FORM 2

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PROVISIONAL SPECIFICATION
(See Section 10 and Rule 13)

TRANSDERMAL ADMINISTRATION OF ANTIBODIES FOR TREATMENT OF SNAKE BITES AND OTHER VENOMOUS OR INFECTIOUS BITES

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The following specification particularly describes the invention and the manner in which it is to be performed

TRANSDERMAL ADMINISTRATION OF ANTIBODIES FOR TREATMENT OF SNAKE BITES AND OTHER VENOMOUS OR INFECTIOUS BITES

RELATED APPLICATION

This application is filed as a Provisional Application with the Indian Patent Office.

FIELD OF INVENTION

The present invention is related to a transdermal patch and the process of developing transdermal administration of a drug that is antibodies or small molecules, for the treatment of snake bites and other venomous or infectious bites. The topical application of drug or antibody formulations is through transdermal patches containing a microneedle array provided with either IgG based anti-snake venoms or IgY based anti-snake venoms or IgG or IgY antibodies to any protein or antigen alone or in combination with peptide(s) or small molecule(s) (synthetic/natural), aimed to treat venomous or infectious bites in humans as well as animals.

BACKGROUND

Snakebite is one of the major public health concerns across the globe. As per WHO 2020, more than 100,000 deaths are reported every year and more than 400,000 people suffer from morbidity due to snakebite alone, globally. Snakebite being one of the most neglected tropical diseases, rural population living in tropical areas is the most affected one by snakebite, where the anti-snake venoms are not available due to limitations in the logistics and cold chain maintenance. Early administration of antivenins is the only way to success in the snakebite treatment without any local tissue damage or amputations.

Envenomation due to snake bites is one of the neglected tropical diseases, though it is associated with huge mortality and morbidity every year globally. According to WHO, India reports highest deaths of snakebite annually compared to other nations. For example, the World Health Organization reports the Indian subcontinent was drastically affected by snake envenoming with the highest mortality of an average of 58,000 annual deaths worldwide estimated from 2000- 2019 [WHO, Departmental News, October 2020]. Snake envenoming as a life-threatening disease is a major public health problem globally for which immediate

remedy is needed on a priority basis. The local toxicity of Cobra (Elapidae) bite associated with tissue damage accompanied by several acute pathological alterations leading to detrimental effects like tissue loss sequel, long-lasting physical disability and tissue damage, and dermo-necrosis depending on the quantity of venom injected and time taken for antivenom administration [Lin *et al*., Toxins 14, 111 2022].

The conventional treatment methods of envenomation include field management like usage of tourniquet, compression of the wound, primary wound suction, and incision, and application of herbal medicine. However, these first-aid techniques are not recommended because of poor outcomes [Juckett & Hancox, Am Fam Physician 65(7): 1367-1374 (2002); Alberts *et al*., Toxicology 43(2): P181-P186 (2004)]. Envenomation by scorpions, spiders, and jellyfish also can cause local and systemic toxicity, neurotoxicity and necrotic ulcers.

Administration of anti-venom or antivenin is the mainstay treatment for envenomation. Such antivenins are manufactured by venom-immunization of equines, typically horses, or immunization of ovines, such as sheep. Antivenins mainly consist of purified Immunoglobulin G (IgG) antibodies that neutralize the venom. These antibodies may be polyclonal or monoclonal. Polyclonal antibodies are effective against wide a range of venoms. Monoclonal antibodies are targeted to the venom of a single toxin [O'Leary & Isbister, Toxicon 54(2): 192-195 (2009)]. Some antivenoms can exhibit poor dose efficacy, and may cause severe side effects like anaphylaxis and serum sickness in some subjects. Available recombinant antivenins can cause fewer adverse reactions than the tradition versions, and can be more potent, paraspecific, and can be manufactured at a lower cost compared to the traditional methods of producing antivenoms [Jenkins & Laustsen, Frontiers in Bioengineering and Biotechnology Vol. 8, Article 703 (2020)].

At present, anti-snake venoms and other anti-venoms typically are given by intravenous (i.v) administration only. Among the limitations with the current treatment options are:

1. Treating the bite victim can require a hospital admission and administration of the treatment by a medically trainer professional.

2. Due to limitations in logistics, and the distance between the location of the bite occurring and a medical facility, it can take a few hours to days for the bite victim to reach a facility where the treatment can be administered.

3. While anti-snake venoms may be available in Primary Health Centres (PHCs) in bite prone geographical areas, the reliability can be reduced due to storage issues and difficulties in cold chain maintenance.

4. Any delay in anti-venom administration can cause severe and irreversible local tissue damage at the bitten area, especially in the case of venomous snakes.

5. At present, the available treatment and route of administration can neutralize only the systemic toxicity of a venom but cannot reverse the necrosis caused at the bitten area.

These limitations are not just restricted to snake-bites, but are applicable to any other venomous bites including bites caused by scorpions, spiders, honeybee, jellyfish, cone snails, and venomous fish, such as lionfish, scorpionfish, and stonefish.

Accordingly, a need exists for a remedy that can be applied readily to a bitten area of a subject by an untrained person immediately or soon after envenomation specifically to prevent local toxicity of venom as well as to prevent or minimize further entry of venom into the circulation system.

Rabies is recognized as one of the global leading causes of death due to infection (*Rabies and Envenoming: A Neglected Public Health Issue*, Report of a Consultative Meeting, World Health Organization, Geneva, 2007). While dog bites are the primary vehicle of transmission, rabies can be present in other domestic as well as wild animals, and can be spread by bites from these animals as well. If untreated, rabies is fatal. While vaccines against rabies are available in developed countries, they are not readily available to the most vulnerable populations (Rabies Fact Sheet, World Health Organization, May 2021). For effective treatment, an approved effective rabies vaccine or rabies immunoglobulin needs to be administered as quickly as possible following suspected infection in order to minimize virus mobility or to prevent virus penetration into the subject's central nervous system. In

addition to rabies, dog bites can cause infection by staphylococcus bacteria, streptococcus bacteria, *Pasteurella multocida* or *Pasteurella canis*.

Accordingly, a need exists for a remedy that can be applied readily by an untrained person immediately or soon after a bite or scratch from an animal, especially if the animal is suspected to have or have been exposed to rabies, of if the wound is contaminated by saliva from the animal, specifically to minimize migration of the virus as well as to prevent or minimize entry of the rabies virus into the circulation system and/or central nervous system.

SUMMARY OF THE INVENTION

The present innovation focuses on a novel scientific technology to treat venomous bites, poisonous bites, and animal bites through a modern transdermal drug delivery system (TDDS). Antivenins mainly consist of IgG or IgY antibodies against four poisonous snakes viz., Naja Naja (Cobra), Bungarus Caeruleus (common Krait), Daboia russelii (Russell's viper) and Echis carinatus (Saw-scaled viper). These antibodies are commercially produced from hyper-immunized horse plasma or chicken eggs by injecting different venoms or antigens along with suitable adjuvants. After establishing the efficacy and safety of these antibody preparations, they can be used in suitable formulations as transdermal patches or ointments or lotions or gels to be applied on local bitten area. Proposed innovation of transdermal patch mediated delivery of anti-snake venoms and other antibodies to the target venom or microbe at bitten areas can have significant impact on reducing the local tissue damage or amputations caused by snakebites or other venomous bites and thereby prolongs the survival time of the victim.

The present invention provides a first-aid and compliments the existing method of treating bites by venomous and non-venomous animals and insects. Usage of drug (antivenin, small molecules etc.) loaded transdermal patches can help in prolonging the survival time before victims reach a hospital or medical facility, and also can help in avoiding amputations or morbidity caused due to venomous and infectious bites and in so doing can help in improving quality of life in human beings as well as animals that are victims to bites by venomous and non-venomous animals and insects across the globe.

In some configurations, the transdermal patches provided herein can be formulated to treat canine bites, such as to prevent rabies, or infections caused by staphylococcus bacteria, streptococcus bacteria, *Pasteurella multocida* or *Pasteurella canis*.

The present invention provides a transdermal patch loaded with IgG or IgY or phytochemical based polyclonal anti-snake venoms against four major venomous snakes which include but not limited to common Cobra, common Krait, Russell's viper and Saw-scaled viper. This transdermal patch loaded with the drug is used as a first aid in the snakebite treatments and prevents systemic circulation of venom toxins thus helping in saving lives of victims. The patch is designed to release the anti-snake venoms into the skin/muscle primarily and allows them to neutralize the free venom toxins at the bitten area. These antibodies are of IgG or IgY based raised in horse or chickens/avians respectively. Horse or chicken/avian are immunized with sub-lethal doses of either individual or cocktail of four major snake venoms or venom peptides along with suitable adjuvants to improve the immune response in the animals. Further these high tier antibodies may be purified from immunized horse plasma (IgG antibodies, FIG. 2) or from eggs of immunized chickens/avians (IgY antibodies, FIG. 3). Further, these purified antibodies can be formulated to ensure the suitability to load into the transdermal patches for easy/sustained release of antibodies into the local tissue at bitten site.

In addition to antibodies, the transdermal patch may also include small molecule drugs, antibiotics, a combination of drugs such as, for example, antivenom peptide and a small molecule, or combinations thereof as appropriate.

The transdermal patches provided herein can be used for immediate administration of an active ingredient, such as an antivenin, to a bite site. The immediate administration can save victims of bites and minimize or prevent local tissue damage. In some configurations, the transdermal patches provided herein can allow an active ingredient to permeate the application site and enter into the bloodstream within 5 minutes of application. This helps in neutralizing the circulating venoms or toxins, thereby lowering venom or toxin induced toxicity. The rapid release of the active ingredient, such as an antivenin, at the bite site also can minimize local tissue damage that otherwise would have been caused by the venom or toxin.

Provided is a transdermal patch for treatment of a subject who is a victim of a venomous or toxic bite. The patch can include a release liner; a microneedle array; a drug matrix containing an IgG or IgY antibody to a venom or toxin in the bite; and a backing layer. The IgG or IgY antibody can react with a venom from a snake selected from among a Naja (Cobra), Bungarus Caeruleus (common Krait), Daboia russelii (Russell's viper) and Echis carinatus (Saw-scaled viper). The IgG or IgY antibody can react with a venom or toxin from a scorpion, spider, honeybee, jellyfish, cone snail, lionfish, scorpionfish, or stonefish. The IgG or IgY antibody can react with a rabies virus.

In some embodiments, the transdermal patch can include a microneedle array that includes a plurality of microneedles, each microneedle having: a base; a tip distal to the base; a body between the base and the tip; and a channel extending substantially from the base through the body portion, the channel being open along at least part of the body portion and forming a channel in fluid communication with at least a portion of the drug matrix. The microneedles can be made of or include a biocompatible metal. The microneedles can be made of or include a biocompatible polymer. The microneedle array can include an adhesive material for securing the microneedle array to the subject's skin in the vicinity of the bite with one or more of the microneedles of the microneedle array inserted into the stratum corneum.

In some embodiments, the drug matrix can be within the body of at least one of the microneedles of the microneedle array. The drug matrix can be (a) lyophilized IgG or IgY antibody; or (b) encapsulated particles of the IgY or IgY antibody; or (c) an emulsion comprising the IgG or IgY antibody; or (d) any combination of (a) to (c). The drug matrix can be (a) an adhesive matrix containing the IgG or IgY; or (b) a drug reservoir containing the IgG or IgY; or (c) a combination of (a) and (b). The drug matrix can include an additional active ingredient selected from among an antibiotic, a chemical permeation enhancer, and combinations thereof. The transdermal patch can include a rate-controlling membrane that controls the rate of release of the additional active ingredient.

The transdermal patch can be contained in a packaging material. The packaging material can include a foil wrapper; a foil-lined pouch, a multi-laminate polymer film pouch, or any combination thereof. The packaging material can include a desiccant and/or an oxygen absorber.

Also provided is a kit that includes the transdermal patch provided herein and instructions for use.

Also provided is a method of treating a bite site of a subject who is a victim of a poisonous or infectious bite. The method includes applying a transdermal patch as described herein to the bite site, and applying pressure to the transdermal patch. The pressure can be applied to or adjacent to the microneedle array of the transdermal patch pressing the microneedle array into the stratum corneum of the skin at the bite site. The pressing of the microneedle array into the stratum corneum of the skin makes holes or micropores in the skin to facilitate delivery of the IgG or IgY transdermally across stratum corneum of the skin.

Brief Description of the Drawings

FIGS. 1A through 1C show side view schematic representations of various embodiments of the transdermal patch provided herein.

FIG. 2 is a schematic representation of the development of anti-snake venoms from horse plasma.

FIG. 3 is a schematic representation of the development of anti-snake venoms from chicken eggs.

FIG. 4 is a photograph of an SDS-PAGE analysis of venoms and purified IgY antibodies from various groups under reducing conditions. (M) Protein Ladder, (CV) Cobra venom, (KV) Krait Venom, (RV) Russell's Viper venom, (SSV) Saw Scaled Viper venom.

FIGS. 5 through 8 are graphs showing cross-reactivity of venoms with anti-venoms. FIG. 5 shows the cross reactivity of Cobra venom (CV) with different anti-venoms. FIG. 6 shows the cross reactivity of Krait venom (KV) with different anti-venoms. FIG. 7 shows the cross reactivity of Russell's viper (RV) with different anti-venoms. FIG. 8 shows the cross reactivity of Saw scaled viper venom (SSV) with different anti-venoms.

FIG. 9 shows a schematic purification using affinity chromatography to produce species-specific antibody.

FIGS. 10 through 13 shows the results of the affinity chromatography purification yielding species-specific antibodies.

DETAILED DESCRIPTION OF THE INVENTION

1. Definitions

As used herein, the singular forms "a", "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise.

As used herein, “about” is a term of approximation and is intended to include minor variations in the literally stated amounts, as would be understood by those skilled in the art. Such variations include, for example, standard deviations associated with techniques commonly used to measure the amounts of the constituent elements or components of an alloy or composite material, or other properties and characteristics. All of the values characterized by the above-described modifier "about," are also intended to include the exact numerical values disclosed herein. Moreover, all ranges include the upper and lower limits.

Any compositions described herein are intended to encompass compositions which consist of, consist essentially of, as well as comprise, the various constituents identified herein, unless explicitly indicated to the contrary.

As used herein, the recitation of a numerical range for a variable is intended to convey that the variable can be equal to any value(s) within that range, as well as any and all sub-ranges encompassed by the broader range. Thus, the variable can be equal to any integer value or values within the numerical range, including the end-points of the range. As an example, a variable which is described as having values between 0 and 10, can be 0, 4, 2-6, 2.75, 3.3 - 4.4, etc.

In the specification and claims, the singular forms include plural referents unless the context clearly dictates otherwise. As used herein, unless specifically indicated otherwise, the word "or" is used in the "inclusive" sense of "and/or" and not the "exclusive" sense of "either/or."

Unless indicated otherwise, each of the individual features or embodiments of the present specification are combinable with any other individual feature or embodiment that are described herein, without limitation. Such combinations are specifically contemplated as being within the scope of the present invention, regardless of whether they are explicitly described as a combination herein.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described.

As used herein, the terms "victim" and "subject" includes members of the animal kingdom including but not limited to human beings.

As used herein, "tissue" includes, but is not limited to an animal tissues, which may be connective, muscle, nervous or epithelial issues, and may include a single tissue or a collection of tissues that form a common function.

As used herein, "transdermally" refers to any type of delivery of an active ingredient that crosses any portion of skin (but not necessarily all of the layers of skin), rather than merely being topically applied to an outer layer of the skin. Transdermally can include systemic delivery (i.e., where the active ingredient is transported across, or substantially through, the dermis such that the active ingredient is delivery into the bloodstream and systemically), as well as intradermal delivery (i.e., where the active ingredient is transported partially through the dermis, e.g., across the outer layer (stratum corneum) of the skin, where the active ingredient is delivered into the skin. That is, transdermal delivery as used herein includes delivery of an active ingredient that is transported across at least a portion of skin.

As used herein, an "active ingredient" refers to a molecule for treating a disease state, and includes small molecules, and macromolecules such as proteins, *e.g.*, immunoglobulins, such as IgG and IgY, DNA, and RNA.

As used herein, a "drug" refers to an active ingredient.

As used herein, a "matrix" refers to a material in which something is enclosed or embedded.

As used herein, the term "hyperimmunization" means repeated exposure to one or more antigens such that an immune response is elevated and maintained above the natural unexposed state.

As used herein, a “hyperimmune state” refers to an elevated immune response in an animal, such as an equine or ovine, or an egg producing animal, that has been hyperimmunized.

As used herein, the term “egg” as used herein refers to a whole egg (table, hyperimmunized or otherwise). The term “egg product” as used herein refers to a whole egg or any product or fraction obtained from a whole egg. In a particular embodiment, the egg product is an egg yolk, for example, an egg yolk powder. In another embodiment, the egg product is an egg white, for example, an egg white powder. In another embodiment, the egg product is obtained from a whole egg, for example, a whole egg powder (e.g. a spray-dried whole egg powder).

As used herein, the term “control egg” refers to an egg obtained from an egg-producing animal that is not maintained in a hyperimmunized state, i.e. an animal that has not been hyperimmunized. The term “control egg product” refers to a control egg or an egg product obtained from a control egg.

As used herein, the term “hyperimmunized egg” refers to a whole egg obtained from an egg-producing animal maintained in a hyperimmune state, i.e. an egg-producing animal that has been hyperimmunized. The term “hyperimmunized egg product” refers to a hyperimmunized egg or any product obtained from a hyperimmunized egg.

In certain embodiments, the hyperimmunized egg product is a concentrate. As used herein the term “concentrate” refers to a hyperimmunized egg product that is at least partially purified, such that the concentration of antibodies in the concentrate is greater than the concentration of antibodies in a hyperimmunized egg.

As used herein, the term “egg powder” refers to a whole egg that has been dried. In some embodiments, the egg powder is spray-dried. In some embodiments, the egg powder is lyophilized.

As used herein, the term “egg-producing animal” means any oviparous animal, and includes any animal that lays an egg, such as avians, fish and reptiles.

As used herein, the term “avian” refers to an animal that is a member of the class *Aves.* Avians include, but are not limited to, chickens, turkeys, geese, ducks, pheasants, quail, pigeons and ostriches.

As used herein, the term “equine” refers to a mammal that is a member of the family Equidae (order Perissodactyla) such as horses.

As used herein, the term “ovine” refers to a mammal that is a member of the genus Bovidae, such as sheep.

As used herein, the term “supranormal levels” means levels in excess of those found in eggs of egg-producing animals that are not hyperimmunized. For example, supranormal levels of an antibody to a particular antigen are levels of the antibody in excess of those found in eggs of egg-producing animals that are not hyperimmunized with the particular antigen.

As used herein, the term "administer" means any method of providing a subject with a substance, including orally, intranasally, parenterally (intravenously, intramuscularly, or subcutaneously), rectally, topically or intraocularly.

As used herein, the term “antigen” refers to a substance that is able to induce a humoral antibody and/or cell-mediated immune response rather than immunological tolerance. The term signifies the ability to stimulate an immune response as well as react with the products of it, e.g., an antibody.

As used herein, an “antibody” is a protein that includes at least one complementarity determining region that binds to a specific target antigen, e.g. antigen A, B, C, D, Co1, Co2, H, or ET-50 disclosed herein. For example, an antibody can include a heavy (H) chain variable region (abbreviated herein as VH), and a light (L) chain variable region (abbreviated herein as VL). In another example, an antibody includes two heavy (H) chain variable regions and two light (L) chain variable regions. In a particular embodiment, the antibody is a polyclonal antibody. The term "polyclonal antibody", as used herein, refers to a population of antibody molecules that that are capable of immunoreacting with different epitopes on a particular antigen. In a particular embodiment, the antibody is an IgY antibody.

2. Transdermal Delivery

Transdermal drug delivery system (TDDS) is a self-contained drug loaded system which delivers drug(s) in reproducible or predetermined rate via skin portal into systemic circulation that last for long time [Prabhakar *et al.*, Journal of Drug Delivery & Therapeutics 3(4): 213-221 (2013)]. Since the first Food and Drug Administration (FDA) approval of Transderm-Scop a scopolamine transdermal patch in 1981, the transdermal delivery market share increased from $12.7 billion to $31.5 billion in the year of 2005 to 2015. Today, more than 35 transdermal products have been approved by USA for several diseases including transdermal patches containing drugs against central nervous system disorders and patches for treatment against Parkinson's (Neupro® (2007)), Alzheimer's ((Exelon® (2007)), depression ((Emsam® (2006)), and deficit hypersensitivity reactions (Daytrana® (2006)) [Singh and Bali, Journal of Analytical Science and Technology 7: 25 (2016); Isaac *et al.*, Therapeutic Advances in Psychopharmacology 2(6): 255-263 (2012)].

While transdermal and topical drug delivery can be used for therapeutic treatment for delivery of some efficacious molecules, the barrier properties of skin can limit the size of the molecules that can penetrate the outermost skin layers, such as the stratum corneum. Typically, only drugs having a low molecular weight, low meting point with high lipophilicity can easily permeate the skin. Thus, many drug molecules do not cross barriers of the skin, particularly the stratum corneum barrier.

To improve permeability, and thus enhance the drug delivery, techniques such as ablation, sonophoresis, iontophoresis, or a combination thereof, have been used. These approaches typically require special equipment to be effective. Chemical permeation enhancers to increase the permeability or porosity of the outermost skin layers have also been developed. Typically, these chemicals interact with one or more skin constituents of the stratum corneum to increase its permeability These can include alcohols, amines, amides, ceramides, esters, fatty acids, glycols, hydrocarbons, lipids, pyrrolidines, sulfoxides, surfactants, terpenes, ureas, and combinations thereof. The mechanism of action and examples of specific types of chemical permeation enhancers for transdermal drug delivery are known in the art (*e.g.*, see Chen *et al*., Asia Journal of Pharmaceutical Sciences 9: 51-64 (2014); and Karande *et al.*, Biochimica et Biophysica Acta 1788: 2362-2373 (2009)).

Transdermal patches are applied on skin. The transdermal patches provided herein can be more effective against local toxicity of venomous bites than current treatment interventions. The transdermal patch is easy for a subject to apply and use, and requires no special training or equipment.

The transdermal patches provided herein can include a microneedle array, alone on in combination with one or more chemical permeation enhancers. The microneedles pierce the skin and provide a point of entry to allow large molecules, such as antibodies, to penetrate the protective outer layers of the skin. The microneedles also can pierce the skin and provide a point of entry for the chemical permeation enhancer to enter and assist with the passage of the antibodies to pass through the stratum corneum.

The transdermal patch provided herein can be applied to the bite site on the skin, and the transdermal patch applies an active ingredient or a combination of active ingredients to the application site, and through the stratum corneum. The active ingredient can be an IgG or IgY antibody, an antibiotic, a chemical permeation enhancer, or any combination thereof. When the patch is applied, and light pressure is applied to the transdermal patch, the microneedle array can puncture the outer layer of the skin, delivering the one or more than one active ingredient through the stratum corneum.

3. Components of Transdermal Patch

The transdermal patch provided herein is a multilaminate design that can include, but is not limited to components including a release liner, microneedle array, a drug matrix, and a backing. Together, these components help in maintaining the stability of the transdermal patch, and can result in the rapid diffusion of active ingredients into the skin without losing the efficacy of the active ingredients loaded into the patch.

Exemplary configurations of the transdermal patch provided herein are shown in FIG. 1A through FIG. 1C. In the embodiment shown in FIG. 1A, the transdermal patch 1000 includes a release liner 100, an adhesive layer 200, a microneedle array layer 300, and a backing layer 400. In this configuration, the adhesive layer 200 serves as both a skin adhesive layer as well as a drug matrix, with the active ingredient embedded in or on the surface of the adhesive. In the embodiment shown in FIG. 1B, the transdermal patch 1000 includes a

release liner 100, a skin adhesive layer 250, a microneedle array layer 300, a drug matrix layer 500, and a backing layer 400. In the embodiment shown in FIG. 1C, the transdermal patch 1000 includes a release liner 100, a skin adhesive layer 250, a microneedle array layer 300, a drug-loaded adhesive layer 600, a rate-controlling membrane 600, a drug matrix layer 500, and a backing layer 400.

A. Backing Layer

The transdermal patch provided herein can include a backing layer. The backing layer can help in providing the physical integrity to the transdermal patch, and can help to protect the drug matrix and the microneedle array.

The backing of the transdermal patch provided herein can be formed of any material known in the art, and typically include materials that result in a flexible structure. Exemplary materials include cellulose acetate, ethyl cellulose, regenerated cellulose, cross-linked polyvinyl alcohol, ethylene-vinyl acetate (EVA) copolymer, polyester, polyethylene, such as low density polyethylene or high density polyethylene, linear low density polyethylene, metallocene polyethylene, polyethylene terephthalate, polypropylene, polyvinyl chloride, polyvinylidene chloride, polyurethane, and combinations, composites, or multi-laminates thereof. Multilayer polymeric films can be used, such as described in U.S. Pat. No. 5,783,269 (Heilmann *et al*., (1998)). The backing can be formulated to be opaque, translucent or transparent. Additives can be included in the material for forming the backing 110, such as anti-oxidants, colorants, plasticizers, tackifiers, and combinations thereof.

B. Drug Matrix

The active ingredient of the transdermal patch can be provided in a drug matrix. The drug matrix can be included within the hollows of the microneedles of the microneedle array. The drug matrix can be included as a drug-loaded adhesive matrix. The drug matrix can be included as a drug reservoir. In some embodiments, the transdermal patch can include the active ingredient within the hollows of the microneedles of the microneedle array, alone or in combination with active ingredient in a drug-loaded adhesive matrix and/or in a drug reservoir.

1. Within the Microneedles

In some embodiments of the transdermal patch provided herein, the active ingredient can be provided within the hollows of the microneedles of the microneedle array. For example, solution of anti-venom IgG or IgY antibodies can be placed into the hollows of the microneedles. In some embodiments, the microneedle array containing the solution of the antibodies can be lyophilized to produce a lyophilized anti-venom IgG or IgY material within the hollows of the microneedles. In some embodiments, the solution of anti-venom IgG or IgY antibodies can include a bioerodable or biodegradable gelling agent that can set to form a solid or semi-solid gel within the hollows of the microneedles. Once the microneedle array punctures the stratum corneum, skin interstitial fluid can dissolve or help to release the anti-venom IgG or IgY antibodies from within the microneedles. Exemplary bioerodable or biodegradable gelling agents include chitin, alginate, gellan gum, polylactic acid (PLA), polyglycolic acid (PGA), poly(D,L-lactide-co-glycolide), polycaprolactone, poly(amides), poly(ester amides), polyhydroxy acids, polyalkanoates, and combinations thereof.

2. Drug-loaded Adhesive Matrix

In some embodiments, the active ingredient can be included in an adhesive matrix. The adhesive matrix can be in fluid communication with the microneedle array. When the microneedle array punctures the stratum corneum, skin interstitial fluid can flow through the microneedles forming a fluid conduit which allows active ingredient within the adhesive matrix to dissolve and flow back past the stratum corneum and into the lower epidermis and/or the dermis. The active ingredient can be mixed with an adhesive material to form the adhesive matrix. For example, a lyophilized form of IgG or IgY anti-venom or anti-toxin antibody and be dispersed into an adhesive material to form the drug loaded adhesive matrix. Micronized or encapsulated lyophilized antibody also can be dispersed throughout the adhesive material to produce the drug-loaded adhesive matrix. Precipitated IgG or IgY anti-venom or anti-toxin antibody also can be dispersed throughout the adhesive material to produce the drug-loaded adhesive matrix. When a high salt concentration is used to precipitate the antibody, residual salt in the precipitated protein can help to act as a driving force when the transdermal patch is activated. When the microneedle array punctures the skin, skin interstitial fluid can flow through the microneedles forming a fluid conduit which allows active ingredient within the adhesive matrix to dissolve, along with any associated salts. The

dissolving salts can result in a creation of an osmotic pressure that can help drive the dissolved active ingredient through the microneedles into the lower epidermis and/or dermis.

In some embodiments the active ingredient is encapsulated. Methods of encapsulating antibodies and other proteins are known in the art and are described, for example, in U.S. Pat. No. 7,105,158 (D'Souza *et al*., 2006). Materials that are biodegradable and nonantigenic can be used as the encapsulating material. Encapsulating materials include, but are not limited to, albumin, poly(lactic-co-glycolic acid), globulin, natural and synthetic polymers, and thermoplastic polymers. Any polymer that is biocompatible and bioerodible can be used for encapsulation. A number of available crosslinking agents such as glutaraldehyde can be used to crosslink the encapsulating material. Additionally, the degree of crosslinking used to form the encapsulated active ingredient can be varied by using different concentrations crosslinking agent whereby the resulting encapsulated materials can exhibit different release rates, thereby creating a prolonged continuous release of the active ingredient.

In another embodiment, the active ingredient can be provided in the form of solid particles adhered to the surface of the adhesive. In particular, these particles may be hydrophilic, so that contact with aqueous fluid exposed at the surface of the treated skin, such as skin interstitial fluid, will cause the particles to dissolve or disintegrate, thus releasing the active ingredient into the skin.

Any adhesive material that causes little or no irritation or sensitization of the skin during the intended period for which the transdermal patch is to be used can be selected for formation of the drug-loaded adhesive matrix. Examples of suitable adhesives include acrylates, silicones, polyisobutylenes, synthetic rubber, natural rubber, and copolymers and mixtures thereof. Further details and examples adhesives that can be selected for use are described, *e.g.*, in U.S. Pat. Nos. 4,584,355 (Blizzard *et al*., 1986); 4,585,836 (Homan *et al*. 1986); 4,591,622 (Blizzard *et al*., 1986); 4,655,767 (Woodard *et al*., 1987); 5,223,261 (Nelson *et al*., 1993); 5,380,760 (Wendel *et al*., 1995); 5,656,286 (Miranda *et al*., 1997); 9,034,370 (Kanios, 2015); and 9,682,068 (Deshmukh *et al*., 2017).

The active ingredient can be mixed with the adhesive prior to forming the transdermal patch, or it can be applied to the adhesive layer of the transdermal patch in a separate process

step. Examples of suitable methods for applying any active ingredient to an adhesive layer are described in U.S. Pat. No. 5,688,523 (Garbe *et al*., 1997); and U.S. Patent Application Publication No. 2003/054025 (Cantor *et al*., 2003).

3. Drug Reservoir

In some embodiments of the transdermal patch provided herein, the drug matrix can be, or can include, a drug reservoir. The size of such a drug reservoir can be selected to deliver a selected amount of active ingredient through the skin. The reservoir can be centrally located with respect to the shape and size of the transdermal patch, and can include on each of its edges an adhesive that can help to secure the drug reservoir in place within the transdermal device. Any known drug reservoir known in the art can be used or adapted for use within the transdermal patch provided herein. Exemplary reservoirs are described in, *e.g.*, U.S. Pat. Nos. 4,751,087 (Wick, 1988); 4,834,979 (Gale, 1989); 6,004,578 (Lee *et al*., 1999); 6,365,178 (Venkateshwaran *et al*., 2002); 6,024,976 (Miranda *et al*., 2000); 6,149,935 (Chiang *et al*., 2000).

The drug matrix can be configured to achieve a desired release rate of the active ingredient to be therapeutically effective. The amount of drug that constitutes a therapeutically effective amount can be readily determined by those skilled in the art with due consideration of the particular drug, the particular carrier, and the desired therapeutic effect.

In some embodiments, the matrix can include a polymeric matrix, for example, a hydrogel, that is capable of swelling and retaining an aqueous liquid.

C. Microneedle Array

The transdermal device provided herein includes a microneedle array for delivery of the active ingredient. Microneedle arrays use microsized dimension needles that can enhance permeation of large molecule drugs, particularly macromolecules like vaccines, proteins, and DNA, through stratum corneum by creating micro-sized channels in the skin [Kalluri *et al*, The AAPS Journal 13(3): 473-481 (2011); Kalluri and Banga, Journal of Drug Delivery Science and Technology 19(5): 303-310 (2009)]. Proteins, such as immunoglobulins, can be loaded in microneedles and can pass through the channel of the needle and then enter into lower epidermis and further diffuse into nearby capillaries. Several proteins like human

recombinant keratinocyte growth factors and human IgG were delivered by using poly lactic-co-glycolic acid and maltose microneedles [Chellathurai *et al.,* Turk J Pharm Sci 18(1): 96-103 (2021); Li *et al*., Int J Pharm 368 (1-2): 109-115 (2009); Jamaledin *et al*., J Clinical Medicine Vol. 9, Article 542, 25 pages (2020)]. Semi-solid needles also can be used, and the drug can be delivered from a reservoir to the penetration area of the needles. The microneedles can create small holes, or pores, or perforations in the outer layer of the skin to prepare the skin for delivery of an active ingredient.

Any microneedle array known in the art can be adapted for use in the transdermal patches provided herein. Exemplary microneedle arrays and how to make them are described, *e.g.*, in Kochhar *et al*. (Journal of Pharmaceutical Sciences 102(11): 4100-4108 (2013)); Kim *et al*. (Journal of Microbiology and Microengineering 14: 597-603 (2004)); Pérennès *et al*. (Journal of Microbiology and Microengineering 16: 473-479 (2006)); Donnelly *et al*. (Advanced Functional Material 22: 4879-4890 (2012)); Kim *et al*. (Microsyst Technolo 13: 231-235 (2007)); Kuo *et al*. (Tamkang Journal f Science and Engineering 7(2): 95-98 (2004)); Ji *et al*. (J Phys. Conf. Ser. 34: 1132 (2006)); and Dardano *et al*. (Materials 8: 8661-8673 (2015)); U.S. Pat. Nos. 7,785,301 (Yuzhakov, 2010) and 8,414,548 (Yuzhakov, 2013); and U.S. Pat. App. Pub. Nos. US2020-0206488 (Lim *et al*., 2020) and US2015-0030642 (Wu *et al*., 2015).

The transdermal patches provided herein allow a subject that is a victim of a bite to adhere the transdermal patch to bite area of the skin, apply manual pressure to the transdermal patch to puncture the skin with the microneedle array, creating pathways or penetration sites through which the active ingredient(s) can pass, and allow the active ingredient(s) to be delivered to the bite area. The active ingredients can be contained within hollow microneedles of the microneedle array. The active ingredient(s) can be loaded into a drug matrix. The drug matrix can be a drug loaded reservoir. The drug matrix can be a drug loaded adhesive. The active ingredients can be contained within hollow microneedles of the microneedle array and in a drug matrix.

In some embodiments, the length of the microneedles of the microneedle array are sufficient to penetrate completely through the stratum corneum. In some embodiments, the length of the microneedles of the microneedle array are sufficient to penetrate into the lower

epidermis layer of the skin. In some embodiments, the length of the microneedles of the microneedle array are sufficient to penetrate into the dermis layer of the skin.

D. Skin Adhesive Layer

The transdermal device provided herein can include a skin adhesive layer. The adhesive layer can be the same as the drug-loaded adhesive matrix described above, or can be a separate distinct layer. The skin adhesive layer can include a skin-contact adhesive. The skin-contact adhesive can be a pressure-sensitive adhesive, and particularly is a pressure-sensitive adhesive that is capable of securely but releasably adhering or bonding to skin of a subject. In some embodiments, the transdermal patch can include more than one skin-contact adhesive, and each skin-contact adhesive can be independently selected with respect to the adhesive material selected and the thickness of the adhesive layer. Examples of suitable adhesives are described above, and include (meth)acrylates, silicones, polyisobutylenes, synthetic rubber, natural rubber, and copolymers and mixtures thereof. In some embodiments, the skin-contact adhesive include polyolefins or (meth)acrylate copolymers.

E. Release Liner

The transdermal patches provided herein can include a release liner. The release liner can be configured to release from the skin-contact adhesive layer, so that the transdermal patch can be adhered to a skin surface of a subject. The release liner is in place during storage or the transdermal device, to protect the adhesive properties of the skin-contact adhesive layer. For use, the release liner is removed, exposing the skin-contact adhesive layer, and when the transdermal device is placed on a bite site, the skin-contact adhesive layer can adhere to the skin of the subject to hold the transdermal patch in position.

Release liners are known in the art and are available from a variety of manufacturers in a wide variety of formulations, sizes, and configuration.. Those skilled in the art can test release liners in simulated use conditions with an adhesive of choice to arrive at a product with the desired release characteristics. Suitable release liners can be made of a material selected from among Kraft paper, supercalendered Kraft paper with or without a coating of clay or polyvinyl alcohol, a biaxially-oriented polyethylene terephthalate film, a biaxially oriented polypropylene film, a polyester film, a high density polyethylene film, a low density

polyethylene film, or laminates or composites of any of these materials. The release liner material can be coated with release agents or low adhesion coatings. Exemplary release agents include silicones. Silicone coated release liners are commercially available.

F. Optional Components

In some embodiments, the transdermal patch provided herein can further include one or more optional additional components. When present, if the optional component is an additional layer, any optional layers can be configured so that the skin-contact adhesive layer is still coupled, directly or indirectly, to a surface of the backing. Exemplary additional layer(s) include, for example, a rate-controlling membrane, a protective barrier layer that prevents interaction between an active ingredient and the backing, or combinations thereof.

A rate-controlling membrane can be present to help control the release rate of active ingredient diffusion into the bite site. As an example, the transdermal patch can include a chemical permeation enhancer to increase the permeability or porosity of the outermost skin layers as an active ingredient, and the rate-controlling membrane can control the rate of release of the chemical permeation enhancer in order to maintain the micropores produced by the microneedle array and to enhance prolonged permeability.

As another example, the transdermal patch can include an antibiotic as optional component, and the rate-controlling membrane can control the rate of release of the antibiotic in order to maintain an efficacious amount at the treatment site, such as to destroy any microbes that may have been introduced via the bite or from a skin surface.

In the embodiment illustrated in FIG. 1C, the transdermal patch includes a drug loaded adhesive and a drug matrix separated by a membrane. The drug matrix can be a drug-loaded reservoir or a drug-loaded adhesive. The membrane can help in controlling the rate of drug diffusion. In other embodiments, a membrane is not present, and the drug matrix can include a drug loaded reservoir, or a drug loaded adhesive layer, or a combination thereof.

4. Packaging

The transdermal patches provided herein can be packages in any packaging known in the art. In some embodiments, each transdermal patches can be packaged individually in a

packaging material. In some embodiments, a transdermal patch can be packaged in foil or a foil-lined pouch. In some embodiments, a transdermal patch can be packaged in a pouch made of a multi-laminate polymer film. The multi-laminate polymer film pouch can have low moisture and/or oxygen permeability. In some embodiments, a transdermal patch can be packaged with a desiccant and/or oxygen absorber.

5. Preparation of Antibodies

A. Equine or Ovine Antibody Production

Methods of producing an antivenin in an equine, such as a horse, or an ovine, such as a sheep are known in the art. For example, see Taherian *et al.* (Tropical Journal of Pharmaceutical Research 17(3): 409-414 (2018)); and Guidlolin *et al*. (African Journal of Biotechnology 9(16): 2446-2455 (2010)).

A schematic depiction of the preparation of anti-sera containing IgG in a horse is shown in FIG. 2. A sera can be prepared by immunizing horses with a composition comprising an antigen selected from among a snake venom antigen, a spider venom antigen, a scorpion venom antigen, a jellyfish venom antigen, a cone snail venom antigen, a venomous fish venom antigen, a rabies antigen, and a bacterial antigen. An adjuvant typically is included in the composition. An immunization schedule is determined for maximum antibody production. Typically, an injection of the composition containing the antigen of interest is administered repeatedly over a period of several weeks. A blood sample can be collected and the serum tested for the titre of antibodies directed against the antigen. When the titre level has reached a targeted value, blood is collected, and the serum is separated out. The anti-antigen antibodies can be separated and purified using techniques known in the art. The IgG anti-antigen antibodies can be used as an active ingredient in the transdermal patches provided herein. The IgG antibodies can target and neutralize a snake venom antigen, a spider venom antigen, a scorpion venom antigen, a jellyfish venom antigen, a cone snail venom antigen, a venomous fish venom antigen, a rabies antigen, or a bacterial antigen.

B. Antibody production in an egg-laying animal

In some embodiments, anti-sera containing IgY is prepared in an egg-laying species. A schematic depiction of the preparation of anti-sera containing IgY in chickens is shown in FIG. 3.

In certain aspects, the present disclosure relates to a method of preparing an antibody in an egg-producing animal directed to a venom, rabies, or a bacteria known to be transferred by an animal bite, the method comprising: a) hyperimmunizing an egg-producing animal with a composition comprising an antigen selected from among a snake venom antigen, a spider venom antigen, a scorpion venom antigen, a jellyfish venom antigen, a cone snail venom antigen, a venomous fish venom antigen, a rabies antigen, and a bacterial antigen; and b) preparing a hyperimmunized egg product from one or more eggs produced by the animal. In some embodiments, the antigen comprises or consists of snake venom antigen selected from among a *Naja Naja* (Cobra) venom, *Bungarus Caeruleus* (common Krait) venom, *Daboia russelii* (Russell's viper) venom, and *Echis carinatus* (Saw-scaled viper) venom. In the methods, the level of antibodies to the antigen in the hyperimmunized egg is increased relative to an egg from an animal that has not been hyperimmunized.

Use of avian antibodies (IgY) further increases the number of antigenic epitopes of toxins available to target, due to avian species' phylogenetic distance from mammalian-derived proteins and proteins of mammalian pathogens. The polyclonal nature of the avian antibodies for multiple epitopes for neutralization or target toxin/antigen translates to improved efficacy, particularly when compared to monoclonal antibodies.

Egg-producing animals produce antibodies in blood and eggs that are specific to particular immunogens. For example, various genera of the class Aves, such as chickens (Gallus domesticus), turkeys, and ducks produce antibodies against antigens associated with avian diseases. U.S. Patent No. 4,748,018 (Stolle *et* al., 1988) discloses a method of passive immunization of a mammal that comprises parenterally administering purified antibody obtained from the eggs of an avian that has been immunized against the corresponding antigen, and wherein the mammal has acquired immunity to the eggs. U.S. Patent No. 5,772,999 (Greenblatt *et al*., 1998), discloses a method of preventing, countering or reducing

chronic gastrointestinal disorders or Non-Steroidal Anti-Inflammatory Drug-induced (NSAID-induced) gastrointestinal damage in a subject by administering hyperimmunized egg and/or milk or fractions thereof to the subject.

One of the advantages of the hyperimmunized egg product is that it would have a higher and more consistent level of antibodies (e.g. IgY antibodies) to one or more of the antigens described herein compared to a control egg product or an egg product from a chicken that has been immunized with the antigen using standard immunization techniques. Typically standard immunization consists of an initial immunization followed by one or two booster immunization at 30 day intervals. In some embodiments, hyperimmunization comprises at least 4, 5, 6, 7, 8, 9 or 10 immunizations with an antigen described herein. In some embodiments, hyperimmunization comprises immunizing an egg producing animal with an antigen described herein at intervals of less than 30 days, less than 25 days, less than 20 days, less than 15 days, less than 10 days, or less than 5 days. In some embodiments, hyperimmunization comprises immunizing an egg producing animal with an antigen described herein at an interval of 1 week, 2 weeks, 3 weeks, 4 weeks, 1 month, 2 months or 3 months. Any of these values can be used to define a range for the interval at which the egg producing animal is immunized. For example, in some embodiments, the egg producing animal is hyperimmunized at an interval ranging from once every 2 weeks to once every 3 months, once per week to once every 3 months, or once every 2 weeks to once per month.

The hyperimmunized egg product can be produced by any egg-producing animal. It is preferred that the animal be a member of the class Aves or, in other words, an avian. Within the class Aves, domesticated fowl are preferred, but other members of this class, such as turkeys, ducks, and geese, are a suitable source of hyperimmune egg product. In a particular embodiment, the egg-producing animal is a chicken.

This special state of hyperimmunization is preferably achieved by administering an initial immunization, followed by periodic boosters with sufficiently high doses of specific antigens or mixtures of antigens. The dosage of the booster can be 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% of the dosage necessary to produce primary immunization of the egg-producing animal. Any of these percentages can be used to define a range for the dosage of the booster immunization. For example, in some embodiments, the

dosage of the booster is 20%-80%, 30%-70%, or 50%-100% of the dosage necessary to produce primary immunization of the egg-producing animal. In a particular embodiment, the dosage of the booster immunization is 50% of the dosage of the primary immunization.

Having knowledge of the requirement for developing and maintaining a hyperimmune state, it is within the skill of the art to vary the amount of antigen administered, depending on the egg-producing animal genera and strain employed, in order to maintain the animal in the hyperimmune state.

The hyperimmune state can be produced by a single antigen or a combination of antigens. Hyperimmunization can be achieved by multiple exposures to multiple antigens, or multiple exposures to a single antigen.

Hyperimmunization Procedure

The following list of steps is an example of a procedure used to bring an egg-producing animal to a heightened state of immunity from which the resultant hyperimmune egg or egg product can be produced.

1. Selecting one or more antigens.
2. Eliciting an immune response in the egg-producing animal by primary immunization with a vaccine containing the antigen(s).
3. Administering booster vaccines of one or more antigens of appropriate dosage to induce and maintain the hyperimmune state.

Step 1: A critical point in this step is that the antigen(s) must be capable of inducing immune and hyperimmune states in the egg-producing animal.

Step 2: For a venom, the venom is diluted to a sub-lethal dosage. For rabies, the vaccine can be either a killed or live-attenuated virus. For bacterial antigens, the antigen can be a killed bacteria or a fraction, fragment, or particle thereof. The vaccine can be administered by any method that elicits an immune response. It is preferred that immunization be accomplished by administering the vaccine through intramuscular or subcutaneous injection. A preferred muscle for injection in an avian is the breast muscle. The minimum dosage of antigen necessary to induce an immune response depends on the

vaccination procedure used, including the type of adjuvants and formulation of antigen(s) used as well as the type of egg-producing animal used as the host. In some applications, a dosage can be in the range of 0.05-5 milligrams of the immunogenic vaccine. Other methods of administration that can be used include intravenous injection, intraperitoneal injection, intradermal, rectal suppository, aerosol or oral administration. The vaccine can be a combination of the antigen and an adjuvant.

It can be determined whether the vaccine has elicited an immune response in the egg-producing animal through a number of methods known to those having skill in the art of immunology. Examples of these include enzyme-linked immunosorbent assays (ELISA), tests for the presence of antibodies to the stimulating antigens, and tests designed to evaluate the ability of immune cells from the host to respond to the antigen.

Step 3: The hyperimmune state is preferably induced and maintained in the target animal by repeated booster administrations of an appropriate dosage at fixed time intervals. The time intervals can be a period of one week to three month intervals over a period of 6-12 months. However, it is essential that the booster administrations do not lead to immune tolerance. Such processes are well known in the art. Methods of preparing the hyperimmunized egg product are described, for example, in U.S. Pat. No. 6,803,035 (Greenblatt *et al*., 2004)), which is incorporated by reference herein in its entirety.

In some embodiments, an antigen as described herein is formulated with an adjuvant. The adjuvant can selected from among Freund's complete adjuvant, Freund's incomplete adjuvant, a saponin, a biodegradable polymer, aluminum hydroxide, mineral oil, a surfactant, and combinations thereof. Exemplary saponins include QS-21 and Quil A . Exemplary biodegradable polymers include chitosan, zymosan, a poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol), such as Pluronic® L121 block copolymer, poly(lactic acid), poly(glycolic acid), poly(lactic-co-glycolic acid), polycaprolactone, and combinations thereof. Exemplary surfactants include polysorbate 80 and sorbitan trioleate.

In a particular embodiment, an antigen as described herein is formulated into a Freund's vaccine. In some embodiments, an antigen as described herein is formulated with an

adjuvant selected from the group consisting of Freund's complete adjuvant, Freund's incomplete adjuvant and QS21. In any exemplary vaccination schedule, in the first vaccination, the egg-producing animal can be given two 0.5 ml doses of the vaccine. One to two weeks later, one 0.5 ml dose of vaccine can be administered to the egg-producing animal as a booster vaccination. An additional booster vaccination can be performed 3 to 6 weeks after the first vaccination. The vaccines can be administered intramuscularly, such as to breast tissue.

It is possible to use other hyperimmunization maintenance procedures or combination of procedures, such as, for example, intramuscular injection for primary immunization and intravenous injection for booster injections. Further procedures include simultaneously administering microencapsulated and liquid antigen, or intramuscular injection for primary immunization, and booster dosages by oral administration or parenteral administration by microencapsulation means. Several combinations of primary and hyperimmunization are known to those skilled in the art.

The hyperimmunized egg from the vaccinated animal comprises antibodies to the antigen used in the vaccination. The antibody can be an IgA, IgM or IgY antibody. In a particular embodiment, the antibody is an IgY antibody. The IgY antibody can react to a portion of a snake venom antigen, a spider venom antigen, a scorpion venom antigen, a jellyfish venom antigen, a cone snail venom antigen, a venomous fish venom antigen, a rabies antigen, or a bacterial antigen. In particular embodiments, the IgY antibody can be polyclonal antibodies, each of which can react with different epitopes of the same antigen. For example, the IgY antibody produced by the methods described herein can react with one or several epitopes of *Naja Naja* (Cobra) venom, *Bungarus Caeruleus* (common Krait) venom, *Daboia russelii* (Russell's viper) venom, or *Echis carinatus* (Saw-scaled viper) venom.

In some embodiments, the hyperimmunized egg or egg product comprises at least 10%, 20%, 30%, 40%, 50%, 100%, 200%, 300%, 400% or 500% more antibody (e.g. IgY antibody) specific to a particular antigen by weight relative to a control egg or control egg product obtained from an egg-producing animal that is not hyperimmunized with the particular antigen. The hyperimmunized egg or hyperimmunized egg product can contain increased levels of antibodies to two or more of the antigens disclosed herein, relative to a

control egg or control egg product obtained from an egg-producing animal that is not hyperimmunized.

Comparisons of antibody titers in hyperimmunized egg products and control egg products can be determined by methods known in the art. For example, in one embodiment, eggs are collected and the antibody titers are monitored by ELISA at regular intervals. To determine antibody titers, total IgY is extracted from eggs using Pierce™ Chicken IgY Purification Kit (Thermo Fisher Scientific, Waltham, MA). Briefly, 2 mL of egg is mixed with five volumes of delipidation reagent and IgY is purified following the manufacturer's instructions. Spray dried egg powder samples are reconstituted in sterile PBS at 1 mg/mL, and filtered through a 0.22 μm membrane filter. Specific antibody titers in the isolated IgY or egg powder samples are measured by ELISA. Flat bottom, 96-well microtiter plates (Corning® Costar®, Corning, NY) are coated with the antigen of interest at an appropriate dilution and incubated overnight at 4 °C. The plates are washed twice with PBS containing 0.05% Tween 20 (Sigma-Aldrich, St. Louis, MO) and blocked with 100 μL/well of PBS containing 1% Bovine Serum Albumin (BSA) and incubated for 1 h at RT. Serially diluted (in PBS with 0.1% BSA) IgY samples from egg powder samples are added to the plates in triplicate wells (100 μL/well) and incubated for 2 h at RT with constant shaking. The plates are then washed with PBS-T and treated with peroxidase-conjugated rabbit anti-chicken IgY (IgG) antibody (1:500; Sigma), incubated for 30 min, followed by color development for 10 minutes with 0.01% tetramethylbenzidine substrate (Sigma) in 0.05 M Phosphate-Citrate buffer, pH 5.0. Bound antibodies are detected by measuring optical density at 450 nm (OD_{450}) using a microplate reader (Bio-Rad, Hercules, CA).

In some embodiments, the hyperimmunized egg or egg product comprises at least 0.0001%, 0.0005%, 0.001%, 0.005%, 0.01%, 0.05%, or 0.1% by weight of an IgY antibody to a specific antigen disclosed herein. Typically, a whole chicken egg weighs approximately 60 grams without the shell, with the egg yolk weighing approximately 20 grams and the egg white weighing approximately 40 grams. In some embodiments, 3 grams of egg yolk contains approximately 20 grams of total IgY, such that a whole egg contains about 150-200 mg total IgY. In some embodiments, at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 25% or 30% of the total IgY in the

hyperimmunized egg or egg product is specific to one of the antigens used for hyperimmunization.

The IgY antibody specific for an antigen disclosed herein is concentrated for its use in the transdermal patch. For example, the IgY antibody can be purified or partially purified and concentrated before formulating into the drug matrix of the transdermal patch. Methods of purifying and concentrating IgY antibodies from egg products are known in the art and are described, for example, in U.S. Pat. No. 5,367,054 (Lee, (1994)), which is incorporated by reference herein in its entirety. The IgY antibody can be purified or partially purified using polyethylene glycol (PEG) precipitation. The IgY antibody can be purified or partially purified by separation of water soluble antibodies into an aqueous layer at different pH by freeze thaw methods followed by NaCl precipitation. The IgY antibody can be purified or partially purified by separation using affinity chromatography.

The IgY antibody also can be purified to yield antigen-specific IgY antibodies. For antibodies that demonstrate cross reactivity with other antigens, antigen-specific antibodies can be obtained using affinity chromatography. For example, to purify IgY antibodies to antigen A from other corss-reacting antibodies, an affinity column can be prepared by conjugating antigen A to a support, placing the conjugated support in a column, and eluting a solution containing the IgY antibodies through the column. IgY antibodies that react with antigen A will adhere to the conjugated support, while IgY antibodies that do not react with antigen A will pass through the column.

After purifying the high titer antibodies from either chicken eggs or horse plasma, these antibodies can be formulated as active ingredients to achieve rapid penetration into the skin followed by including in a drug matrix of the transdermal patches provided herein.

Advantages of the Invention

The transdermal patched provided herein can be used for treating various snake venoms. In addition the invention is also used to treat other acute toxic bites which includes but not limited to**:**

- Other venomous snake bites across the globe

- Honeybee bites
- Scorpion bites
- Dog bites and the likes thereof.

Additional benefits:

1. Polyclonal antibody-loaded transdermal patches can be used for rapid release of the antibody and any additional drug into the blood stream to neutralize the targeted antigen or toxin.
2. Use of such transdermal patches not only helps in lowering the antigen/toxin's systemic circulation but also helps in preventing the local toxicity of the targeted toxins/antigen.
3. Use of polyclonal antibodies allows multiple epitopes for neutralization of target toxin/antigen compared to monoclonal antibodies, and this can translate to improved efficacy.
4. Use of avian antibodies (IgY) further increases the number of antigenic epitopes or toxins available to target, due to avian species phylogenetic distance from mammalian-derived proteins and proteins of mammalian pathogens. Species-specific antibodies are also encompassed by the invention.
5. In addition to antibodies, one or a combination of drugs can be loaded onto the transdermal patch.
6. It is also envisaged that the full antibodies/F(ab)2/F(ab) to the toxin can be administered in the form of intramuscular and/or intravenous injections.

The present invention of transdermal patch loaded with appropriate antibody and/or any other drug will be tested in the following assays:

- Venom neutralization assays with antivenin loaded transdermal patches
- Study of rate of release of antibodies into the skin
- Pharmacokinetics

METHODS

Another aspect of the present disclosure provides a method of treating a bite site of a subject who is a victim of a poisonous or infectious bite by transdermally delivering an active ingredient, such as an IgG or IgY antibody that interacts and/or neutralizes the toxin or infectious agent injected into the subject by the action of the bite. The method can include providing a transdermal adhesive patch provided herein, and applying the transdermal patch to a bite site.

The method can further include adhering at least a portion of a skin-contact adhesive layer of the transdermal patch to a skin surface adjacent to the bite site to anchor the transdermal patch in position. The method can further include applying a pressure to or adjacent to the microneedle array to treat the bite site. The pressure can be applied using one or more fingers, or the palm of the hand. The pressure (e.g., finger pressure or palm pressure) can press the microneedle array into the stratum corneum of the skin to treat the bite site and to make holes or micropores in the skin to facilitate delivery of the active ingredient transdermally across stratum corneum of the skin.

EXAMPLES

The transdermal device will be described in the following examples. However, the following examples are for illustrative purposes only, and the scope of the present invention is not limited thereto.

1. Preparation of anti-snake venom IgY antibodies

IgY antibodies to for species of venomous snakes were prepared.

Venoms: Venoms of *Naja Naja* (Cobra), *Bungarus Caeruleus* (common Krait), *Daboia russelii* (Russell's viper) and *Echis carinatus* (Saw-scaled viper) were purchased from Irula snake catchers Co-Operative Society, Chennai, Tamila Nadu, India. Venom samples were dissolved in sterile phosphate buffered saline (PBS) with pH 7.4 and stored at -20°C until further use.

Adjuvant and other chemicals: Freund's complete adjuvant (FCA), incomplete Freund's adjuvant (IFA) were purchased from Sigma Aldrich (St. Louis, MO).

Animals/Chickens

18 weeks old female Giri Raja chickens were used for preparation of the IgY antibodies. All chickens were vaccinated before initiation of the study. The chickens were maintained at the Department of Poultry Science, Veterinary College, Hebbal, Bangalore, at room temperature with access to standard feed and water.

Immunization of the Chickens

The chickens were divided into 5 groups with 10 chickens in each group. The chickens were maintained in individual pens. Groups 1 to 4 each separately wase injected intramuscularly with sublethal doses of the different snake venoms (dissolved in sterile saline) mixed with Freund's Complete Adjuvant (1:1 ratio v/v). Each Group was inoculated with a different snake venom. Chickens in Group-5 were injected only with saline as a control. Booster immunizations were carried out using the same concentrations of venom samples mixed with Freund's Incomplete Adjuvants (FIA) with 14 days interval between the injections. Concentrations of venom used were in the range between 20µg to 100µg per Kg body weight.

Egg collection

After 14 days of the first immunization, hyperimmunized eggs were collected continuously every day from all the pens/groups separately and stored at 4°C until further use.

Purification of IgY antibodies:

Various protocols have been used for purification of IgY antibodies from the hyperimmunised eggs. One of the methods used included polyethylene glycol (PEG) precipitation. Twenty eggs from each group were broken and the yolk was separated from the egg and rolled on a tissue paper to remove the traces of albumin present. Yolk was diluted 3 times with phosphate buffered saline (PBS) pH 7.4 and mixed with 3.5% PEG-6000 followed by overnight incubation at 4°C.

The supernatant was filtered through a Whatman filter paper after centrifuging the sample at 8000 rpm/30 min at 4°C and mixed with 8% PEG-6000.

The pellet obtained was again dissolved in 100mL of PBS and mixed with 12% PEG-6000. The mixture was centrifuged at 8000 rpm/30 min at 4°C. The final pellet obtained was suspended in 50 mL of PBS and stored at 4°C.

The other method used was separation of water soluble antibodies into aqueous layer at different pH by freeze thaw methods followed by NaCl precipitation. Eggs were broken and yolk was carefully collected without yolk membrane. The yolk was diluted 10 times (1:10 ratio) with distilled water and mixed with a magnetic stirrer for thorough mixing. The pH was adjusted and maintained to be 5.1 using 0.5 M HCl while stirring. After the diluting yolk was homogeneously mixed, it was kept at -20˚C for overnight. Then, the frozen egg yolk was kept at room temperature after complete thawing. The supernatant was carefully filtered using membrane or filter paper. To the clear filtrate, 8.8% NaCl was added into the filtrate while stirring and maintaining the pH at 4.0 using 0.5M HCl. The solution was centrifuged and the IgY precipitate was suspended PBS (or phosphate buffer, 20mM, pH=7.2) to yield a clear solution of antibody. It was further diluted and the protein concentration measured at 280 nm for estimation of IgY yield per egg.

Protein Estimation

Each final precipitate of the IgY samples was dissolved separately in PBS and filtered through 0.4um nitrocellulose membrane filter. The estimated total protein quantity in each sample was determined by measuring at 280 nm using a Nanodrop spectrophotometer (ThermoFisher Scientific, Allentown, PA).

SDS-PAGE analysis

The presence of IgY antibodies in all samples of the test groups (Groups 1 to 4) were confirmed by SDS-PAGE. Each purified sample was analyzed in 12% SDS-PAGE under both reducing and nonreducing conditions by loading equal quantity of protein in the wells followed by staining with coomassie brilliant blue. All samples have shown the confirmation of IgY in both non-reducing and reducing (Heavy Chain + Light Chain) conditions with 180kDa and 65+25 kDa respectively.

ELISA evaluation of IgY against all venoms

Different snake venom of one particular species were dissolved in coating buffer (sodium carbonate/bicarbonate, 50mM, pH=9.6) at a concentration of 20 ug/mL and mixed thoroughly to yield a venom solution. 50µL of the venom solution was dispensed in each well of an ELISA micro titre plate and keep at 4ºC in humid condition for overnight (or at 37ºC for 1.0 hr). All the wells were washed three times with PBS-T (0.05% Tween-20). 10% skimmed milk (in PBST) was prepared as a blocking reagent. A 300µl aliquot of the blocking reagent was dispensed in each well followed by incubation at 37ºC for 1.0 hr. After washing, 50µL of primary antibody, i.e. each of the different IgY anti-venom antibodies, were added at different concentrations and incubated at 37ºC for 1.0 hr. This was followed by washing 3 times with PBST. Then, a 50 µL aliquot of detector antibody conjugated with HRP at a dilution of 1:10000 was added to each well and the plate was incubated at 37ºC for 1.0 hr. 100µg of TMB/ml in 50 mM Phosphate-citrate buffer, pH=5.0 and 0.01% H_2O_2 was prepared for use as a substrate. The substrate was added and allowed to incubate for 10-15 minutes in the dark. The reaction was stopped by the addition of 1.0N sulfuric acid (50 µl/well) and absorbance was read at 450 nm. Results of testing for venom cross-reactivity is shown in FIGS. 5 through 8.

The CV venoms exhibited maximum reactivity with anti-CV antibodies. However, other antibodies also demonstrated significant cross reactivity with the CV venoms. The same pattern of cross-reactivity was observed amongst the other venoms and anti-venoms. Because these produced antibodies were found to cross react to each other, venom-specific antibodies were purified by using sequential affinity chromatography.

Purification of species specific IgY antibodies

Various protocols were adapted to purify the snake specific antibodies from each of the poly specific IgY antibodies purified/produced in hyperimmune chicken eggs. One of the methods included affinity chromatography using iron nanoparticles. A schematic representation of the procedure is shown in FIG. 8. Iron nanoparticles were synthesized from 2M $FeCl_3$ and 1M $FeSO_4.7H_2O$ in presence of 1% chitosan while mixing with 2M NaOH. After thorough washing, different snake venoms of nearly 80 mg each were conjugated using

8% glutaraldehyde to these freshly prepared iron nanoparticles and incubated for overnight at 4°C. 1 mg/ml solution of various IgY antibodies were prepared and passed through set of three different columns conjugated with different types of antigen, so as to absorb the cross reactive antibodies in the column itself. Anti-CV antibodies were passed through columns packed with KV, column packed with RV and subsequently column packed with SSV antigen. The final elute collected and stored as an antibodies very specific to CV antigen. Similar procedure was followed with all other three antibodies and final fractions of species specific antibodies were stored at 4°C until further usage.

The results are shown in FIGS. 10-13. Using the above protocol, significant separation of snake-specific antibodies was achieved.

Dated 26th October 2023

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Claims

We Claim:

1. A transdermal patch for treatment of a subject who is a victim of a venomous or toxic bite, comprising:

a release liner;

a microneedle array;

a drug matrix containing an IgG or IgY antibody to a venom or toxin in the bite; and

a backing layer.

2. The transdermal patch of claim 1, wherein the IgG or IgY antibody reacts with a venom from a snake selected from among a Naja Naja (Cobra), Bungarus Caeruleus (common Krait), Daboia russelii (Russell's viper) and Echis carinatus (Saw-scaled viper).

3. The transdermal patch of claim 1, wherein the IgG or IgY antibody reacts with a venom or toxin from a scorpion, spider, honeybee, jellyfish, cone snail, lionfish, scorpionfish, or stonefish.

4. The transdermal patch of claim 1, wherein the IgG or IgY antibody reacts with a rabies virus.

5. The transdermal patch of any one of claims 1 to 4, wherein the microneedle array comprises a plurality of microneedles, each microneedle having:

a base;

a tip distal to the base;

a body between the base and the tip; and

a channel extending substantially from the base through the body portion, the channel being open along at least part of the body portion and forming a channel in fluid communication with at least a portion of the drug matrix.

6. The transdermal patch of any one of claims 1 to 5, wherein the microneedles comprise a biocompatible metal.

7. The transdermal patch of any one of claims 1 to 6, wherein the microneedles comprise a biocompatible polymer.

8. The transdermal patch of any one of claims 1 to 7, further comprising an adhesive material for securing the microneedle array to the subject's skin in the vicinity of the bite with one or more of the microneedles of the microneedle array inserted into the stratum corneum.

9. The transdermal patch of any one of claims 1 to 8, wherein the drug matrix is within the body of at least one of the microneedles of the microneedle array.

10. The transdermal patch of claim 9, wherein the drug matrix is:

(a) lyophilized IgG or IgY antibody; or

(b) encapsulated particles of the IgY or IgY antibody; or

(c) an emulsion comprising the IgG or IgY antibody; or

(d) any combination of (a) – (c).

11. The transdermal patch of any one of claims 1 to 8, wherein the drug matrix is:

(a) an adhesive matrix containing the IgG or IgY; or

(b) a drug reservoir containing the IgG or IgY; or

(c) a combination of (a) and (b).

12. The transdermal patch of any one of claims 1 to 11, wherein the drug matrix further comprises an additional active ingredient selected from among an antibiotic, a chemical permeation enhancer, and combinations thereof.

13. The transdermal patch of claim 12, further comprising a rate-controlling membrane that controls the rate of release of the additional active ingredient.

14. The transdermal patch of any one of claims 1 to 12 contained in a packaging material.

15. The transdermal patch of claim 14, wherein the packaging material comprises a foil wrapper; a foil-lined pouch, a multi-laminate polymer film pouch, or any combination thereof.

16. The transdermal patch of claim 14 or 15, wherein the packaging material further comprises a desiccant and/or an oxygen absorber.

17. A kit, comprising:

the transdermal patch of any one of claims 1 to 16; and instructions for use.

18. A method of treating a bite site of a subject who is a victim of a poisonous or infectious bite, the method comprising:

applying the transdermal patch of any one of claims 1 to 13 to the bite site; and

applying pressure to the transdermal patch.

19. The method of claim 18, wherein the pressure is applied to or adjacent to the microneedle array of the transdermal patch pressing the microneedle array into the stratum corneum of the skin at the bite site.

20. The method of claim 18 or 19, wherein pressing the microneedle array into the stratum corneum of the skin makes holes or micropores in the skin to facilitate delivery of the IgG or IgY transdermally across stratum corneum of the skin.

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TRANSDERMAL ADMINISTRATION OF ANTIBODIES FOR TREATMENT OF SNAKE BITES AND OTHER VENOMOUS OR INFECTIOUS BITES

ABSTRACT

Provided is a transdermal patch and a method of transdermal administration of a drug that is antibodies or small molecules, for the treatment of snake bites and other venomous or infectious bites. The topical application of drug or antibody formulations is through transdermal patches containing a microneedle array provided with either IgG based anti-snake venoms or IgY based anti-snake venoms or IgG or IgY antibodies to any protein or antigen alone or in combination with peptide(s) or small molecule (synthetic/natural), aimed to treat venomous or infectious bites in humans as well as animals.

Dated 26th October 2023

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