

TITLE OF THE INVENTION

Methods and Apparatus for the Delivery of Hepatitis B Virus (HBV) Vaccines

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority to International Patent Application No. PCT/US2017/067269, filed December 19, 2017, and U.S. Provisional Patent Application No. 62/607,430, filed December 19, 2017, the disclosures of which are incorporated herein by reference in their entireties

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

This application contains a sequence listing, which is submitted electronically via EFS-Web as an ASCII formatted sequence listing with a file name "688097-405 Sequence Listing," creation date of December 10, 2018, and having a size of 46.6 KB. The sequence listing submitted via EFS-Web is part of the specification and is herein incorporated by reference in its entirety.

FIELD

The disclosure is directed to the administration of prophylactic and/or therapeutic Hepatitis B virus (HBV) vaccines to subjects in need thereof, and, more particularly, to the reproducible, consistent, and efficacious delivery of prophylactic and/or therapeutic HBV vaccines, such as nucleic acids encoding HBV antigens, to defined regions in selected tissue site of interest, facilitated by the local application of electrical fields, in a safe, effective, and consistent fashion across heterogeneous recipient populations with minimal user training.

BACKGROUND

Hepatitis B virus (HBV) is a small 3.2-kb hepatotropic DNA virus that encodes four open reading frames and seven proteins. About two billion people are infected with HBV, and approximately 240 million people have chronic hepatitis B infection (chronic HBV), characterized by persistent virus and subvirus particles in the blood for more than 6 months (Cohen et al. *J. Viral Hepat.* (2011) 18(6), 377-83). Persistent HBV infection leads to T-cell exhaustion in circulating and intrahepatic HBV-specific CD4⁺ and CD8⁺ T-cells through chronic stimulation of HBV-specific T-cell receptors with viral peptides and circulating antigens.

As a result, T-cell polyfunctionality is decreased (i.e., decreased levels of IL-2, tumor necrosis factor (TNF)- α , IFN- γ , lack of proliferation, and degranulation). Therapeutic vaccination has the potential to eliminate HBV from chronically infected subjects (Michel et al. *J. Hepatol.* (2011) 54(6), 1286-1296). Immunogenic compositions containing one or more nucleic acid molecules encoding one or more HBV antigens can be used to provide therapeutic immunity to a subject in need thereof, such as a human having chronic HBV infection.

While naked DNA can be delivered to cells *in vivo* and result in gene expression, the efficiency of gene transfer is relatively low and variable. The local application of electrical signals has been shown to enhance the distribution and uptake of macromolecules in living tissue. Application of such electrical signals in tissue in association with the administration of a prophylactic or therapeutic HBV vaccine can have desirable effects on the tissue and/or the agent to be delivered. Specifically, techniques such as electroporation and iontophoresis have been utilized to significantly improve the distribution and/or uptake of nucleic acids, including both DNA and RNA sequences. Potential clinical applications of such techniques include the delivery of nucleic acid sequences for prophylactic and therapeutic immunization, and the delivery of nucleic acid sequences encoding therapeutic proteins or peptides.

The application procedures comprise the administration of an agent of interest to a target tissue site in conjunction with the application of electrical fields of sufficient magnitude and duration to induce the desired effects on the delivery, distribution, and/or potency of the agent. The electrical fields are propagated via two or more electrodes in electrically conductive communication with the tissue. Electrode configurations suitable for use with these techniques include tissue penetrating electrodes, surface contact electrodes, and air gap electrodes. Specific electrode configurations include, but are not limited to, elongate needle or rod electrodes, point electrodes, meander electrodes (i.e., shaped wire), planar electrodes, and combinations thereof. The specific type and arrangement of electrodes is selected based on the target tissue type and the objectives of the procedure. The desired outcome is best achieved when the electrical fields are propagated within the target tissue in the presence of the agent of interest.

A broad range of methods and devices have been described for the application of electrical fields in tissue in the presence of an agent of interest for the purpose of enhancing agent delivery in skin and muscle tissue. The devices include the use of both surface and tissue penetrating electrode systems as well as combinations thereof. In spite of the promise associated

with electrically mediated agent delivery and the potential clinical applications of these techniques, there is still a need for reliable and consistent delivery of nucleic acids of interest to subjects. Significant sources of this variability are due to differences in the technique and skill level of the operator. Other sources of variability that are not addressed by current systems include differences in the physiologic characteristics between subjects that can affect the application of the procedure. Other considerations for the development of suitable devices include their ease of use and the implementation of designs that reduce the frequency and significance of potential user errors.

Given that safe, reliable, accurate, and consistent application of clinical therapies is highly desirable, the development of improved application systems is well warranted. Such development should include a means for minimizing operator-associated variability while providing a means to accommodate the differences in subject characteristics likely to be encountered during widespread clinical application of electrically mediated agent delivery. In other words, specific areas for refinement include the ability to maintain consistent performance across heterogeneous recipient populations and a reduction in the level of training and skill required for effective use by the user. In addition, the device, system or method should be designed to facilitate avoidance of user or device errors and minimize their impact when they occur.

This Background is provided to introduce a brief context for the Summary and Detailed Description that follow. This Background is not intended to be an aid in determining the scope of the claimed subject matter nor be viewed as limiting the claimed subject matter to implementations that solve any or all of the disadvantages or problems presented above.

SUMMARY OF THE DISCLOSURE

The disclosure provides apparatus, kits and methods for the reproducible, consistent, and efficacious delivery of an HBV vaccine comprising a nucleic acid molecule encoding an HBV antigen, to a subject in need thereof, utilizing Electrically Mediated Therapeutic Agent Delivery (EMTAD). In embodiments described herein, the therapeutic agent is an HBV vaccine.

In an aspect of the disclosure, provided is an apparatus for the delivery of an HBV vaccine to a predetermined site within a subject comprising an assembly for controlled

administration of the HBV vaccine to the subject comprising a reservoir containing the HBV vaccine, at least one orifice through which the agent is administered, and a controlled source of energy sufficient to transfer a predetermined amount of the HBV vaccine at a predetermined rate from the reservoir through the orifice to the predetermined site within the subject. In addition, the apparatus can comprise a plurality of penetrating electrodes arranged with a predetermined spatial relationship relative to the orifice, and an electrical signal generator operatively connected to the electrodes.

In another aspect of the disclosure, provided is a kit for the delivery of an HBV vaccine to a predetermined site within a subject, comprising the HBV vaccine and an apparatus comprising an assembly for controlled administration of the HBV vaccine to the subject, wherein the apparatus comprises a reservoir for the HBV vaccine, at least one orifice through which the HBV vaccine is administered, and a controlled source of energy sufficient to transfer a predetermined amount of the HBV vaccine at a predetermined rate from the reservoir through the orifice to the predetermined site within the subject. In addition, the apparatus can comprise a plurality of penetrating electrodes arranged with a predetermined spatial relationship relative to the orifice, and electrical signal generator operatively connected to the electrodes.

An HBV vaccine included in an apparatus or a kit of the present application can comprise:

- a first nucleic acid molecule comprising a first polynucleotide encoding an HBV polymerase antigen having an amino acid sequence that is at least 98% identical to SEQ ID NO: 4, wherein the HBV polymerase antigen does not have reverse transcriptase activity and RNase H activity;

- a second nucleic acid molecule comprising a second polynucleotide encoding a truncated HBV core antigen consisting of the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 14; and

- a pharmaceutically acceptable carrier,

wherein the first nucleic acid molecule and the second nucleic acid molecule are present in the same nucleic acid molecule or in two different nucleic acid molecules.

Other aspects of the disclosure include methods comprising HBV vaccine administration in controlled spatial and temporal relation with Electric Signal Administration (ESA).

Benefits and advantages to certain implementations according to present principles are manifold. Some implementations allow the selection of depth of needle and electrode insertion, allowing insertion into various types of desired tissue, (e.g., dermis, muscle, etc.) across heterogeneous populations of varying body mass and body composition. These implementations also facilitate adaptation of the methods for use in specific target populations, for instance, but not restricted to, human patients having chronic HBV infection and/or an HBV-induced disease. Provided herein are systems and methods comprising design features that render the systems and methods resistant to accidental discharge or potential misuse, e.g., due to dropping, jarring, and/or falling. In some embodiments are provided devices configured for multiple injection depths. Systems and methods according to present principles allow numerous safety interlocks to reduce the frequency and/or impact of user errors. These include features to facilitate proper preparation and configuration of the dose to be administered, ensuring that the device is applied with requisite force to the tissue of the recipient, ensuring that a safety cap has been removed, and so on. Systems, apparatus, kits and methods according to present principles can allow for a highly consistent therapy to be delivered irrespective of administrator or the type of recipient. Systems, apparatus, kits and methods described herein can allow for, e.g., a consistent force profile to be obtained prior to and during delivery of the therapy, so that recipients with varying skin and muscular characteristics can be dosed consistently.

This Summary is provided to introduce a selection of concepts in a simplified form. The concepts are further described in the Detailed Description section. Elements or steps other than those described in this Summary are possible, and no element or step is necessarily required. This Summary is not intended to identify key features or essential features of the claimed subject matter, nor is it intended for use as an aid in determining the scope of the claimed subject matter. The claimed subject matter is not limited to implementations that solve any or all disadvantages noted in any part of this disclosure.

Other aspects, features and advantages of the invention will be apparent from the following disclosure, including the detailed description of the invention and its preferred embodiments and the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

The features of the disclosure are set forth with particularity in the appended claims. A better understanding of the features and advantages of the disclosure will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the disclosure are utilized, and the accompanying drawings of which:

FIG. 1 illustrates potential sources of spatial variability associated with conventional needle syringe injection.

FIG. 2 is an overview of a system according to present principles, including a cartridge assembly **100**, an applicator **400**, and a controller system **700**.

FIGS. 3A-3B show views of aspects of a device described herein. **FIG. 3A** shows a lateral view of a cartridge assembly **100** according to present principles. **FIG. 3B** shows a lateral view of a reservoir for HBV vaccine **101** embodied as a syringe, according to present principles.

FIG. 4 shows various exemplary components of a cartridge assembly **100** according to present principles.

FIGS. 5A-5E show views of aspects of an inner cartridge and cartridge breech in a device described herein. **FIG. 5A** illustrates a top view of an inner cartridge **103** according to present principles. **FIG. 5B** illustrates a bottom view of an inner cartridge **103** and cartridge breech according to present principles. **FIG. 5C** illustrates a detail side perspective view of an inner cartridge **103** according to present principles. **FIG. 5D** shows a lateral view of a cartridge breech **112** according to present principles. **FIG. 5E** illustrates reservoir interlock **120** with improved locking features to prevent the cartridge breech **112** from inadvertently moving forward.

FIG. 6 illustrates details of a cartridge assembly showing a rack **154**, a initiating flag **172**, and a continuing flag **174** according to present principles.

FIGS. 7A-7B show views of aspects of electrodes and/or electrode contacts in a device described herein. **FIG. 7A** shows details of electrode contacts **130** and various electrode contact portions according to present principles. **FIG. 7B** shows details of electrodes **122** and various electrode contacts portions according to present principles.

FIGS. 8A-8B show views of aspects of a force contact interlock system in a device described herein. **FIG. 8A** shows a top view of a force contact interlock system according to the present principle. **FIG. 8B** illustrates details of a force contact interlock system according to present principles.

FIGS. 9A-9D show views of aspects of a device described herein. **FIG. 9A** shows a needle **105** and distal inner cartridge electrodes **137** for tissue insertion according to present principles. **FIG. 9B** illustrates details of a stick shield **134** according to present principles. **FIG. 9C** illustrates an alignment guide **108** and splay shield **168** according to present principles. **FIG. 9D** illustrates stick shield supports integral to outer cartridge cap **106**.

FIGS. 10A-10D show views of aspects of exterior cartridge cap in a device described herein. **FIG. 10A** shows an exterior cartridge cap **110** according to present principles. **FIG. 10B** shows a side view of an exterior cartridge cap **110** according to present principles. **FIG. 10C** shows an exterior cartridge cap **110** in use in an alignment guide **108** and splay shield according to present principles. **FIG. 10D** shows an exterior cartridge cap **110** with extension members designed to hold the inner cartridge **103** in place during handling and loading.

FIGS. 11A-11C show details of stick shields in aspects of a device described herein. **FIG. 11A** shows a stick shield retaining hook **182** of a stick shield **134** according to present principles. **FIG. 11B** shows details of a stick shield **134** according to present principles. **FIG. 11C** shows stick shield supports **132** keeping a stick shield **134** in place according to present principles.

FIG. 12 shows details of an electrode support **124** according to present principles.

FIGS. 13A-13B show views of an applicator in a device described herein. **FIG. 13A** shows a side view of an applicator **400** according to present principles. **FIG. 13B** shows top views of an applicator **400** according to present principles.

FIG. 14 is an exploded view of an applicator **400** according to present principles.

FIG. 15 shows details of a side housing and electroporation electrode connection **496** of an applicator **400** according to present principles.

FIG. 16 is an exploded view of an applicator according to present principles, showing a cartridge loading subassembly **456**.

FIGS. 17A-17B show views of an applicator in a device described herein. **FIG. 17A** is an exploded view of an applicator according to present principles, showing a loading drive subassembly **454**. **FIG. 17B** shows a rack **154** in a loading drive subassembly **454** according to present principles.

FIGS. 18A-18C show views of aspects of an applicator in a device described herein. **FIG. 18A** shows details of a cartridge loading subassembly **456** according to present principles,

showing where the insertion/injection drive assembly of the applicator mates with the cartridge assembly. **FIG. 18B** shows a cross-sectional view of a cartridge assembly according to present principles, showing where the insertion/injection drive assembly of the applicator mates with the cartridge assembly. **FIG. 18C** shows details of cartridge loading, electrode insertion, and injection subassemblies **452** of an applicator **400** according to present principles.

FIG. 19 shows various components of a controller system according to present principles.

FIGS. 20A-20D show views of a device described herein. **FIG. 20A** shows various components of a controller system according to present principles. **FIG. 20B** shows details of an applicator connector port **708** and a tray **710** of a controller system according to present principles. **FIG. 20C** shows details of a stimulator display screen of a controller system according to present principles. **FIG. 20D** shows details of a rear view of a controller system according to the present principles.

FIG. 21 is a flowchart showing a method of operation according to present principles.

FIG. 22 illustrates TriGrid electrode array (cross-section) for intramuscular (IM) delivery, which is comprised of four electrodes arranged in two equilateral triangles to form a diamond shape surrounding a central injection needle.

FIGS. 23A-23B depict the TDS-IM v1.0 TriGrid devices adapted for use in the mouse model (**FIG. 23A**) and the non-human primate (NHP) model (**FIG. 23B**).

FIG. 24 depicts the TDS-IM v2.0 TriGrid device adapted for use in the non-human primate (NHP) model.

FIGS. 25A-25H show the design and optimization of expression cassettes and DNA plasmids encoding HBV pol and core antigens as described in Example 1; **FIG. 25A** is a schematic representation of an expression strategy in which coding sequences of the HBV core and pol antigens are fused in frame; **FIG. 25B** is a schematic representation of an expression strategy in which coding sequences of both the core and pol antigens are expressed from a single plasmid by means of the ribosomal FA2 slippage site; **FIG. 25C** is a schematic representation of an expression strategy in which the core and pol antigens are expressed from two separate plasmids; **FIG. 25D** is a Western blot of core antigen expression in HEK293T cells transfected with a plasmid expressing core with and without the post-transcriptional regulatory element WPRE; expression was tested in cell lysate (left) and supernatant (sup; right) using an α -core

antibody; **FIG. 25E** is a Western blot analysis showing a comparison of core expression in HEK293T cells transfected with a core expressing plasmid including the intron/exon sequence derived from human apolipoprotein A1 precursor (“AI intron”), untranslated R-U5 domain of the human T-cell leukemia virus type 1 (HTLV-1) long terminal repeat (LTR) (“HTLV R”), or triple enhancer composite sequence of the HTLV-1 LTR, synthetic rabbit β -globin intron, and a splicing enhancer (“triple”); the unlabeled lane is purified core protein as a size marker; expression was tested in both lysate (left) and supernatant (sup; right); core antigen expression was highest with the triple enhancer composite sequence; **FIG. 25F** is a Western blot analysis of core antigen secretion using different signal peptides fused to the N-terminus of the HBV core antigen; the most efficient protein secretion was observed with the Cystatin S signal peptide; **FIG. 25G** is a schematic representation of optimized HBV core/pol antigen expression cassettes for each of the three expression strategies illustrated in **FIGS. 25A-25C**; CMVpr: human CMV-IE promoter; TRE: triple enhancer sequence; SP: cystatin S signal peptide; FA2: FMDV ribosomal slippage site; pA: BGH polyadenylation signal; **FIG. 25H** is a Western blot analysis of HBV core and pol antigen expression of pDK vectors containing each of the expression cassettes shown in **FIG. 25G**; lanes 1 and 2: pDK-core; lanes 3 and 4: pDK-pol; lanes 5 and 6: pDK-coreFA2Pol; lanes 7 and 8: pDK-core-pol fusion: the most consistent expression profile for cellular and secreted core and pol antigens was observed when the antigens were encoded by separate vectors;

FIGS. 26A-26B show schematic representations of DNA plasmids according to embodiments of the application; **FIG. 26A** shows a DNA plasmid encoding an HBV polymerase (pol) antigen according to an embodiment of the application; **FIG. 26B** shows a DNA plasmid encoding an HBV core antigen according to an embodiment of the application; the HBV core and pol antigens are expressed under control of a CMV promoter with an N-terminal cystatin S signal peptide that is cleaved from the expressed antigen upon secretion from the cell; transcriptional regulatory elements of the plasmid include an enhancer sequence located between the CMV promoter and the polynucleotide sequence encoding the HBV antigen and a bGH polyadenylation sequence located downstream of the polynucleotide sequence encoding the HBV antigen; a second expression cassette is included in the plasmid in reverse orientation including a kanamycin resistance gene under control of an Amp^r (bla) promoter; an origin of replication (pUC) is also included in reverse orientation;

Like reference numerals refer to like elements throughout. Elements are not to scale unless otherwise noted.

DETAILED DESCRIPTION

The following description and examples illustrate embodiments of the invention in detail. It is to be understood that this invention is not limited to the particular embodiments described herein and as such can vary. Those of skill in the art will recognize that there are numerous variations and modifications of this invention, which are encompassed within its scope.

All terms are intended to be understood as they would be understood by a person skilled in the art. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the disclosure pertains.

The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

Although various features of the invention can be described in the context of a single embodiment, the features may also be provided separately or in any suitable combination. Conversely, although the invention can be described herein in the context of separate embodiments for clarity, the invention may also be implemented in a single embodiment.

In an attempt to help the reader of the application, the description has been separated in various paragraphs or sections, or is directed to various embodiments of the application. These separations should not be considered as disconnecting the substance of a paragraph or section or embodiments from the substance of another paragraph or section or embodiments. To the contrary, one skilled in the art will understand that the description has broad application and encompasses all the combinations of the various sections, paragraphs and sentences that can be contemplated. The discussion of any embodiment is meant only to be exemplary and is not intended to suggest that the scope of the disclosure, including the claims, is limited to these examples. For example, while embodiments of HBV vectors that can be used in the application (e.g., plasmid DNA or viral vectors) described herein may contain particular components, including, but not limited to, certain promoter sequences, enhancer or regulatory sequences, signal peptides, coding sequence of an HBV antigen, polyadenylation signal sequences, etc. arranged in a particular order, those having ordinary skill in the art will appreciate that the

concepts disclosed herein may equally apply to other components arranged in other orders that can be used in HBV vectors useful for the application. The application contemplates use of any of the applicable components in any combination having any sequence that can be used in HBV vectors useful for the application, whether or not a particular combination is expressly described.

The following definitions supplement those in the art and are directed to the current application and are not to be imputed to any related or unrelated case, e.g., to any commonly owned patent or application. Although any methods and materials similar or equivalent to those described herein can be used in the practice for testing of the disclosure, the preferred materials and methods are described herein. Accordingly, the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

In this application, the use of the singular includes the plural unless specifically stated otherwise. It must be noted that, as used in the specification, the singular forms “a,” “an” and “the” include plural references unless the context clearly dictates otherwise. In this application, the use of “or” means “and/or” unless stated otherwise. Furthermore, use of the term “including” as well as other forms, such as “include,” “includes,” and “included,” is not limiting.

Reference in the specification to “some embodiments,” “an embodiment,” “an embodiment” or “other embodiments” means that a particular feature, structure, or characteristic described in connection with the embodiments is included in at least some embodiments, although not necessarily all embodiments, of the inventions.

As used in this specification and claim(s), the words “comprising” (and any form of comprising, such as “comprise” and “comprises”), “having” (and any form of having, such as “have” and “has”), “including” (and any form of including, such as “includes” and “include”) or “containing” (and any form of containing, such as “contains” and “contain”) are inclusive or open-ended and do not exclude additional, unrecited elements or method steps. It is contemplated that any embodiment discussed in this specification can be implemented with respect to any method or composition of the invention, and vice versa. Furthermore, compositions of the invention can be used to achieve methods of the invention.

The term “about” in relation to a reference numerical value and its grammatical equivalents as used herein can include the numerical value itself and a range of values plus or minus 10% from that numerical value. For example, the amount “about 10” includes 10 and any amounts from 9 to 11. For example, the term “about” in relation to a reference numerical value

can also include a range of values plus or minus 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% from that value.

The disclosure provides improved system, kits, methods and apparatus for the reproducible, consistent, and efficacious delivery of HBV vaccines, such as nucleic acids encoding HBV antigens and combinations thereof with Electrically Mediated Therapeutic Agent (e.g., HBV vaccine) Delivery (EMTAD).

In an aspect, the disclosure provides an apparatus for the delivery of an HBV vaccine to a predetermined site within a subject, comprising a cartridge assembly comprising an outer cartridge, an inner cartridge, a reservoir containing the HBV vaccine, wherein a reservoir containment volume is contained within the outer cartridge and configured to receive the reservoir; an applicator comprising a cartridge assembly receiving volume, a needle hub, and an insertion detector, wherein the insertion detector senses loading of the reservoir in the reservoir containment volume; at least one interlock, wherein the interlock facilitates proper execution of the HBV vaccine administration procedure; at least one injection orifice through which the HBV vaccine is administered; a plurality of penetrating electrodes arranged with a predetermined spatial relationship relative to the orifice; an electrical field generator for generating an electrical signal operatively connected to the electrodes; and a controlled source of energy sufficient to transfer a predetermined amount of the HBV vaccine at a predetermined rate from the reservoir through the orifice to the predetermined site within the subject.

In another aspect, the disclosure provides a kit for the delivery of an HBV vaccine to a predetermined site within a subject, comprising the HBV vaccine, and an apparatus comprising a cartridge assembly comprising an outer cartridge, an inner cartridge, a reservoir for the HBV vaccine, wherein a reservoir containment volume is contained within the outer cartridge and configured to receive the reservoir; an applicator comprising a cartridge assembly receiving volume, a needle hub, and an insertion detector, wherein the insertion detector senses loading of the reservoir in the reservoir containment volume; at least one interlock, wherein the interlock facilitates proper execution of the HBV vaccine administration procedure; at least one injection orifice through which the HBV vaccine is administered; a plurality of penetrating electrodes arranged with a predetermined spatial relationship relative to the orifice; an electrical field generator for generating an electrical signal operatively connected to the electrodes; and a controlled source of energy sufficient to transfer a predetermined amount of the HBV vaccine at

a predetermined rate from the reservoir through the orifice to the predetermined site within the subject.

An HBV vaccine included in an apparatus or a kit of the present application can comprise:

- a first nucleic acid molecule comprising a first polynucleotide encoding an HBV polymerase antigen having an amino acid sequence that is at least 98% identical to SEQ ID NO: 4, wherein the HBV polymerase antigen does not have reverse transcriptase activity and RNase H activity;

- a second nucleic acid molecule comprising a second polynucleotide encoding a truncated HBV core antigen consisting of the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 14; and

- a pharmaceutically acceptable carrier,

wherein the first nucleic acid molecule and the second nucleic acid molecule are present in the same nucleic acid molecule or in two different nucleic acid molecules.

In certain aspects of the disclosure, EMTAD can be referred to as the administration of an HBV vaccine to a biological tissue of interest and the earlier, concurrent or subsequent application of electrical signals to biological tissue for the purpose of enhancing movement and/or uptake of the HBV vaccine in said tissue. The process of EMTAD is comprised of two elements: 1) Therapeutic Agent (i.e., HBV vaccine) Administration (TAA), and 2) an Electrical Signal Application (ESA) sufficient to induce the desired EMTAD effect. In the disclosure, TAA can be accomplished, for instance, in a controllable fashion, termed Controlled Therapeutic Agent (e.g., HBV vaccine) Administration (CTAA). The term CTAA used herein can refer to methods or apparatus that provide spatial and/or temporal control over administration of an HBV vaccine relative to the induction of an EMTAD effect. Controllable administration techniques can utilize variations on the conventional needle-syringe (e.g. automatic injection device) and/or various needleless methodologies (e.g. jet injector, transdermal/transcutaneous patch, oral, gel, cream, or inhaled administration). The term ESA used herein can refer to the application of electrical signals to facilitate or enhance the delivery of active agents, e.g., HBV vaccines, by improving movement and/or uptake of said agents within tissue, thus inducing an EMTAD effect. When used to facilitate or enhance delivery of an HBV vaccine, ESA processes such as electroporation, iontophoresis, electroosmosis, electroporabilization, electrostimulation,

electromigration, and electroconvection all represent various modes of EMTAD, one or more of which can be included in the methods described herein.

Specific applications for apparatus, kits and systems described herein include, but are not limited to, the delivery of HBV vaccines containing one or more than one nucleic acid molecules. Traditionally with such applications, EMTAD is initiated by HBV vaccine injection using a conventional needle-syringe. After the agent has been administered, a device suitable for ESA is applied to the subject at a designated location. Finally, an appropriate ESA protocol is utilized to provide the desired facilitation or enhancement to HBV vaccine delivery. With traditional EMTAD, however, the desired spatial and temporal relationship between agent administration and ESA may not be realized.

Spatial Parameters

In some embodiments of the systems, kits, methods and apparatus described herein, HBV vaccine administration is performed using a conventional needle syringe. The need to deliver certain agents with EMTAD brings an additional level of complexity to the issue of TAA. As depicted in **FIG. 1**, in any conventional needle-syringe injection, as the needle **5** is inserted into the tissue, the depth **1** and the angle **2** of insertion relative to the surface of the tissue **3** can be difficult to control. Additionally, the point of needle penetration **4** at the tissue surface **3** may not be representative of the location of the orifice **6** and the region of agent administration **7** within the target tissue. As an illustrative example a transcutaneous intramuscular injection may not correspond to the site of insertion on the skin since the two tissues can often move in relation to one another.

While this conventional approach is generally adequate for the delivery of many different therapeutics that do not require EMTAD, these variables lead to a distribution of the HBV vaccine following injection that is often inconsistent and/or indeterminate and can hamper effective EMTAD. In certain embodiments described herein, the most effective use of EMTAD utilizes a predefined relationship between the HBV vaccine and ESA within the subject. As a result, in the absence of spatial control over TAA in a target tissue, using a conventional needle syringe can result in reduced effectiveness of the EMTAD application, as compared to an apparatus, method or system that provides spatial and temporal control. One illustrative example of this concept is the use of electroporation to facilitate the delivery of an HBV vaccine. Electroporation is typically most effective in enhancing HBV vaccine delivery when TAA and

ESA are co-localized within the target region of tissue. In many cases, if the agent to be delivered and the induced electroporation effect are not co-localized within the target region of tissue, the delivery of said agent is suboptimal.

Another example of the need for adequate spatial control of TAA in EMTAD is iontophoresis. This mode of EMTAD uses electrical fields to cause movement of charged molecules. In order to achieve the desired movement of the agent, the proper spatial relationship between the electrodes and the HBV vaccine must be realized. If a negatively charged agent were placed in close proximity to the location of a positive electrode, little or no movement of the agent through the tissue would be observed. In contrast, localization of the said negatively charged agent near the negative electrode would result in significant movement of the agent through the tissue in the direction of the positive electrode.

As illustrated by the preceding examples, it is important to control the precise location of TAA relative to the application of ESA to achieve the desired effect. As such, embodiments of the apparatus and methods described herein provide control of the precise location of TAA relative to the application of ESA, and are useful to achieve reproducible, consistent, and well-characterized distribution of one or more HBV vaccines.

Temporal Parameters

In the case of conventional needle-syringe injection TAA is that the rate of injection may vary from one operator to another, thereby causing inconsistent agent distribution in the tissue. Additional temporal variability is introduced when multiple device placements are required to complete the EMTAD process. For example, one application of EMTAD calls for the administration of plasmid DNA encoding for a therapeutic protein, followed by generation of an electroporation-inducing electrical field. Using the traditional method of EMTAD, the HBV vaccine is injected with a needle-syringe, followed by placement and activation of the electroporation device. By requiring two separate device placements (the initial needle syringe followed by the ESA device), this procedure is susceptible to inter-subject variability arising from inconsistent temporal application of each device by the operator. Additionally, the use of two separate device placements leads to an unavoidable time interval in between the clinician's placement and activation of each device. This is compounded in the case where multiple application sites are necessary to achieve adequate delivery of the agent to a specifiable region within the target tissue.

These issues are especially critical for agents, such as nucleic acids, that can be degraded or inactivated in the extracellular environment. The degradation of nucleic acids in the HBV vaccine can lead to a reduction in efficacy and consistency in the application of the therapy. Also, the inter-subject rate of degradation of nucleic acids in the HBV vaccine is not constant, thus contributing to the overall therapeutic inconsistency of conventional needle-syringe injection combined with ESA, and more specifically with electroporation therapy.

Due to the inherent difficulty of spatial and temporal variability with conventional needle-syringe injection used in conjunction with ESA, the precise location and timing of TAA relative to ESA is often unknown. As a result, the effective administration and dosing of HBV vaccines with EMTAD can be inconsistent and irreproducible. Though conventional needle-syringe injection is sometimes adequate for HBV vaccine administration, reproducible and consistent delivery of HBV vaccines is significantly enhanced by controlling the spatial and temporal relationship between administration of the HBV vaccine and induction of the desired EMTAD effect.

Thus, while the traditional EMTAD procedure can be adequate for certain applications, temporal and spatial control is highly desirable for clinical applications that typically require a high degree of consistency and reproducibility. In contrast to the conventional EMTAD approach, embodiments of methods, systems and apparatus described herein facilitate CTAA and ESA to provide more advantageous methods and apparatus for the clinical application of EMTAD. The disclosure utilizes various aspects of CTAA in conjunction with ESA to provide reproducible, consistent, and efficacious HBV vaccine delivery. The disclosure describes methods and apparatus to provide spatial and temporal control over administration of an HBV vaccine relative to the application of electrical signals, thereby improving the movement and/or uptake of said vaccine in the target tissue.

In some embodiments are provided methods and apparatus wherein there exists a controllable spatial relationship for the administration of the HBV vaccine relative to the application of electrical signals. Prior to treatment, the optimal location for TAA relative to ESA is determined. This spatial relationship between TAA and ESA is dictated by treatment parameters, including the nature of the agent being administered and the properties of the target tissue to which the agent is administered. In an exemplary embodiment, electrical signals are preferentially applied distal to the site of HBV vaccine administration. In certain other

embodiments, spatial relationship is to apply the EMTAD-inducing electrical signals proximal to the site of agent administration. In certain cases, co-localization between TAA and ESA is preferable. This is often the case when electroporation and/or iontophoresis are utilized for induction of the desired EMTAD effect.

In another aspect of the disclosure, an apparatus described herein provides a controllable temporal relationship for the sequence and timing of TAA relative to ESA. Prior to treatment, the optimal sequence and timing for a combination of TAA and ESA is determined. As with the spatial relationship, the desired temporal relationship between TAA and ESA is dictated by parameters such as the nature of the agent being administered and the properties of the target tissue to which the agent is administered. In certain applications, exposure to the electrical fields associated with ESA may adversely affect the HBV vaccine. In the practice of such applications, generation of such electrical fields is followed by CTAA. However, the typical temporal relationship is CTAA followed by ESA.

The disclosure provides improved methods and apparatus for the reproducible, consistent, and efficacious delivery of HBV vaccines comprising nucleic acid based constructs with EMTAD. This objective is accomplished by controlling the spatial and temporal administration of an HBV vaccine relative to application of electrical signals. In a certain embodiment, EMTAD is initiated by HBV vaccine injection using a conventional needle-syringe. After the agent has been administered, a device suitable for ESA is applied to the subject at a designated location. An appropriate ESA protocol is utilized to provide the desired facilitation or enhancement to HBV vaccine delivery. An exemplary ESA method that has proven to be effective in virtually all cell types is electroporation. Other exemplary methods of electrically mediated delivery include, but are not limited to, iontophoresis, electroosmosis, electropermeabilization, electrostimulation, electromigration, and electroconvection. These terms are used for illustrative purposes only and should not be construed as limitations in the disclosure.

The technique of electroporation utilizes the application of electric fields to induce a transient increase in cell membrane permeability and to move charged particles. By permeabilizing the cell membranes within the target tissue, electroporation dramatically improves the intracellular uptake of exogenous substances that have been administered to the target tissue. The increase in cell membrane permeability and molecular movement due to electroporation offers a method for overcoming the cell membrane as a barrier to HBV vaccine

delivery. The application of electroporation as a technique for inducing EMTAD is advantageous in that the physical nature of the technique allows electroporation to be applied in virtually all tissue types. Accordingly, various aspects and embodiments of the disclosure discuss, but are not limited to, electroporation as a technique for inducing EMTAD.

HBV vaccines

The term "HBV vaccine" is used herein in its broadest sense to include any agent capable of providing a desired or beneficial immune response, therapeutic and/or prophylactic effect against an HBV on a living tissue. Thus, the term includes both prophylactic and therapeutic HBV vaccines, as well as any other category of agent having such desired effects. The scope of the disclosure is sufficiently broad to include the controlled delivery of any HBV vaccines, however categorized. HBV vaccines include, but are not limited to pharmaceutical drugs and vaccines, and nucleic acid sequences (such as supercoiled, relaxed, and linear plasmid DNA, RNA, antisense constructs, artificial chromosomes, or any other nucleic acid-based therapeutic), and any formulations thereof. Such agent formulations include, but are not limited to, cationic lipids, cationic and/or nonionic polymers, liposomes, saline, nuclease inhibitors, anesthetics, poloxamers, preservatives, sodium phosphate solutions, or other compounds that can improve the administration, stability, and/or effect of the HBV vaccine. Additional formulations include agents and additives conferring the ability to control viscosity and electrical impedance of the administered agent.

In a preferred aspect of the application, an HBV vaccine to be used in the invention can comprise:

- a first nucleic acid molecule, preferably a first plasmid DNA vector, comprising a first polynucleotide encoding an HBV polymerase antigen, preferably the HBV polymerase antigen has an amino acid sequence that is at least 98% identical to SEQ ID NO: 4, such as at least 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 4, wherein the HBV polymerase antigen does not have reverse transcriptase activity and RNase H activity;

- a second nucleic acid molecule, preferably a second plasmid DNA vector, comprising a second polynucleotide encoding a truncated HBV core antigen, preferably the truncated HBV core antigen consists of the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 14; and

- a pharmaceutically acceptable carrier, and

wherein the first nucleic acid molecule and the second nucleic acid molecule are present in the same nucleic acid molecule, such as the same plasmid DNA vector, or in two different nucleic acid molecules, such as two separate plasmid DNA vectors.

As used herein, each of the terms “HBV core antigen,” “HBcAg” and “core antigen” refers to an HBV antigen capable of inducing an immune response, e.g., a humoral and/or cellular mediated response, against an HBV core protein in a subject. Each of the terms “core,” “core polypeptide,” and “core protein” refers to the HBV viral core protein. Full-length core antigen is typically 183 amino acids in length and includes an assembly domain (amino acids 1 to 149) and a nucleic acid binding domain (amino acids 150 to 183). The 34-residue nucleic acid binding domain is required for pre-genomic RNA encapsidation. This domain also functions as a nuclear import signal. It comprises 17 arginine residues and is highly basic, consistent with its function. HBV core protein is dimeric in solution, with the dimers self-assembling into icosahedral capsids. Each dimer of core protein has four α -helix bundles flanked by an α -helix domain on either side. Truncated HBV core proteins lacking the nucleic acid binding domain are also capable of forming capsids.

In an aspect of the application, an HBV antigen to be used in the invention is a truncated HBV core antigen. As used herein, a “truncated HBV core antigen,” refers to an HBV antigen that does not contain the entire length of an HBV core protein but is capable of inducing an immune response against the HBV core protein in a subject. For example, an HBV core antigen can be modified to delete one or more amino acids of the highly positively charged (arginine rich) C-terminal nucleic acid binding domain of the core antigen, which typically contains seventeen arginine (R) residues. A truncated HBV core antigen of the application is preferably a C-terminally truncated HBV core protein which does not comprise the HBV core nuclear import signal and/or a truncated HBV core protein from which the C-terminal HBV core nuclear import signal has been deleted. In an embodiment, a truncated HBV core antigen comprises a deletion in the C-terminal nucleic acid binding domain, such as a deletion of 1 to 34 amino acid residues of the C-terminal nucleic acid binding domain, e.g., 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, or 34 amino acid residues, preferably a deletion of all 34 amino acid residues. In a preferred embodiment, a truncated HBV core antigen comprises a deletion in the C-terminal nucleic acid binding domain, preferably of all 34 amino acid residues.

An HBV core antigen useful in the application can be a consensus sequence derived from multiple HBV genotypes (e.g., genotypes A, B, C, D, E, F, G, and H). As used herein, “consensus sequence” means an artificial sequence of amino acids based on an alignment of amino acid sequences of homologous proteins, e.g., as determined by an alignment (e.g., using Clustal Omega) of amino acid sequences of homologous proteins. It can be the calculated order of most frequent amino acid residues, found at each position in a sequence alignment, based upon sequences of HBV antigens (e.g., core, pol, etc.) from at least 100 natural HBV isolates. A consensus sequence can be non-naturally occurring and different from the native viral sequences. Consensus sequences can be designed by aligning multiple HBV antigen sequences from different sources using a multiple sequence alignment tool, and at variable alignment positions, selecting the most frequent amino acid. Preferably, a consensus sequence of an HBV antigen is derived from HBV genotypes B, C, and D. The term “consensus antigen” is used to refer to an antigen having a consensus sequence.

An exemplary truncated HBV core antigen useful for the application lacks the nucleic acid binding function, and can be capable of inducing an immune response in a mammal against at least two HBV genotypes. Preferably a truncated HBV core antigen is capable of inducing a T cell response in a mammal against at least HBV genotypes B, C and D. More preferably, a truncated HBV core antigen is capable of inducing a CD8 T cell response in a human subject against at least HBV genotypes A, B, C and D.

Preferably, an HBV core antigen useful for the application is a consensus antigen, preferably a consensus antigen derived from HBV genotypes B, C, and D, more preferably a truncated consensus antigen derived from HBV genotypes B, C, and D. An exemplary truncated HBV core consensus antigen according to the application consists of an amino acid sequence that is at least 90% identical to SEQ ID NO: 2 or SEQ ID NO: 14, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9%, or 100% identical to SEQ ID NO: 2 or SEQ ID NO: 14. SEQ ID NO: 2 and SEQ ID NO: 4 are core consensus antigens derived from HBV genotypes B, C, and D. SEQ ID NO: 2 and SEQ ID NO: 14 contain a 34-amino acid C-terminal deletion of the highly positively charged (arginine rich) nucleic acid binding domain of the native core antigen.

In a particular embodiment of the application, an HBV core antigen is a truncated HBV antigen consisting of the amino acid sequence of SEQ ID NO: 2. In another particular embodiment, an HBV core antigen is a truncated HBV antigen consisting of the amino acid sequence of SEQ ID NO: 14.

Examples of polynucleotide sequences encoding a truncated HBV core antigen consisting of the amino acid sequence of SEQ ID NO: 2 include, but are not limited to, a polynucleotide sequence at least 90% identical to SEQ ID NO: 1, such as or at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 95.5%, at least 96%, at least 96.5%, at least 97%, at least 97.5%, at least 98%, at least 98.5%, at least 99%, at least 99.1%, at least 99.2%, at least 99.3%, at least 99.4%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8%, at least 99.9% or 100% identical to SEQ ID NO: 1, preferably about 98%, about 99% or 100% identical to SEQ ID NO: 1.

Examples of polynucleotide sequences encoding a truncated HBV core antigen consisting of the amino acid sequence of SEQ ID NO: 14 include, but are not limited to, a polynucleotide sequence at least 90% identical to SEQ ID NO: 15, such as or at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 95.5%, at least 96%, at least 96.5%, at least 97%, at least 97.5%, at least 98%, at least 98.5%, at least 99%, at least 99.1%, at least 99.2%, at least 99.3%, at least 99.4%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8%, at least 99.9% or 100% identical to SEQ ID NO: 15, preferably about 98%, about 99% or 100% identical to SEQ ID NO: 15.

In particular embodiments of the application, a HBV vaccine to be used in the invention comprises a non-naturally occurring nucleic acid molecule encoding a truncated HBV core antigen, and the non-naturally occurring nucleic acid molecule comprises the polynucleotide sequence of SEQ ID NO: 1 or SEQ ID NO: 15.

As used herein, the term “HBV polymerase antigen,” “HBV Pol antigen” or “HBV pol antigen” refers to an HBV antigen capable of inducing an immune response, e.g., a humoral and/or cellular mediated response, against an HBV polymerase in a subject. Each of the terms “polymerase,” “polymerase polypeptide,” “Pol” and “pol” refers to the HBV viral DNA polymerase. The HBV viral DNA polymerase has four domains, including, from the N terminus to the C terminus, a terminal protein (TP) domain, which acts as a primer for minus-strand DNA

synthesis; a spacer that is nonessential for the polymerase functions; a reverse transcriptase (RT) domain for transcription; and a RNase H domain.

In an embodiment of the application, an HBV antigen comprises an HBV Pol antigen, or any immunogenic fragment or combination thereof. An HBV Pol antigen can contain further modifications to improve immunogenicity of the antigen, such as by introducing mutations into the active sites of the polymerase and/or RNase H domains to decrease or substantially eliminate certain enzymatic activities.

Preferably, an HBV Pol antigen useful in the application does not have reverse transcriptase activity and RNase H activity, and is capable of inducing an immune response in a mammal against at least two HBV genotypes. Preferably, an HBV Pol antigen is capable of inducing a T cell response in a mammal against at least HBV genotypes B, C and D. More preferably, a HBV Pol antigen is capable of inducing a CD8 T cell response in a human subject against at least HBV genotypes A, B, C and D.

Thus, in some embodiments, an HBV Pol antigen useful in the application is an inactivated Pol antigen. In an embodiment, an inactivated HBV Pol antigen comprises one or more amino acid mutations in the active site of the polymerase domain. In another embodiment, an inactivated HBV Pol antigen comprises one or more amino acid mutations in the active site of the RNaseH domain. In a preferred embodiment, an inactivated HBV pol antigen comprises one or more amino acid mutations in the active site of both the polymerase domain and the RNaseH domain. For example, the “YXDD” motif in the polymerase domain of an HBV pol antigen that can be required for nucleotide/metal ion binding can be mutated, e.g., by replacing one or more of the aspartate residues (D) with asparagine residues (N), eliminating or reducing metal coordination function, thereby decreasing or substantially eliminating reverse transcriptase function. Alternatively, or in addition to mutation of the “YXDD” motif, the “DEDD” motif in the RNaseH domain of an HBV pol antigen required for Mg^{2+} coordination can be mutated, e.g., by replacing one or more aspartate residues (D) with asparagine residues (N) and/or replacing the glutamate residue (E) with glutamine (Q), thereby decreasing or substantially eliminating RNaseH function. In a particular embodiment, an HBV pol antigen is modified by (1) mutating the aspartate residues (D) to asparagine residues (N) in the “YXDD” motif of the polymerase domain; and (2) mutating the first aspartate residue (D) to an asparagine residue (N) and the first glutamate residue (E) to a glutamine residue (N) in the “DEDD” motif of the RNaseH domain,

thereby decreasing or substantially eliminating both the reverse transcriptase and RNaseH functions of the pol antigen.

In a preferred embodiment of the application, an HBV pol antigen is a consensus antigen, preferably a consensus antigen derived from HBV genotypes B, C, and D, more preferably an inactivated consensus antigen derived from HBV genotypes B, C, and D. An exemplary HBV pol consensus antigen according to the application comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 4, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 4, preferably at least 98% identical to SEQ ID NO: 4, such as at least 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 4. SEQ ID NO: 4 is a pol consensus antigen derived from HBV genotypes B, C, and D comprising four mutations located in the active sites of the polymerase and RNaseH domains. In particular, the four mutations include mutation of the aspartic acid residues (D) to asparagine residues (N) in the “YXDD” motif of the polymerase domain; and mutation of the first aspartate residue (D) to an asparagine residue (N) and mutation of the glutamate residue (E) to a glutamine residue (Q) in the “DEDD” motif of the RNaseH domain.

In a particular embodiment of the application, an HBV pol antigen useful for the application comprises the amino acid sequence of SEQ ID NO: 4. In other embodiments of the application, an HBV core antigen useful in the application consists of the amino acid sequence of SEQ ID NO: 4.

Examples of polynucleotide sequences encoding a HBV pol antigen comprising the amino acid sequence of SEQ ID NO: 4 include, but are not limited to, a polynucleotide sequence at least 90% identical to SEQ ID NO: 3 or SEQ ID NO: 16, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 3 or SEQ ID NO: 16, preferably 98%, 99% or 100% identical to SEQ ID NO: 3 or SEQ ID NO: 16. In particular embodiments of the application, a HBV vaccine useful for the application comprises a non-naturally occurring nucleic acid molecule encoding a HBV pol antigen, and the non-naturally occurring nucleic acid molecule comprises the polynucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 16.

As used herein the term “fusion protein” or “fusion” refers to a single polypeptide chain having at least two polypeptide domains that are not normally present in a single, natural polypeptide.

In an aspect of the application, an HBV antigen to be used in the invention can comprise a fusion protein comprising a truncated HBV core antigen operably linked to an HBV Pol antigen, or an HBV Pol antigen operably linked to a truncated HBV core antigen, preferably via a linker. As used herein, the term “linker” refers to a compound or moiety that acts as a molecular bridge to operably link two different molecules, wherein one portion of the linker is operably linked to a first molecule, and wherein another portion of the linker is operably linked to a second molecule. As used herein, the term “operably linked” refers to a linkage or a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. For example, a regulatory sequence operably linked to a nucleic acid sequence of interest is capable of directing the transcription of the nucleic acid sequence of interest, or a signal sequence operably linked to an amino acid sequence of interest is capable of secret or translocate the amino acid sequence of interest over a member.

For example, in a fusion protein containing a first polypeptide and a second heterologous polypeptide, a linker serves primarily as a spacer between the first and second polypeptides. In an embodiment, a linker is made up of amino acids linked together by peptide bonds, preferably from 1 to 20 amino acids linked by peptide bonds, wherein the amino acids are selected from the 20 naturally occurring amino acids. In an embodiment, the 1 to 20 amino acids are selected from glycine, alanine, proline, asparagine, glutamine, and lysine. Preferably, a linker is made up of a majority of amino acids that are sterically unhindered, such as glycine and alanine. Exemplary linkers are polyglycines, particularly (Gly)₅, (Gly)₈; poly(Gly-Ala), and polyalanines. One exemplary suitable linker is (AlaGly)_n, wherein n is an integer of 2 to 5.

Preferably, a fusion protein useful in the application is capable of inducing an immune response in a mammal against HBV core and HBV Pol of at least two HBV genotypes. Preferably the fusion protein is capable of inducing a T cell response in a mammal against at least HBV genotypes B, C and D. More preferably, the fusion protein is capable of inducing a CD8 T cell response in a human subject against at least HBV genotypes A, B, C and D.

In an aspect of the application, a fusion protein useful in the application can comprise a truncated HBV core antigen having an amino acid sequence at least 90%, such as at least 90%,

91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9%, or 100% identical to SEQ ID NO: 2 or 14, a linker, and a HBV Pol antigen having an amino acid sequence at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9%, or 100%, identical to SEQ ID NO: 4.

Preferably, a fusion protein useful in the application comprises a truncated HBV core antigen consisting of the amino acid sequence of SEQ ID NO: 2 or 14, a linker comprising (AlaGly)_n, wherein n is an integer of 2 to 5, and a HBV Pol antigen having the amino acid sequence of SEQ ID NO: 4. More preferably, a fusion protein useful in the application comprises the amino acid sequence of SEQ ID NO: 20.

In some embodiments of the application, a fusion protein useful in the application further comprises a signal sequence. Preferably, the signal sequence has the amino acid sequence of SEQ ID NO: 6 or SEQ ID NO: 19. More preferably, the fusion protein comprises the amino acid sequence of SEQ ID NO: 21.

Examples of polynucleotide sequences encoding a fusion protein useful in the application include, but are not limited to, a polynucleotide sequence at least 90% identical to SEQ ID NO: 1 or SEQ ID NO: 15, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 1 or SEQ ID NO: 15, preferably 98%, 99% or 100% identical to SEQ ID NO: 1 or SEQ ID NO: 15, operably linked to a linker coding sequence at least 90% identical to SEQ ID NO: 22, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 22, preferably 98%, 99% or 100% identical to SEQ ID NO: 22, which is further operably linked a polynucleotide sequence at least 90% identical to SEQ ID NO: 3 or SEQ ID NO: 16, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 3 or SEQ ID NO: 16, preferably 98%, 99% or 100% identical to SEQ ID NO: 3 or SEQ ID NO: 16. In particular embodiments of the application, a HBV vaccine useful in the application comprises a non-naturally occurring nucleic acid molecule encoding a fusion protein, and the non-naturally occurring nucleic acid

molecule comprises SEQ ID NO: 1, operably linked to SEQ ID NO: 22, which is further operably linked to SEQ ID NO: 3.

In an aspect of the application, in an HBV vaccine to be used in the application, the first and second nucleic acid molecules are first and second plasmid DNA vectors, respectively, and each of the first and second plasmid DNA vectors comprises an origin of replication, an antibiotic resistance gene, and from 5' end to 3' end, a promoter sequence, an enhancer sequence, a signal peptide coding sequence, the first polynucleotide sequence or the second polynucleotide sequence, and a polyadenylation signal sequence. In a preferred embodiment of the application, a DNA plasmid is an expression vector suitable for protein expression in mammalian host cells. Expression vectors suitable for protein expression in mammalian host cells include, but are not limited to, pcDNATM, pcDNA3TM, pVAX, pVAX-1, etc. Preferably, the expression vector is based on pVAX-1, which can be further modified to optimize protein expression in mammalian cells. pVAX-1 is a commonly used plasmid in DNA vaccines, and contains a strong human intermediate early cytomegalovirus (CMV-IE) promoter followed by the bovine growth hormone (bGH)-derived polyadenylation sequence (pA). pVAX-1 further contains a pUC origin of replication and a kanamycin resistance gene driven by a small prokaryotic promoter that allows for bacterial plasmid propagation. Preferably, the plasmid DNA vector comprises a codon optimized kanamycin resistance gene having a polynucleotide sequence at least 90% identical to SEQ ID NO: 12, preferably 100% identical to SEQ ID NO: 12.

In a specific embodiment, an HBV vaccine to be used in the invention comprises:

- a) a first plasmid DNA vector comprising, from 3'-end to 5'-end, the promoter sequence comprising the polynucleotide sequence of SEQ ID NO: 7, the enhancer sequence comprising the polynucleotide sequence of SEQ ID NO: 8, the signal peptide coding sequence comprising the polynucleotide sequence of SEQ ID NO: 5, the first polynucleotide sequence comprising the polynucleotide sequence of SEQ ID NO: 3, and the polyadenylation signal sequence comprising the polynucleotide sequence of SEQ ID NO: 11;
- b) a second plasmid DNA vector comprising, from 3'-end to 5'-end, the promoter sequence comprising the polynucleotide sequence of SEQ ID NO: 7, the enhancer sequence comprising the polynucleotide sequence of SEQ ID NO: 8, the signal peptide coding sequence comprising the polynucleotide sequence of SEQ ID NO: 5, the second polynucleotide sequence

comprising the polynucleotide sequence of SEQ ID NO: 1, and the polyadenylation signal sequence comprising the polynucleotide sequence of SEQ ID NO: 11; and

c) a pharmaceutically acceptable carrier,

wherein each of the first plasmid DNA vector and the second plasmid DNA vector further comprises a kanamycin resistance gene having the polynucleotide sequence of SEQ ID NO: 12, and an origin of replication having the polynucleotide sequence of SEQ ID NO: 10, and

wherein the first plasmid DNA vector and the second plasmid DNA vector are in the same composition or two different compositions.

In those embodiments of the application in which an HBV vaccine comprises a first vector, such as a first DNA plasmid, and a second vector, such as a second DNA plasmid, the amount of each of the first and second vectors is not particularly limited. For example, the first DNA plasmid and the second DNA plasmid can be present in a ratio of 10:1 to 1:10, by weight, such as 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, or 1:10, by weight. Preferably, the first and second DNA plasmids are present in a ratio of 1:1, by weight.

Compositions and immunogenic combinations of the application can comprise additional polynucleotides or vectors encoding additional HBV antigens and/or additional HBV antigens or immunogenic fragments thereof. However, in particular embodiments, the compositions and immunogenic combinations of the application do not comprise certain antigens. In preferred embodiments, an HBV vaccine to be used in the invention does not contain a nucleic acid molecule encoding an HBV antigen selected from the group consisting of a Hepatitis B surface antigen (HBsAg), an HBV envelope (Env) antigen, and an HBV L protein antigen, nor an HBV antigen selected from the group consisting of a Hepatitis B surface antigen (HBsAg), an HBV envelope (Env) antigen, and an HBV L protein antigen.

An HBV vaccine useful in the application can also comprise a pharmaceutically acceptable carrier. A pharmaceutically acceptable carrier is non-toxic and should not interfere with the efficacy of the active ingredient. Pharmaceutically acceptable carriers can include one or more, such as water, glycols, sugar, oils, amino acids, alcohols, preservatives, emollients, stabilizers, coloring agents and the like.

Other examples of HBV vaccines useful in the application are described in International Patent Application entitled “Hepatitis B Virus (HBV) Vaccines and Uses thereof” filed on the same day as this application with the Attorney Docket Number 688097-403WO1, the contents of which are hereby incorporated by reference in their entireties.

Kits/Systems

In a general aspect, the invention relates to a kit or system for the controlled delivery of a HBV vaccine to a predetermined tissue site within a subject in need thereof, comprising the HBV vaccine and an apparatus for administering the HBV vaccine to the predetermined tissue site via electroporation. For example, the kit or system can have a prepacked container, such as a syringe, containing a premeasured amount of the HBV vaccine, which can be loaded to an apparatus described here for the subsequent administration of the HBV vaccine.

Methods of Inducing an Immune Response

In another general aspect, the invention relates to a method of inducing an immune response against hepatitis B virus (HBV) in a subject in need thereof, comprising administering to the subject an immunogenically effective amount of an HBV vaccine using an apparatus, kit or system of the application. Any of the apparatus, kit or system of the application described herein can be used in the methods of the application. As used herein, the term “infection” refers to the invasion of a host by a disease causing agent. A disease causing agent is considered to be “infectious” when it is capable of invading a host, and replicating or propagating within the host. Examples of infectious agents include viruses, e.g., HBV and certain species of adenovirus, prions, bacteria, fungi, protozoa and the like. “HBV infection” specifically refers to invasion of a host organism, such as cells and tissues of the host organism, by HBV.

As used herein, “inducing an immune response” when used with reference to the methods described herein encompasses causing a desired immune response or effect in a subject in need thereof against an infection, e.g. HBV infection. “Inducing an immune response” also encompasses providing a therapeutic immunity for treating against a pathogenic agent, e.g., HBV. As used herein, the term “therapeutic immunity” or “therapeutic immune response” means that the vaccinated subject is able to control an infection with the pathogenic agent against which the vaccination was done, for instance immunity against HBV infection conferred by vaccination with HBV vaccine. In an embodiment, “inducing an immune response” means producing an immunity in a subject in need thereof, e.g., to provide a therapeutic effect against a

disease, such as HBV infection. In certain embodiments, “inducing an immune response” refers to causing or improving cellular immunity, e.g., T cell response, against HBV. In certain embodiments, “inducing an immune response” refers to causing or improving a humoral immune response against HBV. In certain embodiments, “inducing an immune response” refers to causing or improving a cellular and a humoral immune response against HBV.

As used herein, the term “protective immunity” or “protective immune response” means that the vaccinated subject is able to control an infection with the pathogenic agent against which the vaccination was done. Usually, the subject having developed a “protective immune response” develops only mild to moderate clinical symptoms or no symptoms at all. Usually, a subject having a “protective immune response” or “protective immunity” against a certain agent will not die as a result of the infection with said agent.

Typically, the administration of an HBV vaccine according to embodiments of the application will have a therapeutic aim to generate an immune response against HBV after HBV infection or development of symptoms characteristic of HIV infection, e.g., for therapeutic vaccination.

As used herein, “an immunogenically effective amount” or “immunologically effective amount” means an amount of a composition, polynucleotide, vector, or antigen sufficient to induce a desired immune effect or immune response in a subject in need thereof. In an embodiment, an immunogenically effective amount means an amount sufficient to induce an immune response in a subject in need thereof. In another embodiment, an immunogenically effective amount means an amount sufficient to produce immunity in a subject in need thereof, e.g., provide a therapeutic effect against a disease such as HBV infection. An immunogenically effective amount can vary depending upon a variety of factors, such as the physical condition of the subject, age, weight, health, etc.; the particular application, e.g., providing protective immunity or therapeutic immunity; and the particular disease, e.g., viral infection, for which immunity is desired. An immunogenically effective amount can readily be determined by one of ordinary skill in the art in view of the disclosure.

In particular embodiments of the application, an immunogenically effective amount refers to the amount of a composition or immunogenic combination (such as an HBV vaccine) which is sufficient to achieve one, two, three, four, or more of the following effects: (i) reduce or ameliorate the severity of an HBV infection or a symptom associated therewith; (ii) reduce the

duration of an HBV infection or symptom associated therewith; (iii) prevent the progression of an HBV infection or symptom associated therewith; (iv) cause regression of an HBV infection or symptom associated therewith; (v) prevent the development or onset of an HBV infection, or symptom associated therewith; (vi) prevent the recurrence of an HBV infection or symptom associated therewith; (vii) reduce hospitalization of a subject having an HBV infection; (viii) reduce hospitalization length of a subject having an HBV infection; (ix) increase the survival of a subject with an HBV infection; (x) eliminate an HBV infection in a subject; (xi) inhibit or reduce HBV replication in a subject; and/or (xii) enhance or improve the prophylactic or therapeutic effect(s) of another therapy.

In other particular embodiments, an immunogenically effective amount is an amount sufficient to reduce HBsAg levels consistent with evolution to clinical seroconversion; achieve sustained HBsAg clearance associated with reduction of infected hepatocytes by a subject's immune system; induce HBV-antigen specific activated T-cell populations; and/or achieve persistent loss of HBsAg within 12 months. Examples of a target index include lower HBsAg below a threshold of 500 copies of HBsAg International Unit (IU) and/or higher CD8 counts.

As general guidance, an immunogenically effective amount when used with reference to a DNA plasmid can range from about 0.1 mg/mL to 10 mg/mL of DNA plasmid total, such as 0.1 mg/mL, 0.25 mg/mL, 0.5 mg/mL, 0.75 mg/mL, 1 mg/mL, 1.5 mg/mL, 2 mg/mL, 3 mg/mL, 4 mg/mL, 5 mg/mL, 6 mg/mL, 7 mg/mL, 8 mg/mL, 9 mg/mL, or 10 mg/mL. Preferably, an immunogenically effective amount of DNA plasmid is less than 8 mg/mL, more preferably less than 6 mg/mL, even more preferably 3-4 mg/mL. An immunogenically effective amount can be from one vector or plasmid, or from multiple vectors or plasmids. An immunogenically effective amount can be administered in a single composition, or in multiple compositions, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 compositions (e.g., tablets, capsules or injectables), wherein the administration of the multiple capsules or injections collectively provides a subject with an immunogenically effective amount. It is also possible to administer an immunogenically effective amount to a subject, and subsequently administer another dose of an immunogenically effective amount to the same subject, in a so-called prime-boost regimen. This general concept of a prime-boost regimen is well known to the skilled person in the vaccine field. Further booster administrations can optionally be added to the regimen, as needed.

According to embodiments of the application, an immunogenic combination comprising two DNA plasmids, e.g., a first DNA plasmid encoding an HBV core antigen and a second DNA plasmid encoding an HBV pol antigen can be administered to a subject by mixing both plasmids and delivering the mixture to a single anatomic site. Alternatively, two separate immunizations each delivering a single expression plasmid can be performed. In such embodiments, whether both plasmids are administered in a single immunization as a mixture or in two separate immunizations, the first DNA plasmid and the second DNA plasmid can be administered in a ratio of 10:1 to 1:10, by weight, such as 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, or 1:10, by weight. Preferably, the first and second DNA plasmids are administered in a ratio of 1:1, by weight.

In some embodiments, a subject to be treated according to the methods of the application is an HBV-infected subject, particular a subject having chronic HBV infection. Acute HBV infection is characterized by an efficient activation of the innate immune system complemented with a subsequent broad adaptive response (e.g., HBV-specific T-cells, neutralizing antibodies), which usually results in successful suppression of replication or removal of infected hepatocytes. In contrast, such responses are impaired or diminished due to high viral and antigen load, e.g., HBV envelope proteins are produced in abundance and can be released in sub-viral particles in 1,000-fold excess to infectious virus.

Chronic HBV infection is described in phases characterized by viral load, liver enzyme levels (necroinflammatory activity), HBeAg, or HBsAg load or presence of antibodies to these antigens. cccDNA levels stay relatively constant at approximately 10 to 50 copies per cell, even though viremia can vary considerably. The persistence of the cccDNA species leads to chronicity. More specifically, the phases of chronic HBV infection include: (i) the immune-tolerant phase characterized by high viral load and normal or minimally elevated liver enzymes; (ii) the immune activation HBeAg-positive phase in which lower or declining levels of viral replication with significantly elevated liver enzymes are observed; (iii) the inactive HBsAg carrier phase, which is a low replicative state with low viral loads and normal liver enzyme levels in the serum that may follow HBeAg seroconversion; and (iv) the HBeAg-negative phase in which viral replication occurs periodically (reactivation) with concomitant fluctuations in liver enzyme levels, mutations in the pre-core and/or basal core promoter are common, such that HBeAg is not produced by the infected cell.

As used herein, “chronic HBV infection” refers to a subject having the detectable presence of HBV for more than 6 months. A subject having a chronic HBV infection can be in any phase of chronic HBV infection. Chronic HBV infection is understood in accordance with its ordinary meaning in the field. Chronic HBV infection can for example be characterized by the persistence of HBsAg for 6 months or more after acute HBV infection. For example, a chronic HBV infection referred to herein follows the definition published by the Centers for Disease Control and Prevention (CDC), according to which a chronic HBV infection can be characterized by laboratory criteria such as: (i) negative for IgM antibodies to hepatitis B core antigen (IgM anti-HBc) and positive for hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), or nucleic acid test for hepatitis B virus DNA, or (ii) positive for HBsAg or nucleic acid test for HBV DNA, or positive for HBeAg two times at least 6 months apart.

According to particular embodiments, an immunogenically effective amount refers to the amount of a composition or immunogenic combination which is sufficient to treat chronic HBV infection.

In some embodiments, a subject having chronic HBV infection is undergoing nucleoside analog (NUC) treatment, and is NUC-suppressed. As used herein, “NUC-suppressed” refers to a subject having an undetectable viral level of HBV and stable alanine aminotransferase (ALT) levels for at least six months. Examples of nucleoside/nucleotide analog treatment include HBV polymerase inhibitors, such as entacavir and tenofovir. Preferably, a subject having chronic HBV infection does not have advanced hepatic fibrosis or cirrhosis. Such subject would typically have a METAVIR score of less than 3 for fibrosis and a fibroscan result of less than 9 kPa. The METAVIR score is a scoring system that is commonly used to assess the extent of inflammation and fibrosis by histopathological evaluation in a liver biopsy of patients with hepatitis B. The scoring system assigns two standardized numbers: one reflecting the degree of inflammation and one reflecting the degree of fibrosis.

It is believed that elimination or reduction of chronic HBV may allow early disease interception of severe liver disease, including virus-induced cirrhosis and hepatocellular carcinoma. Thus, the methods of the application can also be used as therapy to treat HBV-induced diseases. Examples of HBV-induced diseases include, but are not limited to cirrhosis, cancer (e.g., hepatocellular carcinoma), and fibrosis, particularly advanced fibrosis characterized by a METAVIR score of 3 or higher for fibrosis. In such embodiments, an immunogenically

effective amount is an amount sufficient to achieve persistent loss of HBsAg within 12 months and significant decrease in clinical disease (e.g., cirrhosis, hepatocellular carcinoma, etc.).

Methods according to embodiments of the application further comprise administering to the subject in need thereof another immunogenic agent (such as another HBV antigen or other antigen) or another anti-HBV agent (such as a nucleoside analog or other anti-HBV agent) in combination with a composition of the application.

The ability to induce or stimulate an anti-HBV immune response upon administration in an animal or human organism can be evaluated either *in vitro* or *in vivo* using a variety of assays which are standard in the art. For a general description of techniques available to evaluate the onset and activation of an immune response, see for example Coligan et al. (1992 and 1994, Current Protocols in Immunology; ed. J Wiley & Sons Inc., National Institute of Health). Measurement of cellular immunity can be performed by measurement of cytokine profiles secreted by activated effector cells including those derived from CD4⁺ and CD8⁺ T-cells (e.g. quantification of IL-10 or IFN gamma-producing cells by ELISPOT), by determination of the activation status of immune effector cells (e.g. T cell proliferation assays by a classical [3H] thymidine uptake), by assaying for antigen-specific T lymphocytes in a sensitized subject (e.g. peptide-specific lysis in a cytotoxicity assay, etc.).

The ability to stimulate a cellular and/or a humoral response can be determined by antibody binding and/or competition in binding (see for example Harlow, 1989, Antibodies, Cold Spring Harbor Press). For example, titers of antibodies produced in response to administration of a composition providing an immunogen can be measured by enzyme-linked immunosorbent assay (ELISA). The immune responses can also be measured by neutralizing antibody assay, where a neutralization of a virus is defined as the loss of infectivity through reaction/inhibition/neutralization of the virus with specific antibody. The immune response can further be measured by Antibody-Dependent Cellular Phagocytosis (ADCP) Assay.

Target Tissues

Target tissues well suited for EMTAD by use of methods, apparatus and systems described herein include both healthy and diseased cells located in, for instance, the epidermis, dermis, hypodermis, connective, and muscle tissue. The technique can also be utilized for application in healthy or diseased organs that must be accessed via minimally invasive or other surgical methods. Such target tissues include the liver, lungs, heart, blood vessels, lymphatic,

brain, kidneys, pancreas, stomach, intestines, colon, bladder, and reproductive organs. In some embodiments, a desired therapeutic effect can be derived by use of a method, or apparatus described herein to deliver an amount of agent to cell types normally located within the target tissues as well as other cell types abnormally found within said tissues (e.g. chemotherapeutic treatment of tumors).

As discussed previously, and depicted in **FIG. 1**, traditional EMTAD suffers from a lack of precision and reproducibility in the spatial and temporal relationship between the administration of the HBV vaccine and the electrical signal. In contrast to the traditional EMTAD approach, the disclosure describes methods and apparatus for combined CTAA and ESA to provide a more advantageous clinical application of EMTAD. The disclosure utilizes various aspects of CTAA in conjunction with ESA to provide reproducible, consistent, and efficacious HBV vaccine delivery. The methods and apparatus provided herein provide spatial and temporal control over administration of an HBV vaccine relative to the application of electrical signals, thereby improving the movement and/or uptake of said agent in the target tissue.

Methods

In an aspect, the disclosure described herein provides systems, kits and apparatus for use in methods for controlled administration of an HBV vaccine to a subject in need thereof followed by ESA. As used herein, “subject” means any animal, preferably a mammal, most preferably a human, to whom will be or has been treated by a method according to an embodiment of the application. The term “mammal” as used herein, encompasses any mammal. Examples of mammals include, but are not limited to, cows, horses, sheep, pigs, cats, dogs, mice, rats, rabbits, guinea pigs, non-human primates (NHPs) such as monkeys or apes, humans, etc., more preferably a human.

In another aspect, the disclosure described herein provides systems, kits and apparatus for use in methods for controlled administration of an HBV vaccine preceded by ESA. In a further aspect, the disclosure described herein provides systems and apparatus for use in methods for controlled administration of an HBV vaccine accompanied by ESA. These methods include, but are not limited in scope or sequential relationship to, the determination of treatment parameters, subject preparation procedures, CTAA, ESA, and additional measures.

Determination of Treatment Parameters

In some embodiments, treatment parameters are based on the desired amounts and/or duration of dosing of the HBV vaccine. HBV vaccine dosing can depend, for instance, on the particular indication or treatment application (such as the type and location of the target tissue), as well as various subject parameters (such as age and body mass). Dosing of the HBV vaccine can be controlled by parameters pertaining to administration of the HBV vaccine and ESA. Exemplary controllable parameters pertaining to CTAA include, but are not limited to, agent volume, agent viscosity, and injection rate. Exemplary controllable parameters pertaining to ESA include, but are not limited to, the characteristics of the electrical signals, the tissue volume exposed to the electrical signals, and the electrode array format. The relative timing and location of CTAA and ESA are parameters providing further control over HBV vaccine dosing.

Subject Preparation

In embodiments described herein, methods described herein can include a subject preparation step. The subject preparation can include, but is not limited to, antiseptic cleansing and anesthetic administration, including local or regional, nerve block, spinal block, epidural block, or general anesthesia. In an exemplary case of intramuscular (IM) ESA, protocols to minimize the effects of electrical stimulation of the muscle can be included in a method described herein, for instance, including thermal control (e.g. cooling the muscle), administration of anesthetics, and/or alternative stimulation patterns sufficient for mitigation of discomfort. It is to be understood that the selected subject preparation techniques do not adversely affect therapeutic efficacy, if acceptable alternatives exist. For example, it has been shown that in some cases, the intramuscular administration of amide based anesthetics can have an undesirable effect on intramuscular delivery plasmid DNA-based therapies, putatively due to the mild myotoxicity of these agents, which can inhibit the muscle cells ability to express the protein encoded by the administered DNA sequence.

CTAA and ESA

In some embodiments described herein, is a method wherein CTAA and ESA are combined, enabling consistent and reproducible HBV vaccine delivery. In some cases, are provided apparatus or kits suitable for CTAA, including for instance apparatus comprising at least one of automatic injection devices and jet injectors.

The disclosure provides methods, kits and apparatus enabling the transcutaneous deployment of a plurality of elongate electrodes to a target depth in a safe and consistent manner

with respect to a target site of agent distribution in recipients with heterogeneous skin thickness and composition in order to support the application of electrical fields in tissue to enhance the intramuscular, intradermal, and/or subcutaneous administration of therapeutic or prophylactic agents such as nucleic acids, pharmaceuticals, antibodies, peptides, proteins, or combinations thereof.

Systems and methods according to present principles enable the consistent transcutaneous deployment of a plurality of elongate, tissue penetrating electrodes to a pre-determined target tissue site in order to propagate electrical fields in the skin, subcutaneous tissue and/or skeletal muscle. The disclosure provided herein is designed to enable a user with minimal training to consistently deploy the electrodes to a target depth while maintaining the proper spatial relationship among the plurality of electrodes, even when the procedure is applied at sites with varying skin characteristics. Such variation can be due either to variation in skin characteristics at different sites within an individual or among heterogeneous recipient populations. In other words, systems and methods according to present principles should allow a consistent profile irrespective of the administrator or the recipient. In certain embodiments, the deployment of the electrodes is accompanied by the insertion of one or more injection needles which are configured for the administration of HBV vaccines to the target region of tissue and arranged in a pre-determined spatial relationship with the electrodes to be used for ESA. In an exemplary embodiment, the electrodes are arranged such that any electrical signals from said electrodes are preferentially applied distal to the site of HBV vaccine administration by the insertion of one or more injection needles. In another embodiment, the electrodes are arranged such that any electrical signals are preferentially applied proximal to the site of HBV vaccine administration by the insertion of one or more injection needles.

Aspects of the disclosure can be used singly or in combination to support the transcutaneous insertion of electrodes for the *in vivo* application of electrical fields to enhance the intramuscular, intradermal, and/or subcutaneous administration of nucleic acids, small molecule drugs, antibodies, peptides, proteins, and combinations thereof. In some embodiments, the electrode deployment and subsequent electrical field propagation are performed in coordinated fashion with the distribution of the agent of interest to the target tissue site. In an exemplary embodiment, the administration of the agent and the application of one or more electrical fields are performed in a controlled and monitored fashion such that the probability of

achieving spatial and temporal co-localization of the distribution of the agent with the site of electric field application is maximized.

In general terms, the disclosure provides methods and apparatus for the transcutaneous deployment of electrodes to a predetermined site within the skin, subcutaneous tissue, and/or skeletal muscle of a recipient in conjunction with the administration of an agent of interest and the local application of electrical fields to improve the delivery, uptake, and/or biological effect of the agent. In some embodiments, the disclosure has been implemented such that set up and usage of the device can be performed effectively and reliably by users with minimal training. In another embodiment, the disclosure also comprises the implementation of numerous interlocks, sensors, and feedback loops to reduce the frequency and/or potential impact of potential user errors committed during the set up and use of the device. Referring to **FIG. 2**, an embodiment of an apparatus described herein comprises a "cartridge assembly" **100** detachably interfaced to an "applicator" **400** which is configured to connect to a controller **700** which acts as source of electrical energy for electrode activation and subsequent electrical field generation, as well as diagnostics and other control routines. The controller **700** further provides a user interface, tray, holster for the applicator **400**, and various other features are described. As seen in **FIG. 2**, a reservoir **101** of suitable uniform size and general shape can be inserted in the cartridge assembly **100** in a method of use.

Details of a cartridge assembly **100** present in some embodiments of an apparatus described herein, are described for instance, in **FIGS. 3A-12**, along with corresponding cooperating portions of the applicator **400**, in **Figs. FIGS. 13A-18C**, followed by, where relevant, portions of the controller **700**, in **FIGS. 19-20D**. Remaining portions of the applicator **400** are described next, followed by remaining portions of the controller **700**.

Referring in addition to **FIGS. 3A-4**, in some embodiments described herein, the cartridge assembly **100** can comprise a support structure configured to interface with the applicator **400** and accommodating two or more elongate electrodes **122** mounted on the structure to form an array. To avoid unwanted propagation of electrical currents within the device, the design and materials of the electrode mounting structure should be specified such that there is an adequate dielectric barrier between electrodes of opposite polarity within the device. The distal region **137** of the elongate electrodes are engaged with the mounting structure using

standard mechanical features and/or bonding agents appropriate for the material composition of the electrode mount structure and the electrodes.

In an exemplary embodiment, the cartridge outer housing structure **102** is configured to interface with a fluid reservoir **101** containing the HBV vaccine where the reservoir **101** and cartridge housing structure **102** are configured to operatively connect to at least one injection orifice (needle **105**) through which the HBV vaccine is administered into the target tissue. In some embodiments, this configuration facilitates the co-localization of the distribution of the agent of interest with the site of electrical field application. In another embodiment, this configuration facilitates the implementation of a pre-determined spatial relationship between the apparatus for ESA and CTAA. In yet another exemplary embodiment, a syringe **101** is inserted into a cartridge **100**, wherein upon loading of the cartridge into an applicator **400**, the syringe **101** moves forward to mate with the needle hub **152** and to connect the cartridge to the needle.

Certain embodiments of the disclosure can include the use of syringes, vials, ampoules, cartridges or equivalent structures for storing one or more HBV vaccines. In some embodiments, the reservoirs can comprise at least one of glass and plastic, with the material selected for compatibility with the agent of interest. Coatings can be applied to the reservoir used to provide desirable lubricious or protective properties. As disclosed above, the electrodes **122** can be hollow and in some cases configured with injection orifices that can be operatively connected to the fluid reservoirs. Alternatively, the injection orifice can comprise one or more hypodermic needles and/or needle free injection ports positioned relative to the electrodes. Selection of the type and size of injection orifice can dependent on the desired route of administration, tissue distribution, and physical characteristics of the agent of interest. In a certain embodiment, the cartridge structure is designed to ensure a pre-determined spatial relationship between the injection orifice and electrodes in their deployed state such that the distribution of the agent of interest occurs substantially in the tissue bounded by the conductive regions of the plurality of electrodes. To minimize the need for handling of sharps by the user, in a certain embodiment, a cartridge **100** designed for use with hypodermic needles is configured to allow the hypodermic needle to be mated to the cartridge at the time of manufacture rather than the more common convention of mating the needle to the syringe at the time of use. In certain embodiments, an aspect of the disclosure can incorporate features in the cartridge and/or the needle to ensure retention of the needle during manufacture, distribution, handling, and use as well as features to

ensure that proper mating of the reservoir to the needle prior to the use. In some embodiments, such features can minimize the risks of leakage of the agent from the reservoir or the reservoir orifice interface due to breakage or improper mating.

In certain embodiments, the cartridge can include a tissue contact interface located at the distal end of the subassembly. In certain embodiments, the tissue contact interface comprises a substantially planar structure oriented perpendicular to the elongate orientation of the electrodes and having one or more apertures configured to allow passage of the electrodes through the tissue contact interface. For embodiments incorporating an integrated reservoir and injection orifice, the interface also has apertures to accommodate injection orifice or needle free injection. To minimize the risk of contamination of the electrodes and injection needle as well as the occurrence of unintended sharps exposure to the user or recipient, in a certain embodiment, the apertures accommodating passage of the electrodes and injection needle are of a size suitable to prevent accidental contact with the electrodes and injection needle. Most commonly, the tissue contact interface is comprised of one or more plastics suitable for at least short term tissue contact.

To avoid potential cross contamination of biological material between recipients, the cartridge assembly **100** can be configured for single use. In some cases, the cartridge includes one or more mechanical, electrical, and/or identification elements which restrict use of the cartridge to a single administration. Examples of mechanical elements of this nature include for instance, but are not restricted to, lockouts and/or detents which secure the electrode mount structure and/or the stick shield (see below) in the deployed state after use. Examples of electrical elements include for instance, fuses or links configured in series with one or more electrodes which are deactivated by the source of electrical energy at the conclusion of the first use of the cartridge. Examples of identification elements include, for instance, serialized radio frequency identification devices, bar codes, or quick response codes configured to be read by the applicator and/or the source of electrical energy. The identification information for a specific cartridge can then be used to prevent accidental or intentional re-use of that cartridge by the applicator and/or the source of energy. In an embodiment, one or more redundant features are incorporated to minimize the potential for re-use of the cartridges.

In an embodiment of an apparatus described herein, as shown in **FIG. 2**, the applicator **400** comprises a support structure configured to interface with the cartridge assembly **100**, a user

interface **410-418 (FIG. 13B)**, electrically conductive electrical connections configured to provide operative connection between the conductive contact region located on the distal region of the elongate electrodes and the source of electrical energy when the electrodes are deployed into the target tissue of the recipient. In some cases, the user interface comprises a handle, one or more display features designed to convey information to the user, and one or more features capable of accepting user input. In certain embodiments, the display features are configured to convey the operating status of the device during its set up and use as well as relevant warning / error messages. Such displays can comprise mechanical features, lights, alphanumeric displays, and/or electronic display screens. In some cases, the features capable of accepting user input are configured to allow the user, at the appropriate stage of the procedure, to de-activate safety features within the device preventing accidental discharge, make selections regarding particular parameters of the procedure (e.g., the intended depth of injection), and to initiate procedure administration and can include buttons, triggers, mechanical slides, and/or levers.

In certain embodiments, the applicator **400** also includes actuation mechanisms which interface with the cartridge assembly and which are configured for transcutaneous deployment of the electrodes, positioning of the injection orifice relative to the target tissues, discharge of the agent of interest from the reservoir through the orifice and into the target tissue site, and/or conveying electrical signals from an electric field generator such as a controller **700** to the cartridge **100**. The applicator **400** can be configured such that the energy to actuate the mechanisms is supplied by the user, or more preferably, the apparatus may incorporate one or more inanimate sources of energy operatively connected to the actuation mechanisms within the applicator. Such inanimate sources of energy include for instance, electromechanical devices (solenoids, motors, lead screws), mechanical components (springs and related devices), and compressed gases.

An exemplary implementation of a cartridge assembly **100** is as described in **FIG. 3A**, a cartridge assembly **100** includes a reservoir loading port **140** and a reservoir containment volume **142** to receive and contain a reservoir **101** of a medicament. A cartridge assembly **100** is required because, for a device in which an electrical field is to be generated and used as part of a therapy, an electric field generator such as a controller **700** is required to electrically interface with the device containing the electrodes configured to contact the target tissue. As the controller can be configured for multiple uses, and the reservoir **101** can be intended for single

use, the cartridge assembly **100** can be present to hold the electrodes **122** and, the reservoir **101**, to interface with the re-usable device, and for the cartridge assembly **100** to be configured for single use. Thus, the applicator **400** can be a reusable component and the cartridge assembly **100** can be configured for single use. The cartridge assembly **100** can also be rendered in a condition to prevent subsequent use if errors or tampering occur in the insertion of a reservoir **101**, or if defects are present.

The term reservoir **101** can refer to a syringe, vial, or any other device which can contain a medicament or HBV vaccine and which can interface with a device having an orifice, such as a needle, shown in **FIG. 4** as a needle **105** having a needle hub **152**. The reservoir **101**, for a given type of cartridge assembly **100**, generally has a common shape and size. Various components within the cartridge assembly **100** allow for a leeway in exact sizing and/or manufacturing tolerances, but generally a common shape and size are required to reduce the risk that drugs not labeled for use with cartridge **100** are erroneously delivered with the device. If an appropriately sized reservoir **101** is not provided by the user, one or more interlocks within the cartridge assembly **100** can be unable to deactivate, and the system can be rendered unusable until a proper-sized reservoir **101** is inserted.

As seen in **FIG. 3B**, the reservoir **101** generally can be equipped with a plunger and a port **156** for drug egress. A removable cap **158** can also be provided to maintain the sterility and integrity of the agent until such time as the reservoir is to be inserted and used. The port for drug egress can be proximal to the needle hub **152** in use, and the plunger can be opposite this port for drug egress. As an alternative to an open port for drug egress, in some embodiments, the reservoir can be configured with a septum component which covers and seals the end of the container opposite the plunger. The septum can be made of elastomeric compounds such as silicone or butyl rubber, with the specific formulation and coating of the material selected for stability and compatibility with the agent contained within the reservoir. The septum component is typically held in place by a crimp seal or other fastening mechanism. This septum seal configuration obviates the need for a removable cap, but requires that needle **105** be equipped with a suitable piercing member such as a needle, spike, or other features to access the fluid contained in the reservoir. Specific implementations for this configuration include dual sided needle configurations and spike vial adapters.

The cartridge assembly **100** is not only configured for receiving the reservoir **101** but also for being received by an applicator **400** in an applicator cartridge assembly receiving port **401** (**FIG. 2**). Thus, the cartridge assembly **100** includes a device allowing the applicator **400** to pull and retain the same within an interior volume, at least partially. In certain embodiments, the device is one or more racks on the surface(s) of the cartridge assembly **100** that engage a corresponding motorized pinion assembly in the applicator **400**. In other implementations, the applicator **400** can interface with the cartridge assembly **100** without the need to pull the same into an interior volume. In yet other implementations, other techniques can be employed to cause the cartridge assembly **100** to engage the applicator **400**, e.g., motorized tracks or brackets and the like onto which the cartridge assembly **100** can interface.

The applicator **400** is further provided with interface elements allowing the same to control certain actions within the cartridge assembly **100**. In particular, the applicator **400** can be configured to control needle insertion, medicament delivery, electrode insertion, and electrode activation using various subsystems. In some cases these steps are linked, so that a single action of applicator **400** initiates multiple of these steps. In some implementations, all of these steps but the medicament delivery and electrode activation are caused by a single action, as described in the exemplary implementation below.

Upon appropriate activation, such as the use of electrical or optical signals conveyed to mechanical, electrical, or optical elements of the cartridge assembly **100**, the applicator **400** can be enabled to test subsystems within the cartridge assembly **100**, ensuring that the same are operating properly and are properly configured for medicament delivery with electric field application. For example, such subsystems include that the applicator **400** can test to ensure that the cartridge assembly **100** has not been previously used, that the reservoir has been properly placed within the cartridge assembly, that appropriate force applied against the body of a subject as applied through an alignment guide / splay shield **108**, a test that a depth has been affirmatively selected by the user, and that an exterior cartridge cap **110** has been removed. Moreover, the applicator **400** can be configured to monitor the status of the cartridge functions during execution of the procedure. For example, such subsystems include that the applicator **400** can test to ensure that that electrodes **122** are properly deployed within a subject prior to commencing with administration of the medicament, that the plunger in the reservoir has been appropriately actuated prior to application of the electrical fields, that the user has maintained

appropriate force applied against the body of the subject during the administration procedure, and so on.

In addition to the subsystems that are operated by the applicator **400**, the cartridge assembly **100** can incorporate appropriate subsystems, including those that interact with the applicator **400** and those that do not so interact, so as to accomplish the goals of the medicament delivery and electric field application therapy. These include a subsystem for causing needle and electrode insertion, a subsystem for protecting users from sharps following therapy administration, a subsystem for providing different depths of needle/electrodes insertion, a subsystem for ensuring that adequate force has been applied against the tissue of the recipient prior to allowing initiation of the procedure and subsequently during application of the administration procedure and so on. While often described in the context of deployable needles and electrodes, it is noted here that such are not strictly required, and that systems with non-deployable or fixed needles and electrodes also benefits from systems and methods according to present principles, including the subsystems described.

In one exemplary implementation, as shown in **FIG. 4**, the cartridge assembly **100** includes an outer cartridge **102**, in some cases termed a housing. The outer cartridge **102** is terminated at a distal end by an outer cartridge cap **106**. The outer cartridge **102** includes an inner cartridge containment volume **150**, for receiving an inner cartridge **103**, which is received and moves in a slidable manner in relationship to the outer cartridge **102**. The inner cartridge **103** includes a reservoir containment volume **142** in which the reservoir **101** can be situated. The inner cartridge **103** engages with an inner cartridge cap **104** at a distal end. The inner cartridge cap **104** has a number of functions, including to lock electrodes **122** in place (the inner cartridge **103** itself has seams that the electrodes **122** are placed into) and to provide a bearing surface for a stick shield **134**. The inner cartridge cap **104** locks onto the inner cartridge **103**.

A cartridge breech **112** is received in a portion of the reservoir containment volume **142** in the inner cartridge **103**, in a portion opposite that of the inner cartridge cap **104**. A reservoir detection cap **118** engages the cartridge breech **112** through a reservoir detection spring **116**. A cartridge lock ring **114** locks the system in place, including the cartridge breech **112** to the inner cartridge **103**. The reservoir detection spring **116** also serves to push the reservoir **101** into engagement with the needle hub **152**, and also serves to accommodate tolerances in the size of reservoir **101**.

A reservoir interlock **120** provides a mechanical interlock to prevent inadvertent or unwanted actuation of cartridge functions. In particular, the reservoir interlock **120**, also termed a first reservoir insertion trigger, is placed below the inner cartridge **103** and has fingers that extend through slots or holes defined in the inner cartridge **103** (see **FIG. 5B**). The fingers prevent the cartridge breech **112** from slidably moving relative to the inner cartridge **103**, and in particular from moving within the inner cartridge **103** towards the inner cartridge cap **104** before a reservoir has been inserted into a reservoir containment volume **142**.

When a reservoir **101** is properly inserted in the reservoir containment volume **142**, the reservoir interlock **120** is pushed down and the fingers are pushed down, no longer extending into the reservoir containment volume **142**. This pushing down or depression of the reservoir interlock **120** may also be configured to provide an audible, tactile, or haptic “click” that can inform the user of proper insertion. Once depressed, the cartridge breech **112**, no longer blocked by the fingers of reservoir interlock **120**, is then permitted to move, and in particular is permitted to move in the direction towards the inner cartridge cap **104**.

The cartridge breech **112** is caused to move such by the action of the spring cap / cartridge interface **470** when the cartridge assembly **100** is inserted in the applicator **400** in a fashion described below. When the cartridge breech **112** moves far enough forward, it locks in place, securing the reservoir **101** in the reservoir containment volume **142** and ensuring that it is properly positioned relative to the needle hub **152** to ensure an intact fluid pathway from the reservoir **101** to the orifice of needle **105**.

For embodiments of the device where the injection needle is incorporated into the cartridge **100**, the use of standard “off the shelf” single use hypodermic injection needles can be employed within the device. However, the operational and reliability characteristics of the device can be improved through the incorporation of customized design elements that are not present in hypodermic needles intended for conventional parenteral administration procedures. Specific aspects of the needle hub **152** can include the material from which it is comprised, the inclusion of retention features to prevent the needle hub **152** from becoming dislodged from inner cartridge **103** during distribution and use, and the orientation of any bevel features in the needle relative to the hub.

Conventional single use disposable injection needles are commonly comprised of injection molded polypropylene thermoplastic. However, for many applications, the impact

strength, tensile strength, and flexural strength of polypropylene may not be adequate to ensure integrity of the hub when subjected to the forces characteristic of needle deployment and injection with this device. Specific failures of concern include failure in the hub wall due to impact or injection forces as well as failure of the hub needle joint due to same. While adjustments in the design of the hub, including its geometry and wall thickness can be utilized to address prevent these failures, it is not always feasible to modify the design sufficiently to prevent hub failure while ensuring that the hub retains the dimensional properties required for proper mating to a conical male luer slip connectors as described in the relevant standard published by the International Standards Organization (ISO) ISO 80369-7:2016 Small-bore connectors for liquids and gases in healthcare applications -- Part 7: Connectors for intravascular or hypodermic application. Specifically, given the forces that the syringe needle and hub are subjected to during deployment, in certain embodiments, a material with improved impact strength, tensile strength, and flexural strength is used. One example is the use of injection molded polycarbonate plastics (such as ZELUX® GS, Makrolon, or Lexan) or copolyesters (such as Eastman Tritan™ Copolyester MX731, MX711, and MX 730). When assessed according to ISO 180:2000 Plastics — Determination of Izod impact strength, a notched impact strength of at least 70 kJ/m² is considered suitable for this application. When assessed according to the ISO 527-1:2012 Plastics -- Determination of tensile properties -- Part 1: General principles, a tensile strength of at least 30 MPa is considered suitable for this application. When assessed according to ISO 178:2010 Plastics -- Determination of flexural properties, a flexural strength of at least 50 MPa is considered suitable for this application. In some embodiments, the specific resin selected exhibits compatibility with the intended method of sterilization (e.g., gamma radiation) without exhibiting detrimental changes in its physical properties that could compromise its function.

For embodiments where a custom injection needle is utilized, one or more mechanical features are included which are not ordinarily present on conventional syringe hubs that enable the device to be inserted into inner cartridge **103**. Such features can include tabs, snaps, or ridges with corresponding mechanical features located on inner cartridge **103**. In some cases, the features are implemented such that the hub mates with the inner cartridge **103** in a consistent orientation. Combined with a needle manufacturing process that is capable of consistently orienting the bevel or other needle orifice feature, this insures that biases in injection location or

medicament distribution due to the location and design of the orifice can be accounted for in the design of the device. For example, needles with asymmetrical penetrating tip features (e.g., a bevel cut) can exhibit a directional bias during deployment into tissue due to interaction between the tissue and the asymmetrical penetration feature on the needle. If the electrodes have a symmetrical penetrating tip feature (e.g., a trocar tip) then the electrodes would not exhibit a corresponding bias in their deployment characteristics. Therefore, mounting feature for needle hub **152** on inner cartridge **103** can include an offset in the position of the injection orifice on needle **105** relative the electrodes **122** prior to deployment to account for the expected deployment characteristics of the asymmetrical bevel of the needle. The precise dimension of the offset can depend on the nature of the target tissue and the expected range of penetration depths, but in certain embodiments the needle is offset by 0.5-1 mm for each 10 mm of penetration depth. When using electrodes and injection needles with differing tip profiles or where the tip profiles must be consistently oriented with one another, such features are advantageous for insuring co-localization of the medicament distribution with the application of the electrical fields.

The incorporation of a syringe detection cap **118** mounted to a syringe detection spring **116** ensures that the cartridge assembly **100** can accept and properly position the syringe **101** relative to needle hub **152** across the range of manufacturing tolerances expected for syringe **101**. The applicator **400** causes the cartridge breech **112** to move forward during the loading procedure when the spring cap / cartridge interface **470** within the applicator **400** moves distally, relative to the cartridge assembly **100**. This action occurs when the cartridge assembly **100** is loaded into the applicator **400** and the cartridge assembly **100** is pulled into the applicator **400**, e.g., by the action of a loading mechanism, e.g., a rack-and-pinion mechanism described below.

The movement of the cartridge breech **112** can act as a second interlock. In particular, in one implementation, as seen in **FIGS. 5C-5D**, a line of sight is visible and detectable, by an appropriately configured sensor, within the cartridge assembly receiving volume **403**, through a set of reservoir locking holes **144'** (**FIG. 5D**). This line of sight is visible when the cartridge assembly **100** is loaded into the applicator **400**. The visible light of sight, or occlusion of the same, can act as part of a second interlock that must be deactivated for the controller **700** to allow activation and triggering of the device, including needle and electrode insertion, medicament delivery, and electrode activation.

For example, in one implementation, the reservoir locking holes **144'** (**FIG. 5D**) must be occluded for the device to operate. If a visible line of sight is present, e.g., as detected by an IR or visible light emitter and detector paired within the cartridge assembly receiving volume **403** of the applicator, the device can be rendered inoperable and generate and display an error message on the applicator display **404** and/or controller **700**, including the controller display **712**, to notify the user of the state of the device as well as the recommended steps to be performed to address the error.

Thus, in this implementation, one error state can be that no reservoir **101** was loaded into the cartridge **100** or that the syringe was not properly seated into the inner cartridge **103**. In this case, the reservoir interlock **120** cannot be depressed as there is no reservoir **101** to perform this action. In this case, the cartridge breech **112** cannot be moved forward, in the distal direction, towards the inner cartridge cap **104**. The construction of these components can be such that an open breech state results in an open line of sight **146** through first reservoir locking holes **144'** (**FIG. 5D**). An ancillary check is that the cartridge breech **112** cannot be closed, and this can manifest itself as an inability of the cartridge to move the required distance backward or distally into the cartridge assembly receiving volume **403**. As these conditions are defined by the system to be an error state, the same can be identified and used in the generation of an error message, e.g., with an appropriate message to the user on a user interface on the applicator display **404** and/or controller **700**, including the controller display **712**. A similar error state may occur if the reservoir **101** is improperly loaded, or if the reservoir interlock **120** is damaged. Generally in this case, an appropriate error message can be accompanied by instructions to the user to remove the cartridge, reinstall a new reservoir, and attempt to reintroduce the cartridge assembly **100** into the applicator **400**.

Another error state can be that the cartridge breech **112** is moved forward manually by the user without a reservoir **101** present, such occurring by the user manually pushing the reservoir interlock **120** out of the reservoir containment volume **142**. This situation can also be defined as an error state, and the same can be detected because another (second) set of reservoir locking holes **144** (**FIG. 5C**) are placed on a portion of the outer cartridge **102**. If no reservoir is in place, but the cartridge breech **112** is moved forward by the action of the reservoir interlock **120** being depressed, then the reservoir locking holes **144'** (**FIG. 5D**) align with the reservoir locking holes **144** (**FIG. 5C**), again creating an open line of sight **146** and a subsequent error

state. This error state may also occur if the reservoir interlock **120** is damaged and its fingers are no longer within the reservoir containment volume **142**. In a certain embodiment, this error state need not be not remediable by attempting to reinstall a reservoir, as a reservoir may not fit in the reservoir containment volume **142** with the cartridge breech **112** locked. In a certain embodiment, a new cartridge assembly **100** is required.

In contrast, if an appropriately sized reservoir **101** is positioned properly in place, then the reservoir detection cap **118** is pushed back against the reservoir detection spring **116**, and the movement of the reservoir detection cap **118** occludes the reservoir locking holes **144** (**FIG. 5C**) and the reservoir locking holes **144'** (**FIG. 5D**). In this case there is no error state, allowing the device to operate. The occlusion, and detection of the occlusion, occurs within the body of the applicator **400**, after the cartridge assembly **100** is inserted, and thus is insusceptible to user attempts to defeat this interlock, whether intentional, accidental, or caused by a defect. It is noted that this “no error” state still occurs even if the user intentionally or inadvertently closes the cartridge breech **112** themselves during handling of the cartridge **100**.

Depending on which error state occurs, the cartridge assembly **100** can remain usable or not. If the cartridge breech **112** has been locked into place, the cartridge assembly **100** is rendered unusable. If, however, the cartridge breech **112** has not been locked into place, then the cartridge assembly **100** can be removed from the applicator **400** and a new reservoir **101** inserted.

While the above-noted set of two interlocks (one mechanical using reservoir interlock **120** and one using a light emitter and collector and reservoir lockout holes **144** and **144'**) have been found particularly useful in some implementations, it is to be understood that other types of interlocks can also be employed (**FIGS. 5C-5D**). For example, instead of having an error state occur when the reservoir locking holes **144** are not occluded, the error state can be configured to occur (via a change in reservoir locking hole location and program logic) when the reservoir locking holes **144** are occluded (and the clear line of sight **146** then corresponding to the non-error state). The reservoir interlock **120** can incorporate additional mechanical flag features that are recessed in the cartridge when the interlock is active, but become visible to an appropriately configured sensor when the syringe **101** has been properly inserted and the reservoir interlock **120** is depressed into its released position. In other variations, other ways can be employed to determine if a line of sight is present, e.g., optical, acoustic, electrical, or the like, so long as a

suitable emitter and collector can be positioned within the applicator **400**. Other ways may also be employed to determine if the reservoir **101** is properly loaded, e.g., mechanical techniques, as it is to be understood by one of ordinary skill in the art given this teaching. Depending on implementation, if an error state is detected, the applicator **400** can be prevented from operating either with the cartridge assembly **100** in place, or the applicator **400** can be prevented from even accepting the cartridge assembly **100** in the first place. For example, the cartridge can be designed such that the reservoir interlock **120** includes mechanical tab or locking features which extend from one or more of the cartridge surfaces until a syringe **101** is properly inserted into the cartridge **100**. The mechanical tabs are designed to interact with a corresponding detent feature located in the applicator **400** such that loading of the cartridge **100** into the applicator **400** is physically blocked unless the reservoir interlock **120** is released. This mechanical interaction would provide feedback to the user or the system that an error condition must be resolved before proceeding with the loading of the cartridge **100** into applicator **400**. In other variations, more or less than two interlocks can be provided, although the same can be correspondingly associated with a different safety profile.

In certain implementations, other features can also be employed in the above determinations, or to enhance the above determinations. For example, where a motor is employed to pull the cartridge assembly **100** into the applicator **400**, sensors can be employed as described below to detect the spatial position of the cartridge assembly **100** during the insertion process. Put another way, the applicator **400** may detect where the cartridge assembly **100** is within the cartridge assembly receiving volume **403**. In some cases, such may allow determination of additional error states either directly, or by prompting the activation of additional sensors to assess the state of the device. For example, in a used cartridge, the cartridge breech **112** is locked into position. If the used cartridge assembly **100** is attempted to be re-used, the optical detector detects the cartridge assembly **100** at a different point than it would for an unused cartridge assembly **100**. The use of an electrical motor for one or more system functions also provides the opportunity to monitor its operational status including the voltage and current levels supplied to the motor as well as the number of revolutions that the motor has performed during a specific operation. Measurement of these quantities during system operation can be used as a primary or secondary method for detecting potential or actual fault conditions. For example, the use of mechanical interlocks designed to block the loading

procedure when the cartridge **100** is not properly configured can be coupled with sensors and logical circuits monitoring the motor to ensure proper loading of the cartridge **100** into applicator **400**. For example, the interaction of the mechanical features described above that are designed to prevent the loading of cartridge **100** without a properly inserted reservoir **102** would result in increased load on the motor drive mechanism, resulting in a higher current draw. Detection of the elevated current draw by the motor would prompt the loading procedure to be halted and a fault condition displayed to the user, for example on stimulator display **712** and/or applicator display **404**.

Since it is possible that medicaments not intended for administration by this method could be contained within reservoirs of similar size and configuration as that intended for use by this delivery method. Therefore, an additional aspect of the system is the incorporation of one or more methods to ensure that the reservoir **101** inserted into the cartridge **100** by the user is specifically, intended for use with the device. The implementation of such features would reduce the risk that an incorrect medicament is administered to a given subject. Customarily, specific information in the user instructions and labeling of the medicament includes the route and method of administration. However, to further reduce the potential for user errors, the incorporation of mechanical, optical, and/or electrical features within the reservoir and device can be desirable. In an embodiment, the syringe can be designed to incorporate one or more unique mechanical features that are not present in other reservoirs which can be similar to those designed for use with this delivery method. For example, the reservoir can be specified to incorporate a rib or other elongate feature on the flange or barrel of the reservoir. In this embodiment, a corresponding mating feature would be included on the reservoir interlock **120** such that the reservoir interlock would be deactivated only if a reservoir with the appropriate mating feature were properly inserted into the device. In the event that it is not feasible to directly implement the feature in the design of the reservoir, an alternative embodiment would include the placement of a secondary mechanical component on the reservoir that would be unique to reservoirs intended for use with the device. For example, a ring or other appropriately configured feature designed to slide over the barrel of the reservoir can be used to “key” the reservoir for use with the device by mating with a corresponding feature in the outer cartridge **102**, inner cartridge **103**, reservoir interlock **120**, reservoir detection cap **118**, or other suitable feature within the cartridge **100**. An additional embodiment a custom label of suitable size, color,

and/or electrical conductivity applied to a pre-determined location on the outer surface of reservoirs that are intended for use with the device. Corresponding optical or electrical sensors in applicator **400** would be configured to assess the presence or absence of the label on the surface of the reservoir in order to verify that the medicament inserted into the cartridge is intended for use with the device. The detection method would comprise the use of optical or electrical signals applied to the surface of the label in order to assess its presence or absence. In this way, reservoirs containing medicaments not intended for use with the device (and therefore missing the relevant label) could be detected and excluded from potential misuse.

A configuration of sensors is now described to perform the cartridge loading and syringe detection determination described above, such sensors further forming a portion of a cartridge loading subassembly within the application **400**. In more detail, and referring in addition to **FIGS. 6, 17B, and 18C**, an exemplary way of detecting where the cartridge assembly **100** is located is by use of a cartridge loading sensor **436** and a cartridge loaded sensor **438**, which forms a portion of a loading drive subassembly **454**, the subassembly **454** further including cartridge guide rails **442** and a loading motor **444** which has a connection to a pinion gear assembly **448** that pulls the cartridge assembly **100** into the cartridge assembly receiving volume **403** via racks **154** on the base of the outer cartridge **102**. In more detail, when the cartridge loading sensor **436** detects an initiating flag **172** on the cartridge assembly **100** (see **FIG. 6**), the motor can be caused to initiate loading. When the cartridge loaded sensor **438** detects the same flag, loading can be caused to cease. A continuing flag **174** can be employed that is required to be present for loading to continue.

The first "teeth" of the rack **154** can be configured to provide a tactile sensation (or audible or haptic) for the user when they are inserting the cartridge assembly **100** into the cartridge assembly receiving volume **403**. Such configuration may include the shape and/or size of the rack teeth **154** as well as the amount of flexion permitted by their positioning in the outer cartridge. By adapting the rack teeth implementation, the desired degree of tactile feedback can be achieved while ensuring that it does not provide a significant force against the loading motor **444** from receiving and loading the cartridge assembly **100**.

Referring in addition to **FIG. 17A**, and as noted above, the cartridge assembly **100** is inserted into a cartridge assembly receiving volume **403** within the applicator **400**. While various ways can be employed to perform this insertion, one way that has been found particularly

useful is by way of a pinion gear assembly **448** engaging racks **154** on the outer cartridge **102**. The use of more than one rack provides additional stability, particularly torsional stability during the loading phase. Referring also to the insertion/injection drive assembly **456** of **FIG. 18A**, in addition to drawing the cartridge assembly **100** within the cartridge assembly receiving volume **403**, the insertion action further compresses an electrode / needle insertion spring **472** through a spring cap / cartridge interface **470**. The electrode / needle insertion spring **472** is used as the primary driving force for the needle and electrode insertion during medicament delivery.

This hybrid motor/spring action provides numerous benefits. The motor drive is beneficial as it is highly controllable and allows the cartridge **100** to be loaded into the applicator **400** in a semi-automated fashion with minimal input of mechanical force required by the user. As described above, the implementation of motor drive based mechanisms provides monitoring of the operational status of the system. For instance, conveying the current draw and revolution count to the logical and control circuitry in the system provides a supplementary method for detection and diagnosis of potential fault conditions. Despite these advantages, in certain cases electric motors can be poorly adapted to exerting the necessary linear force over sufficiently brief time scales that is most desirable for effective transcutaneous deployment of arrays comprising a plurality of elongate electrodes and, in selected embodiments, hypodermic injection needles. In particular, the penetration of dermal tissues is most consistently achieved by the application of a large linear force over a brief time scale. In some embodiments, the most favorable insertion characteristics are achieved when the penetrating electrodes, and when present, injection needle contact the skin at higher velocity. This is because sharps travelling at increased velocity at the point of skin contact result in less tissue deformation as they cut or penetrate the tissue. Therefore, in some embodiments, rapidly accelerating the sharps prior to contact with the skin is desired. In some embodiments, multiple electrodes and an injection needle are frequently utilized. In another embodiment, the velocity of the electrodes is at least 50 mm/second prior to contact with the skin. In yet another embodiment, the velocity of the electrode is at least 500 mm/second prior to contact with the skin. This deployment approach minimizes the discomfort perceived by the subject during the electrode penetration and is most favorable for maintaining a consistent spatial relationship between the plurality of electrodes. In contrast to electromechanical motors, spring driven mechanisms exhibit a more favorable discharge profile that is capable of imparting the rapid impulse force to the electrodes and injection needle that is

desirable for transcutaneous electrode implantation. In particular, the force exerted by a compression spring is at its peak at initial discharge. This is favorable for transcutaneous deployment where a high velocity at the point of skin contact is favorable and, due to the viscoelasticity of skin tissue, the greatest force is required for penetration of the skin, particularly when contacting the skin with a plurality of electrodes and/or injection needles. In addition, a spring based mechanism is capable of generating this force from a simple, durable, and compact form factor that can be readily integrated into a handheld device format. However, a disadvantage of spring based mechanisms is that they typically require the input of substantial mechanical force by the user in order to prime them for operation, especially for springs with high force constants and/or large displacements. The use of a hybrid motor and spring mechanism, as described in the disclosure, achieves the desired deployment force characteristics while being simple for the user to operate. While the hybrid motor and spring mechanism is a preferred embodiment, depending on implementation, other hybrid mechanisms incorporating two or more drive mechanisms wherein one is capable of generating a rapid impulse force and the other is capable of priming the impulse force mechanism, e.g., a pump capable of compressing gas into a chamber and then discharging the compressed gas in order to apply an impulse force for deployment of the electrodes and, where applicable, hypodermic needle(s) can also be used.

In any case, once loaded, the desired depth electrode deployment and/or agent administration is affirmatively selected by the physician or other medicament administrator and the same transmitted to the applicator **400**. Referring in addition to **FIG. 13B**, the depth can be selected by depth selection buttons **409** (or other equivalent interface such as a toggle switch or sliding switch) and the result displayed on injection depth selection indicators **408** (or, again, other equivalent interface). The available injection depths are conveyed to the user by appropriate labeling of the applicator **400** and/or the cartridge **100**. In some embodiments, any labeling regarding the injection depth is located on the cartridge **100** and remains visible to the user following installation in the applicator **400**. For example, the available injection depths can be labeled on the upper surface of the alignment guide / splay shield **108**. In order to avoid circumstances in which the user forgets or neglects to select a depth of injection, it is preferable that the device does not allow the user to proceed with the administration procedure until such affirmative selection is made. This can be accomplished by the implementation of appropriate

control logic within the system such that subsequent elements of the device set up or usage are not accessible until a valid depth selection has been entered by the user. In certain embodiments, when the user is prompted to affirmatively select the injection depth, the controller display may convey to the user information regarding the proper methods for assessing the subject and determining the appropriate injection depth for the selected administration site.

Referring in addition to **FIGS. 18A-18B**, upon proper cartridge assembly insertion, the spring cap / cartridge interface **470** engages and also presses against the spring cover hole **471** and tabs **491**. While a large spring force presses against the inner cartridge **103**, the same is prevented from moving forward by engagement of a set of retaining posts **488** against walls **494** of the outer cartridge **102**. However, the force exerted by **470** splays apart tabs **491** (which prevent inadvertent rotation of the lock ring during handling) thereby allowing rotation of the cartridge lock ring **114** by the motor drive mechanism. The rotation of the cartridge lock ring **114** causes rotation of the retaining posts **488**. The retaining posts **488** can be rotated into either the channels for first depth **490** or the channels for second depth **492**. The length of the channels for first depth **490** correspond to one of the choices of depths, and the length of the channels for second depth **492** corresponds to the other, with one or the other depths being selected by the user using buttons **409**. For example, the length of channels **490** can be in the range of 20-30 mm and the length of the channels **492** can be in the range of 12-20 mm. The requirement of a user to affirmatively select a depth provides yet another interlock. Without an affirmative selection, the applicator may not allow activation / needle insertion. The rotation of the cartridge lock ring **114** is thus caused by a clockwise or a counterclockwise rotation directed by the applicator **400** according to the dictates of the user. Requiring rotational motion of a set of posts into such channels to achieve deployment of the electrodes and, if present, injection needle greatly reduces the chances for accidental discharge, even upon violent jarring or falling.

In more detail, the rotation of the cartridge lock ring **114** is transmitted to the cartridge assembly **100** by the retaining posts **488**, which are disposed upon proper cartridge assembly **100** insertion into slots on an insertion mechanism gear drive ring **478**. In **FIG. 18A**, the slots on the insertion mechanism gear drive ring **478** are disposed at 3 o'clock and 9 o'clock positions. The insertion mechanism gear drive ring **478** is mounted to a partial insertion gear ring **479**, which is driven by an insertion mechanism drive motor **482**. Driving the partial insertion gear ring **479** causes the insertion mechanism gear drive ring **478** to rotate either clockwise or counter-

clockwise. A flag **481** on a ring **480** and accompanying insertion mechanism position sensor **483** are employed to determine the position of the insertion mechanism gear drive ring **478**, and is further used to accurately return the same to the 3 o'clock and 9 o'clock positions when the applicator **400** is to be re-used with another cartridge.

The above implementation provides various advantages. For example, the user has to actively perform a step of selecting the depth before proceeding with the administration procedure. In so doing, the user has to assess the proper depth of injection for the selected injection site and prepare the site as noted in a guide or instructions for use document. As noted, the insertion mechanism, requiring rotational motion to deploy, is significantly hardened against accidental deployment due to falling, dropping, jarring, and so on.

Variations are to be understood by a person skilled in the art. For example, while two channels and two retaining posts are employed for each depth, one channel and one retaining post may also be used. Various types of motors and mechanisms can be employed for conveying the rotational motion necessary to rotate the posts into the channels. Other variations are also to be understood, including the use of solenoids and the like. In lieu of the use of channels, motor driven deployment can be used to provide variable depths, which depths can be controlled simply by how far the motor is controlled to drive the deployment. Preferably, in this context, the hybrid drive mechanism described would be configured such that the impulse discharge mechanism (e.g., the spring) is used for initial deployment through the dermis and then the motor drive mechanism is used to advance the electrodes to their desired depth.

One or more interlocks can be in place which must be deactivated before the insertion mechanism gear drive ring **478** is caused to rotate, rotating retaining posts **488** into the channels.

First, a force detection interlock subsystem can be in place that requires the device to be applied to the subject at a force of greater than a predetermined amount prior to allowing the administration procedure to be initiated. This force can be measured by an appropriate mechanical or electromechanical system and the result fed back into the controller **700** and used as an interlock to prevent activation of the device where insufficient force is provided. In some embodiments, the controller **700** is capable of conveying the state of the force detection interlock to the user through visual, haptic, or auditory signals so that a state of inadequate force can be corrected and the user may proceed with administration. In the event that the user attempts to proceed with the administration in a state of inadequate force contact (e.g., by depressing a

trigger **407** or other activation button), additional visual, haptic, or auditory signals can be provided by the applicator **400** or controller **700** to notify the user that the interlock must be deactivated prior to proceeding with administration.

The detection of the force applied to the subject can be accomplished in a number of ways. Referring to the particular implementation of **FIGS. 4 and 8A-8B**, the alignment guide / splay shield **108** is equipped with a force contact pickup **128**. The alignment guide / splay shield **108** can be mechanically biased in a distal direction (towards the subject) using one or more force contact springs **126**, of which four are shown in **FIG. 4**. The force contact pickup **128** changes its position by virtue of the force applied to the alignment guide / splay shield **108**. In so doing, it also changes the state of an electrical circuit formed by the force contact pickup **128**, a set of first pads **162**, a set of second pads **164**, and a flexible circuit **160**. In particular, by testing for continuity between one or more pads **162** and one or more respective pads **164**, it can be determined how far backward or proximal the alignment guide / splay shield **108** has been moved by applied force, and thus if sufficient force exists for proper delivery. The state of the circuit is read by the applicator **400** using sensor contacts **434** (see **FIG. 16**). If sufficient force is indicated, the force contact interlock is deactivated, allowing the user to operate the device.

In one implementation, at the first contact point, the system may not register that any particular force has been applied. At the second contact point, the system may register that it is at partial (but not sufficient) pressure. At the third contact point, the system may register that the prescribed level of pressure required to proceed with procedure administration has been achieved, and the interlock may deactivate. Preferably, the status of the force contact circuit is provided to the user via the applicator display **404**.

Variations are to be understood by a skilled person in the art. For example, the force contact interlock may form an electrical lock that is deactivated either within the controller **700** or within the applicator **400** itself. In another variation the force contact circuit can be configured to provide information regarding the status of the device throughout the procedure administration. In particular, the system may include a feedback loop between the force contact circuit and the controller **700** wherein a reduction in the force applied by the user precipitates the generation of a visual, haptic, or auditory signal by the controller **700** and/or applicator **400** so that a state of reduced force can be corrected. In a certain embodiment, a feedback loop exists between the force contact circuit and the controller **700** such that the detection of a change in the

applied force prompts the system to initiate a check as to whether the electrodes remain properly deployed into the tissue of the subject, e.g., via an impedance or resistance check between the electrodes. If the check is passed, then the procedure proceeds normally. In the event that the position of the electrodes is no longer acceptable, then the procedure can be aborted and the user notified of the state of the device through the generation of a visual, haptic, or auditory signal by the controller **700** and/or applicator **400**. This feedback loop is of particular significance during the injection of the medicament. By monitoring the position of the device and the state of the electrodes, the feedback loop between the force contact circuit and electrode resistance/impedance monitor, the system may detect if the electrodes (and therefore the injection needle) are no longer in the subject, allowing system halt operation of the injection drive mechanism **456** to cease depressing the reservoir plunger **484** and terminating the injection of the medicament. While this embodiment is most readily implemented with a motor driven injection drive **456**, other variations can be implemented in the case of manually operated or spring drive mechanisms, wherein the activation of mechanical interlocks can be used to halt actuation of the reservoir plunger following detection of a fault condition. This feature can be particularly useful in stopping the HBV vaccine from inadvertently spraying out into the environment when the applicator **400** is removed from the tissue of the subject prior to completion of a medicament delivery. For medicaments or therapeutic agents that are potentially hazardous for exposure to users or the environment, such a design may avoid inadvertent discharges / exposures.

Since the quantity of the dose delivered to the subject in a partial dose situation can be critical to inform the decision making of the clinician regarding further treatment, it is preferable that the injection drive mechanism **456** include appropriate sensor and control features to determine the position of the injection drive plunger **484** at the time the injection stroke was halted due to the detection of a fault condition or other circumstance in which halting the injection stroke is necessary. Preferably this is achieved through monitoring of the revolution count of the motor used to drive the injection drive plunger **484**, but other methods for monitoring the position of the injection drive plunger **484** including optical or electrical sensors can be employed. Based on the known dimensions of the injection drive plunger **484**, the depth of insertion, and the reservoir **101**, the use of appropriate logic and control circuits can translate the position of the injection drive plunger into an estimate of the volume of medicament

remaining in the syringe at the point at which the injection stroke was terminated. Such information can be conveyed to the user via the display **712**.

In addition to providing a termination feature to terminate the injection stroke in the event that a fault condition is detected, the use of a motor injection drive **456** can also provide a supplementary detector for detection of a fault condition or other operational issue. Specifically, the incorporation of appropriate measurement and logic circuits to monitor the current drawn by the motor during the injection stroke can be used to confirm that the injection has been administered within defined specifications. Expected ranges for the current drawn by the motor can be established for the various stages of the injection stroke including the initial run up of the injection drive plunger **484** before it contacts the plunger stopper **159**, the initial interface between the injection drive plunger **484** and the plunger stopper **159**, actuation of plunger stopper **159** forward in the barrel of the reservoir **101**, and conclusion of the injection stroke as the plunger stopper **159** contacts the end of the barrel of reservoir **101**. By correlating the position of the injection drive plunger **484** with the measured current drawn by the motor to the expected values during each phase of the injection of the agent, the system is capable of identifying potential fault conditions and conveying them to the user. For example, if the user inadvertently inserted a reservoir **101** in which the plunger stopper **159** had been partially actuated (and therefore did not contain the full intended dose of medicament) into cartridge **100**, the system could detect that the expected increase in current drawn by the motor injection drive **456** did not occur when the injection drive plunger **484** reached the outer tolerance for plunger positioning. Under this circumstance the system could terminate the administration procedure and notify the user of the detected fault. Additional fault conditions including (but not limited to) faulty components in the applicator **400**, breakage of the reservoir **101**, and/or a defective cartridge **100** would be potentially detectable via this method.

Another ‘interlock’ to facilitate proper execution of the administration procedure is provided by the alignment guide / splay shield **108**, which is illustrated in **FIG. 9C**. In this figure, the alignment guide / splay shield **108** is shown with splay features **168** and a hole **170** defined therein for slidable movement of a stick shield **134**. The stick shield **134** is illustrated in **FIG. 9B**, and electrode holes **167** are also illustrated. For transcutaneous insertion of electrodes and, where relevant, injection needles, having a consistent skin interface facilitates deployment of electrodes into the target tissue while maintaining the desired spatial relationship between the

members of the plurality. In particular, proper deployment is most consistently achieved when the skin is positioned perpendicularly to the direction of deployment. In addition, misalignment of the electrodes and injection needle are reduced when the skin is placed into tension in the orientation perpendicular to the direction of deployment. As can be seen, the splay shield **108** includes mechanical features including ribs and edges to engage with the skin and place it into tension perpendicular to the direction of deployment. Combined with the force contact circuit pick up system described above which ensures a consistent force applied to the skin, the alignment guide / splay shield **108** ensures that the skin is oriented and placed under tension in the direction perpendicular to the direction of electrode deployment. While this embodiment utilizes mechanical rib features to engage the skin at the device interface, numerous other designs and features could be utilized for engagement including the use of alternative materials with a high coefficient of friction when placed in contact with skin (e.g., rubber insets, adhesive patches, and the like) or alternative mechanical features including molded textures, cut outs, and sawtooth features capable of placing the skin into tension.

As can be seen, the alignment guide / splay shield **108** has a preferential direction defined. As described above, spatial and temporal co-localization of the medicament distribution and electric field application are desirable. Notably, the inherent structural properties of skeletal muscle lead to a characteristic ellipsoid distribution pattern of intramuscular injections where the major axis of the ellipsoid aligns with the striations of the muscle fibers. For applications involving intramuscular injections, it is therefore favorable to arrange the electrodes to generate an ellipsoid electric field profile. In order to ensure that the electrode array and resultant electric field profile are properly oriented relative to the striations of the target skeletal muscle, the use of an alignment guiding feature is of particular utility for intramuscular administration. The objective of the alignment guiding feature is to facilitate placement of the device such that the orientation of the electrode array is most favorable relative to the muscle striations and the resulting medicament distribution following injection. For an arm injection, the alignment guide / splay shield **108** would desirably wrap around the arm horizontally like an arm band. A similar orientation would be desired for the leg with the splay shield wrapping around the leg horizontally. It has been found that having the alignment guide / splay shield **108** configured in this manner results in >98% accurate medicament deliveries by users even in the absence of verbal instructions. The preferred direction shown and resulting skin placement is particularly

useful for intramuscular injections, because the diamond shaped array of electrodes (see the array of distal ends of electrodes **137** in **FIG. 9A**, which roughly matches the shape of the stick shield **134** and its associated holes **167**) is then oriented properly to deliver a medicament and to cause electroporation of the medicament along the preferred muscular striation direction. A primary feature is that, when aligned properly, the skin should be flush with the skin interface where the orifice is located, e.g., where the needle emerges. In this way, a consistent interface to the skin is obtained.

Where the alignment relative to the target muscle is improper, the arc generally causes a visually evident gap, e.g., 2-5 mm, to occur between the alignment guide / splay shield **108** and the skin. The visually evident gap can be employed as a reminder to the user to reorient the applicator **400**. The applicator **400** can be less stable against the skin of the subject. Alignment guide features wherein the distance between edges of the “wings” of the feature are at least 1.3 times the vertical height of the feature are preferable for facilitating proper placement. In addition, in some cases the design of the feature is such that the tissue interface can be placed flush against the skin in the desired orientation while exhibiting at least a 2 mm air gap when placed at a 90 degree angle relative to the desired orientation.

Variations on the design of the alignment guide are to be understood by a skilled person in the art. For example, instead of an arc shaped alignment guide / splay shield **108**, a “V” shaped one can be used. Other variations are also to be understood with the key characteristic being that the device can be placed directly against the skin in the desired orientation whereas a visually apparent gap between the tissue interface and the skin of the recipient of 2 mm or greater is present when the device is misaligned relative to the striations of the target muscle.

As noted above, the orientation of the alignment guide / splay shield **108** is related in some implementations to the shape of the electrode array, because the diamond symmetry of the electrode array has a certain distribution associated with it, and this distribution should bear a certain predetermined relationship to the shape of the alignment guide / splay shield **108**. Referring to **FIGS. 9A-9B**, the electrodes, and more particularly the distal portions **137**, are shown, which are four in number. The electrodes are positioned in a diamond shaped array, which can mean they have second order symmetry, i.e., are twofold symmetric, in that they can be rotated and at two different positions appear the same. The electrodes extend in this array from holes **167** defined in the stick shield **134**. The use of a diamond shaped array is particularly

useful because it is generally desired for intramuscular injections as discussed above. To reiterate, the second order symmetric array leads to a second order symmetric applied electric field. This type of applied electric field can have a preferred direction along the striations of the muscles if the alignment guide / splay shield **108** is oriented properly. Thus the alignment guide / splay shield **108** and the second order electric field orientation work together in a synergistic fashion to accomplish medicament propagation along the direction of muscle striation.

Broadly speaking, in a diamond shape, or other second-order symmetric shape, there is generally a major (long) axis and a minor (short) axis. The axes can bear a predetermined relationship with a preferred axis of the alignment guide / splay shield **108**. For example, if the alignment guide / splay shield **108** is thought of as having wings, with the arcuate shape of the wings encircling the arm, then a line segment connecting the center of the wings can be perpendicular to the major axis of the diamond shaped array.

Variations are to be understood by a skilled person in the art. For example, while a diamond is one possible shape for an electrode array, another exemplary shape is a rectangle, which is also second-order symmetric. The electrodes can be placed into the appropriate positions but can be moved to within a predetermined or desired tolerance, e.g., within 5%, 10%, and so on. Electrodes can be in various other shapes so long as they create a second order rotationally symmetrical electric field.

In another variation, for non-intramuscular injections, other order symmetries can be used. For example, for skin, there is generally no preferred direction of medicament propagation and so even a circular array of electrodes can be employed for intradermal injections.

Other considerations of electrode arrays are as follows. The simplest array configuration comprises two electrodes connected to the opposite poles of the electrical energy source. As disclosed in U.S. patents 5,873,549 and 6,041,252 (incorporated herein by reference in their entirety), utilization of three or more simultaneously active electrodes arranged in a multi-element array can be used to increase target volumes of tissue and improve the uniformity in electrical field propagation within the target tissue. A wide range of geometrical electrode arrangements and activation patterns have been developed for electric field application in tissue. These include electrodes arranged in linear, rectangular, circular, or triangular configurations capable of propagating electrical fields in a volume of tissue of roughly ellipsoid, cuboid, cylindroid, or spheroid shape. Most commonly, the electrodes are arranged parallel to one

another and configured for transcutaneous insertion in an orientation substantially perpendicular to the skin surface. In order to ensure that the target volume and shape of tissue is affected by the application of electrical field, it is desirable that the intended spatial relationship between each of the electrodes within the array is achieved following transcutaneous insertion. Specifically, unintended changes in the inter-electrode spacing should be avoided as they can cause changes in the magnitude of the electrical fields propagated within the target tissue, potentially leading to negative consequences for the safety and/or efficacy of the procedure.

Another interlock involves use of an exterior cartridge cap **110**. In particular, the alignment guide / splay shield **108** is covered while stored with an exterior cartridge cap **110**. The exterior cartridge cap **110** is configured to not just generally protect the distal end of the device until use but also to serve as an interlock feature itself. While protection of the distal end of the cartridge assembly **100** serves the purpose of protecting the needle and electrodes from the environment, a commonly-encountered problem is that users often forget to remove such caps. One solution is to make the cap a bright color that is different from the color(s) of the other components in cartridge **100**, so as to notify the user of its presence and thus remind the user to remove it. Another part of this solution is to include an extension or reminder tab **190**, as shown by the arcuate section adjacent a rectangular section, the arcuate section visible even when the cartridge assembly **100** is placed up against a subject. As this reminder tab **190** is visible, it can serve as a reminder to remove the exterior cartridge cap **110** even when the remainder of the exterior cartridge cap **110** is not visible.

Referring in more detail to **FIGS. 10A-10B**, the exterior cartridge cap **110** includes an outer surface facing distally, towards the subject, and an inner surface facing the alignment guide / splay shield **108**. On the inner surface are provided a number of hooks **176** which engage a corresponding wall **180** defined on the alignment guide / splay shield **108**. The hooks hold the exterior cartridge cap **110** in place.

However, if the exterior cartridge cap **110** were inadvertently left in place, and the applicator **400** pressed up against an insertion site with force, the force of the alignment guide / splay shield **108** against the exterior cartridge cap **110** may tend to cause the exterior cartridge cap **110** to “pop off” by being pushed away from its hooked position by the alignment guide / splay shield **108**. However, chamfered surfaces **178** are provided on the hooks **176** which tend to act against the stick shield **134** when pressure is applied, causing the hooks **176** to splay outward,

increasing their retaining force against the alignment guide / splay shield **108**, and preventing the exterior cartridge cap **110** from popping off. In addition, the chamfered surface acts further against the stick shield, preventing the alignment guide / splay shield **108** from moving relative to the stick shield **134**. If the alignment guide / splay shield **108** cannot move relative to the stick shield **134**, the force detection interlock cannot be deactivated, as the force contact pickup **128** cannot move to the third force contact point discussed above, where full pressure is detected (or for that matter even to the second force contact point). The controller can provide a report when this happens to the controller. For example, a user interface indication such as “You need to remove outer cartridge cap.” can be displayed, the same caused by detection of a trigger pull performed in the absence of adequate force.

While certain interlocks have been described above, more or less interlocks can be provided in any given implementation. For example, other ways can be employed for force detection and use as triggering signals for interlocks. Other types of interlocks may also be employed, including trigger locks, safety switches, and the like. Yet other types are to be understood by one of ordinary skill in the art, given this teaching.

Once a depth of insertion is affirmatively selected by the user, and the exterior cartridge cap **110** is removed, and the proper force is detected by the force interlock, activation of the trigger causes clockwise or counterclockwise rotation of the cartridge lock ring **114**, with the direction of rotation dependent on the depth of insertion selected by the user. The rotation causes the retaining posts **488** to move into the channels of selected depth, which in turn causes the needle and electrodes to deploy. In particular, the inner cartridge **103**, cartridge breech **112**, reservoir **101**, inner cartridge cap **104**, needle hub **152**, needle **105**, electrodes **122**, and associated elements to move forward under the influence of the electrode / needle insertion spring **472**. In the present embodiment, these elements are rigidly connected to each other and thus move forward as a unit.

To further minimize the risk of sharps injuries to the user, it is preferable that the cartridge assembly also incorporates a mechanism for sheathing the electrodes and any injection needles following their removal from the recipients' tissue. This can be accomplished through the incorporation of a stick shield feature that houses the electrodes and injection needle (if present) prior to their use and then extends over the electrodes and injection needle (if present) following their deployment and removal from the tissue of the subject. Commonly, the tissue

contact interface comprises the distal surface of the shield feature. Although the use of manually operated shield features can be considered, preferably, the device incorporates a mechanism to automatically extend the shield feature over the electrodes and further to engage a locking feature to maintain the shield feature in the extended state once it has been removed from the tissue of the recipient. Examples include a shield feature slidably engaged with the cartridge outer housing and operatively connected to a source of stored energy, such as a spring, which slides the shield forward upon withdrawal of the electrodes from the recipients' tissue. Alternatively, the mechanism can be contained within the applicator and configured to reverse the electrode deployment step, thereby retracting the electrodes and any associated injection needles behind the tissue contact interface. This can be accomplished using simple electromechanical mechanisms for linear motion such as motors or solenoids.

In a particular implementation, and referring back to **FIG. 4** as well as to **FIGS. 11A** and **11C**, the stick shield **134** is configured to stay in position while the medicament delivery happens, but the movement forward of the inner cartridge **103** and other associated components during medicament delivery causes compression of a stick shield spring **138**. This compressed stick shield spring **138** then relaxes as the applicator is removed from the subject, as the stick shield **134** is caused to move forward when the applicator **400** is removed from the subject, covering the needle and electrodes, and protecting the subject and others against the uncovered sharps. Notably, this feature also prevents the visualization of the sharps features during withdrawal, which can facilitate acceptance from subjects experiencing anxiety related to needles.

While the relaxed spring would otherwise be capable of recompressing, and in particular if the user moved the stick shield **134** proximally, it is prevented from doing so by the action of stick shield support arm **132**, which are mounted to the interior of the outer cartridge **102** and which serve a ratcheting function as the stick shield **134** moves forward. In particular, and referring to **FIGS. 11A** and **11C**, stick shield support arms **132** move over various sequential retaining walls, and can do so easily when the stick shield **134** is moving distally. However, in the proximal direction, the stick shield **134** is prevented from moving because the stick shield support arms **132** abut the retaining walls in this direction, in a ratcheting fashion, and do not allow passage.

An initial set of retaining walls **188** prevent proximal movement of the stick shield **134** prior to use of the cartridge assembly **100**. A set of first depth retaining walls **184** prevent proximal movement of the stick shield **134** after discharge at a first selected depth. A set of second depth retaining walls **186** prevent proximal movement of the stick shield **134** after discharge at a second selected depth. The stick shield **134** is prevented from complete removal from the cartridge assembly **100** by the action of the stick shield retaining hooks **182** acting against the inner cartridge **103**.

Variations are to be understood by a skilled person in the art. For example, in one implementation the stick shield support arms **132** can be provided by stamped metal support arms that interface with the outer cartridge **102**. In some embodiments, the support arm features are directly integrated as injection molded plastic features in the outer cartridge cap **106** and/or alignment guide / splay shield **108**. The specific material should be selected to achieve sufficient rigidity to provide support to the elongate electrodes while retaining sufficient elasticity to prevent fracture of the component under load. Material selection should also consider the intended method of sterilization for the electrode array (e.g., gamma radiation, steam sterilization, ethylene oxide, or e-beam) to ensure that the features retain adequate material properties following sterilization. In an exemplary embodiment, the features, as implemented, should be capable of holding the stick shield **134** against proximal movement when at least 5 N, but more preferably at least 15 N of force applied. Other ways of providing a ratcheted function to prevent proximal movement of the stick shield may also be employed including a gear rack implemented on stick shield **134** and a corresponding ratchet feature implemented in outer cartridge cap **106**.

Referring to **FIG. 4**, each elongate electrode **122** within the array comprises a distal portion **137** and a proximal portion **135** that are in conductive communication. The distal portion is configured for tissue penetration and electric field propagation in tissue and the proximal portion is configured with a conductive contact region capable of reliably achieving conductive communication with the electrical connections contained within the applicator with a suitable source of electrical energy. The source of electrical energy can, by temporal variation in the power applied to individual electrodes, cause a spatially and temporally varying electric field within the body of the subject, generally confined to a volume around the region of medicament distribution, and the same can be used to advantage in electroporation therapy.

The relevant characteristics of the electrodes include shape, diameter, tip profile, length, material composition, and conductivity, the specifics of which are selected based on the intended application. Most commonly, the electrodes comprise electrically conductive elongate rods with a curvilinear cross section with diameter 0.1 - 1.5 mm. The electrodes can be solid core or hollow. Most commonly hollow electrodes incorporate one or more orifices and an operative connection to a fluid reservoir, thereby allowing for the administration of the agent of interest or other associated medicaments including anesthetics, surfactants, proteins, adjuvants, or enhancers from the electrode itself. Depending on the application, appropriate metallic electrode materials include, but are not limited to titanium, gold, silver, aluminum, copper, tantalum, tungsten, molybdenum, tungsten, stainless steel, MP35N and alloys thereof. Electrodes may also be comprised of electrically conductive ceramics or plastics. To minimize unwanted electrochemical reactions at the tissue electrode interface, it is often desirable that one or more of the electrodes are coated in a conductive material providing improved electrochemical stability compared to the electrode material itself. Such coating materials include, but are not limited to, platinum, iridium, palladium, osmium, gold, silver, titanium, and alloys thereof.

As described above, the tip of the distal portion is configured for tissue penetration. Common tip profiles include, but are not limited to, trocar, bevel, cone, blade, lance, and taper. For transcutaneous electrode deployment, tip profiles with one or more cutting edges are preferred. For certain applications where the penetrating tip profile can be undesirable for generating the required electrical fields, the tip can be comprised of a non-conductive material fixed to the distal tip of the electrode or the penetrating tip can be covered in an adherent coating or tubing comprised of a biocompatible electrically insulating material such as poly(p-xylylene) polymer, polyolefin, polyvinyl chloride, polyurethane, polyester, polyimide, silicone rubber, thermoplastic elastomer/rubber, ethylene tetrafluoroethylene, fluorinated ethylene propylene, and/or perfluoroalkoxy plastic.

Proximal to the penetrating tip are one or more conductive regions configured for the propagation of electrical fields in tissue. In order to confine the propagation of electrical fields to the target region of tissue, commonly, especially for subcutaneous and intramuscular administration, at least a portion of the electrode length configured for penetration in the tissue is covered in an adherent coating or tubing comprised of a biocompatible electrically insulating material such as poly(p-xylylene) polymer, polyolefin, polyvinyl chloride, polyurethane,

polyester, polyimide, silicone rubber, thermoplastic elastomer/rubber, ethylene tetrafluoroethylene, fluorinated ethylene propylene, and/or perfluoroalkoxy plastic. In this way, electrodes can be configured to only activate at a particular depth within the tissue.

Collectively, the number of electrodes and their inter-electrode distance as well as the penetration depth of the electrodes and the length of their conductive region define the volume of tissue in which the electric fields is applied. These parameters are selected based on the specific objectives for the administration procedure, including target tissue site as well as the volume, dose, and viscosity of the agent to be delivered as well as the variation in skin and subcutaneous tissue thickness in the intended recipient population. Generally, for intradermal administration in human recipients, arrays comprise 2-16 electrodes of diameter 0.2 - 0.7 mm, an inter-electrode distance of 2-8 mm, a depth of electrode penetration of 0.5 - 4 mm, and a conductive length of 0.5-4 mm. Generally, for subcutaneous administration in human recipients, arrays comprise 2-8 electrodes of diameter 0.3-0.8 mm, an inter-electrode distance of 4-10 mm, a depth of electrode penetration of 5-15 mm, and a conductive length of 2-8 mm. Generally for intramuscular administration in human recipients, arrays comprise 2-8 electrodes of diameter 0.3 - 1.2 mm, with an inter-electrode distance of 4-12 mm, a depth of electrode penetration of 10-60 mm, and a conductive length of 2 - 20 mm.

In order to avoid undesirable visualization and exposure to the electrodes during the usage of the device, the cartridge assembly **100** is preferably configured such that the electrodes **122** are recessed within the device prior to their insertion into the tissue of the recipient. Most commonly, this is accomplished by providing the outer housing structure **102** slidably engaged with the electrode mount support, which in one implementation comprises an inner cartridge **103** having seams formed therein in which the electrodes are disposed. Prior to use, the electrodes are recessed within the outer housing **102**. During use, the sliding engagement of the electrode mount structure, e.g., the inner cartridge **103**, with the outer housing allows the electrodes **122** to slide forward relative to the distal tip of the outer housing **102**, thereby deploying the electrodes **122** from the outer housing **102**. The length of the sliding engagement between the electrode mount structure and the outer housing corresponds to the maximum desired depth of electrode penetration for the given application.

As can be seen in **FIG. 4**, and the electrodes **122** have a proximal portion **135** and a distal portion **137**. The proximal portion is separated from the distal portion by a shoulder or bend **139**.

The shoulder or bend **139** is secured by and between the inner cartridge cap **104** and the inner cartridge **103**. The shoulder or bend **139** provides a number of functions. First, while the electrodes at the distal end of the cartridge assembly **100** are required to form an array of a certain size and shape as described above, putting the electrodes **122** in the desired array size and shape at the proximal end of the cartridge assembly **100** is impractical due to the presence of the reservoir **101**. In other words, the electrodes **122** have to bend "out-of-the-way" to make room for the reservoir **101**. In addition, having the bend, particularly when the bend is locked in place frictionally between the inner cartridge **103** and the inner cartridge cap **104**, provides a surface for the electrodes **122** to abut against when the force of the needle and electrodes insertion recoils in the distal direction as the electrodes **122** interact with the tissue during their deployment. The bend, can resistant to axial rotations, which is beneficial so that the electrodes do not exhibit significant torsional movement and rotate away from their electrical contact pads **130** (described below). In addition, in contrast to prior art techniques in which multiple electrodes interfaced with axially separated coaxial rings, requiring a different configuration for each electrode, the present system allows a common electrode type to be used for all four electrodes.

Systems and methods according to present principles provide for the propagation of an electrical field in the skin, subcutaneous tissue, and/or skeletal muscle of a recipient which facilitates the intracellular delivery of an HBV vaccine within said recipient's tissue. In this aspect, the apparatus includes two or more elongate electrodes **122** arranged in a predetermined spatial relationship and configured for deployment into the target tissue in which the agent of interest has been or is to be distributed. The desired enhancement in agent activity is contingent on achieving co-localization of the site of agent distribution with the propagation of electrical fields of sufficient magnitude to induce the desired physiological effects. Therefore it is desirable that the apparatus consistently achieve electrode deployment to the target depth and with specified intraelectrode spacing. Specifically, this ensures that the electrical fields are propagated at the proper tissue site, and, since the magnitude of the electrical fields propagated within the tissue is a function of the intraelectrode spacing, a safe and effective electric field intensity. However, the variation in the thickness, density, and composition of skin and subcutaneous tissue between sites in a given recipient and across recipient populations can lead to significant variation in electrode deployment characteristics. For example, recipients with increased

thickness of skin are at increased susceptibility for electrode distortion and/or insufficient depth of penetration which can affect co-localization with the site of agent distribution.

Systems and methods according to present principles further facilitate consistent transcutaneous insertion of electrode arrays comprising 2-16 electrodes of diameter 0.2 - 1.3 mm with an inter-electrode distance of 2-12 mm, and a depth of electrode penetration of 0.5 - 60 mm in recipients with skin of heterogeneous thickness, composition, and condition. In order to permit transcutaneous insertion, tissue penetrating electrodes are most commonly elongate and configured with a tissue penetrating tip and a tissue contact region in the distal portion and an electrical contact region in the proximal portion. Given their elongate configuration, they are susceptible to bowing, bending, and/or buckling (collectively termed distortion) when subjected to the compression forces generated during transcutaneous insertion into the recipient. Such distortions during electrode insertion are undesirable as they can lead to improper electrode insertion characterized by insufficient depth of penetration and/or excessive changes in intraelectrode spacing. Of note, humans and animals exhibit significant intra- and inter-species differences in the thickness, composition, and condition of skin, any of which can have significant impact on the forces which the electrodes are subjected to during transcutaneous insertion. In some embodiments, devices for transcutaneous insertion of elongate electrodes are designed to withstand the forces present under the most stringent conditions of electrode insertion that is to be encountered in the target population of recipients. The occurrence of electrode distortion can be partially mitigated by electrode material selection as well as reducing the length and increasing the diameter of the electrodes within the array. However, material selection can be constrained by issues of performance, cost, manufacturability, and biocompatibility. In addition, electrodes of reduced length may preclude adequate coverage of a target population with heterogeneous tissue thickness while larger diameter electrodes are associated with increased discomfort and tissue trauma. Thus, the disclosure is designed to provide methods and apparatus to facilitate the transcutaneous insertion of electrodes independent of these variables.

In a first embodiment of the disclosure the subassembly cartridge housing the electrode array incorporates one or more support dynamic support members in physical contact with one of the electrodes during tissue insertion and configured to constrain movement of the electrodes perpendicular to the direction of insertion and maintain the desired spatial relationship between

the electrodes. For example, and referring to **FIG. 12**, an electrode support feature **124** is illustrated in which holes **192** are provided for electrodes and a hole **194** is provided for passage of the needle. The tendency of the elongate electrodes to distort or buckle under load is exacerbated by any bending, eccentricity, or bowing of the electrode that may have been introduced during manufacture or assembly. Thus, it is advantageous to employ a device such as the electrode support feature **124** to constrain the electrodes to prevent, or correct, any bending, eccentricity, or bowing they might exhibit at rest, and to prevent unwanted perpendicular motion of the electrode as a result of the loading that occurs during deployment into tissue.

In the context of the disclosure, a dynamic support member is defined as a structural element which provides sliding engagement with the elongate electrodes during insertion into the tissue of the recipient and which is configured to undergo a change in position, size, and/or conformation during electrode insertion in order to maintain the desired spatial relationship of the electrodes as they are deployed to the target tissue depth. Since the electrodes are subjected to the largest loading forces at the initial contact with the skin, the engagement of the dynamic support member with the electrodes comprising the array is preferably affected prior to initial contact with the tissue of the recipient and to continue to provide support as the electrodes deploy to the full depth of penetration. It is also favorable that the dynamic support member be designed to provide support to the electrodes and injection needle while minimizing losses in electrode penetration force due to friction.

While the primary function of the dynamic support member is to maintain the desired spatial relationship of the plurality of the electrodes within the array relative to one another and to the injection needle, it is also desirable for the design of the dynamic support member to include features capable of stabilizing the array as a whole. This is preferably accomplished by providing additional structural support features which constrain lateral movement of the support member. In a certain embodiment, these support features are integrated into a sharps protection shield, which also serves to protect the user against accidental exposure to the electrodes and/or injection needle. Disclosed below are various embodiments of dynamic support members consistent with the disclosure.

An embodiment of the disclosure, described above as electrode support feature **124**, involves the use of a planar support structure **124** positioned perpendicularly relative to the elongate orientation of the electrodes. The planar support structure is configured with one or

more apertures **192** which correspond to the positions of said electrodes in their specified spatial relationship. The size, shape, and position of the apertures are configured to allow the support structure to slide smoothly along the elongate length of the electrodes within the array while constraining unwanted motion perpendicular to the direction of electrode deployment. Commonly, the apertures can be configured as holes or slots **192** in the planar structure with adequate clearance for the electrode (at least 10% larger than the largest cross section of the electrode, including any coatings or other adherent materials). However, if more substantial support is required for specific electrodes, one or more of the apertures may comprise tubular structures **196** arranged perpendicularly to the planar support structure. Apertures comprising such tubular structures increase the surface area contacting the electrode and thereby increase the support provided to the elongate electrodes. The planar support structure can be made from any material with appropriate structural characteristics including metal, polymer, ceramic, or composite materials and can be formed, machined, molded, or produced with other methods. To avoid unwanted electrical interactions with the electrodes, it is preferable that the interface between the electrodes and the planar support structure is not electrically conductive. The material and manufacturing method should also be selected to minimize the amount of friction at the interface between the electrodes and the dynamic support member. Due to a number of factors including their low cost, ease of manufacturability, and favorable electrical properties, the electrode support structure is commonly made of a thermoplastic such as polycarbonate, polystyrene, polypropylene, acrylic, or polyethylene. The specific material should be selected to achieve sufficient rigidity to provide support to the elongate electrodes while retaining sufficient elasticity to prevent fracture of the component under load. Material selection should also consider the intended method of sterilization for the electrode array (e.g., gamma radiation, steam sterilization, ethylene oxide, or e-beam) to ensure that the dynamic support member is compatible. The specific dimensions and design of the support structure depend on the properties of the selected material. However, it is desirable to minimize the dimensions of the support structure so that it does not excessively limit the distance that the electrodes can be deployed or to interfere with other functional properties of the device. Rigid planar structures of 0.5 mm – 2 mm thickness are typically sufficient

Another embodiment of a dynamic support member is the use of a compression spring with apertures accommodating the electrodes and constraining their lateral movement. The

compression spring can be made from metal, polymer, or elastomeric materials, and can be formed, machined, molded, or produced with other methods. At rest, the spring is uncompressed or minimally compressed with the electrodes inserted into the apertures along the spring's length. As the electrodes are deployed, the spring compresses in the direction of deployment, with the apertures accommodating the sliding movement of the electrodes perpendicular to the spring coils. This embodiment is of particular utility when combined with a shield or sheath used to house the penetrating electrodes / needles following removal from the tissue site. In these embodiments, the force imparted to the spring by the forward deployment of the electrodes can be used to deploy a sheath or shield over the electrodes as the device is removed from the tissue.

In the implementation of **FIG. 4**, the stick shield spring **138** can be used to partially support the electrode support **124**, and in particular the radius of the spring can be configured to match (or be just slightly greater than) the radius of the wall **198** of the electrode support **124**. In this way, the electrode support **124** can be inserted into the middle of the stick shield spring **138** during use. The electrode support **124** may then slide within the interior of the stick shield **134**. By being placed in the center of the spring, the electrode support **124** naturally maintains a position in the center of the spring, which provides a desired "halfway point" for support of the electrodes, roughly halfway between their point of support at the inner cartridge cap **104** and their point of penetration at the tissue interface.

Other spring-based electrode supports are contemplated. For example, a formed compression spring is positioned in the region of the electrodes, to provide adaptive electrode support. Formed compression springs can be shaped and proportioned to conform closely to the relative positions of the electrodes, in order to restrict their lateral motion. An optional biasing element can be positioned in conjunction with a formed compression spring, and may serve to bias the electrodes outward against formed compression spring. Formed compression springs can be made from metal, polymer, or elastomer materials, and can be formed, machined, molded, or produced with other methods. The biasing element can be made from metal, polymer, or elastomer materials, and can be formed, machined, molded, or produced with other methods.

Electrode supports based on other mechanisms are also contemplated, for example telescoping tubes. Telescoping tubes serve to support electrodes during insertion into the subject. The telescoping tubes can be sized to move freely relative to each other, or can be sized to move only when an axial force is applied to them.

Other support structures are contemplated, for example support structures based on movable, flexible, or pivoting support members. Lateral support members can attach to the electrodes at optional hinge features. Lateral support members can be formed integrally with the structure housing the electrodes or can be separate components attached by conventional methods (snaps, welding, adhesives, fasteners, etc.).

Another embodiment is the use of a compressible matrix material in which the electrodes are embedded. As the electrodes are deployed, the material compresses in the direction of deployment, providing lateral support along the direction of travel. Examples of compressible matrix materials include cellulose, foamed plastic or rubber polymers such as microcellular plastics, foamed silicone or foamed polychloroprene, or carbon foam matrices. Since the materials are designed to contact the electrodes and/or injection needle (if applicable), the materials should be selected to be compatible with indirect tissue contact.

The above described structures thus support transcutaneous deployment of a plurality of elongate electrodes at tissue depths of up to 60 mm while maintaining the desired spatial relationship among the plurality of electrodes and, in specific embodiments, the orifice of a hypodermic injection needle. Such support members engage the plurality of elongate electrodes during transcutaneous insertion, and constraining deflection of the electrodes in one or more directions perpendicular to their elongate orientation. Another aspect of the disclosure provides methods and apparatus for utilizing the application of biocompatible lubricious compounds to the surfaces of the plurality of electrodes in order to reduce the applied force required to achieve consistent transcutaneous deployment of a plurality of elongate electrodes to depths of up to 60 mm. In an exemplary embodiment, biocompatible silicone compounds such as Dow Corning 360 Medical Fluid or Dow Corning MDX4-4159 can be applied by conventional spray or dip coating to the plurality of the electrodes comprising the array in order to improve the insertion characteristics of the electrodes. The specific selection of the coating and application conditions, such as coating method and thickness depends on the number, size, composition, and tip configuration of the electrodes as well as the target tissue in which the electrodes are deployed.

Referring to **FIGS. 4** and **7A-7B**, the proximal portions **135** of the electrodes **122** can be positioned on the exterior of the inner cartridge **103** of the cartridge assembly **100** (**FIG. 7B**). In this way, when the inner cartridge **103** is slidably disposed within the outer cartridge **102** (**FIG. 7A**), the proximal portions **135** contact corresponding electrode contacts **130**, and in particular

outer cartridge interior portions **133** of the electrode contacts **130**, these interior portions **133** configured for power communication with the proximal portions **135**. Due to the length of the proximal portions **135**, and the length of the outer cartridge interior portions **133**, the electrical communication can be made at a number of locations along their continuous interface, no matter the longitudinal position of the inner cartridge with respect to the outer cartridge. The electrode contacts **130** further include outer cartridge exterior contacts **131**, configured for power communication with corresponding connections **496** on the applicator **400** (see **FIG. 18C**). The electrode contacts **130** are comprised of a conductive material sufficient to convey an electrical signal. In certain embodiments, the design and material selection ensure that the contact provide for adequate engagement to ensure an electrically conductive interface with the corresponding electrode over the range of expected manufacturing variation in electrode and contact position while not inhibiting or interfering with the forward travel of the electrodes **122** mounted on the inner cartridge **103**. The design must also permit this engagement to persist when exposed to the expected storage conditions over the labeled shelf life of the product. In a certain embodiment, electrical contacts **130** are made of stamped or formed metal with appropriate temper to facilitate engagement with the electrode and may include coatings such as gold or copper to ensure the integrity of the electrical contacts and avoid corrosion. In addition to ensuring that electrical contact can be maintained with the electrodes at a number of locations along their continuous interface, the incorporation of the electrode contacts into outer cartridge **102** in this configuration ensures that any wear due to the sliding interaction between the electrodes **122** and the electrode contacts **130** occurs within the cartridge **100** designed for single use, thereby allowing for a static interface between the outer cartridge contacts **131** and applicator electrical contacts **496** which minimizes potential mechanical wear on the electrode connections **496** of the applicator designed for multiple uses. This configuration has the benefit of extending the useful functional life of the multi-use applicator.

Remaining portions of the applicator **400** are now described. These portions are generally those that are independent of operation with the cartridge assembly **100**. Referring first to **FIG. 13A**, the applicator **400** includes a handle **402** and a multi-conductor cable **406** designed to carry power and control signals as well as the electrical signals to be applied to the tissue. The cable **406** is generally terminated in a connector with a corresponding connector interface in the controller **700**. The applicator **400** further includes a user interface **404**, in which aspects of the

procedure can be viewed by the user, and in which the user can direct the applicator to perform various functions, in particular, depth selection. The applicator **400** further includes a procedure activation trigger **407**, which is used by the subject to initiate the procedure.

Referring in addition to **FIG. 13B**, the applicator **400** includes a procedure countdown timer **410**, which informs the user of the remaining duration of the procedure, and further impliedly indicates to the user that they should not remove the applicator **400** from the subject until such time as the countdown trigger has counted down to zero. A power indicator **418** is provided to indicate a satisfactory and powered connection with the controller **700**. A procedure fault indicator **414** is provided to indicate to the user if a fault has occurred, e.g., one of the interlocks described above has not been deactivated. An application placement indicator **412** is provided to inform the user if a proper pressure has been obtained against the tissue of the subject, allowing the procedure to commence.

FIG. 14 indicates a number of structural components of the applicator **400**, including a connector **426** (not to scale) for connection to the controller **700**, a top housing **420**, side housings **422** and **424**, and an inner protective shell **432**. A front cap **430** is provided, along with an end cap **428**. Various electromechanical subassemblies **450** are also provided, several of which have been described above.

FIG. 15 illustrates a more detailed view of the applicator **400**, indicating cartridge pressure sensor contacts **434** and subassemblies **452** corresponding to the cartridge loading, electrode insertion, and injection functions as well as the associated sensors.

FIG. 16 illustrates a more detailed view of the group of subassemblies **452**, including the loading drives subassemblies **454** and the cartridge loading subassembly **456**. The operation of the subassemblies has been described above.

FIG. 17A illustrates a more detailed view of the loading drive subassembly **454**, the general to operation of which has been described above. Here it is noted that the loading is triggered by a flag on the cartridge assembly **100**, and that detection of the flag leads to the system being triggered at switch **464**. The loading drives subassembly is mounted to the applicator housing using brackets **466** and **468**. The action of the motor **444** is transmitted to the pinion gear assembly **448** by the motor drive shaft **462**.

FIG. 17B illustrates a more detailed view of the insertion/injection drive assembly **456**. Many of these components have been described above. Here it is noted that the operative

components are mounted to the applicator housing by a mounting bracket **476**. Following insertion of the electrodes **122** and the needle **105**, the plunger of the needle **105** is depressed by the injection drive plunger **484**, whose action ejects the medicament from the orifice of the needle. The injection drive plunger is driven by an injection drive motor and gear assembly **486**.

Generally the injection drive plunger, driven by the injection drive assembly **486**, moves forward until such point at which it can no longer move forward, i.e., distally, indicating that it has reached the end of each stroke. This indication is generally given by the current used in the injection drive motor **486** rising substantially, indicating that the reservoir plunger has reached the end of the reservoir and that the injection has been completed. However, in some cases, the number of turns of the motor can be employed to determine how much medicament has been delivered. Such a feature could be used to support metered dosing of medicament or in order to notify the user in cases where the volume of delivery was not within the expected total, e.g., where the full volume of reservoir **101** was expected to be injection, but based on the position of the plunger rod, it was not emptied. These situations may arise where the user failed to hold the applicator **400** against the body of the subject until the procedure was completed, e.g., until the countdown timer had counted down to zero. As noted above, in a mid-procedure timeframe, where the force against the subject is no longer being detected, an impedance check can be performed to determine if the electrodes are still within the subject. If they are not, then it can be presumed that the applicator was prematurely removed, and a signal can be sent to the injection drive motor **486** to cease injection, limiting the amount of medicament ejected outside of the subject.

Referring to **FIG. 19**, the controller system/assembly **700** can be seen including an electrical field controller / generator **750** and a handle **702** and an applicator cradle **706**. The controller can be configured for both table top as well as cart mounted use. In the cart mounted configuration it is the inclusion of a storage bin **704** is favorable. Details of the controller in the cart mounted configuration are shown in **FIGS. 20A-20D**, including wheel locks to secure the controller assembly against movement in an operational setting, a tray **710** for placing supplies including subject preparation supplies, reservoirs/vials/syringes, and so on. An applicator connector port **708** is illustrated to connect the applicator **400** to the controller assembly **700**. A display **712** displays status of an administration procedure, particularly with regard to the IFU attached.

A cartridge eject button **714** is provided to cause ejection of the cartridge assembly **100** from the applicator **400**. Menu navigation buttons **716** allow navigation and manipulation of components as seen on the display **712**. A mute button **718** is provided to mute alerts or other audible indicators if desired. A power button **722** is provided to power the unit, and the same is activatable if the main power switch **726** (**FIG. 20D**) has been turned on.

The display also includes a battery indicator **720**, which provides an indication of battery level where a battery backup system is provided. Such a battery backup system can be included in the controller/generator **750** to accommodate situations in which power loss prevents a main power source from powering the unit. Such may also be employed as a backup where a procedure has been started under main power, but where a main power loss has been encountered. In this case, logic and control circuitry is implemented to provide for essentially seamless transition from mains to battery power so that the procedure can be finished using the battery backup. It is favorable for the controller to include battery monitoring circuitry that is capable of monitoring whether the battery has sufficient charge to complete the procedure following loss of mains power. In some embodiments, the controller also includes display to notify the user in the event that the current charge status of the battery is not sufficient to complete the procedure in the event of mains power loss.

Referring to **FIG. 20D**, in which a rear view of the controller **750** is illustrated, the same can be seen to include a USB port **724**, a main power switch **726**, and a main power input **728**.

Referring to **FIG. 21**, in one method of use, as illustrated by the flowchart **800**, the controller/generator **750** is powered and its program automatically started (step 802). The applicator is connected (step 804), and an indication or instruction to the user to perform this action can be displayed on the display screen **712**, if the applicator has not already been connected. The system may perform a self test (step 806), the self test not only ensuring proper operation of the controller/generator **750** but also ensuring correct connection of the applicator **400** to the controller/generator **750**.

The program may cause the display screen to provide instructions to the user on preparation of the site of administration (step 808). This step may include ensuring that the correct medicament agent is being delivered, that the same is not expired, that contraindications have been reviewed, and that warnings/precautions have been followed.

The user then removes the reservoir cap and inserts the reservoir **101** in the cartridge assembly **100** (step 810). In some embodiments, the user experiences an audible, tactile, or haptic click (step 811) indicating proper placement of the reservoir in the cartridge assembly.

The user then inserts the cartridge assembly **100** into the applicator **400** (step 816). An error status is then tested for (step 818), e.g., for proper reservoir placement, and if one is detected, the procedure is stopped, an error message is displayed, and the user is instructed to take remedial action. If the error state can be corrected, e.g., the user has inserted the reservoir improperly but not engaged or closed the cartridge breech, then the user can be instructed to remove the cartridge and reinsert the reservoir properly (state 820). In some cases, the cartridge is automatically ejected, and in other cases the user may have to push the "cartridge eject" button to accomplish the same. In other error states, e.g., where the cartridge breech has been closed, the user can be instructed to use a new cartridge.

In any case, once the device set up is completed and a "no error" state has been achieved, the user can proceed to administration of the medicament and electroporation therapy (step 822).

The primary function of the controller is to generate the electrical fields required to achieve the desired delivery of the medicament, to control the operation of the system during set up and use, to monitor the state of the system during set up and use, and to convey the state of the system to the user during set up and use. In some embodiments, the controller is capable of providing recommendations and instructions for use of the device both in the context of user training as well as during resolution of fault conditions during ordinary usage.

The controller system and controller method of operation can be fully implemented utilizing any number of computing devices including microprocessors, microcontrollers, programmable logic controllers. Typically, instructions are laid out on computer readable media, generally non-transitory, and these instructions are sufficient to allow a processor in the computing device to implement the method of the disclosure. The computer readable medium can be a hard drive or solid state storage having instructions that, when run, are loaded into random access memory. Inputs to the application, e.g., from the plurality of users or from any one user, can be by any number of appropriate computer input devices. For example, users may employ a keypad, keyboard, mouse, touchscreen, joystick, trackpad, other pointing device, or any other such computer input device to input data relevant to the calculations. Data may also be input by way of an inserted memory chip, hard drive, flash drives, flash memory, optical

media, magnetic media, or any other type of file – storing medium. The outputs can be delivered to a user by way of a video graphics card or integrated graphics chipset coupled to a display that maybe seen by a user. Alternatively, the system may output one or more formats of electronic document or a printer can be employed to output hard copies of the results. Given this teaching, any number of other tangible outputs is also be understood to be contemplated by the disclosure. For example, outputs can be stored on a memory chip, hard drive, flash drives, flash memory, optical media, magnetic media, or any other type of output. It should also be noted that the disclosure can be implemented on any number of different types of computing devices, e.g., personal computers, laptop computers, notebook computers, net book computers, handheld computers, personal digital assistants, mobile phones, smart phones, tablet computers, and also on devices specifically designed for these purpose. In one implementation, a user of a smart phone or wi-fi – connected device downloads a copy of the application to their device from a server using a wireless Internet connection. An appropriate authentication procedure and secure transaction process may provide for payment to be made to the seller. The application may download over the mobile connection, or over the WiFi or other wireless network connection. The application may then be run by the user. Such a networked system may provide a suitable computing environment for an implementation in which a plurality of users provide separate inputs to the system and method. In the below system where control of an applicator is contemplated, the plural inputs may allow plural users to input relevant data at the same time.

TABLE OF ELEMENTS

REF #	PART
100	Cartridge assembly
101	Reservoir
102	Outer cartridge, aka housing
103	Inner cartridge
104	Inner cartridge cap
105	Needle
106	Outer cartridge cap
108	Alignment guide / splay feature
110	Exterior (safety) cartridge cap
112	Cartridge breech
114	Cartridge lock ring
116	Reservoir detection spring
118	Reservoir detection cap
120	Reservoir interlock, aka reservoir insertion trigger

122	Electrodes
124	Electrodes support
126	Force contact springs
128	Force contact pickup
130	Electrode contacts
131	Exterior Outer cartridge electrode portions for coupling to applicator
132	Stick shield supports
133	Interior Outer cartridge electrode portions for coupling to inner cartridge
134	Stick shield
135	Proximal Inner cartridge electrode portions for coupling to outer cartridge
137	Distal Inner cartridge electrode portions for tissue insertion
138	Force contact flexible circuit
139	Electrode shoulder or bend
140	Reservoir loading port
142	Reservoir containment volume
144 & 144'	Reservoir lockout holes (1 st or 2 nd set)
146	Optical line of sight
148	Insertion detector, e.g., emitter/collector IR sensor within applicator
150	Inner cartridge containment volume
152	Needle hub
154	Rack
156	Egress port
158	Reservoir cap
159	Plunger stopper
160	Flexible circuit
162	First set of pads
164	second set of pads
166	Stick Shield nubs
167	Stick shield holes
168	Splay feature
170	Alignment guide hole for stick shield
172	Initiating flag
174	Continuing flag
176	Exterior cartridge cap hooks
178	Exterior cartridge cap chamfer surfaces
180	Hook engaging wall of alignment guide / splay shield 108
182	Stick shield retaining hooks
184	First depth retaining wall
186	Second depth retaining wall
188	Initial or rest retaining wall
190	Reminder tab
192	Electrode support feature electrode holes
194	Electrode support feature needle hole
196	Electrode support feature electrode hole support structure

198	Electrode support feature wall
400	Applicator
401	Applicator cartridge assembly receiving port
402	Handle
403	Cartridge assembly receiving volume, which is defined by a housing
404	User interface
406	Multi-conductor cable
407	Trigger
408	Injection depth selection indicator(s)
409	Injection depth selection button(s) (could be toggle or other forms in another implementation)
410	Procedure countdown timer
412	Application placement indicator
414	Procedure fault indicator
416	Procedure complete indicator
418	Power indicator
420	top housing
422	First side housing
424	Second side housing
426	Electrical connector
428	End cap
430	Front cap
432	Inner protective shell
433	Electrical contacts for motor drive 444
434	Cartridge force sensor contacts
435	Connectors for switch
436	Cartridge loading sensor
438	Cartridge loaded sensor
440	Guide or track
442	Cartridge guide rails
444	Loading drive motor
446	Motor trigger connector
448	Pinion gear assembly
450	Electromechanical subassemblies
452	Cartridge loading, electrode insertion, and injection subassemblies
454	Loading drive subassembly
456	Cartridge loading subassembly
462	Motor drive shaft
464	System trigger switch
466	gear cover bracket
468	Mounting bracket
470	Spring cover / cartridge interface
471	Spring cover hole
472	Electrodes/needle insertion spring
474	Insertion gear bushing

476	Mounting bracket
478	Insertion mechanism gear drive ring
479	Insertion gear ring
480	Flag holder
481	Insertion mechanism flag
482	Insertion mechanism drive motor
483	Insertion mechanism position sensor
484	Injection drive plunger
486	Injection drive motor and gearing
488	Retaining posts (retaining feature)
490	Channels for first depth
491	Lock tabs
492	Channels for second depth
494	Abutment wall
496	Applicator electroporation electrode contacts
700	Controller Assembly
702	Handle
704	Storage bin
706	Applicator cradle
708	Applicator connector port
710	Tray
712	Display screen
714	Eject cartridge button
716	Menu navigation buttons
718	Mute button
720	Battery indicator
722	Power button
724	USB port
726	Main power switch
728	Main power port
750	Electrical Field Generator

EXAMPLES

The following examples of the invention are to further illustrate the nature of the invention. It should be understood that the following examples do not limit the invention and the scope of the invention is to be determined by the appended claims.

Example 1: Generation of HBV Core and Pol Antigen Sequences and Plasmid Optimization

T-cell epitopes on the hepatitis core protein are considered important for elimination of hepatitis B infection and hepatitis B viral proteins, such as polymerase, may serve to improve the breadth of the response. Thus, hepatitis B core and polymerase proteins were selected as antigens for the design of a therapeutic hepatitis B virus (HBV) vaccine.

Derivation of HBV Core and Polymerase Antigen Consensus Sequences

HBV pol and core antigen consensus sequences were generated based on HBV genotypes B, C, and D. Different HBV sequences were obtained from different sources and aligned separately for core and polymerase proteins. Original sequence alignments for all subtypes (A to H) were subsequently limited to HBV genotypes, B, C, and D. Consensus sequences were defined for each protein alignment in each subtype separately and in all joint BCD sequences. In variable alignment positions, the most frequent amino acid was used in the consensus sequence.

Optimization of HBV Core Antigen

The HBV core antigen consensus sequence was optimized by a deletion in the native viral protein. In particular, a deletion of thirty-four amino acids corresponding to the C-terminal highly positively charged segment was made, which is required for pre-genomic RNA encapsidation.

Optimization of the HBV Pol Antigen

The HBV pol antigen consensus sequence was optimized by changing four residues to remove reverse transcriptase and RNaseH enzymatic activities. In particular, the aspartate residues (D) were changed to asparagine residues (N) in the “YXDD” motif of the reverse transcriptase domain to eliminate any coordination function, and thus nucleotide/metal ion binding. Additionally, the first aspartate residue (D) was changed to an asparagine residue (N) and the first glutamate residue (E) was changed to a glutamine residue (A) in the “DEDD” motif of the RNaseH domain to eliminate Mg²⁺ coordination. Additionally, the sequence of the HBV pol antigen was codon optimized to scramble the internal open reading frames for the envelope proteins, including the S protein and versions of the S protein with the N-terminal extensions pre-S1 and pre-S2. As a result, open reading frames for the envelope proteins (pre-S1, pre-S2, and S protein) and the X protein were removed.

Optimization of HBV Core and Pol Antigen Expression Strategies

Three different strategies were tested to obtain maximum and equal expression of both core and pol antigens from plasmid vectors: (1) fusion of HBV core and pol antigens in frame

with a small AGAG inserted between the coding sequences to produce a single Core-Pol fusion protein (FIG. 25A); (2) expression of both core and pol antigens from one plasmid by means of a ribosomal slippage site, particularly the FA2 slippage site from foot-and-mouth disease (FMDV) to produce a bicistronic expression vector expressing individual core and pol proteins from a single mRNA (FIG. 25B); and (3) two separate plasmids encoding for HBV core and pol antigens, respectively (FIG. 25C).

In vitro Expression Analysis

The coding sequences of consensus HBV core and pol antigens according to each of the above three expression strategies were cloned into the commercially available expression plasmid pcDNA3.1. HEK-293T cells were transfected with the vectors and protein expression was evaluated by Western blot using a HBV core-specific antigen.

Optimization of Post-Transcriptional Regulatory Elements

Four different post-transcriptional regulatory elements were evaluated for enhancement of protein expression by stabilizing the primary transcript, facilitating its nuclear export, and/or improving transcriptional-translational coupling: (1) Woodchuck HBV post-transcriptional regulatory element (WPRE) (GenBank: J04514.1); (2) intron/exon sequence derived from human apolipoprotein A1 precursor (GenBank: X01038.1); (3) untranslated R-U5 domain of the human T-cell leukemia virus type 1 (HTLV-1) long terminal repeat (LTR) (GenBank: KM023768.1); and (4) composite sequence of the HTLV-1 LTR, synthetic rabbit β -globin intron (GenBank: V00882.1), and a splicing enhancer (triple composite sequence). The enhancer sequences were introduced between a CMV promoter and the HBV antigen coding sequences in the plasmids. No significant difference was observed by Western blot in the expression of the core antigen when expressed from a plasmid in the presence and absence of the WPRE element (FIG. 25D). However, when core antigen expression in HEK293T transfected cells from plasmids having the other three post-transcriptional regulatory sequences was evaluated by Western blot, the triple enhancer sequence resulted in the strongest core antigen expression (FIG. 25E).

Selection of Signal Peptide for Efficient Protein Secretion

Three different signal peptides introduced in frame at the N-terminus of the HBV core antigen were evaluated: (1) Ig heavy chain gamma signal peptide SPIgG (BAA75024.1); (2) the Ig heavy chain epsilon signal peptide SPIgE (AAB59424.1); and (3) the Cystatin S precursor signal peptide SPCS (NP_0018901.1). Signal peptide cleavage sites were optimized *in silico* for

core fusion using the Signal P prediction program. Secretion efficiency was determined by analyzing core protein levels in the supernatant. Western blot analysis of core antigen secretion using the three different signal peptides fused at the N-terminus demonstrated that the Cystatin S signal peptide resulted in the most efficient protein secretion (FIG. 25F).

DNA Vaccine Vector Optimization

The optimized expression cassettes containing the triple composite enhancer sequence upstream of the HBV antigen coding sequence with an N-terminal Cystatin S signal peptide sequence were cloned in the DNA vaccine vector pVax-1 (Life Technologies, Thermo Fisher Scientific, Waltham, MA). The expression cassette in pVax-1 contains a human CMV-IE promoter followed by the bovine growth hormone (BGH)-derived polyadenylation sequence (pA). Bacterial propagation is driven by the pUC ori replicon and kanamycin resistance gene (Kan R) driven by a small eukaryotic promoter. The pUC ori replication, KanR expression cassette, and expression cassette driven by the CMV-IE promoter are all in the same orientation within the plasmid backbone. However, a marked reduction in core antigen expression was observed in the pVax-1 vector as compared to the expression level observed in the pcDNA3.1 vector.

Several strategies were employed to improve protein expression: (1) reversal of the entire pUCori-KanR cassette into counterclockwise orientation (pVD-core); and (2) changing the codon usage of the KanR gene along with replacement of the Kan promoter with the Amp promoter from the pcDNA3.1 vector (pDK-core). Both strategies restore core antigen expression, but core antigen expression was highest with the pDK vector, which contained the codon-adjusted Kan R gene, AmpR promoter (instead of KanR promoter), and inverse orientation of the pUCori-KanR cassette.

The four different HBV core/pol antigen optimized expression cassettes as shown in FIG. 25G were introduced into the pDK plasmid backbone to test each of the three expression strategies illustrated in FIGS. 25A-25C. The plasmids were tested *in vitro* for core and pol antigen expression by Western blot analysis using core and pol specific antibodies. The most consistent expression profile for cellular and secreted core and pol antigens was achieved when the core and pol antigens were encoded by separate vectors, namely the individual DNA vectors pDK-core and pDK-pol (FIG. 25H). A schematic representation of the pDK-pol and pDK-core vectors is shown in FIGS. 26A and 26B, respectively.

Example 2. Electroporation mediated intramuscular administration of nucleic acid based biopharmaceuticals with TriGrid Delivery System (TDS-IM) device

The intracellular delivery of nucleic acid sequences in the skeletal muscle of the upper or lower limb in a subject can be enhanced with the use of an exemplary device, *e.g.* TriGrid Delivery System (TDS-IM) model II, as provided herein. In some embodiments, the TDS-IM device is used in conjunction with agents approved for investigational use with the TDS-IM device. In an exemplary embodiment, the approved agent is nucleic acids, *i.e.*, DNA or RNA. In some embodiments, the use of TDS-IM device is restricted to a subject in need thereof. In some embodiments, the use of TDS-IM device is restricted to a subject enrolled in an open clinical trial of electroporation mediated intramuscular nucleic acid delivery.

To start up the system for administration, the main power of the device is connected to the stimulator and the system battery is adequately charged for use. The main power switch is turned on and the front panel power button is depressed. The applicator is connected to the applicator connector. Proper connection of the applicator is confirmed by the illumination of the applicator power indicator. The start screen appears once the system completes all self-checks. The OK button is pressed to proceed with the procedure administration.

To insert a syringe into a TDS cartridge, the syringe cap is removed from the syringe, and the syringe flange is aligned with the TDS cartridge syringe loading port. The syringe should snap into place and be fully seated in the TDS cartridge. Once the syringe is loaded, the OK button is pressed on the pulse stimulator to continue. The cartridge cap should remain affixed to the cartridge until the cartridge is loaded into the applicator.

To insert the syringe loaded cartridge into the applicator, the cartridge is aligned with the applicator with the cartridge syringe loading port facing upward. When the cartridge is inserted into the applicator, and the cartridge is automatically drawn into its fully loaded position in the applicator. Successful loading of the cartridge is indicated in the stimulator. Once the cartridge is loaded, the applicator is returned to its cradle, and an appropriate injection site on the subject is selected. In some embodiments, the injection site for intramuscular nucleic acid delivery is medial deltoid muscle at approximately three finger widths below the edge of acromion process (shoulder bone). In an exemplary embodiment, the injection depth at medial deltoid is about 0.75"–1.25" (19–30 mm). In some embodiments, the injection site for intramuscular nucleic acid delivery is vastus lateralis muscle (outer thigh) at approximately the midpoint between the

hip and the knee. In an exemplary embodiment, the injection depth at vastus lateralis is about 1.0”–1.5” (25–38 mm). Once the injection site is selected, the applicator depth selection button followed by the injection depth selection button corresponding to the injection site/depth are pressed. In some embodiments, the injection depth selection indicator turns to solid illumination, confirming the selected injection depth. In some embodiments, the depth selection button on the right side corresponds to a deeper injection depth. In an embodiment, wherein the initially selected injection site is to be changed, the selection button corresponding to the other injection depth is pressed, and the other injection depth is selected when the the select injection depth screen returns.

To start administration of an approved agent via TDS-IM device to the subject, the cartridge cap is removed and discarded. The device is aligned with and firmly pressed against the target injection site. When the device is firmly pressed against the target injection site, all four bars of the applicator placement indicator illuminates, and the procedure countdown timer illuminates with “8” seconds, indicating the time remaining in the administration procedure. The applicator trigger is depressed for the agent to be administered while the pressure is consistently maintained. When the procedure countdown timer reaches “0,” the electrical stimulation is delivered. Once the administration procedure is completed, the procedure complete indicator illuminates, and the device can be withdrawn from the injection site. The device may not be withdrawn from the injection site until the procedure is completed or a procedure fault indicator illuminates. In some embodiments, wherein the device detects a problem during the administration procedure, the device aborts the administration procedure and illuminates the procedure fault indicator. In an exemplary embodiment, wherein the device aborts the administration procedure and the device promptly is removed from the subject, the stimulator display of the device provides further instructions.

To eject the cartridge from the applicator after completion of the agent administration to the subject in need thereof, the eject button located on the stimulator is depressed. The applicator automatically advances the cartridge to the position where it can be manually removed from the applicator. Once the cartridge stops moving, the sides of the cartridge as indicated by arrows can be grasped for pulling the cartridge out of the applicator. After the cartridge is removed, completion of the full injection can be verified by inspecting the syringe plunger

position. To turn off the device, the applicator is placed in the holster, and the front panel power button is pressed for 5 seconds.

Example 3. TDS-IM Electroporation to Mouse and Non-human Primate

TriGrid Delivery System – Intramuscular (TDS-IM) electroporation technology is used to enhance the delivery of DNA-based constructs in muscle tissue. The TriGrid electrode array for intramuscular (IM) delivery is comprised of four electrodes arranged in two equilateral triangles to form a diamond shape surrounding a central injection needle (**FIG. 22**). Integration of the agent delivery and the electric field propagation into a single device assures that induction of the electroporation effect occurs at the site of agent distribution and allows injection of the plasmid construct and application of the electric pulses in a single step. In this manner, IM delivery of plasmid DNA is achieved in an effective and reproducible manner.

The TriGrid electrode arrays for each animal model are scaled to efficiently deliver the DNA plasmids, such as those made in Example 1, to the selected muscle regardless of size and overlying tissue characteristics. The primary electroporation device parameters that are scaled for use in each animal model are the TriGrid size (i.e., electrode spacing), applied voltage (250 V/cm where cm is the TriGrid size), electrode diameter, and the electrode penetration depth. Secondary adaptations to the device include variation in syringe volume, plasmid DNA volume, and hypodermic needle gauge/length.

The mouse studies were performed with a TDS-IM v1.0 device using an electrode array with a 2.5 mm spacing between the electrodes. This adjustment in electrode array size is to accommodate the smaller size of the mouse muscle. **FIG. 23A** depicts a TDS-IM v1.0 TriGrid version adapted for use in the mouse model. The non-GLP monkey studies were also performed with a TDS-IM v1.0 device, but with a 6 mm electrode array spacing. **FIG. 23B** depicts a TDS-IM v1.0 TriGrid version adapted for use in the non-human primate (NHP) model.

The device used in the GLP monkey study was a TDS-IM v2.0 device, which is also intended for human clinical study. This device has a 6 mm electrode spacing and the same activation conditions as the TDS-IM v1.0 device. The electrode materials of the TDS-IM v2.0 device are identical with the TDS-IM v1.0 device used in the NHP study described above, but the Cartridge support structure and syringe used for administration are different. Also, the insertion depth for humans and non-human primates is different. Therefore, the TDS-IM v2.0 device was fitted with a spacer in order to adapt the TDS-IM v2.0 device for use in the much

smaller NHP muscles. Figure 24 depicts the TDS-IM v2.0 TriGrid version adapted for use in the non-human primate (NHP) model.

In each animal study, the animals were anesthetized to immobilize them prior to the electroporation procedure administration. The administration site was located using regional bony anatomical landmarks as reference points and the hair was removed over the selected site with an electric hair clipper followed by an aseptic swab. The TDS-IM array was inserted percutaneously into the selected muscle with the major axis of the diamond configuration oriented in parallel with the muscle fibers. Following electrode insertion, the injection was initiated to distribute the DNA in the muscle. Following completion of the IM injection, a 250 V/cm electrical field was locally applied for a total duration of 400 ms at a 10% duty cycle (i.e., voltage is actively applied for a total of 40 ms of the 400 ms duration). Once the electroporation procedure was completed, the TriGrid™ array was removed and the animals were recovered. The variable components for each TDS-IM version and/or animal model are as follows in Table 1 and Table 2.

Table 1: TDS-IM version and TriGrid details

TDS-IM Version	Animal Model	TriGrid size (mm)	Electrode diameter (in)	Conductive length (mm)	Effective Penetration depth (mm)	DNA Injection Volume (cc)	Syringe Volume (cc)	Injection needle gauge	Injection method
TDS-IM v1.0	mouse	2.5	0.030	3.2	3.2	0.020	3/10 cc	30	manual
TDS-IM v1.0	NHP	6.0	0.021	5.0	15.5	1.0	1.0 cc	22	automated
TDS-IM v2.0	NHP	6.0	0.023	5.0	9.0*	1.0	1.0 cc	22	automated
*NHP body weight and muscle size required a shorter penetration depth; a 10mm depth limiter was used to shorten the penetration depth to 9mm.									

Table 2: TDS-IM version and Pulse Stimulator details

TDS-IM Version	Animal Model	Applied electric field (V/cm)	Applied Voltage range (V)	Applied Current limits (A)	Applied current limit (A/sec)	Total pulse number	Active stimulation duration (ms)	Maximum sequence duration (ms)
TDS-IM v1.0	mouse	250	59.4 - 65.6	N/A* - 4	0.16	6	40.8	369.3
TDS-IM v1.0	NHP	250	142.4 - 157.6	0.6 - 4	0.16	6	40.8	369.3
TDS-IM v2.0	NHP	250	142.4 - 157.6	0.6 - 4	0.16	6	40.8	369.3
*low current limit disabled to accommodate mouse model characteristics								

Example 4. TDS-IM Electroporation to human

A TDS-IM v2.0 device is used in the clinical study. The device which is essential the same as that used for the GLP monkey. The insertion depth for humans and non-human primates is different. Therefore, the TDS-IM v2.0 device was fitted with a spacer in order to adapt the TDS-IM v2.0 device for use in the much smaller NHP muscles. The stimulation conditions were essentially the same as those used for GLP monkeys, with the exception that the penetration depth about 19 mm, instead of 9 mm as in the GLP monkeys.

While preferred embodiments of the disclosure have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the disclosure. It should be understood that various alternatives to the embodiments described herein, or combinations of one or more of these embodiments or aspects described therein can be employed in practicing the disclosure. It is intended that the following claims define the scope of the disclosure and that methods and structures within the scope of these claims and their equivalents be covered thereby.

Claims

1. An apparatus for the controlled delivery of an HBV vaccine to a predetermined tissue site within a subject in need thereof, comprising:
 - a cartridge assembly comprising an outer cartridge, an inner cartridge, a reservoir containing the HBV vaccine, wherein a reservoir containment volume is contained within the outer cartridge and configured to receive the reservoir;
 - an applicator comprising a cartridge assembly receiving volume, a needle hub, and an insertion detector, wherein the insertion detector senses loading of the reservoir in the reservoir containment volume;
 - at least one interlock, wherein the interlock facilitates proper execution of the HBV vaccine administration procedure;
 - at least one injection orifice through which the HBV vaccine is administered;
 - a plurality of penetrating electrodes arranged with a predetermined spatial relationship relative to the orifice;
 - an electrical field generator for generating an electrical signal operatively connected to the electrodes; and
 - a controlled source of energy sufficient to transfer a predetermined amount of the HBV vaccine at a predetermined rate from the reservoir through the orifice to the predetermined site within the subject,wherein the HBV vaccine comprises:
 - a first nucleic acid molecule comprising a first polynucleotide encoding an HBV polymerase antigen, wherein the HBV polymerase antigen comprises an amino acid sequence that is at least 98% identical to SEQ ID NO: 4 and wherein the HBV polymerase antigen does not have reverse transcriptase activity and RNase H activity;
 - a second nucleic acid molecule comprising a second polynucleotide encoding a truncated HBV core antigen consisting of the amino acid sequence of SEQ ID NO: 2; and
 - a pharmaceutically acceptable carrier,wherein the first nucleic acid molecule and the second nucleic acid molecule are present in the same nucleic acid molecule or in two different nucleic acid molecules,

preferably the electrodes are a plurality of elongate electrodes and the interlock prevents inadvertent actuation of cartridge function.

2. The apparatus of claim 1, wherein at least one interlock is selected from the group consisting of a mechanical interlock, a light emitter/collector, a cartridge breech, a force interlock, an alignment guide and a splay shield, a trigger lock, and a safety switch.

3. The apparatus of claim 1 or 2, wherein the apparatus comprises at least two sets of interlocks, wherein a first interlock is a mechanical interlock, and a second interlock is selected from the group consisting of a light emitter/collector, a cartridge breech, a force interlock, an alignment guide and a splay shield, a trigger lock, and a safety switch, preferably the mechanical interlock is a reservoir interlock further comprising (i) at least one reservoir lockout hole, or (ii) a tab extending from one or more of the cartridge surface, wherein the tab ensures that reservoir is properly inserted into the cartridge, and preferably the cartridge breech provides an optical line of sight through at least one reservoir lockout hole.

4. The apparatus of claim 3, wherein the apparatus further comprises a third interlock that is a force interlock, wherein the force interlock senses a force applied against the predetermined tissue site of the subject and prevents administration of the HBV vaccine to the subject when insufficient force is provided, preferably the force interlock further forms an electrical lock within the applicator.

5. The apparatus of any one of claims 1-4, wherein the apparatus further comprises at least one sensor, preferably selected from the group consisting of a cartridge loading sensor, a cartridge loaded sensor, a cartridge force sensor, an insertion mechanism position sensor, an insertion detector, an optical detector, and an electrical sensor.

6. The apparatus of claim 5, wherein the sensor detects a reservoir label and verifies the HBV vaccine.

7. An apparatus for the controlled delivery of an HBV vaccine to a predetermined tissue site within a subject comprising:

a cartridge assembly comprising a housing, a reservoir containing the HBV vaccine, wherein a reservoir containment volume is contained within the housing and configured to receive the reservoir;

an applicator comprising a cartridge assembly receiving volume, a needle hub, and an insertion detector, wherein the insertion detector senses loading of the reservoir in the reservoir containment volume; at least one injection orifice through which the HBV vaccine is administered;

a plurality of penetrating electrodes arranged with a predetermined spatial relationship relative to the orifice;

an electrode support structure comprising a hole and a wall, wherein the electrode support structure prevents inadvertent perpendicular motion of the electrodes;

an electrical field generator for generating an electrical signal operatively connected to the electrodes; and

a controlled source of energy sufficient to transfer a predetermined amount of the HBV vaccine at a predetermined rate from the reservoir through the orifice to the predetermined site within the subject,

wherein the HBV vaccine comprises:

a first nucleic acid molecule comprising a first polynucleotide encoding an HBV polymerase antigen, wherein the HBV polymerase antigen comprises an amino acid sequence that is at least 98% identical to SEQ ID NO: 4 and wherein the HBV polymerase antigen does not have reverse transcriptase activity and RNase H activity;

a second nucleic acid molecule comprising a second polynucleotide encoding a truncated HBV core antigen consisting of the amino acid sequence of SEQ ID NO: 2; and

a pharmaceutically acceptable carrier, and

wherein the first nucleic acid molecule and the second nucleic acid molecule are present in the same nucleic acid molecule or in two different nucleic acid molecules.

8. The apparatus of claim 7 , wherein the electrode support structure provides an operative connection between a conductive contact region located on the distal region of the electrodes and the controlled source of energy when the electrodes are deployed into the predetermined tissue site within the subject, preferably the electrode support structure comprises at least one hole for passage of the electrodes and a hole for passage of an injection needle .
9. The apparatus of claim 7 or 8, wherein the electrode support structure is a planar support structure positioned perpendicularly relative to the elongate orientation of the electrodes, preferably the planar support structure comprises at least one aperture configured to allow passage of the electrode through the predetermined tissue site.
10. The apparatus of any one of claims 7 to 9, wherein the electrode support structure is an adaptive electrode support, preferably the adaptive electrode support is a compression spring made from a metal, a polymer or an elastomeric material.
11. The apparatus of any one of claims 7 to 10, wherein the electrode support structure comprises at least one of a telescoping tube, a stick shield spring, and at least one lateral support member attached to the electrodes with at least one optional hinge feature.
12. The apparatus of any one of claims 1-11, wherein the HBV vaccine is capable of inducing an immune response in a mammal against at least two HBV genotypes, preferably the HBV vaccine is capable of inducing a T cell response in a mammal against at least HBV genotypes B, C and D, and more preferably the HBV vaccine is capable of inducing a CD8 T cell response in a human subject against at least HBV genotypes A, B, C and D.
13. The apparatus of any one of claims 1-12, wherein the first non-naturally occurring nucleic acid molecule is present in a first plasmid DNA vector, and the second non-naturally occurring nucleic acid molecule is present in a second plasmid DNA vector.
14. The apparatus of claim 13, wherein the HBV vaccine comprises:

- a) a first plasmid DNA vector comprising, from 3'-end to 5'-end, the promoter sequence comprising the polynucleotide sequence of SEQ ID NO: 7, the enhancer sequence comprising the polynucleotide sequence of SEQ ID NO: 8, the signal peptide coding sequence comprising the polynucleotide sequence of SEQ ID NO: 5, the first polynucleotide sequence comprising the polynucleotide sequence of SEQ ID NO: 3, and the polyadenylation signal sequence comprising the polynucleotide sequence of SEQ ID NO: 11;
- b) a second plasmid DNA vector comprising, from 3'-end to 5'-end, the promoter sequence comprising the polynucleotide sequence of SEQ ID NO: 7, the regulatory sequence comprising the polynucleotide sequence of SEQ ID NO: 8, the signal peptide coding sequence comprising the polynucleotide sequence of SEQ ID NO: 5, the second polynucleotide sequence comprising the polynucleotide sequence of SEQ ID NO: 1, and the polyadenylation signal sequence comprising the polynucleotide sequence of SEQ ID NO: 11; and
- c) a pharmaceutically acceptable carrier,

wherein each of the first plasmid DNA vector and the second plasmid DNA vector further comprises a kanamycin resistance gene having the polynucleotide sequence of SEQ ID NO: 12, and an origin of replication having the polynucleotide sequence of SEQ ID NO: 10, and wherein the first plasmid DNA vector and the second plasmid DNA vector are in the same composition or two different compositions.

15. The apparatus of any one of claims 1-14, wherein the electrical signal operably connected to the electrodes comprises an electrical field strength of 100 V/cm to 400 V/cm, preferably 250 V/cm; a voltage of 50 to 200 V, preferably about 150 V; and a current of 0.5 to 5 A/sec, preferably 0.6 to 4 A/sec, more preferably 0.16 A/sec.

16. The apparatus of any one of claims 1 to 14, wherein said apparatus is capable of electroporation of the HBV vaccine in a subject in need thereof, preferably in a human.