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(54) Title: NON-INVASIVE AGENT APPLICATOR

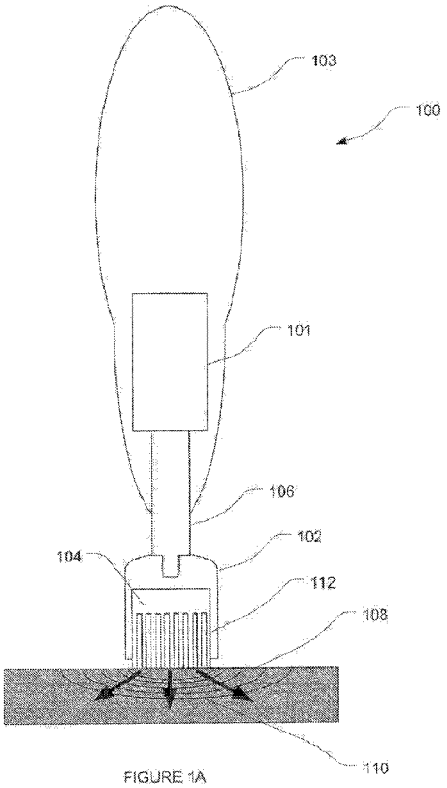

FIGURE 1A

(57) Abstract: There are disclosed systems and methods for non-invasive delivery of an agent to biological tissues. Delivery of the agent to the tissues is by ultrasound and delivery induces an immune response in the subject. In some embodiments the systems and methods use agent carrier body including a tissue contacting surface for non-invasively engaging tissues under treatment. The tissue contacting surface can be at least partly defined by a plurality of protrusions that are in fluid communication with one or more reservoirs forming part of the agent carrier body. The protrusions may extend outward from an inside of a void and terminate at said tissue contacting surface.

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Non-invasive Agent Applicator

Field of the invention

The present invention relates to the application of an agent to a target site. In a preferred form, the invention uses ultrasonic energy to transport an agent contained within an agent carrier body having a plurality of micro-scale structures within it to the target site non-invasively. In this preferred form, at the target site, penetration of the agent into the target site is enabled or enhanced through sonophoretic mechanisms.

Background of the invention

WO 2007/143796 discloses a method of delivering a molecule and/or particle to a target site using a device that includes generating ultrasound for enhancing the penetration of a molecule and/or particle into the target tissue.

The device of WO 2007/143796 includes an electro-conductive polymeric gel material that is loaded with a molecule and/or particle such as a pharmaceutical or ink etc. Application of an electric field to the electro conductive polymer gel releases substantially bound molecules or particles within the polymer gel matrix and, ultimately, such molecules or particles are transported through such polymer gel by ultrasound to the target tissue surface. At the target tissue surface, penetration of the molecule and/or particle into the tissue is enabled or enhanced through sonophoretic mechanisms.

One difficulty relating to this delivery mechanism is that the structure of the polymer gel can degrade over time, for example due to loss of moisture, which results in reduced propagation of the molecule and/or particle by ultrasound. Additionally, gels are poor transmitters of ultrasound reducing the efficacy of the sonophoretic process. Furthermore, it can be time consuming and non-trivial to properly load an applicator with small volumes of the molecule and/or particle loaded polymeric gel.

In light of these problems, an improved device and mechanism for delivering an agent to a target tissue is sought.

Reference to any prior art in the specification is not, and should not be taken as, an acknowledgment or any form of suggestion that this prior art forms part of the common general knowledge in Australia or any other jurisdiction, or that this prior art could reasonably be expected to be ascertained, understood and regarded as relevant, or combined with other prior art by a person skilled in the art.

Summary of the invention

The present disclosure provides an agent carrier for non-invasive delivery of an agent to biological tissues. Delivery of the agent to the tissues can be by one or more modalities. The modality of delivery can be characterised by a transportation stimulus or stimuli that causes transportation of the agent through the agent carrier. In a preferred form, the transportation stimulus also enhances or permits penetration of the agent into the tissue. Preferred embodiments use only ultrasound as the transportation stimulus.

The stimulus may enhance penetration of the agent into the tissue by among other things, increasing the rate or depth (or both) of movement of an agent into tissue that would otherwise without the stimulus, diffuse into tissue at a slower rate or to a lesser depth (or both). The stimulus may alternatively permit or enable penetration of the agent into the tissue by among other things, enabling the movement of an agent into tissue that would otherwise without the stimulus, not be able to move into tissue or would diffuse in negligible amounts into tissue.

In preferred embodiments the agent carrier includes an agent carrier body configured to retain agent within the agent carrier body. The agent carrier body has a tissue contacting surface for engaging tissues under treatment, wherein application of the transportation stimulus causes transportation of the agent through the agent carrier body to the tissue contacting surface.

The agent to be delivered can include one or more molecules or particles or one or more molecules and particles in any combination. The agent can be a fluid or can be carried in a fluid medium, e.g. by being dissolved, suspended or dispersed in a fluid medium, such as water, oil, an emulsion, a gel or the like. To give but a few examples, the agent can include, proteins (including amino acids, peptides, polypeptides), vaccines, nucleic acids, monoclonal and polyclonal antibodies, nanoparticles or molecular machines. In preferred embodiments the agent is a pharmaceutical or pharmaceutical composition. The pharmaceutical or one or more active pharmaceutical components of a pharmaceutical composition may be, without limit, any one of: a synthesised compound; a naturally occurring compound; or a biopharmaceutical. The purpose of the delivery of the pharmaceutical or pharmaceutical composition to the biological tissues can be for any desired clinical reason including: treating, curing or mitigating a disease, condition, or disorder; attenuating, ameliorating, or eliminating one or more symptoms of a particular disease, condition, or disorder; preventing or delaying

the onset of one or more a disease, condition, or disorder or a symptom thereof; diagnosing a disease, condition, or disorder, or any agent intended to affect the structure or any function of the body. In other embodiments the agent can be an agent used for cosmetic purposes such as for cleansing, beautifying, promoting attractiveness, or altering the appearance of the body. The agent could also be a marker agent used for creating human or machine perceptible makings, e.g. ink or other. Other types of agents may also be used.

The transportation stimulus is the driving force for moving the agent through the agent carrier to the tissue-contacting surface, and may enhance and/or enable the penetration of the agent from the tissue-contacting surface into the tissue.

In some embodiments the tissue can be any human or animal biological tissue, including mucous membranes, skin and teeth. Preferably the tissue is ocular tissue or oral mucosa. In some embodiments the tissue is any plant tissue.

In an aspect there is provided an agent carrier body including a tissue contacting surface for non-invasively engaging tissues under treatment, the tissue contacting surface being at least partly defined by a plurality of protrusions. The protrusions may be in fluid communication with one or more reservoirs forming part of the agent carrier body. Each agent reservoir may comprise a void formed within the agent carrier body. The protrusions may extend outward from an inside of a void and terminate at said tissue contacting surface. The void may be formed by a peripheral structure, where at least part of said peripheral structure may terminate at the tissue contacting surface.

In some embodiments the peripheral structure terminates in a common plane with the protrusions. In others at least some of said protrusions defining the tissue contacting surface extend outward from the void beyond the peripheral structure. In some embodiments, the protrusions may terminate in a plane and the peripheral structure may terminate short of the plane such that the protrusions extend beyond the peripheral structure.

The agent carrier body may include one or a multiplicity of micro channels extending at least partially through the agent carrier body to the tissue contacting surface enabling transportation of the agent to a tissue surface. The micro channels may extend through the agent carrier body to fluidly connect to an agent reservoir.

The agent carrier body of these aspects can include a stack of layers including a tissue-contacting layer, which includes the tissue-contacting surface, and at least one other layer. The tissue-contacting layer preferably has holes extending through it to define at least a portion of the micro channels in the body. In some embodiments a plurality of layers have holes formed therein to enable agent to be transported from one layer to the next. Preferably holes formed in one layer of the plurality of layers are aligned with holes in an adjacent layer so that a plurality of holes in a plurality of layers cooperate to form the micro channels. In some embodiments the holes decrease in diameter and increase in number from the first layer to the tissue-containing layer. The micro channels may have a varying cross-section along their length.

In some embodiments a reservoir for storing agent is at least partly (and optionally fully) formed in the agent carrier body.

The micro channels and/or agent reservoir(s) and/or protrusions are defined by internal exposed surfaces within the agent carrier body. Preferably these internal exposed surfaces are configured to possess predetermined hydrophilic, hydrophobic, and/or electro-conductive properties. In this case, at least part of the internal exposed surfaces could be modified or treated to configure their hydrophilic, hydrophobic, and/or electro-conductive properties.

The agent carrier body may include a port to enable loading of the agent carrier body and/or reservoir(s) with agent.

The agent carrier body can further include a stimulus generator, operable to generate transportation stimulus. The stimulus generator preferably includes an ultrasonic transducer.

In some embodiments, the agent carrier preferably includes a housing configured to mechanically support an agent carrier body, of any type described herein. The housing can include a mounting arrangement configured to be mounted to the agent applicator device. The mounting arrangement preferably enables selective attachment and removal of the agent carrier to and from the agent applicator device, such that the agent carrier can be replaced.

The agent carrier housing also may include a recess or other mounting formation formed therein for receiving the agent carrier body. In some embodiments the agent

carrier body can be selectively attached to, or removed from, the recess or mounting formation such that the agent carrier body can be replaced.

The agent carrier can include a port to enable loading of the agent carrier body and/or reservoir(s) with agent.

The agent carrier can further include a stimulus generator, operable to generate a transportation stimulus. The stimulus generator preferably includes an ultrasonic transducer. At least part of the stimulus generator can be formed as part of the agent carrier body.

In a preferred embodiment the agent carrier or agent carrier body is a consumable applicator tip adapted for one-time use as part of an agent applicator device.

In another aspect of the disclosure there is provided a non-invasive agent applicator device comprising an agent carrier and/or an agent carrier body as described herein.

The agent carrier or agent carrier body can be coupled directly or indirectly to a handle unit to facilitate hand held operation of the agent applicator device. The handle unit preferably includes a mounting arrangement configured to cooperate with a complementary mounting arrangement of the agent carrier and/or agent carrier body.

The handle unit may include an ultrasonic generator to generate ultrasonic waves that are transmitted to the attached agent carrier and/or agent carrier body.

Preferably the agent carrier is a consumable applicator tip adapted for one-time use.

The agent carrier preferably includes an agent carrier body including a tissue contacting surface for non-invasively engaging tissues under treatment, the tissue contacting surface being at least partly defined by a plurality of protrusions.

The agent carrier may include one or more agent reservoirs for carrying said agent, wherein said protrusions are in fluid communication with one or more reservoirs forming part of the agent carrier. Each agent reservoir may at partly (or wholly) comprise a void formed within the agent carrier body.

Also disclosed herein is a method of dispensing an agent from an agent carrier described herein. The method comprises holding the agent within an agent carrier, said agent carrier including a solid agent carrier body. The method can further comprise engaging a tissue contacting surface of the agent carrier body with a tissue surface of the biological tissue. The method can further comprise dispensing agent from the agent

carrier to the tissue surface by applying at least one transportation stimulus to cause transportation of the agent through the agent carrier body to the tissue surface.

In some forms the method further includes applying the transportation stimulus to the tissue via the agent carrier to enhance or enable penetration of the agent into the biological tissue.

Holding the agent within an agent carrier can include holding at least some agent within the carrier body.

In preferred embodiments the agent carrier body terminates at its tissue contacting surface in a plurality of protrusions. In this case, engaging a tissue contacting surface of the agent carrier body with a tissue surface of the biological tissue, includes engaging the tissue surface of the biological tissue with the protrusions of the agent carrier body. Such engagement preferably does not involve mechanically penetrating any layer of biological tissue with the protrusions.

In another aspect of the disclosure there is provided a method of dispensing an agent from an agent carrier, an agent carrier body, or an agent applicator device as described previously, the method including: contacting the tissue-contact surface of the agent carrier with a tissue surface; and dispensing agent from the agent carrier body to the tissue surface and into the target tissue.

In some embodiments of any of the above methods the step of dispensing the agent includes generating ultrasonic waves for agent transport to the tissue contact surface. Even more preferably the method includes propagating ultrasonic waves through the agent carrier to the tissue. This aids the delivery of the agent through the tissues via sonophoresis.

In some embodiments of any of the above methods the step of dispensing the agent can include applying an electrical voltage across the agent carrier body to cause agent transport to the tissue contact surface. The electric voltage can also provide for the transport of agent into and through the tissue via iontophoresis. Even more preferably the method includes propagating an electric current through the agent carrier to the tissue.

In yet another aspect of the present disclosure there is provided a method of dispensing an agent from an agent carrier, an agent carrier body or an agent applicator device as described herein. The method including, contacting the tissue contacting surface of the

agent carrier body with a tissue surface; and dispensing agent from the agent carrier to the tissue surface. The step of dispensing the agent preferably includes generating ultrasonic waves to cause or facilitate agent transportation to the tissue-contacting surface. The method can include the application of ultrasonic waves to the tissue surface to non-invasively cause or facilitate agent penetration of the agent into and through the tissue via sonophoresis.

The method further includes propagating ultrasonic waves through the agent carrier or agent carrier body to the tissue.

In another aspect the present disclosure provides a method of loading agent into any one of an agent carrier, agent carrier body, an agent applicator device as described herein. The method includes, exposing the agent carrier body to the agent to enable filling either of both of, a reservoir or micro channels in fluid communication with said reservoir, with said agent.

The method can include applying a negative pressure to the agent carrier or agent carrier body to draw agent into the micro channels or agent reservoirs in fluid communication with the micro channels. The method can include applying a positive pressure to the agent carrier or agent carrier body to inject the agent into the micro channels or agent reservoirs in fluid communication with the micro channels.

The step of filling the micro channels or agent reservoirs with the agent can include the application of ultrasonic energy to the agent carrier or agent carrier body to draw agent into the agent carrier or agent carrier body.

In some embodiments, the voids and/or micro channels in the agent carrier body are loaded by virtue of capillary forces when the agent carrier is in contact with the agent.

Embodiments of the present invention may advantageously be used in the non-invasive delivery of agent to delicate tissues, such as mucous membranes (including the conjunctiva, buccal mucosa and labial mucosa), the cornea and the external coats of the eye.

In a first aspect, the present invention provides a method of delivering an agent to a tissue, including: applying said agent using an agent carrier, agent carrier body or agent applicator of any one of the aspects or embodiments described herein, wherein ultrasound is the transportation stimulus; and configuring the operational parameters of the application to enhance or cause delivery of said agent to a selected depth within

such tissue. The operational parameters configured may include (but are not limited to) any one or more of:

Application pressure;
Ultrasonic frequency;
Ultrasonic power level;
Ultrasonic waveform;
Ultrasonic application duration;
Ultrasonic application duty cycle; and
Ultrasound direction.

Preferably the operational parameters are selected to deliver a chosen amount of agent to a selected depth within tissue. The person skilled in the art will appreciate that the optimal operational parameters needed to achieve the desired immunological response by application of agent to specific types of tissue and using a specific agent carrier design can be determined by empirical testing, including clinical testing in subjects.

The method may involve delivering the agent to or beyond any one or more of the following tissues or tissue layers:

Mucous Membrane;
Epithelium
Sub-epithelium
Mucosa;
Sub-mucosa
Mucous membrane vasculature
Cornea;
Corneal epithelium
Bowman's membrane
Corneal stroma
Corneal Endothelium
Conjunctiva;
Tenon's Fascia;
Episclera:
Sclera;
Choroid;
Choriocapillaris;

Bruch's membrane;
Retinal Pigment Epithelium;
Neural retina;
Retinal blood vessels;
Internal Limiting Membrane;
Vitreous;
Skin epidermis; and
Skin dermis.

In another aspect of the present invention there is provided is a method of delivering an agent to a selected depth range within tissue of a subject, including the steps of:

applying ultrasound to said agent using an agent carrier body, agent carrier or agent applicator, wherein ultrasound is the transportation stimulus; the agent carrier body comprising a tissue contacting surface for engaging the tissue; and

configuring the operational parameters of the agent applicator to enhance or enable delivery of said agent to a selected depth within the tissue

wherein delivery of the agent induces an immune response in the subject.

In another aspect of the present invention there is provided is a method of delivering an agent to one or more selected layers of a tissue of a subject, including the steps of:

applying ultrasound to said agent using an agent carrier body, agent carrier or agent applicator, wherein ultrasound is the transportation stimulus; the agent carrier comprising a tissue contacting surface for engaging the tissue; and

configuring the operational parameters of the agent applicator to enhance or enable delivery of said agent to the one or more layers of the tissue

wherein delivery of the agent induces an immune response in the subject.

Preferably this method is performed in accordance with a method according to an embodiment of the previous aspect of the invention.

In a further aspect of the invention, there is provided a system for delivering an agent to a selected depth range within a tissue of a subject, the system including:

an agent contained in an agent carrier body, agent carrier or agent applicator; the agent carrier body comprising a tissue contacting surface for engaging the tissue; and

a means for applying an ultrasonic signal to the agent, wherein ultrasound is the transportation stimulus;

wherein the system is configured to enhance or enable delivery of said agent to a selected depth range within such tissue, and delivery of the agent induces an immune response in the subject.

In a further aspect of the invention, there is provided a system for delivering an agent to one or more selected layers of a tissue in a subject, the system including:

an agent contained in an agent carrier body, agent carrier or agent applicator; the agent carrier body comprising a tissue contacting surface for engaging the tissue; and

a means for applying an ultrasonic signal, wherein ultrasound is the transportation stimulus;

wherein the system is configured to enhance or enable delivery of said agent to the one or more layers of the tissue

and delivery of the agent induces an immune response in the subject.

In another aspect of the present invention there is provided is a method of inducing an immune response in a subject, including the steps of

applying ultrasound to an agent contained within an agent carrier body, agent carrier or agent applicator, wherein ultrasound is the transportation stimulus; the agent carrier body comprising a tissue contacting surface for engaging the tissue; and

configuring the operational parameters of the agent applicator to enhance or enable delivery of said agent to a selected depth range within the tissue

wherein delivery of the agent induces an immune response in the subject.

In another aspect of the present invention there is provided is a method of inducing an immune response in a subject, including the steps of

applying ultrasound to an agent contained within an agent carrier body, agent carrier or agent applicator, wherein ultrasound is the transportation stimulus; the agent carrier comprising a tissue contacting surface for engaging the tissue; and

configuring the operational parameters of the agent applicator to enhance or enable delivery of said agent to one or more selected layers of a tissue

wherein delivery of the agent induces an immune response in the subject.

In another aspect of the present invention there is provided is an agent for use in inducing an immune response in a subject, wherein the agent is contained within an agent carrier body or agent carrier or agent applicator, the agent carrier body comprising a tissue contacting surface for engaging the tissue; and the agent is delivered to a selected depth range within a tissue.

In another aspect of the present invention there is provided is an agent for use in inducing an immune response in a subject, wherein the agent is contained within an agent carrier or agent carrier body or agent applicator, the agent carrier comprising a tissue contacting surface for engaging the tissue; and the agent is delivered to one or more selected layers of a tissue.

In yet another aspect of the present invention there is provided use of an agent in the preparation of a medicament for inducing an immune response in a subject, wherein the agent is contained within an agent carrier or agent carrier body or agent applicator, the agent carrier comprising a tissue contacting surface for engaging the tissue; and the agent is delivered to a selected depth range within a tissue.

In yet another aspect of the present invention there is provided use of an agent in the preparation of a medicament for inducing an immune response in a subject, wherein the agent is contained within an agent carrier or agent carrier body or agent applicator, the agent carrier comprising a tissue contacting surface for engaging the tissue; and the agent is delivered to one or more selected layers of a tissue.

The agent in these aspects of the invention is delivered to a selected depth range, or to one or more selected layers of a tissue according to the methods described herein and by configuring the operational parameters of the agent applicator.

In a further aspect of the invention, there is provided a system for delivering an agent to a tissue to induce an immune response in a subject, the system including:

an agent contained within an agent carrier body, agent carrier or agent applicator; the agent carrier body comprising a tissue contacting surface for engaging the tissue; and

a means for applying an ultrasonic signal, wherein ultrasound is the transportation stimulus;

wherein the system is configured to enhance or enable delivery of said agent to a selected depth range within the tissue,

and delivery of the agent induces an immune response in the subject.

In a further aspect of the invention, there is provided a system for delivering an agent to a tissue to induce an immune response in a subject, the system including:

an agent contained within an agent carrier body, agent carrier or agent applicator; the agent carrier body comprising a tissue contacting surface for engaging the tissue; and

a means for applying an ultrasonic signal, wherein ultrasound is the transportation stimulus;

wherein the system is configured to enhance or enable delivery of said agent to one or more selected layers of a tissue,

and delivery of the agent induces an immune response in the subject.

The immune response induced in these aspects of the invention can be a mucosal immune response, a systemic immune response, or both. Preferably, at least a mucosal immune response is induced, and optionally a systemic immune response is also induced.

As can be seen, in each of the aspects and embodiments of the invention described herein, the target delivery site in a tissue may be defined as either being a particular layer or layers of a tissue, or alternatively be defined as a depth range. For example, the delivery of the agent may be defined in terms of being delivered to the Bowman's membrane of the cornea (ie a layer) or may be defined in terms of being delivered to a depth of approximately 5 to 15μM (ie a depth range). The skilled person would be aware of what depth any given target layer is in any given tissue. The immune response induced in these aspects of the invention can be a mucosal immune response, a systemic immune response, or both. Preferably, at least a mucosal immune response is induced, and optionally a systemic immune response is also induced. It is considered that by selectively configuring the operational parameters of the agent applicator presently described, the amount of agent delivered to a selected depth or one or more layers of a tissue may be controlled. For example, in some embodiments of the present and previous aspects of the invention, there is provided delivery of the agent to induce at least a mucosal immune response by controlling the delivery of the agent such that

the majority of the agent is delivered into the the epithelial and sub-epithelial layer of the mucous membrane.

Accordingly, in some embodiments of the present and previous aspects of the invention, delivery of the agent induces at least a mucosal immune response. The agent may be applied using the operational parameters described herein, and preferably a sufficient dose of agent remains resident in the mucous membrane, at least temporarily, in order to induce an immune response in the mucous membrane. More specifically, a sufficient dose of agent remains resident at least temporarily in one or more of the epithelial or sub-epithelial layers of the mucous membrane.

Accordingly there is provided a method of inducing at least a mucosal immune response in a subject, including the steps of

applying ultrasound to an agent within an agent carrier body, agent carrier or agent applicator, wherein the ultrasound is the transportation stimulus; the agent carrier body comprising a tissue contacting surface for engaging the tissue; and

configuring the operational parameters of the agent applicator to enhance or enable delivery of said agent to the epithelial layer or both the epithelial layer and sub-epithelial layers of the mucous membrane,

wherein delivery of the agent induces at least a mucosal immune response.

In another aspect of the present invention there is provided is an agent for use in inducing at least a mucosal immune response in a subject, wherein the agent is contained within an agent carrier body or agent carrier or agent applicator, the agent carrier body comprising a tissue contacting surface for engaging the tissue; and the agent is delivered into the epithelial layer, or into the epithelial and sub-epithelial layers of a tissue, wherein delivery of the agent induces at least a mucosal immune response.

In another aspect of the present invention there is provided is use of an agent in the preparation of a medicament for inducing at least a mucosal immune response in a subject, wherein the agent is contained within an agent carrier body or agent carrier or agent applicator, the agent carrier body comprising a tissue contacting surface for engaging the tissue; and the agent is delivered to the into the epithelial layer, or into the epithelial and sub-epithelial layers of a tissue, wherein delivery of the agent induces at least a mucosal immune response.

The agent in these aspects of the invention is delivered to the epithelial and sub-epithelial tissue according to the methods described herein, and by configuring the operational parameters of the agent applicator.

In a further aspect of the invention, there is provided a system for delivering an agent to a tissue to induce at least a mucosal immune response in a subject, the system including:

an agent contained within an agent carrier body, agent carrier or agent applicator, the agent carrier body comprising a tissue contacting surface for engaging the tissue; and

a means for applying an ultrasonic signal, wherein ultrasound is the transportation stimulus;

wherein the system is configured to enhance or enable delivery of said agent into the epithelial layer, or into the epithelial and sub-epithelial layers of the tissue,

and delivery of the agent induces at least a mucosal immune response in the subject.

Whereas delivery of an agent to the epithelial layer or the epithelial and sub-epithelial layer can induce at least a mucosal immune response and potentially a systemic immune response, controlling delivery of the agent through those layers to layers beneath the sub-epithelial layer can more assuredly induce a systemic immune response. For example, in some embodiments of the present and previous aspects of the invention, there is provided delivery of the agent to induce a systemic immune response is by controlling the amount of agent delivered to be into and through the epithelial and sub-epithelial layers of a tissue to underlying tissue.

Accordingly there is provided a method of inducing a systemic immune response in a subject, including the steps of

applying ultrasound to an agent within an agent carrier body, agent carrier or agent applicator, wherein the ultrasound is the transportation stimulus; the agent carrier body comprising a tissue contacting surface for engaging the tissue; and

configuring the operational parameters of the agent applicator to enhance or enable delivery of said agent into and through epithelial and sub-epithelial layers of a tissue to underlying tissue

wherein delivery of the agent induces a systemic immune response in the subject.

In another aspect of the present invention there is provided is an agent for use in inducing a systemic immune response in a subject, wherein the agent is contained within an agent carrier body or agent carrier or agent applicator, the agent carrier body comprising a tissue contacting surface for engaging the tissue; and the agent is delivered into and through epithelial and sub-epithelial layers of a tissue to underlying tissue, wherein delivery of the agent induces a systemic immune response in the subject.

In another aspect of the present invention there is provided use of an agent in the preparation of a medicament for inducing a systemic immune response in a subject, wherein the agent is contained within an agent carrier body or agent carrier or agent applicator, the agent carrier body comprising a tissue contacting surface for engaging the tissue; and the agent is delivered into and through epithelial and sub-epithelial layers of a tissue to underlying tissue, wherein delivery of the agent induces a systemic immune response in the subject.

The agent in these aspects of the invention is delivered into and through the epithelial and sub-epithelial layers of a tissue to underlying tissue according to the methods described herein, and by configuring the operational parameters of the agent applicator.

In a further aspect of the invention, there is provided a system for delivering an agent to a tissue to induce a systemic immune response in a subject, the system including:

an agent contained within an agent carrier body, agent carrier or agent applicator; the agent carrier body comprising a tissue contacting surface for engaging the tissue; and

a means for applying an ultrasonic signal, wherein ultrasound is the transportation stimulus;

wherein the system is configured to enhance or enable delivery of said agent into and through epithelial and sub-epithelial layers of a tissue to underlying tissue,

and delivery of the agent induces an immune response in the subject.

In some embodiments of the present and previous aspects of the invention, the agent induces both a mucosal immune response and systemic immune response.

The methods of the invention described herein can also include one or more of the steps:

- loading the agent carrier body and/or agent carrier with agent;
- providing the agent carrier body or agent carrier holding the agent;
- bringing a tissue contacting surface of the agent carrier body or agent carrier into direct or indirect contact with said tissue; and
- dispensing the agent from the agent carrier body or agent carrier to the tissue surface, wherein the step of dispensing the agent preferably includes generating ultrasonic signal to cause or facilitate transportation of the agent to the tissue-contacting surface.

By indirect contact it would be understood that a substance such as a gel may be placed in between the agent carrier body and the tissue.

As would be understood by the skilled person, the delivery of agent to one selected layer, may not be absolute. For example, the operational parameters of the agent applicator may be configured to deliver a sufficient amount of the agent (and by 'sufficient amount' it would be understood to mean an amount sufficient to induce an immune response) to, for example, the epithelium. But a small amount of the agent may also end up in the sub-epithelium. This small amount of 'overflow' is not contemplated to be delivery to both the epithelium and sub-epithelium in accordance with the invention. Rather, if it is intended that a sufficient amount of agent be delivered to both the epithelium and sub-epithelium, it is required that that specific operational parameters of the agent applicator would need to be configured in order to specifically achieve delivery of a sufficient amount of the agent to those layers. Similarly, delivery of the agent into and through, for example, the epithelium and sub-epithelium layer of tissue may result in some of the agent remaining in either or both of those layers; but for the purposes of the invention, a sufficient amount of agent will be delivered to the underlying tissue.

In some embodiments of the present and previous aspects of the invention, delivery of an agent induces immunity against infections and infectious agents that gain access to the body via mucous membranes.

The agent carrier, agent carrier body or agent applicator described in each of the above aspects and embodiments of the invention is as described in any one of the aspects or embodiments described herein. For example, as described herein, the tissue contacting surface of the agent carrier body may be at least partly defined by a plurality of protrusions. The agent carrier body may also include a stack of layers including the

tissue-contacting layer and at least one other layer. And the operational parameters of each aspect or embodiment of the invention are preferably selected from those listed in the first aspect, and more preferably, are selected to deliver a chosen amount of agent to a selected depth range within tissue, or to one or more selected layers of a tissue.

The tissues and tissue layers described in each of the above aspects and embodiments of the invention are as listed above in the first aspect. The selection of a tissue and the specific layers thereof to deliver the agent to may be on the basis of the immune response to be achieved.

The delivery or use of the agent in each of the aspects and embodiments of the invention is preferably non-invasive.

As used herein, except where the context requires otherwise, the term "comprise" and variations of such term, such as "comprising", "comprises" and "comprised", are not intended to exclude further things, additives, components, integers or steps. Also, as used herein, except where there is express wording to the contrary, specifying anything after the words 'include' or 'for example' or similar expressions does not limit what else is included.

Brief description of the drawings

Further aspects of the present invention and further embodiments of the aspects described in the preceding paragraphs will become apparent from the following description, given by way of example and with reference to the accompanying drawings. In the drawings:

Figure 1A shows a schematic cross-sectional block diagram of an agent applicator device according to one embodiment, being applied to a tissue surface and provides an illustration of the overall components of one exemplary agent applicator device.

Figure 1B shows a more detailed cross sectional view of the agent carrier body of the embodiment shown in Figure 1A and previously described in the Applicant's Australian patent application 2013901606.

Figure 1C shows a similar agent carrier body to that of Figure 1B that includes an ultrasonic transducer.

Figure 2 provides a cross sectional block diagram of an embodiment of a handle assembly of the agent applicator device and its basic component parts.

Figure 3 is a cross sectional view through an agent carrier that takes the form of a single use applicator tip including an agent carrier body of the type previously described in the Applicant's Australian patent application 2013901606. As will be appreciated, any agent carrier body as generally described herein, and as exemplified in any one of figures 8A to 10 or 23 to 30A may be used as an alternative.

Figures 4A, 4B, and 4C provide illustrations of various embodiments of a single layer agent carrier body with different micro-channel, and or reservoir arrangements previously described in the Applicant's Australian patent application 2013901606.

Figure 4D provides an illustration of an embodiment of a first surface and a tissue contact surface of a single layer agent carrier body.

Figures 4E, 4F, 4G, and 4H provide illustrations of various embodiments of a multiple layer agent carrier body with different micro-channel and reservoir arrangements.

Figure 4I provides an illustration of the embodiment shown in Figure 4H of a first surface and a second surface of a first layer of the agent carrier body, and a first surface and a tissue contact surface of the second layer of the agent carrier body.

Figure 4J provides illustrations of further example embodiments of agent reservoir contacting layer of an agent carrier body that can store additional agent and replenish the micro-channels as they are depleted of agent during the course of usage.

Figures 5A, 5B and 5C provide illustrations of various embodiments of the agent carrier body each of which has a differently configured surface contact layer.

Figure 5D provides an illustration of two exemplary types of micro-protrusions that extend from the agent carriers shown in Figures 5B and 5C.

Figure 6 provides an illustration of an embodiment of an agent carrier body having a stacked layer arrangement and an agent filling port.

Figure 7A and 7B provide illustrations of embodiments of the holes, and the channels defined by the holes, in an agent carrier body that has a stacked layer structure.

Figures 7C to 7E provides magnified images of the holes and micro-channels created by the micro-manufacturing process.

Figures 8A and 8B are schematic representations of an alternative embodiment of an agent carrier body, according to an aspect of the present invention, and respectively illustrate plan and perspective views thereof.

Figures 8C and 8D are schematic representations of an alternative embodiment of an agent carrier body layer having micro channels formed through it, and respectively illustrate plan and perspective views thereof.

Figures 8E and 8F are schematic representations of an alternative embodiment of an agent carrier body layer having a reservoir formed therein, and respectively illustrate plan and perspective views thereof.

Figures 8G and 8H are schematic representations of an agent carrier body formed by the agent carrier body layer of figures 8E and 8F stacked with the agent carrier body layer of figures 8C and 8D, and respectively illustrate the agent carrier body in unfilled and filled configurations.

Figure 9A and is an electron micrograph of a portion of an agent carrier body of any one of figures 8A to 8H.

Figure 9B and is an electron micrograph of a single protrusion of an agent carrier body of any one of figures 8A to 8H.

Figure 10 illustrates a series of four mask designs, each suitable for forming a respective agent carrier body (or layer thereof) in embodiments of the present invention.

Figures 11A to 11C provide an illustration of a various embodiments in which an agent reservoir is provided in an agent carrier in a location external to the agent carrier body. As will be appreciated any agent carrier body as generally described herein, and as exemplified in any one of figures 8A to 10 or 23 to 30A may be used with such an embodiment.

Figure 12A to 12E illustrate steps in various embodiments of charging or recharging methods that can be used in embodiments of the present invention. As will be appreciated any agent carrier body as generally described herein, and as exemplified in any one of figures 8A to 10 or 23 to 30A may be used with such an embodiment.

Figures 13A and 13B illustrate an exploded view and cross sectional view through agent carrier according to one embodiment. The agent carrier can be used to carry any agent carrier body as generally described herein, and as exemplified in any one of figures 8A to 10 or 23 to 30A.

Figure 14 shows plots for the evaluation of the uptake of FPV-HIV-GFP vaccine 24 hours post lip delivery, illustrating I-Ad APC MHC-II cells containing the fluorescent GFP

antigen of the vaccine detected in the top right hand quadrant indicated by the arrow. Each dot represents a single cell.

Figure 15 illustrates plots for the evaluation of recruitment of antigen uptake by different dendritic cell subsets to the respective draining lymph nodes 24 hours post lip delivery. The proportion of dendritic cells, identified as being MHC-11+, and either CD11b+ (left two columns) or CD11c+ (right two columns) are indicated in the top right hand quadrant (refer to arrows).

Figure 16 illustrates plots for the evaluation of the uptake of FPV-HIV-GFP vaccine 24 hours post lip delivery in cervical, mediastinal and mesenteric nodes I-Ad APC MHC-II cells containing the fluorescent GFP antigen of the vaccine are detected in the top right hand quadrant indicated by the arrow.

Figure 17 contains photographs showing the following phases of the experiments performed. The phases illustrated include: Loading the microchips with the agent to be administered (top left), Ultrasonic system settings (top right) and lip delivery to the mice (bottom photos).

Figure 18 illustrates plots enabling the evaluation of the magnitude of HIV-specific splenic CD8 T cells using IFN-γ intracellular staining. The FACS data were analyzed using Cell Quest Pro or FlowJo analysis. The box indicates the percentage of HIV-specific splenic CD8 T cells expressing IFN-γ following Lip/i.m. (top 3 mice), i.n./i.m. (middle 2 mice) and booster only (bottom 3 mice) vaccinations.

Figure 19 illustrates plots enabling evaluation of HIV-specific splenic CD8 T cells using tetramer staining. The FACS data were analysed using Cell Quest Pro or FlowJo analysis. The box indicates the percentage of HIV-specific splenic CD8 T cells following different routes of vaccine delivery. Lip/i.m. (top three mice), i.n./i.m. (middle two mice) and booster only (bottom two mice).

Figure 20 shows plots illustrating HIV-specific splenic CD8 T cell responses observed with the four different microchips of figure 10. Data represent HIV-specific CD8 T cell numbers measured by tetramer staining (data represent one mouse from each group).

Figure 21 illustrates plots enabling evaluation of HIV-specific splenic CD8 T cell responses using tetramer staining. The FACS data were analyzed using Cell quest Pro software. Plots represent three animals per group microchip 1 (top) & 2 (middle) prime-boost immunization data compared to oral delivery (bottom). The upper right quadrants

(arrows) indicate the % of HIV-specific CD8 T cells observed following each vaccine strategy.

Figure 22 illustrates plots enabling evaluation of the magnitude of HIV-specific CD8 T cell responses using IFN-γ intra cellular cytokine staining. The FACS data were analysed using Cell quest Pro software. Plots represent three animals per group microchip 1 (top) & 2 (middle) prime-boost immunization data compared to oral delivery (bottom). The upper right quadrants (red arrows) indicate the % of HIV-specific CD8 T cells expressing IFN-γ.

Figure 23 illustrates a series of mask designs for creation of various agent carrier bodies (or layers thereof).

Figures 24 to 28 illustrate a series of electron micrographs of agent carrier bodies and regions thereof of various embodiments.

Figures 29 to 30A illustrate diagrammatically two hybrid agent carrier bodies according to an embodiment of the present invention.

Figure 31A to 31D illustrates plots of the displacement (nm) and velocity (m/s) during operation of five types of agent applicators useable with embodiments of the present invention having different tips.

Figure 32 illustrates the tip displacement of the MP4 and AMO 1 agent applicators when driven at selected frequencies over a range of drive voltages.

Figure 33 illustrates the results of experiment 5 and show a control mouse in the left image and images of the expression of mCherry fluorescent antigen assessed at 3h, 6h, and 9h post vaccination in another mouse

Figure 34 illustrates the results of experiment 5 and show a control mouse in the left image and images of the expression of mCherry fluorescent antigen assessed at 3h, 6h, and 9h and 24h post vaccination in another mouse.

Figures 35 to 38 illustrate the results of experiment 6, specifically figures 35 and 36 illustrate the HIV-specific tetramer results and figures 37 and 38 illustrate the IFN-γ staining results from the experiment.

Detailed description of the embodiments

In the following description, for the purposes of explanation, numerous specific details are set forth in order to provide a thorough understanding of the present invention. It will be apparent, however, that the present invention may be practiced without these specific details. In other instances, well-known structures and devices are shown in block diagram form in order to avoid unnecessary obscuring.

[0022] This description follows the following outline:

1. Overview
2 General principles and Micro-Channel Embodiments
3 Protrusion-based embodiments
4 Hybrid and alternative embodiments
5. Loading and use examples
6. Trial results
7. References

1. Overview

Background to the present embodiments

The delivery of drugs, including macromolecules larger than approximately 500 Daltons and hydrophilic drugs, to the body without using hypodermic injections, ingestion or surgery has long been a desired goal in medicine.

A myriad of drug delivery devices using a variety of technologies have been developed to achieve this ("drug delivery devices"), however, these have been unable to non-invasively deliver to the body a large range of drugs in a safe, practical, predicable and effective way. Historically, the transdermal route, has been the primary focus of non-invasive drug delivery applications.

The advantages of delivering drugs to the body without ingestion, includes bypassing the degradation of drugs by the acid and or alkaline regions of the gastrointestinal tract and enzymes in the gastro-intestinal tract and avoiding their metabolism by the liver enzymes as well as removal of the dyspeptic side effects of drugs. Advantages of delivering macromolecules or hydrophilic drugs to the body without hypodermic injections include decreased or elimination of pain, local trauma and side effects,

increased patient compliance, and lowering the incidence of needle contamination, disease transmission and needle misuse. Delivering drugs to the body using implanted devices requires surgery which will have potential risks of complications from the procedure itself (including anaesthetic risk) and the potential risk of complications from the introduction of a foreign body.

Drug delivery devices may be applied to skin for both targeted applications and as a portal for systemic drug delivery. The primary barrier for transdermal transport of hydrophilic molecules and/or molecules larger than approximately 500 Daltons is the outermost layer of the epidermis, the stratum corneum, which is typically 10–20 μm in thickness. The stratum corneum is a nonviable cell layer that is comprised of highly-crosslinked keratinocytes embedded in a continuous matrix of skin lipids. Drug delivery devices are needed to overcome these natural semipermeable barriers to deliver the drugs. Drug delivery devices for the skin commonly use microneedles and/or iontophoresis as the primary means of delivering drugs to such tissues.

Another application site for drug delivery devices, less commonly used as a portal for systemic drug delivery, is mucosal membranes. The primary barrier for trans-mucosal transport of hydrophilic molecules and macromolecules is the epithelial layer. Drug delivery devices for trans-mucosal delivery commonly use nasal sprays, inhalants and/or iontophoresis as the primary means of delivering drugs.

The following technology (either solely or in any combination) is presently used in drug delivery devices:

Iontophoresis

Drug delivery devices that deliver an agent to the body using a process known as iontophoresis operate by generating an electric current that results from the application of electrodes which create and maintain a potential difference between the device and the target tissue. Ionic forms of the drug to be delivered are transported in the electric current and thereby gain access to the target tissue. Devices that deliver an agent to the body using iontophoresis commonly have a continuous layer of drug containing fluid in which the electrode within the device is bathed. The application time tends to be long, in many cases, hours.

Agents that can be delivered to the body using iontophoresis must be both hydrophilic and have an electrical charge. Iontophoresis is not capable of delivering neutral molecules and/or particles including large proteins and vaccines.

Microneedles

Microneedles are discrete protrusions that function to pierce one or more layers of tissue. Depending on their application, microneedles can be partly or fully hollow or solid. Microneedles used in Drug delivery devices commonly function as: 1) structures that can increase permeability within tissue when combined with certain external stimuli; 2) structures incorporating an agent that dissolves into tissue; 3) hollow conduits for injection of agent into tissue; and/or 4) structures designed to scrape surface tissue or expose internal tissue. Microneedles are commonly incorporated into patches that are applied to skin either with adhesives or are mechanically engaged. They may also contain compounds to enhance the penetration of agent through tissue or applied to tissue after it has been pre-treated with permeation enhancer compounds.

Sonophoresis

Drug delivery devices that deliver an agent to the body using a process known as sonophoresis operate through applying ultrasound to tissue that both increases the permeability of tissues and provides kinetic energy to the agent. The increase in permeability of tissues through ultrasound results from a number of phenomena including any one or more of the following: 1) cavitation though generation and oscillation of gas bubbles; 2) thermal effects from an increase in temperature causing induction of convective transport; or 3) mechanical effects through occurrence of stresses due to pressure variation induced by ultrasound. Low frequency ultrasound, generally in the range of 20-200 kHz, but preferably below 100kHz, has been found to be more effective for sonophoresis than higher frequencies of ultrasound. The prime method of sonophoretic transport through skin requires power sufficient to create cavitation.

Drug delivery devices that deliver an agent to the body using sonophoresis commonly have a layer of fluid containing the agent in which the source of the ultrasound is bathed or is placed in close proximity. These devices also sometimes include various kinds of microneedles where the microneedles are bathed in such fluid. In each of the aforementioned devices, because fluids attenuate the power of ultrasound more than solid materials, and the volume of fluid on which the ultrasound acts is large with

respect to solid structures within or around it, the ultrasound energy is considerably attenuated by the time the wavefront approaches the tissue surface. This ultrasonic wavefront is partially reflected from the tissue surface back into the fluid layer which further disrupts the efficiency of the ultrasound resulting in the need for more power to be applied to the fluid. These techniques have some potential drawbacks, for example, ultrasound applied to tissue can, depending upon the magnitude of power, cause localised damage from cavitation and thermal effects. The threshold for damaging tissue from ultrasonic power depends on a variety of factors including the type of tissue, the thickness of tissue, the health of the tissue and whether the tissue is intact. For example, the skin is capable of tolerating more ultrasonic power being applied to it than mucous membranes and ocular tissues. Furthermore, ultrasound applied to an agent may, depending upon the magnitude of power, cause the agent, or molecules within it, to cleave or denature or otherwise be damaged from cavitation, thermal or mechanical effects. Agents which are known to have a low tolerance to ultrasonic cavitation, mechanical forces or temperatures above 40 degrees centigrade include vaccines, proteins and other biologics.

Overview of the embodiments

In summary, preferred embodiments of the present invention use low frequency ultrasound at low power to transport an agent, contained within an agent carrier body having micro-scale structures within it, for delivering the agent non-invasively to tissues.

As will be appreciated, ultrasound will be applied over one or more frequency bands or over a frequency spectrum having several bands. Preferably the band(s) correspond to a resonant frequency of the agent applicator device including the agent carrier body, and optionally one or more harmonics of the resonant frequency. In some forms of the present invention, the ultrasound applied is of a low frequency, between 20kHz to 100kHz, most preferably the frequency of the ultrasonic energy is between 20kHz and 40kHz. This is particularly preferred for use with the mucous membranes, eyes and other delicate tissues. However, in other embodiments the agent applicator device may have a resonant frequency lower than this, and the devices describe herein may be operated with a primary resonant frequency at the tip of the agent carrier body of around 10kHz. In testing, agent applicators suitable for use with embodiments of the present invention have been operated at frequencies in any one or more of the following frequency bands, a band centred at or about 10kHz; 20kHz, 22kHz, 28kHz, 28.19kHz,

and 38kHz and/or frequency bands of 20-25kHz, 25-30 kHz, 38-40kHz, 40-45kHz, 40 to 60kHz, 40-80 kHz, 140-160kHz.

For other tissues, such as skin, the ultrasonic frequency may be outside these ranges.

In preferred forms of the present invention, the ultrasonic power used is relatively low, typically in the range 0.05 to 3.5 Wcm^{-2}. Higher intensity ultrasonic power may be needed in some applications. In these cases it may be necessary to pulse or otherwise control the duty cycle of the ultrasonic energy to prevent tissue damage, (e.g. from thermal effects) and/or to prevent damage to the agent.

The ultrasonic energy applied from the agent applicator causes reciprocating motion of the tissue contacting surface in the agent carrier body. In typical embodiments the displacement of the tissue contacting surface from its mean position may be between about 100nm and 2200nm. Embodiments may operate with a displacement more than 200nm. Embodiments may operate with a displacement less than 2100nm. Embodiments may operate with a displacement more than 400nm. The embodiments used in the experiments operated with a displacement less than 500nm, and more specifically less than 400nm.

Figure 31A illustrates plots of the displacement (nm) and velocity (m/s) during operation of two types of agent applicator, MP1 and MP4, with two types of tip assembly, e.g. agent carrier, “Tip assembly 1” and “Tip assembly 2” at a range of frequencies between 0 and 200kHz.

Figure 31B illustrates plots of the displacement (nm) and velocity (m/s) during operation of three types of handle unit useable in an agent applicator according to an embodiment, AMO1(Model no. Sov37706); AMO2(Model no. Sov39302) and ALCON1 (Model no. Turbo Sonic-375), without a tip assembly, e.g. agent carrier, at a range of frequencies between 0 and 200kHz.

Figure 31C illustrates plots of the displacement (nm) and velocity (m/s) during operation of three handle units, AMO1; AMO2 and ALCON1, with a second type of tip assembly, e.g. agent carrier, at a range of frequencies between 0 and 200kHz.

Figure 31D illustrates plots of the displacement (nm) and velocity (m/s) during operation of three types of agent applicator, AMO1; AMO2 and ALCON1, with a third type of tip assembly, e.g. agent carrier, at a range of frequencies between 0 and 200kHz.

Figure 32 illustrates the tip displacement of the MP4 and AMO 1 agent applicators when driven at selected frequencies over a range of drive voltages. The MP4 system was driven through an RF amplifier at 22kHz over a range of input voltages of between 50 to 400V peak to peak. The AMO1 was driven at 28.19kHz over the same voltage range.

The studies of the devices were performed using a laser doppler vibrometer model MSA400 from Polytec instruments in Germany.

As can be seen by selecting the frequency (or frequency band) of operation desired oscillation parameters can be chosen. These parameters will vary depending on the particular agent carrier used. In preferred embodiments these devices will be operated in a frequency band that corresponds to one or more of the peaks in motion as illustrated in the plots.

In typical embodiments the velocity of motion of the tissue contacting surface may be between about 0.01m/s and 0.4m/s. Embodiments may operate with a velocity more than 0.03m/s. Embodiments may operate with a velocity less than 0.36m/s. Embodiments may operate with a velocity more than 0.06m/s. The embodiments used in the experiments operated with a displacement less than 0.05m/s.

Embodiments of the present invention may advantageously be used in the delivery of agent to delicate tissues, such as mucous membranes (including the conjunctiva, buccal mucosa and labial mucosa), the ocular tissues.

The ultrasonic power and/or frequency parameters in embodiments may be increased or decreased for a variety of reasons including to control the depth of penetration of the agent into tissue. As an example, the ultrasonic power and/or frequency parameters used for delivering agent to the epithelial surface cells of a mucous membrane, may be lower than power and/or frequency parameters used for delivering agent to the rich blood vessel capillary beds and deeper connective tissue layers that lie below the epithelial surface.

It is intended that in the preferred embodiments the agent carrier body does not penetrate any layer of the tissue surface. Although some superficial cell damage may occur in using the preferred embodiments of the present invention, it is not intended and is not relied upon in order to achieve delivery of the agent to the target tissue. Maintaining an intact tissue surface as much as possible may serve to more accurately control the depth of penetration of the agent into tissue layers.

The various micro-scale structures within the agent carrier body described herein, amongst other things serves the purpose of making direct contact between the agent carrier body and the tissue surface to propagate ultrasonic energy, thereby minimizing the extent of any continuous layer fluid within the agent carrier body and between the agent carrier body and the target tissue (which tend to attenuate ultrasonic waves).

One group of embodiments first described in the Applicant's International patent application PCT/AU2014/050027, (the contents of which are incorporated herein by reference for all purposes), include an agent carrier body having microstructures that form a plurality of micro channels surrounded by rigid walls for delivery of various agents. The micro channels are typically in the range of approximately 25 to 100 μm across when measured transverse to the direction of delivery, may have a length of between approximately 0.5mm to 2mm. Any suitable cross-sectional and/or longitudinal geometry can be used.

In use, each channel contains the agent in a fluid column within the channel and the ultrasonic energy is directly applied to each fluid column and the walls surrounding the fluid column. The ultrasonic wave is generated to be longitudinal in nature, i.e. it propagates along the channel. In some embodiments, by using the micron scale architecture of the microstructures, the wave front that impacts the fluid column is concentrated within each micro channel thus reducing attenuation of ultrasound. Reflection of ultrasonic waves at tissue surface is minimized by having direct contact of the device, and most preferably the agent carrier body, with the tissue surface so as not to permit the presence of a fluidic space between them. This further assists molecules to efficiently move toward the target tissue under the influence of ultrasound along the ultrasonic wavefront path. The ultrasonic waves are also carried in the agent carrier body, and specifically in the walls defining the micro channels. Since they do not attenuate the ultrasonic energy as much as fluids do, they efficiently transmit the sonophoretic power to the target tissue directly.

In preferred embodiments, the tissue-contacting surface of the device is not separated from the tissue by a continuous layer of fluid. The tissue-contacting surface of the agent carrier body presents a surface that has areas of solid body and liquid agent (i.e. the openings of the micro channels), in some embodiments approximating a solid-liquid "checker board"-like array. This arrangement may facilitate the sonophoretic ability of the device since the faces of the solid walls directly contact the tissue. In such

embodiments the device architecture might be conceptualized as a large number of individual micron-scale sonophoretic delivery devices tightly packed and joined together.

Another group of new embodiments include a plurality of micro-scale structures that is formed by micron-scale protrusions that together define the tissue contacting surface of the agent carrier body. These protrusions contact the target tissue and the agent to be delivered surrounds them. In preferred forms, the agent carrier body has a peripheral structure, typically a wall, that surrounds the protrusions and contains the agent in use. This embodiment has a lower ratio of microstructures to fluid within the agent carrier body compared to an agent carrier body comprised of micro-channels. Preferably these embodiments maintain direct contact between the ultrasonic source and the target tissue via the protrusions, and possibly also the peripheral structure. The longitudinally directed ultrasonic waves are conducted by the protrusions and the fluid between. The protrusions act by facilitating the transport of drugs toward the target tissue. Waveform interference from fluid in adjacent spaces between protrusions is minimised by the presence of the protrusions, which serve to at least block propagation of waveforms.

Another group of new embodiments present a hybrid device, having at least one region having multiple micron-scale protrusions and at least one other region having micro channels surrounded by rigid walls. Typically a region or regions having micro channels surrounded by rigid walls will form part of a peripheral structure bounding a region that has micron-scale protrusions.

It should be appreciated that methods and systems of the present invention may use an agent carrier having an agent carrier body that falls into to any of the above groups.

Molecules that are known to the inventors to possibly be delivered to the body using sonophoresis include 1) molecules having any kind of electric charge or have a neutral (including overall neutral) electrical charge and 2) small or large molecules (including monoclonal antibodies of approximately 150,000 Daltons) and 3) molecules that are hydrophilic or hydrophobic or lipophilic.

The present inventors have additionally realized that delivering vaccines primarily to mucous membrane epithelia using the present invention creates new opportunities to induce mucosal immunity to prevent or treat diseases or conditions whose origin is by initial infection at mucous membranes including, but not limited to influenza, HIV/AIDS, Human Papilloma Virus, tuberculosis and other pathogens. Mucosal immunity also

offers opportunities to treat or alleviate autoimmune diseases, cancers, allergies or the like. Several studies have demonstrated that stimulation of the mucosal immune response can result in production of protective B and T cells in both mucosal and systemic environments so that infections may be confined to the area of entry and prevented from gaining access to other tissues in the body. In particular, mucous membranes produce a special type of antibody called secretory IgA or sIgA. Moreover, it is believed that antibodies and cytotoxic T cells generated through mucosal immunity are more effective than antibodies and cytotoxic T cells generated through systemic immunity for pathogens that gain entry to the body through mucous membranes.

Several exemplary embodiments of the various aspects of the invention are described with reference to an exemplary agent applicator device for delivering an agent non-invasively to a target tissue surface site via a transportation modality, which preferably uses only ultrasonic waves. In these exemplary embodiments, at the target tissue surface site, penetration of the agent into the target tissue surface site is enabled or enhanced through sonophoretic mechanisms. Preferably, target tissue surface sites are mucous membranes including, but not limited to, conjunctival, vaginal, urethral, inner ear, tracheal and bronchial mucosa, anal, oral, and nasal tissues. A target tissue surface can also include the cornea.

2 General principles and Micro-Channel Embodiments

The system comprises an agent applicator device that is preferably hand-held and used for delivering an agent to a target tissue. The preferred form of agent applicator device includes a handle coupled to an applicator tip. The applicator tip includes an agent carrier body that has micro channels formed in it through which the agent is delivered from within the applicator tip to a target tissue surface. The agent carrier body may be integrated within the applicator tip, or may be a separate component (such as a cartridge) that is attachable to the applicator tip.

The applicator tip may include a reservoir that holds an agent. The reservoir may form part of the agent carrier body, or may be a separate component that is in fluid communication with the agent carrier body.

An ultrasonic transducer forming part of the handle or applicator tip generates ultrasonic energy (waves) which causes the agent to be moved through the micro channels in the agent carrier body, egress through terminal pores of the micro channels at a tissue contacting surface of the agent carrier body and onto the target tissue surface. The

ultrasonic waves also enhance and/or permit agent uptake into the target tissue through sonophoresis.

Figure 1A is a highly schematic diagram illustrating a first embodiment of an agent applicator device according to the present invention that is useable with any agent carrier or agent carrier body described herein. In this example, an agent applicator device 100 includes an applicator tip 102 coupled to an applicator handle 103 (entire device not shown). The applicator handle 103 includes an ultrasonic generator 101. The applicator tip 102 is connected to the handle 103 so that ultrasonic energy from the transducer 101 is transmitted to it via a coupling rod 106. As will be appreciated the application of ultrasound will be generally in accordance with the parameters set out in the overview above. The tissue contact surface of the applicator tip 102 is brought into contact with a target tissue surface 108. The ultrasonic generator is then activated, which results in the propagation of ultrasonic waves 110 via the coupling rod 106, through the applicator tip 102 and the agent carrier body 104 and into the target tissue 108. In this embodiment, agent is stored in the agent carrier body 104 and is transported to the target tissue surface 108 via micro channels 112 that have been fabricated within the agent carrier body 104. Ultrasonic waves assist in the transport of agent from the agent carrier body 104 to the target tissue surface 108 via the micro channels 112. Ultrasonic waves also enhance and/or permit the penetration of the agent into the target tissue 108 via sonophoretic effects on tissue ultrastructure.

In this example, the agent carrier body 104 may be of any type described generally herein, and as exemplified in any one of figures 8A to 10 or 23 to 30A. However, to illustrate the principle of operation of an agent carrier body Figure 1B provides a more detailed view of an agent carrier body 104 of the type previously described in the Applicant's Australian patent application 2013901606, 1A applied to the tissue surface 108. The agent carrier body 104 has a tissue-contacting surface 114. In this example it includes with micro channels 112 fabricated within the agent carrier body 104 that extend from within the interior of the agent carrier body 104 to the tissue-contacting surface 114. The micro channels 112 terminate as pores 116 at the tissue-contacting surface 114. Agent is provided from the agent carrier body 104, through the channels 112 where it egresses through the pores 116 in the tissue-contacting surface 114, and on to the tissue surface 108. As an alternative the agent carrier body 104 may be of any

type described generally herein, and as exemplified in any one of figures 8A to 10 or 23 to 30A.

In this example, ultrasound 110 is generated and conducted through the agent carrier body 104. This causes agent 118 stored within the channels 112 to be released from the channels 112 and on to the tissue surface 108. The penetration of agent into the tissue 108 is enhanced and/or permitted by the use of ultrasound, which provides a sonophoretic effect on the tissue.

In the embodiment of figure 1A, the applicator handle 103 has an ultrasonic transducer 101, which generates ultrasonic waves 110 that are transmitted through the applicator tip 102 to the agent carrier body104 via the coupling rod 106. However, in alternative embodiments the applicator tip 102 can be fabricated to include within its structure, a system that is capable of generating ultrasonic waves itself without the need for an external ultrasonic transducer. Figure 1C illustrates an alternative embodiment in which the agent carrier body 104 additionally includes an ultrasonic transducer 124.

It is preferred that the inner surface(s) of the channel112 are functionalised. The inner surface 113 of the channels 112 may be functionalised with compounds or molecules having hydrophobic or hydrophilic properties or a combination of both moieties. Alternatively, the surface 113 of the channels 112 may be functionalised by contacting the surface of the channels with small molecules that are adsorbed to the surface of the channels, exposing specific functional groups that have the desired physical and/or chemical properties. The small molecules may be adsorbed through chemisorption or physisorption to the internal surface of the channels. Alternatively, or in addition to changing the water/oil affinity, the inner surfaces of the micro-channels and/or agent reservoirs may be functionalised by enabling them to become electro-conductive.

Figure 2 provides an illustration of an embodiment of the handle assembly 200 of an agent applicator device, usable with an agent carrier body of any type described generally herein, and as exemplified in any one of figures 8A to 10 or 23 to 30A. The handle assembly 200 includes a main housing 202, which contains an ultrasonic transducer 204. The transducer is powered by a battery 206 (or alternatively by an external power supply) and is configured to generate ultrasonic waves and transmit them to a coupling rod 208 that terminates in a connector 210. The connector 210 can be of any type for example a screw thread or bayonet fitting or the like, that enables the

handle assembly 200 to engage with an agent carrier (through either direct or indirect engagement).

Figure 3 is a schematic cross section of an agent carrier in the form of an applicator tip 300 that may be used with the handle assembly 200 of Figure 2. The applicator tip 300 includes a housing 301 having a first end 302 and a second end 303. The first end 302 includes a mounting mechanism 305 such as a bayonet fitting or screw thread or the like, that makes a mechanical connection with a connector 210 of the handle assembly 200.The applicator tip 300 further includes a recess 304 at its second end 303 that is arranged to accept the agent carrier body 104 or an agent carrier body of any type described generally herein, and as exemplified in any one of figures 8A to 10 or 23 to 30A . The applicator tip 300 is configured, in use, to carry agent to the tissue-contacting surface 306 of the agent carrier body 104 and deliver it as required to tissue being treated by application of ultrasonic waves. In some embodiments the applicator tip 300 can include an agent reservoir, which is fluidically in contact with the micro channels formed in the agent carrier body 104.

Figures 4A, 4B, 4C, and 4D provide illustrations of various embodiments of single layer agent carrier bodies, and Figures 4E, 4F, 4G, 4H provide illustrations of various embodiments where an agent carrier body is created from stacked agent carrier layers.

The agent carrier body 400 is formed of a layer(s) of solid material and possesses a number or network of micro channels that may be a variety of geometric shapes and sizes. These micro channels can be used to store or retain an agent and also to deliver agent from within the agent carrier body 400 to a tissue- contacting surface 406 of the agent carrier body 400. The micro channels can be created by a micro-fabrication technique. For instance, in embodiments where the agent carrier body 400 is formed from silicon, the micro channels can be formed by lithography, etching and/or other processes. In embodiments made from metal, plastics or polymers the micro channels can be created by other techniques including the use of lasers of various types and wavelengths and molding and extrusion technologies. The use of these micro-fabrication techniques are particularly desirable as they provide the advantages of retained agent volume accuracy, the benefits of predicable micro-fluidics and further permits refinements such as specialised surface chemical treatment to either or both the exposed tissue-contacting surface and the internal walls lining the micron-scale cavities

402 of the agent carrier body 400. These benefits can be used, for example, to further enhance agent loading, retention and delivery to a target tissue.

The tissue-contacting surface 406 has a series of openings, fenestrations or pores 404. A wide variety of shapes and sizes of pores can be on the order of 10 to 100 μm, but other embodiments may have pore sizes up to 1000μm. The micro channels 402 extend from the pores 404 in the tissue contact surface 406 at least partially through the agent carrier body 400. The micro channels 402 can be used for both retention of the agent and transportation of the agent to a tissue surface.

The pores 404 may have a patterned appearance and exhibit a range of geometries, for example: close packed hexagon structures, arrayed squares with assorted densities, mixed polygon mosaics, spirals, lines etc. The desired geometries are physically etched into the agent carrier body 400 so as to create arrays of micro channels 402 for retention and/or transport of an agent. The micro channels may be in a variety of shapes for example cylindrical, conical etc.

The walls of the micro channels 402 and/or other internal surfaces within the agent carrier body 400 may be treated such that: they have hydrophilic or hydrophobic characteristics that may be the same or opposite in nature to each other and/or the areas between the pores 404 of the tissue-contacting surface 406. The walls of the micro channels 402 and/or other internal surfaces within the agent carrier body 400 may be treated such that they conduct electric charge or can generate a local electric field that may have the same or opposite polarity to each other and/or the areas between the pores 404 of tissue contacting surface 406.

The agent carrier body 400 can be formed from a unitary piece of material. However, in alternative embodiments the agent carrier body may include a number of layers that are stacked. The use of micro-fabricated solid material as single or multiple layers to create an agent carrier body allows for improved acoustic transmission and thus improved delivery of agent to a target tissue site by ultrasound.

The dimensions and internal lining characteristics of the micro channels 402 and/or other internal surfaces within the agent carrier body 404, and the dimensions and number of layers comprising the agent carrier, will be tailored to suit the agent and the target tissues, and will vary as a consequence of agent properties, dose and formulation requirements, ultrasonic power and heat generation, and the duration of use.

Figure 4B shows another embodiment, similar to that of Figure 4A, except that the micro channels 402' are interconnected by internal linking channels 408. Such a structure provides some level of agent storage in addition to channels 402' alone.

Figure 4C represents a further embodiment in which the single layer agent carrier body 400" has micro-channels 402" which terminate as pores 404" in the tissue-contacting surface 406" at one end of the micro-channels 402", and connect at their other end to an agent reservoir 410.

Figure 4D provides surface views of a single layer agent carrier body shown in any one of Figures 4A to 4C. The agent carrier body 400" has a first surface 411 and a second surface 412 which is the tissue-contacting surface. As previously discussed, micro-channels extend from within the agent carrier body 400 (from a reservoir 410 or linking channel 408 if present) and terminate as pores 404 in the tissue-contacting surface 412.

In alternative embodiments, the agent carrier body has a stacked layer structure and includes at least two layers. More preferably, one or more layers have additional micro-reservoir volumes formed within them and which are in fluid communication with the micro-channels for holding agent prior to application to the tissues being treated. The micro-reservoir volume may be a single volume or a plurality of small volumes, e.g. each of which is contiguous with one or a group of micro-channels. There may be a single large reservoir volume in the layer furthest from the tissue-contacting layer that is fluidically connected with the channels. Alternatively, there may be multiple micro-reservoir volumes, with each of the micro-reservoir volumes being in fluid communication.

Figures 4E, 4F, and 4G correspond with Figures 4A, 4B, and 4C respectively, except that the agent carrier body 413 includes a first layer 414, 414', 414" and second layer 416, 416', 416". The first layer 414, 414', 414" is as generally described with respect to the single layer embodiment of Figures 4A, 4B, and 4C, except instead of having a tissue contacting surface 422, the first layer has an interface surface 415 including pores or blind holes that defines a portion of the micro channels that extend through the first and second layers when the layers are stacked together. The second layer 416, 416', 416" includes a first surface 420 that contacts the interface surface 415 of the first layer 414, 414', 414" and a tissue-contacting surface 422 having pores 426 that are formed by micro channels 424. As can be seen the micro channels 424 extend from within the first layer, through the second layer 416, 416', 416", and terminate at the

tissue-contacting surface 422 of the second layer 416, 416', 416" as pores 426. In this way, the holes in the first layer 414, 414', 414" and second layer 416, 416', 416" are aligned to form the micro channels 424 so that the first layer 414, 414', 414"and second layer 416, 416', 416" are connected permitting fluid continuity in the system.

Figure 4H illustrates a further alternative embodiment of a double stacked layer agent carrier body 413 in which the first layer 414"' contains an open-ended agent reservoir 425 that provides agent directly into the micro-channels of the second layer 416"'.

Figure 4I provides surface views of the various layers of a double layered agent 413" carrier shown in Figure 4H. The first layer 414" has a first surface 430 and a second surface 432. The second layer 416" has a first surface and a second surface (which are the same and are generally represented as 434). The agent reservoir 425, is formed by a recess formed in first layer 414" that extends partially into it. The second surface 432 of the first layer 414" is aligned and placed over the interface surface of the second layer 416" such that substantially all of the micro-channels 424 formed in the second layer are fluidically connected with the agent reservoir 425 in the first layer 414".

Figure 4J provides illustrations of further embodiments of agent reservoirs formed in an agent carrier body that can store additional agent and replenish the micro-channels as they are depleted of agent during the course of usage. The reservoirs may connect to micro-channels in the same agent carrier body layer as shown for example in Figure 4G or connect to micro-channels in a contiguous layer in the agent carrier body as shown for example in Figure 4H. Agent carrier body 438 includes a reservoir formed by two annular ring shaped reservoir volumes 440 and 442 and includes a conduit 444 extending through a port 446. When a vacuum is applied to the port 446, or the port 446 is injected with agent, a negative pressure or a positive pressure respectively is applied to the reservoir 440, 442. A layer of this type is arranged in a stack of layers to form the agent carrier body, the first layer overlies its adjacent layer such that any holes in the adjacent layer fluidically connect to the reservoir volumes to allow agent to travel via micro channels through the layers and to the tissue-contacting surface.

Agent carrier body 448 is another embodiment in which the reservoir consists of a number of concentric rings each fluidically connected to each other. It will be appreciated that other arrangements of the agent reservoir volumes within a layer are possible without departing from the invention.

Generally, the holes in a lower or intermediate layer of an agent carrier body extend through the whole thickness of that layer and in combination with subsequent fluidically connected holes in other layers, form a micro channel that extends from the tissue-contacting surface in the surface contact layer of the agent carrier. It will be appreciated that in certain instances the holes only extend partway into a particular layer; this can be the case for the first layer as illustrated for example in Figures 4E – 4G.

As stated previously, it is preferred that the inner surface(s) of the micro channels and other internal surfaces of the agent carrier, such as those of the agent reservoirs, may be functionalised.

In the embodiments illustrated in Figures 5A, 5B and 5C the agent carrier body includes six layers including a surface tissue-contact layer and five layers stacked on top of one another overlying the surface contact layer.

Figure 5A shows an embodiment of an agent carrier body 500 having six stacked layers 501.1, 501.2, 501.3, 501.4, 501.5, 501.6 The first end of the agent carrier body is a surface tissue-contact surface 502 on layer 501.6 that contacts the tissues being treated. On top of this layer there are a plurality of additional layers and a top most layer 501.1. In this embodiment the agent reservoirs and micro channels for holding and delivering agent (not shown) may extend through some or all of the layers 501.1-501.6 of the agent carrier 500. In some embodiments, the channels extend from the tissue-contact surface 502 in layer 501.6, through intermediate layers 501.5 to 501.2, and terminate in the top-most layer 501.1.

Figure 5B shows an alternative embodiment of a six stacked layer agent carrier body 505 to that shown in Figure 5A. In this embodiment, the surface tissue-contact layer 501.6 includes a number of micro-protrusions 506, which in this example are micro - tubules. Figure 5C shows a further alternative agent carrier body 510 having a similar overall arrangement but in which the micro-protrusions 506' are micro-needles. The micro-protrusions are hollow, and included channels formed therein that form a part of the system of minor channels for delivering the agent. As noted below, the use of microneedles is not preferred, so as to maintain an intact tissue surface, as far as possible.

Micro protrusions, such as micro-needles and microtubules can be created by secondary fabrication consisting of etching the tissue contact surface 502 of a tissue-contacting layer 501.6 such that the areas between the pores are largely removed. This

leaves a wall around each pore of the required protrusion to surround each pore. The micro- needles and microtubules can be of any shape desired. For example, Figure 5D shows the micro-protrusions as having a cylindrical shape (micro-tubules 510) and other micro-protrusions as having a frustoconical shape (micro-needles 508). In other embodiments, not illustrated, the surface 502 tissue contact layer 501.6 can be provided with other surface treatments, or surface engaging structures, such as a saw tooth structure, ripples, rings or the like to help the agent carrier body interface with the target tissue.

In a preferred embodiment each layer is disc shaped or cylindrical in shape. Preferably the layers have a thickness of from about 0.3mm to about 1.0mm, and even more preferably each layer has a thickness of about 0.5mm. It is preferred that each layer has a diameter of from about 3mm to about 10mm, and even more preferably has a diameter of about 5mm. The thickness dimension and the diameter dimension may vary between layers. While the layers and overall shape of the agent carrier body have been described as being disc shaped or cylindrical in cross sectional shape, as in Figure 3, other shapes could be employed without departing from the ambit of the invention, e.g. rectangular, square, or other polygon, oval etc. Furthermore, while it is preferred that the overall shape of the agent carrier body is of constant cross section the overall shape of agent carrier body could change along its length e.g. the agent carrier body could be shaped as a frustum (whether conical or otherwise pyramidal), or a prism etc. The overall shape and/or the shape of components of the agent carrier and the agent carrier body can modified in order to maximise the efficiency of the device which is dependent on the transportation modality or combination of transportation modalities employed.

Figure 6 provides an illustration of an agent carrier 600 having an agent carrier body 601 with stacked arrangement. The stack includes a bottom most layer 602, four intermediate layers 604, a top most layer 606.The bottom most layer 602 has micro-tubules 608 extending to form the tissue-contact surface 610. The agent carrier 600 additionally includes a port 612. In this embodiment the port 612 is part of the first layer 606. The port 612 is connected with micro-channels formed in the agent carrier body 601, preferably via an agent reservoir volume in the first layer 606 so that fluid can flow between them. The port 612 is configured to connect to a vacuum line or pressure injector so that a negative or positive pressure respectively can be applied to the port 612. This allows the agent to be loaded into the agent carrier from an external source.

On application of a vacuum to the port 612, agent is drawn through the pores in the microtubules in the tissue contact surface 610, through micro-channels into the stack of layers of the agent carrier body 601 to fill the micro channels and the reservoir volumes. Alternatively, agent can be injected into the agent carrier via the port 612. Using either method, the agent carrier can be charged with an agent.

Figure 6 also shows a closure or seal 614 applied to the port 612, and a closure or seal 616 applied over the surface contact layer 610. The seal 616 seals the surface of the surface contact layer 610 to maintain sterility and any vacuum that is created within the micro channels. Similarly, seal 614 seals the port 612 for similar purposes. It is preferred that this seal layer is a plastic film.

The embodiment of Figure 6 also includes an additional layer 618 and an ultrasonic transducer 620. Layer 618 may be a simple insulation layer that serves to cover the fenestrations in the top layer (if the micro-channels extend the entire way through the top layer) to prevent the egress of fluids and/or to prevent release of a contained vacuum.

The transportation modality may use an electric field to cause a charged agent to be transported. The electric field can be provided by applying a voltage to an electrode in the agent carrier using an internal battery in the agent applicator device or by an external power supply. In a preferred form an electrode is located within the agent applicator device, a second external electrode, also connected to the agent applicator device power supply, can be located in such a way that the target tissue effectively becomes an electrode opposite in polarity to that of the internal electrode. The polarity of the electrodes can be selected such that the internal electrode is of the same polarity as the electric charge on the agent. The voltage established between the two electrodes transports an electrically charged agent through the agent carrier to the tissue-contacting surface and can enhance and/or permit the transport of the charged agent into the tissue via iontophoresis. Embodiments of the invention can use multiple delivery modalities using ultrasonic waves and electric current used in combination either alternately or simultaneously. Accordingly, Layer 618 can additionally be modified to include, or alternatively be, a material that serves as an electrode. The electrode can be positively or negatively charged and is used to generate a static or dynamic electric field. In the case where the top surface of the adjacent agent carrier layer does not have pores and the adjacent agent carrier layer is made from a material that is not electro-

conductive, there is no direct contact between the electrode and the ions or charged agents contained within the micro channels or reservoirs however, ions and charged agents of the same polarity as that existing on the electrode will be repelled. If the adjacent agent carrier layer is made from a material that is electro-conductive and the adjacent agent carrier layer does not have holes, there is electrical conductivity established with the ions or charged agents contained within the micro channels or reservoirs. This scenario is functionally equivalent to the case where the surface of the adjacent agent carrier layer does have pores (and is not dependent on the electro-conductivity of the adjacent agent carrier layer) and the electrode is in direct contact with the ions or charged agents contained within the micro channels or reservoirs, where a further electrode, opposite in polarity to layer 618 can be placed on, or adjacent to, the target tissue. To complete the electric circuit, the electrode placed on or adjacent to the target tissue may be connected to the agent carrier; applicator handle; or other component of the application device (not shown). An applied voltage can provide the energy required to cause an electrically charged agent of the same polarity as the electrode of layer 618, to flow in the fluid contained in the micro channels of an agent carrier body 601 to migrate through the agent carrier, out of the pores to the tissue surface to be delivered into the tissue by iontophoresis.

This provides an alternative embodiment whereby the agent carrier is able to generate an electric voltage to facilitate the flow of an electric current to transport electrically charged agents through the agent carrier and out of the pores to the tissue.

In some embodiments the agent carrier body includes (as with layer 618), or is itself an electrode to facilitate the transport of a charged agent through the agent carrier and out of the pores to the target tissue. The electrode may be located adjacent to the stack of layers, or may be an electrode layer that is integrated within the stack of layers (as with layer 618).

In the above embodiment, ultrasonic energy and/or electrical voltage provide the energy required to move the agent through the agent carrier to its tissue contact surface where sonophoresis and/or iontophoresis enable the agent to be delivered into the target tissue.

As will be appreciated in the above embodiments, a layer including the tissue contacting surface e.g. 422, 422', 422" 502, 610 can be a layer including a tissue contacting

surface being at least partly defined by a plurality of protrusions, such as those described in any one of figures 8A to 10 and 23 to 28.

Figures 7A and 7B provide an illustration of an embodiment of the holes, and the channels defined by the holes, in a stack of layers forming the agent carrier body according to an embodiment of the present invention. Figure 7A provides an illustration of a stack of layers 700 that includes two layers, 702 and 704. Layer 702 is a layer that is further from the tissue-contacting surface than layer 704. The layer 702 includes a plurality of holes 706; the layer 704 includes a plurality of holes arranged as a cluster of holes 708. These layers 704, 702 are arranged adjacent to each other in the stack of layers 700 such that each cluster of holes 708 in layer 704 is aligned with a hole 706 in layer 702. The holes in the layer 704 are more numerous and smaller than the holes in layer 702. To facilitate alignment in the layers during device fabrication each layer 702, 704 can be provided with a datum point or structure 707, 707' which define the alignment of the layer. Layers can then be aligned with their respective datum points 707, 707' arranged in a predetermined fashion (e.g. aligned with each other) to achieve correct alignment of holes in respective layers 702, 704, thereby forming micro-channels that extend through multiple layers of a stack 700.

Figure 7B provides a further illustration of the variation and alignment between holes of different sizes in different stack layers of the agent carrier body. Hole 706' is a magnified version of hole 706. The hole 706' overlies a first cluster of holes 708 (shown in dotted lines) in the next adjacent stack layer. Hole 708' is a magnified version of hole 708. The hole 708' overlies a corresponding cluster of holes 710 (shown in dotted lines) in the next adjacent stack layer. Similarly Hole 710' is a magnified version of hole 710. The hole 710' overlies a corresponding cluster of holes 712 (shown in dotted lines) in the next adjacent stack layer. Hole 712' is a magnified version of hole 712 and so on until the final layer.

Multiple layers can be arranged such that progressing from the top most layer, through the intermediate layers, to the surface contact layer, the diameter of the holes decreases and the number of holes may be increased. Each subsequent layer includes a cluster of holes that is in alignment with a hole in the adjacent subsequent layer. For example, a first layer (which may be the top most layer or an upper one of the intermediate layers) has a number of holes. This first layer overlies a second layer, wherein the second layer has clusters of holes that are arranged beneath the holes in

the first layer. This second layer may overlie a third layer and each hole in each of the cluster of holes in the second layer overlies a further cluster of smaller holes in the third layer (additional layers may also be provided in this manner).

The channels define a flow path for the agent through the agent carrier body to the tissue surface. The channels are defined initially by the diameter of the holes in the first hole possessing layer. Subsequent layers have clusters of holes that are aligned with the holes in this first hole possessing layer. Therefore, progressing from the first hole possessing layer through subsequent layers, the channels become multi-furcated into numerous branches. It will be understood that these numerous branches all form a part of the channel.

Figures 7C, 7D, and 7E show magnified images detailing examples of micro-channels created by a micro-manufacturing process. Figure 7C and 7D (7D showing a higher magnification of 7C) shows a layer in which the holes have square cross-sections. Figure 7E shows a layer that includes holes having square and hexagonal cross-sections.

3. Protrusion-based embodiments

The following series of embodiments include a plurality of microstructures formed from micron-scale protrusions that together define the tissue contacting surface of the agent carrier body. These micro-scale structures contact the target tissue and the agent to be delivered surrounds them. In preferred forms the agent carrier body has a peripheral structure, typically a wall, that surrounds the protrusions and contains the agent in use. This embodiment has a lower ratio of microstructures to fluid within the agent carrier body compared to an agent carrier body comprised of micro-channels. Preferably these embodiments maintain direct contact between the ultrasonic source and the target tissue via the protrusions, and possibly also the peripheral structure. As will be appreciated the application of ultrasound will be generally in accordance with the parameters set out in the overview above.

More specifically figures 8A to 10 and 23 to 30A illustrate several embodiments that employ an agent carrier body including a tissue contacting surface for engaging tissues under treatment, the tissue contacting surface being at least partly defined by a plurality of protrusions. The protrusions preferably extend outward from an inside of a void and terminate at said tissue contacting surface. The void may be formed by a peripheral

structure, where at least part of said peripheral structure may terminate at the tissue contacting surface.

In some embodiments the peripheral structure terminates in a common plane with the protrusions. In others at least some of said protrusions defining the tissue contacting surface extend outward from the void beyond the peripheral structure. In some embodiments, the protrusions may terminate in a plane and the peripheral structure may terminate short of the plane such that the protrusions extend beyond the peripheral structure.

In such embodiments it should be noted that the protrusions of the preferred embodiments do not act as microneedles. Unlike microneedles, the protrusions of the preferred embodiments are not intended to penetrate any layer of tissue. The function of protrusions includes engaging the target tissue by applying pressure resulting in a frictional force on the surface. This aids the positioning of the device (e.g. on the slippery surface of mucous membranes) and enhances the sonophoretic effect.

As will be appreciated, the agent carrier bodies exemplified in these figures, can be used in place of any agent carrier body illustrated herein e.g. agent carrier body 104, 810, 902, 903, 903', 903", 903"', 903"" and 1302, or with any of the agent carriers described herein, e.g. agent carriers 300, 800, 800' and 900, 900', 900", 900"', 900"".

In the preferred embodiments, protrusions include the following properties:

They do not have a needle-like tip, that is, they do not narrow to a point such that their width does not decrease to near zero at the tip.

The cross-section is relatively constant, at least near their tip, and most preferably along their whole length. In most cases the width will not narrow by more than 20%, and preferably less than 10% towards its tip.

they typically have a tip width greater than 10μm. Thus the scale of the protrusions also differs generally from that of microneedles.

They do not enter an intact epithelial surface of the target tissue.

They aid in stabilizing the device by the frictional force they apply when the device is placed in contact with the tissue. This is particularly advantageous on mucous membranes that tend to have a low friction surface due to local mucous secretions.

They generally have a height to width aspect ratio (across their shortest cross sectional width) of between 1:1 to 10:1. Whilst higher aspect ratios may be used it is difficult to achieve acceptable strength that they can withstand handling, loading and application of ultrasonic energy without damage. As will be appreciated cross sectional shape will greatly affect the strength of them and will be chosen accordingly.

In preferred embodiments the protrusions occupy more than 5% of the volume surrounding them in which agent is carried. This percentage needs to be high enough so that the capillary force or other forces retain the agent within the agent carrier body against gravity or other forces caused by normal handling. In embodiments used with water-like agents, will typically have a density of projections of greater than 5% and most preferably greater than 10%. It should also be appreciated that as the agents become thicker, e.g. protein rich agents, the density of protrusions, or their size and/or wall surface area, can be lowered.

Figures 8A to 8G illustrate schematic representations of alternative embodiments of an agent carrier body and agent carrier body layers which include multiple protrusions that together define the tissue contacting surface of the agent carrier body.

In this embodiment the agent carrier body 750, can be used for delivery of an agent to a tissue via a transportation stimulus. The agent carrier body 750 includes a tissue contacting surface 752 for engaging tissues under treatment. In this example the tissue contacting surface is defined, at least partly by a plurality of protrusions 754.

The protrusions 754 may be of any shape, but in the present example are generally cylindrical. Preferably the protrusions have a constant cross sectional shape along their height. The protrusions 754 extend outward from an inside of a void 756 that is formed within the agent carrier body 750. The outward ends 758 at least partly define the define the tissue contacting surface of the agent carrier body 750,

The void 756 is formed by a peripheral structure 760, which in this case takes the form or a peripheral wall or rim. The rim 760 also defines part of the tissue contacting surface 752.

The peripheral structure 760 in this embodiment terminates in a common plane with the protrusions, to define a planar tissue contacting surface 752. However, in other embodiments the at least some of said protrusions 754 can extend beyond, and/or stop short of the peripheral structure so that tissue contacting surface 752 is not planar. In

some embodiments the protrusions 754 may all extend beyond the peripheral structure 760.

The void 754 acts as a reservoir to hold agent within the agent carrier body 750. However unlike previous embodiments this reservoir is located on the tissue contacting surface side of the agent carrier body.

The protrusions 754 are located within the reservoir so that they are in fluid communication with the agent in the reservoir. This allows the protrusions 754 to act on the agent within the agent carrier body 750 and transmit the transportation stimulus into the agent, whereas in the embodiments above the walls of the micro channels acted on the agent within the agent carrier body.

Embodiments of this type generally have more volume for holding agent than embodiments described above. By having a larger filling volume, the possibility of air entrapment may also be reduced. These improved filling properties may give certain embodiments improved filling accuracy and repeatability, which contributes to an increase in dose accuracy, that may be important in medical applications. Furthermore the improved filling may lead to better ultrasonic energy transmission as dampening by retained air spaces is reduced.

It is preferred that the inner surface(s) of the void 754 are functionalised. The inner surface of the void 754 and the protrusions 752 may be functionalised with compounds or molecules having hydrophobic or hydrophilic properties or a combination of both moieties. Alternatively, the surface of the void 754 and the protrusions 752 may be functionalised by contacting the surface of the channels with small molecules that are adsorbed to the surface of the channels, exposing specific functional groups that have the desired physical and/or chemical properties. The small molecules may be adsorbed through chemisorption or physisorption to the internal surface of the channels. Alternatively, or in addition to changing the water/oil affinity, the inner surfaces of the micro-channels and/or agent reservoirs may be functionalised by enabling them to become electro-conductive. In a preferred form loading of the agent carrier body is performed by virtue of capillary forces when the agent carrier is in contact with the agent.

Figures 8C and 8D show an agent carrier body layer 750'. In general the agent carrier body layer 750' is the same as the agent carrier body 750 and like features are like numbered. However the agent carrier body layer 750' additionally includes one or more

micro channels 762 extending through it. The micro channels 762 extend through the agent carrier body layer so that the reservoir 756 may be fluidly connected to an adjacent agent carrier body layer as in previous embodiments. In this example, four micro channels are used.

Figures 8E and 8F are schematic representations of an agent carrier body layer having a reservoir formed therein. The agent carrier body layer 764 is generally cylindrical in form and includes a peripheral wall 766 that defines a reservoir volume 770 within it. In use the agent carrier body layer 764 is stacked on the agent carrier body layer 750' such that the outer rim 768 of the wall 766 contacts the back of the agent carrier body layer 750' such that a reservoir volume 770 closed. The micro channels 762 in the agent carrier body layer 750' allow agent within the reservoir volume 770 to pass into the reservoir 756 for dispensing.

Figures 8G and 8H are schematic representations of an agent carrier body formed by the agent carrier body layer of figures 8E and 8F stacked with the agent carrier body layer of figures 8C and 8D to form an agent carrier body 780. The agent carrier body 780 includes a stack of layers including the tissue-contacting layer 750' which includes the tissue contacting surface 752 and one other layer 764. More layers could also be stacked to form an agent carrier body.

In figure 8H the agent carrier body 780 is shown filled with agent. In this configuration the agent is filled to the tissue contacting surface 752.

Figure 9A and is an electron micrograph showing a portion of an agent carrier body (or layer thereof) of the type schematically illustrated in figures 8A to 8H. Figure 9A shows part of three pillars 782 that operate as protrusions 754. The surface 784 is the base of the void 756 from which the pillars 782 extend. Figure 9B and is an electron micrograph showing a close up portion of another pillar 786. As can be seen these embodiments from their respective scales, the pillars 782 and 786 are around 200 micrometres wide and a similar height. However in other embodiments different heights and widths may be used.

Figure 10 illustrates a series four mask designs, each suitable for forming a respective agent carrier body (or layer thereof). The masks are used in a micromachining process for forming the protrusions and peripheral structure of a tissue contacting surface of an agent carrier. The protrusions are to be arranged in a pattern, in this example in a regular array.

In figure 10 the mask for each device (Devices 1 to 4) includes a first mask section 792 for defining a square peripheral wall. Device 1 includes an array of 25 mask sections 794 arranged in a 5x5 array to create a 5x5 array of protrusions. Device 2 includes an array of 16 mask sections 794 arranged in a 4x4 array to create a 4x4 array of protrusions. Device 3 includes an array of 9 mask sections 794 arranged in a 3x3 array to create a 3x3 array of protrusions. Device 4 also includes an array of 16 mask sections 794 arranged in a 4x4 array to create a 4x4 array of protrusions. As can be seen, the protrusions of Device 4 are spaced more widely than that of Device 2. The height of the protrusions in embodiments of the present invention can be chosen to create a void having a desired volume. In some embodiments the protrusions can be greater than 200μm in height. In some forms they can be less than 1mm in height. In some embodiments the protrusions can be greater than 300μm in height. In some forms they can be less than 800μm in height. In some embodiments the protrusions can be greater than 400μm in height. In some forms they can be less than 700μm in height. In some embodiments the protrusions can be greater than 500μm in height. In some forms they can be less than 600μm in height. In some embodiments heights greater than 1mm or less than 200μm could be used.

Figure 23 illustrates a series (a) through (g) of mask designs for creation of various agent carrier bodies (or layers thereof). Figures (a) to (e) are embodiments with round protrusions 794, whereas figures (f) and (g) have protrusions 794 that are cross-shaped in plan-view. The embodiments are summarised in the following table.

Example	Array	Protrusion width μm	Protrusion separation μm	Protrusion shape
a	7x7	100	200	round
b	6x6	100	300	round
c	5x5	100	400	round
d	5x5	200	200	round
e	4x4	200	400	round

f	4x4	450	100	cross 200μm arm length, 50μm arm thickness
g	3x3	650	100	cross 300μm arm length, 50μm arm thickness

It will be appreciated that these embodiments are not exhaustive in any way and many alternative embodiments, having different protrusion dimensions, separations, cross sectional shapes can be devised. It should also be noted that, whilst these embodiments are contained within a square rim designated by reference numeral 792, other shapes can be used. Furthermore, the array of protrusions need not be a regular array or have even density or distribution across the chip. All protrusions 794 used in an embodiment need not have the same cross sectional shape.

Examples (f) and (g) have cross shaped protrusions 794. The cross shaped protrusions have the advantage that they have an increased wall surface area compared to round protrusions, but a reduced cross sectional area, thus maximising agent storage volume. The geometry of cross shaped protrusions also have relatively good mechanical properties, insofar as each arm acts as a buttresses to support the transversely extending arm.

Figures 24 to 28 illustrate a series of electron micrographs of agent carrier bodies and regions thereof. Figures 24(a) to (c) show a first embodiment. This embodiment has a 4x4 array of cross shaped protrusions 782 extending upward from a void 784. The void 784 is surrounded by a peripheral wall 785, as in previous embodiments. In use agent to be delivered is retained in the agent carrier body by using the void 784 as a reservoir. As can be seen the protrusions 782 are cross shaped in cross sectional shape over their whole height although their width changes. The changes, particularly near their tip are relatively small, such that the topmost surface, which forms the tissue contacting surface of the agent carrier body, is substantially flat. In this embodiment the peripheral wall 785 is around half a millimetre high, and most specifically 484.89μm. The protrusions are substantially the same length. The width of the cross profile in this embodiment is 31.11μm. This is compared with a nominal cross section, as defined by the mask of 50μm and represents almost a 40% tapering of the protrusion. However, as can be seen the protrusions are not sharp like microneedles despite the small thinning

towards the top. This flat surface and the plurality of densely packed protrusions prevent mechanical damage or penetration of the surface by these embodiments.

Figures 24(d) is a further embodiment also having cross shaped protrusions 782 surrounded by a generally square peripheral rim 785. In this example the protrusions are arranged in a 3x3 pattern.

Figures 25 (a) to (e) are electron micrographs of several embodiments with circular cross section. Like features are like numbered and will not be described in detail. As will be observed however, despite a narrowing of the protrusions 782 towards their tip, the tip is flat and not pointed like a microneedle. Again in this embodiment the protrusions are about half a millimetre high (525.25 μm, as shown in picture (b). The diameter of the tip of a protrusion, as illustrated in figure 25 is 184.02μm.

Figures 26 (a) to (d) are electron micrographs of several additional embodiments having cross shaped (a) and circular cross section (b) to (d). In this example the sides of the projections 782 are more vertical than previous embodiments. That is, their sides taper less than the previous embodiments. This is most noticeable in picture (a) to (c).

Figures 27 (a) to (d) are electron micrographs of several additional embodiments having protrusions with a circular cross section. Again in these examples the sides of the projections 782 are more vertical than previous embodiments. The height of the peripheral wall and protrusions are again around half a millimetre. Two final embodiments are shown in figures 28(a) and (b). These examples are a 4x4 array and 5x5 array respectively.

In this micrograph the protrusions also taper at their bottom. The tapering is a result of the etching process used during manufacturing, and is largely unintentional. However, in some embodiments, this tapering can be used to advantage as it increases the volume of the void in which agent is held.

4 Hybrid and alternative embodiments

Figures 29 and 30 illustrate two hybrid embodiments of the agent carrier body. In figure 29 the agent carrier body 104 includes a plurality of micro channels 112 arranged around its peripheral edge in the rim 785. It also has an array of protrusions 782 formed within a central void 784.

Figure 30 and 30A illustrates an alternative embodiment, which can be viewed as a protrusion-based embodiment, but with a textured, or profiled rim. In this case the agent

carrier body has a peripheral rim 785 which is castellated. The rim 785 has a series of channels or fenestrations 785A that extend through the rim 785 from the peripheral edge to the void 784. The void 784 also contains protrusions 782 in a 3x3 array. To illustrate the arrangement better a cross section along line 30A-30A is provided in figure 30A.

An agent carrier having a plurality of agent carrier bodies, perhaps arranged in a pattern such as an array, could also be provided.

5. Loading and use examples

Figures 11A, 11B, and 11C illustrate an embodiment in which the agent reservoir is provided within the agent carrier as a separate component to the agent carrier body.

Figure 11A illustrates a portion of an agent applicator according to a further embodiment of the present invention. In this figure there is illustrated an embodiment of an applicator tip 800 attached to a coupling rod 802,for coupling the applicator tip 800 to a handle portion of a hand-held agent applicator device. The applicator tip 800 includes an agent reservoir 804 formed within the tip's housing 803. The housing 803 also includes a recess area 806 for receiving an agent carrier body. The agent reservoir 804 includes a port 808. The port 808 may be configured for a number of different uses. In certain embodiments the port 808 may be used to inject the agent reservoir 804 with an agent. In other embodiments the port 808 may be used to apply a vacuum to the agent reservoir 804 to draw agent into the reservoir 804.

Figure 11B provides applicator tip 800' with an agent carrier body 810 inserted into the recess area 806 (not shown due to the presence of the agent carrier body 810). As will be appreciated from the description in Figure 11A, the agent reservoir 804' may be filled with an agent by suction applied to the port 808' whereby the agent is drawn through the agent carrier body 810 via its micro channels for storage/holding in the reservoir 804. Alternatively, port 808' may be used to directly inject the agent reservoir 804' with an agent which then fills both the reservoir 804' and the micro channels in the agent carrier 810 with the agent.

Figure 11C provides a further embodiment of an applicator tip 800" as generally described above, and accordingly corresponding features have been like numbered with the addition of double prime to indicate the change of embodiment. The applicator tip 800" is connected to coupling rod 802". It includes an agent reservoir 804" and a

stacked agent carrier body 810". In other respects it is the same as the previous examples.

Figures 12A, 12B, 12C, 12D, and 12E provide illustrations of mechanisms, modifications and methods of charging an agent carrier with agent and/or other substances that assist in the loading, retention and delivery of agent by the system.

The loading mechanisms, generally illustrated in Figures 12A to 12E, may also be used alone, or in combination, as methods for lining the surface of the agent carrier or its cavities with hydrophilic or hydrophobic moieties prior to loading an agent, or with moieties that can conduct electric charges and/or participate in generating or propagating electric fields prior to loading an agent.

Figure 12A provides an illustration of an embodiment of a method for charging an agent carrier with an agent. In this embodiment, the applicator tip 900 containing the agent carrier body 902 is connected to a hand-held agent applicator device (not shown) via its coupling rod 908. The agent carrier body 902is at least partially immersed in a container 904 containing an agent 906. Ultrasonic vibration created by an ultrasonic transducer of the agent applicator device is coupled, via the coupling rod 908 to the applicator tip 900, and through it, to the agent carrier body 902. The vibration expels air from the micro channels and at least partially fills the micro channels and/or agent reservoirs within the agent carrier body 902 with agent 906.

Figures 12B provides an illustration of another embodiment of a method for charging an agent carrier with an agent. In this embodiment, the agent carrier is a removable applicator tip 900'. The applicator tip 900' and/or a separate agent carrier body 903 are at least partially immersed in a container 904' containing an agent 906'. Ultrasonic vibration created by an external source 910 is applied to the container 904', which expels air from the micro channels and/or agent reservoirs of the agent carrier contained in the applicator tip 900' (not shown) and/or the separated agent carrier body 903 and at least partially fills the micro channels and/or agent reservoirs of the agent carrier within the applicator tip 900'and/or the separated agent carrier body 903 with agent 906'. In other embodiments loading may be performed by simple immersion of the agent carrier or agent carrier body without application of ultrasonic vibration.

Figure 12C provides an illustration of a vacuum chamber 912. Vacuum is applied at the port 914 to remove air from the chamber 912 and the air within the micro channels and/or agent reservoirs of an agent carrier held within an applicator tip 900" or a

separated agent carrier body 903'.When the vacuum is complete, a valve controlling the agent entry port 916 is opened so that agent stored in chamber 917 is drawn into the chamber 912 through the agent entry port 916 and into the micro channels and/or agent reservoirs in the agent carrier body 902" in the applicator tip 900" and/or the separated agent carrier body 903'. Ingress of agent occurs via the pores in the tissue-contact surface of the agent carrier(s). Once charged with agent, the applicator tip 900" and/or the separated agent carrier body 903' is removed from the agent containing fluid and a seal layer may be applied over exposed surfaces.

Figure 12D provides another embodiment of a method in which a vacuum is used to charge an agent carrier body 903"' with agent 906"'. Agent 906"' is held within a container 904"'. The agent carrier 903"' is placed within the container 904"' and at least partially submerged so that the pores of the tissue contact surface 920 of the agent carrier body 903"' are in the agent solution 906"'. A vacuum is applied to port 918 to draw agent solution up through the micro channels in the agent carrier 903"' so that the micro channels and/or agent reservoirs are at least partially filled with the agent solution 906"'.

In an alternative embodiment of a method for charging an agent carrier body with agent, an agent can be directly injected into the port so that the air in the agent carrier (i.e. in the micro channels and/or agent reservoirs) is expelled and replaced by the agent.

Figure 12E provides a similar method to that in Figure 12D except an applicator tip 900"" having an agent carrier body 902"' is to be charged with agent. The applicator tip 900"" is illustrated in cross section to illustrate that the applicator tip includes a reservoir 921 within its housing that is separate from any reservoir formed within the agent carrier body 902"'. The applicator tip 900"" includes a vacuum port 922 that provides access to the reservoir 921. As above, a vacuum is applied at the vacuum port 922 which draws agent solution up through the micro channels in the agent carrier body 902"" so that the micro channels and/or agent reservoirs in either the agent carrier body 902"' or applicator tip's 900"" housing are at least partially filled with the agent solution 906"".

In an alternative embodiment of a method for charging an agent carrier or applicator tip having an agent carrier with agent, agent can be directly injected into a port so that the air in the agent carrier (e.g. in the micro channels and/or agent reservoirs) is expelled and replaced by the agent.

As will be appreciated, the loading techniques described above can be used with suitable micro-channel, hybrid or protrusion based agent carrier bodies described herein or devised. However, agent carrier bodies or agent carriers which permit direct access to an agent reservoir may be loaded by directly placing agent into the reservoir, e.g. by pipetting the agent onto the reservoir. One example of such a mechanism was used in the experiments described below. In this example the agent was pipetted into the void on the tissue contacting surface of the agent carrier body of a protrusion-based agent carrier body. In a similar manner, agent may be pipetted to a reservoir on the back of the agent carrier body for delivery via micro channels to the tissue contacting surface.

The agent carrier may be provided as either empty agent carriers or as charged agent carriers that are filled with an agent. Where empty agent carriers are provided, an end user will need to charge the agent carrier with agent prior to use.

The invention also relates to a method of charging the agent carrier with an agent and discharging agent from the agent carrier.

The method of discharging agent from the agent carrier or dispensing agent to a tissue surface includes applying the agent carrier to a tissue surface and dispensing agent from the agent carrier to the tissue surface. Preferably the process of dispensing the agent includes applying ultrasonic waves to the tissue surface to facilitate penetration of the agent into the tissue through sonophoresis.

As will be appreciated from the foregoing the agent carrier or an agent carrier body itself can be an item separable from the agent applicator device. In a preferred form the agent carrier or agent carrier body is a single use item that is removable or interchangeable. This aids in the sterility required for medical usage and facilitates among other things cleaning and sterilising of the hand-held agent applicator device between patients. The solid physical nature of the preferred embodiments facilitates mounting and handling of the agent carrier in circumstances where they are replaceable. Moreover, the use of a solid material for the agent carrier body to contain the agent facilitates loading of an agent into an agent carrier, packaging, handling of agent carrier bodies pre-loaded with agent. Importantly, the use of solid materials for the agent carrier body facilitate the propagation of ultrasonic waves that are used to move an agent through the agent carrier and enhances and/or permits the entry of an agent into the target tissue by sonophoresis.

Figures 13A and 13B illustrate one embodiment of an agent carrier in the form of an applicator tip. The applicator tip 1300 is generally speaking equivalent to the applicator tip 102 shown in figure 1. In this example the agent carrier 1300 takes the form of an applicator tip with a removable and interchangeable agent carrier body.

The agent carrier 1300 includes the following main components: An agent carrier body 1302, and a tip housing 1303 that includes a tip body 1304 and an agent carrier body retaining cap 1306.

The agent carrier body 1302 is generally rectilinear in plan view, and in this example it is square. The agent carrier body 1302 may be made in accordance with any one of the examples given above or aspects described herein. The agent carrier body 1302 has a tissue contacting surface 1304.

The tip body 1304 serves to both connect the agent carrier 1300 to an agent applicator device and conduct transmission stimulus, in the form of ultrasonic energy to the agent carrier body 1302. To achieve this, the tip body 1304 is provided, on a first end thereof, with a mounting mechanism 1305 in the form of a screw thread. The mounting mechanism 1305 is used to make a mechanical connection with a corresponding connector of a handle assembly. The second end of the tip body 1304 is shaped to operate as a horn to conduct ultrasonic energy, via mating surface 1307, to the agent carrier body 1302.

The agent carrier body retaining cap 1306 serves to retain the agent carrier body 1302 and hold it in contact with the mating surface 1307. The agent carrier body retaining cap 1306 has an aperture 1310 formed in it, through which the tissue contacting surface 1308 of the agent carrier body 1302 is exposed in use. The agent carrier body retaining cap 1306 is mounted to the tip body 1304 using a screw thread.

As will be appreciated there are many morphological and mechanical variations can be made in such a system. For example the shape of the components, including the agent carrier body, and its associated tissue contacting surface may be varied. The present square embodiment is particularly convenient when the agent carrier body is made from a semiconductor material and its manufacturing process most conveniently outputs square components. The shape of the tip body can be varied to optimise transmission of ultrasonic energy if ultrasonic energy is used as a transportation stimulus. The shape of the aperture thorough which the tissue contacting surface of the agent carrier body is

exposed can be varied. In some cases it may differ from the shape of the tissue contacting surface of the agent carrier body.

The method of engagement of the agent carrier retaining cap with the tip body can be varied widely to use any convenient type of mechanism. In this example engagement is by screw thread, however the agent carrier retaining cap could be press fit onto the tip body, or engaged with snap fasteners, or a bayonet fitting, to give a non-exhaustive list or alternatives. Similarly the mounting mechanism of the agent carrier body can be varied to use any known coupling mechanism.

An agent carrier having a plurality of agent carrier bodies, perhaps arranged in a pattern such as an array, could also be provided.

6. Trial results and treatment methodologies

In a further aspect of the present invention there is provided methods for delivering an agent to a living tissue, e.g. animal, plant or human. It is considered that by selectively choosing the operational parameters of the non-invasive agent applicator presently described, the amount of agent delivered to a selected depth within tissue may be controlled.

The controlled operational parameters may include one or more of:

Application pressure;

Ultrasonic frequency;

Ultrasonic waveform;

Ultrasound direction;

Ultrasonic power level;

Ultrasonic application duration; and

Ultrasound duty cycle.

The person skilled in the art will appreciate that the optimal operational parameters needed to achieve the desired immunological response by application of agent to specific types of tissue and using a specific agent carrier design can be determined by empirical testing, including clinical testing in subjects. In particularly preferred forms, the operational parameters can be chosen to control the delivery of an agent to a desired depth in the target tissue. An example of this would be setting system

parameters such that trans-epithelial delivery of an agent predominantly into the stroma of the cornea would occur. Advantageously, this presents the opportunity to deliver a drug, vaccine or other agent to a selected tissue depth where it is known to be most efficacious.

In this regard, the present invention in one form provides a method of controlling the amount of agent delivered to a selected depth range within tissue, or to one or more selected layers of a tissue, using an agent carrier, agent carrier body or agent applicator of any one of the aspects or embodiments described herein.

In a preferred form the method is used to preferentially induce an immune response in a mucous membrane, preferably at least a mucosal immune response. In addition, a systemic immune response may also be induced. This method includes controlling the amount of agent delivered to a depth range within a mucous membrane so that a sufficient dose of agent remains resident (at least temporarily) in a depth of such tissue in order to induce an immune response, preferably a mucosal immune response. Moreover, it is believed that extending duration of the application of ultrasound, (at a low power level) may enhance the ability to induce a mucosal immune response by increasing the delivered dose selectively to more superficial tissue layers.

In an another form, the method is used to deliver an agent to induce a systemic immune response through controlling the amount of agent delivered into and through the epithelial and sub-epithelial tissue layers to the underlying. Said agent is delivered at a sufficient dose to cause said response. As detailed earlier in the specification, some agent may remain in the epithelial and/or sub-epithelial tissue layers, but a sufficient amount passes through those layers in order to induce the systemic immune response.

Embodiments that selectively control the amount of agent being delivered to a tissue depth range or to one or more selected layers of a tissue to induce mucosal immunity may be used for the treatment or prevention of infections that gain access to the body via mucous membranes including, for example only, influenza, HIV/AIDS, human papilloma virus, tuberculosis, measles, mumps and whooping cough.

Systemic immunity is beneficial for blood borne infections. Hepatitis C virus, HIV/AIDS, malaria and tetanus serve as examples where systemic immunity may be preferred.

A combination method of use can be used which delivers the agent to multiple depths of tissue either simultaneously or sequentially. This may be used among other things, to seek to induce both systemic and mucosal immunity.

Experimental testing

A series of experiments were conducted using mice to determine if an agent can be successfully delivered to tissues using embodiments of the present invention. The transportation stimulus in each case was ultrasonic energy only. In the present experiments a viral vaccine was administered using an embodiment of the present invention, using ultrasonic energy only, applied to the inside of the lip to determine whether the agent was presented to the immune system, and may induce an immune response. Researchers noted that no damage occurred to the mucous membrane of the lip by the application of the device for the period required to achieve a systemic immune response in Experiment 2.

In addition to the methodologies described in the examples below, mucosal immunity can be monitored or confirmed by detection of specific, secretory IgA antibodies, given this is the dominant antibody isotype of the mucosal immune system. This class of antibody is found in humans in two isotypic forms, IgA1 and IgA2; in mucosal secretions, it is a dimeric form that is produced. This makes it more stable and a good marker of mucosal immunity.

6.1 Experimental Summary

Experiment 1

Mice were vaccinated with an embodiment of the present invention illustrated in Figure 7c using two agent carrier bodies (termed “microchips” in the experimental discussions) totalling around 2-5 $x10^6$ plaque forming units (pfu) of the fluorescent labelled recombinant poxviral vector-based HIV vaccine per mouse.

The proportion of antigen presenting cells taking up the vaccine antigen (0.025-0. 068 vs 0.025-0.022), and the proportion of dendritic cells recruited to the draining lymph nodes (0.25-0.54 vs 0.22-0.49) were similar in immunised and unimmunised mice, respectively (Figures 1 and 2). The key conclusion was that an immune response was not induced using only two microchips.

Experiment 2

A full heterologous prime-boost vaccination using recombinant poxviruses expressing HIV antigens was conducted using three microchips per mouse prime, and the responses were compared to mice primed intranasally (i.n.) (positive control), and to mice not primed with any vaccine (negative control). All mice were given an intramuscular (i.m.) booster vaccination two weeks after the priming vaccination.

The magnitude of the systemic immune responses (responses in the blood compartment) induced by different vaccination routes were evaluated by determining the percent of HIV-specific CD8 T cells in spleen. One of the mice vaccinated using an embodiment of the present invention had an immune response that exceeded the positive control thus demonstrating proof of concept.

Experiment 3

In a further experiment a preliminary prime-boost vaccination experiment was conducted using embodiments of the present invention illustrated in figure 10. Mice were primed with the lip delivery system using three microchips according to each embodiment (around 2-5 $x10^6$ pfu) of FPV-HIV per mouse, followed by an intramuscular booster vaccination. The percent of HIV-specific CD8 T cells was used to assess the magnitude of the immune responses induced. Data indicated that microchips 1 (1% of cells) and 2 (0.6%) performed slightly better than microchips 3 and 4 (0.5%). It was also noted that during loading and delivery the microchips 1 and 2 performed much more effectively than microchips 3 and 4.

Experiment 4

Full prime-boost vaccination experiment was performed using the microchips 1 and 2 of figure 10. In this experiment one of the mice in each of the groups vaccinated generated an immune response that exceeded the intranasal positive control, whereas the other two mice in each group had responses similar to the oral vaccine negative control group.

Table 1 summarises the experimental parameters and outcomes of each of Experiments 1 to 4.

Table 1: Summary of the prime-boost vaccination experiments conducted on the original microchip, and microchips 1 and 2.

Chip identification where relevant [a]	Priming: route, dose FPV-HIV[b]	Booster: route dose VV-HIV[c]	% HIV-specific CD8+ T cells (tetramer test)[d]			Magnitude of HIV-specific CD8+ T cell response (ICS test)[d]		
			M#1	M#2	M#3	M#1	M#2	M#3
Original Mc (x3) Test group	Lip ~2-5 x 10^6 pfu	i.m. 1 x 10^7 pfu	15.1	1.03	1.06	10.5	0.73	0.78
Positive control	i.n. 1 x10^7 pfu	i.m. 1 x 10^7 pfu	8.94	9.33		6.85	6.14	
Negative control		i.m. 1 x 10^7 pfu	1.36	1.40		1.03	0.78	
Mc1 (x3) Test group	Lip ~2-5 x 10^6 pfu	i.m. 1 x 10^7 pfu	0.38	15.5	0.67	0.06	1.5	0.08
Mc2 (x3) Test group	Lip ~2-5 x 10^6 pfu	i.m. 1 x 10^7 pfu	0.81	0.73	9.45	0.12	0.08	2.0
Negative control	Oral 5 x 10^6 pfu	i.m. 1 x 10^7 pfu	1.17	0.45	2.87	0.8	0.05	0.35

[a] (x3) – refers to the number of microchips of vaccine administered to each mouse, thus "x3" means that three microchips were applied;

Mc – is an abbreviation of "microchip" and is used to designate which type was used in each test;

[b] Dose, is represented in plaque forming units (pfu) of the priming vaccine, fowl pox virus expressing HIV antigens (FPV-HIV) are provided. The route of vaccination delivery; is indicated as follows:

"Lip" designates that administration was made using an embodiment of the present invention applied to the tissues of the lip of the subject;

"i.n." designates intranasal delivery;

"oral" designates delivery directly into the mouth

[c] The booster vaccine is vaccinia virus expressing HIV antigens (VV-HIV), and in all cases this was delivered using intramuscular (i.m.) route

[d] In both cases, systemic immune response was investigated.

M# represents mouse number.

Experiment 5

This experiment seeks to determine the uptake of a vaccine delivered to a subject using an embodiment of the present invention. In this example delivery was made to the lip. Extra experiments were also performed to assess intra dermal – (i.d.) uptake. Nude mice were vaccinated using 3x microchips with microchip 1 design in figure 10 . The microchips contained fluorescent-labelled recombinant poxviral vector-based HIV vaccine expressing mCherry fluorescent antigen. Live animal imaging was performed

3h, 6h, 9h and 24h post vaccination, and fluorescent vaccine uptake and expression was assessed over time. Data indicated that lip delivery was effective and that also i.d. delivery was also effectively performed. The microchip device #1, has an excellent vaccine uptake, and antigen expression profile was detected as early as 3h post delivery.

Experiment 6

An embodiment of the present invention was tested by performing heterologous prime-boost vaccination using recombinant poxviruses expressing the HIV antigens using microchip device #1 of figure 10. 3 microchip doses per BALB/c mouse were used in the prime (around 2-5 $x10^6$ pfu of FPV-HIV per mouse) followed by an intramuscular (i.m.) $1x10^7$ VV-HIV booster vaccination two weeks after the priming vaccination. The responses were compared to mice not primed-boosted with any vaccine (unimmunised control) (Table 3). The magnitude of the systemic immune responses (responses in the blood compartment) and mucosal responses in gut mucosae (Peyer's patches) were evaluated by determining the percent of HIV-specific CD8 T cells in spleen and Peyer's patches respectively as well as intracellular cytokine staining, and measurement of ant-viral cytokine IFN-γ.

This experiment shows that an embodiment of the present invention used on the lip tissue can induce systemic response and also mucosal immunity. Consistent (80% efficacy) of CD8 T cell immune responses following prime-boost vaccination was observed. Collectively the data also suggests that the method can successfully be used as a lip/ i.d. prime-boost needle free delivery strategy.

Table 3: Summary of the systemic and mucosal immune responses induced following lip/ i.m. prime-boost vaccination.

Chip identification where relevant [a]	Priming: route, dose FPV-HIV[b]	Booster: route dose VV-HIV[c]	% HIV-specific CD8+ T cells **(tetramer test)**[d]					Magnitude of HIV-specific CD8+ T cell response (**ICS test**)[d]				
			M1	**M2**	**M3**	**M4**	**M5**	**M1**	**M2**	**M3**	**M4**	**M5**
Negative control Spleen	nil	nil	0.20	0.11				0.31	0.24			
Mc1 (x3) Test group Spleen	Lip ~2-5 $x10^6$ pfu	i.m. 1 $x10^7$ pfu	3.89	1.01	8.39	8.97	12.4	3.24	1.43	8.12	8.39	12.6
Negative control	nil	nil	0.07					0.07				

Gut - PP[e]												
Mc1 (x3) Test group Gut - PP[e]	Lip ~2-5 x10[6] pfu	i.m. 1 x10[7] pfu	1.20	1.26	1.21			1.22	1.4	1.33		

[a] x3 – refers to administering 3 chips of vaccine per mouse; Mc – microchip type is indicated; test, and negative control within a group of experiments is also indicated

[b] Dose, in plaque forming units (pfu) of the priming vaccine, fowl pox virus expressing HIV antigens (FPV-HIV) are provided. Route of vaccination delivery; lip using the MuPharma system

[c] The booster vaccine is vaccinia virus expressing HIV antigens (VV-HIV), and in all cases this was delivered using intramuscular (i.m.) route

[d] In both cases, the systemic and mucosal immune response was investigated using tetramer staining and Intra-cellular cytokine staining (ICS).

[e] Indicates Peyer's Patches.

M# represents mouse number.

6.2 Experimental detail

Experiment 1

Aims: To determine whether the lip delivery system using the embodiment of figure 7c induced antigen uptake in the draining lymph nodes (LN), the antigen presentation and immune cell recruitment was monitored 24 hours post vaccination as follows:

1. Uptake of the vaccine antigens was monitored in cervical, mediastinal and/or mesenteric lymph nodes following administration of a number of microchips of a fluorescently labelled vaccine - recombinant fowl pox virus expressing HIV antigens together with green fluorescent protein (FPV-HIV-GFP);

2. To evaluate whether antigen presenting cells (APC) are recruited to these LN the relative number of dendritic cells (DCs) and macrophages at these sites were identified by the staining for characteristic cell surface markers

Methods:

1. Mice were immunised with FPV-HIV-GFP and responses were evaluated 24 hours post vaccination. In these experiments, mice were also kept as either

a) unimmunised controls (Figures 14 and 15), or

b) controls vaccinated with only FPV-HIV (i.e. no GFP fluorescent antigen, Figure 16).

Mice were given the vaccination with two microchips, one to the left and one to the right lip (around 5 x10^6 pfu per mouse).

2. At 24h the different draining LN were harvested, pooled, and single cell suspensions were prepared in complete medium (Ranasinghe et al., 2011, Ranasinghe et al., 2006, Ranasinghe et al., 2007, Ranasinghe et al., 2013) 3. 1x10^6 cells were aliquoted and stained with the different cell surface markers. [Antigen presenting MHC-II cells were stained with antibody to the I-A^d APC cell surface marker

Antibodies to cell surface markers CD11b-PE and CD11c-PerCP were used to identify DCs, (Figure 15) and antibody to cell surface marker F4/80-PE Cy7 was used to identify macrophages (data not shown)] (Ranasinghe et al 2013)

4. Different cell subsets were analysed based on the fluorescent-labelled cell surface marker expressed on the cell surface using flow cytometry analysis (FACS). These experiments were repeated three times, combined results are presented in Figure 14 to 16

5. In these experiments singe colour controls (SS) and fluorescent minus one (FMO) controls were also used to set up the gating and perform the correct analysis of the different cell subsets.

Results and conclusions:

Figures 14 to 16 illustrate graphically the outcomes of the experiments. In this regard, Figure 14 shows plots for the evaluation of the uptake of FPV-HIV-GFP vaccine 24 h post lip delivery, illustrating I-A^d APC MHC-II cells containing the fluorescent GFP antigen of the vaccine detected in the top right hand quadrant indicated by the arrow. Note in this and other FACS plots, each dot represents a single cell.

Figure 15 illustrates plots for the evaluation of recruitment of antigen uptake by different dendritic cell subsets to the respective draining lymph nodes 24 h post lip delivery. The proportion of dendritic cells, identified as being MHC-11+, and either CD11b+ (left two columns) or CD11c+ (right two columns) are indicated in the top right hand quadrant (refer to arrows).

Figure 16 illustrates plots for the evaluation of the uptake of FPV-HIV-GFP vaccine 24 h post lip delivery in cervical, mediastinal and mesenteric nodes (repeat experiment 3) I-Ad APC MHC-II cells containing the fluorescent GFP antigen of the vaccine are detected in the top right hand quadrant indicated by the arrow. (Note that the top three graphs show the gating strategy).

As can be seen, no differences in the antigen uptake and presentation (Figures 14 & 16) or the DC subsets recruited to the draining lymph nodes (Figure 15) were detected between the mice that received the FPV-HIV- GFP vaccine and the controls. The data indicated that;

i) Vaccine delivery applied at a dose of two microchips per mouse (dose ~ 2-5x10^6 pfu) was not effective.

ii) Thus, to obtain any immune outcomes, a minimum of 3 chips or more per mouse were used in the subsequent prime-boost vaccination experiments.

Experiment 2

In this next experiment an evaluation of the efficacy of lip delivery with the same microchip as experiment 1, using prime-boost vaccination was performed.

Aims: To test whether lip prime followed by intramuscular (i.m.) booster vaccination can induce effective HIV-specific CD8 T cell immunity compared to intranasal prime (i.n.)/i.m. booster vaccination strategy using:

1. HIV gag-specific tetramer staining.

2. Intracellular cytokine staining (ICS) of IFN-γ in HIV-specific CD8 T cells.

Methods:

Figure 17 are photographs showing the following phases of the experiments performed. The phases illustrated include: Loading the microchips (top left), Ultrasonic system settings (top right) and lip delivery to the mice (bottom photos). The experimental method was performed as follows.

1) Priming vaccination with FPV-HIV

a. Vaccine (~600-800 μl of the stock) was sonicated (i.e. output: 30%; 3 cycles for 10 seconds per cycle) as for routine i.n. delivery. 300-400 μl/well of the sonicated virus was added into two wells of a 48 well plate.

b. Microchips were soaked in FPV-HIV (5x 108 PFU/ml) in a 48 well plate (Figure 17 top left). It was assumed that each microchip could absorb and expel 5µl, thus the dose per microchip was calculated to be $2.5x10^6$ pfu.

c. Six microchips per well were submerged in liquid without any overlap and incubated for 30 minutes on ice (Figure 17 top left).

d. One microchip was taken out to test whether the chips were loaded with virus, by placing the loaded microchip in a well containing PBS. If the microchip floated it meant the chip was not loaded, but if it sank it was considered to be loaded.

e. Controls: positive control two mice were immunised i.n. (20 µl/mouse $1x10^7$ pfu) and two mice were vaccinated with i.m. booster ($1x10^7$ pfu) only to solely test its effect.

f. Test group: three mice were immunised for the lip/i.m. group as follows. The microchip was mounted to the agent applicator, similar to that illustrated in figure 1,that was connected to the power source. Ultrasonic gel was used between the arm and the microchip for better contact). Power was switched on.

g. Microchip was pressed firmly onto the inner lip region of an anesthetised mouse. (Figure 17 bottom)

h. Output switch was turned on an ultrasonic energy was applied for 30 seconds, to deliver the virus into the lip region. At this time point the instrument settings were transducer drive voltage V = 95-160; P = 2800-3200 mW.

i. To check whether the virus has been delivered from the microchip, the chip was placed in PBS as before. If the chip floated it suggested that the virus was successfully expelled from the chip. 80% of the time the chip floated suggesting that the vaccine was expelled. If two microchips failed to deliver the vaccine correctly, the mouse was discarded and a new mouse was immunised.

j. This was repeated for 3 microchips per mouse, using one new chip each time.

2) Intramuscular booster vaccination using 10^7 PFU VV-HIV

a. Booster vaccination was performed two weeks post FPV-HIV priming vaccination

b. Booster vaccine was prepared for 9 mice total 9 x 10^7 PFU in 900 µl of PBS.

c. Virus was sonicated exactly as done for the FPV-HIV.

d. Mice were anesthetized with isoflurane using a nose cone and 50µl of VV-HIV per quadriceps muscle was delivered i.m.

3) Preparation of spleen samples for analysis

7-14 days post booster vaccination spleens were harvested from each mouse, and single cell suspensions were prepared as described in Ranasinghe et al (2006).

The magnitude of the HIV-specific CD8 T cell responses was assessed with tetramer staining and intracellular cytokine staining, using 4 x 10^6 spleen cells from each mouse according to the plate scheme in Tables 2 and 3 as follows:

a. Tetramer staining was performed as described in (Ranasinghe et al., 2011, Ranasinghe et al., 2006, Ranasinghe et al., 2007, Ranasinghe et al., 2013)

- Cells were stained for 45 min at room temperature with K^dGag197-205-APC tetramer and anti-CD8α FITC in FACS buffer.
- Cells were washed and fixed in 0.5% PFA prior to analysis using FACS.

b. Intra cellular cytokine staining (ICS) for IFN-γ was also performed as described (Ranasinghe et al., 2011, Ranasinghe et al., 2006, Ranasinghe et al., 2007, Ranasinghe et al., 2013)

- Cells were stimulated over night with K^dGag197-205 peptide for 1h at 37 °C + 5% CO2
- Brefeldin A was added to each well and incubated for further 5 hours at 37 °C
- Cells were surface stained for 25 mins at 4 °C with anti-CD8α FITC in FACS buffer.
- Cells were fixed/permeabilized using IC/fix and IC/perm from eBioscience
- Cells were then intracellular stained with anti-IFN-γ, for 25 mins at 4 °C (Table 2)
    - Positive stain –anti IFN-γ APC in in IC Perm
    - Single colour controls and FMO's.

Table 2: Plate Scheme for Tetramer Staining.

	1	2	3	4	5	6	7	8	9
A ss cont	Unstain	FITC	APC						
B	LIP 1	LIP 2	LIP 3	i.n. 1	i.n. 2	Boost only 1	Boost only 2	FMO CD8	FMO tetramer

Table 3: Plate Scheme for ICS.

	1	2	3	4	5	6	7	8	11
A ss cont	Unstain	FITC	APC						
B stimulated	LIP 1	LIP 2	LIP 3	i.n. 1	i.n. 2	Boost only 1	Boost only 2	FMO CD8	FMO IFN-g
C Unstim	LIP 1	LIP 2	LIP 3	i.n. 1	i.n. 2	Boost only 1	Boost only 2		

SS = Single colour control, FMO = Fluorescent minus one

Results and conclusions:

Figure 18 shows plots illustrating the evaluation of the magnitude of HIV-specific splenic CD8 T cells using IFN-γ intracellular staining. The FACS data were analyzed using Cell Quest Pro or FlowJo analysis. The box indicates the percentage of HIV-specific splenic CD8 T cells expressing IFN-γ following Lip/i.m. (top 3 mice), i.n./i.m. (middle 2 mice) and booster only (bottom 3 mice) vaccinations. Figure 19 illustrates plots enabling evaluation of HIV-specific splenic CD8 T cells using tetramer staining. Cells were stained as described in materials and methods. The FACS data were analysed using Cell Quest Pro or FlowJo analysis. The box indicates the percentage of HIV-specific splenic CD8 T cells following different routes of vaccine delivery. Lip/i.m. (top three mice), i.n./i.m. (middle two mice) and booster only (bottom two mice).

The HIV-specific tetramer (**Figure 18)** and IFN-γ staining (**Figure 19)** data indicated that unlike the i.n./i.m. delivery strategy that gave highly consistent results (Figure 18 - range 8.94 - 9.33%), the lip/i.m. delivery strategy did not yield consistent outcomes (Figure 19 – range 1.03 - 15.1%). Whilst it appears that this is due to the inconsistency of the priming of the mice during lip delivery (Note: see also lip/i.m. compared to i.m. booster only), one mouse (mouse 1) showed an immune response that exceeded that of the i.n./i.m. delivery strategy, indicating that a response is possible using embodiments of the present invention.

Data also revealed that 3x lip or 4x lip microchip delivery was more effective than 5x lip microchip delivery (data not shown). These experiments were performed twice and data

were found to be very similar between the experiments (Experiments 4 & 5). Data are representative of one experiment.

Experiments 3:

A further experiment was performed to test prime-boost vaccination strategy to assess the efficacy of lip delivery using each of the protrusion-based embodiments of the present invention illustrated in figure 10.

Aims: To test whether these microchips can load and deliver the vaccine more effectively to the lip compared to microchips of figure 7c using HIV gag-specific tetramer staining (Figure 20).

1) Priming vaccination with FPV-HIV

a. Vaccine was sonicated and 300-400 ml per well was added into a 48 well plate as before.

b. The microchips were connected to the device, then 5-7µl of vaccine was loaded onto the tissue contacting surface of the microchip using a pipette and immediately delivered to the lip of the mouse. Unlike the microchip of figure 7c, these improved microchips were NOT soaked in FPV-HIV for 30 min.

c. Controls: for the positive control, two mice were immunised i.n. (20 ml/mouse); for the negative controls, two mice were immunised orally and two mice were kept as controls for the i.m. booster only to test the effect of i.m. vaccination only. (similar to figure 5)

2) i.m. booster vaccination and evaluation of immune responses using tetramer staining

a) These were performed exactly as described in experiment 2.

Results and conclusions:

1) Unlike the microchip of figure 7c, direct pipetting of the vaccine onto the chips made it extremely easy to determine whether the new microchips were properly loaded. Similarly, once the vaccination was performed, the microchip was placed on a piece of tissue to determine whether the vaccine had been properly expelled. If the microchip was dry it meant the vaccine was delivered. We also tested the above loading by visualising the empty, loaded and used microchips under a microscope.

2) It was observed that microchips 1 & 2 (Figure 10 top) loaded and discharged the vaccine much more effectively (without leakage) compared to microchips 3 and 4 (Figure 10 bottom). Even though loading was much more effective, the vaccine leaked

out of microchip 3 (in particular) and 4 as soon as the device was held against the lip, prior to turning on the output switch, making it more of an oral delivery.

3) The preliminary HIV-specific tetramer data further confirmed that microchip 1 performed better than 3 & 4. Hence, it was decided to repeat the prime-boost vaccination experiments with microchips 1 and 2 of figure 10, including an oral prime/i.m. booster immunization strategy as a control to validate the data in experiment 4, below.

Experiment 4

In this experiment vaccination using a 3x lip/ i.m. vaccination strategy using microchips 1 & 2 of figure 10 was tested in a similar manner to previous experiments.

Aim: Test the efficacy 3x lip/i.m, vaccination strategy compared to 1x oral/i.m. prime-booster vaccination using:

a) HIV gag-specific tetramer staining **(Figure 21)** and

b) Intracellular cytokine staining (ICS) of IFN-γ **(Figure 22)**

Methods:

Vaccination and analysis were performed exactly as in experiment 3 with 3 mice per group. 1x oral prime/ i.m. booster vaccination was also performed as an additional control to assess whether the priming was related to oral delivery or lip delivery (oral dose = 5x 106 FPV-HIV). The HIV-specific CD8 T cell responses were measured in the spleen 14 days post booster vaccination using tetramer staining and intracellular IFN-γ staining. The experiments were performed two times.

Results and Conclusion:

Figure 21 illustrates plots enabling evaluation of HIV-specific splenic CD8 T cell responses using tetramer staining. The FACS data were analysed using Cell quest Pro software. Plots represent three animals per group microchip 1 (top) & 2 (middle) prime-boost immunization data compared to oral delivery (bottom). The upper right quadrants (red arrows) indicate the % of HIV-specific CD8 T cells observed following each vaccine strategy.

Figure 22 illustrates plots enabling evaluation of the magnitude of HIV-specific CD8 T cell responses using IFN-γ intra cellular cytokine staining. The FACS data were analysed using Cell quest Pro software. Plots represent three animals per group

microchip 1 (top) & 2 (middle) prime-boost immunization data compared to oral delivery (bottom). The upper right quadrants (red arrows) indicate the % of HIV-specific CD8 T cells expressing IFN-γ.

As can be seen the HIV-specific splenic CD8 T cell responses observed with microchip 1 - mouse 2 and microchip 2 - mouse 3 (red arrows) were greatly elevated compared to oral delivery (bottom 3 mice Figures 21 & 22), these results clearly indicated that the responses observed were due to lip uptake not oral uptake.

Data indicated that if the delivery was uniform/consistent the microchip 1 and 2 could induce good HIV-specific CD8 T cell immunity in the blood compartment.

The positive responses detected with the microchips made in accordance with Figure 10 were very much similar to the positive responses detected with the microchip of figure 7c used in experiments 1 and 2). However, they present greater ease of loading.

Data from experiments, suggest that if uniformity/consistency could be attained, lip delivery could be more effective than oral or intranasal delivery.

Discussion

Molecules that are known to the inventors to possibly be delivered to the body using sonophoresis include 1) molecules that have any kind of electric charge or have a neutral (including overall neutral) electrical charge and 2) small or large molecules (including monoclonal antibodies of approximately 149,000 Daltons) 3) molecules that are hydrophilic or hydrophobic or lipophilic.

The present inventors have additionally realized that delivering vaccines to mucous membrane epithelia using the present invention creates new opportunities to prevent or treat diseases including, but not limited to influenza, HIV/AIDS and tuberculosis through inducing mucosal immunity in addition to systemic immunity. It is believed that mucosal antibodies are more effective than systemic antibodies in creating immunity to pathogens that infect through mucous membranes. Systemic immunity is generally induced by delivering vaccines to the body by an injection although there is evidence to suggest that stimulation of the mucosal immune response can result in production of protective B and T cells to create both mucosal and systemic immunity

Experiment 5

This experiment evaluates the uptake of the viral vector-based vaccines following lip and/or intradermal (i.d.) delivery, using an embodiment of the present invention.

The microchips were cut from a 6-inch Silicon wafer, and made using a mask featuring microchip 1 of Figure 10. The completed sizes of the chips were 3mm with1mm in thickness, with open etched areas and free standing pillar (hairbrush bristle-like arrangement) as previously discussed. The microchips have a depth of 500- 600μm and a maximum sidewall variation of ±10% of the etch depth.

Aims: To determine whether the lip and /or i.d antigen uptake was effective using an embodiment of the present invention. Nude mice were vaccinated with recombinant FPV-HIV expressing a fluorescent tag protein (mCherry) and uptake and expression of proteins were monitored for 24h post vaccination as described in Townsend et al (in preparation for publication).

Method: Three nude mice (n = 3) were immunised with FPV-HIV-mCherry and uptake/expression of antigens were evaluated up to 24 hours post vaccination. In these experiments, 1 mouse was also kept as either a) unimmunised control or b) control vaccinated with only FPV-HIV (i.e. no mCherry fluorescent antigen. Figures 33 and 34 show images of the live animals using FPV-HIV expressing mCherry antigen. In both figures the leftmost animal is the unimmunised mouse not given any vaccine. In Figure 33 the rightmost three images indicate the uptake and expression of mCherry antigen at 3h, 6h, and 9h post vaccination following lip delivery in a single animal. Similarly, in figure 34 the rightmost four images indicates a mouse given FPV-HIV-mCherry i.d. into the ear and expression of mCherry assessed at 3h, 6h, and 9h and 24h post vaccination. Although the figures are representative of one mouse tracked over time the experiment was performed using three mice (n=3) and repeated two times. As noted above, the mice were given the vaccination with 3x microchips, to the lip or the ear for i.d. delivery (around 2-5 $x10^6$ pfu per mouse).

Conclusion: The data indicated that:

Vaccine uptake via lip using 3x microchips per mouse (dose ~ 2-5$x10^6$ pfu) is effective. Uptake and peak antigen expression can be detected as early as 3h, as can be seen in the second from left images in figures 33 and 34. This is favourable compared to some

alternative vaccination strategies in which peak expression may be detected at 6-12h post delivery ((Trivedi et al., 2014), Townsend et al in preparation for publication.)

Delivery of vaccines intradermally (i.d.) using 3x microchips per mouse was demonstrated.

Experiment 6

This experiment evaluates the efficacy of using an embodiment of the present invention that involves lip delivery using the same microchip as in Experiment 5.

Aims: To test whether lip prime, followed by intramuscular (i.m.) booster vaccination can induce effective HIV-specific mucosal and systemic CD8 T cell immunity, using HIV gag-specific tetramer staining and Intracellular cytokine staining (ICS) of IFN-γ.

Methods:

1) Priming vaccination with FPV-HIV
   a. Vaccine (~600-800 µl of the stock) was sonicated (i.e. output: 30%; 3 cycles for 10 seconds per cycle).
   b. Five mice were immunised for the lip/i.m. group as follows. Each microchip was mounted to an agent applicator device. Ultrasonic gel can be used between the actuator rod of the applicator and the microchip for better ultrasonic coupling to the agent carrier.
   c. The microchip was loaded with ~3 µl of vaccine, and pressed firmly onto the lip region of the subject, which in each sae was an anesthetised BALB/c mouse.
   d. Ultrasound was applied for 30 seconds, to deliver the virus into the lip region. Transducer output was set at 10, V = 1.52.
   e. *This was repeated for 3 microchips per mouse*
2) Intramuscular booster vaccination using 10^7 PFU VV-HIV
   a. Booster vaccination was performed two weeks post FPV-HIV priming vaccination.
   b. Virus was sonicated exactly as done for the FPV-HIV.
   c. Mice were anesthetized with isoflurane using a nose cone and 50 µl of VV-HIV per quadriceps muscle was delivered i.m. (total 10^7 pfu)
3) Preparation of spleen and Peyer's Patch samples for analysis

14 days post booster vaccination spleens and Peyer's patches (PP) were harvested from each mouse, and single cell suspensions were prepared as described in (Ranasinghe et al., 2006; Ranasinghe et al., 2013(Xi et al., 2012).

The magnitude of the HIV-specific CD8 T cell responses was assessed with tetramer staining and intracellular cytokine staining, using 4 x 10^6 spleen cells from each mouse as follows:

a. Tetramer staining was performed as described in (Ranasinghe et al., 2011, Ranasinghe et al., 2006, Ranasinghe et al., 2007, Ranasinghe et al., 2013)
   - Cells were stained for 45 min at room temperature with $K^dGag_{197-205}$-APC tetramer and anti-CD8α FITC in FACS buffer.
   - Cells were washed and fixed in 0.5% PFA prior to analysis using FACS.

b. Intra cellular cytokine staining (ICS) for IFN-γ was also performed as described (Ranasinghe et al., 2011, Ranasinghe et al., 2006, Ranasinghe et al., 2007, Ranasinghe et al., 2013).
   - Cells were stimulated overnight with $K^dGag_{197-205}$ peptide for 1h at 37 °C + 5% CO_2
   - Brefeldin A was added to each well and incubated for further 5 hours at 37 °C
   - Cells were surface stained for 25 mins at 4 °C with anti-CD8α FITC in FACS buffer.
   - Cells were fixed/permeabilized using IC/fix and IC/perm from eBioscience
   - Cells were then intracellular stained with anti-IFN-γ, for 25 mins at 4 °C

Results and conclusions:

Each experiment was performed twice and data illustrated in figures 35 to 37 are representative of one experiment. Figures 35 and 36 illustrate the HIV-specific tetramer results and figures 37 and 38 illustrate the IFN-γ staining results from the experiment.

More specifically figure 35 illustrates the HIV-specific splenic CD8 T cells using tetramer staining. Spleen cells were stained as described above. The FACS data were analysed using FlowJo analysis. The box indicates the percentage of HIV-specific splenic CD8 T cells following vaccination. The top five plots indicate the Lip/i.m. immunised mice and the lower plots two unimmunised control mice.

Figure 36 illustrates the HIV-specific gut (mucosal) CD8 T cells using tetramer staining. Cells from Peyer's patches were stained as described above. The FACS data were analysed using FlowJo analysis. The box indicates the percentage of HIV-specific splenic CD8 T cells following vaccination. In the plots the top row illustrate results for

Lip/i.m. immunised mice pooled two mice per group, over 5 mice total. The bottom row is pooled data for two unimunised control mice.

Figure 37 illustrates the magnitude of HIV-specific splenic CD8 T cells using IFN-γ intracellular staining. The staining was performed as described above and the FACS data were analyzed FlowJo analysis. Each of the top plots show results for a Lip/i.m immunised mouse. The box indicates the percentage of HIV-specific splenic CD8 T cells expressing IFN-γ The bottom plots represent data for two unimmuniized control mice.

Figure 38 illustrates the magnitude of HIV-specific gut-specific (mucosal) CD8 T cells using IFN-γ intracellular staining. Cells from Peyer's patches were stained as describe above. The FACS data were analyzed FlowJo analysis. Each plot represents data from two pooled mice with five mice total being used. The box indicates the percentage of HIV-specific splenic CD8 T cells expressing IFN-γ following Lip/i.m immunization. The bottom plot represents the two unimmunised control mice.

Results

1. Live imaging data demonstrate good uniform uptake and expression of recombinant vector-based vaccines following lip and i.d. delivery using an embodiment of the present invention.
2. Data from prime-boost experiments indicate that, lip priming can induce effective mucosal (gut-specific) and systemic HIV-specific CD8 T cell immunity.
3. Data also suggest that, the apparatus has the potential to be used in a lip/i.d. needle free prime-boost strategy. I.d. delivery (into skin in the context of humans) also has the potential to improve mucosal immunity. (e.g. replacing i.m. to i.d. booster).

The results indicate that in BALB/c mice the methods performed can induce consistent immune outcomes (Table 3 and Fig 35-38). The data indicates consistency in priming efficacy as 4/5 mice were shown to respond effectively to lip priming.

7. References:

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It will be understood that the invention disclosed and defined in this specification extends to all alternative combinations of two or more of the individual features mentioned or evident from the text or drawings. All of these different combinations constitute various alternative aspects of the invention.

CLAIMS

1. A method of delivering an agent to one or more selected layers of a tissue of a subject, including the steps of:

applying ultrasound to said agent within an agent carrier body, agent carrier or agent applicator, wherein ultrasound is the transportation stimulus; the agent carrier comprising a tissue contacting surface for engaging the tissue; and

configuring the operational parameters of the agent applicator to enhance or enable delivery of said agent to the one or more layers of the tissue

wherein delivery of the agent induces an immune response in the subject.

2. A method of inducing an immune response in a subject, including the steps of:

applying ultrasound to an agent within an agent carrier body, agent carrier or agent applicator, wherein ultrasound is the transportation stimulus; the agent carrier body comprising a tissue contacting surface for engaging the tissue; and

configuring the operational parameters of the agent applicator to enhance or enable delivery of said agent to one or more selected layers of a tissue

wherein delivery of the agent induces an immune response in the subject.

3 A method as claimed in claim 1 or 2 wherein the immune response is at least a mucosal immune response.

4. A method as claimed in claim 3 wherein delivery of the agent to induce at least a mucosal immune response is by controlling the amount of agent delivered into the epithelial layer, or into the epithelial and sub-epithelial layers of the mucous membrane.

5. A method as claimed in claim 1 or 2 wherein the immune response is a systemic immune response.

6. A method as claimed in claim 5 wherein delivery of the agent to induce a systemic immune response is by controlling the amount of agent delivered into and through the epithelial and sub-epithelial tissue.

7. A method as claimed in any one of the preceding claims further including one or more steps of:

- loading the agent carrier body and/or agent carrier with agent;
- providing the agent carrier body or agent carrier holding the agent;
- bringing a tissue contacting surface of the agent carrier body or agent carrier into direct or indirect contact with said tissue; and
- dispensing the agent from the agent carrier body or agent carrier to the tissue surface, wherein the step of dispensing the agent includes generating an ultrasonic signal to enable or enhance transportation of the agent to the tissue-contacting surface.

8. A system for delivering an agent to one or more selected layers of a tissue in a subject, the system including:

an agent contained in an agent carrier body, agent carrier or agent applicator; the agent carrier body comprising a tissue contacting surface for engaging the tissue; and

a means for applying an ultrasonic signal, wherein ultrasound is the transportation stimulus;

wherein the system is configured to enhance or enable delivery of said agent to the one or more layers of the tissue

and delivery of the agent induces an immune response in the subject.

9. A system for delivering an agent to a tissue to induce an immune response in a subject, the system including:

an agent contained within an agent carrier body, agent carrier or agent applicator; the agent carrier body comprising a tissue contacting surface for engaging the tissue; and

a means for applying an ultrasonic signal, wherein ultrasound is the transportation stimulus;

wherein the system is configured to enhance or enable delivery of said agent to one or more selected layers of a tissue,

and delivery of the agent induces an immune response in the subject.

10 A system as claimed in claim 8 or 9 wherein the immune response is a mucosal immune response.

11. A system as claimed in claim 10 wherein delivery of the agent to induce at least a mucosal immune response is by controlling the amount of agent delivered into the epithelial layer, or into the epithelial and sub-epithelial layers of the mucous membrane.

12. A system as claimed in claim 8 or 9 wherein the immune response is a systemic immune response.

13. A system as claimed in claim 12 wherein delivery of the agent to induce a systemic immune response is by controlling the amount of agent delivered into and through the epithelial or sub-epithelial layers of the mucous membrane.

14. The method or system as claimed in any one of the preceding claims wherein the operational parameters configured include any one or more of:

Application pressure;
Ultrasonic frequency;
Ultrasonic power level;
Ultrasonic waveform;
Ultrasonic application duration;
Ultrasonic application duty cycle; and
Ultrasound direction.

15. A method as claimed in claim 1 or 2, or a system as claimed in claim 8 or 9 which involves delivering the agent to or beyond any one or more of the following tissues or tissue layers:

Mucous Membrane;
Epithelium
Sub-epithelium
Mucosa;
Sub-mucosa
Mucous membrane vasculature
Cornea;
Corneal epithelium
Bowman's membrane
Corneal stroma
Corneal Endothelium

Conjunctiva;
Tenon's Fascia;
Episclera:
Sclera;
Choroid;
Choriocapillaris;
Bruch's membrane;
Retinal Pigment Epithelium;
Neural retina;
Retinal blood vessels;
Internal Limiting Membrane;
Vitreous;
Skin epidermis; and
Skin dermis.

16. A method or system as claimed in any one of the preceding claims wherein the agent carrier or agent carrier body comprises a tissue contacting surface for engaging tissues under treatment, the tissue contacting surface being at least partly defined by a plurality of protrusions.

17 The method or system as claimed in claim 16 wherein the agent carrier includes one or more agent reservoirs for carrying said agent, wherein said protrusions are in fluid communication with one or more reservoirs forming part of the agent carrier.

18, The method or system as claimed in claim 17 wherein each agent reservoir comprises a void formed within the agent carrier body.

19. The method or system as claimed in claims 16 or 17 wherein the protrusions extend outward from an inside of a void and terminate at said tissue contacting surface.

20. The method or system as claimed in any one of claims 18 or 19 wherein the void is formed by a peripheral structure, wherein at least part of said peripheral structure terminates at the tissue contacting surface.

21. The method or system as claimed in claim 20 wherein:

the peripheral structure terminates in a common plane with the protrusions.

22. The method or system as claimed in claim 20 wherein at least some of said protrusions defining the tissue contacting surface extend outward from the void beyond the peripheral structure.

23. The method or system as claimed in claim 22 wherein the protrusions terminate in a plane and the peripheral structure terminates short of the plane such that the protrusions extend beyond the peripheral structure.

24. The method or system as claimed in any one of the preceding claims wherein the agent carrier body includes a stack of layers including:

a tissue-contacting layer which includes the tissue contacting surface; and

at least one other layer.

25. The method or system as claimed in claim 24 wherein at least the tissue contacting layer has at least one hole extending through it to define at least a portion of a respective micro channel in the agent carrier body.

26. The method or system as claimed in claim 25 wherein a micro channel enables agent to be transported from one layer to the next.

27. The method or system as claimed in any one of the preceding claims wherein the agent carrier conducts the transmission stimulus.

28. The method or system as claimed in claim 27 wherein the agent carrier body conducts the transmission stimulus.

29. The method or system as claimed in any one of the preceding claims wherein the agent carrier body includes one or a multiplicity of micro channels extending at least partially through the agent carrier body to the tissue contacting surface enabling transportation of the agent to a tissue surface.

30. The method or system as claimed in claim 29 wherein the micro channels extend through the agent carrier body to fluidly connect to an agent reservoir.

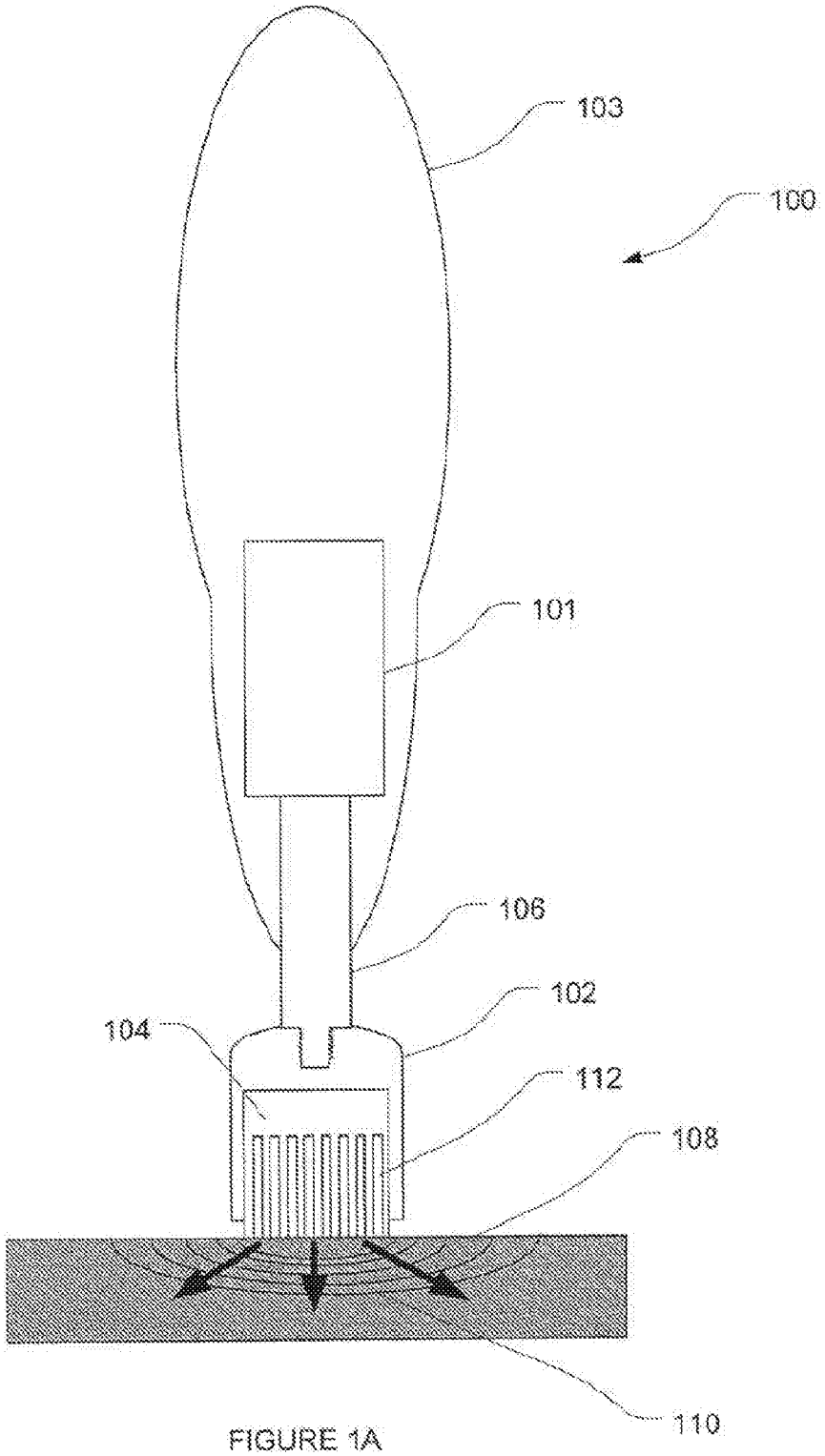


FIGURE 1A

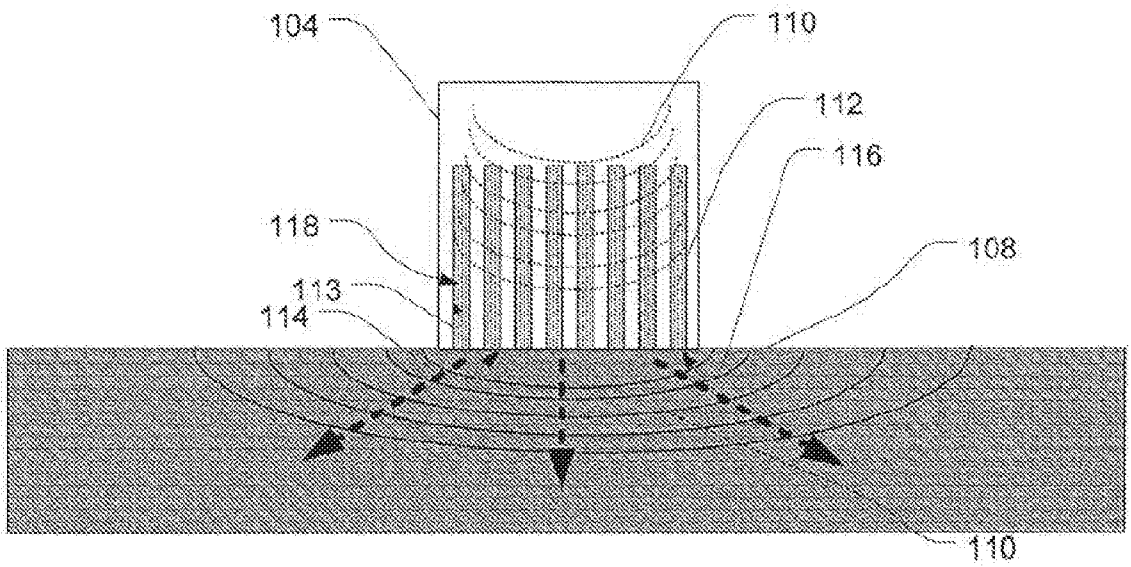


FIGURE 1B

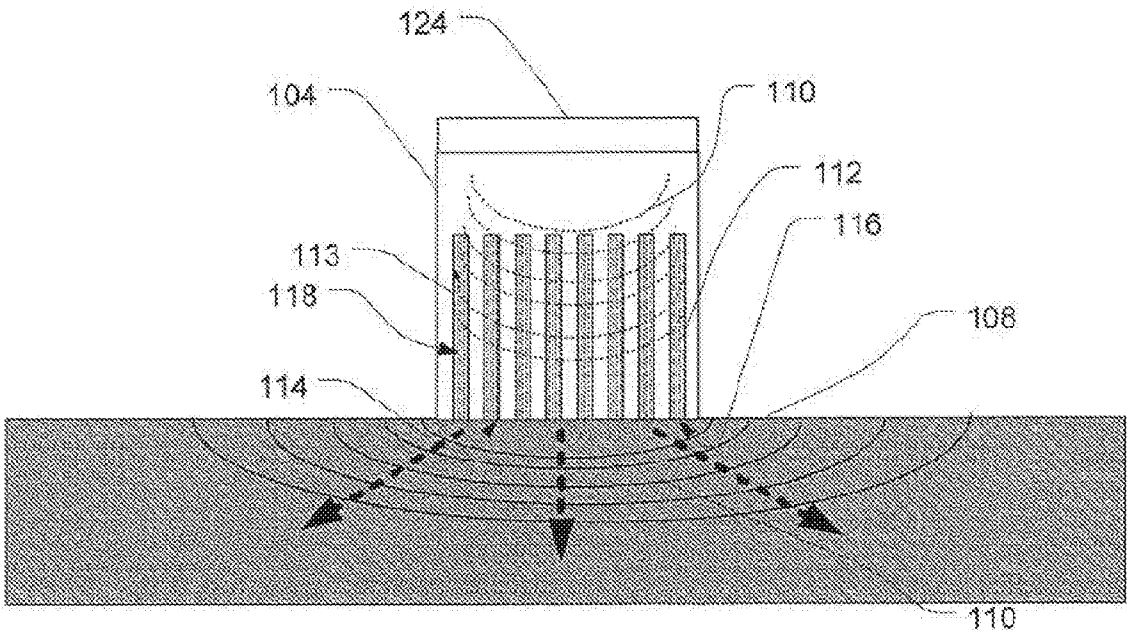


FIGURE 1C

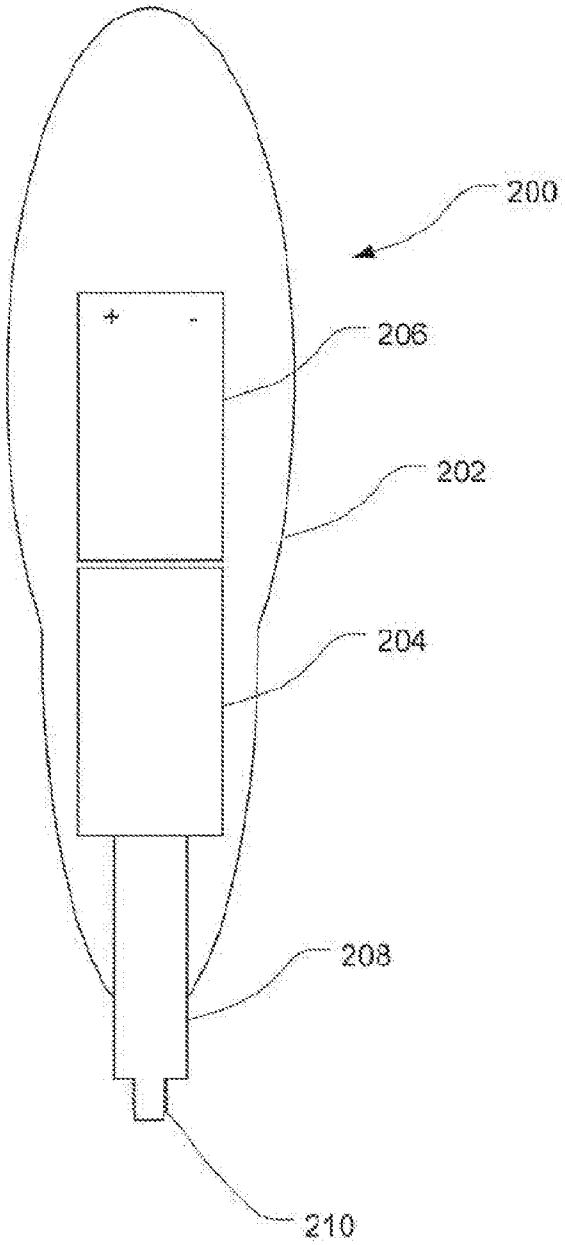


FIGURE 2

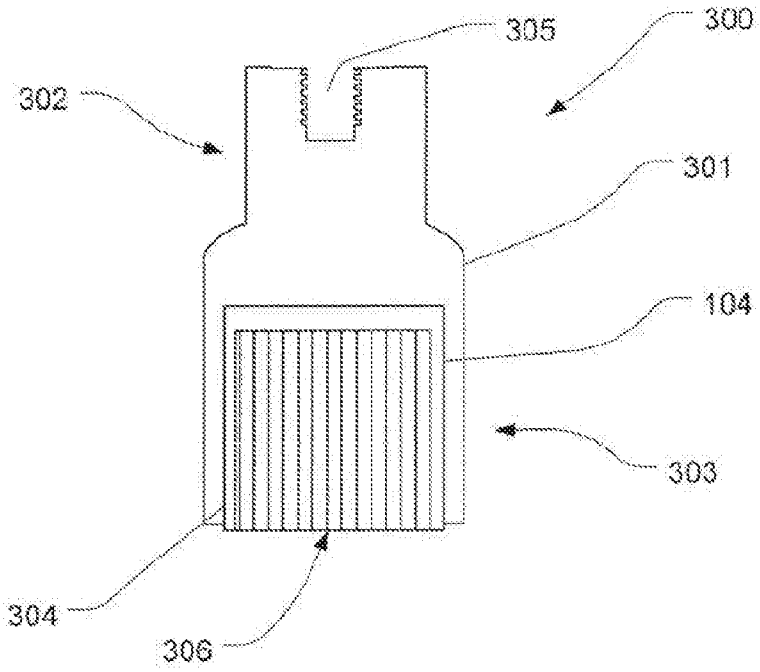


FIGURE 3

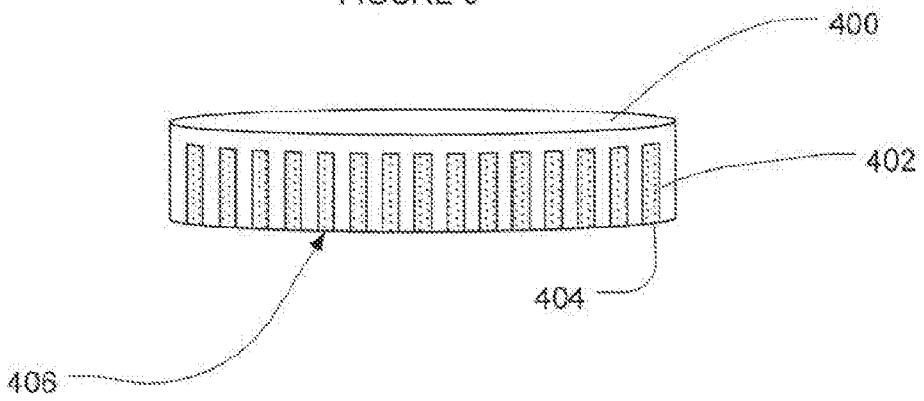


FIGURE 4A

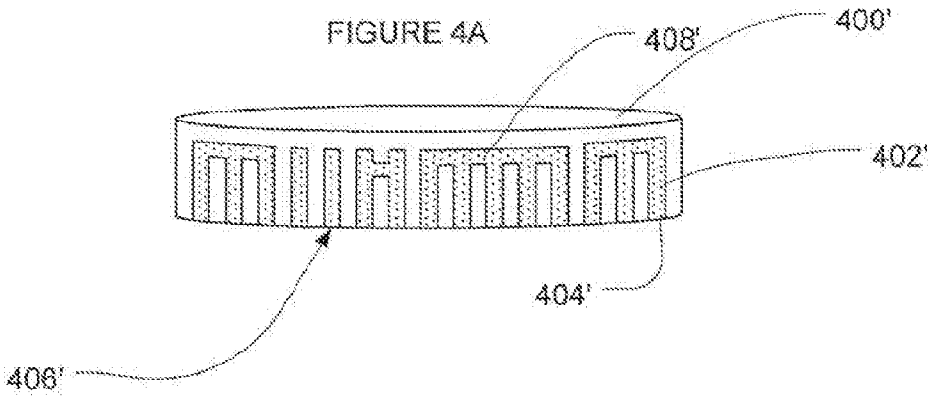


FIGURE 4B

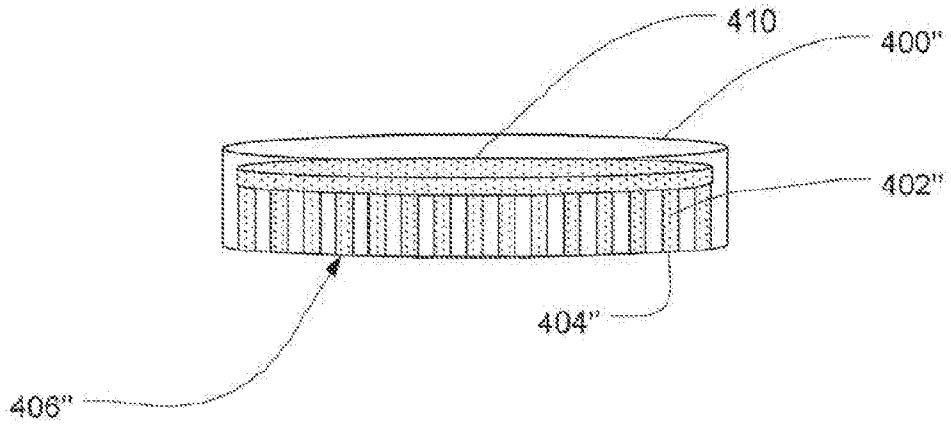


FIGURE 4C

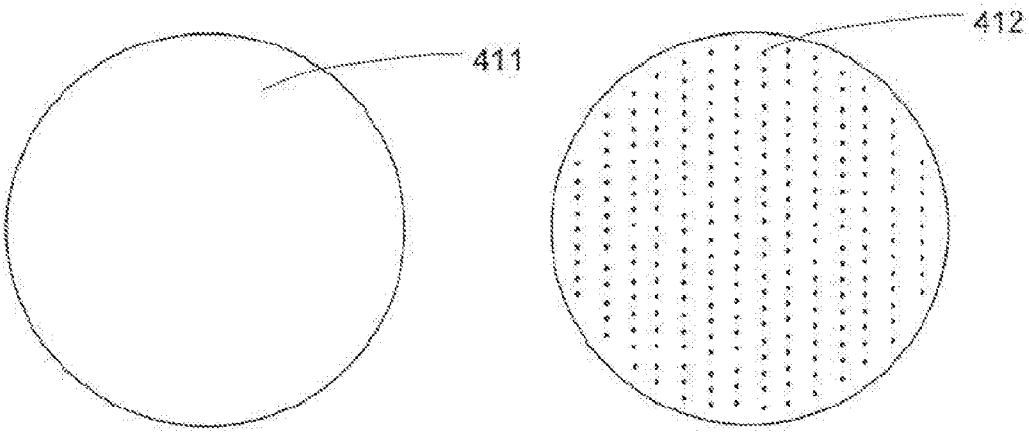


FIGURE 4D

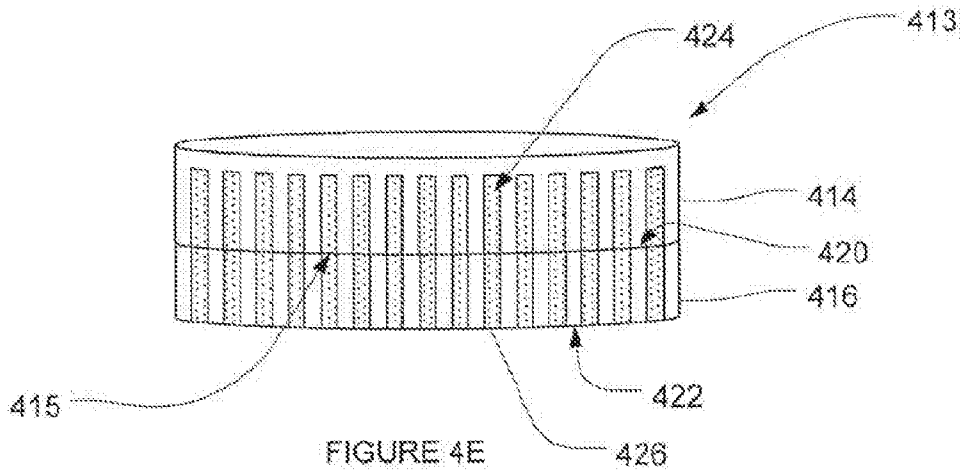


FIGURE 4E

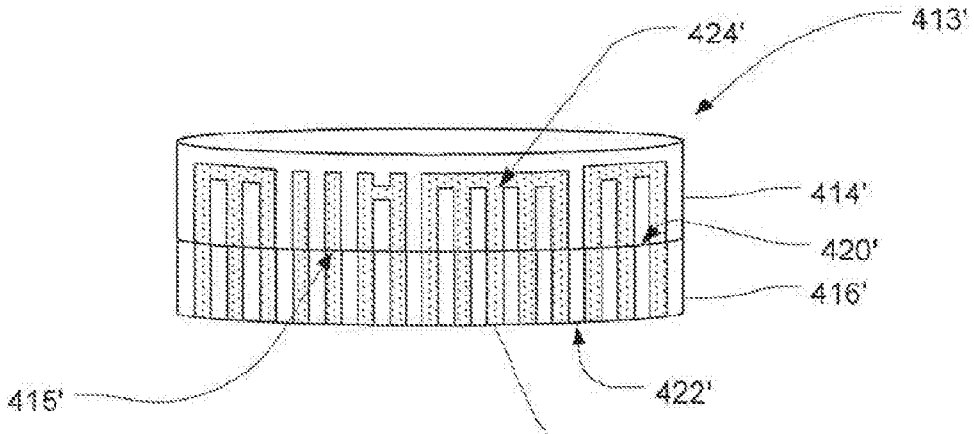


FIGURE 4F

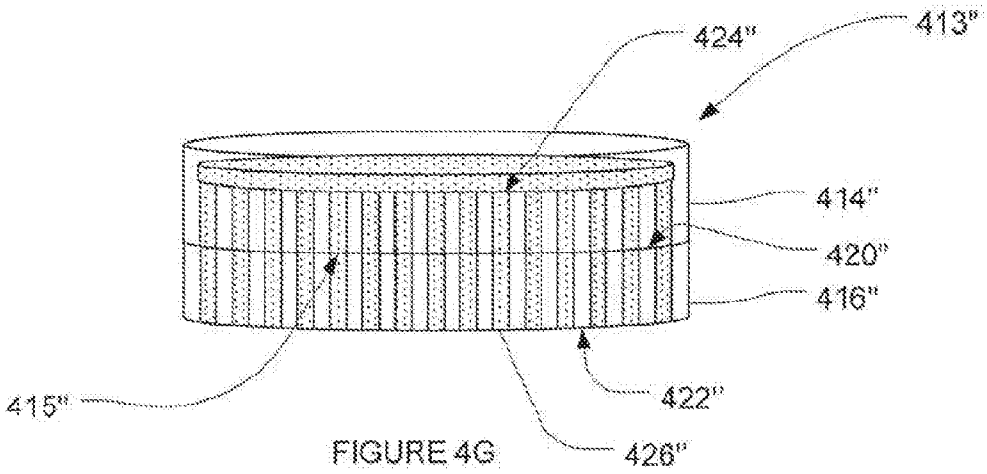


FIGURE 4G

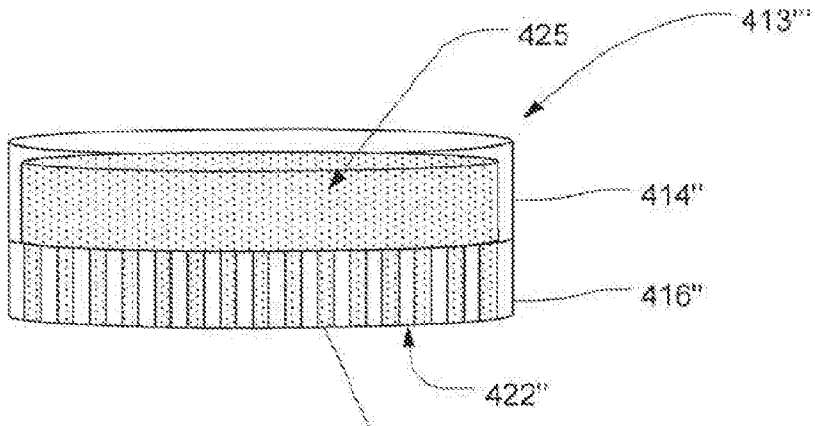


FIGURE 4H

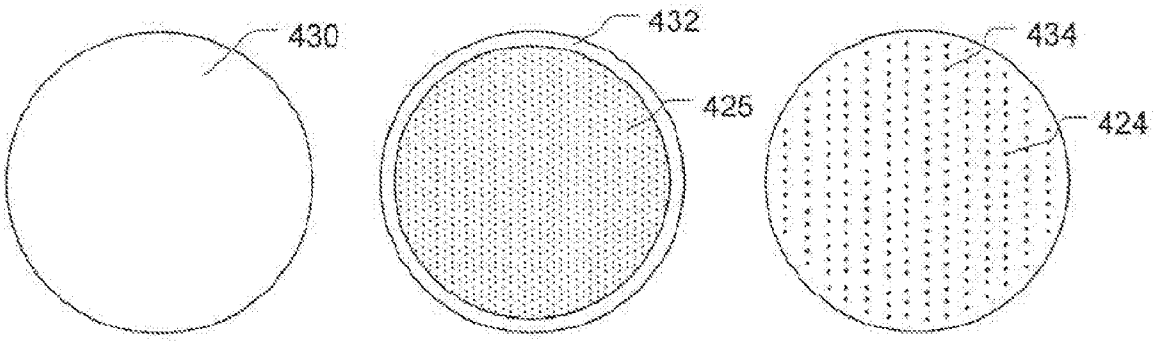


FIGURE 4I

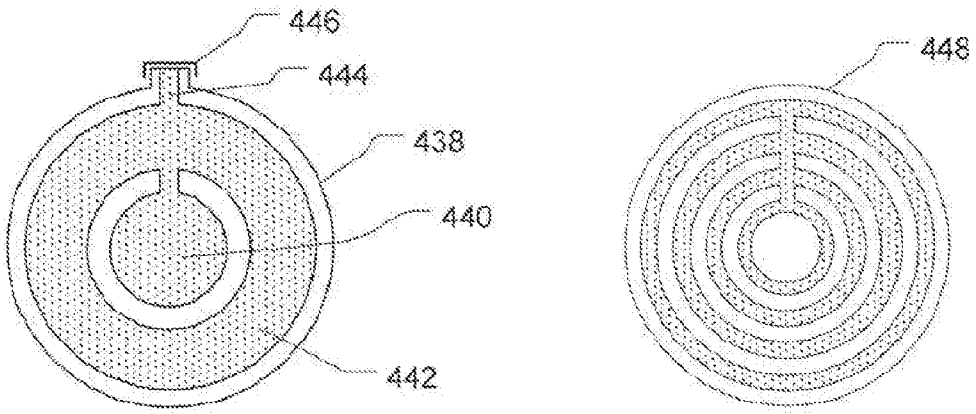


FIGURE 4J

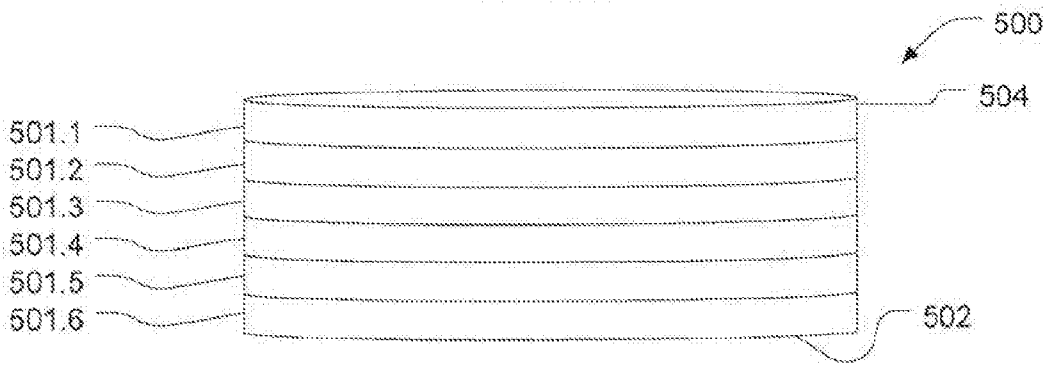


FIGURE 5A

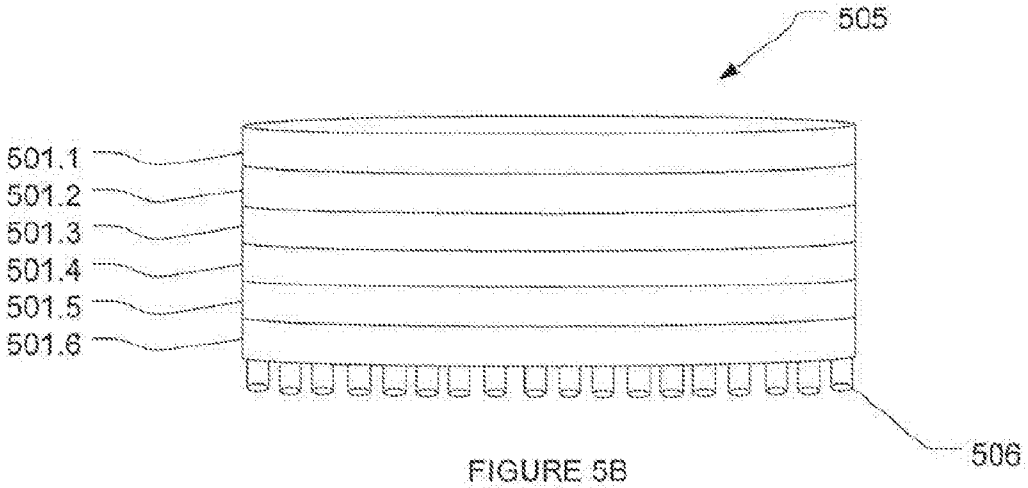


FIGURE 5B

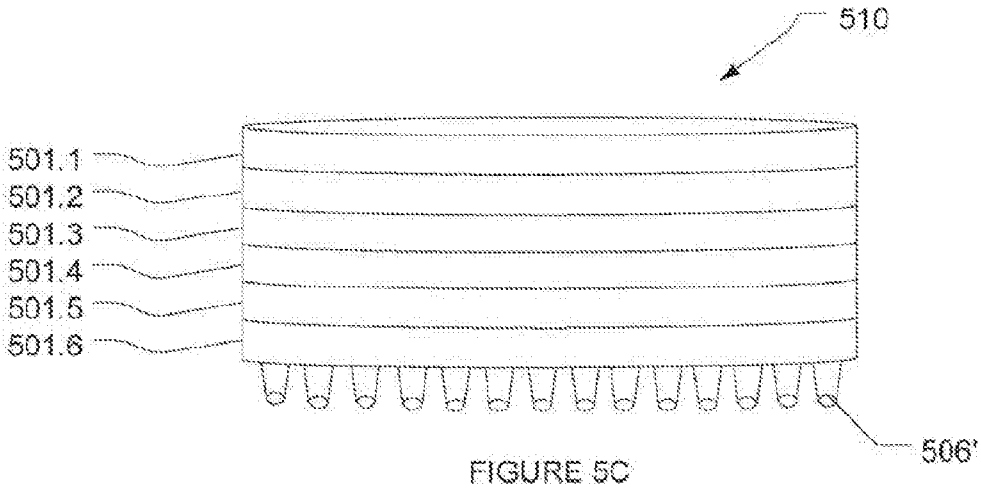


FIGURE 5C

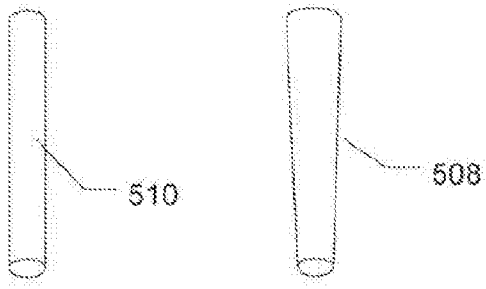


FIGURE 5D

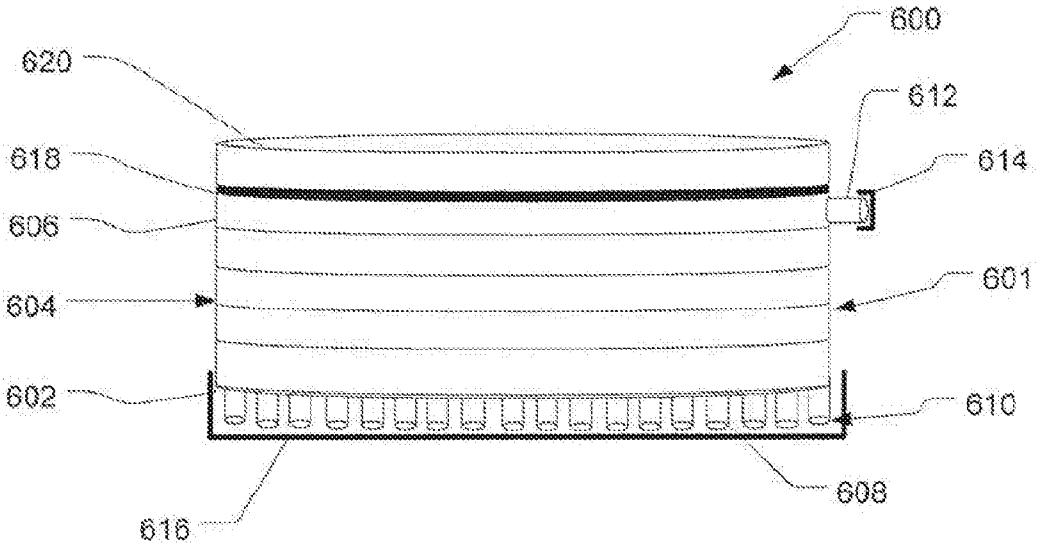


FIGURE 6

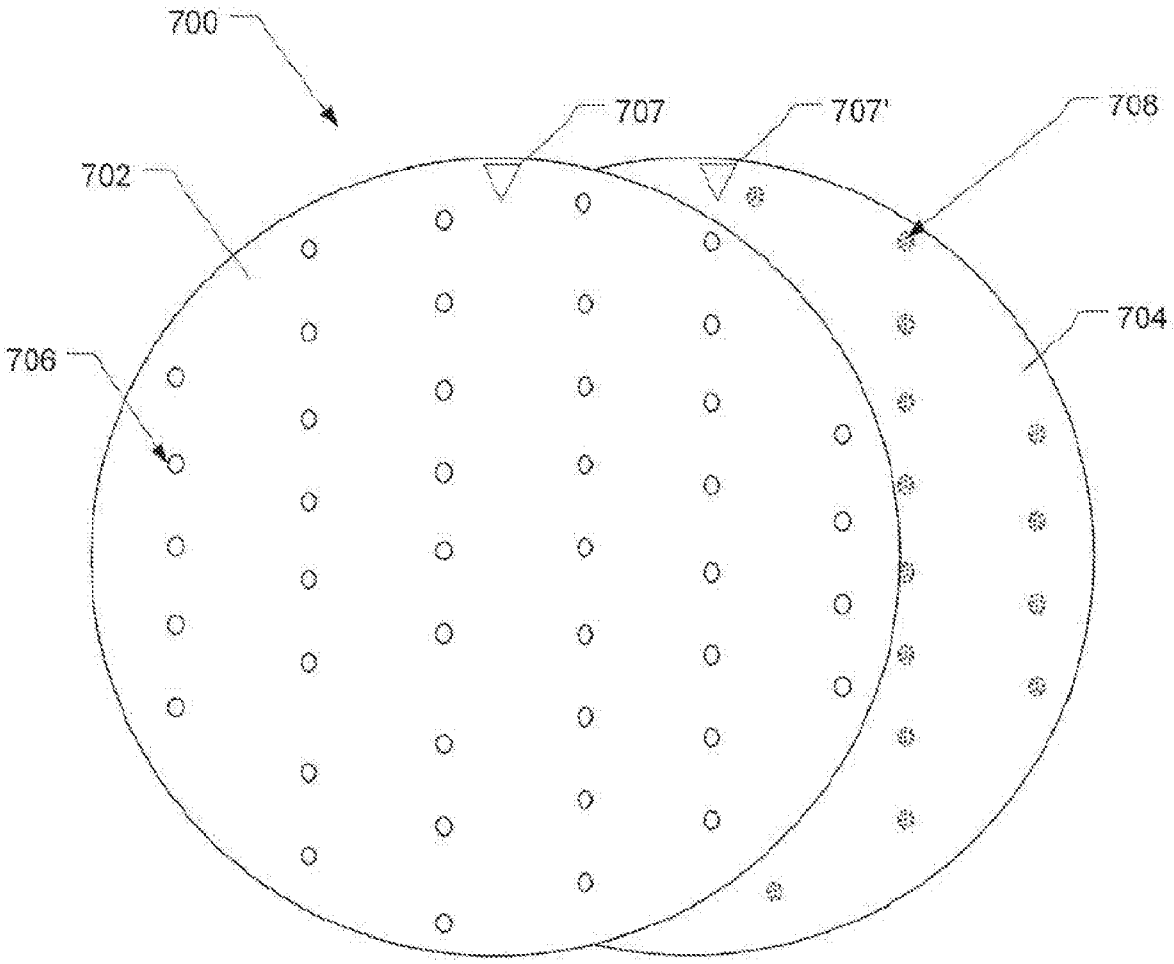


FIGURE 7A

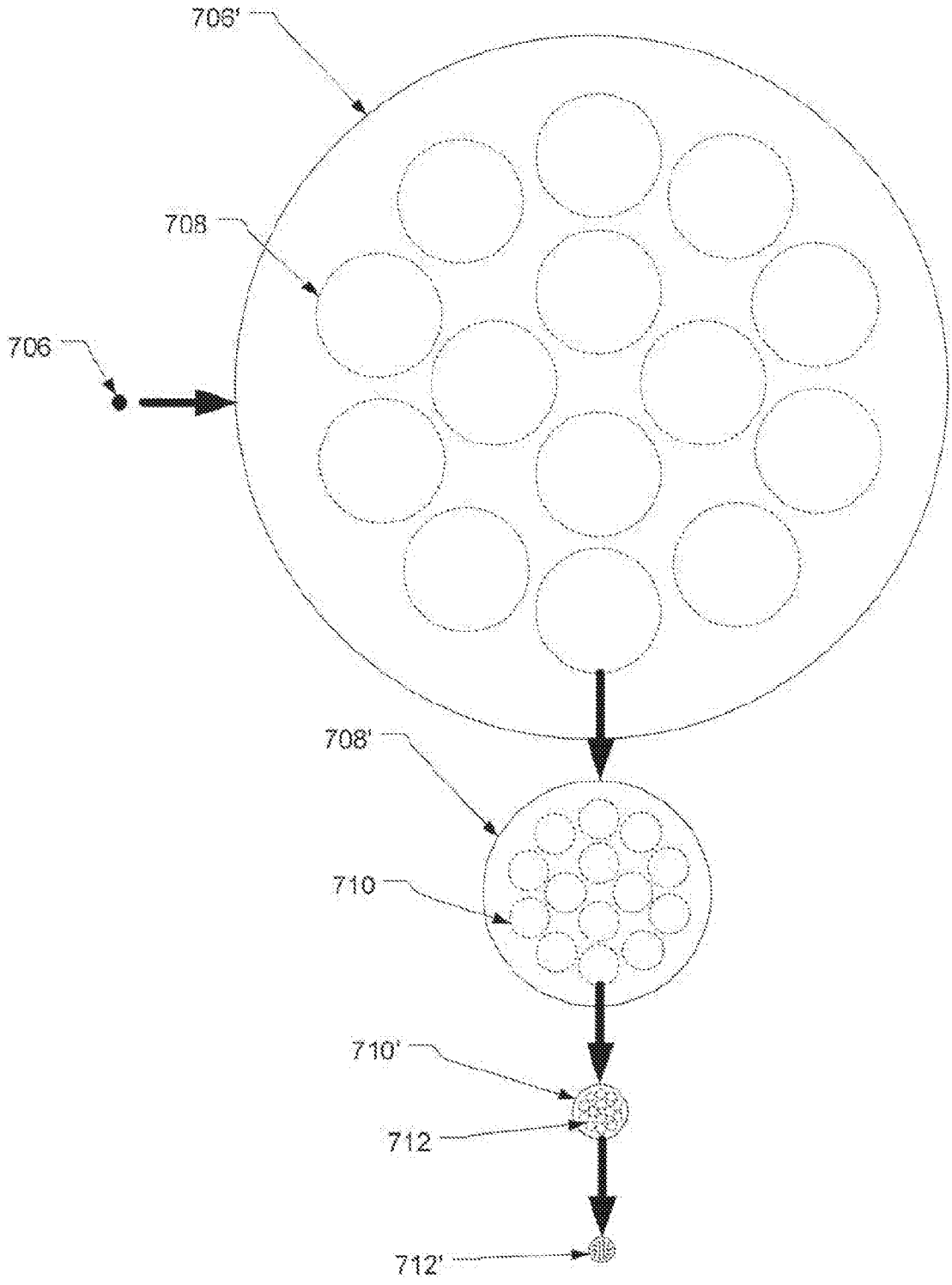


FIGURE 7B

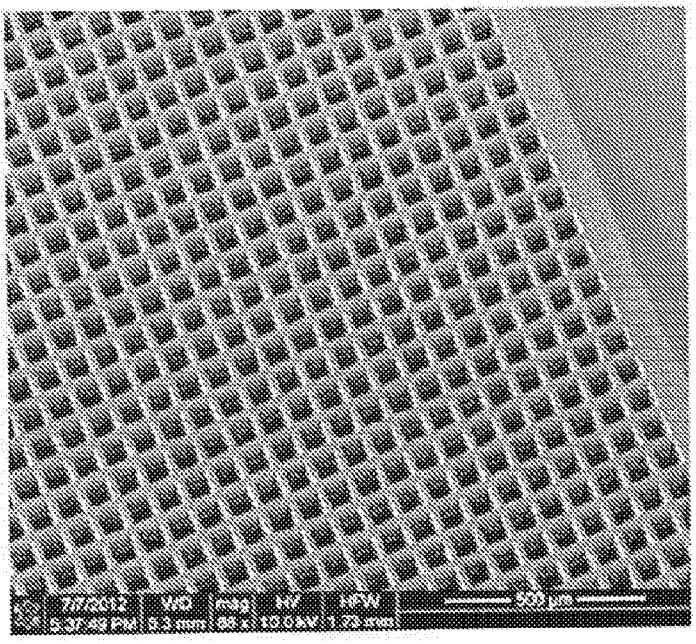


FIGURE 7C

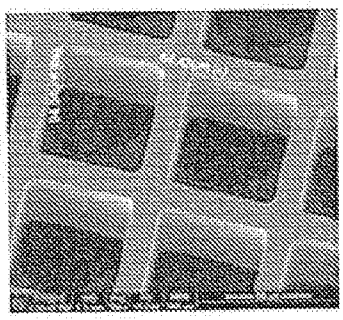


FIGURE 7D

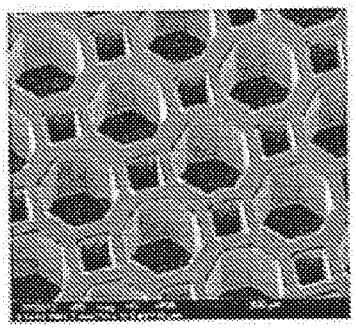


FIGURE 7E

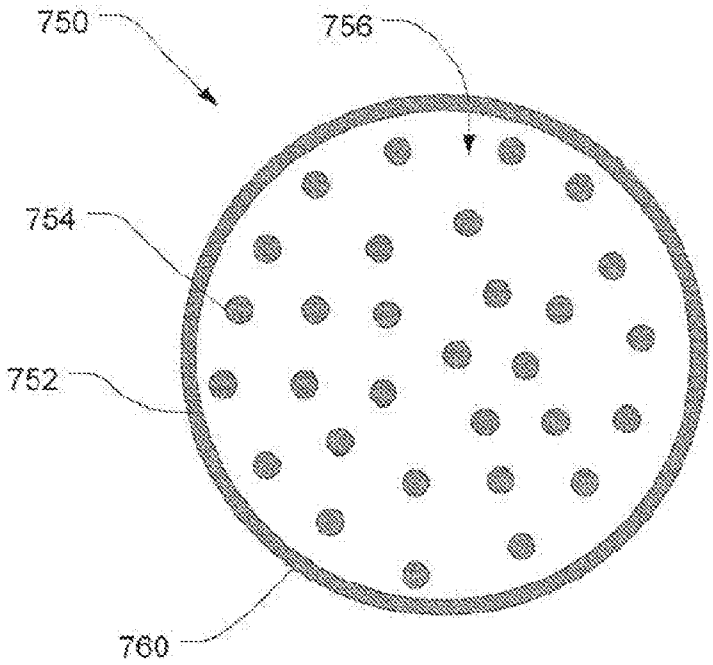


FIGURE 8A

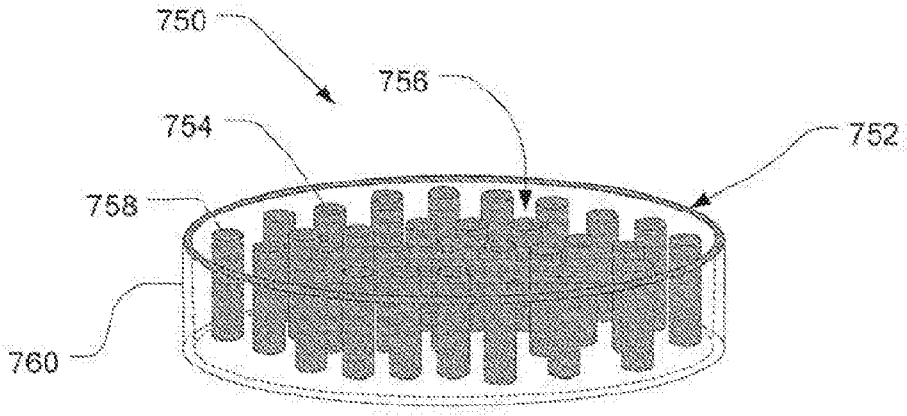


FIGURE 8B

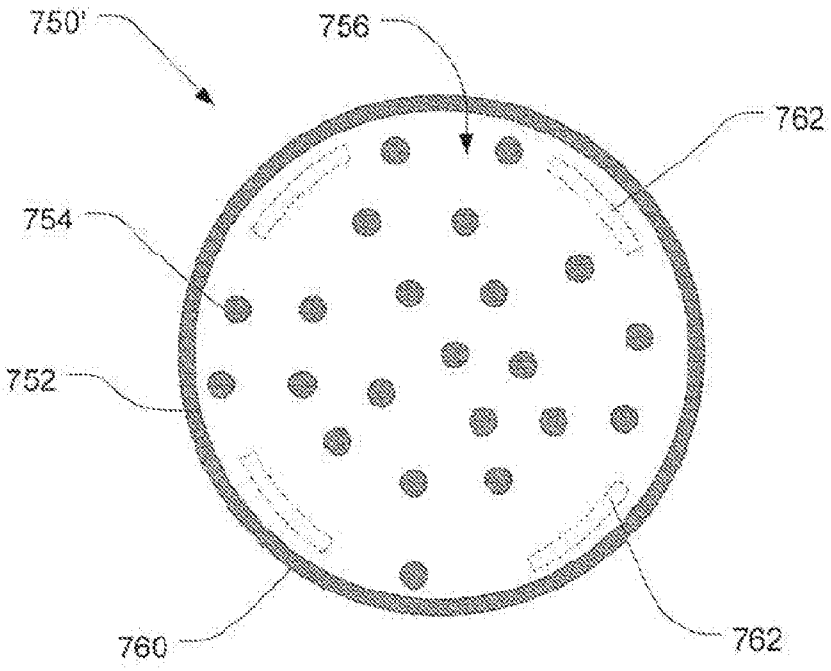


FIGURE 8C

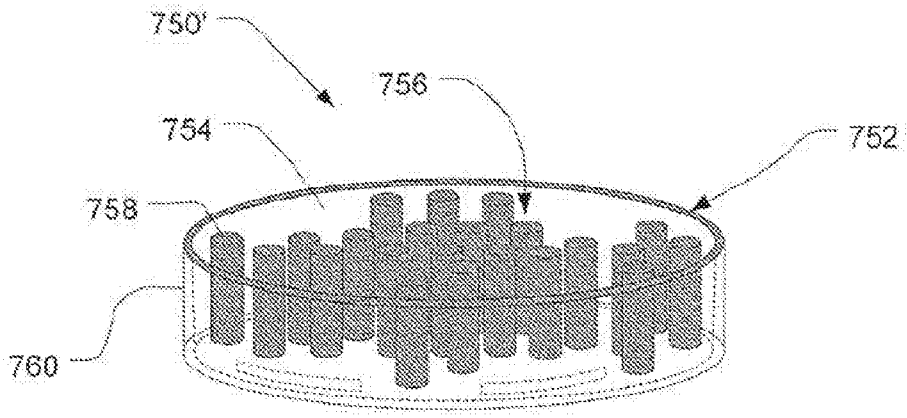


FIGURE 8D

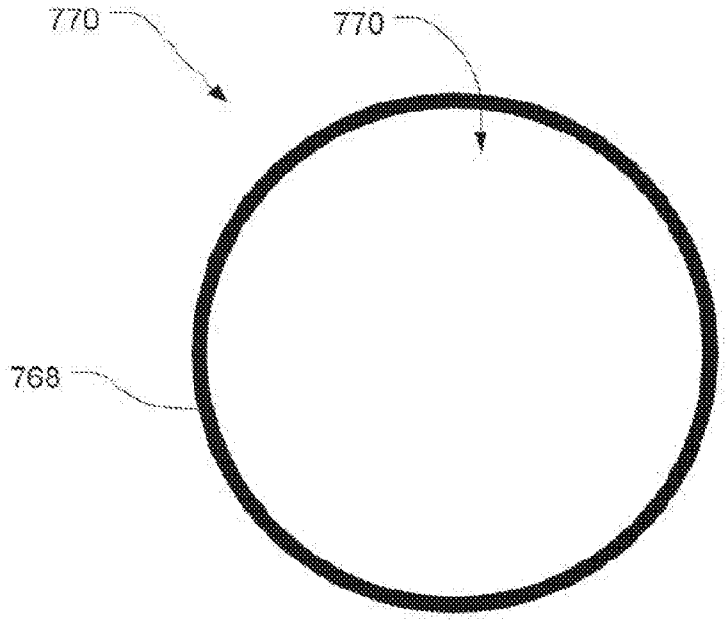


FIGURE 8E

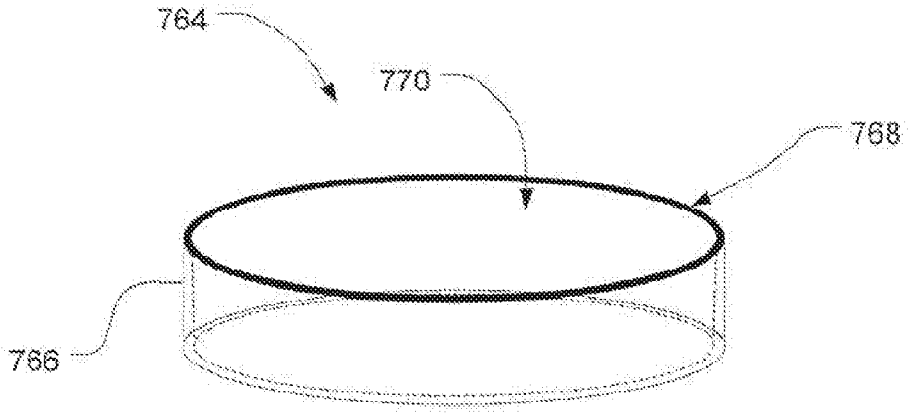


FIGURE 8F

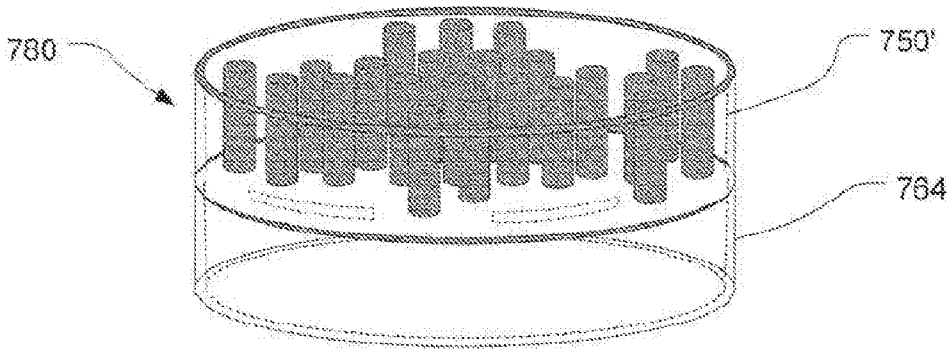


FIGURE 8G

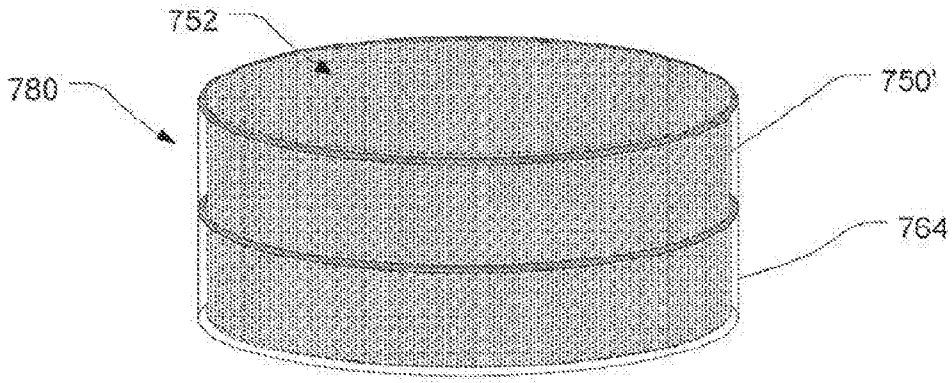


FIGURE 8H

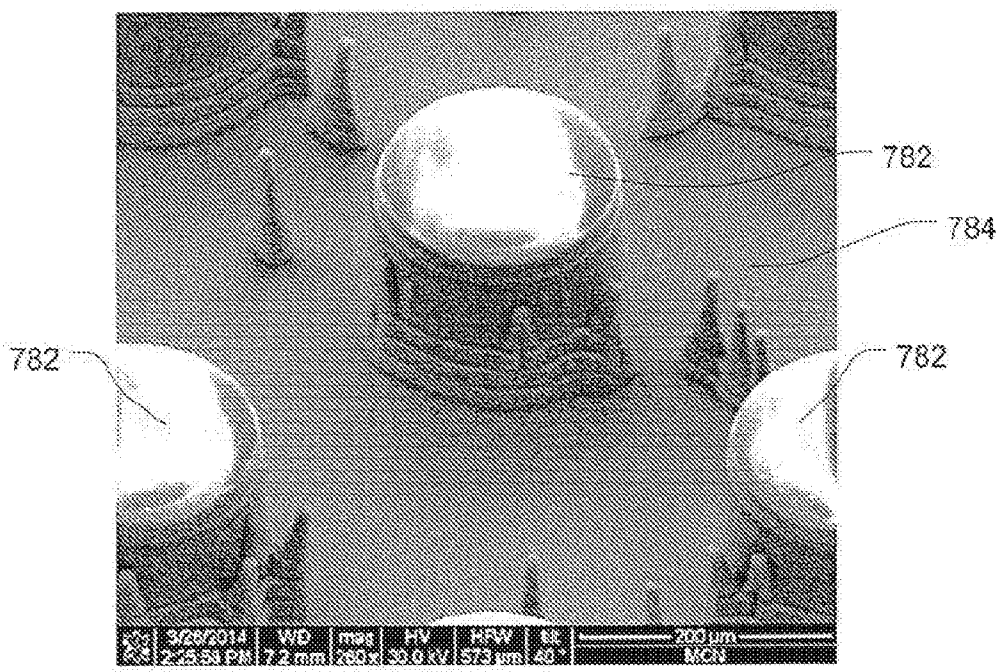


FIGURE 9A

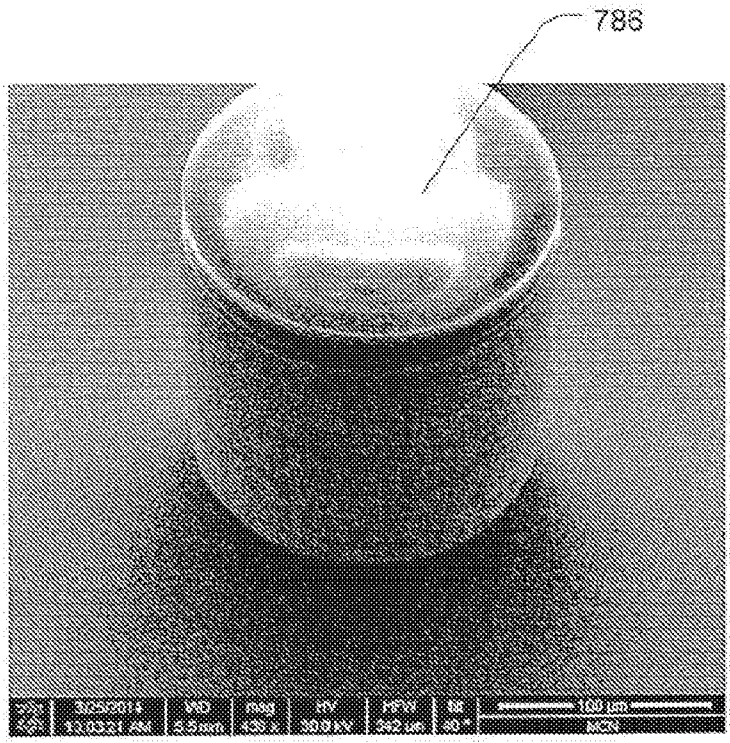


FIGURE 9B

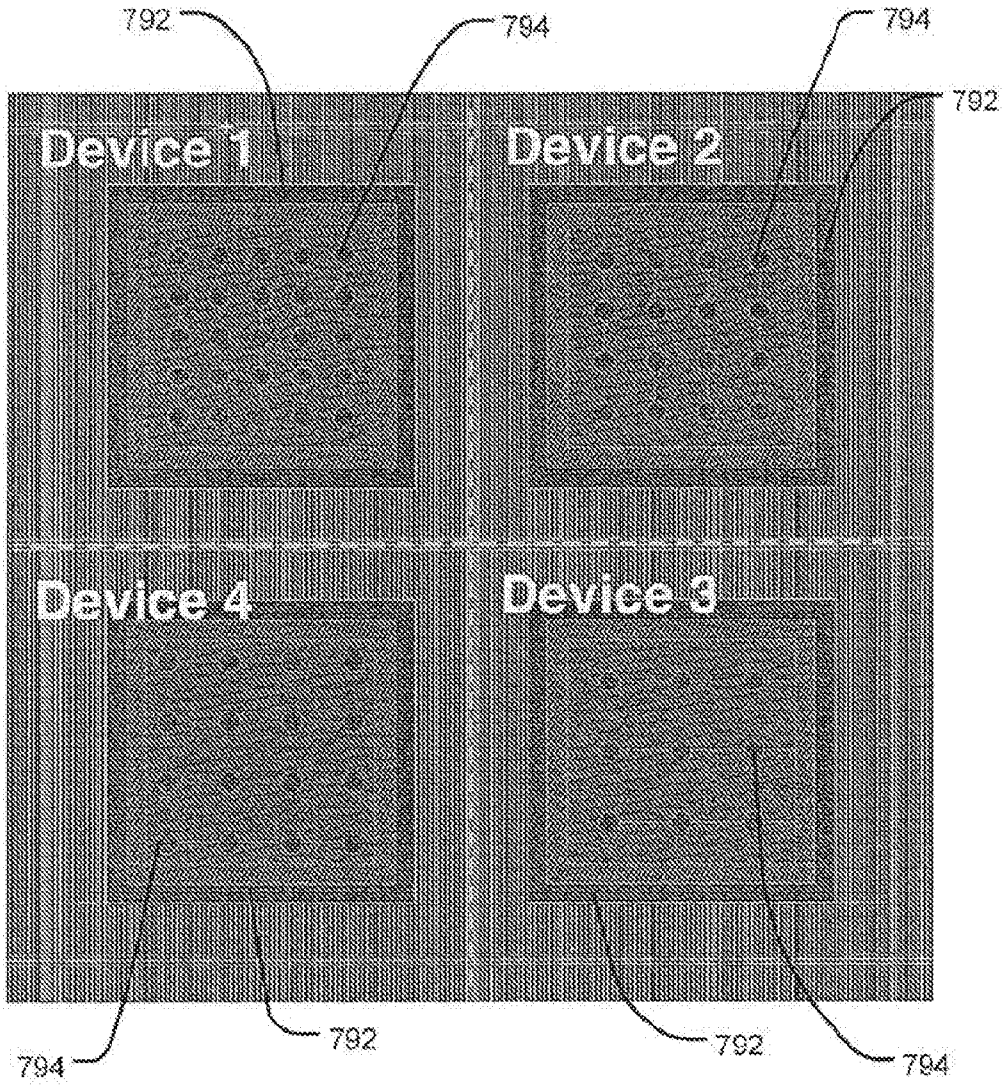

792
794
794
792
Device 1
Device 2
Device 4
Device 3
794
792
792
794

FIGURE 10

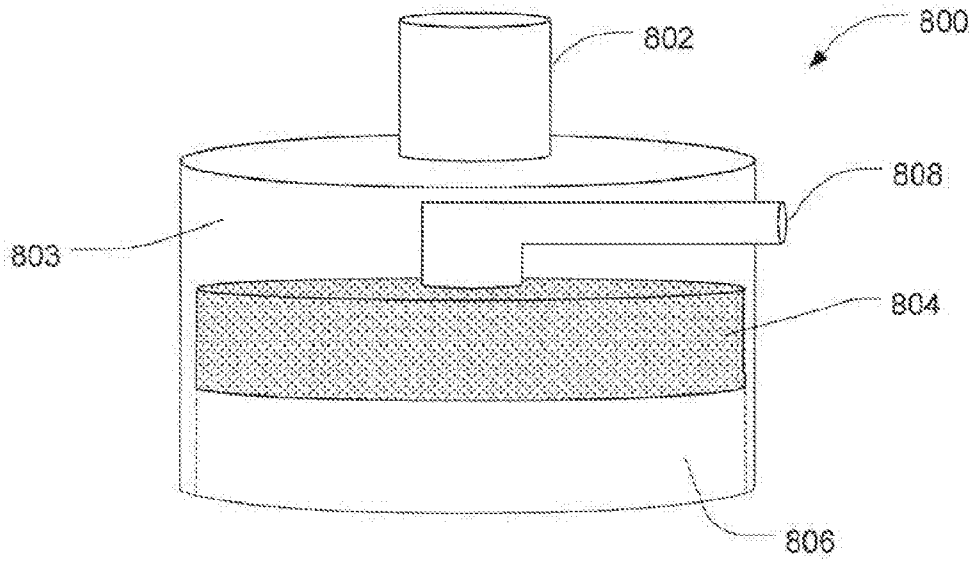


FIGURE 11A

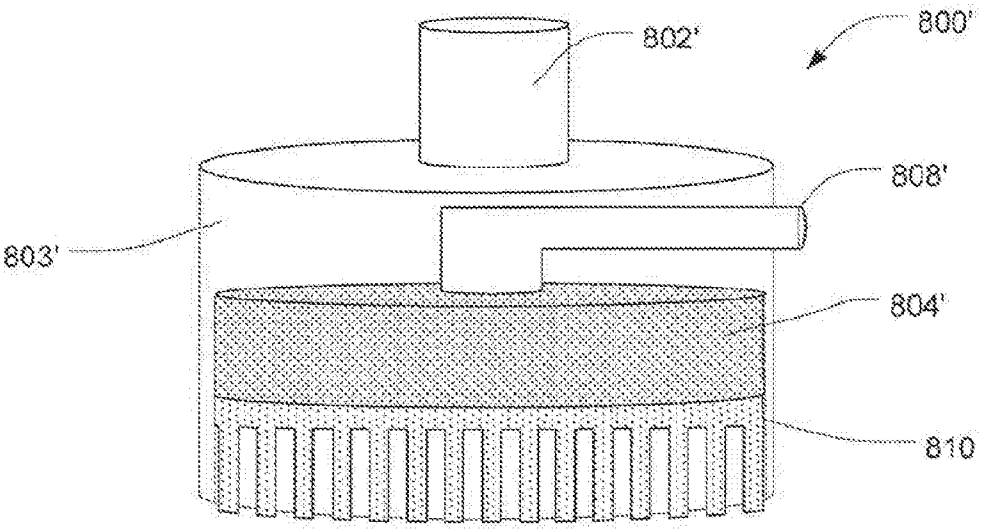


FIGURE 11B

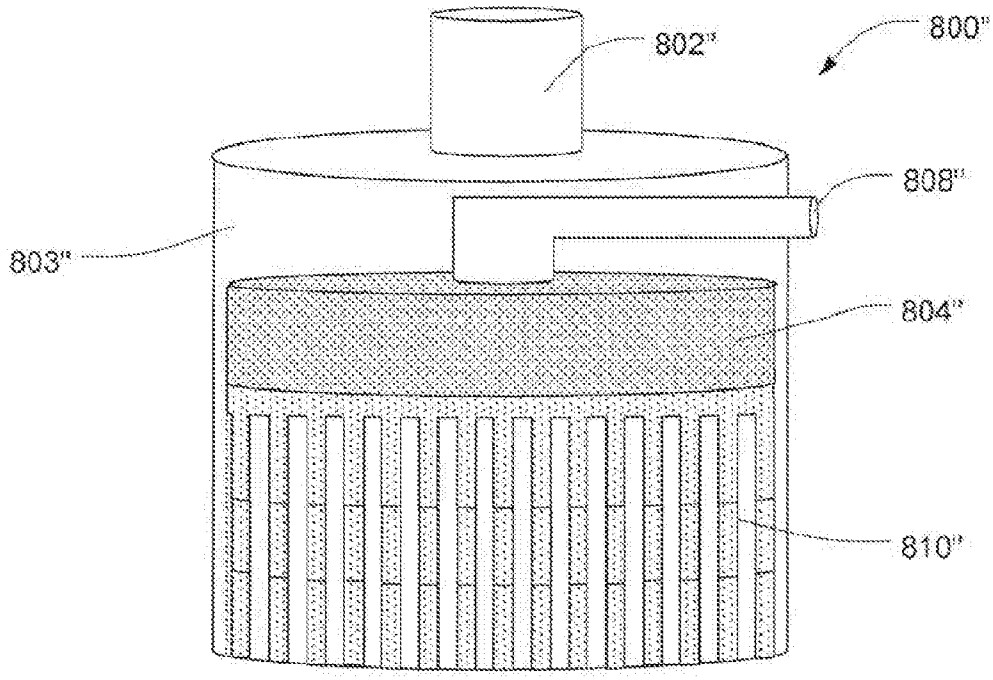


FIGURE 11C

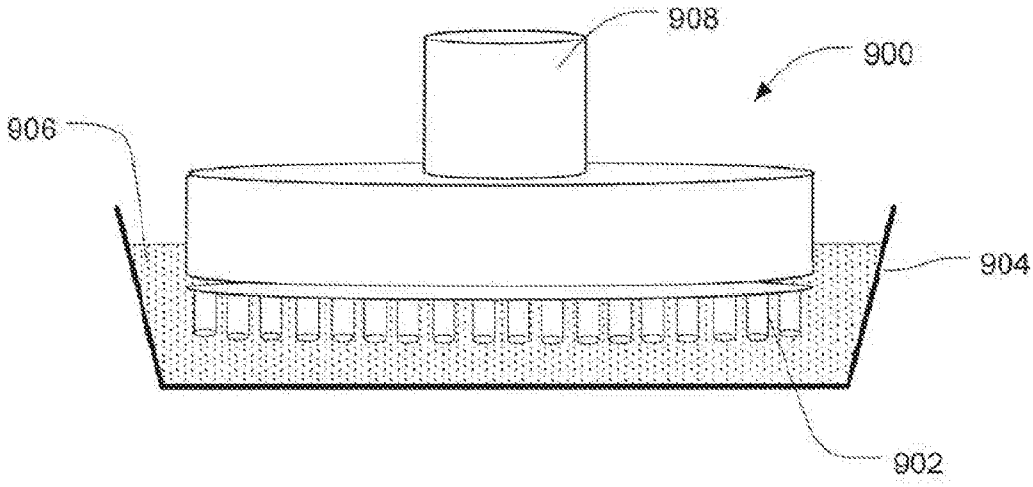


FIGURE 12A

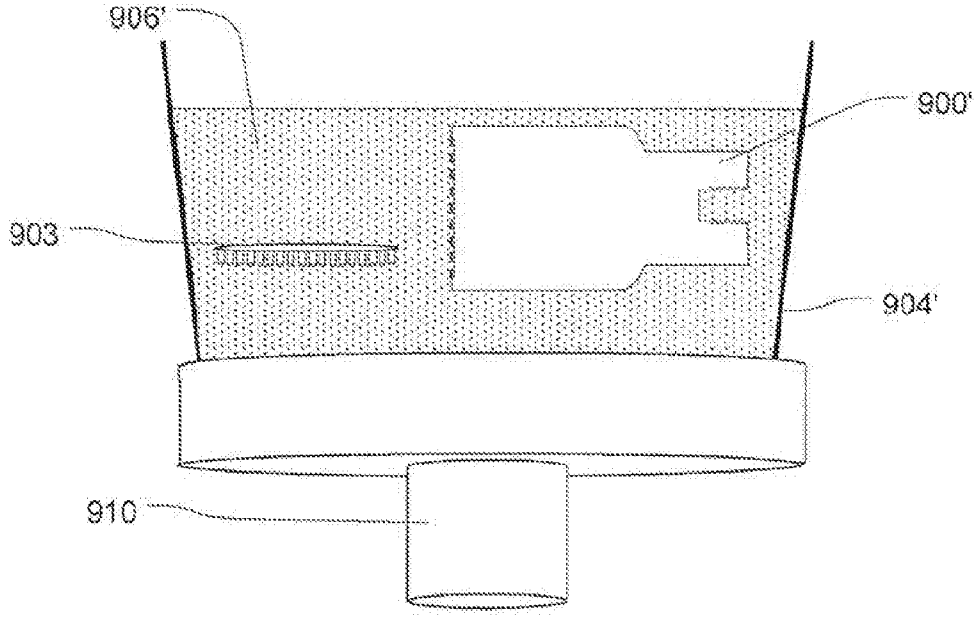


FIGURE 12B

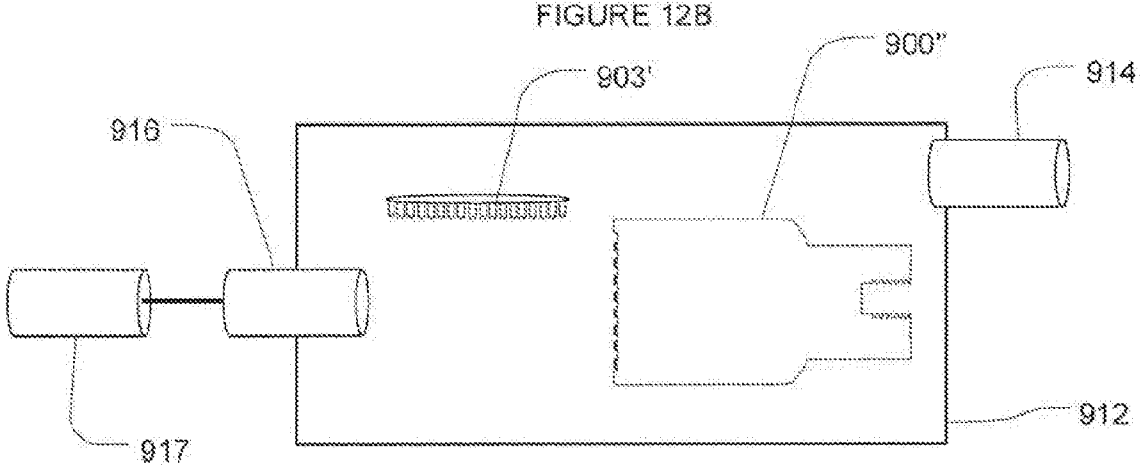


FIGURE 12C

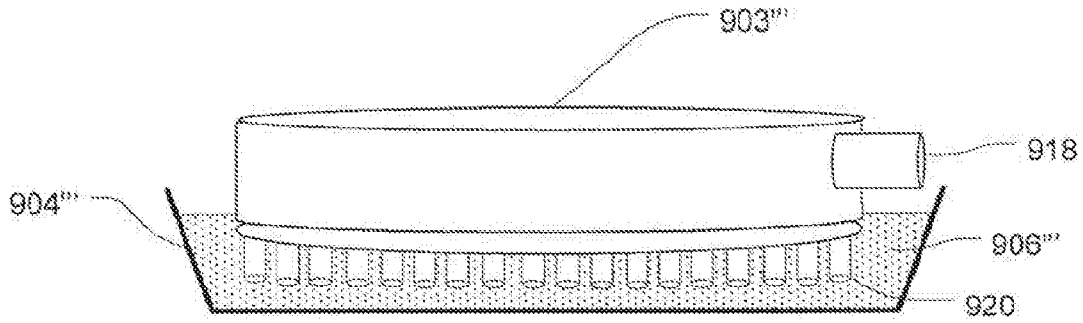


FIGURE 12D

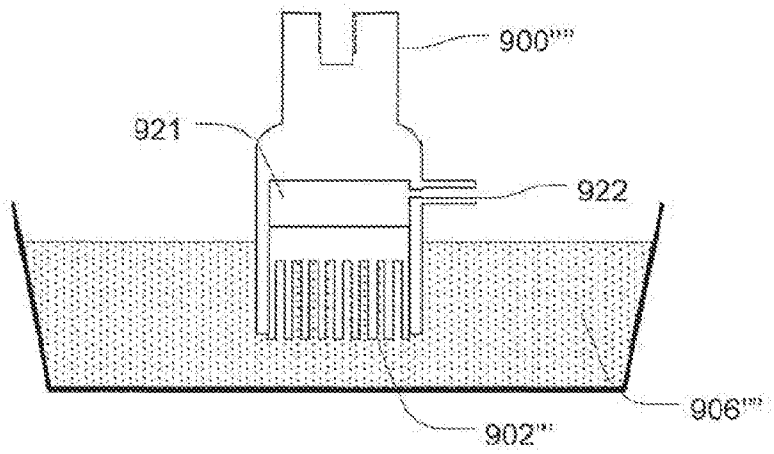


FIGURE 12E

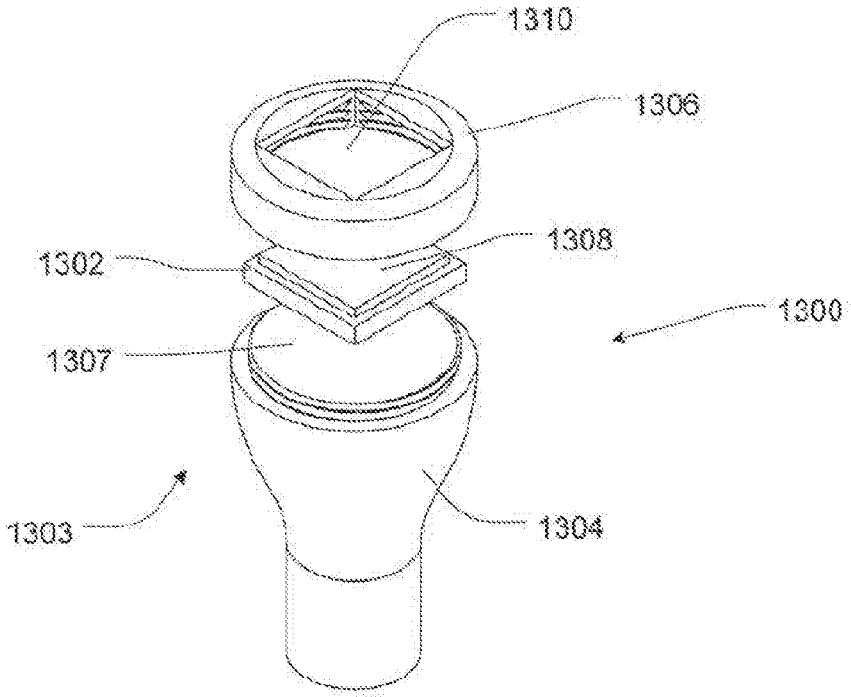


FIGURE 13A

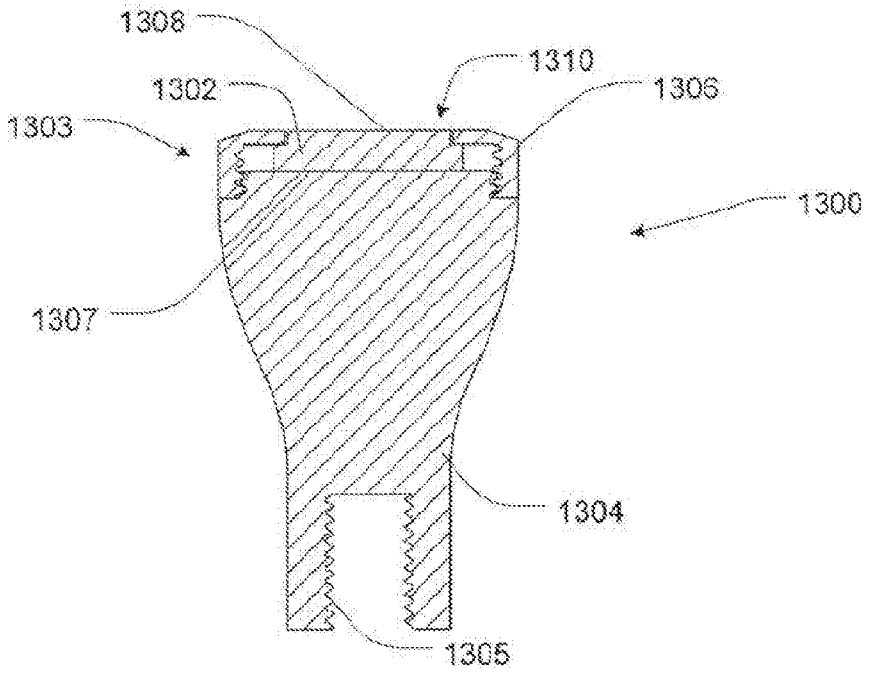


FIGURE 13B

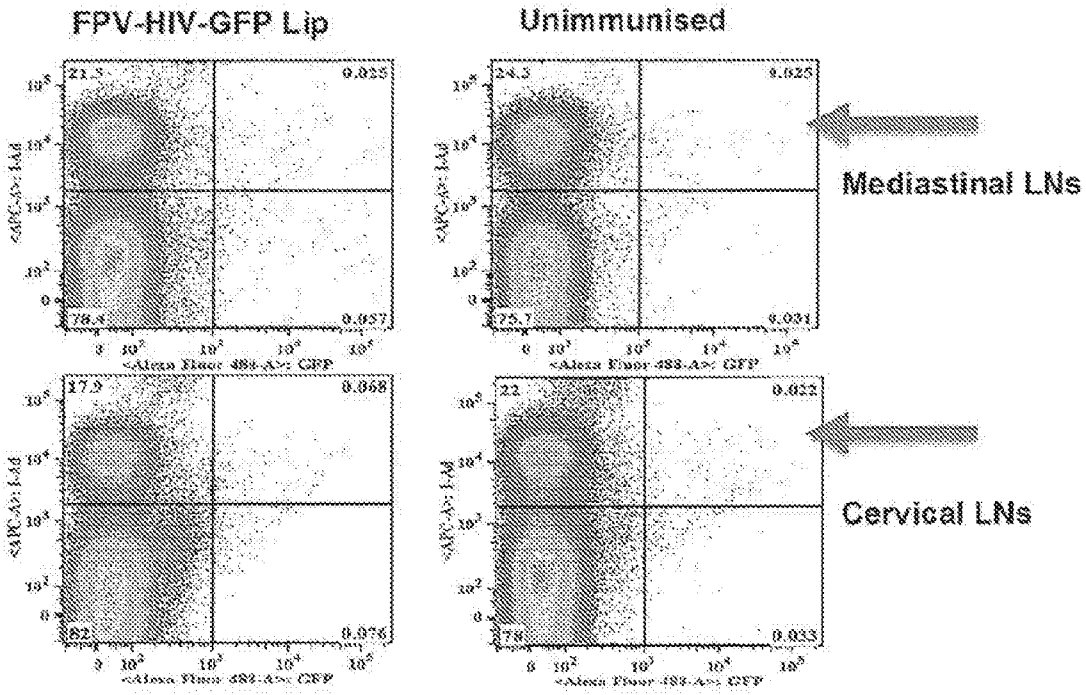


FIGURE 14

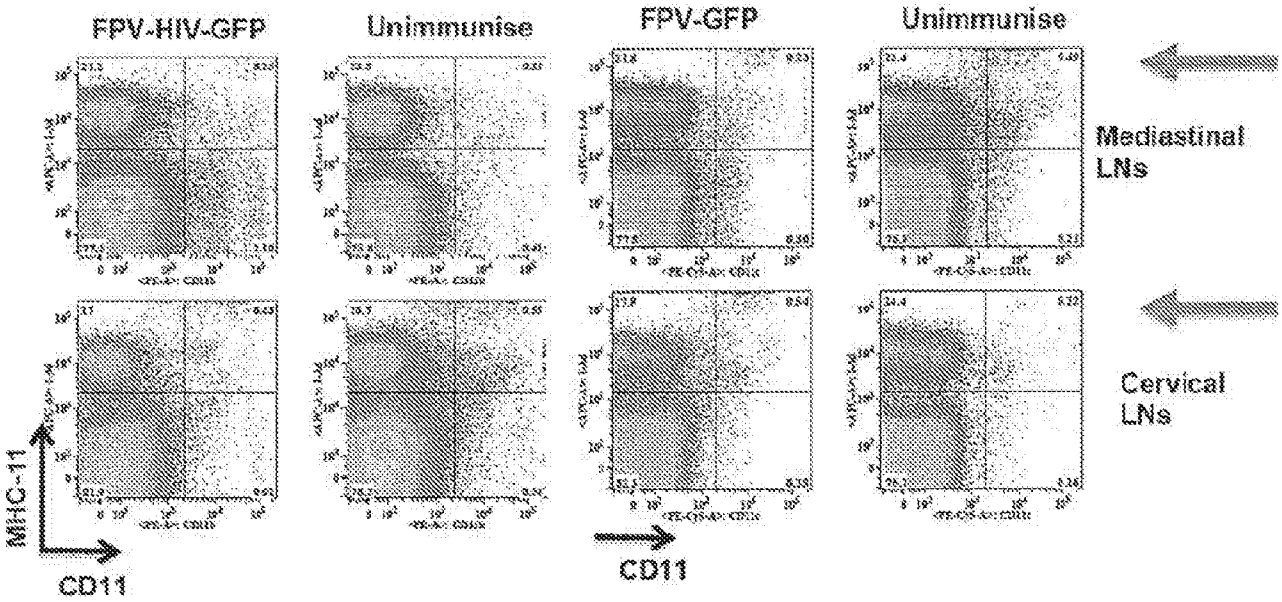


FIGURE 15

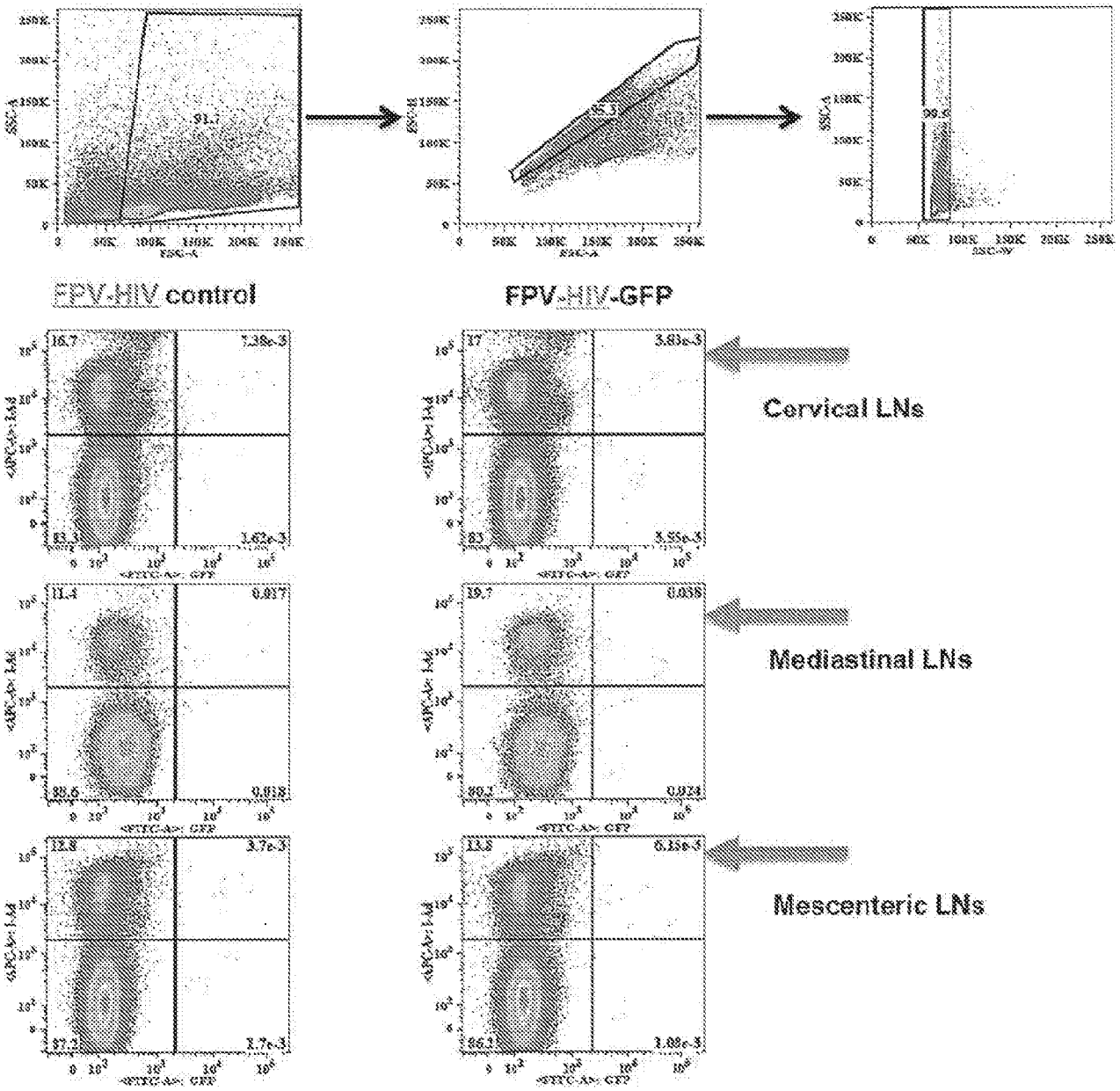

FPV-HIV control
FPV-HIV-GFP
Cervical LNs
Mediastinal LNs
Mescenteric LNs

FIGURE 16

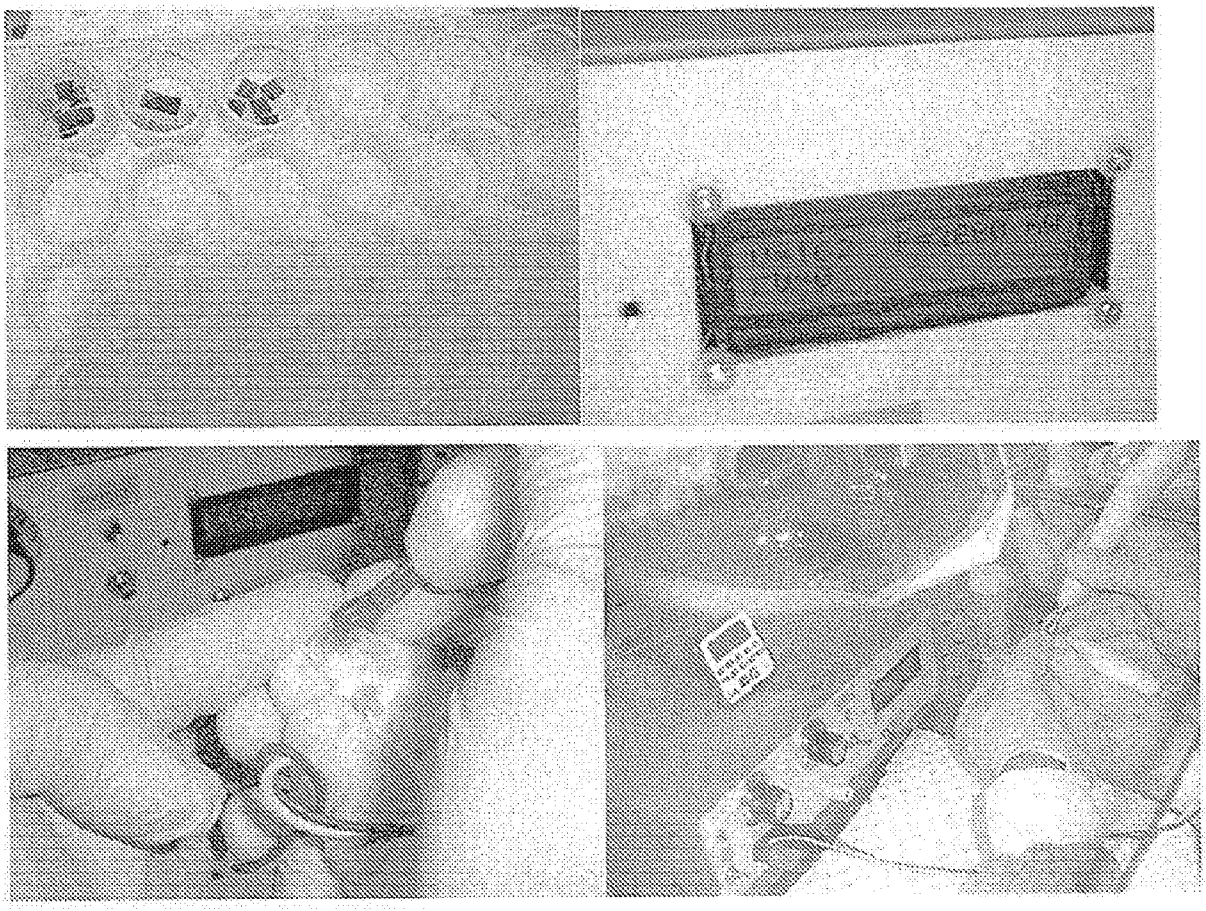


FIGURE 17

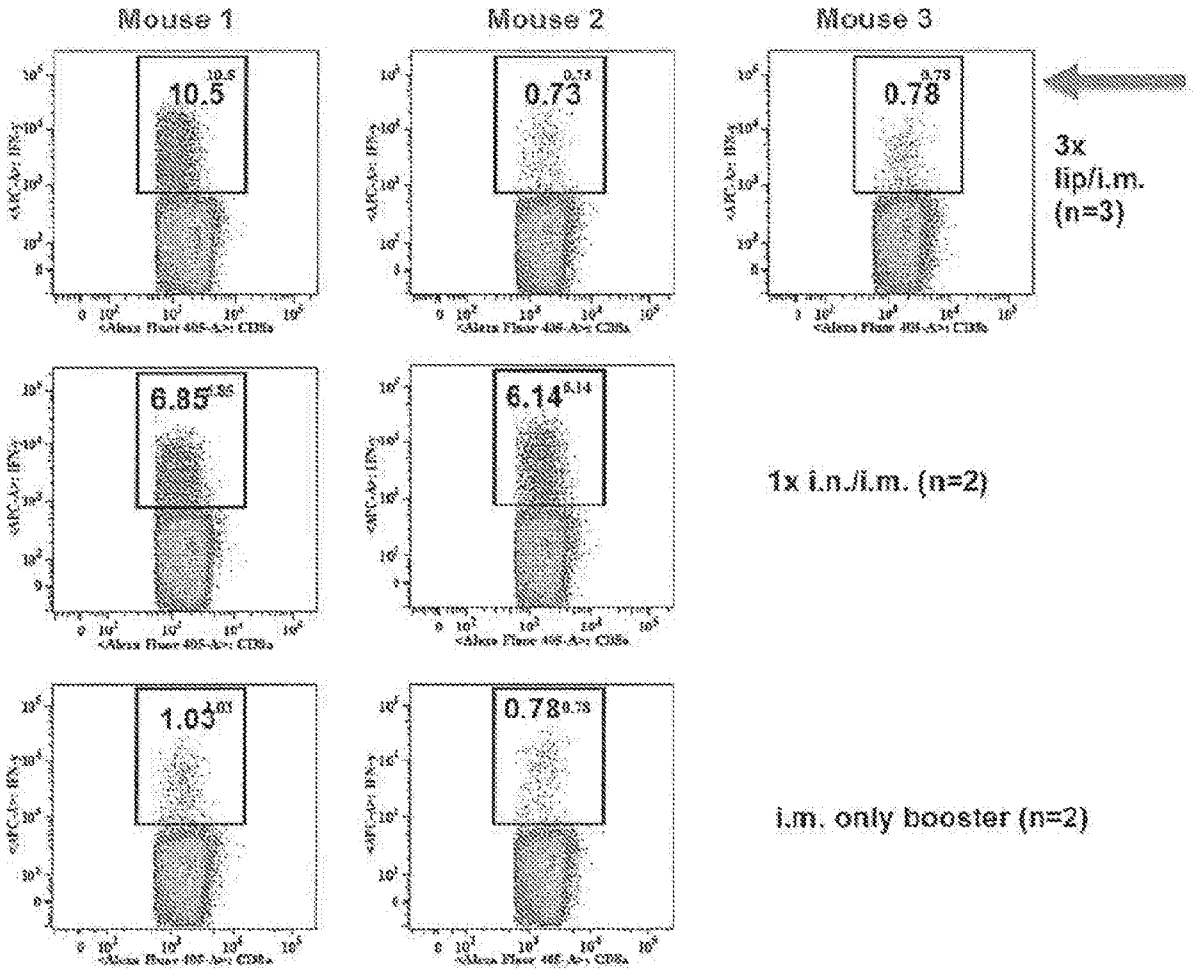

Mouse 1
Mouse 2
Mouse 3
10.5
0.73
0.78
3x
Iip/i.m.
(n=3)
6.85
6.14
1x i.n./i.m. (n=2)
1.03
0.78
i.m. only booster (n=2)

FIGURE 18

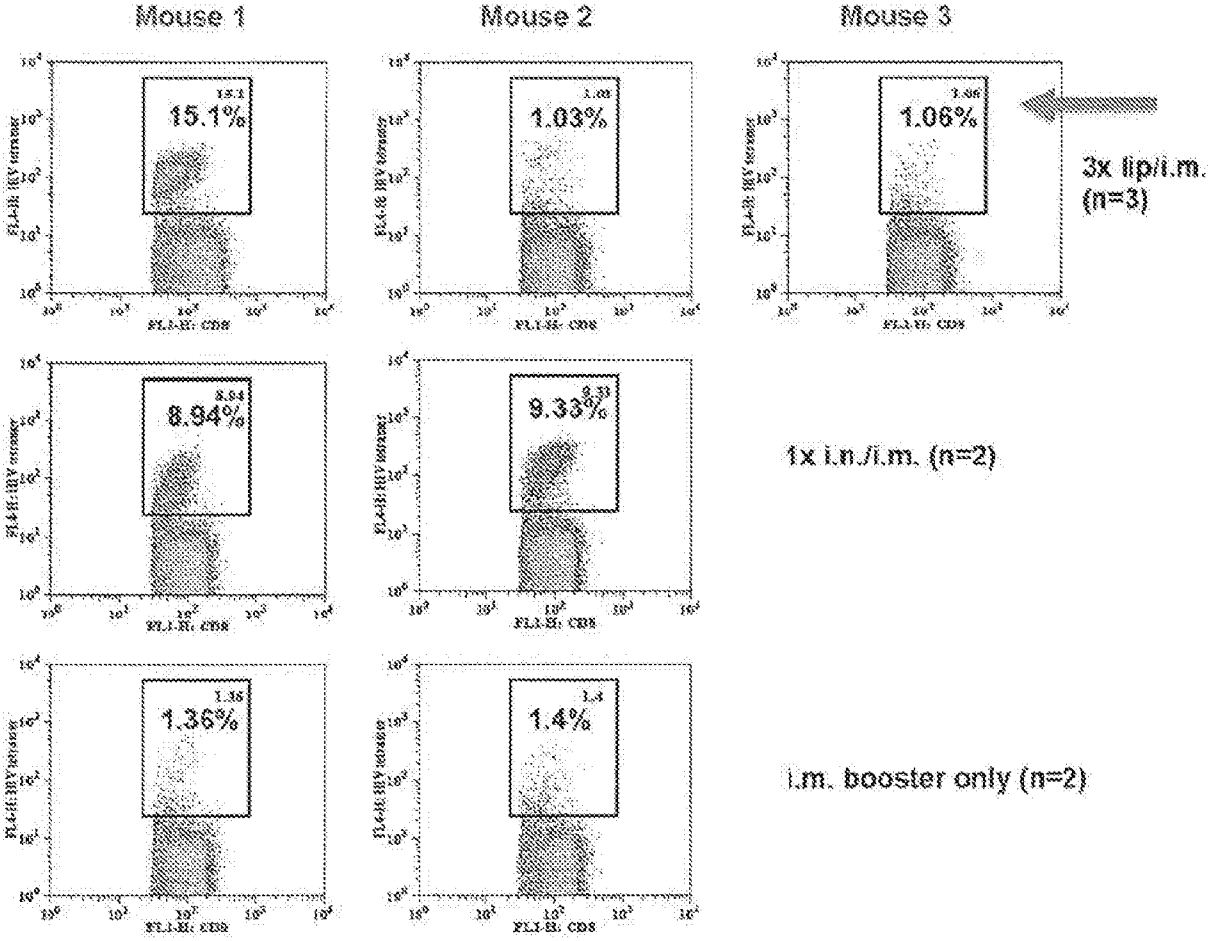

Mouse 1
Mouse 2
Mouse 3
15.1%
1.03%
1.06%
3x Iip/i.m.
(n=3)
8.94%
9.33%
1x i.n./i.m. (n=2)
1.36%
1.4%
i.m. booster only (n=2)

FIGURE 19

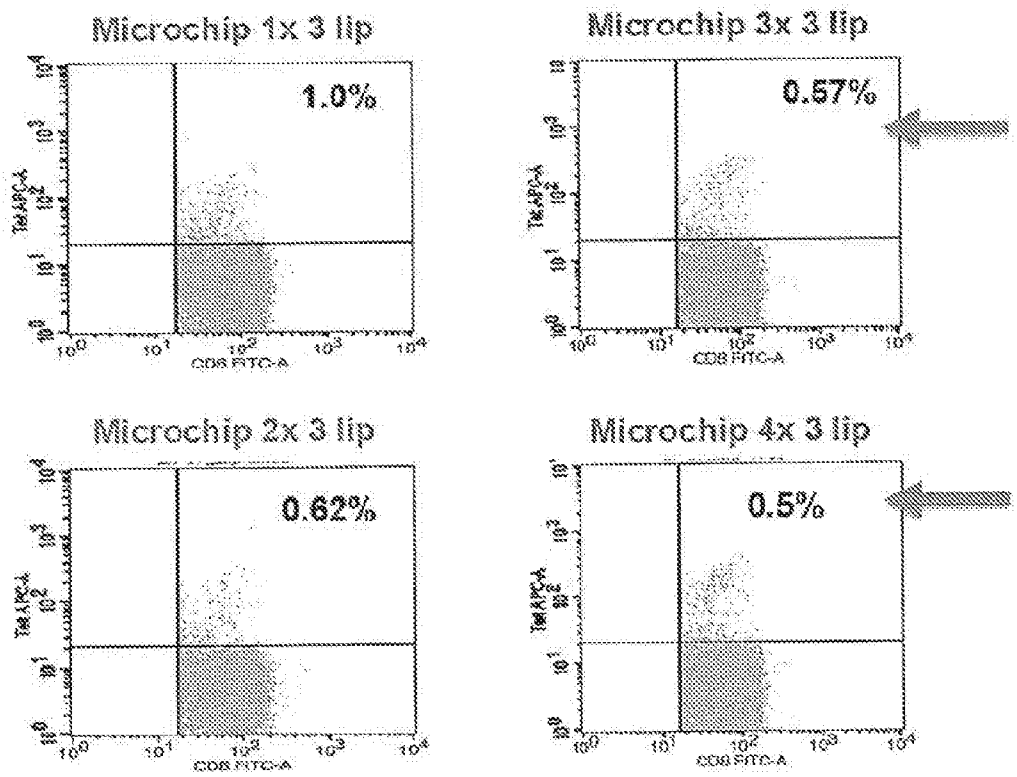


FIGURE 20

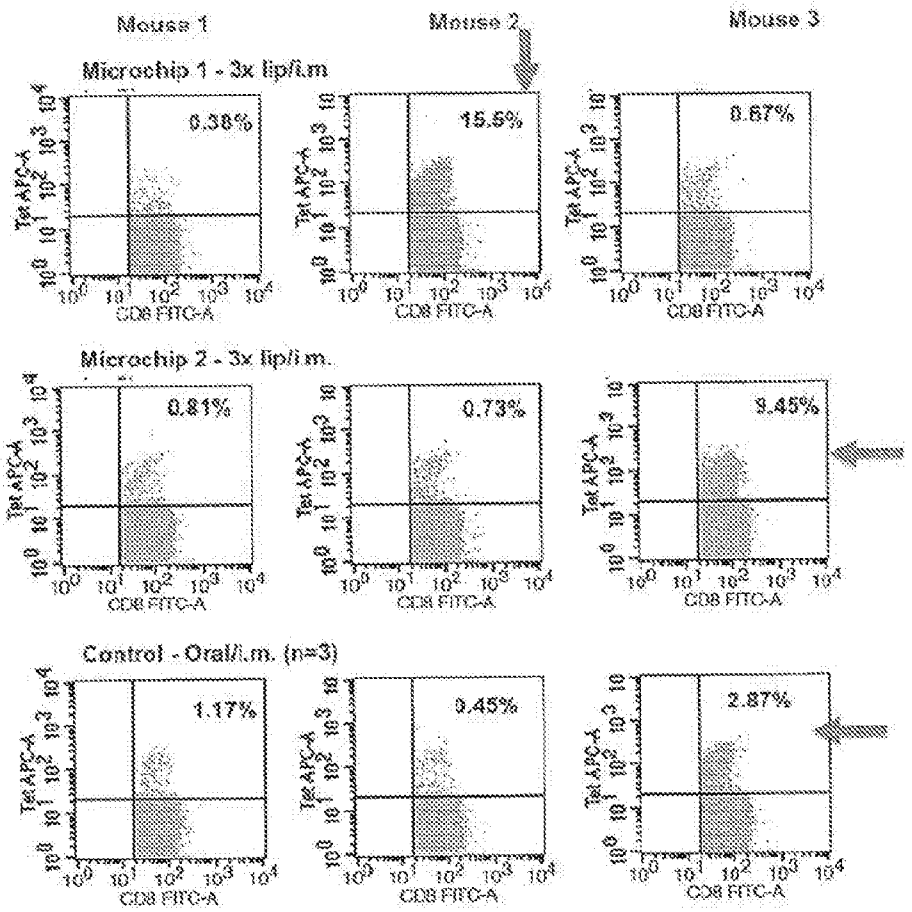


FIGURE 21

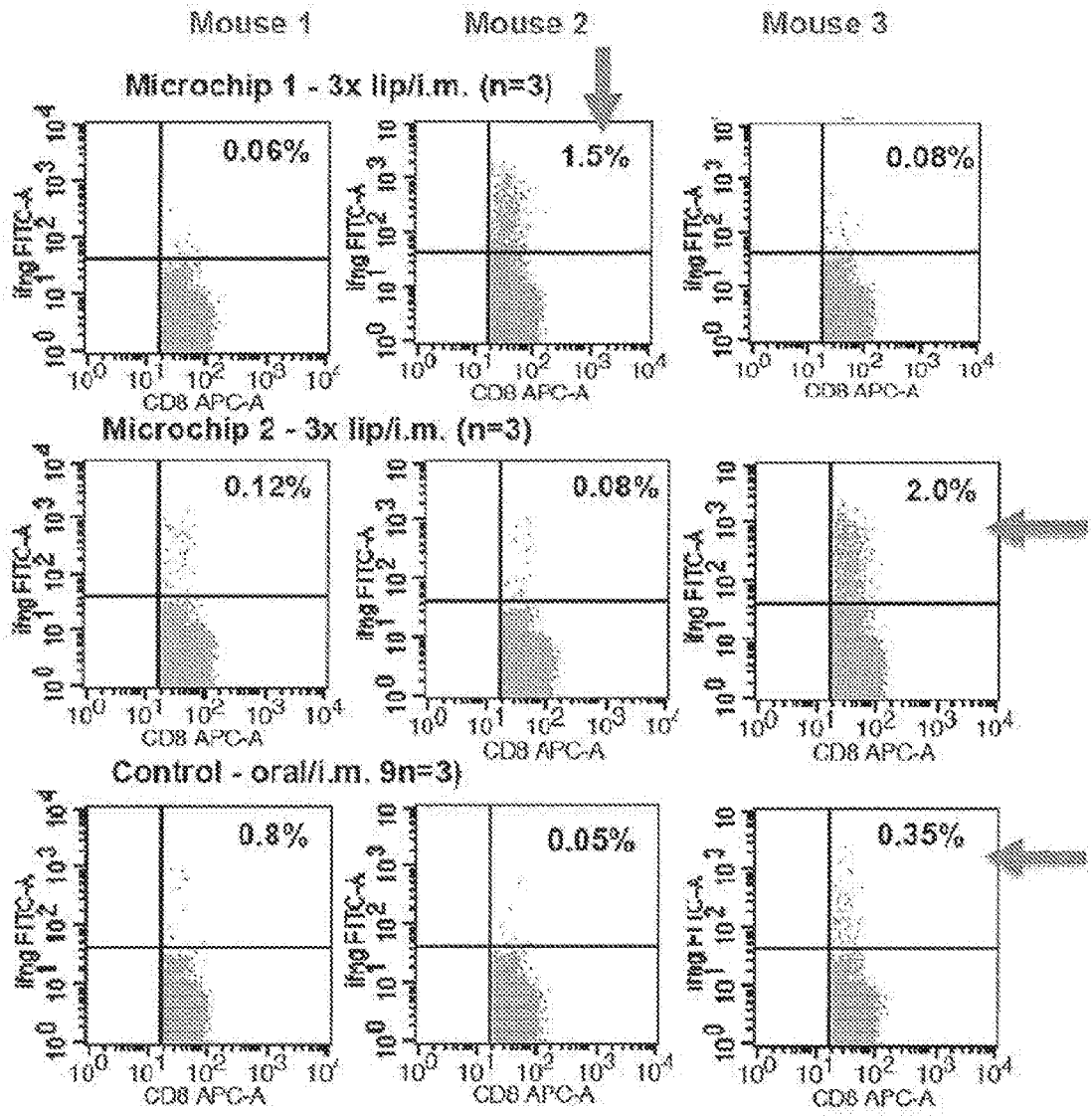

Mouse 1
Mouse 2
Mouse 3
Microchip 1 - 3x lip/i.m. (n=3)
0.06%
1.5%
0.08%
Microchip 2 - 3x lip/i.m. (n=3)
0.12%
0.08%
2.0%
Control - oral/i.m. 9n=3)
0.8%
0.05%
0.35%
IFNg FITC-A
CD8 APC-A

FIGURE 22

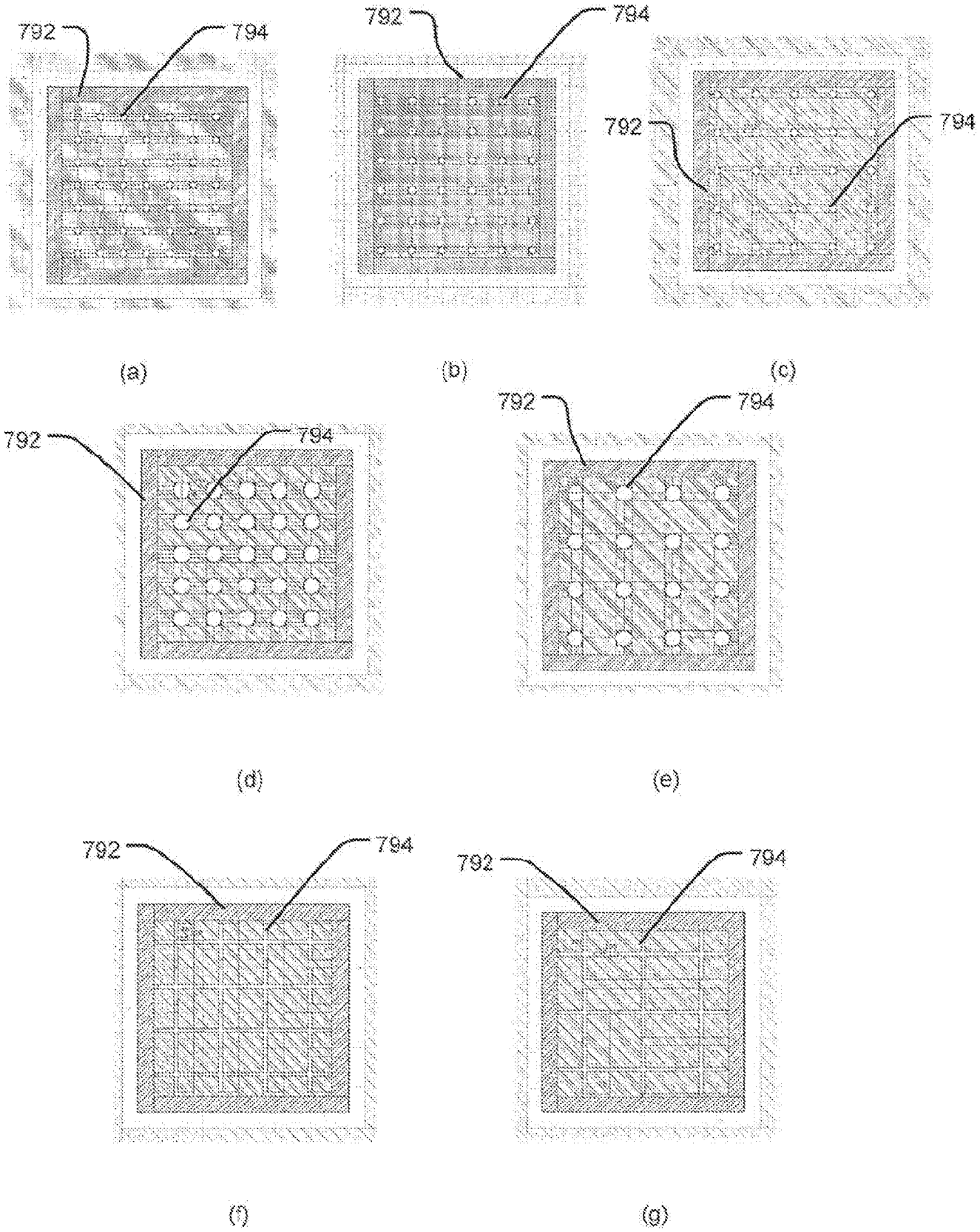


FIGURE 23

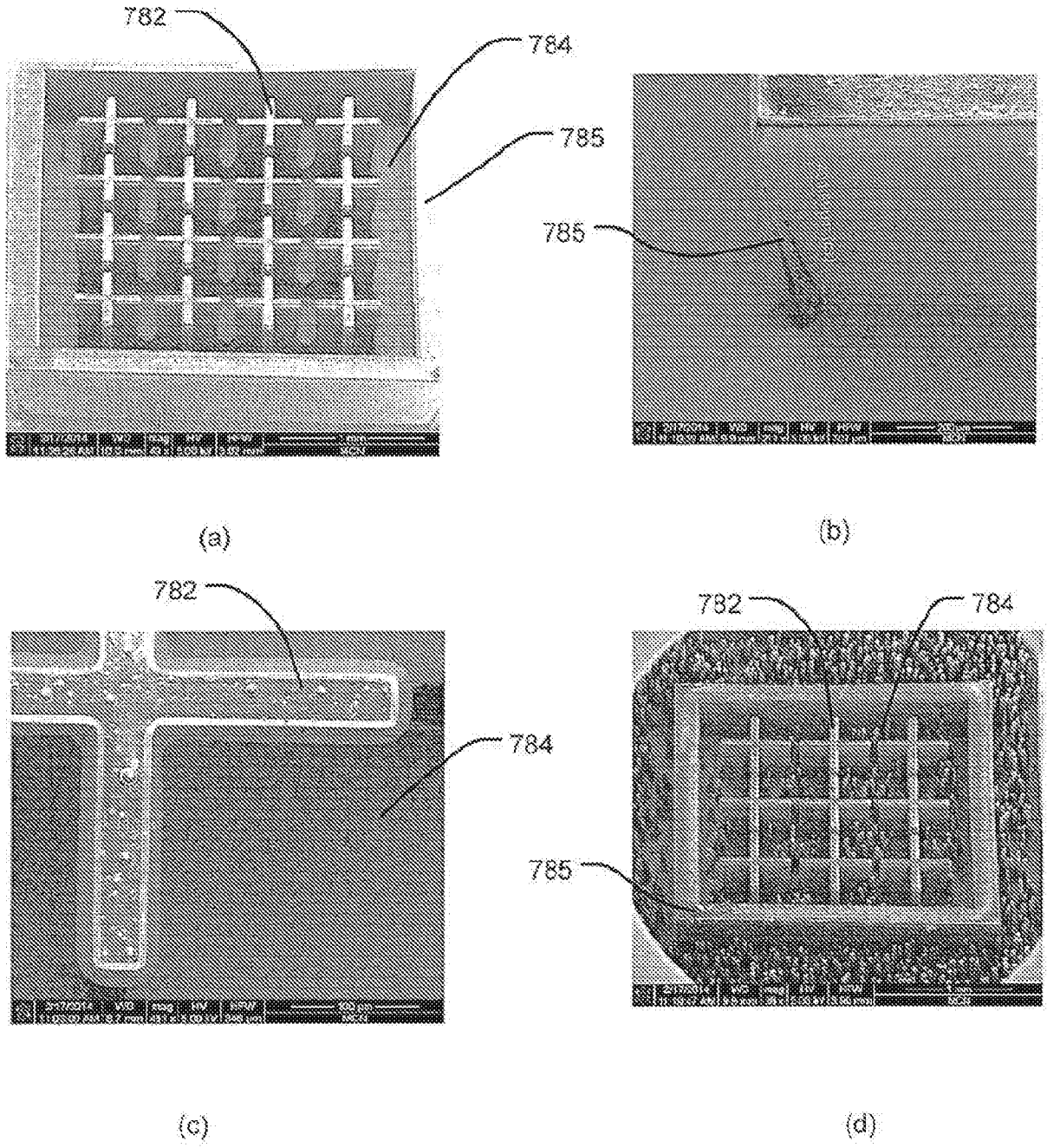


FIGURE 24

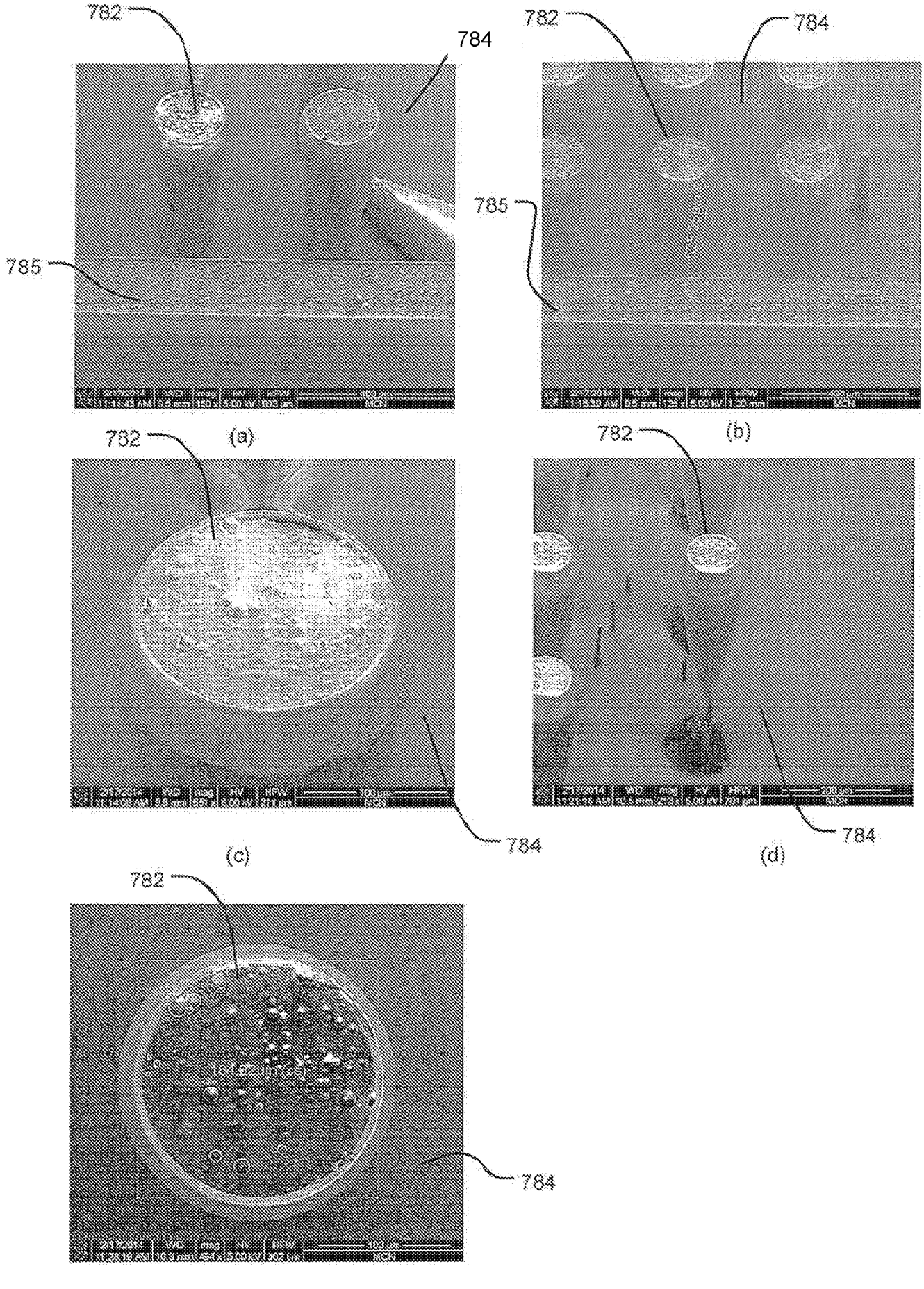


FIGURE 25

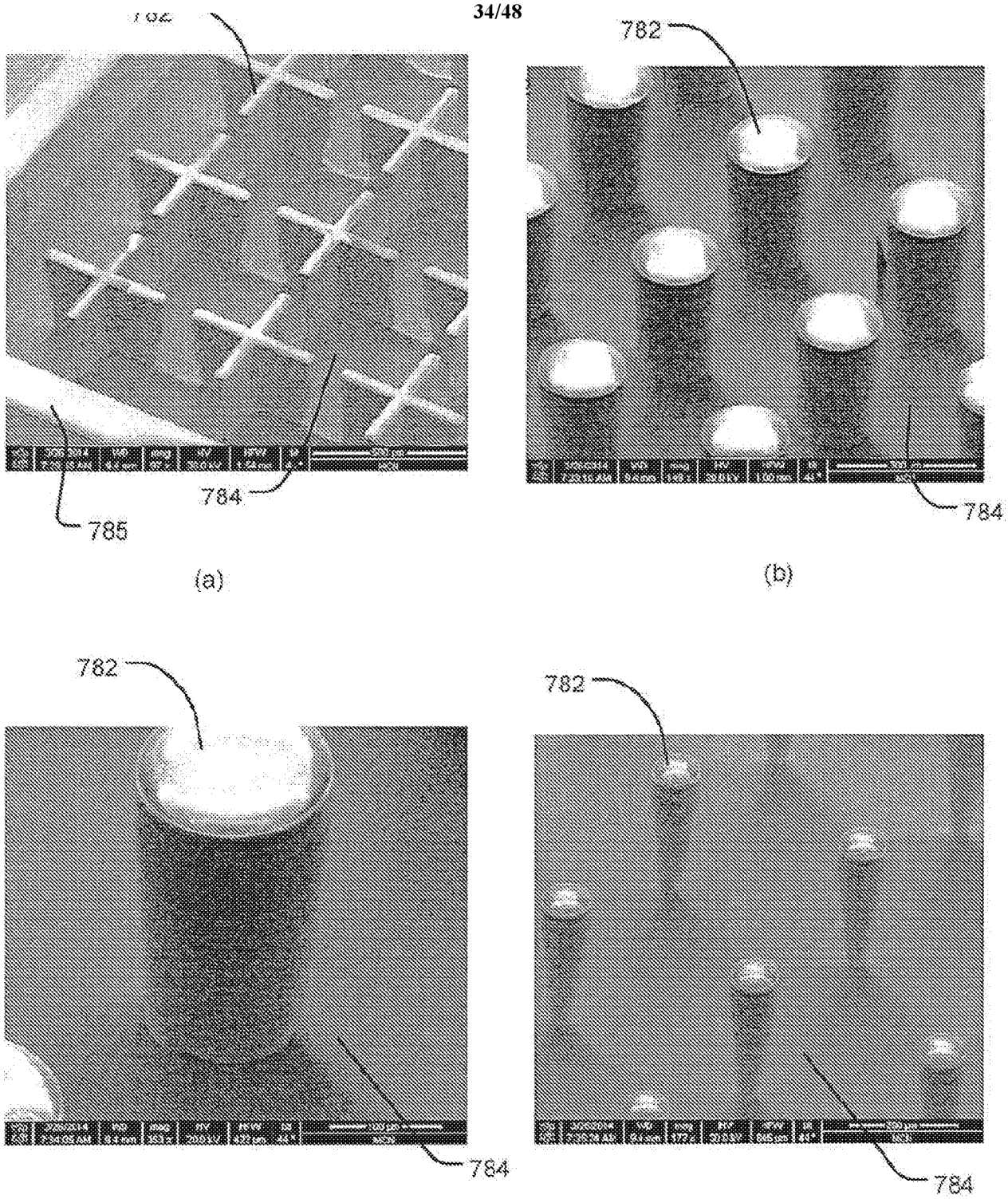


FIGURE 26

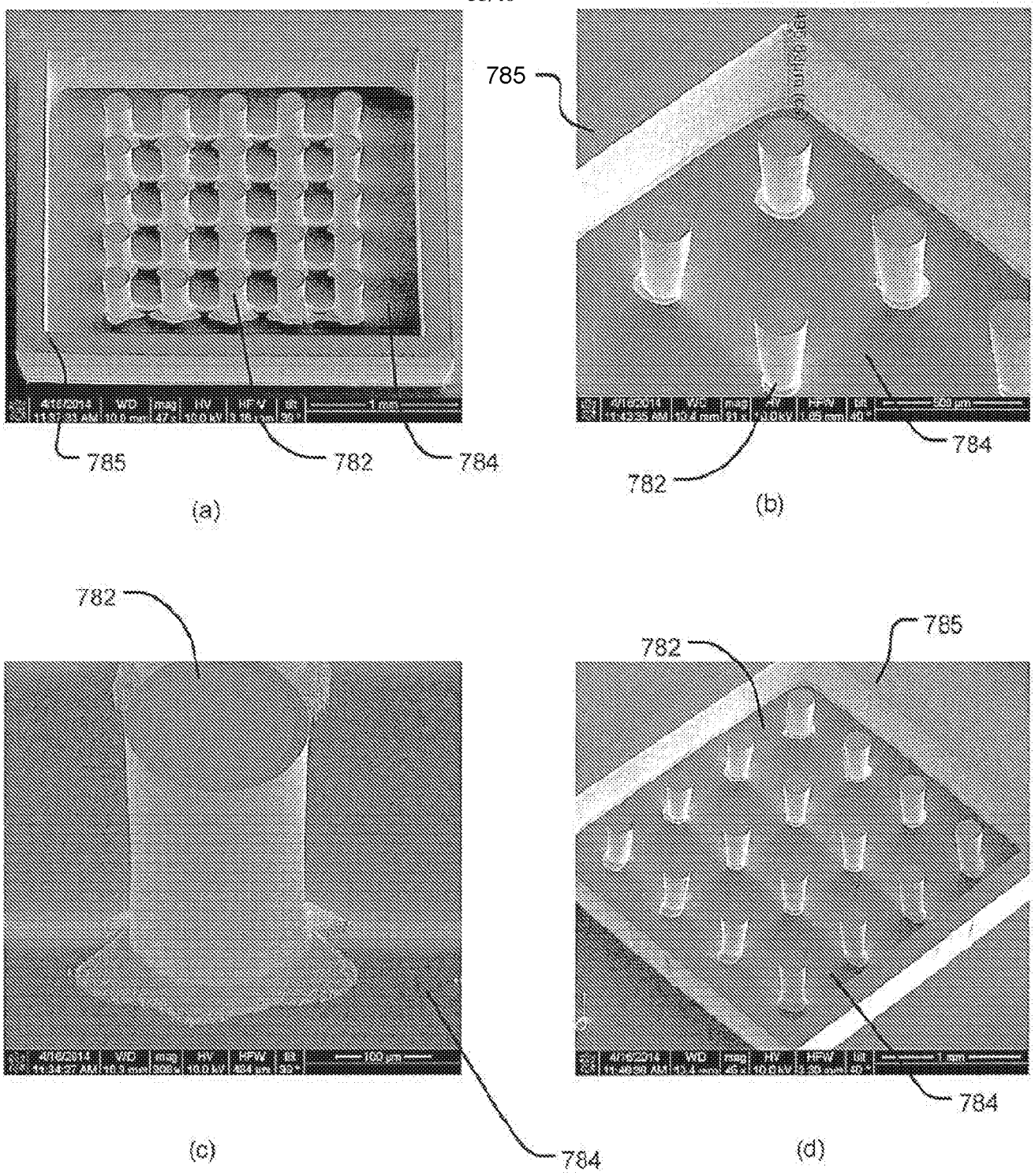
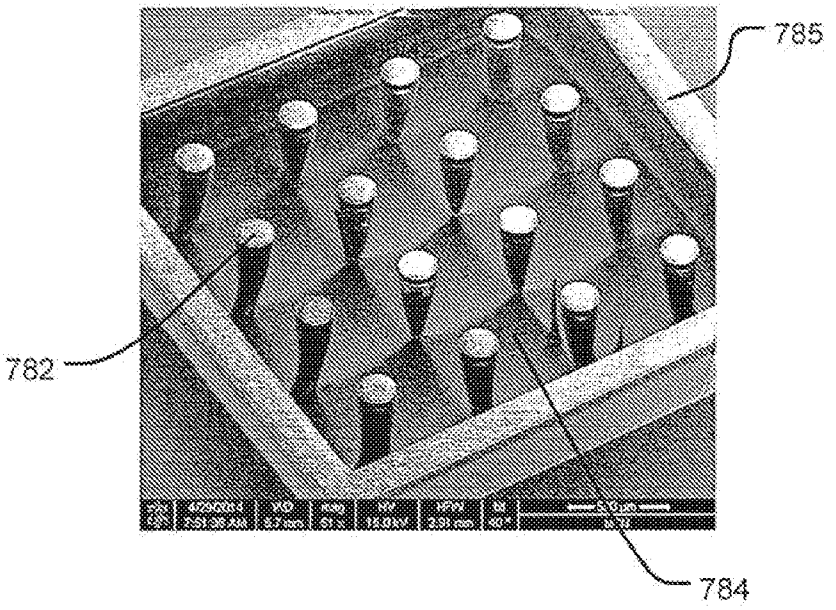
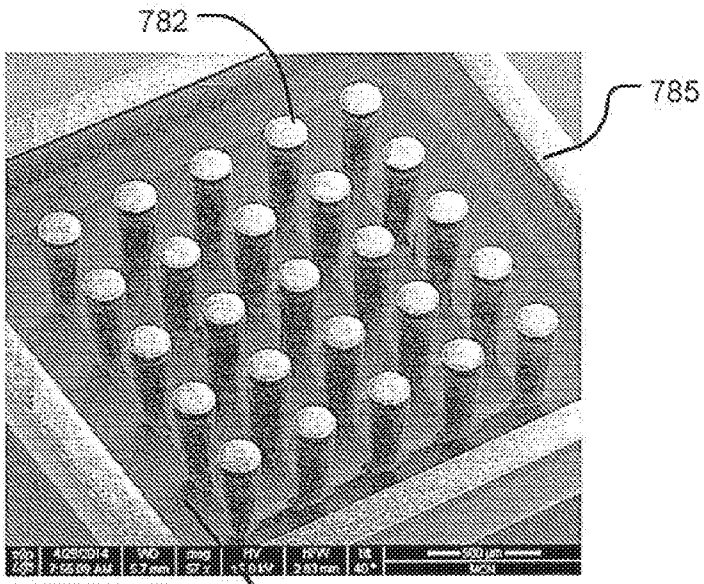


FIGURE 27



(a)



(b)

FIGURE 28

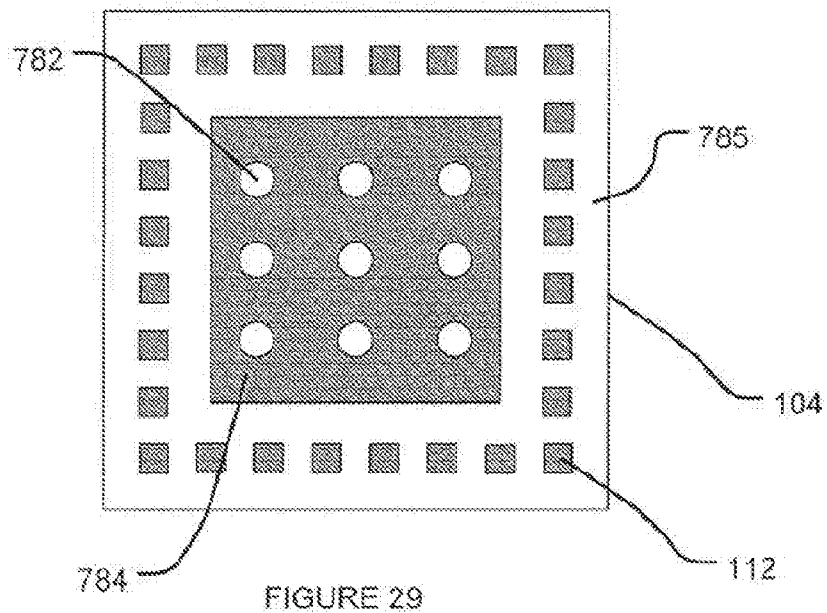


FIGURE 29

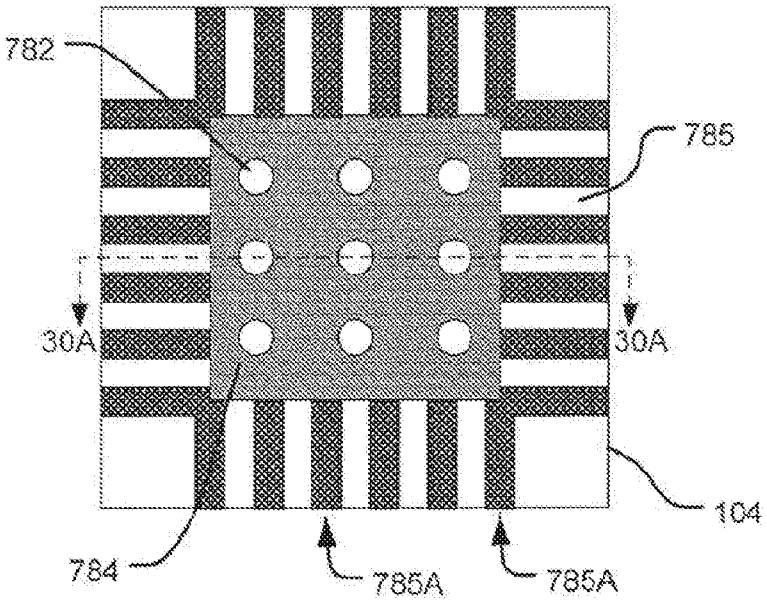


FIGURE 30

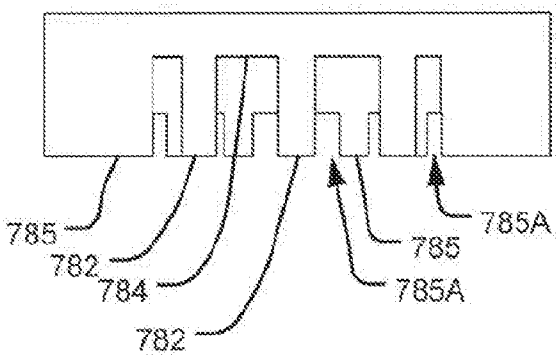


FIGURE 30A

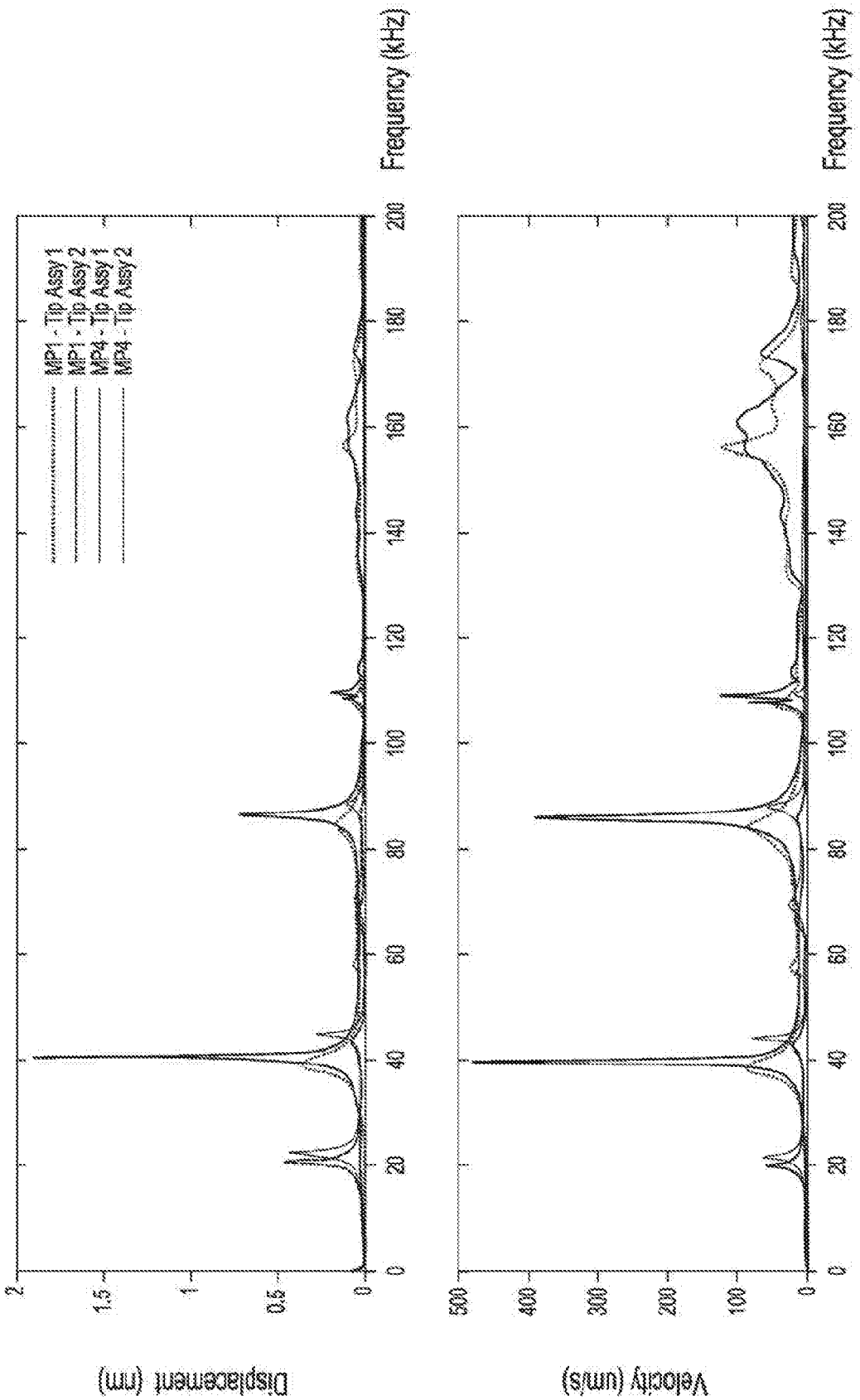

Displacement (nm)
Frequency (kHz)
MP1 - Tip Assy 1
MP1 - Tip Assy 2
MP4 - Tip Assy 1
MP4 - Tip Assy 2
Velocity (um/s)
Frequency (kHz)

Figure 31A

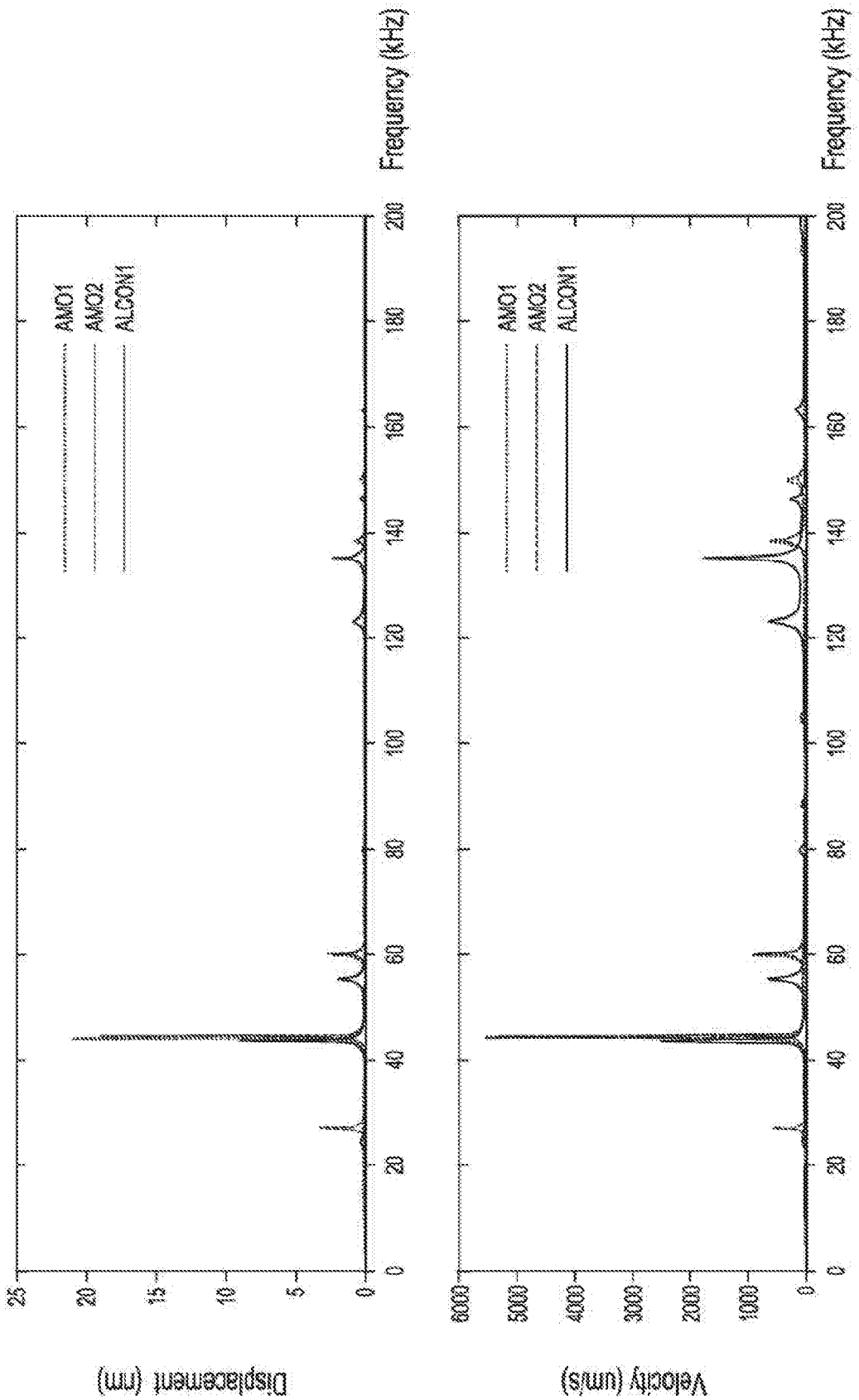

Displacement (nm)
Frequency (kHz)
AMO1
AMO2
ALCOW1
Velocity (um/s)
Frequency (kHz)
AMO1
AMO2
ALCOW1

Figure 31B

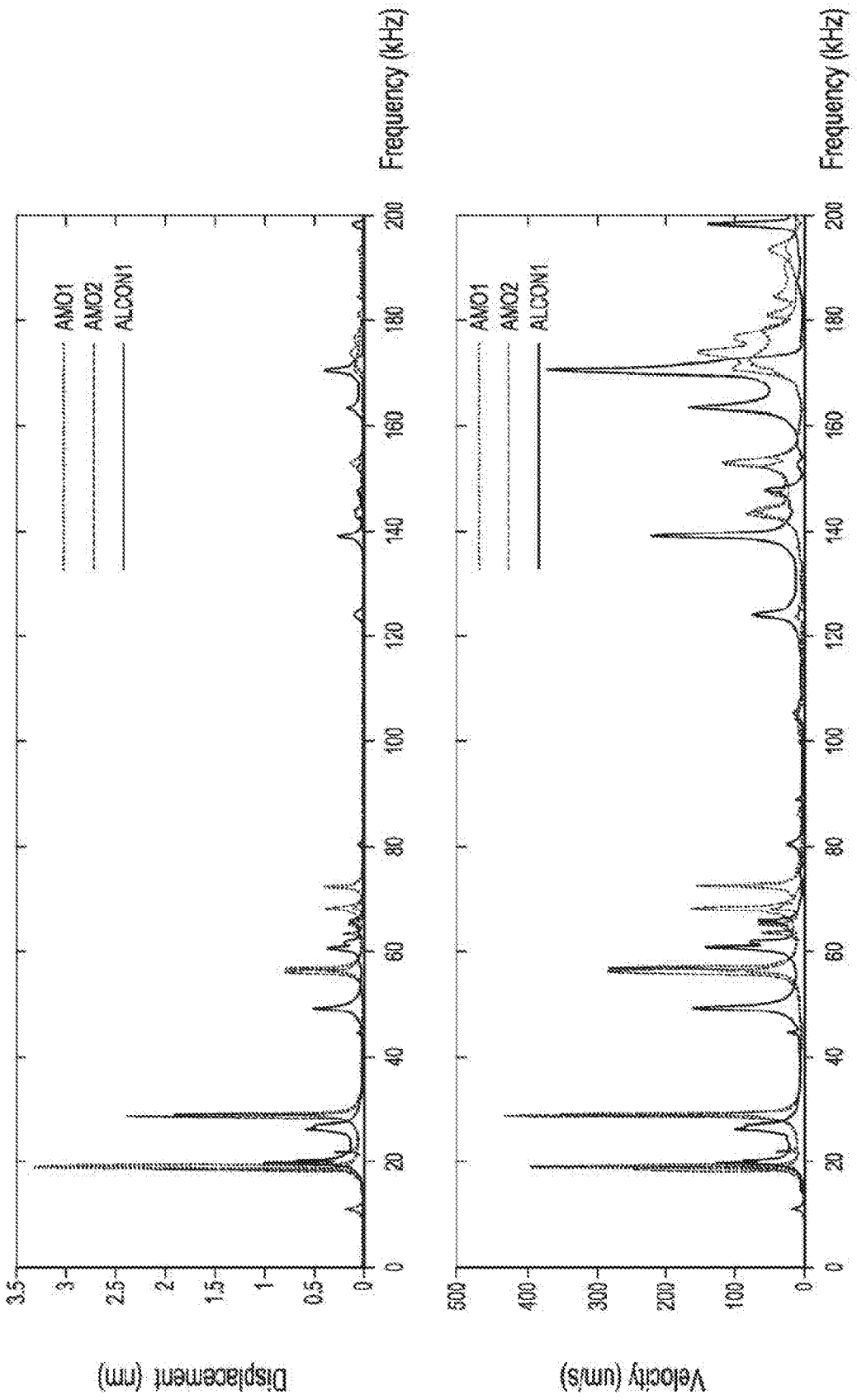

Displacement (nm)
Frequency (kHz)
AMO1
AMO2
ALCON1
Velocity (um/s)
Frequency (kHz)
AMO1
AMO2
ALCON1

Figure 31C

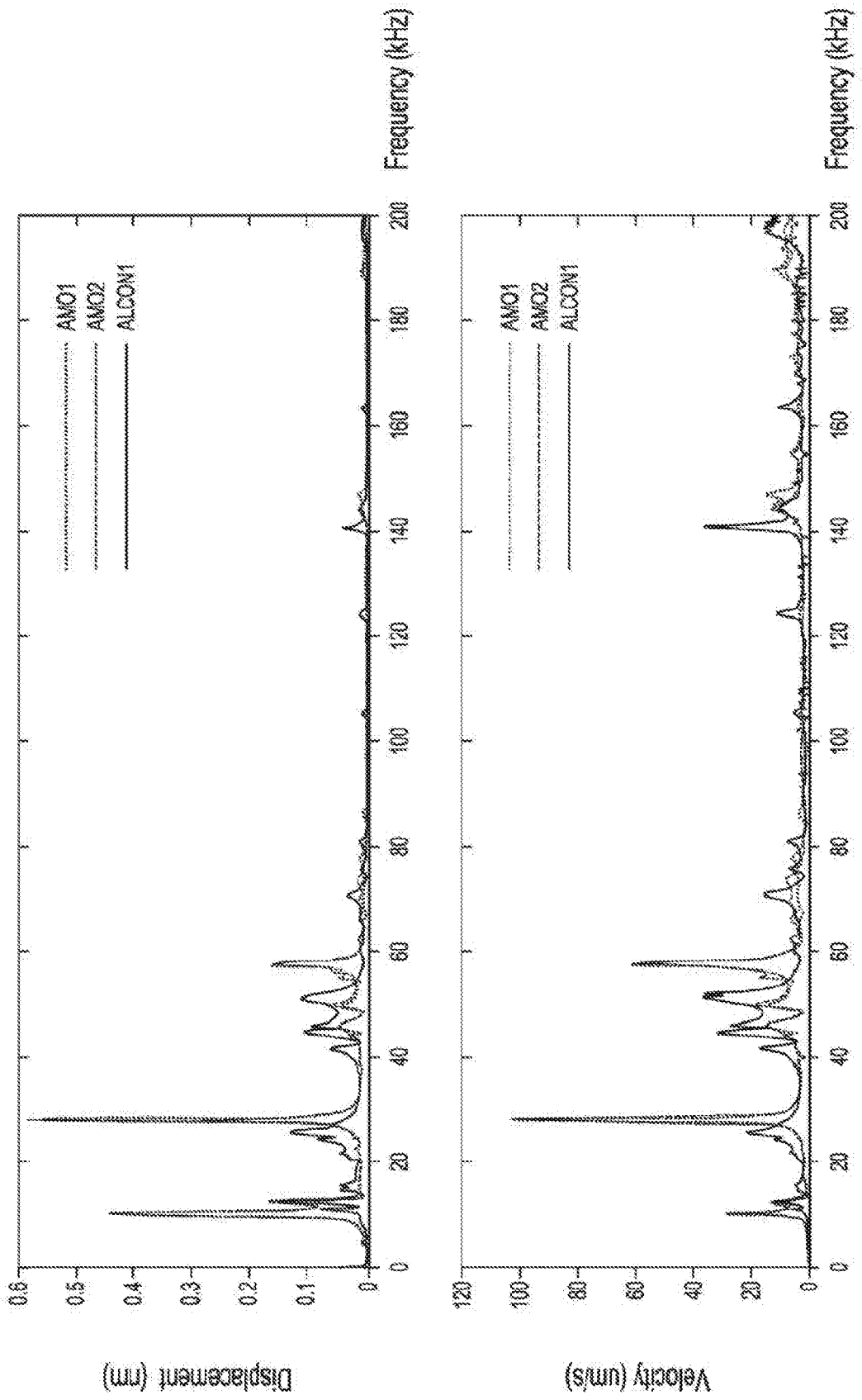

Displacement (nm)
Velocity (um/s)
Frequency (kHz)
AMO1
AMO2
ALCOM1
0
20
40
60
80
100
120
140
160
180
200
0.1
0.2
0.3
0.4
0.5
0.6

Figure 31D

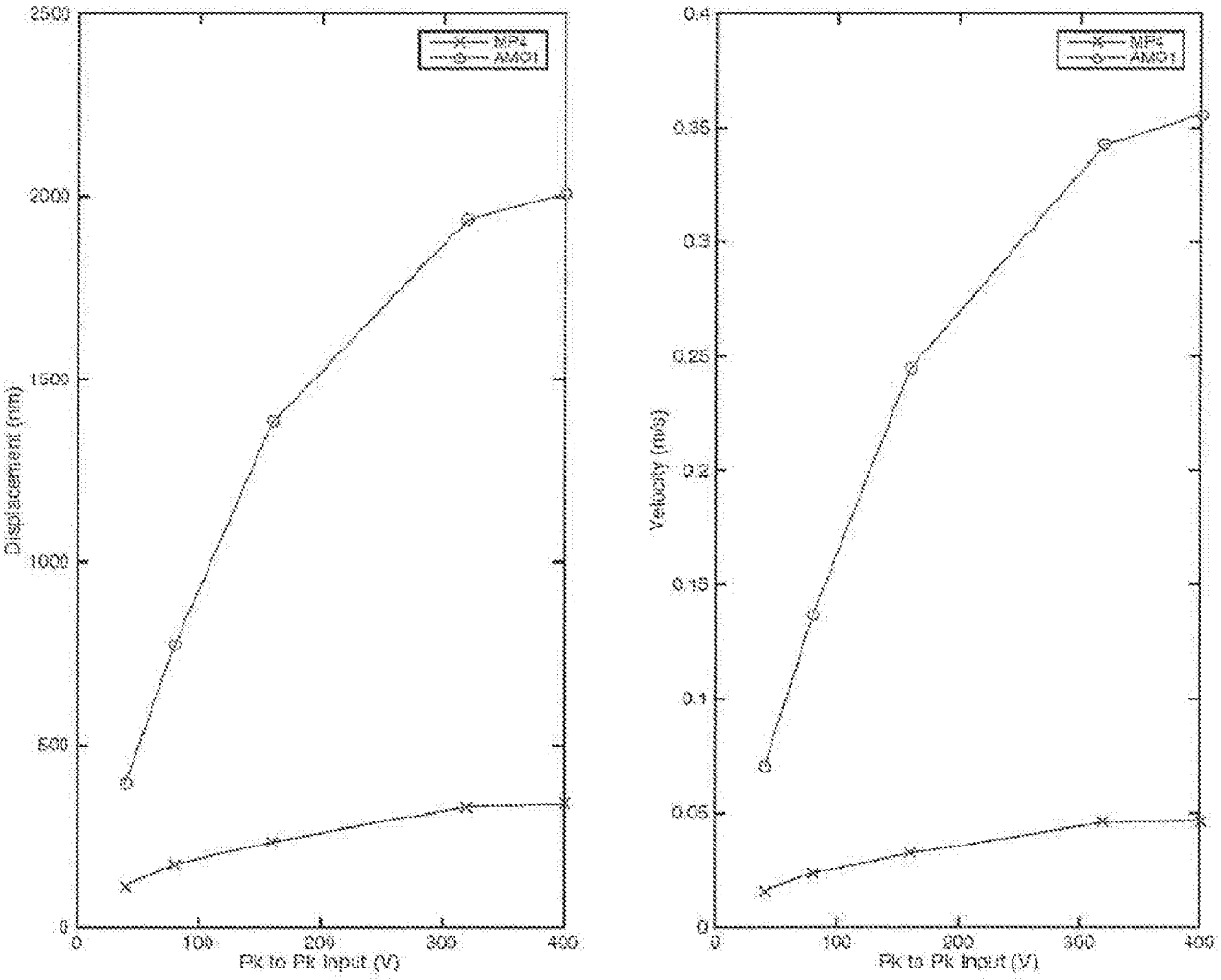

MP1
AMO1
Displacement (nm)
Pk to Pk Input (V)
Velocity (m/s)
Pk to Pk Input (V)

Figure 32

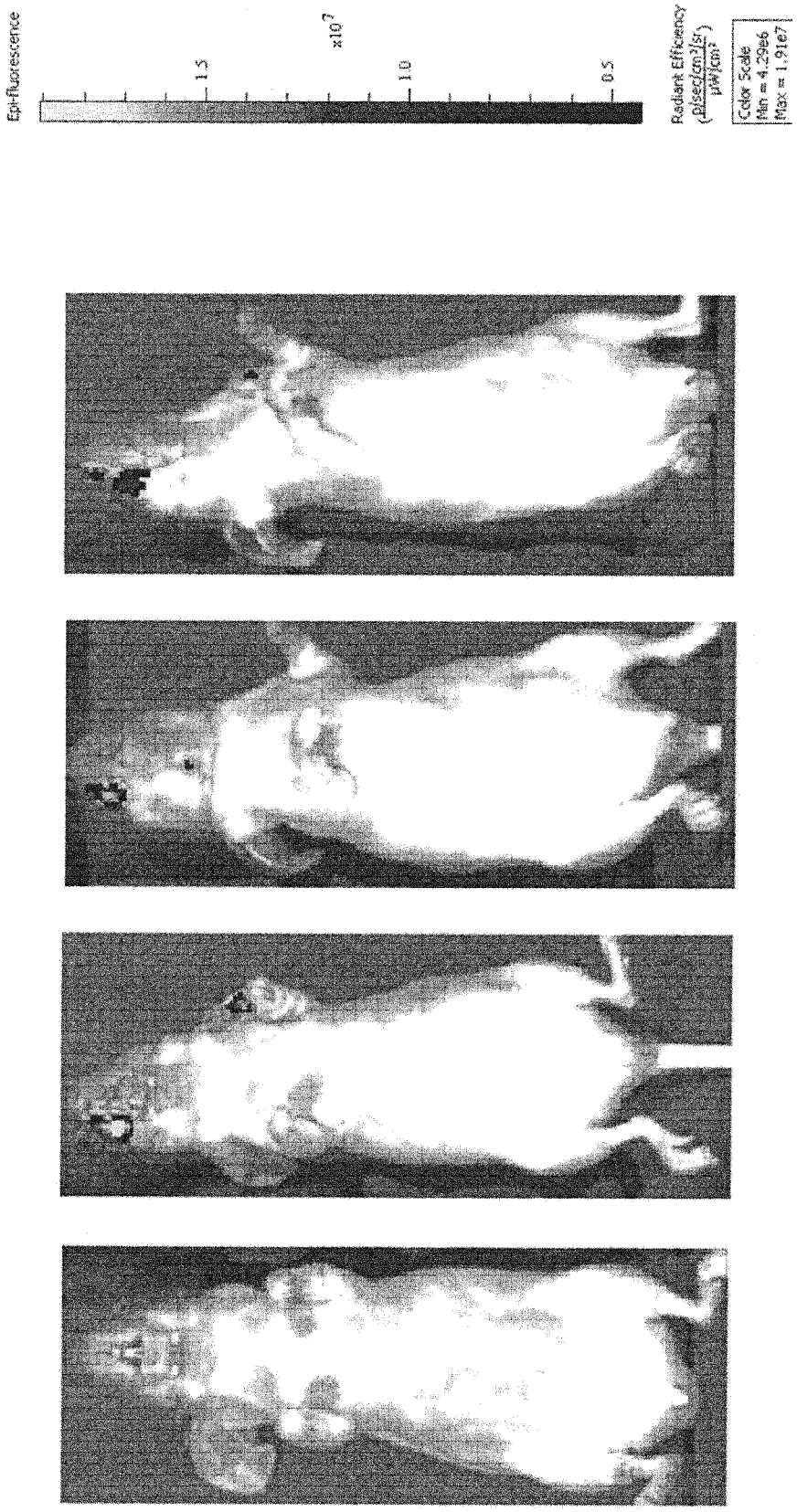
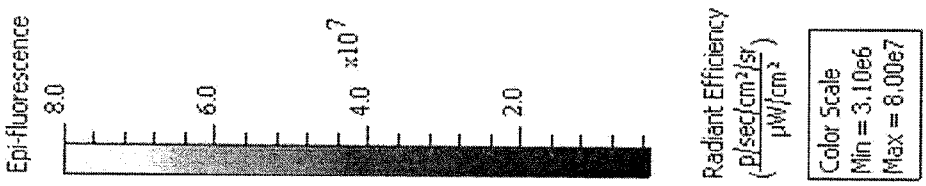

Epi-fluorescence
1.5
x10^7
1.0
0.5
Radiant Efficiency
(photons/cm²/sr)
µW/cm²
Color Scale
Min = 4.29e6
Max = 1.91e7

FIGURE 33


Epi-fluorescence
8.0
6.0
4.0
2.0
x10^7
Radiant Efficiency
(p/sec/cm²/sr / µW/cm²)
Color Scale
Min = 3.10e6
Max = 8.00e7

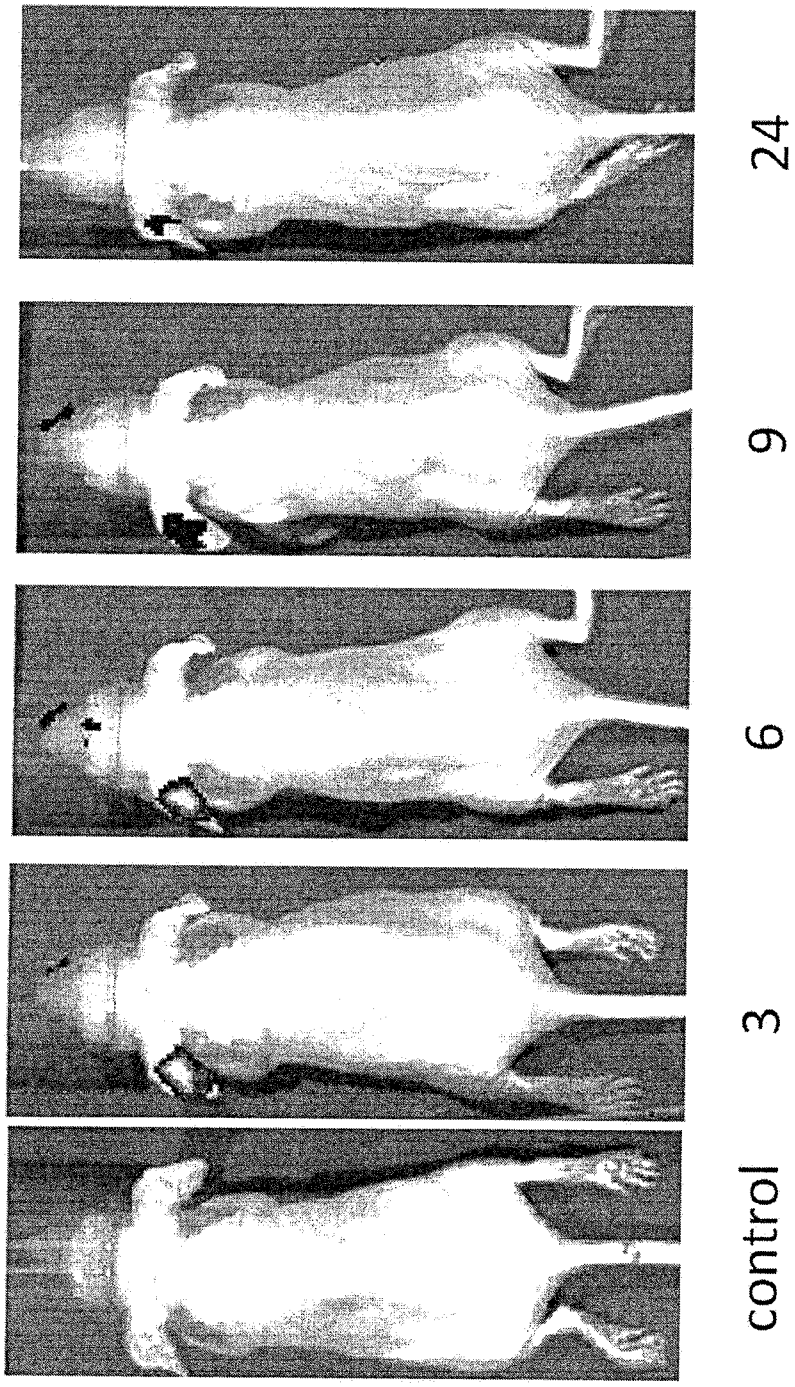

control
3
6
9
24

FIGURE 34

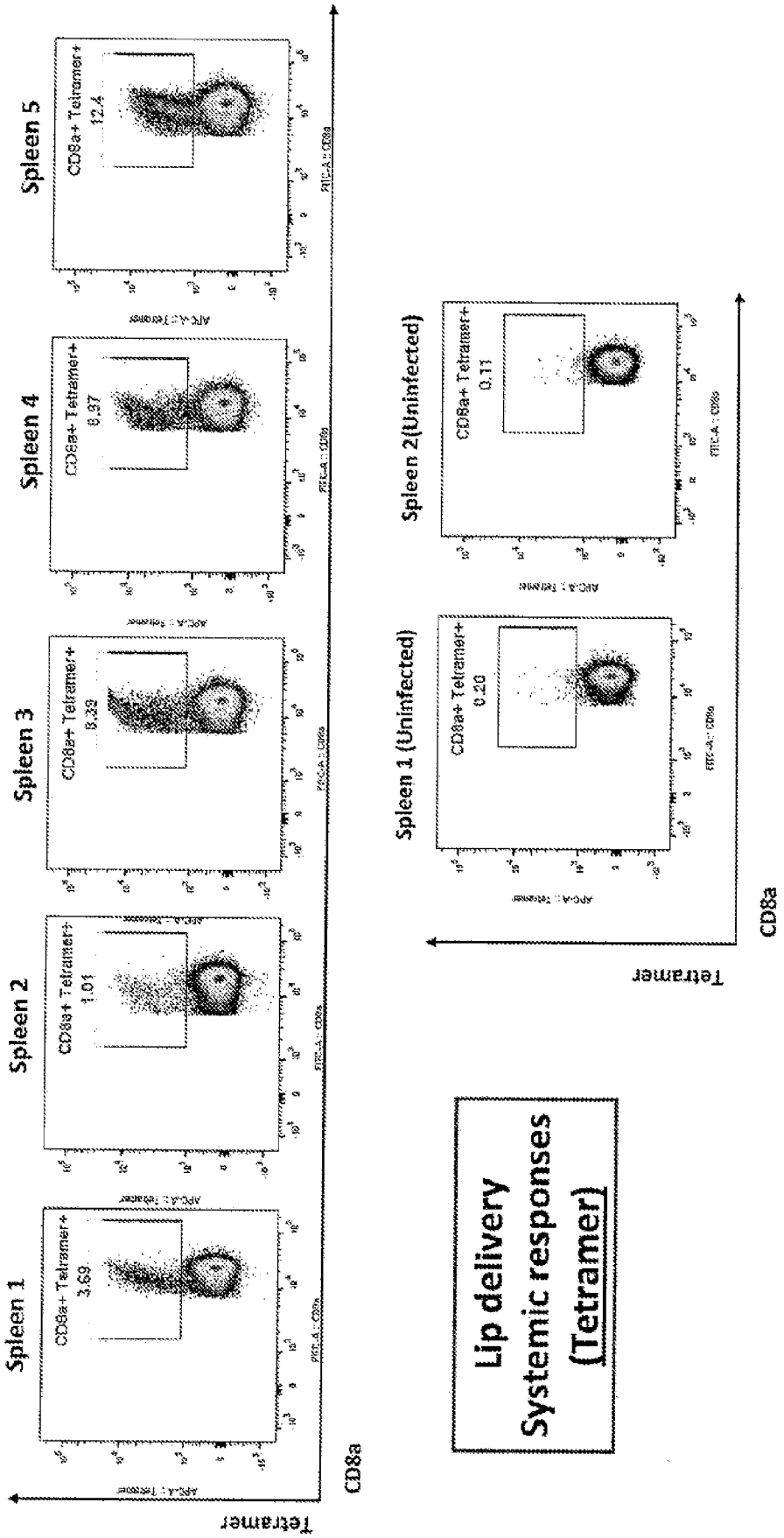

Spleen 1
CD8a+ Tetramer+
3.69
Spleen 2
CD8a+ Tetramer+
1.01
Spleen 3
CD8a+ Tetramer+
8.39
Spleen 4
CD8a+ Tetramer+
8.37
Spleen 5
CD8a+ Tetramer+
12.4
Tetramer
CD8a
Spleen 1 (Uninfected)
CD8a+ Tetramer+
0.20
Spleen 2(Uninfected)
CD8a+ Tetramer+
0.11
Tetramer
CD8a
Lip delivery
Systemic responses
(Tetramer)

FIGURE 35

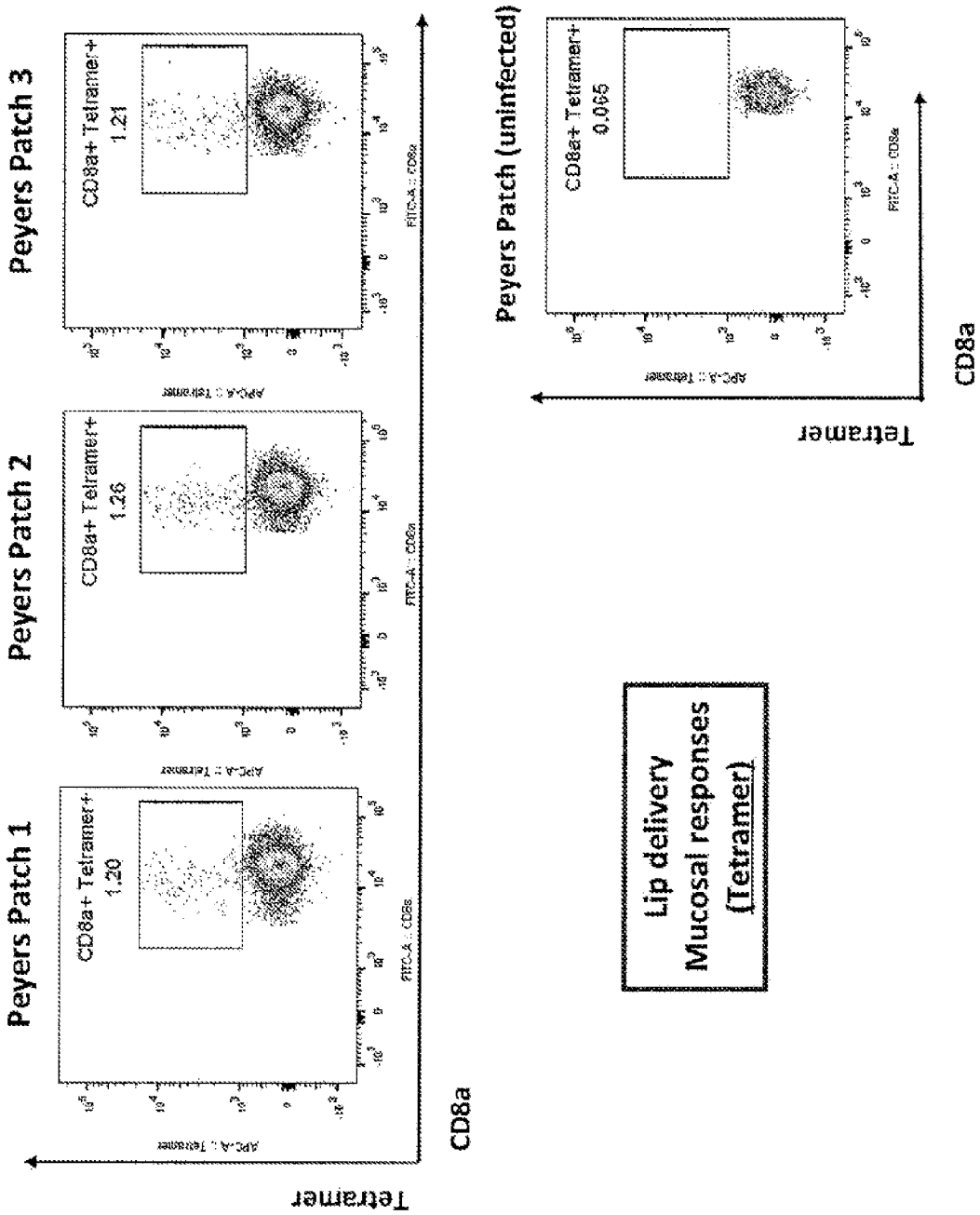

Peyers Patch 1
CD8a+ Tetramer+
1.20
Peyers Patch 2
CD8a+ Tetramer+
1.26
Peyers Patch 3
CD8a+ Tetramer+
1.21
Tetramer
CD8a
Peyers Patch (uninfected)
CD8a+ Tetramer+
0.005
Tetramer
CD8a
Lip delivery
Mucosal responses
(Tetramer)

FIGURE 36

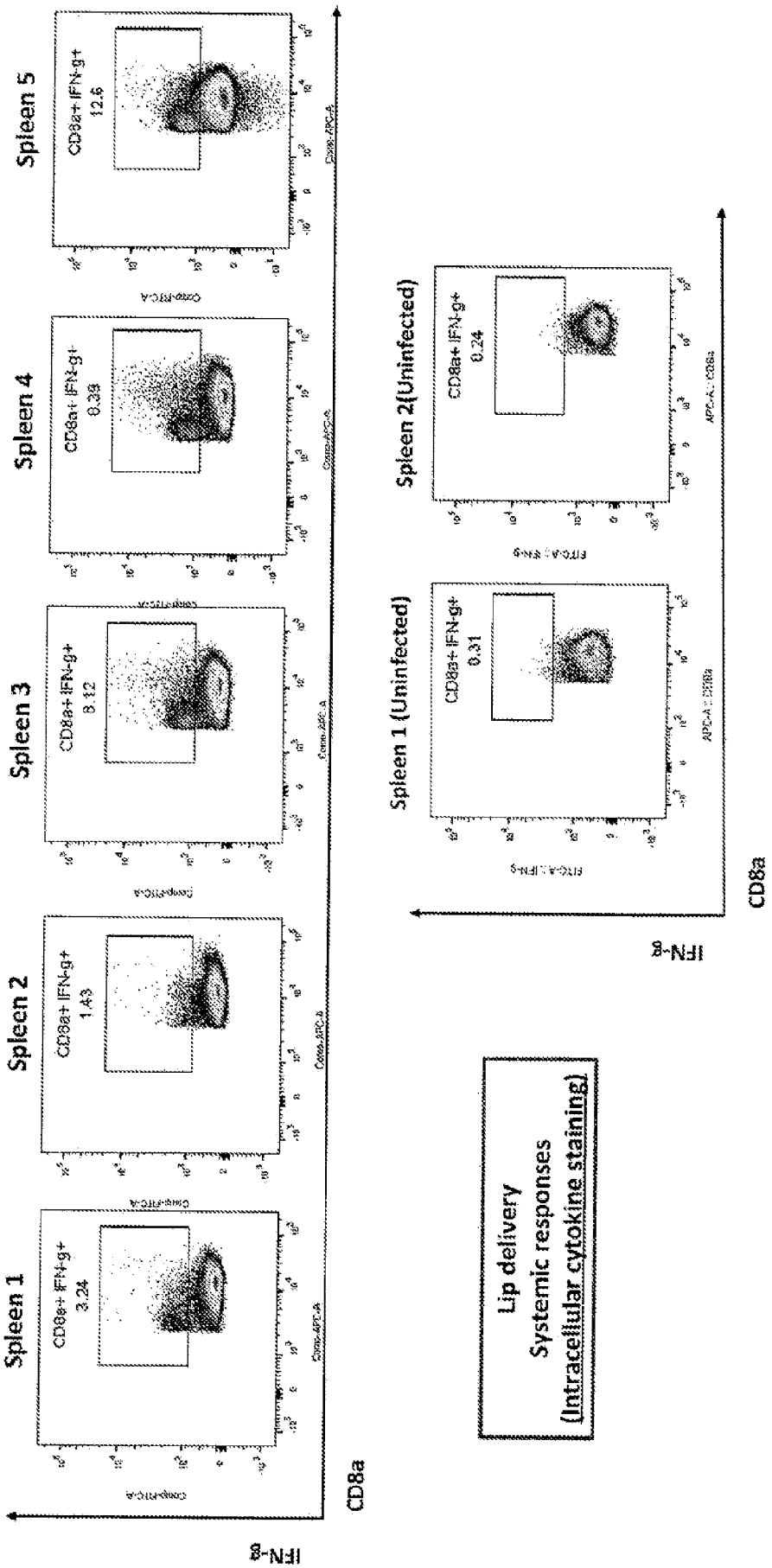

Spleen 1
CD8a+ IFN-g+
3.24
Spleen 2
CD8a+ IFN-g+
1.43
Spleen 3
CD8a+ IFN-g+
8.12
Spleen 4
CD8a+ IFN-g+
8.38
Spleen 5
CD8a+ IFN-g+
12.6
Spleen 1 (Uninfected)
CD8a+ IFN-g+
0.31
Spleen 2(Uninfected)
CD8a+ IFN-g+
0.24
IFN-g
CD8a
Lip delivery
Systemic responses
(Intracellular cytokine staining)

FIGURE 37

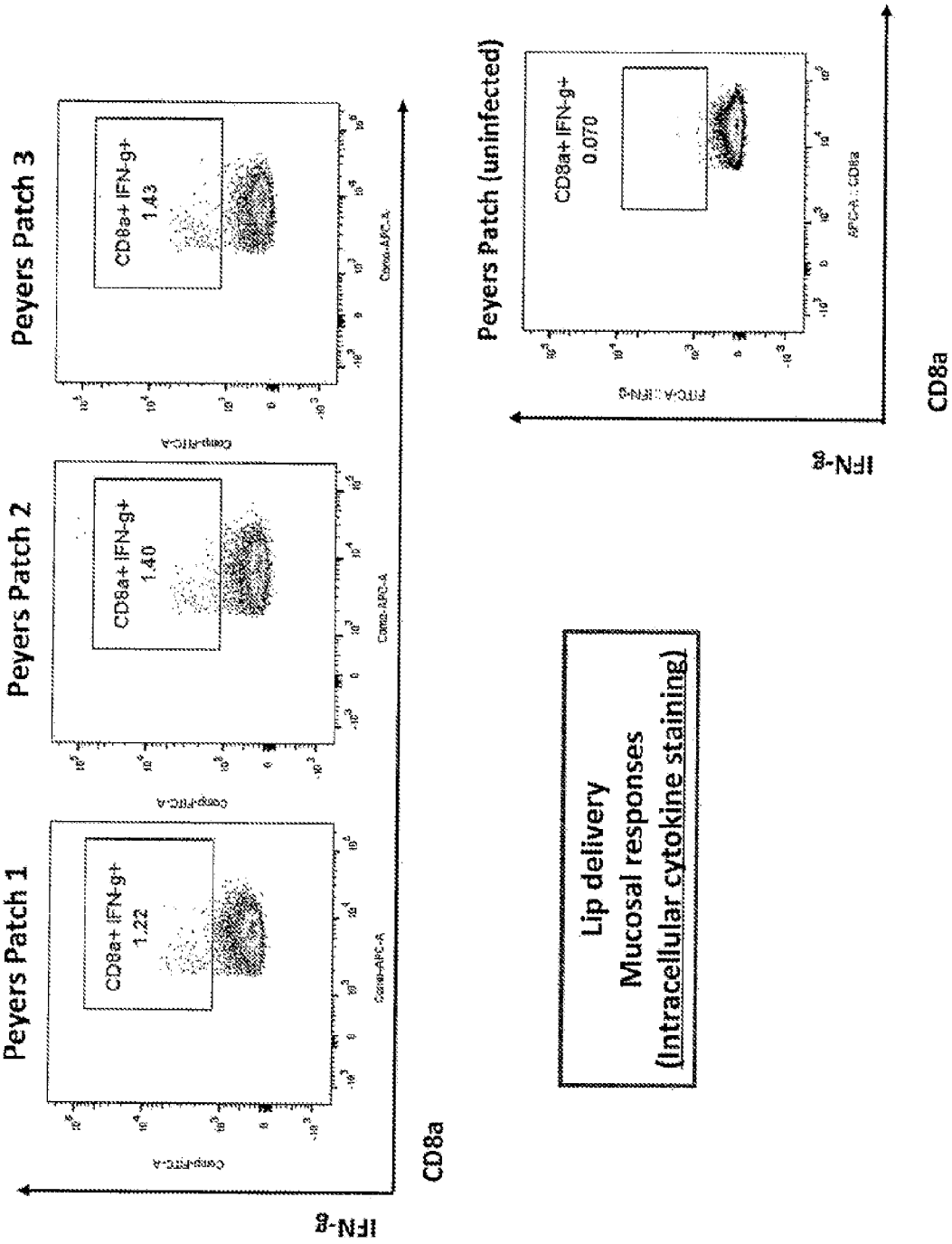

Peyers Patch 1
CD8a+ IFN-g+
1.22
Peyers Patch 2
CD8a+ IFN-g+
1.40
Peyers Patch 3
CD8a+ IFN-g+
1.43
IFN-g
CD8a
Peyers Patch (uninfected)
CD8a+ IFN-g+
0.070
IFN-g
CD8a
Lip delivery
Mucosal responses
(Intracellular cytokine staining)

FIGURE 38