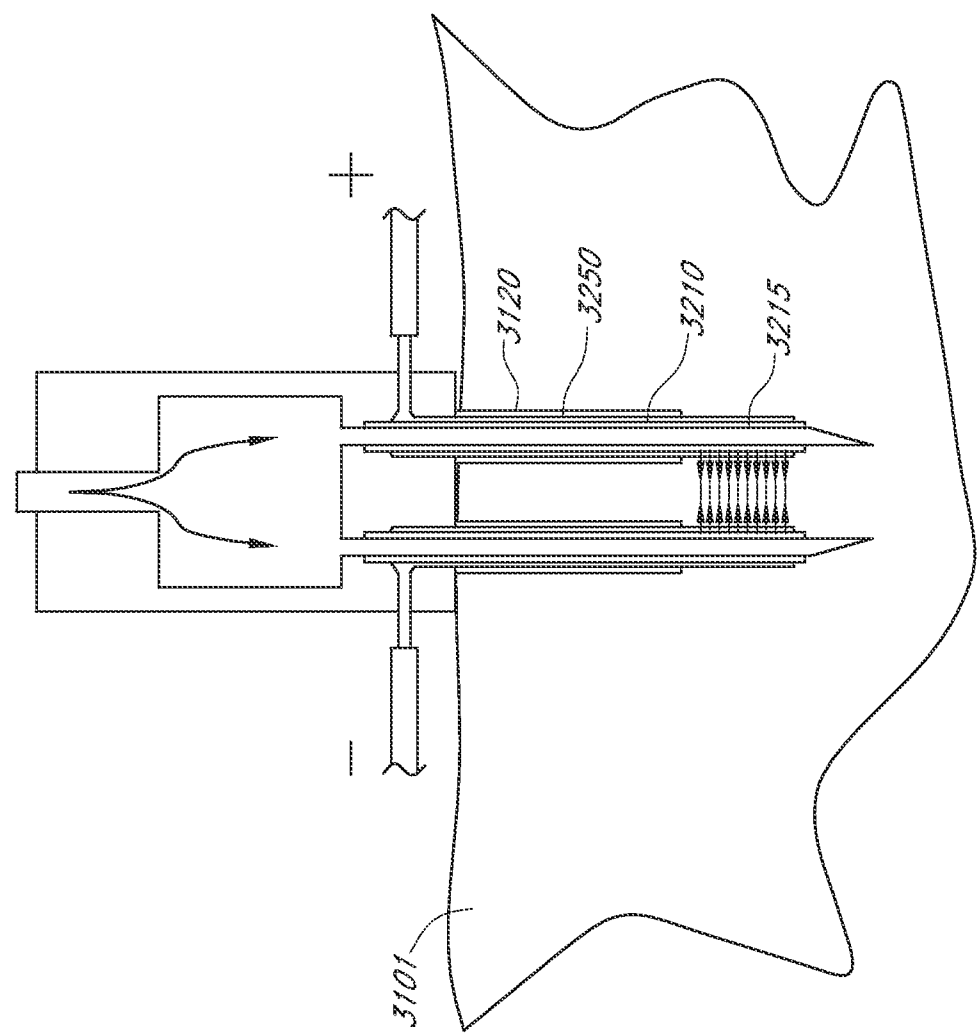


FIG. 32A

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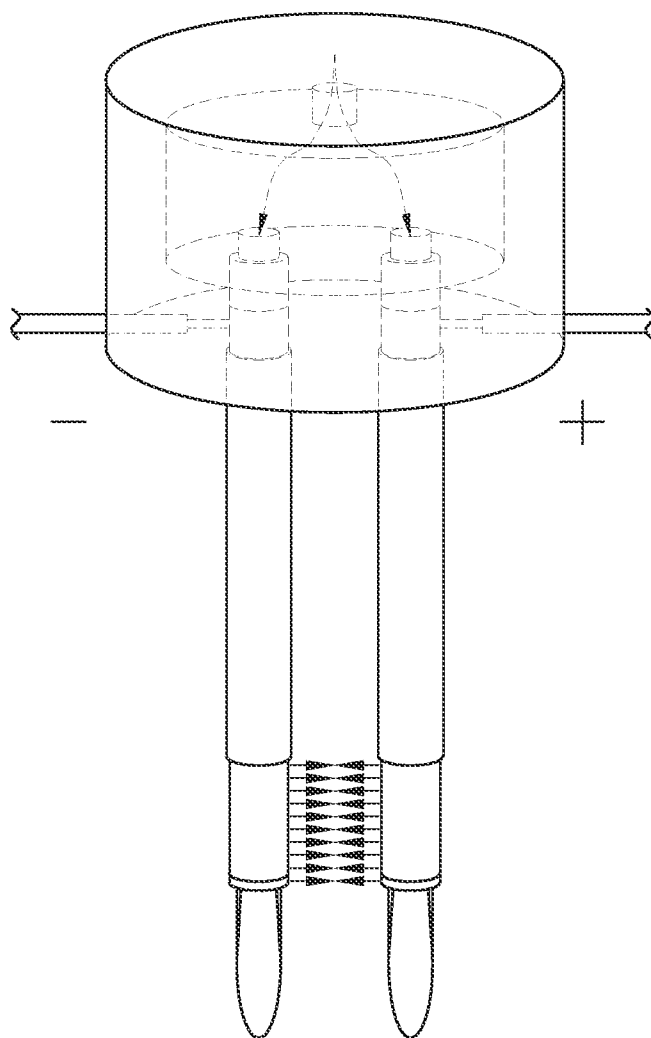


FIG. 32B

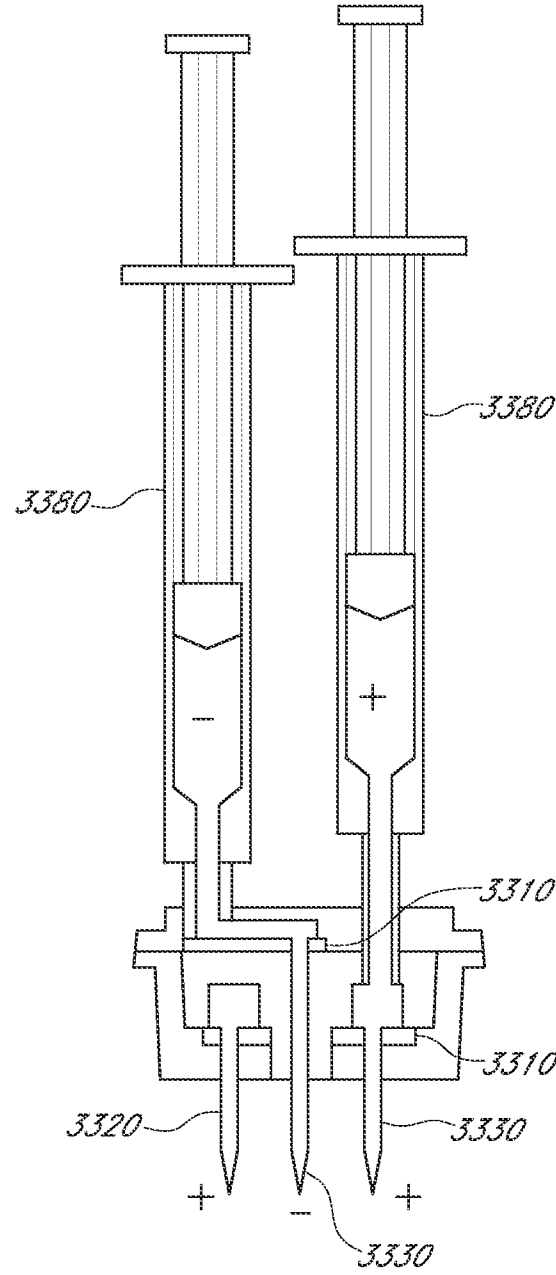


FIG. 33A

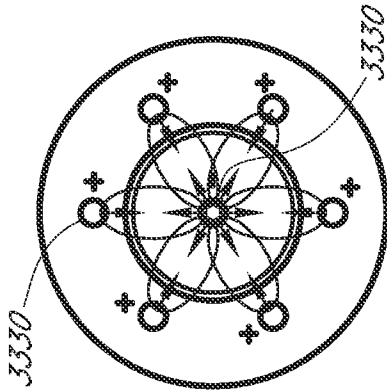


FIG. 33B

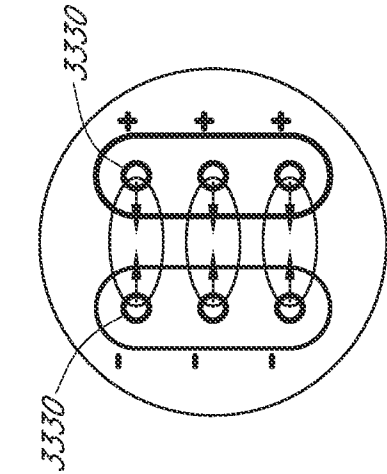


FIG. 33C

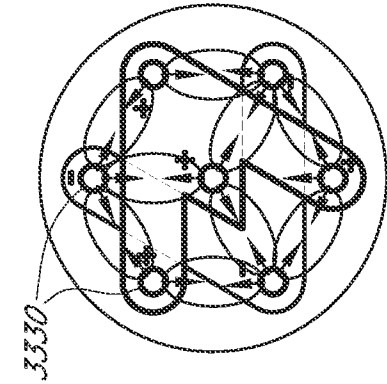


FIG. 33D

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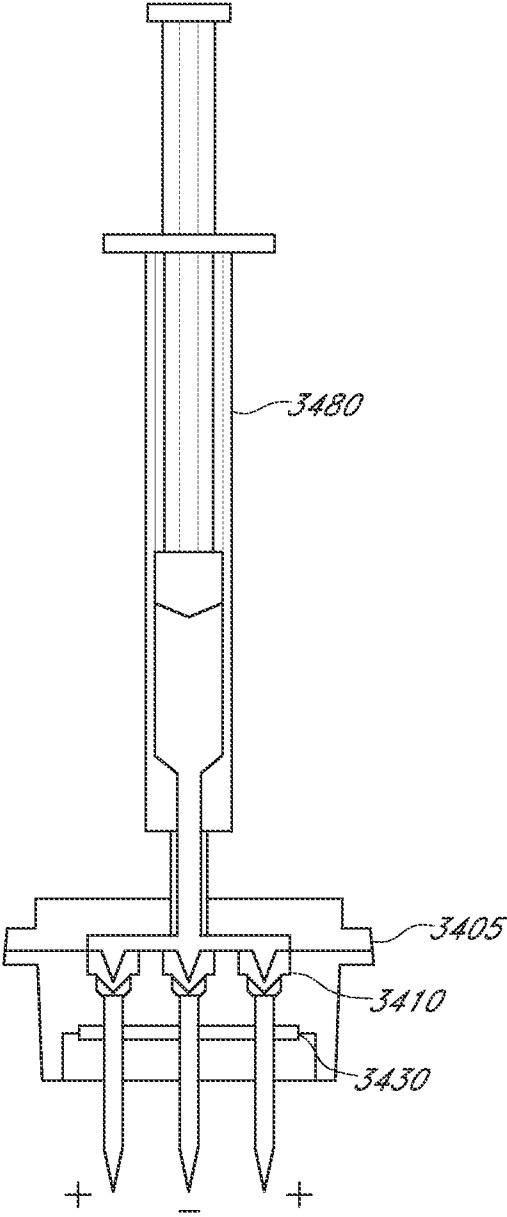


FIG. 34A

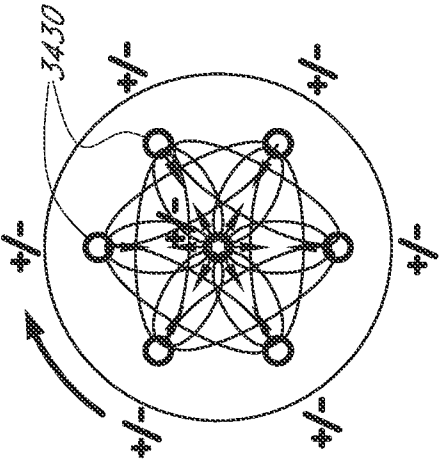


FIG. 34B

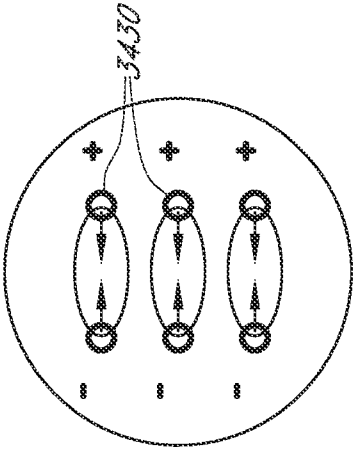


FIG. 34C

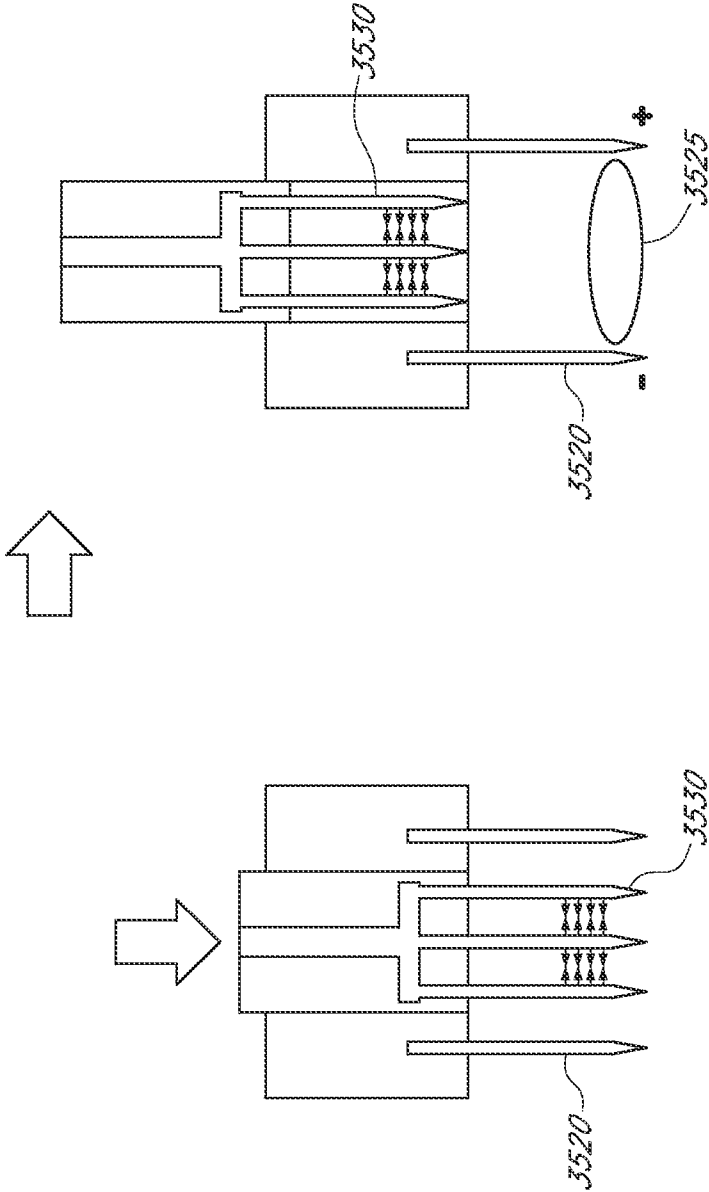


FIG. 35C

FIG. 35A

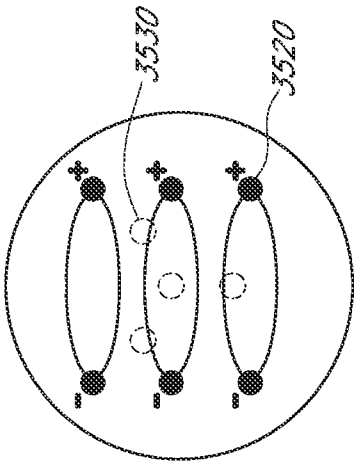


FIG. 35D

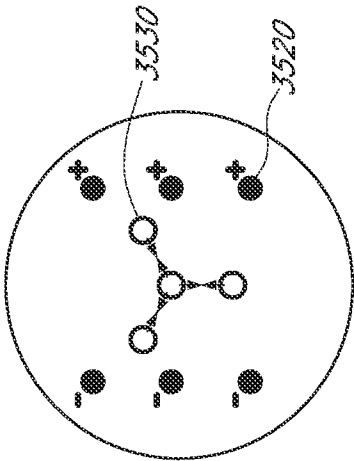


FIG. 35B

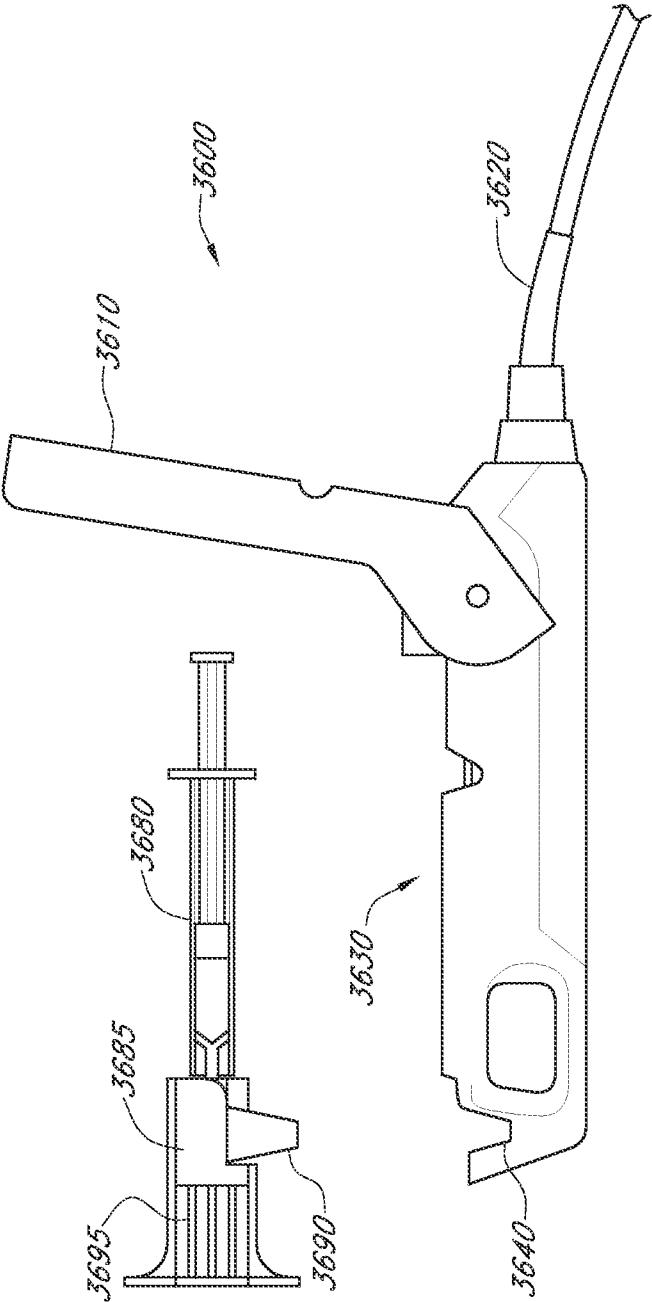


FIG. 36

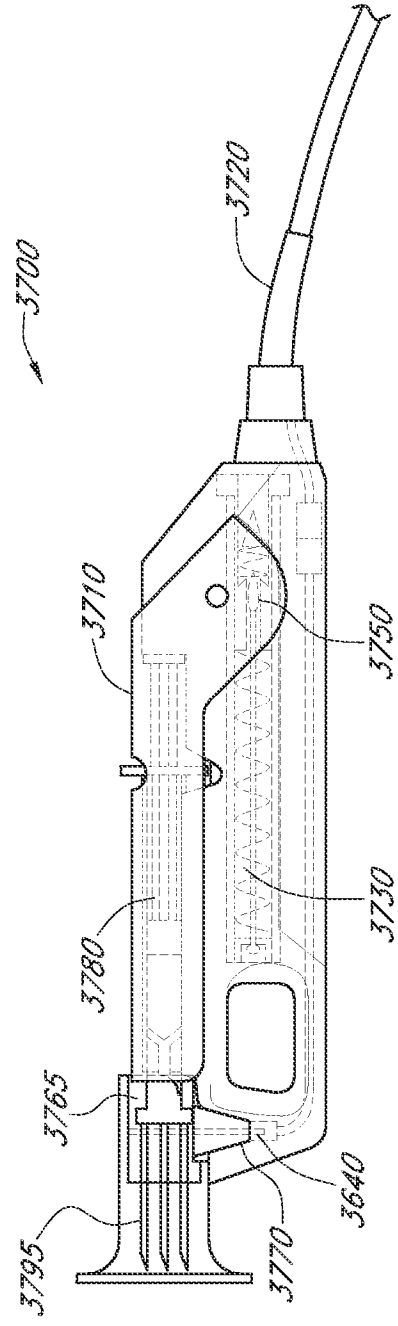


FIG. 37

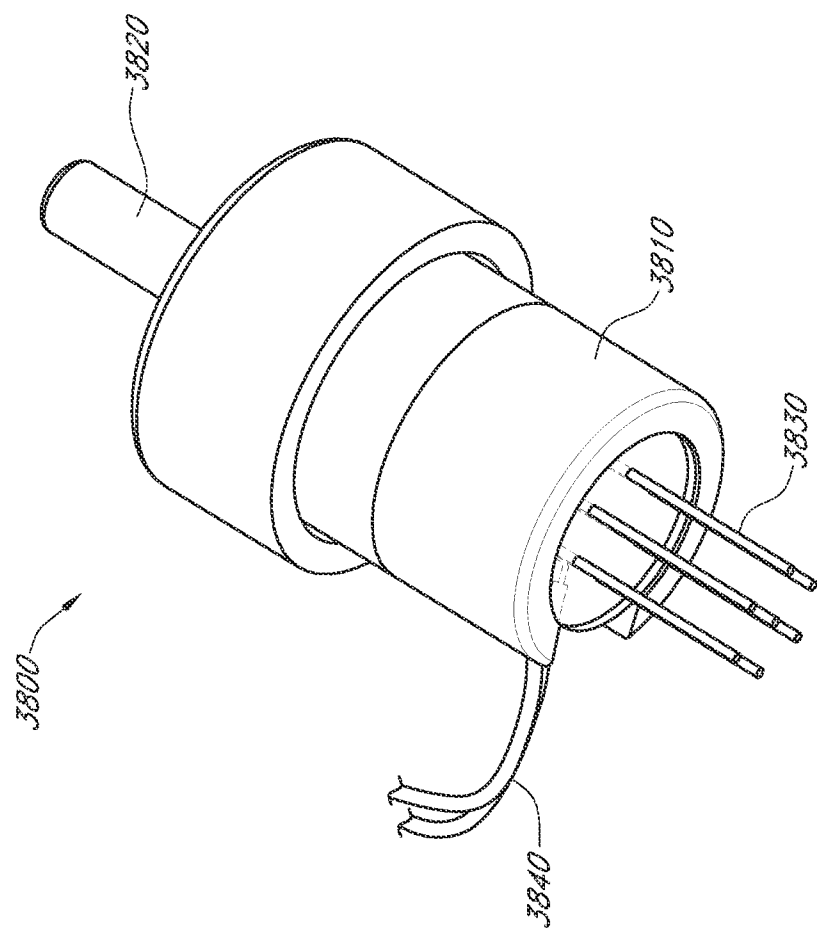


FIG. 38A

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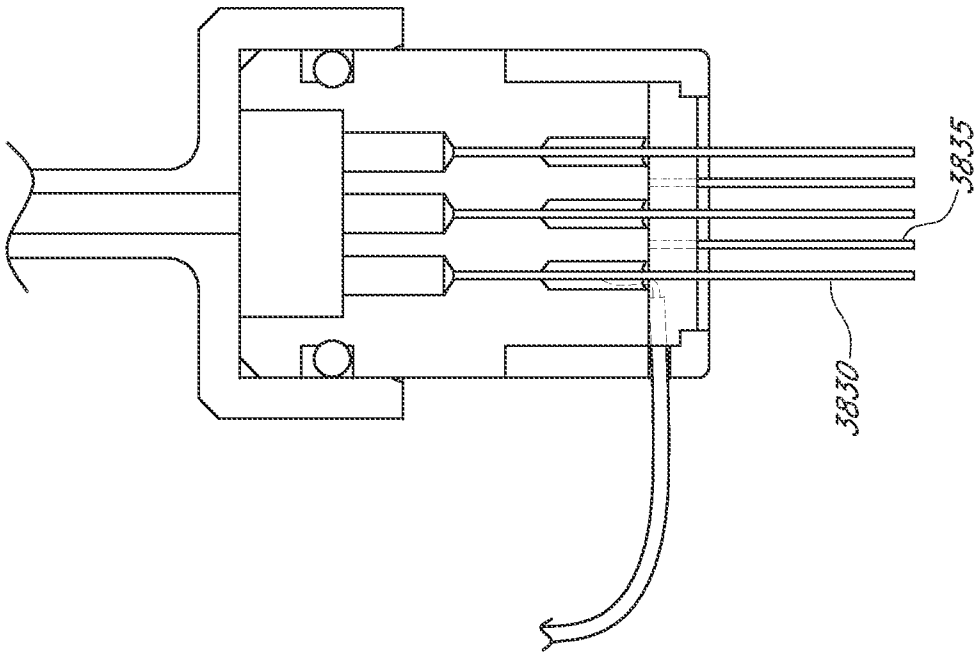


FIG. 38C

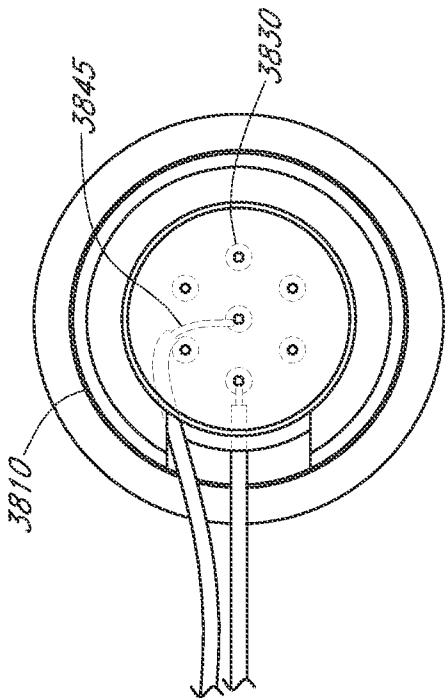


FIG. 38B

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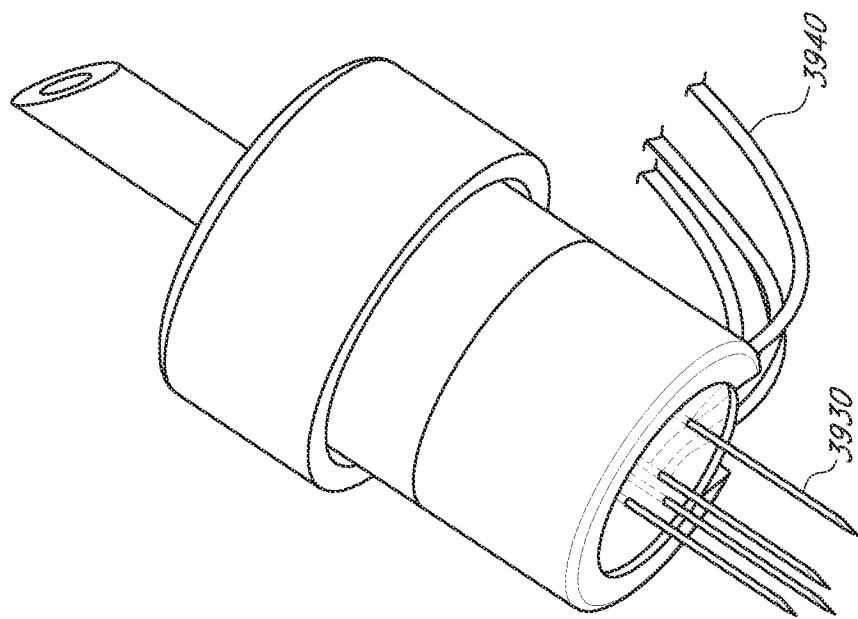


FIG. 39A

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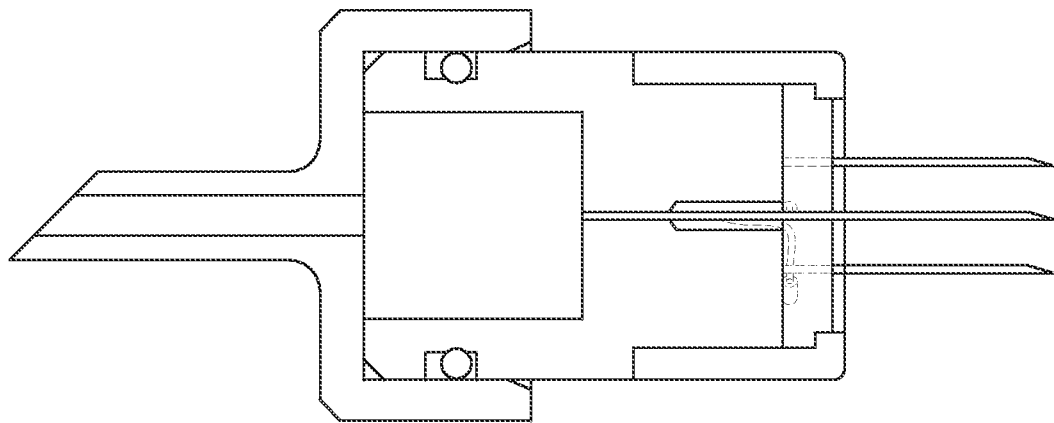


FIG. 39C

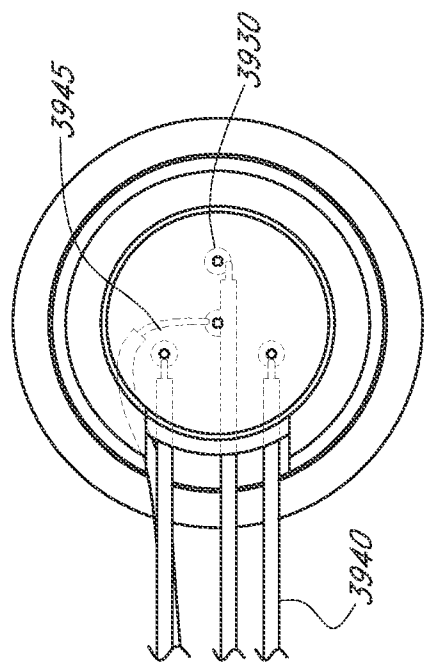


FIG. 39B

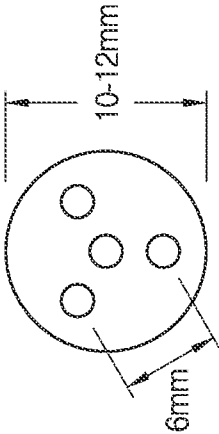
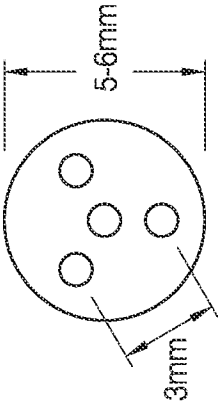
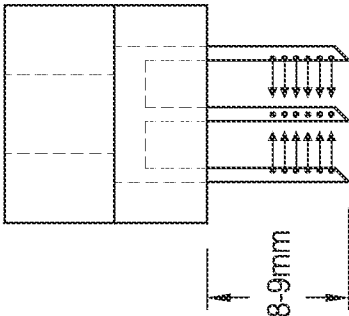
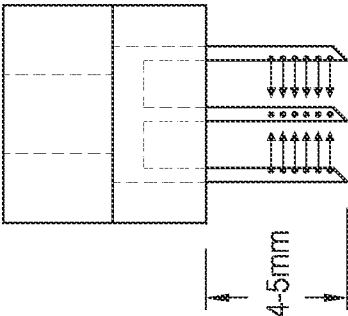


FIG. 40A

FIG. 40B

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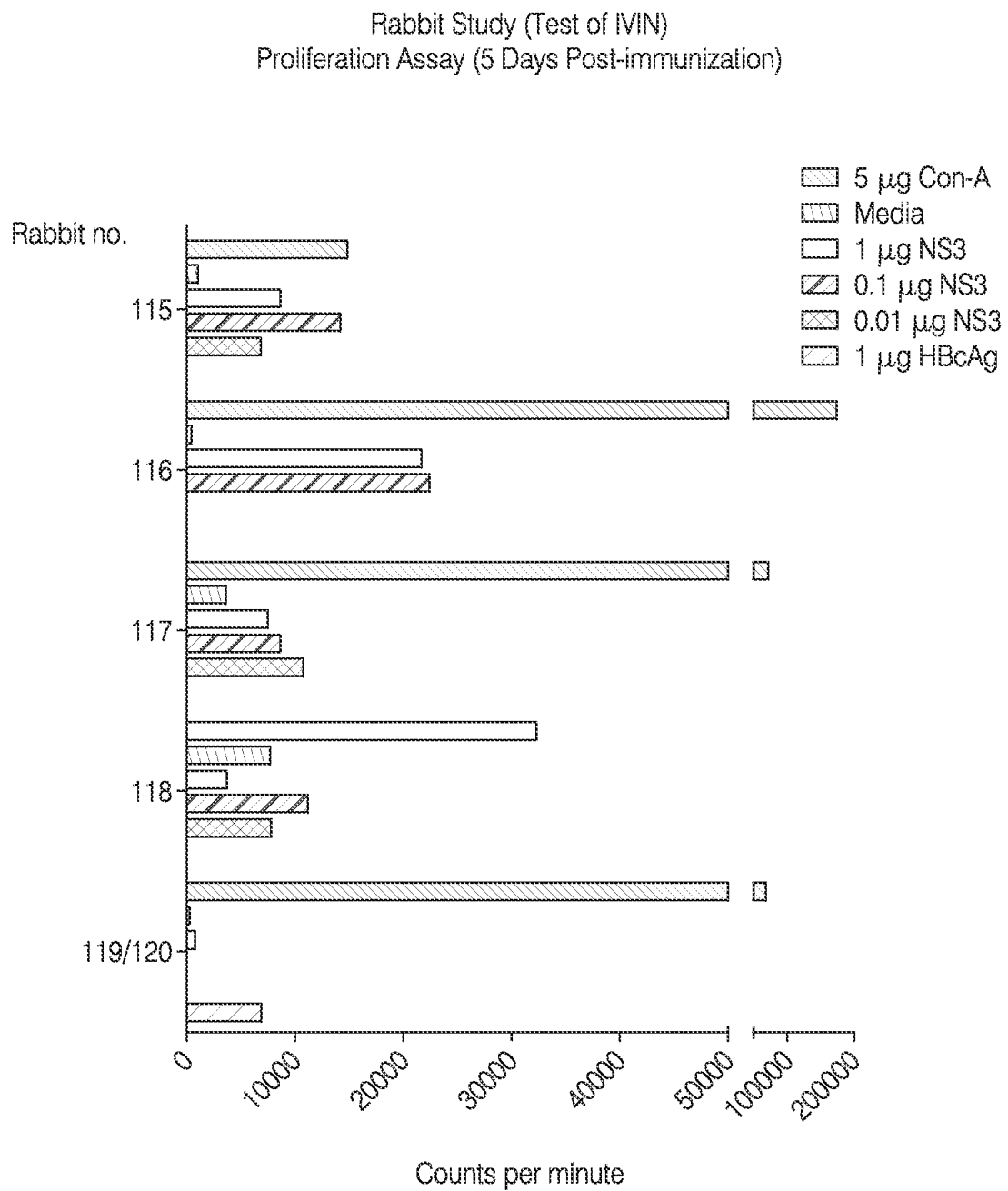


FIG. 41

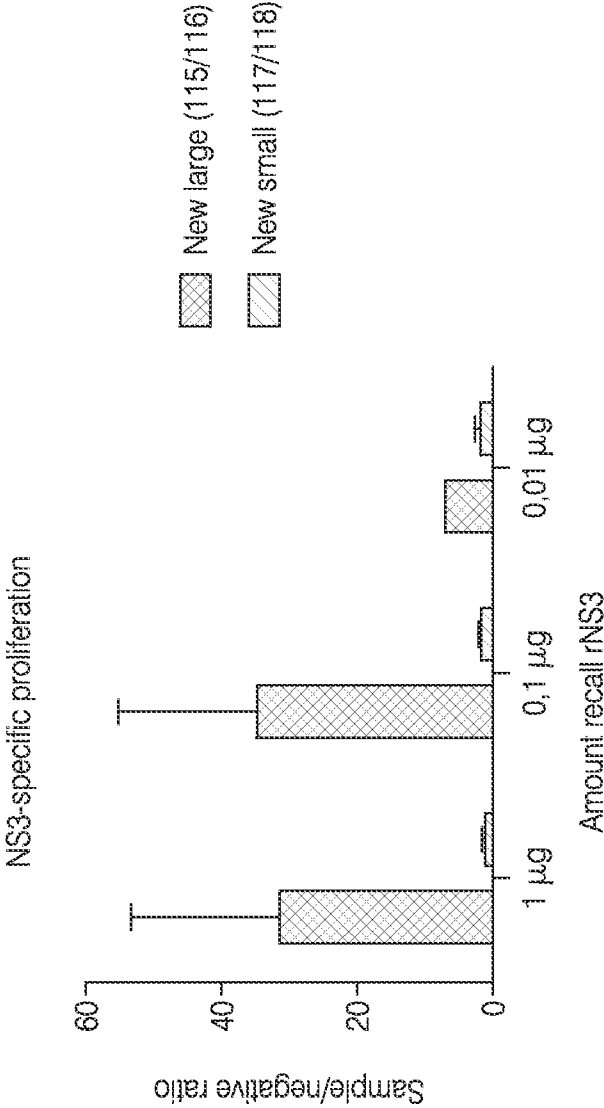


FIG. 42

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Regular needle
(0,9mg/0,3mL;115L)

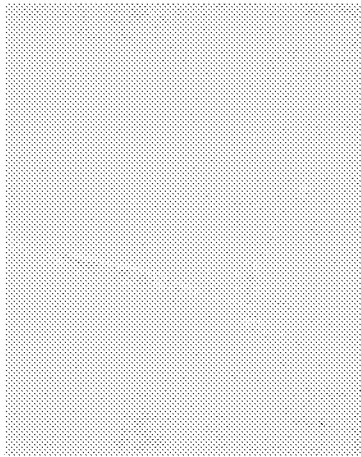


FIG. 43A

HIP/small
(0,9mg/0,3mL;117R)



FIG. 43B

HIP/large
(0,9mg/0,3mL;115R)

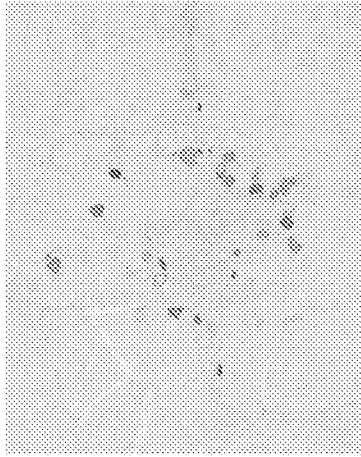


FIG. 43C

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FIG. 44A



FIG. 44B

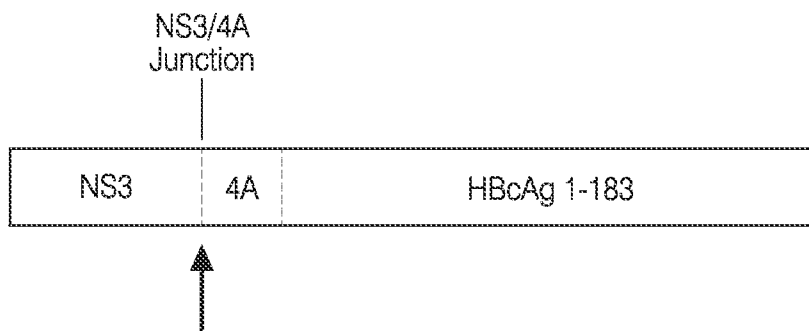


FIG. 44C

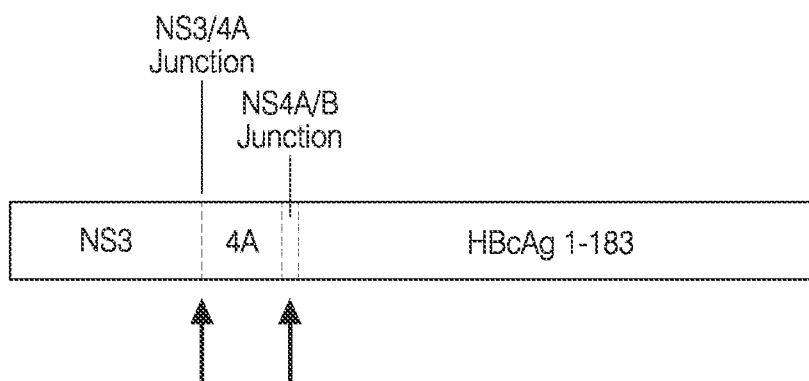


FIG. 44D

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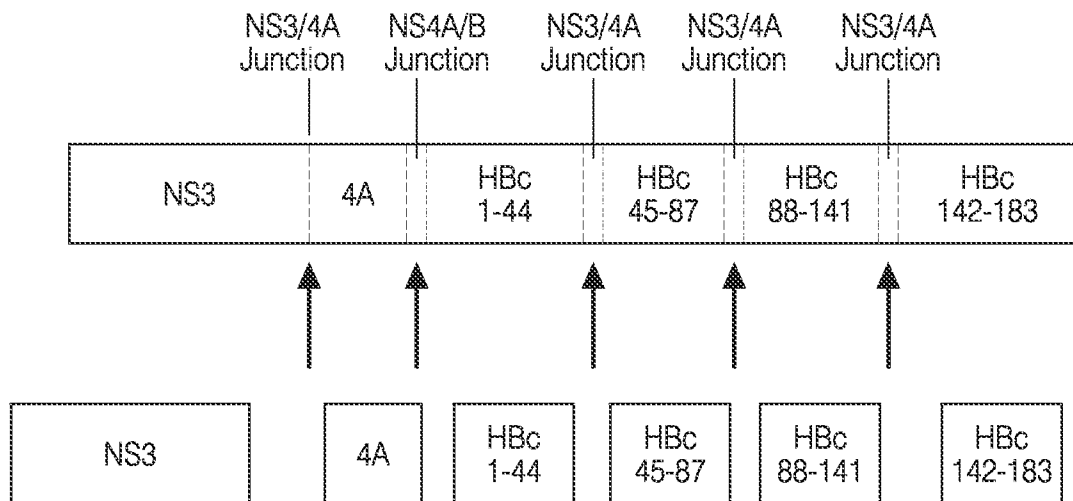


FIG. 44E

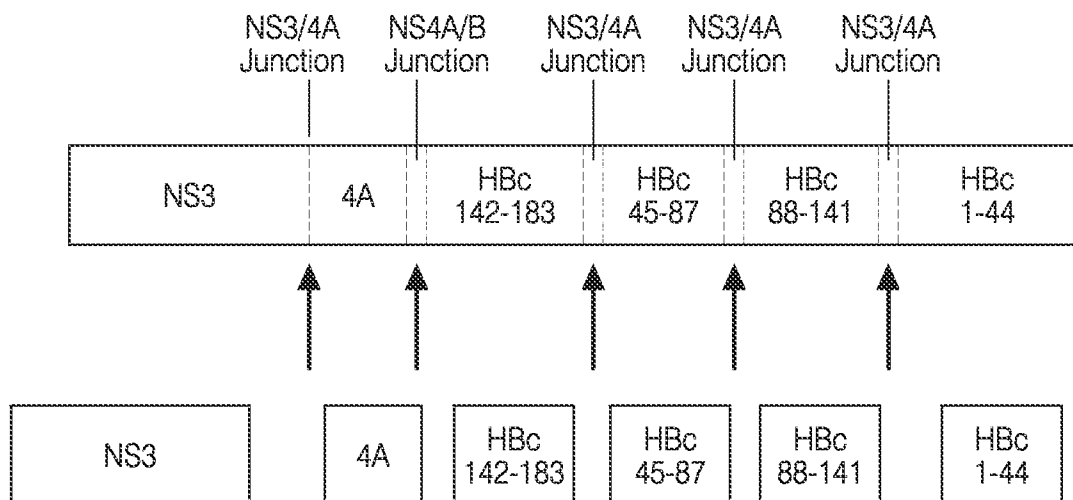


FIG. 44F

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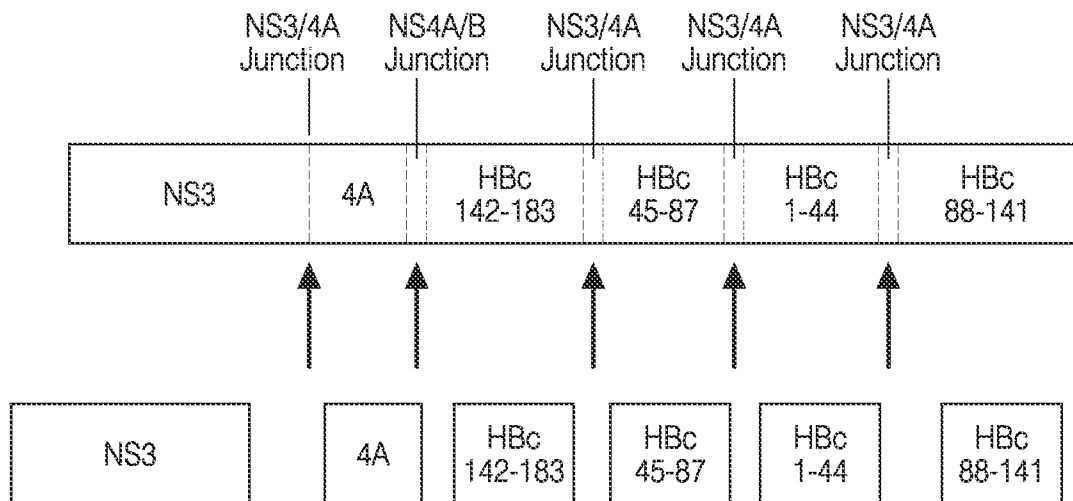


FIG. 44G

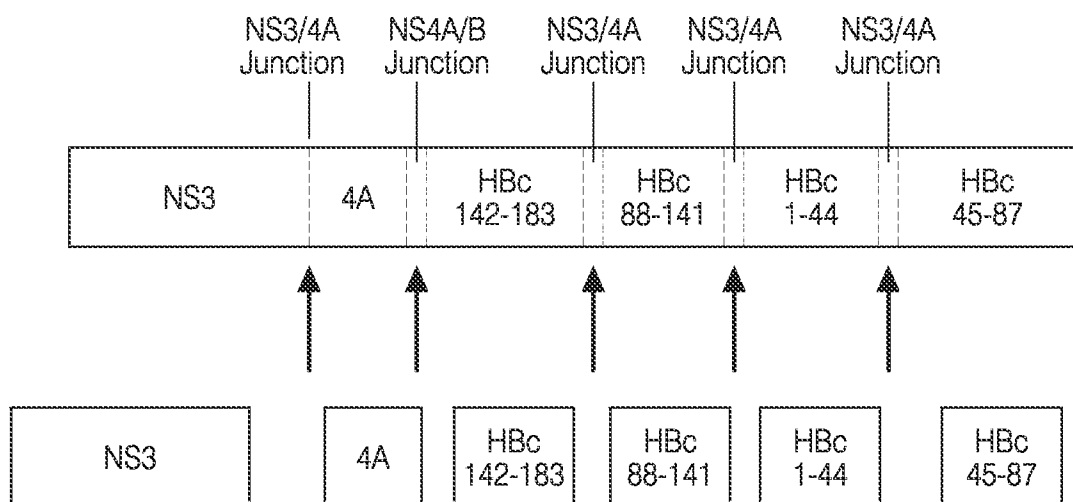


FIG. 44H

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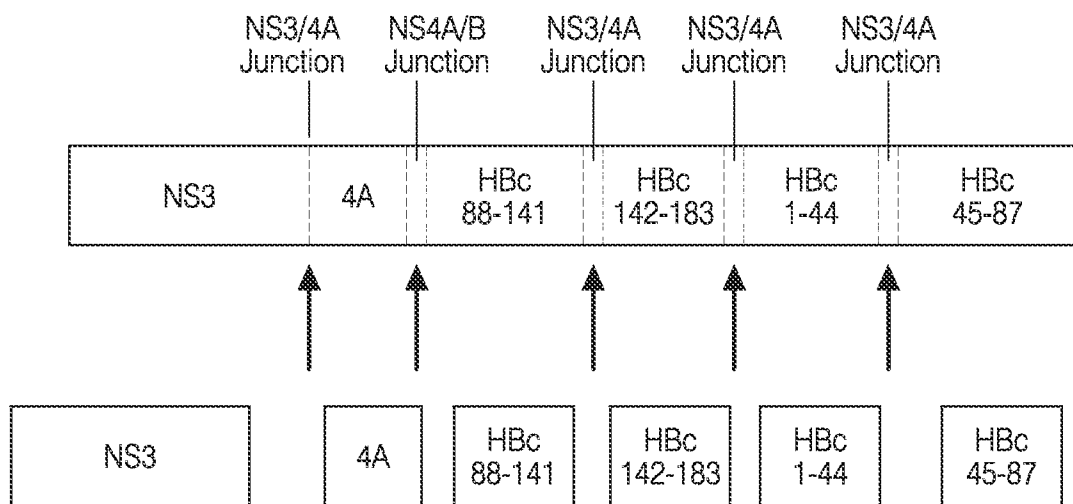


FIG. 44I

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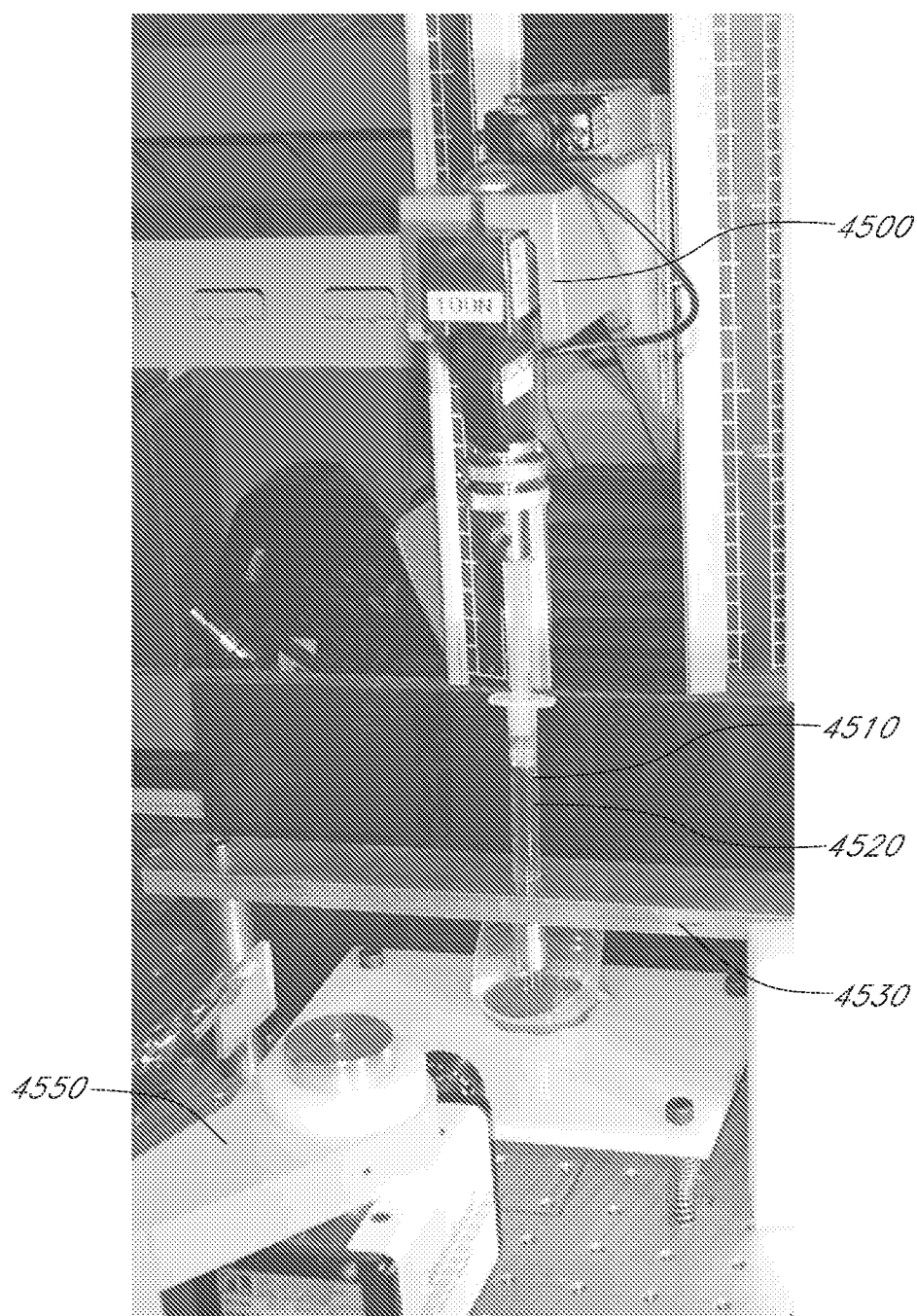


FIG. 45

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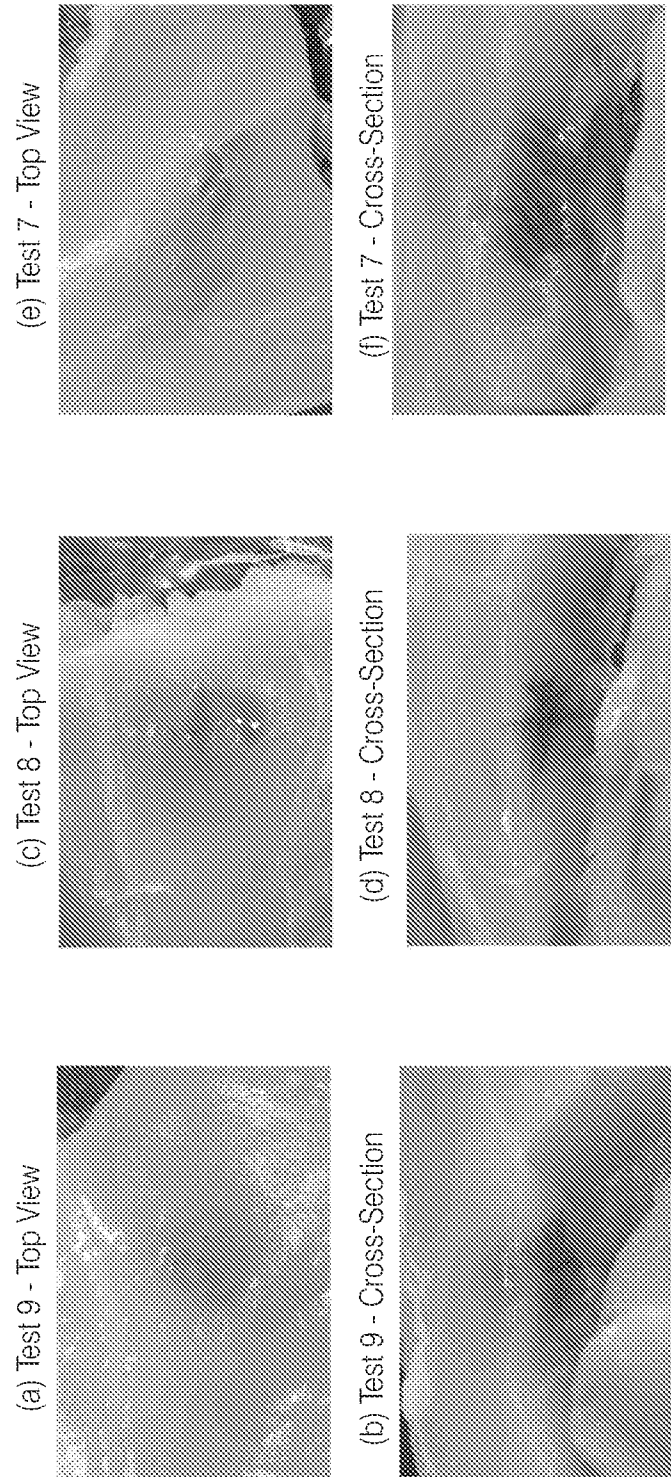


FIG. 46

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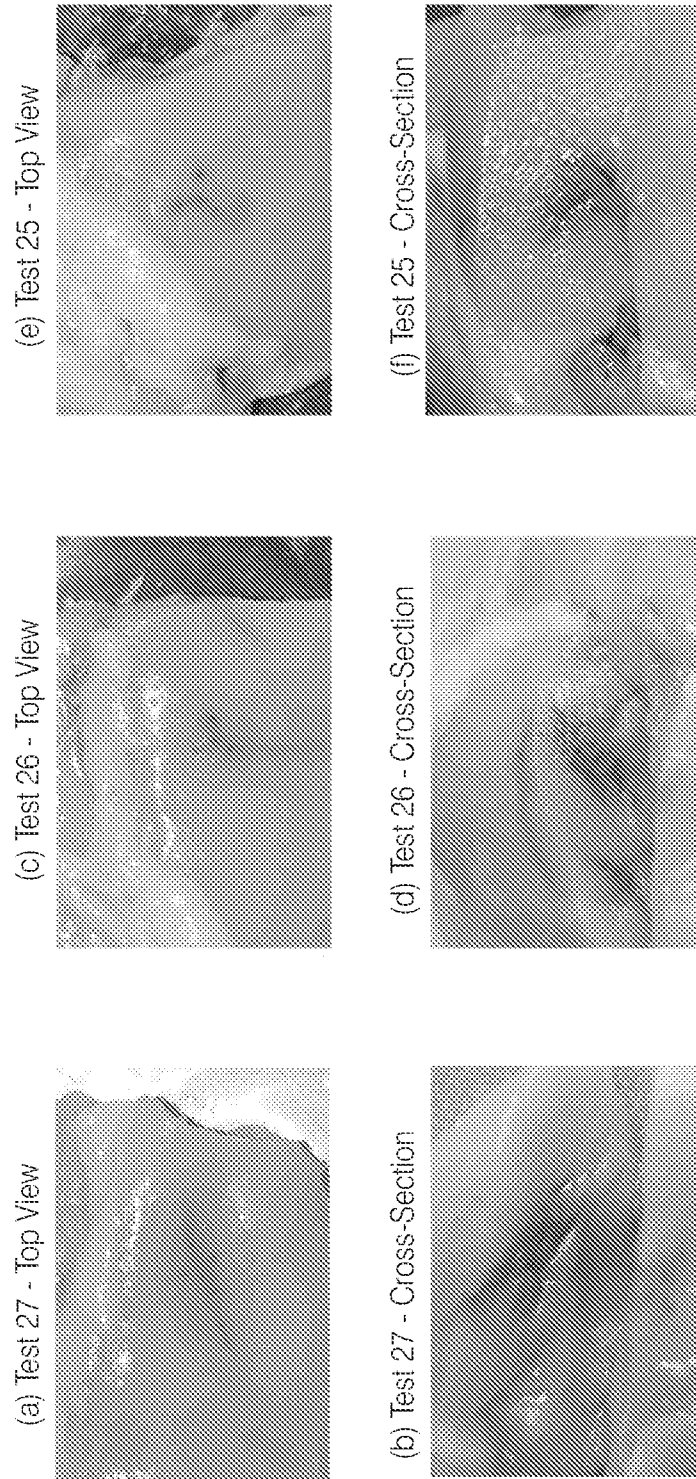


FIG. 47

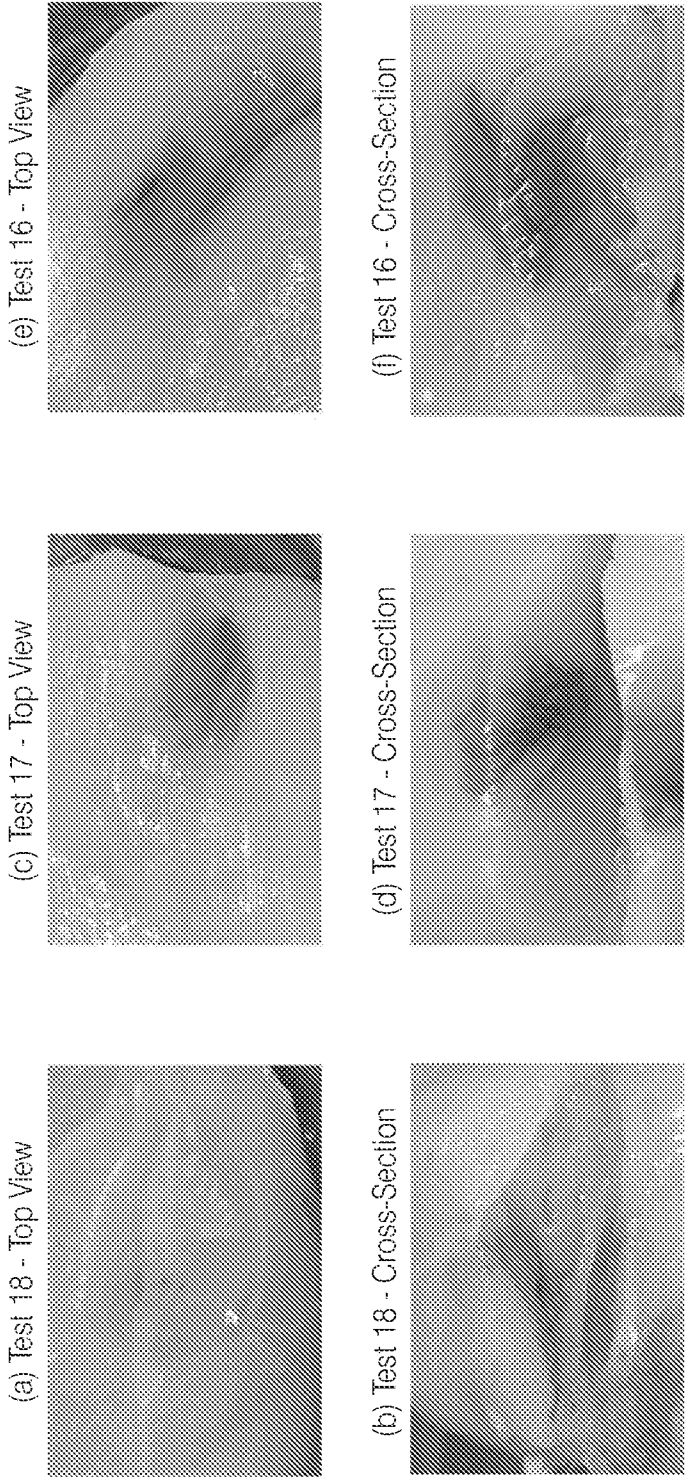


FIG. 48

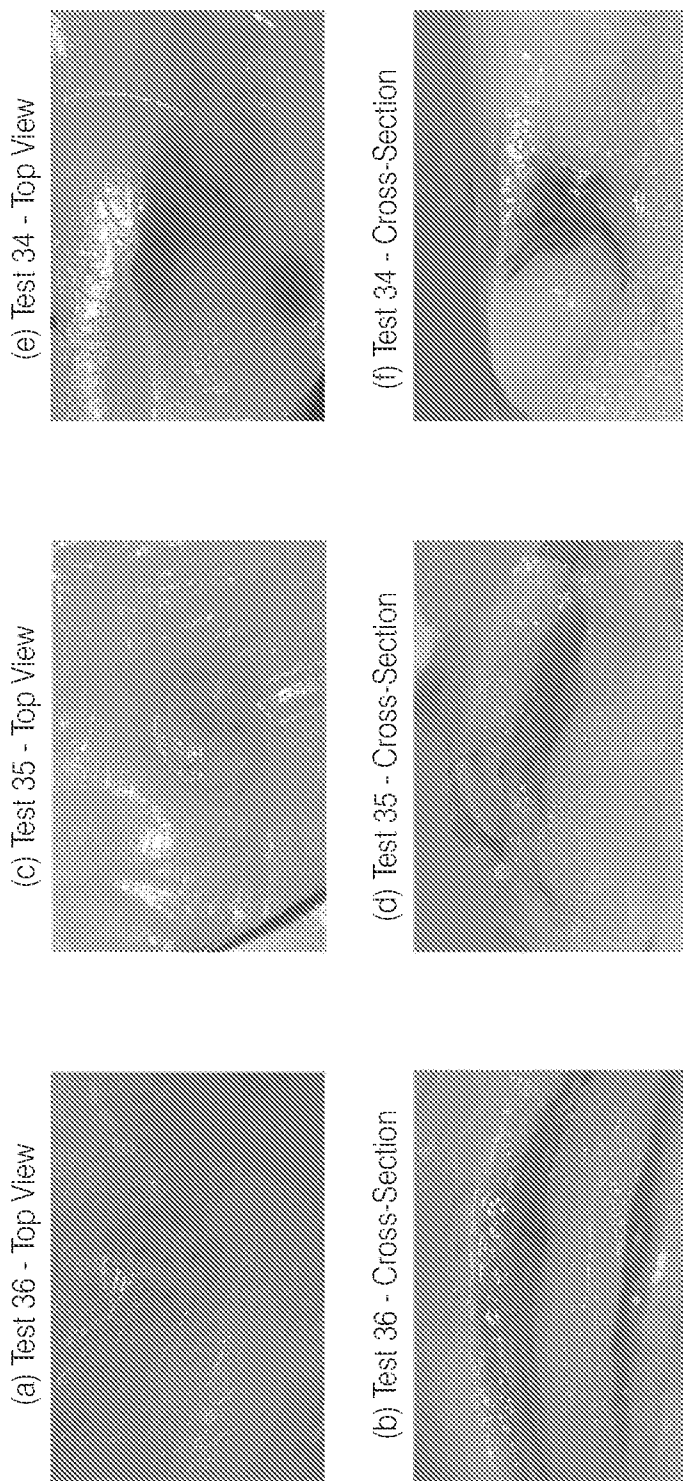


FIG. 49

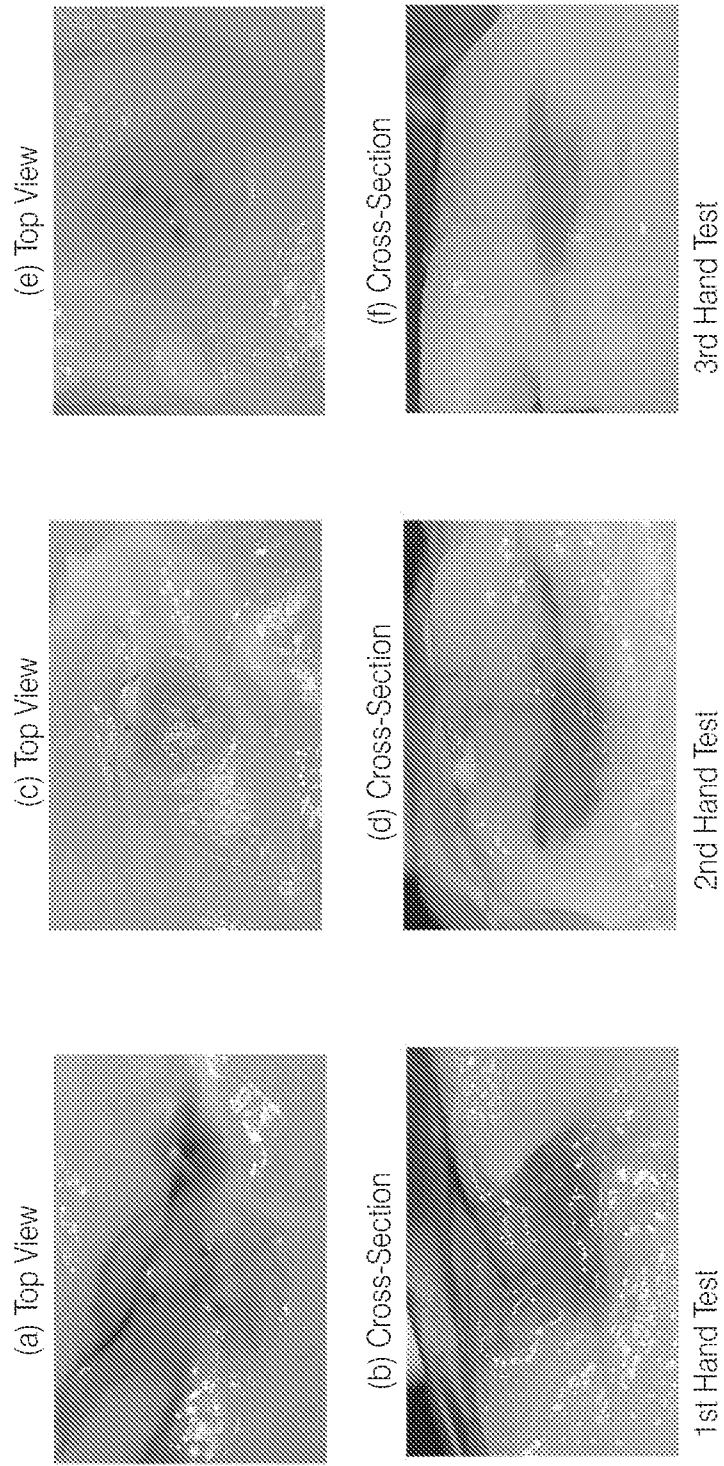


FIG. 50

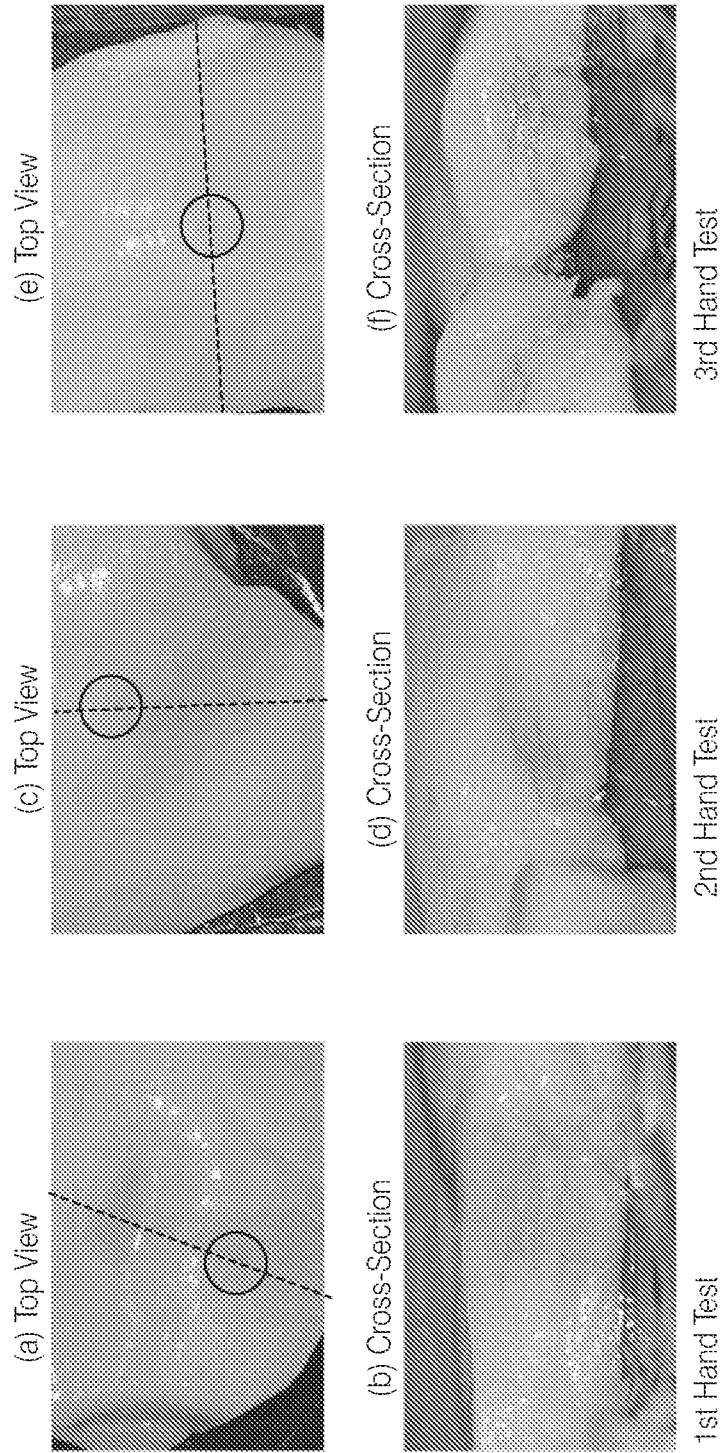


FIG. 51

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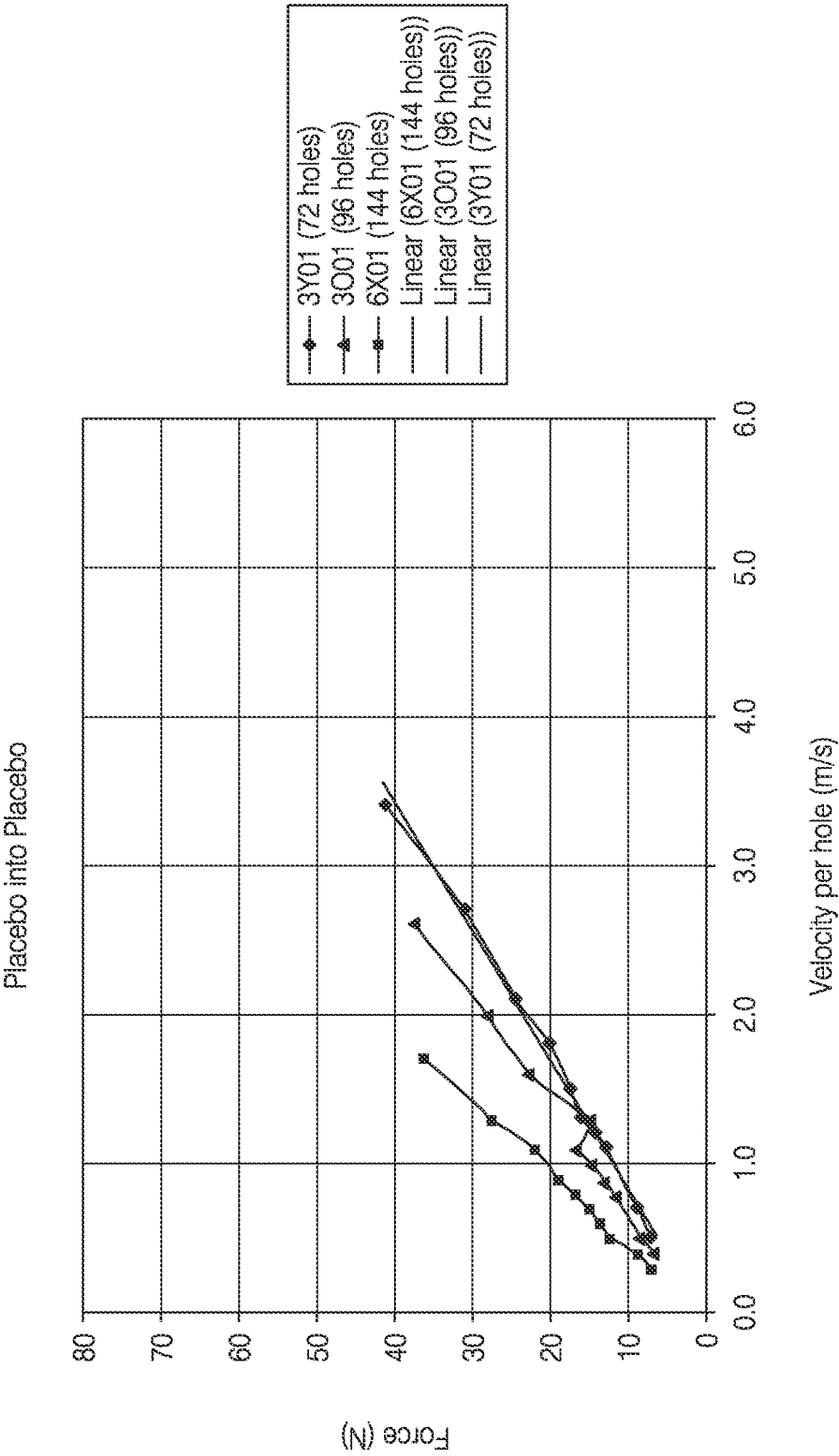


FIG. 52

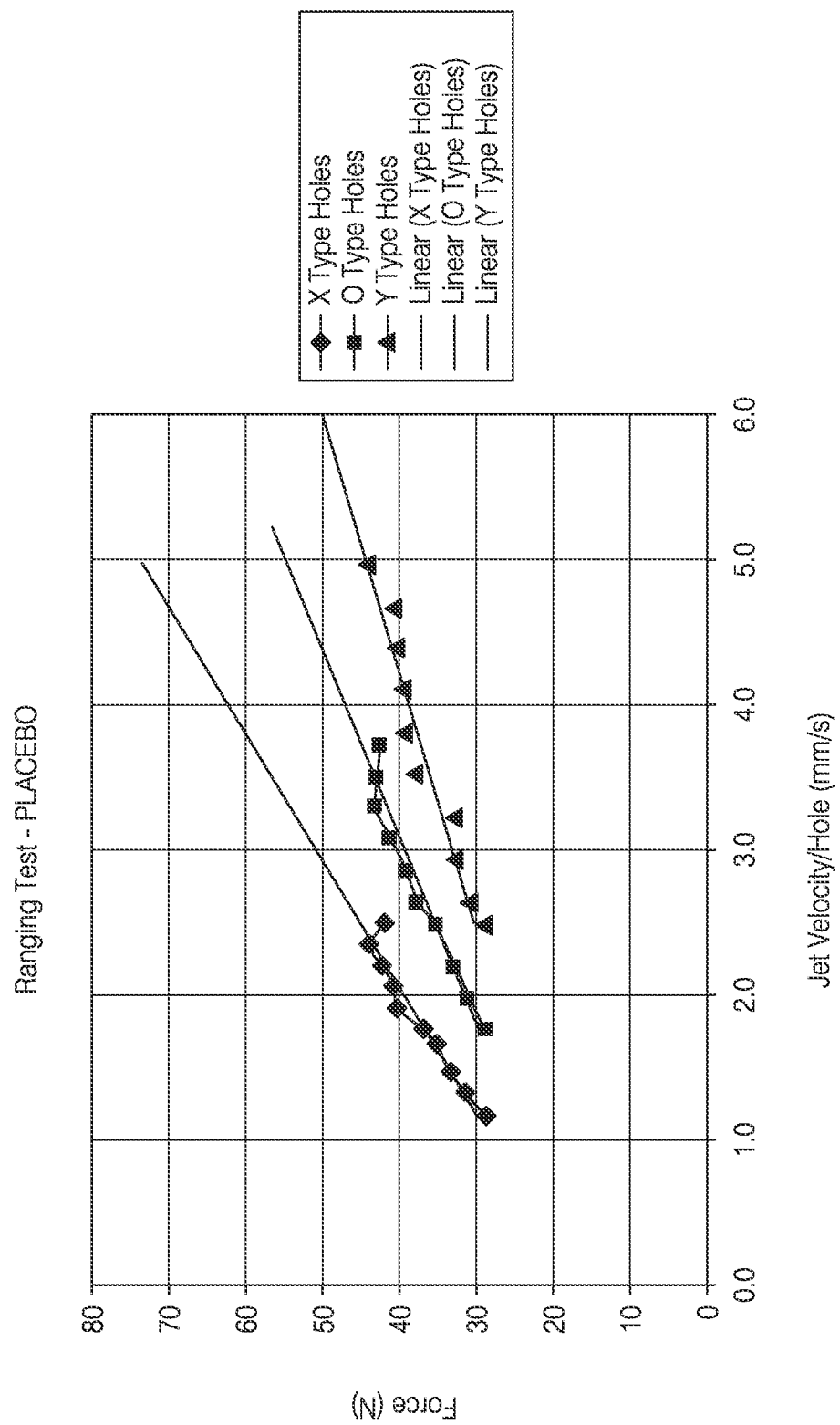


FIG. 53

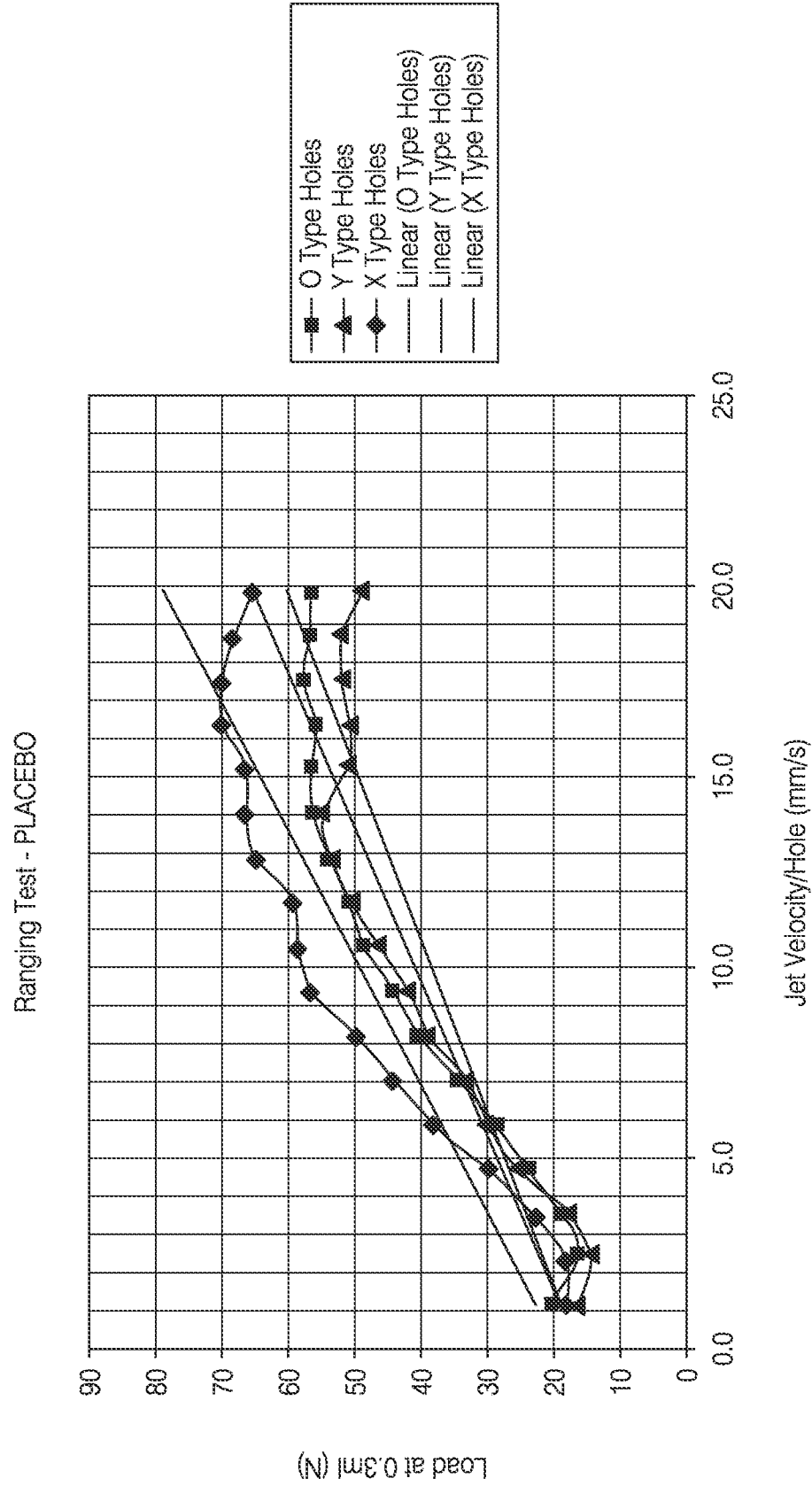


FIG. 54

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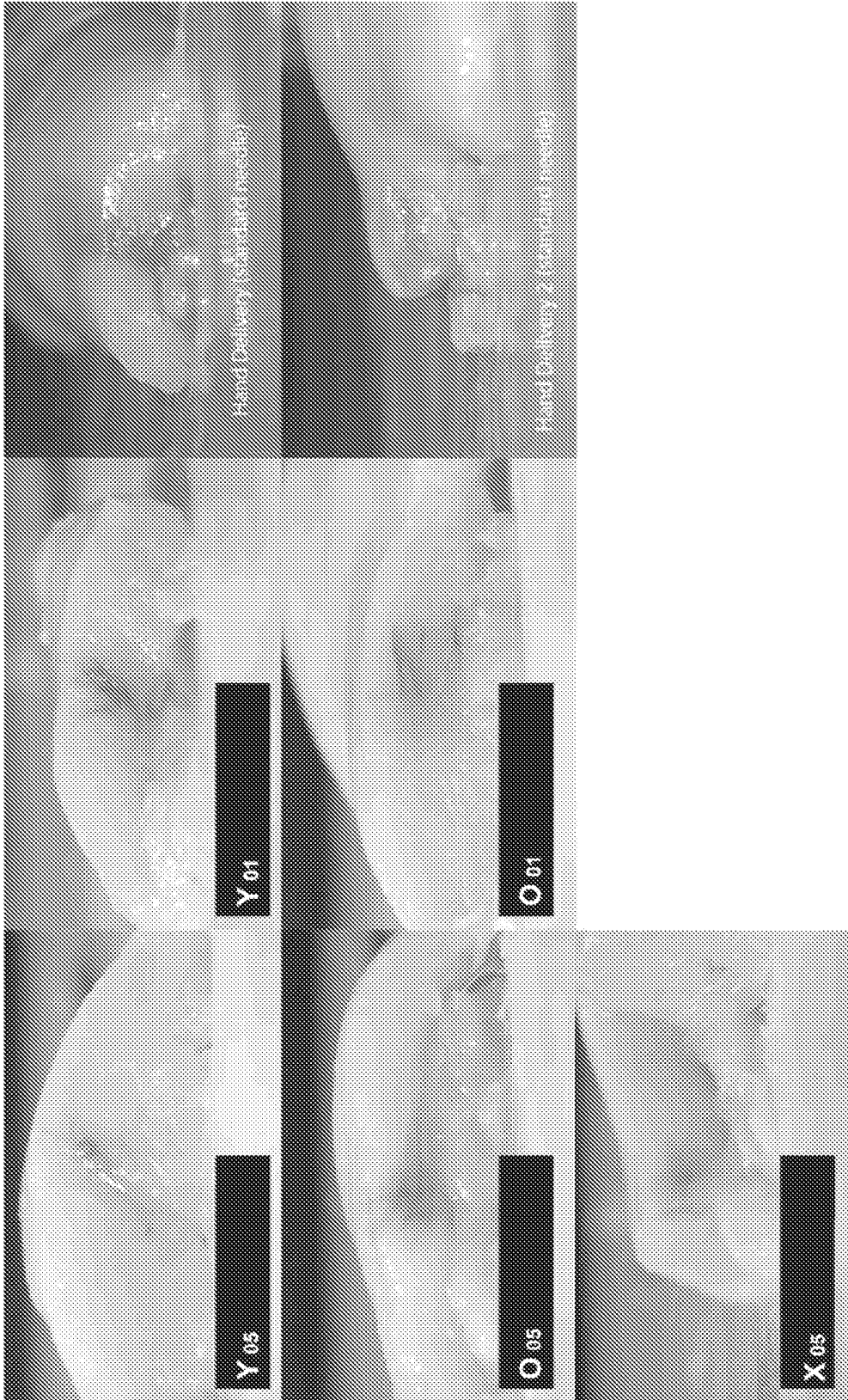


FIG. 55

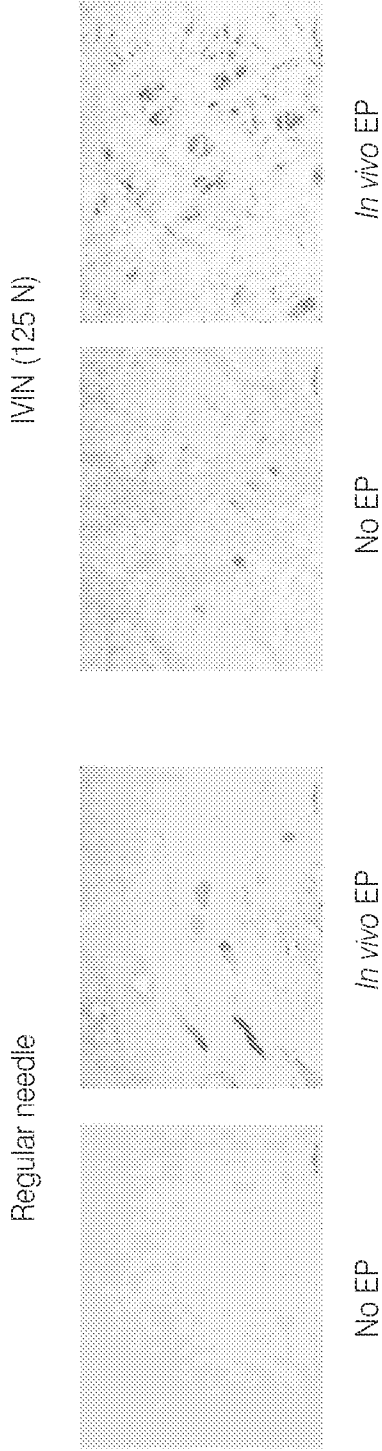


FIG. 56A1 FIG. 56A2 FIG. 56B1 FIG. 56B2

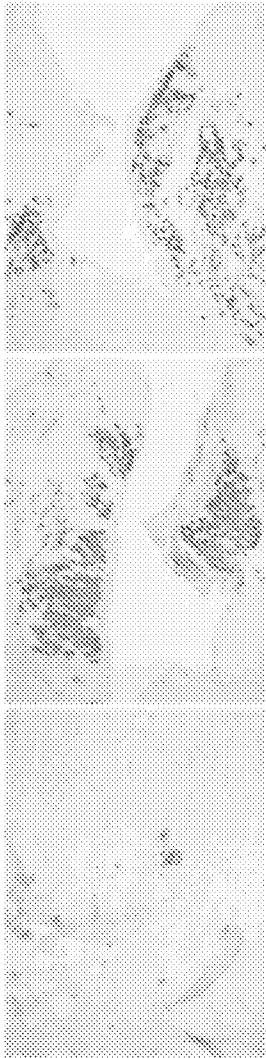


FIG. 57A1 FIG. 57B1 FIG. 57C1



Regular needle/EP IVIN 75N/EP IVIN 125N/EP
FIG. 57A2 FIG. 57B2 FIG. 57C2

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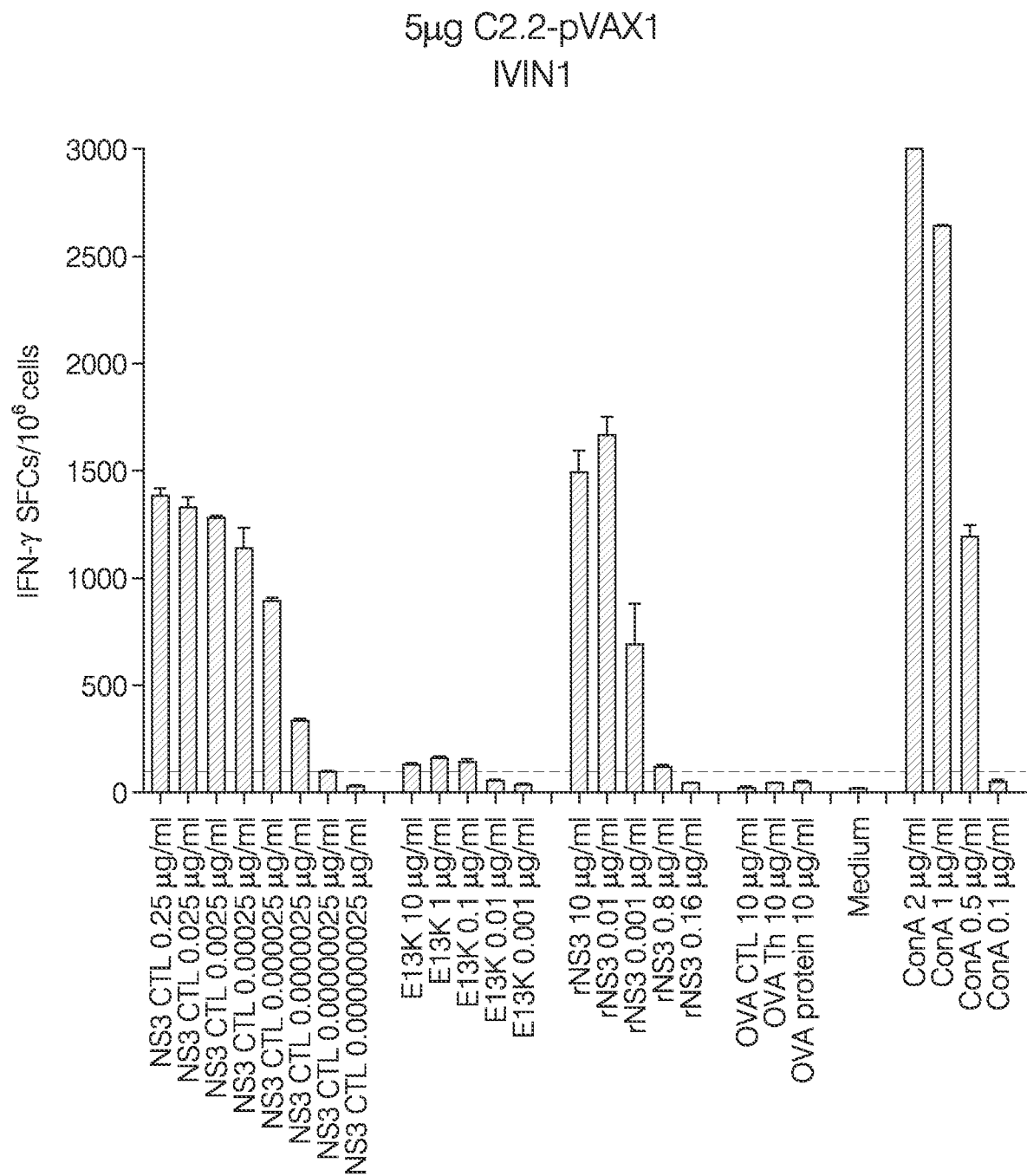


FIG. 58A

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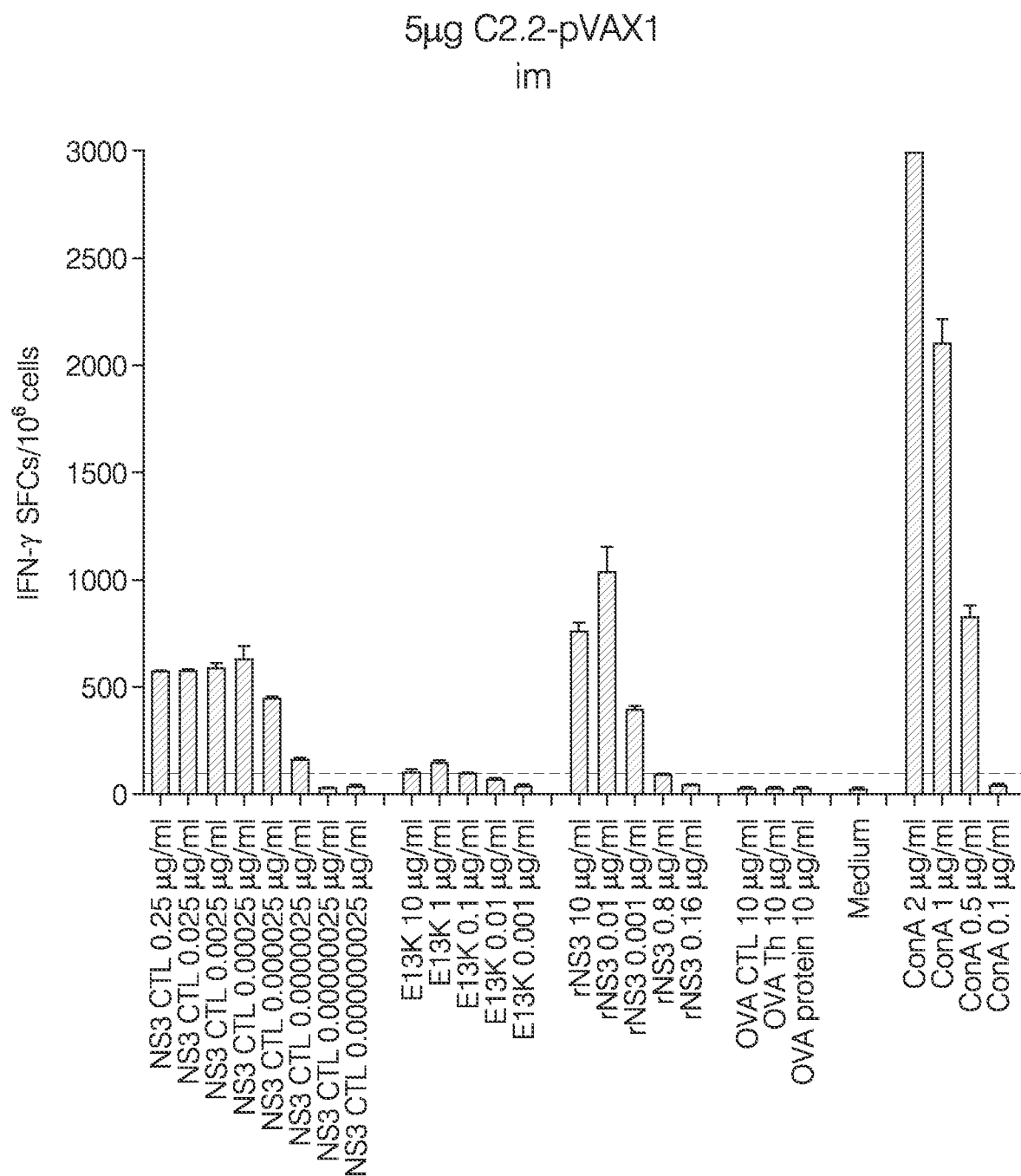


FIG. 58B

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5 μ g C2.2-pVAX1
IVIN1-EP

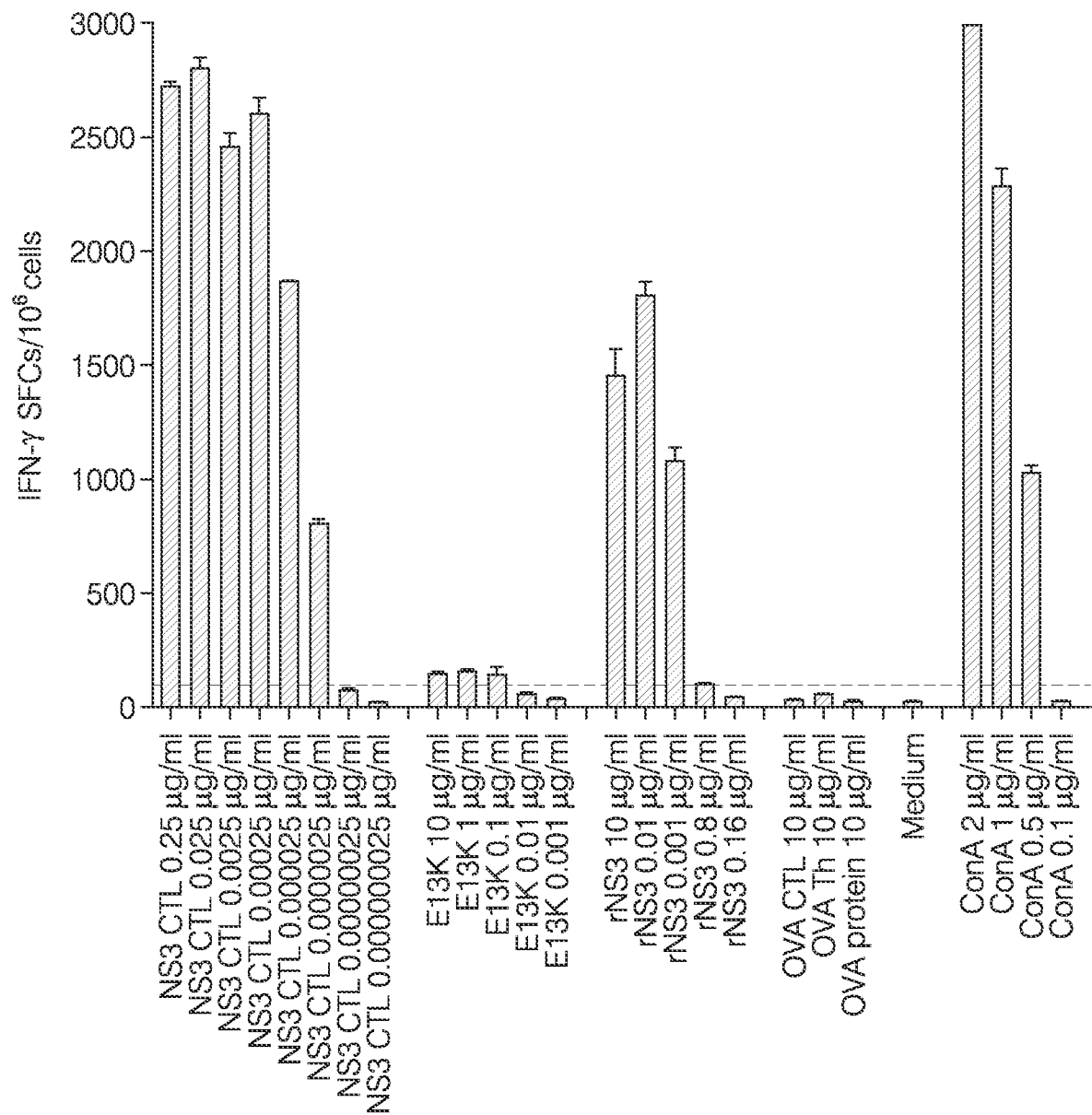


FIG. 58C

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5 μ g C2.2-pVAX1
im-EP

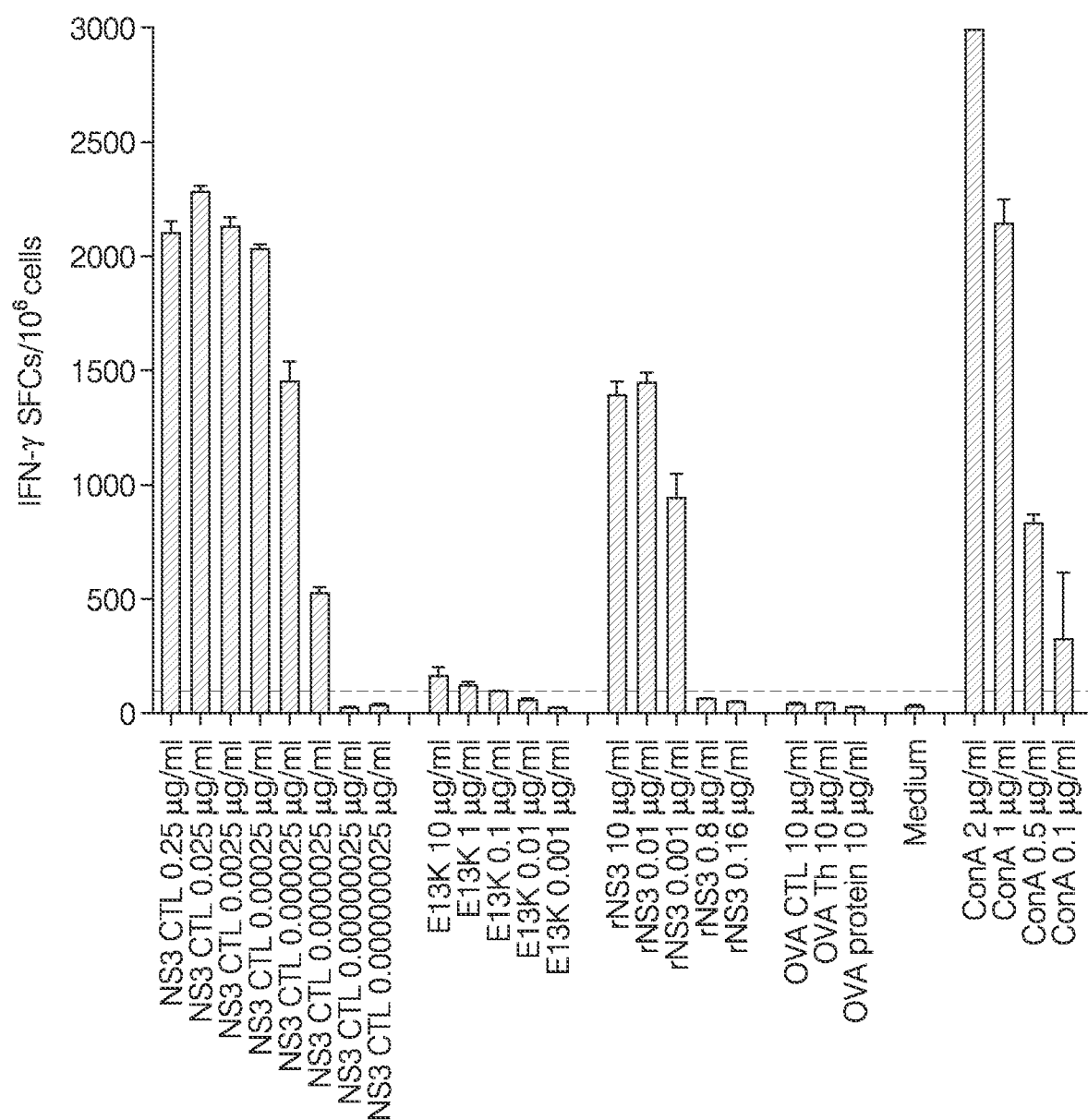


FIG. 58D

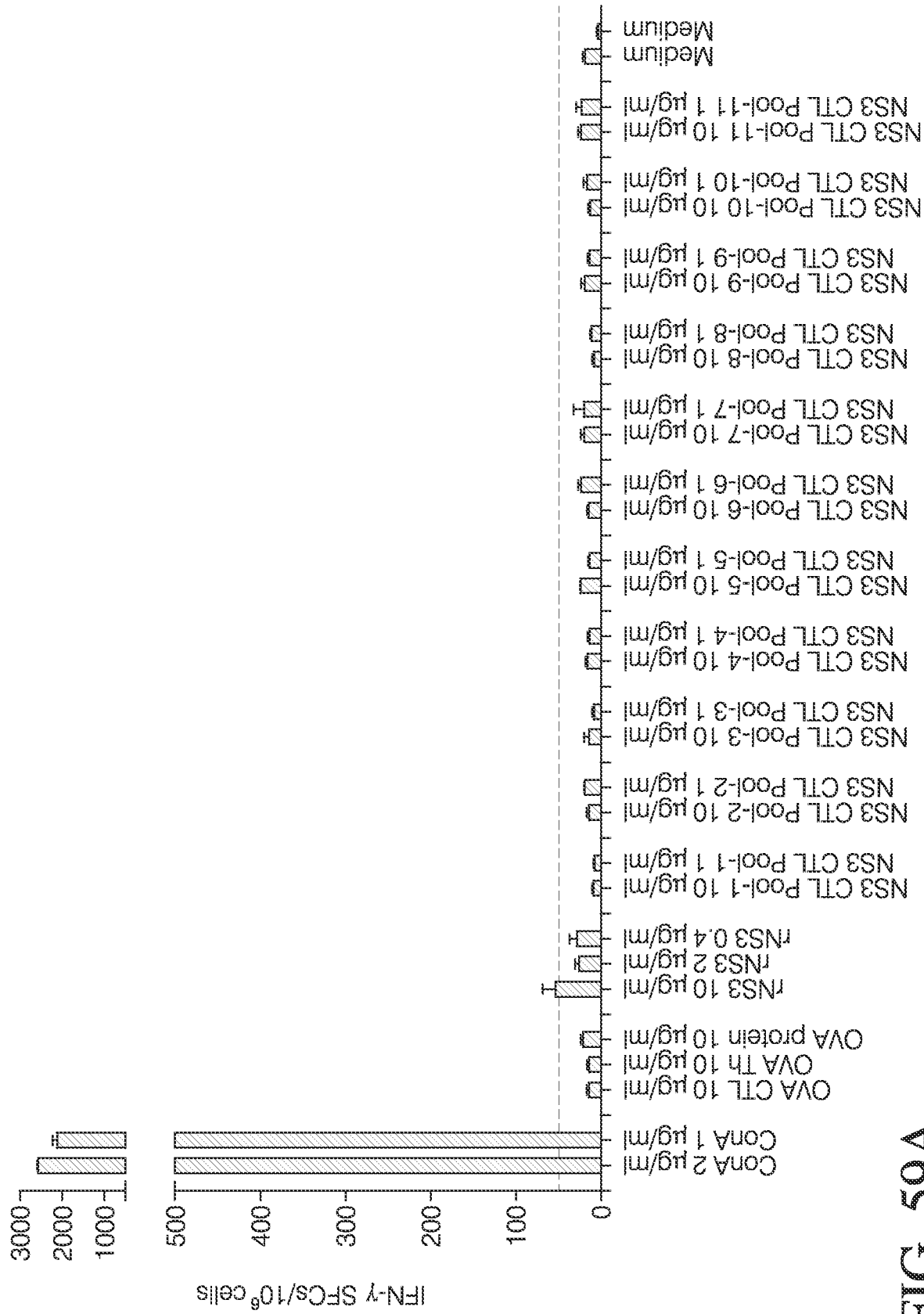


FIG. 59A

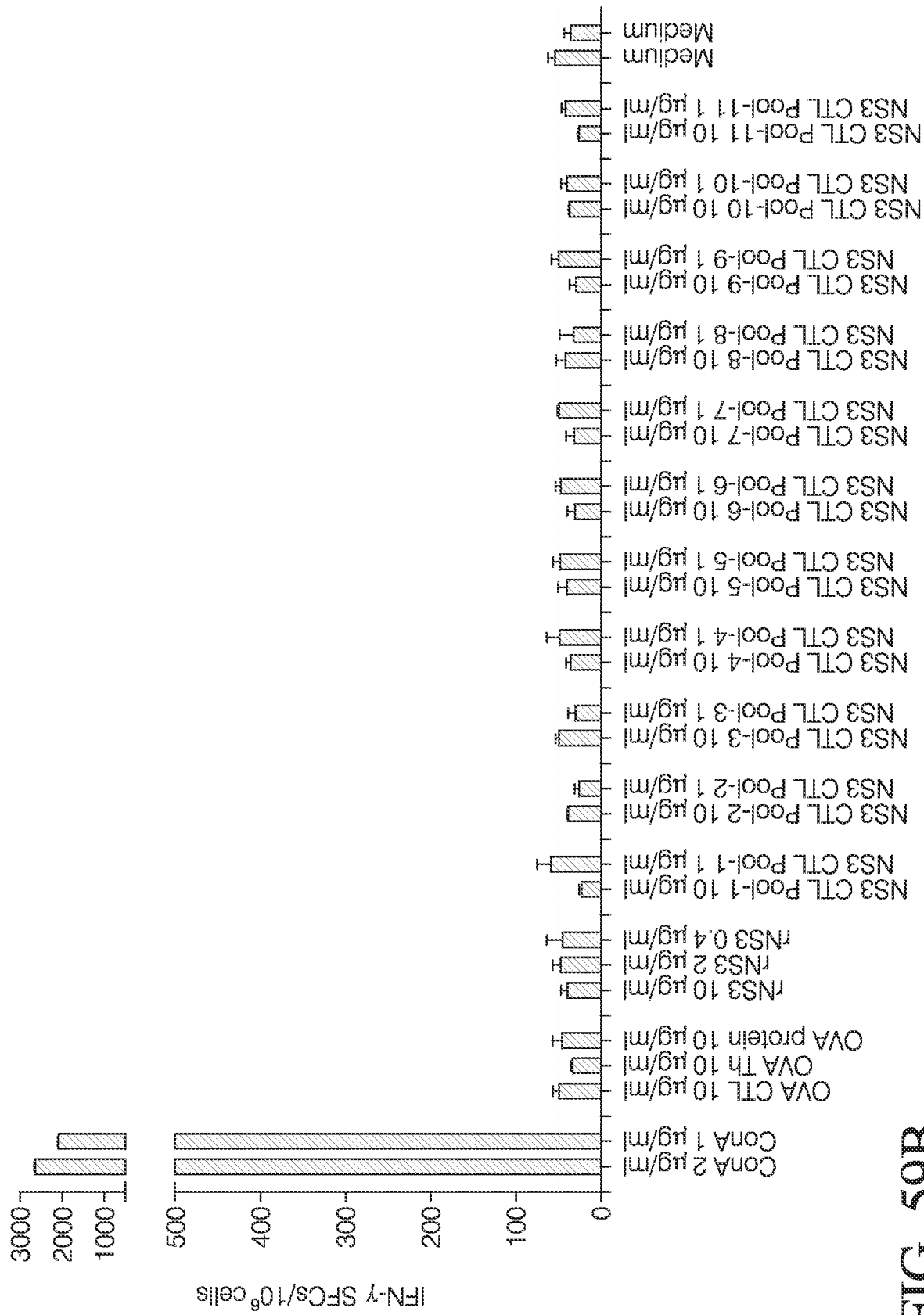


FIG. 59B

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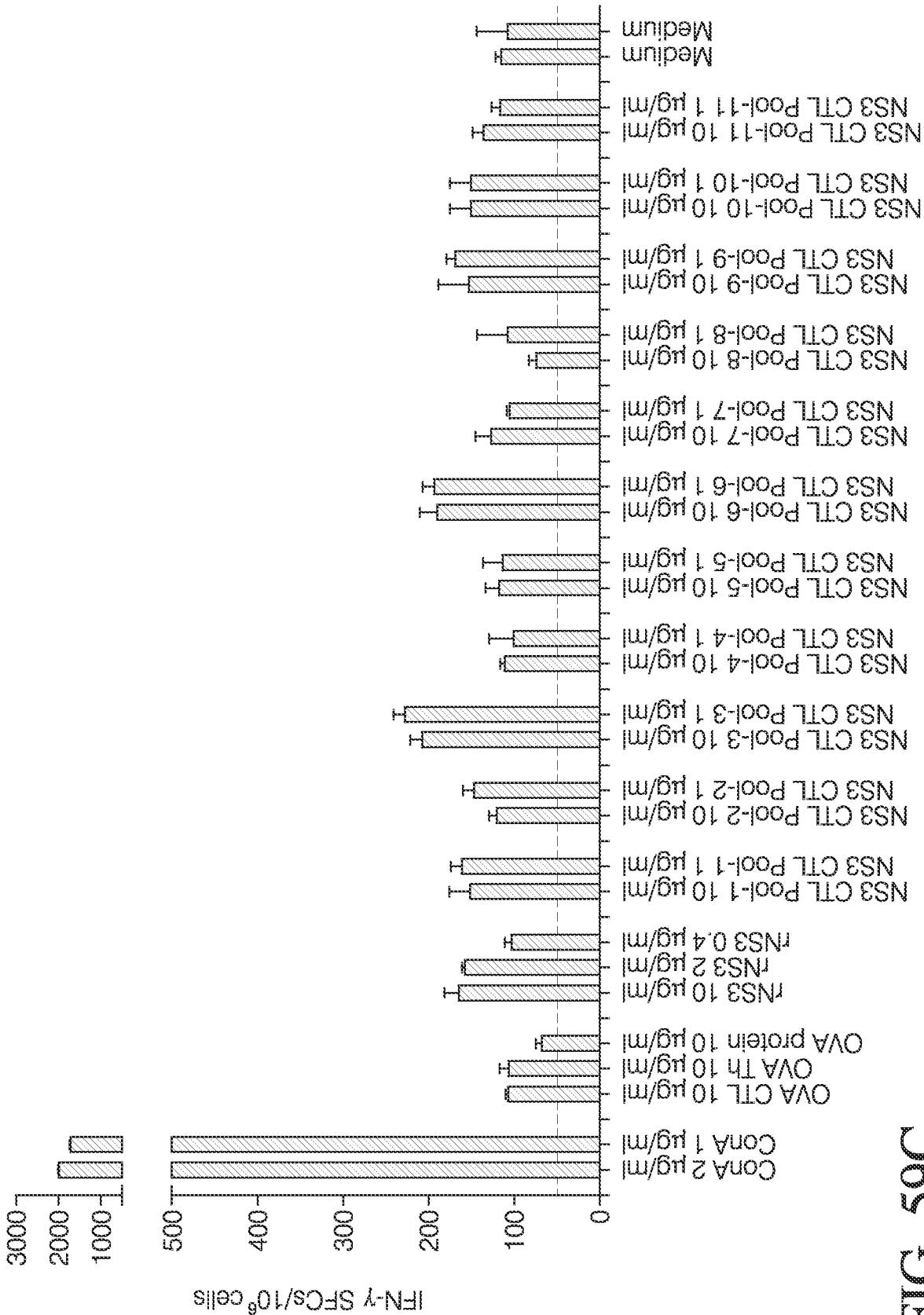


FIG. 59C

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Ex vivo frequency of NS3-specific Cd8+ T cells

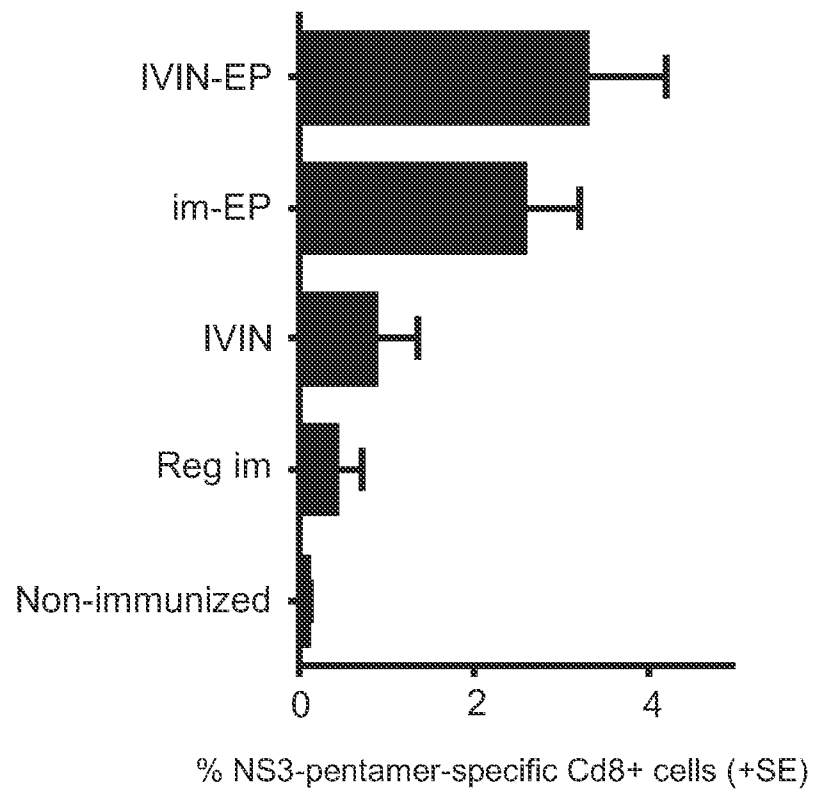


FIG. 60

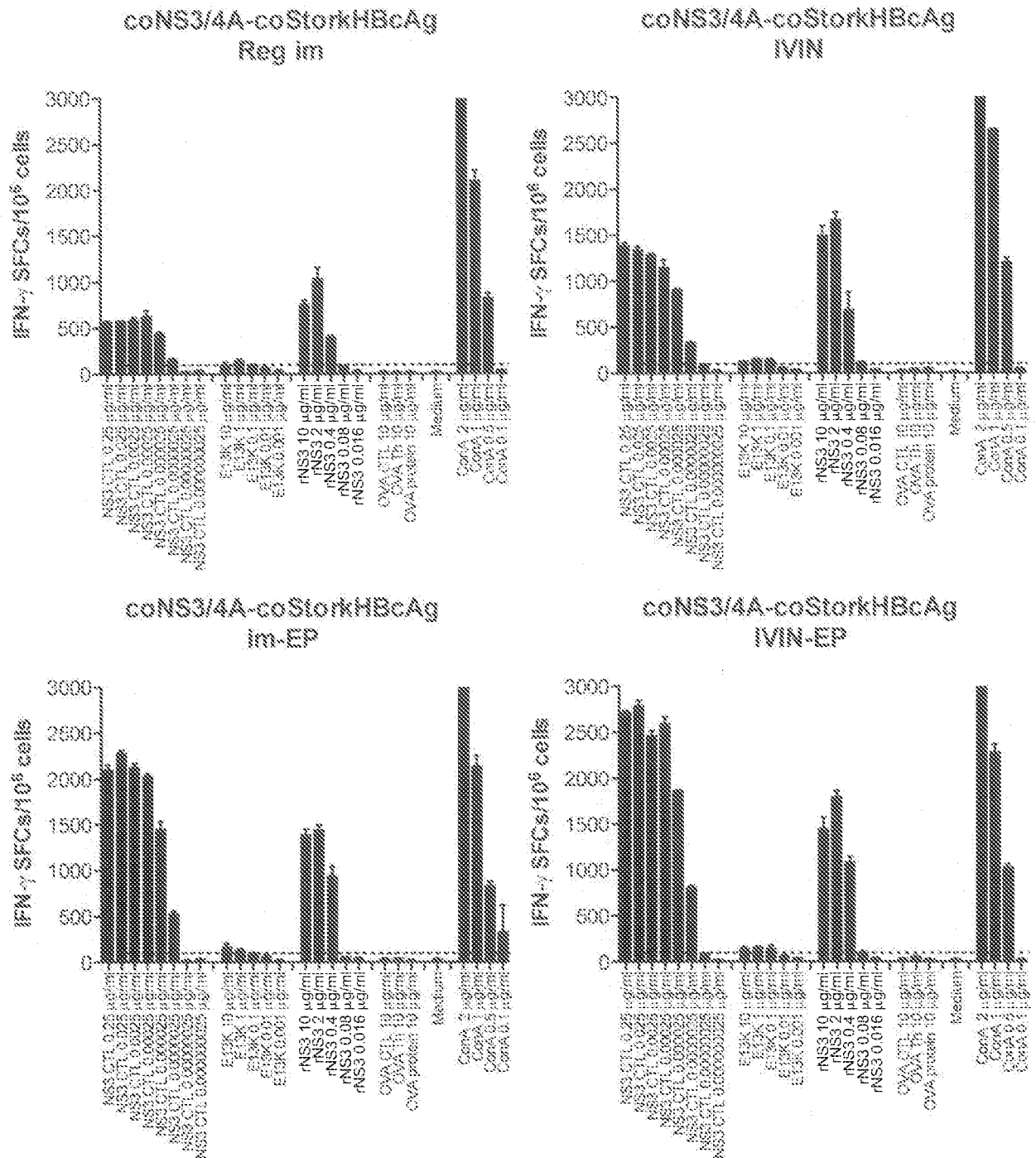


FIG. 67

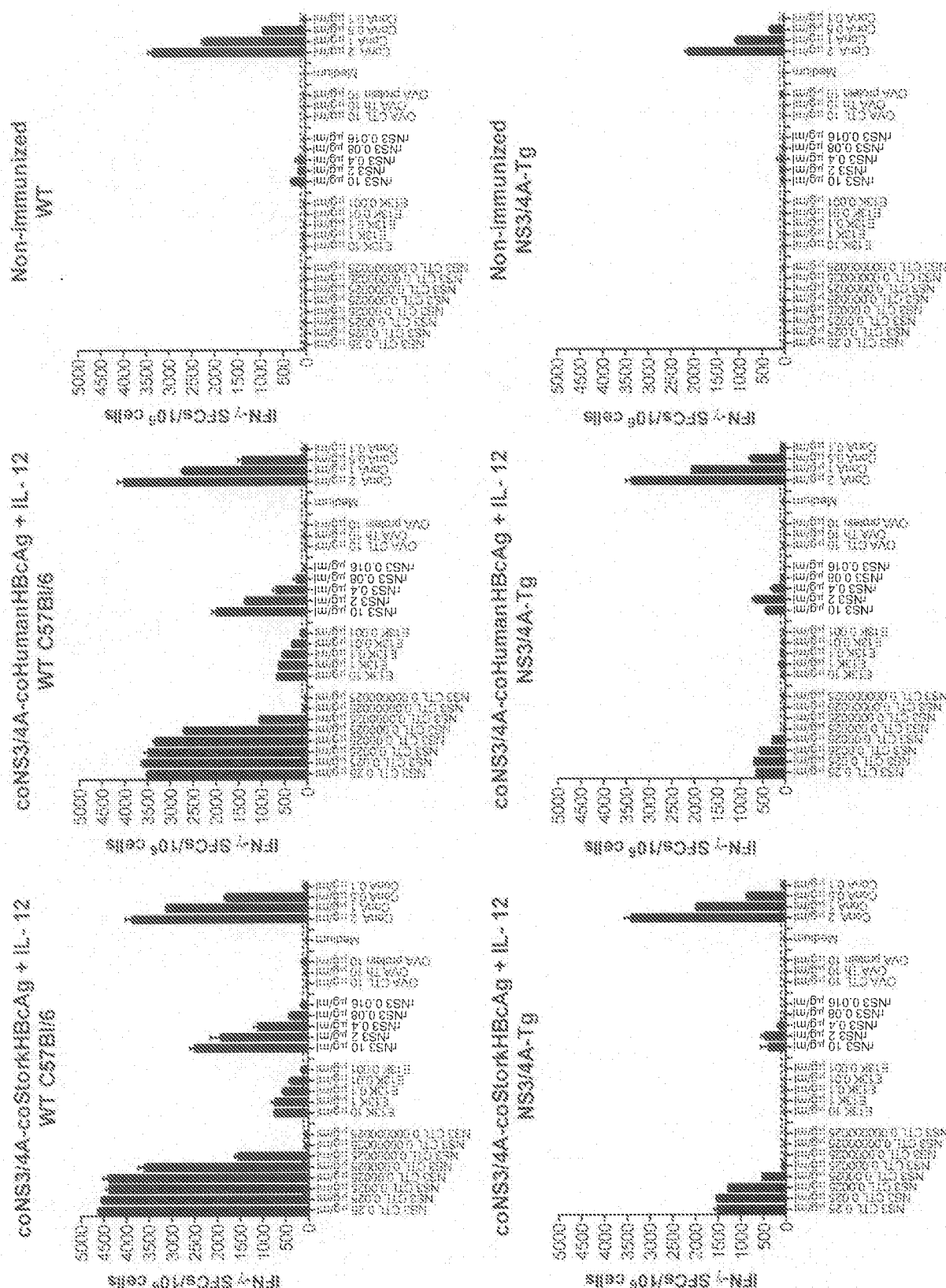


Figure 63

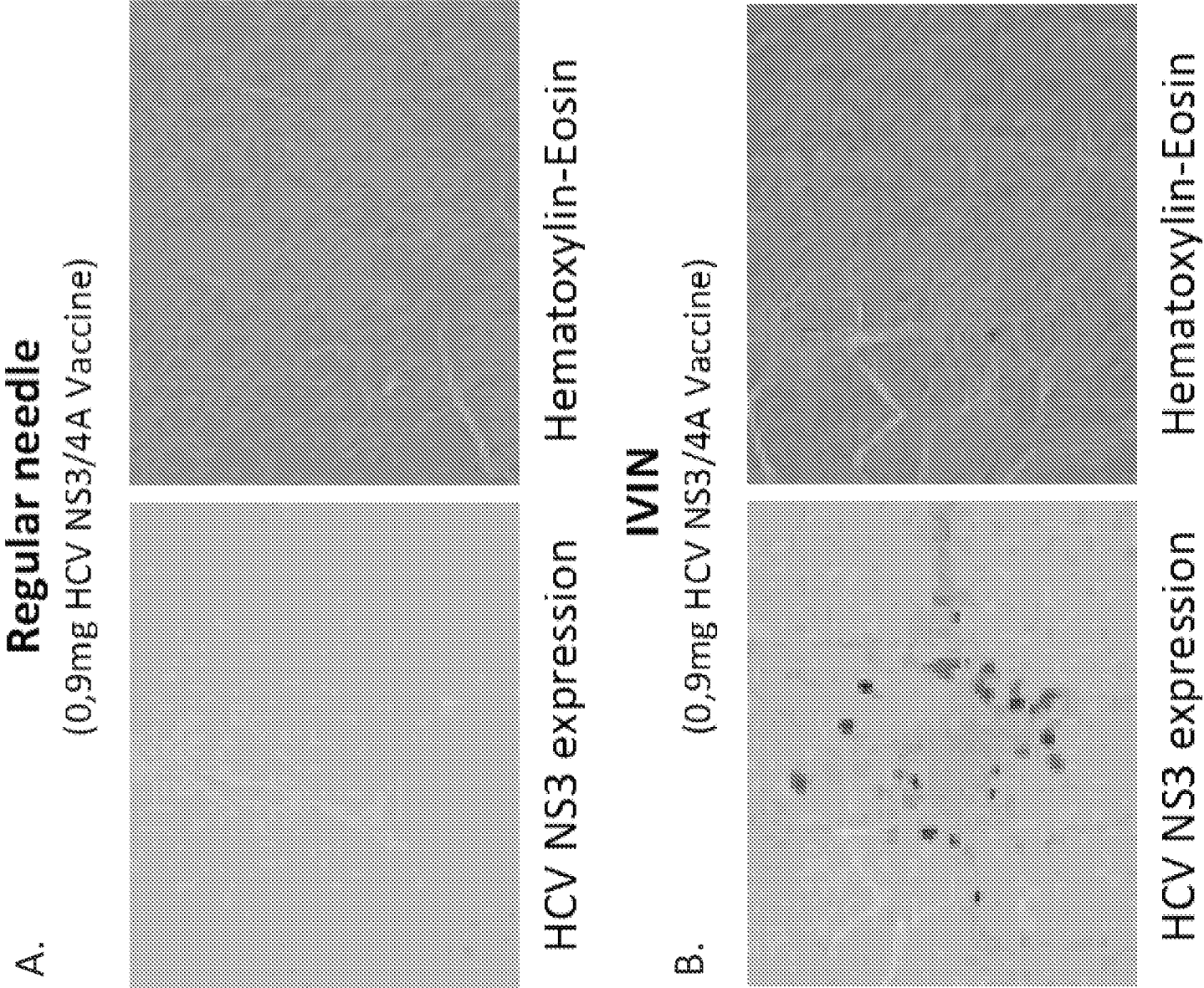


Figure 64

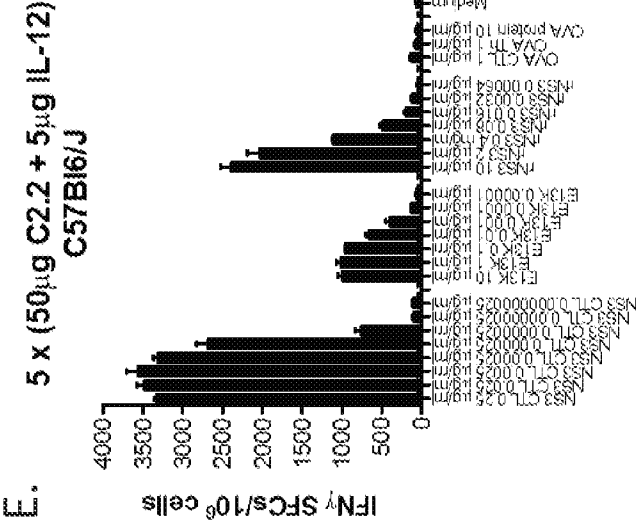
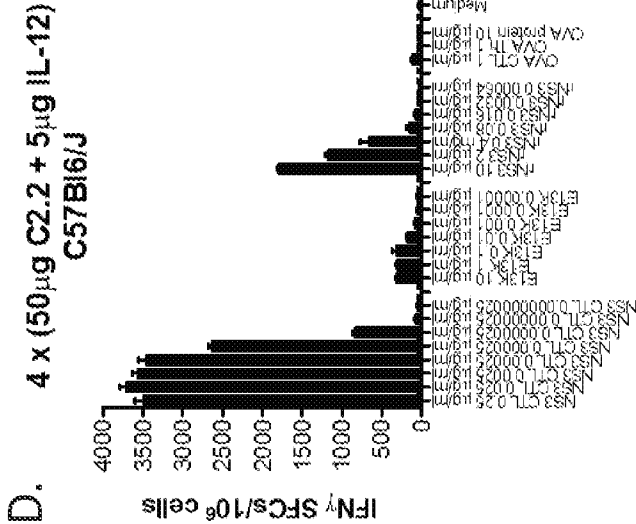
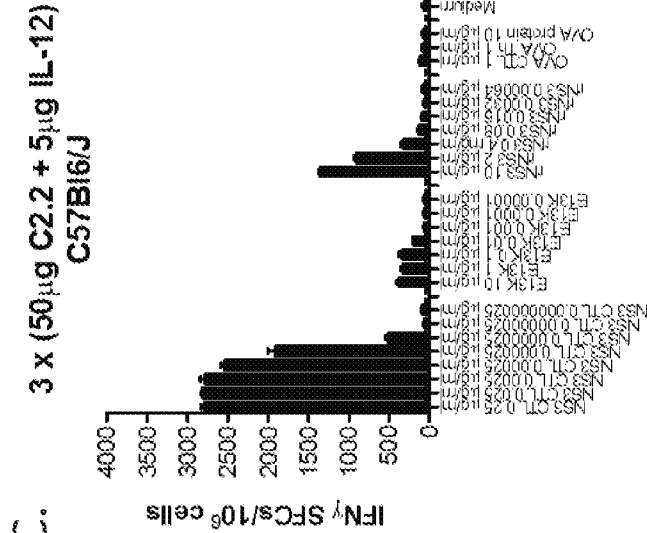
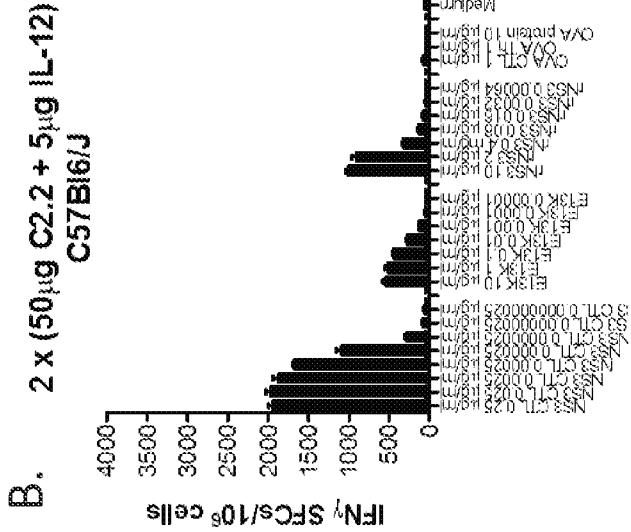
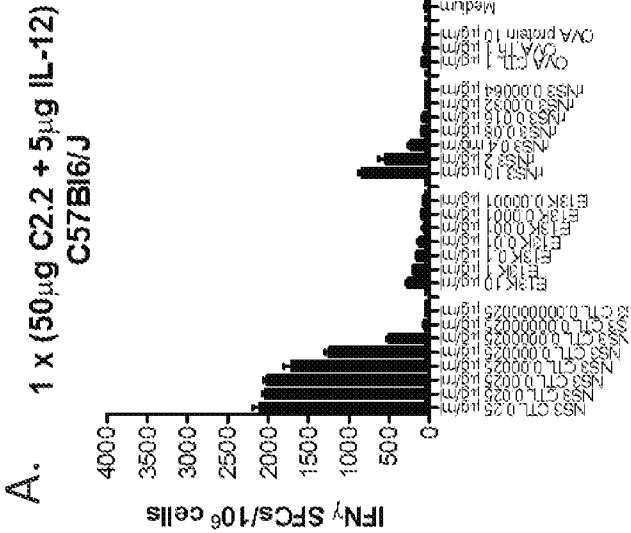
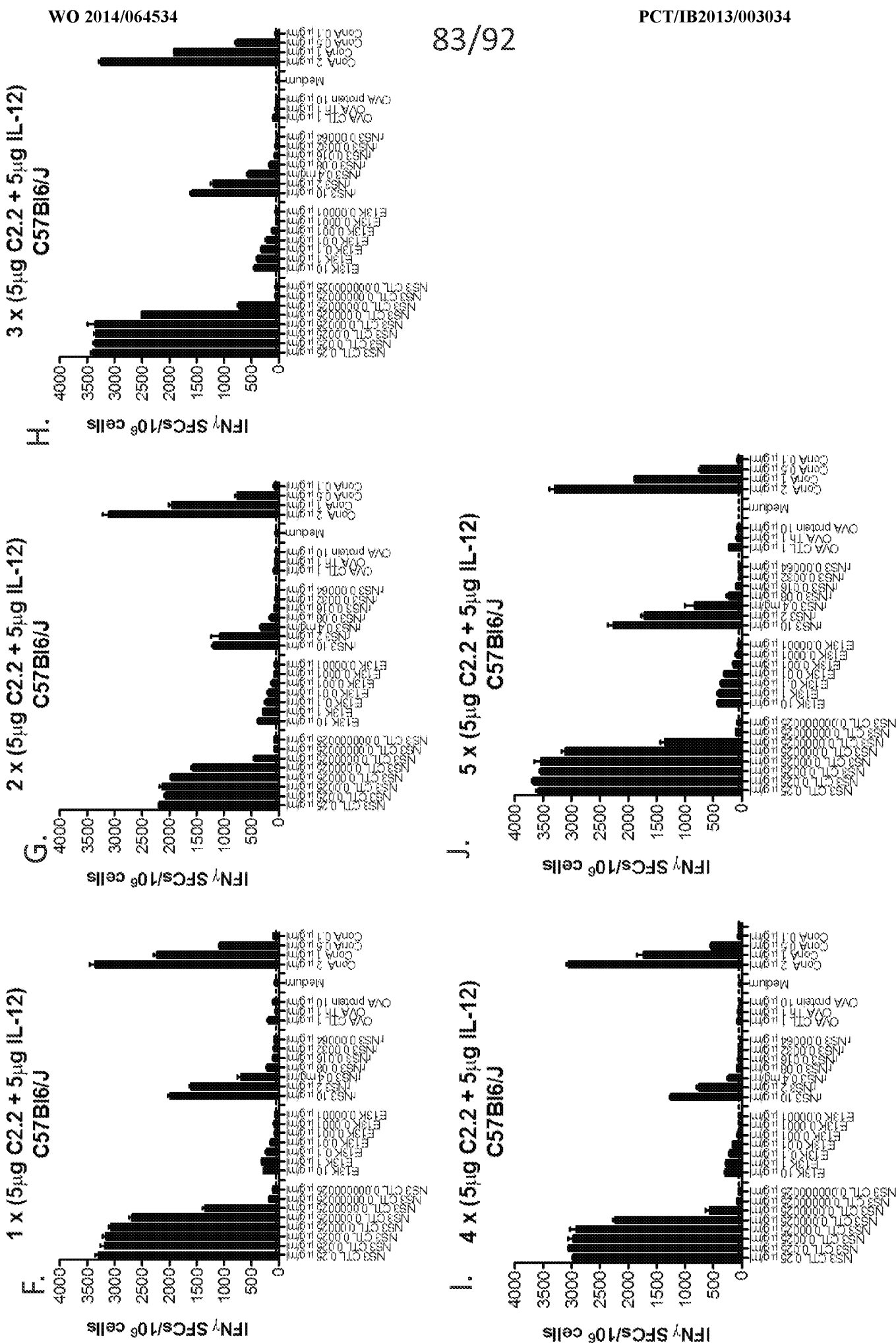


Figure 64 (cont.)



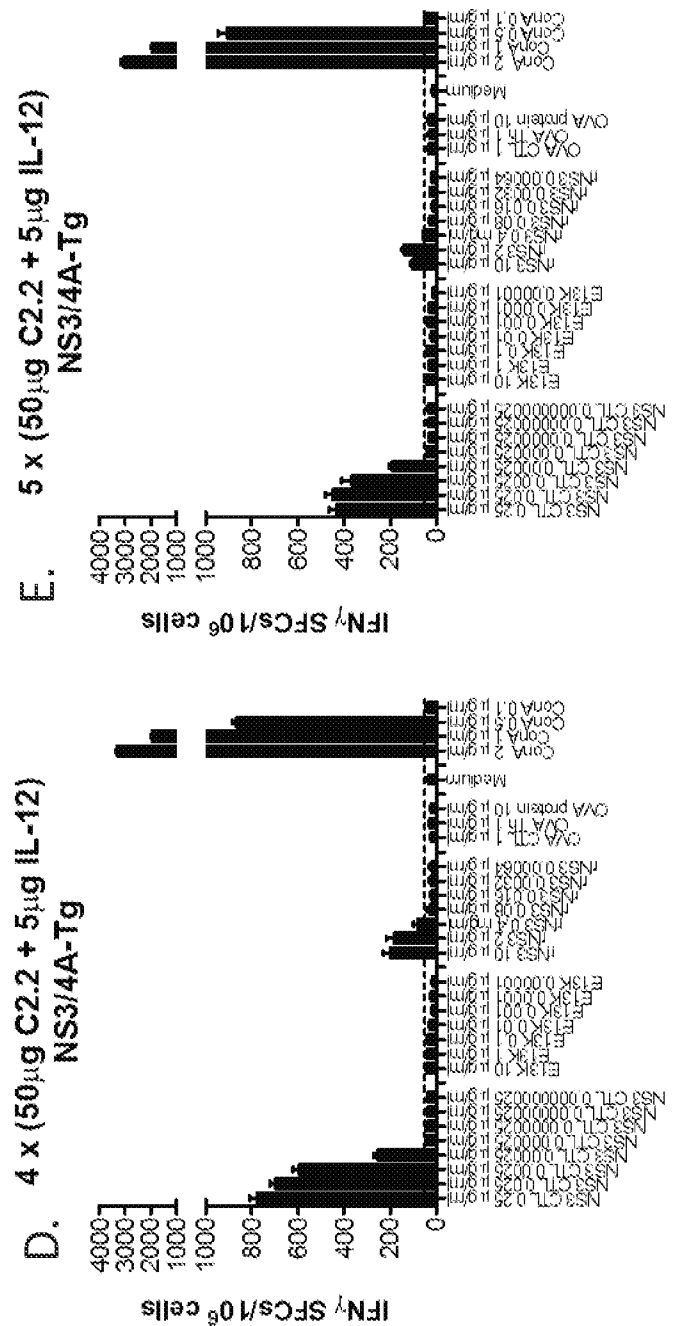
D. 4 x (50 μ g C2.2 + 5 μ g IL-12)

E. 5 x (50 μ g C2.2 + 5 μ g IL-12)

NS3/4A-Tg

IFN γ SFCs/10 5 cells

Medium
OVA protein 10 μ g/ml
OVA CTL 1 μ g/ml
OVA CTL 10 μ g/ml
NS3 0.000025 μ g/ml
NS3 0.00005 μ g/ml
NS3 0.0001 μ g/ml
NS3 0.0002 μ g/ml
NS3 0.0004 μ g/ml
NS3 0.0008 μ g/ml
NS3 0.0016 μ g/ml
NS3 0.0032 μ g/ml
NS3 0.0064 μ g/ml
NS3 0.0128 μ g/ml
NS3 0.0256 μ g/ml
NS3 0.0512 μ g/ml
NS3 0.1024 μ g/ml
NS3 0.2048 μ g/ml
NS3 0.4096 μ g/ml
NS3 0.8192 μ g/ml
NS3 1.6384 μ g/ml
NS3 3.2768 μ g/ml
NS3 6.5536 μ g/ml
NS3 13.1072 μ g/ml
NS3 26.2144 μ g/ml
NS3 52.4288 μ g/ml
NS3 104.8576 μ g/ml
NS3 209.7152 μ g/ml
NS3 419.4304 μ g/ml
NS3 838.8608 μ g/ml
NS3 1677.7216 μ g/ml
NS3 3355.4432 μ g/ml
NS3 6710.8864 μ g/ml
NS3 13421.7728 μ g/ml
NS3 26843.5456 μ g/ml
NS3 53687.0912 μ g/ml
NS3 107374.1824 μ g/ml
NS3 214748.3648 μ g/ml
NS3 429496.7296 μ g/ml
NS3 858993.4592 μ g/ml
NS3 1717986.9184 μ g/ml
NS3 3435973.8368 μ g/ml
NS3 6871947.6736 μ g/ml
NS3 13743895.3472 μ g/ml
NS3 27487790.6944 μ g/ml
NS3 54975581.3888 μ g/ml
NS3 109951162.7776 μ g/ml
NS3 219902325.5552 μ g/ml
NS3 439804651.1104 μ g/ml
NS3 879609302.2208 μ g/ml
NS3 1759218604.4416 μ g/ml
NS3 3518437208.8832 μ g/ml
NS3 7036874417.7664 μ g/ml
NS3 14073748835.5328 μ g/ml
NS3 28147497671.0656 μ g/ml
NS3 56294995342.1312 μ g/ml
NS3 112589990684.2624 μ g/ml
NS3 225179981368.5248 μ g/ml
NS3 450359962737.0496 μ g/ml
NS3 900719925474.0992 μ g/ml
NS3 1801439850948.1984 μ g/ml
NS3 3602879701896.3968 μ g/ml
NS3 7205759403792.7936 μ g/ml
NS3 14411518807585.5872 μ g/ml
NS3 28823037615171.1744 μ g/ml
NS3 57646075230342.3488 μ g/ml
NS3 115292150460684.6976 μ g/ml
NS3 230584300921369.3952 μ g/ml
NS3 461168601842738.7904 μ g/ml
NS3 922337203685477.5808 μ g/ml
NS3 1844674407370955.1616 μ g/ml
NS3 3689348814741910.3232 μ g/ml
NS3 7378697629483820.6464 μ g/ml
NS3 14757395258967641.2928 μ g/ml
NS3 29514790517935282.5856 μ g/ml
NS3 59029581035870565.1712 μ g/ml
NS3 118059162071741130.3424 μ g/ml
NS3 236118324143482260.6848 μ g/ml
NS3 472236648286964521.3696 μ g/ml
NS3 944473296573929042.7392 μ g/ml
NS3 1888946593147858085.4784 μ g/ml
NS3 3777893186295716170.9568 μ g/ml
NS3 7555786372591432341.9136 μ g/ml
NS3 15111572745182864683.8272 μ g/ml
NS3 30223145490365729367.6544 μ g/ml
NS3 60446290980731458735.3088 μ g/ml
NS3 120892581961462917470.6176 μ g/ml
NS3 241785163922925834941.2352 μ g/ml
NS3 483570327845851669882.4704 μ g/ml
NS3 967140655691703339764.9408 μ g/ml
NS3 1934281311383406679529.8816 μ g/ml
NS3 3868562622766813359059.7632 μ g/ml
NS3 7737125245533626718119.5264 μ g/ml
NS3 15474250491067253436239.0528 μ g/ml
NS3 30948500982134506872478.1056 μ g/ml
NS3 61897001964269013744956.2112 μ g/ml
NS3 123794003928538027489912.4224 μ g/ml
NS3 247588007857076054979824.8448 μ g/ml
NS3 495176015714152109959649.6896 μ g/ml
NS3 990352031428304219919299.3792 μ g/ml
NS3 1980704062856608439838598.7584 μ g/ml
NS3 3961408125713216879677197.5168 μ g/ml
NS3 7922816251426433759354395.0336 μ g/ml
NS3 15845632502852867518708790.0672 μ g/ml
NS3 31691265005705735037417580.1344 μ g/ml
NS3 63382530011411470074835160.2688 μ g/ml
NS3 126765060022822940149670320.5376 μ g/ml
NS3 253530120045645880299340641.0752 μ g/ml
NS3 507060240091291760598681282.1504 μ g/ml
NS3 1014120480182583521197362564.3008 μ g/ml
NS3 2028240960365167042394725128.6016 μ g/ml
NS3 4056481920730334084789450257.2032 μ g/ml
NS3 8112963841460668169578900514.4064 μ g/ml
NS3 16225927682921336339157801028.8128 μ g/ml
NS3 32451855365842672678315602057.6256 μ g/ml
NS3 64903710731685345356631204115.2512 μ g/ml
NS3 129807421463370690713262408230.5024 μ g/ml
NS3 259614842926741381426524816461.0048 μ g/ml
NS3 519229685853482762853049632922.0096 μ g/ml
NS3 1038459371706965525706099265844.0192 μ g/ml
NS3 207691874



F. 1 x (5 μ g C2.2 + 5 μ g IL-12)
NS3/4A-Tg

IFN γ SFCs/ 10^6 cells

G. 2 x (5 μ g C2.2 + 5 μ g IL-12)
NS3/4A-Tg

IFN γ SFCs/ 10^6 cells

H. 3 x (5 μ g C2.2 + 5 μ g IL-12)
NS3/4A-Tg

IFN γ SFCs/ 10^6 cells

I. 4 x (5 μ g C2.2 + 5 μ g IL-12)
NS3/4A-Tg

IFN γ SFCs/ 10^6 cells

J. 5 x (5 μ g C2.2 + 5 μ g IL-12)
NS3/4A-Tg

IFN γ SFCs/ 10^6 cells

60
60
e
n
bo
x
L

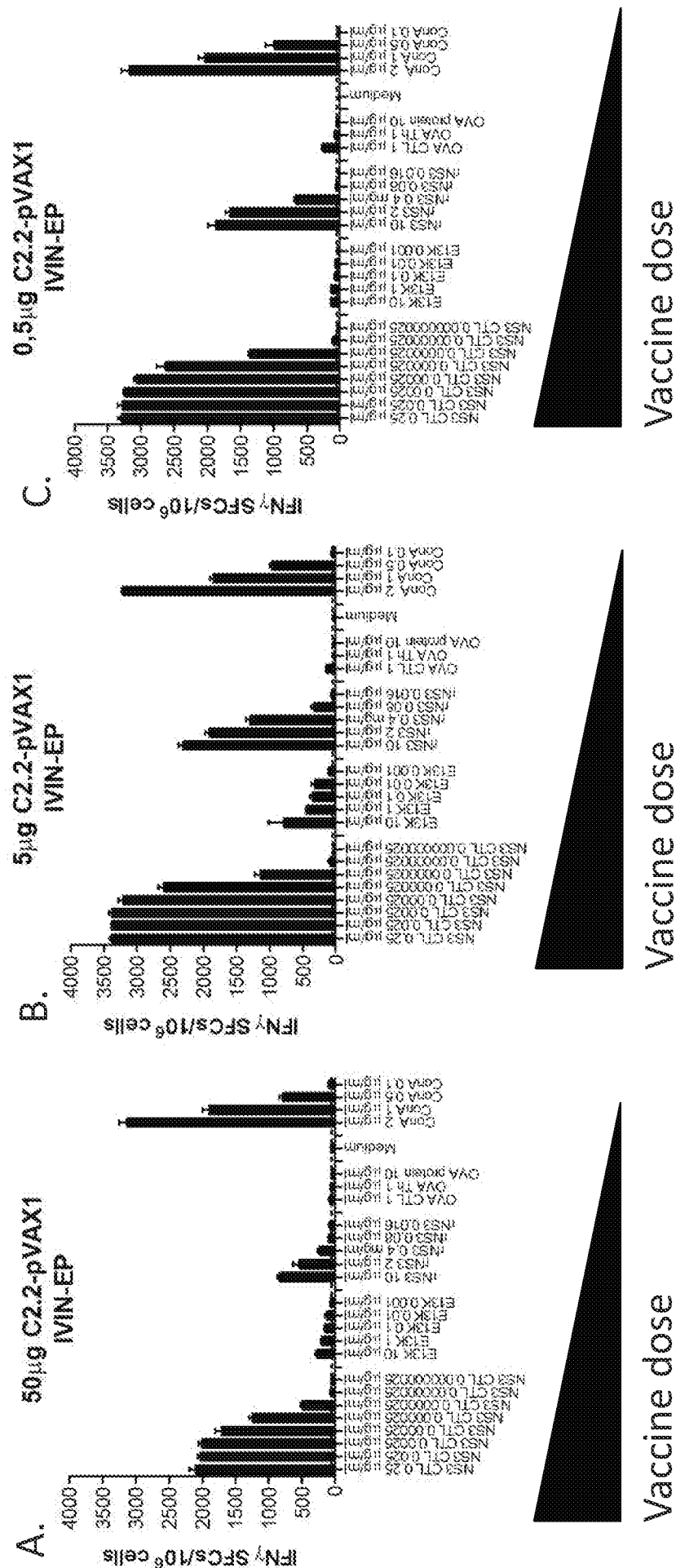
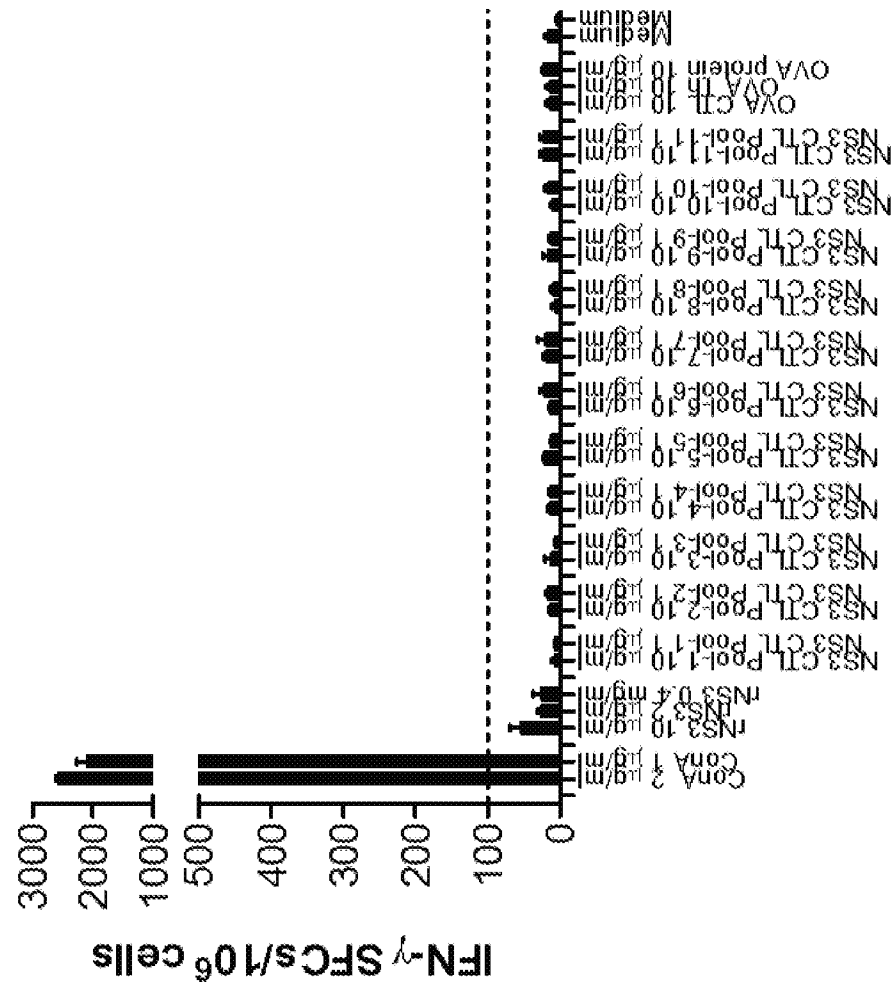


Figure 67

A. Non-immunized (Puck)



B. 1 x Skin (Timon)

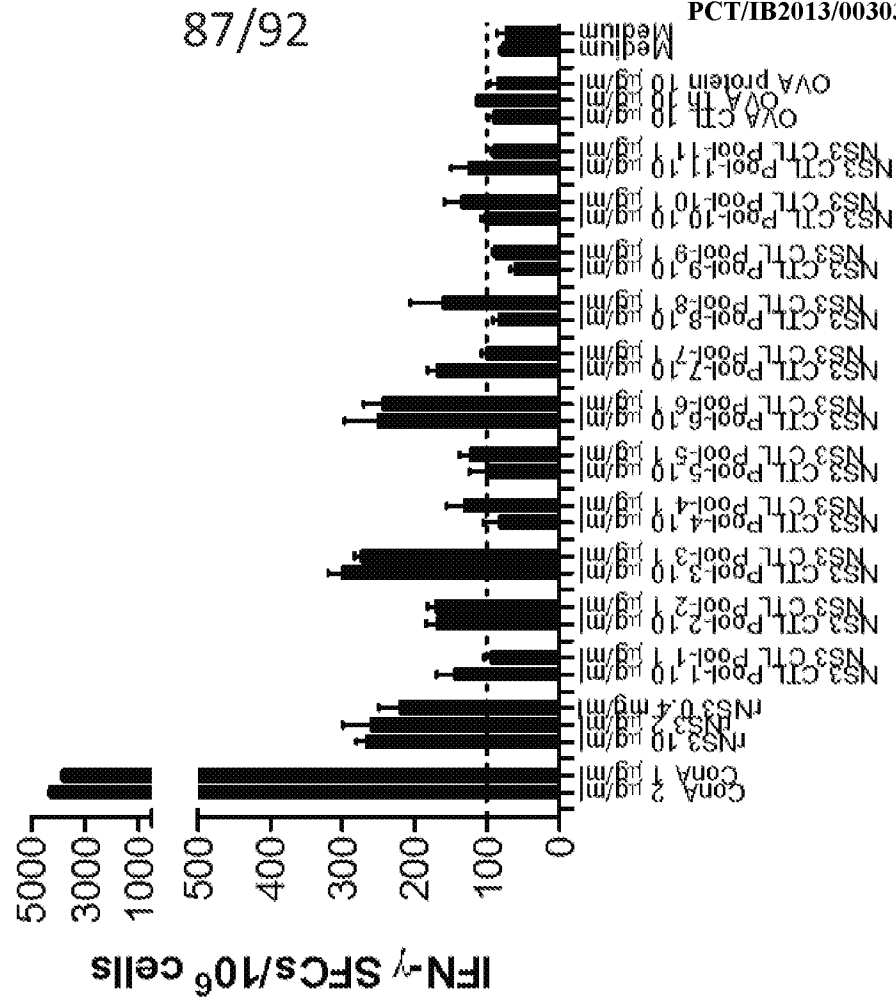
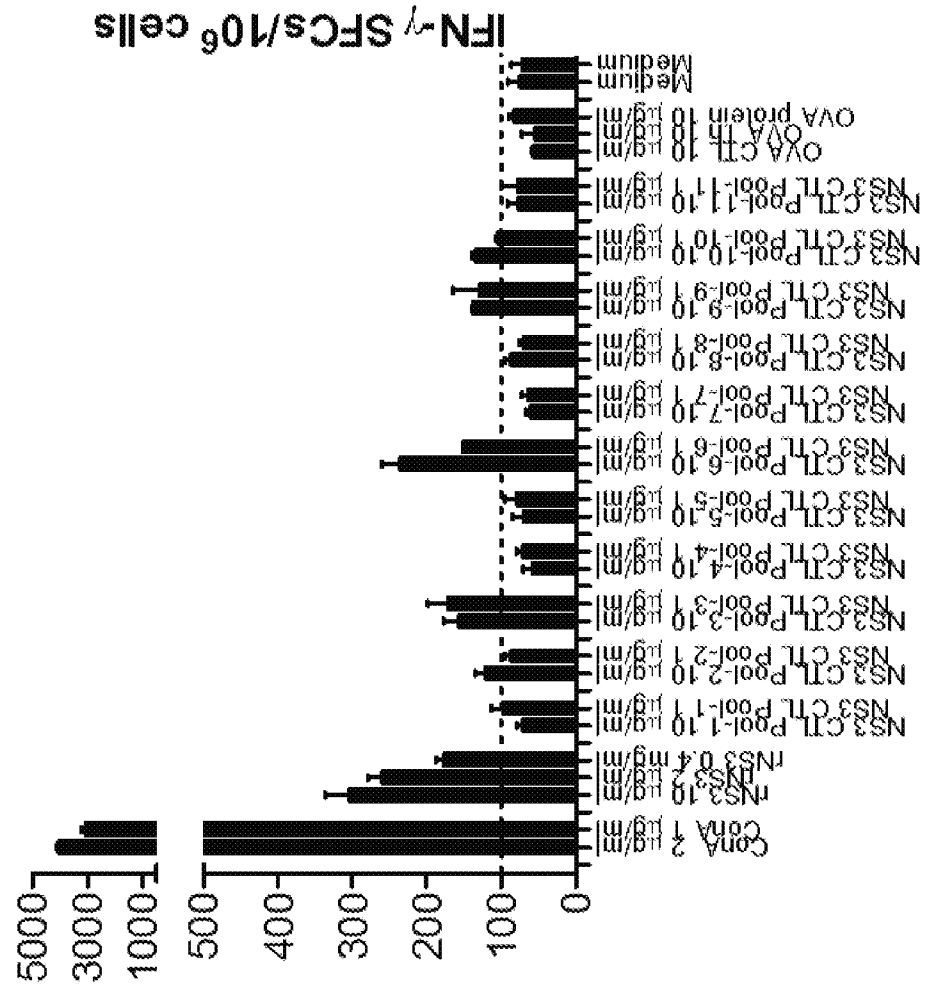


Figure 67 (cont.)

C. 1 x Skin (Hamlet)



D. 2 x Skin (Hamlet)

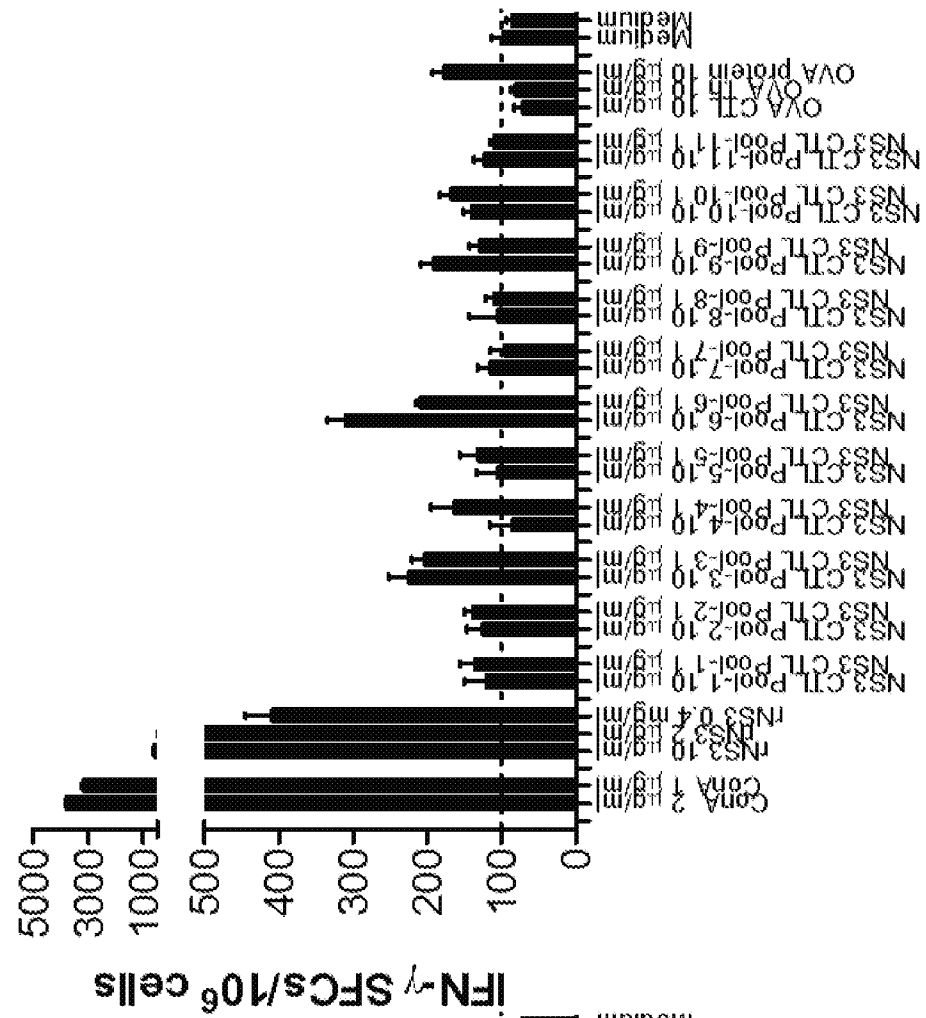
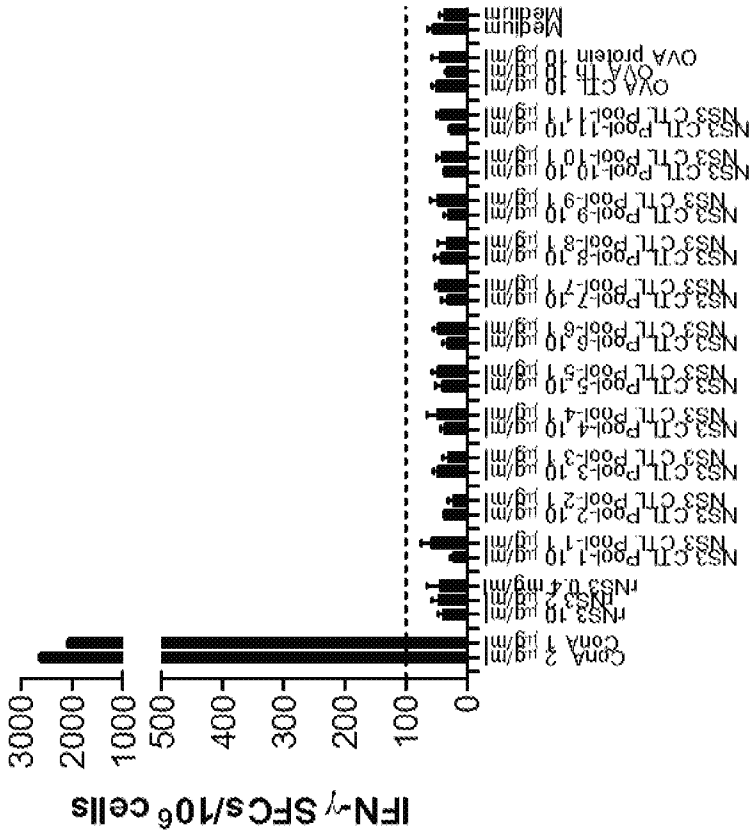


Figure 67 (cont.)

E. Non-immunized (Yorrick)



F. 1 x IM (Romeo)

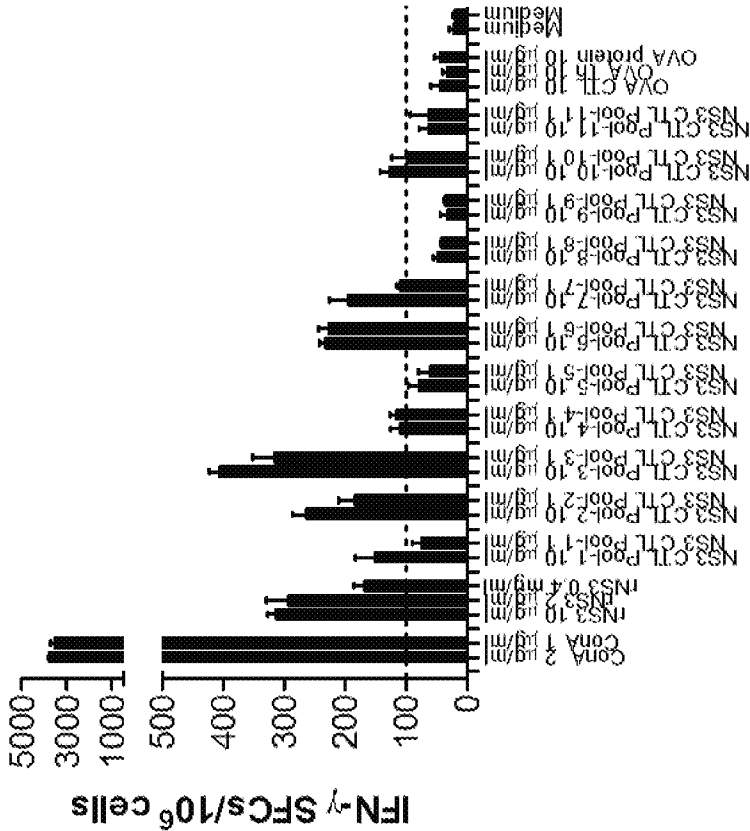
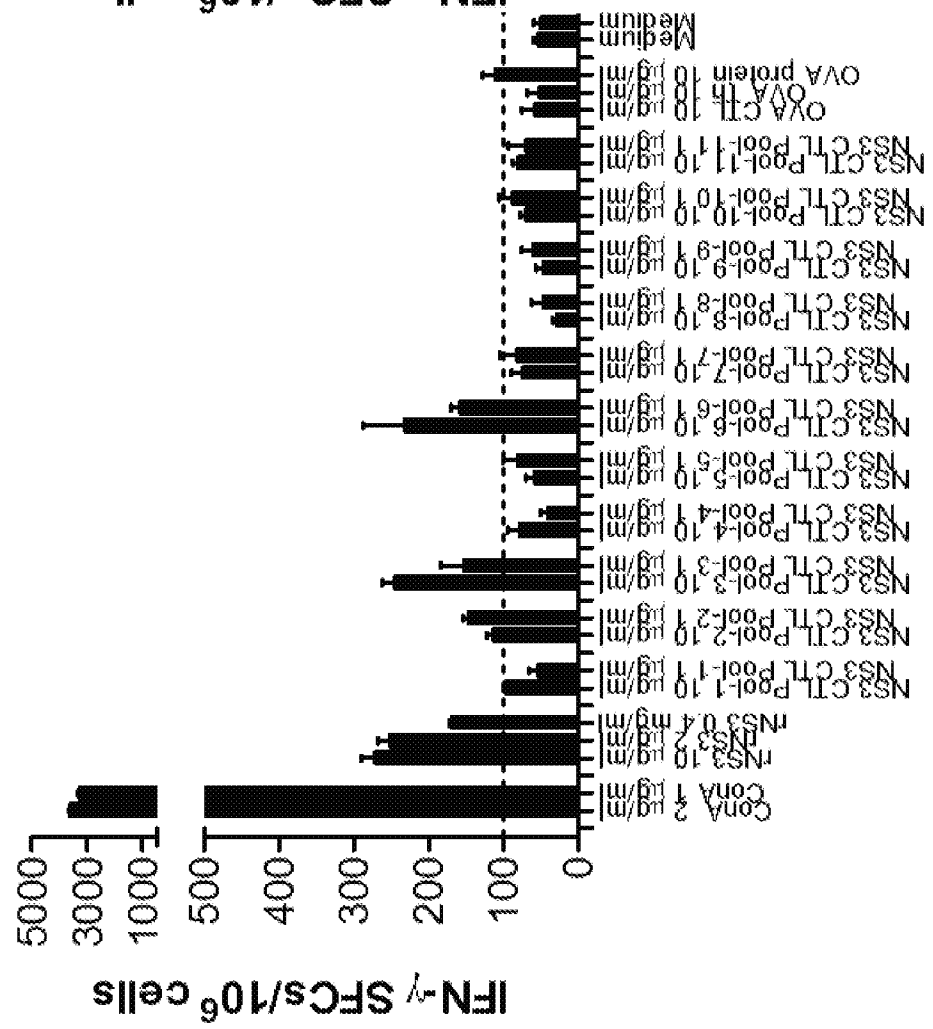
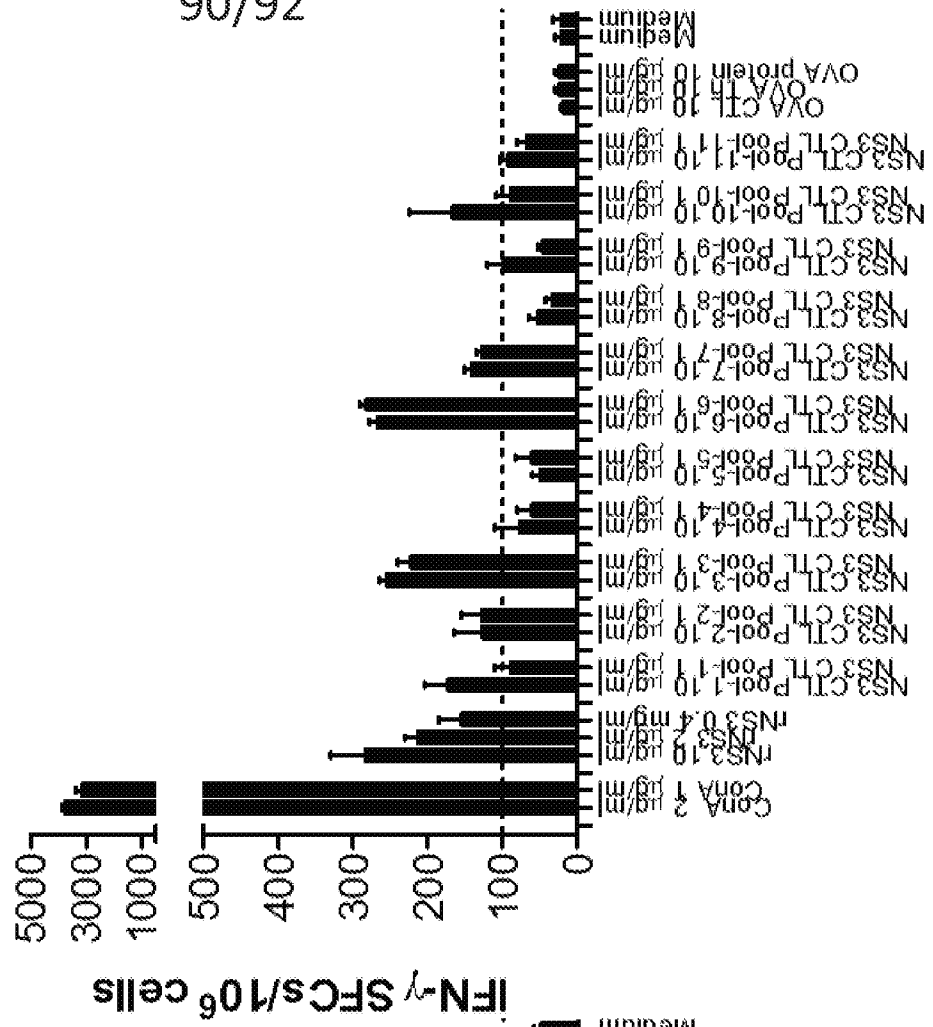


Figure 67 (cont.)

G. 1 x IM (Othello)



H. 1 x IM + 1 x Skin (Othello)



A.

5 μ g coHBcAg
5 μ g IL-12
im/EP
B(-/-)

IFN- γ SFCs/ 10^6 cells

2 μ g/ml CTL
0.2 μ g/ml CTL
0.02 μ g/ml CTL
0.0002 μ g/ml CTL
0.0002 μ g/ml CTL
0.002 μ g/ml CTL
0.02 μ g/ml CTL
0.2 μ g/ml CTL
0.002 μ g/ml CTL
0.02 μ g/ml Pool1
0.02 μ g/ml Pool1
2 μ g/ml Pool1
10 μ g/ml rHbc
0.1 μ g/ml rHbc
0.01 μ g/ml rHbc
2 μ g/ml Th
0.2 μ g/ml Th
0.02 μ g/ml Th
10 μ g/ml rHbc
1 μ g/ml rHbc
0.1 μ g/ml rHbc
0.01 μ g/ml rHbc
2 μ g/ml Pool2
0.02 μ g/ml Pool2
0.02 μ g/ml Pool2
2 μ g/ml Pool3
0.2 μ g/ml Pool3
0.02 μ g/ml Pool3
0.002 μ g/ml Pool3
OVA CTL 2 μ g/ml
OVA Th 2 μ g/ml
rOVA 10 μ g/ml
ConA 1 μ g/ml
ConA 0.5 μ g/ml
Medium

B.

5 μ g coHBcAg
5 μ g IL-12
IVN/EP
B(-/-)

IFN- γ SFCs/ 10^6 cells

2 μ g/ml CTL
0.2 μ g/ml CTL
0.02 μ g/ml CTL
0.0002 μ g/ml CTL
0.0002 μ g/ml CTL
0.002 μ g/ml CTL
0.02 μ g/ml CTL
0.2 μ g/ml CTL
0.002 μ g/ml CTL
0.02 μ g/ml Pool1
0.02 μ g/ml Pool1
2 μ g/ml Pool1
10 μ g/ml rHbc
1 μ g/ml rHbc
0.1 μ g/ml rHbc
0.01 μ g/ml rHbc
2 μ g/ml Th
0.2 μ g/ml Th
0.02 μ g/ml Th
10 μ g/ml rHbc
1 μ g/ml rHbc
0.1 μ g/ml rHbc
0.01 μ g/ml rHbc
2 μ g/ml Pool2
0.02 μ g/ml Pool2
0.02 μ g/ml Pool2
2 μ g/ml Pool3
0.2 μ g/ml Pool3
0.02 μ g/ml Pool3
0.002 μ g/ml Pool3
OVA CTL 2 μ g/ml
OVA Th 2 μ g/ml
rOVA 10 μ g/ml
ConA 1 μ g/ml
ConA 0.5 μ g/ml
Medium

Figure 69 HBcAg-CTL precursor frequency

