US011484493B2

(12) **United States Patent**
Friedman

(10) **Patent No.: US 11,484,493 B2**
(45) **Date of Patent: Nov. 1, 2022**

(54) **TRANSDERMAL DELIVERY FORMULATIONS**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **17/565,556**

(22) Filed: **Dec. 30, 2021**

(65) **Prior Publication Data**

US 2022/0142947 A1 May 12, 2022

Related U.S. Application Data

(63) Continuation of application No. PCT/US2021/058611, filed on Nov. 9, 2021.

(60) Provisional application No. 63/235,880, filed on Aug. 23, 2021, provisional application No. 63/161,696, filed on Mar. 16, 2021, provisional application No. 63/111,160, filed on Nov. 9, 2020.

(51) **Int. Cl.**

A61K 31/12	(2006.01)
A61K 9/00	(2006.01)
A61P 7/00	(2006.01)
A61K 47/12	(2006.01)
A61K 31/366	(2006.01)
A61K 47/10	(2017.01)
A61K 31/121	(2006.01)
A61P 37/06	(2006.01)
A61K 9/06	(2006.01)
A61K 31/05	(2006.01)
A61K 31/352	(2006.01)
A61K 31/4375	(2006.01)
A61K 31/593	(2006.01)

(52) **U.S. Cl.**

CPC ***A61K 9/0014*** (2013.01); ***A61K 9/06*** (2013.01); ***A61K 31/05*** (2013.01); ***A61K 31/12*** (2013.01); ***A61K 31/121*** (2013.01); ***A61K 31/352*** (2013.01); ***A61K 31/366*** (2013.01); ***A61K 31/4375*** (2013.01); ***A61K 31/593*** (2013.01); ***A61K 47/10*** (2013.01); ***A61K 47/12*** (2013.01); ***A61P 7/00*** (2018.01); ***A61P 37/06*** (2018.01)

(58) **Field of Classification Search**

CPC A61K 31/12
USPC 514/680, 681
See application file for complete search history.

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(57) **ABSTRACT**

A transdermal formulation for the delivery of a nitric oxide booster or nitric oxide precursor to a subject is provided. The formulation can be applied to the treatment of various diseases or conditions by enhancing systemic level of nitric oxide.

19 Claims, 3 Drawing Sheets

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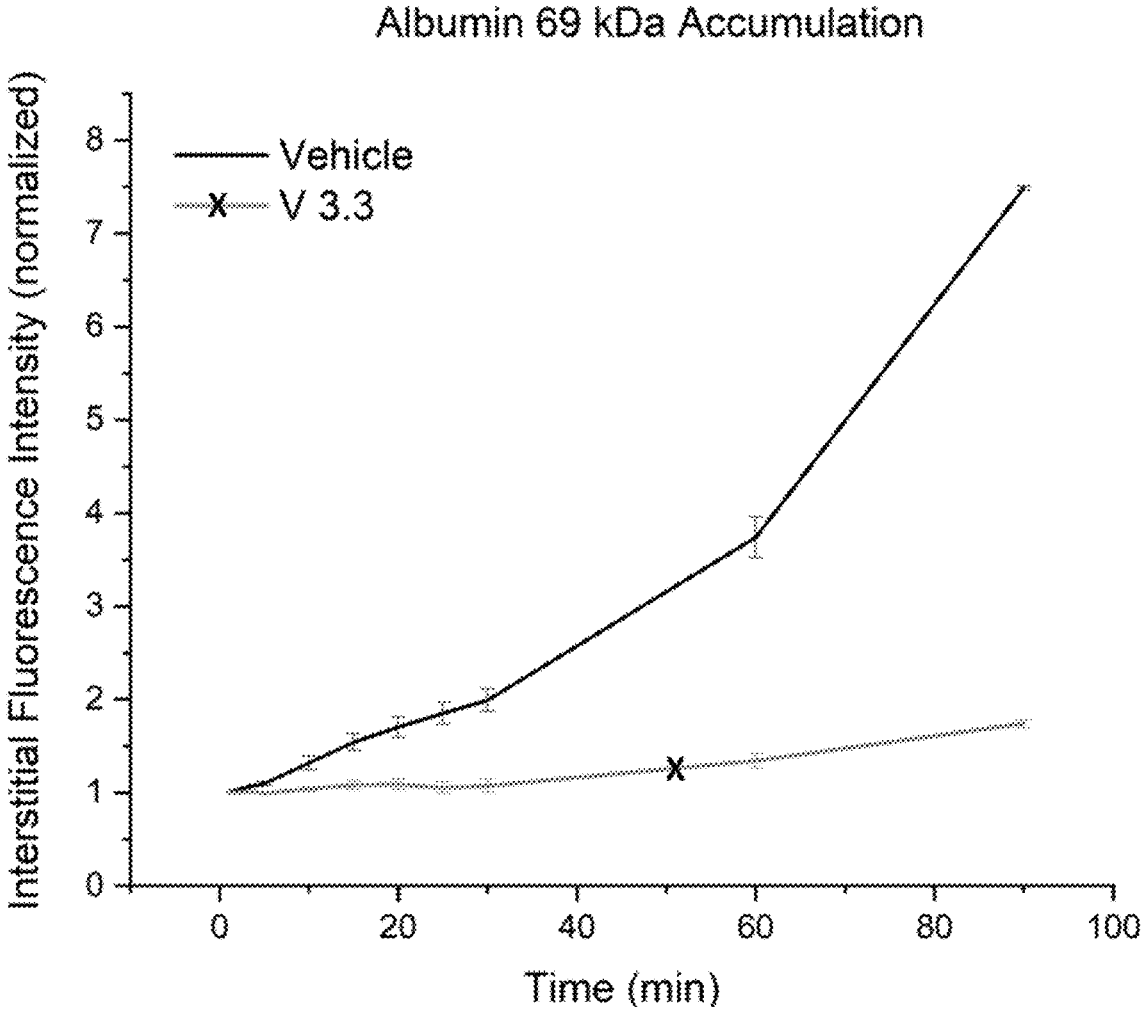
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Figure 1

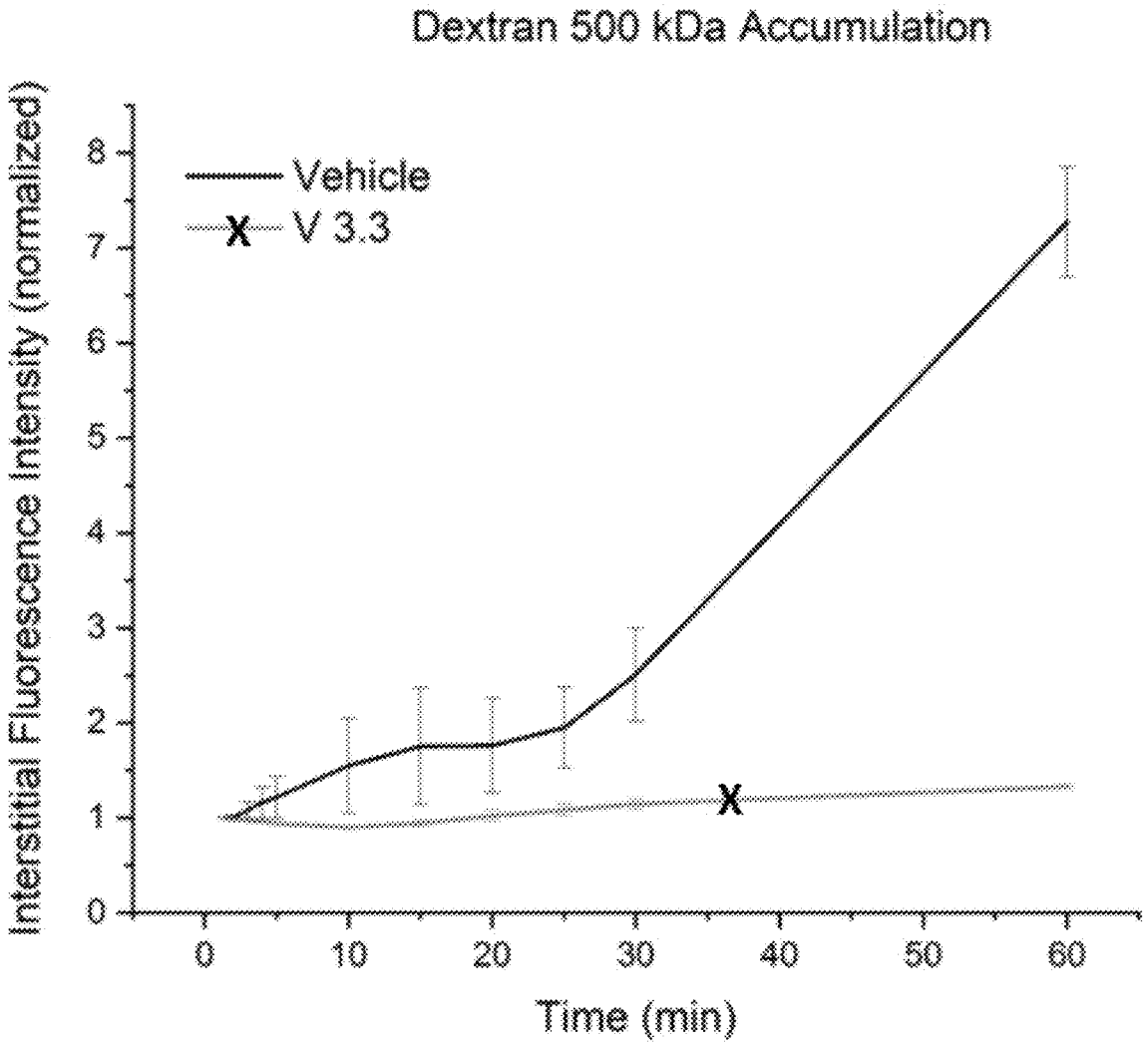
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Figure 2

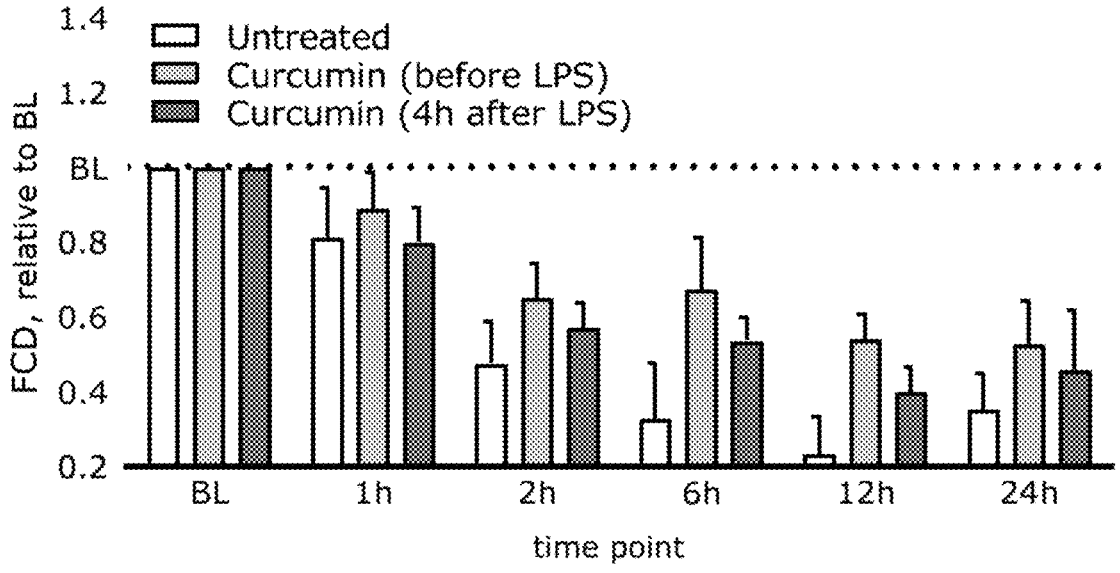
1.4
1.2
BL
0.8
0.6
0.4
0.2
FCD, relative to BL
Untreated
Curcumin (before LPS)
Curcumin (4h after LPS)
BL
1h
2h
6h
12h
24h
time point

Figure 3

TRANSDERMAL DELIVERY FORMULATIONS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a Continuation of International Application No. PCT/US2021/058611 filed on Nov. 9, 2021, which claims the benefit priority to Provisional Application Nos. 63/111,160, filed Nov. 9, 2020; 63/161,696 filed Mar. 16, 2021; and 63/235,880 filed Aug. 23, 2021, the disclosures of all of which are hereby incorporated by reference in their entireties.

TECHNICAL FIELD

Disclosed herein are formulations for sustained transdermal delivery of active agents that enhance levels of nitric oxide in the vasculature and/or limit the production of reactive oxygen species, thus providing new methods of both preventing and treating endothelial dysfunction and its many clinical consequences.

BACKGROUND

Delivery of many therapeutic agents remains a challenge due to bioavailability issues that preclude achieving therapeutic levels either systemically or locally at a targeted site. Poor bioavailability arises from various factors including low solubility, low uptake from the gut, and rapid elimination from the circulation due to rapid breakdown by the liver (first pass limitations). As a result, many promising therapeutics with the appropriate activity cannot be directly delivered in an effective manner to afflicted sites such as the lungs, arthritic joints, inner ear, sinuses etc. Meanwhile, oral or IV routes of administration often do not provide adequate therapeutic levels at the targeted local site.

Nitric oxide (NO) has known systemic benefits including the capability of reversing inflammation, preventing and reversing endothelial dysfunction, repolarizing activated macrophages, deactivating activated platelets, protecting/restoring the endothelial lining of blood vessels (glycocalyx) and antimicrobial/antiviral activity. Proinflammatory insults result in enhanced production of reactive oxygen species (ROS) which drives the development and continuation of endothelial dysfunction. The enhanced levels of ROS set in motion multiple events that cause dysregulation of the vasculature. The ROS degrades the glycocalyx which results in loss of vascular integrity, enhanced cells (monocytes, red blood cells, neutrophils and platelets) access to and adhesion to the endothelial surface, loss of sheer stress mediated NO production and loss of superoxide dismutase which limits ROS. Enhanced ROS causes endothelial nitric oxide synthase (eNOS) to stop production of NO in endothelium and actually start generating more ROS. Enhancing NO levels either with direct supplementation or enhanced production can dramatically reverse this cycle and restore vascular homeostasis. However, systemic delivery of NO is a challenge due to the short lifetime of the NO molecule and limited access to the circulation. A need exists for delivery system that provides efficient and effective enhancement of NO levels in the vasculature and/or the intrinsic regulation of NO levels and bioactivity.

SUMMARY

The delivery system and methods disclosed herein address such a need. The strategy presented in this patent

document both enhances NO levels/production directly and reduces ROS production which also results in restoration of NO levels. NO is essential for normal vascular health and function. The presented strategy also restores the integrity of the glycocalyx which allows for the resumption of flow mediated regulation of NO activity and the preservation of vascular integrity. Through these mechanisms, the presented formulations/strategy can be used to assist in preventing or delaying the development of endothelial dysfunction, limit or reverse endothelial dysfunction, and prevent the development of the many clinical consequences of untreated endothelial dysfunction.

The agent delivered via the formulation disclosed herein is able to enhance systemic nitric oxide levels in the endothelium, either by boosting the NO production or by releasing NO into the circulation. By upregulating the systemic NO level or delivering an effective amount of NO to the circulation, the formulation and kit disclosed herein can be applied to the treatment of various conditions or diseases. Examples include transdermal treatment of acute and chronic inflammatory conditions, transdermal prevention and reversal of endothelial dysfunction, risk reduction of cytokine storm or cytokine storm phenomena (release of an abnormal level or a higher than normal level of pro-inflammatory cytokines) in patients with conditions that create underlying endothelial dysfunction, for example COVID-19 or SARS-CoV-2 infection, topical treatment of dermatological conditions, anti-aging skin treatments, ophthalmological conditions, aerosol based treatments of pulmonary conditions, topical treatments of infections, treatment of red blood cells to improve storage properties and reverse storage lesions thus improving safety and efficacy of stored red blood cells, loading red blood cells with therapeutic agents prior to transfusion, stabilizing red blood cells via intravenous (IV) delivery of RBC stabilizing agents, IV interventions to treat cytokine storm phenomena and related acute inflammatory crises, aerosol treatment and prevention of acute respiratory distress syndrome (ARDS) and other conditions that destroy lung tissue through excessive oxidative damage and the ensuing inflammation, and loading medical sponges with therapeutic agents for use, for example in the ear, nose, mouth, rectum, and vagina. Sustained transdermal systemic delivery can also be achieved to harness the therapeutic potential of NO for various chronic diseases and conditions.

An aspect of the present disclosure provides a transdermal formulation for enhancing systemic nitric oxide (NO), comprising:

(a) an effective amount of an NO booster and/or an NO precursor, wherein the NO booster increases systemic production of NO, and wherein the NO precursor comprises an NO releasing agent or derives or produces a NO releasing agent;

(b) a solvent in an amount sufficient to dissolve the effective amount of the NO booster or the NO precursor; and optionally

(c) a fatty acid,

wherein upon administration the effective amount of the NO booster and/or the NO released from the releasing agent is delivered transdermally.

NO boosters useful in the formulation of the present invention include polyphenols, flavonoids, stilbenoid, secosteroid and other phytochemicals and natural products which promote formation of NO. In some embodiments, the NO booster comprises at least one of an agent selected from the group consisting of polyphenol, flavonoid, stilbenoid, secosteroid, and natural products of the like. In some embodi-

ments, the NO booster includes at least one of curcumin, demethoxycurcumin, bisdemethoxycurcumin, quercetin, berberine, resveratrol and vitamin D. In some embodiments, the NO booster includes quercetin and at least one curcuminoid (e.g. curcumin).

NO precursors useful in the formulation comprises a S-nitrosothiol-containing molecule, or a thiol-containing molecule and a nitrite source. In some embodiments, the formulation includes a S-nitrosothiol-containing molecule as a NO precursor. In some embodiments, the formulation includes, as the NO precursor, a thiol-containing molecule, a nitrite source (e.g. nitrite loaded nanoparticles), and optionally an acid source physically separate from the nitrite source, wherein contact of the acid source with the nitrite source nitrosates a thiol group of the thiol-containing molecule. In some embodiments, the nitrite source and the acid source are separately enclosed in permeable or frangible pouches.

In some embodiments, the polyol is polyethylene glycol having a molecular weight ranging from 100 to about 1000. In some embodiments, the fatty acid is myristic acid.

In some embodiments, the formulation provides continued release of the NO booster or the NO releasing agent from the NO precursor (e.g. S-nitrosothiol-containing molecule) over a period of about about 8, about 10, about 15, about 20, about 24 or about 48 hours.

In some embodiments, the formulation further includes a thickener which keeps the transdermal formulation in a semi-solid or solid form. In some embodiments, the thickener is petroleum jelly, cocoa butter or polyalkylene glycol of MW of more than 2k Da.

Another aspect of this invention provides a kit or transdermal delivery system, incorporating the transdermal formulation disclosed herein. In some embodiments, the transdermal delivery system is a kit or nebulizer.

A further aspect provides a method of increasing systemic NO level in a subject. The method includes administering to the subject the transdermal formulation disclosed herein.

A further aspect provides a method of treating a disease or condition by administering to the subject the transdermal formulation disclosed herein. In some embodiments, the disease or condition is associated with endothelial dysfunction.

A further aspect provides a method of incorporating an agent into a cell by contacting the cell with the formulation disclosed herein.

DESCRIPTION OF THE DRAWINGS

FIG. **1** shows the comparison of vascular leakage for albumin 69 kDa accumulation between LPS treated rat subsequently treated with only vehicle and LPS treated rat subsequently treated with formulation V3.3 as a function of time.

FIG. **2** shows the comparison of vascular leakage for dextran 500 kDa accumulation between LPS treated rat subsequently treated with only vehicle and LPS treated rat subsequently treated with formulation V3.3 as a function of time.

FIG. **3** shows how topical V4.3 prevents the steep drop in functional capillary density that occurs with LPS induced endotoxemia.

DETAILED DESCRIPTION OF THE INVENTION

Embodiments of this disclosure provide transdermal formulations and methods of enhancing systemic NO levels. Because NO has known systemic benefits including the capability of reversing inflammation, preventing and reversing endothelial dysfunction, repolarizing activated macrophages, deactivating activated platelets, protecting the endothelial lining of blood vessels and antimicrobial/antiviral activity, the formulation of this disclosure can be applied to the treatment of various diseases and conditions. The treatment is based on transdermal delivery of agents that can either improve nitric oxide production in the endothelium or directly release NO from appropriate S-nitrosothiol containing molecules.

The transdermal formulation described herein also has the advantage of reduced side effects in comparison with conventional oral administration of active agents. Oral curcumin for instance can result in GI upset (hypermotility, increased acid production in stomach) especially when chronically used. Delivery of the agent over skin or mucosa bypasses the GI tract and achieves desirable therapeutic effect while minimizing side effects.

While the following text may reference or exemplify specific embodiments of a formulation, a kit or a method relating to the treatment or prevention of a disease, it is not intended to limit the scope of the formulation, kit or method to such particular reference or examples. Various modifications may be made by those skilled in the art, in view of practical and economic considerations, such as the specific form of the formulation and the amount or frequency of administration of the formulation for treating or preventing a disease or condition.

The articles "a" and "an" as used herein refer to "one or more" or "at least one," unless otherwise indicated. That is, reference to any element or component of an embodiment by the indefinite article "a" or "an" does not exclude the possibility that more than one element or component is present.

The term "about" as used herein refers to the referenced numeric indication plus or minus 10% of that referenced numeric indication. In some embodiments, "about" refers to the referenced numeric indication plus or minus 5% of that referenced numeric indication.

The term "agent" or "active agent" refers to a molecule or compound that prevents, alleviates or ameliorates symptoms of disease, prolongs the survival of the subject being treated, or reaches a desirable/acceptable medical or sanitary condition. An agent in the NO booster increases systemic production of NO in a subject. An agent in the NO precursor releases NO or undergoes a reaction to generate another agent, which after being transdermally delivered into blood circulation releases NO.

The term "body cavity" includes any opening and/or surface area within the opening on a subject's body. Non-limiting examples of body cavity include nose, nasal sinuses, mouth, ears, rectum, vagina, open wound, sore, buccal cavity and mucosal surface (e.g. gum).

The term C_1-30 alkyl includes, branched or nonbranched, alkyl groups having any number of carbons ranging from 1 to 30. Non-limiting examples include methyl, ethyl, propyl, and butyl.

The term "cytokine storm" refers to dysregulated abnormal systemic release of pro-inflammatory cytokines leading to diseases and has also been referred to as "cytokine release syndrome" or "inflammatory cascade". Often, a cytokine storm or cascade is referred to as being part of a sequence because one cytokine typically leads to the production of multiple other cytokines that can reinforce and amplify the immune response. Generally, these pro-inflammatory mediators have been divided into two subgroups: early

mediators and late mediators. Early mediators, such as e.g., tumor-necrosis factor, interleukin-1, interleukin-6, are not sufficient therapeutic targets for re-establishing homeostatic balance because they are resolved within the time frame of a patient's travel to a clinic to receive medical attention. In contrast, the so-called "late mediators" have been targeted because it is during this later "inflammatory cascade" that the patient realizes that he or she has fallen ill.

The term "inflammatory disease or disorder" may refer to any disease, disorder or syndrome in which an excessive or unregulated inflammatory response results in a transient inflammatory condition, damage to host tissue, or loss of tissue function. "Inflammatory diseases" also refer to pathological conditions mediated by granulocyte influx and/or neutrophil chemotaxis and transient inflammatory conditions including "brain fog" due to chemotherapy and leaky gut syndrome.

The term "long Covid" refers to side effects or symptoms attributed to Covid that become manifest well after the seeming recovery from the primary infection. Nonlimiting examples of long Covid symptoms include include brain fog, fatigue, achiness, clotting issues, myocarditis, and edema.

The term "NO booster" refers to an agent or a mixture of agents that increase systemic production of NO in a subject. The NO booster itself does not release NO.

The term "NO precursor" refers to an agent or a mixture that directly or indirectly through a derivative releases NO. The NO precursor may be or include an agent containing a NO releasing moiety and is transdermally delivered into blood circulation of a subject before releasing NO. Nonlimiting examples of such a NO releasing agent include S-nitroso-Glutathione (GSNO), S-nitroso-N-acetylcysteine (SNAC), S-Nitroso-N-acetylpenicillamine (SNAP), and S-nitroso-human serum albumin (SNO-HAS). Alternatively, a NO precursor may include an agent that leads to a derivative containing a NO releasing moiety, and the derivative releases NO after being transdermally delivered into blood circulation. Nonlimiting examples of agents that lead to NO releasing derivatives include glutathione, N-acetyl cysteine (NAC), N-acetylpenicillamine, and cysteine, which can be nitrosated at the thiol group to produces a S-nitrosothiol-containing derivative.

The term "semisolid" refers to a flexible and deformable solid form. Free flowing liquid and rigid solid forms are excluded from semisolid. Non-limiting examples include gels, ointments, creams, emulsions, microemulsions, nanoemulsions, pastes, balms, lotions, and mousses.

The term "subject" encompasses any animal, but preferably a mammal, e.g., a human, a non-human primate, a dog, a cat, a horse, a cow, or a rodent. More preferably, the subject is a human.

The term "pharmaceutically acceptable salts" means salts of compounds of the present invention which are pharmaceutically acceptable and possess the desired pharmacological activity. Non-limiting examples of such salts include acid addition salts formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, and phosphoric acid; or with organic acids such as 1,2-ethanedisulfonic acid, 2-hydroxyethanesulfonic acid, 2-naphthalenesulfonic acid, 3-phenylpropionic acid, 4,4'-methylenebis (3-hydroxy-2-ene-1-carboxylic acid), 4-methylbicyclo [2.2.2]oct-2-ene-1-carboxylic acid, acetic acid, aliphatic mono- and dicarboxylic acids, aliphatic sulfuric acids, aromatic sulfuric acids, benzenesulfonic acid, benzoic acid, camphorsulfonic acid, carbonic acid, cinnamic acid, citric acid, cyclopentanepropionic acid, ethanesulfonic acid, fumaric acid, glucoheptonic acid, gluconic acid, glutamic acid, glycolic acid, heptanoic acid, hexanoic acid, hydroxynaphthoic acid, lactic acid, laurylsulfuric acid, maleic acid, malic acid, malonic acid, mandelic acid, methanesulfonic acid, muconic acid, o-(4-hydroxybenzoyl)benzoic acid, oxalic acid, p-chlorobenzenesulfonic acid, phenyl-substituted alkanoic acids, propionic acid, p-toluenesulfonic acid, pyruvic acid, salicylic acid, stearic acid, succinic acid, tartaric acid, tertiarybutylacetic acid, and trimethylacetic acid. Pharmaceutically acceptable salts also include base addition salts which may be formed when acidic protons present are capable of reacting with inorganic or organic bases. Acceptable inorganic bases include sodium hydroxide, sodium carbonate, potassium hydroxide, aluminum hydroxide and calcium hydroxide. Non-limiting examples of acceptable organic bases include ethanolamine, diethanolamine, triethanolamine, tromethamine, and N-methylglucamine. It should be recognized that the particular anion or cation forming a part of any salt of this invention is not critical, so long as the salt, as a whole, is pharmacologically acceptable. Additional examples of pharmaceutically acceptable salts and their methods of preparation and use are presented in *Handbook of Pharmaceutical Salts: Properties, and Use* (P. H. Stahl & C. G. Wermuth eds., Verlag Helvetica Chimica Acta, 2002).

The term "therapeutically effective amount" or "effective amount" refers to an amount of an active agent effective to prevent, alleviate or ameliorate symptoms of disease, prolong the survival of the subject being treated, or reach a desirable/acceptable medical or sanitary condition. Determination of a therapeutically effective amount or effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

The term "treating" or "treatment" of any disease or condition refers, in some embodiments, to ameliorating the disease or disorder (i.e., arresting or reducing the development of the disease or at least one of the clinical symptoms thereof). In some embodiments "treating" or "treatment" refers to ameliorating at least one physical parameter, which may not be discernible by the subject. In some embodiments, "treating" or "treatment" refers to modulating the disease or disorder, either physically, (e.g., stabilization of a discernible symptom), physiologically, (e.g., stabilization of a physical parameter), or both. In some embodiments, "treating" or "treatment" refers to delaying the onset of the disease or disorder, or even preventing the same. For instance, a treatment can be a "prophylactic treatment" is to be construed as any mode of treatment that is used to prevent progression of the disease or is used for precautionary purpose for persons at risk of developing the condition.

The term "transdermal" or "transdermally" refers to delivery, administration or application of a formulation containing an active agent by means of direct contact with skin or mucosa and then transport of the agent through the skin or mucosa into blood circulation of a subject. Such delivery, transport, administration or application is also known to include dermal, percutaneous, transmucosal and buccal path. As used herein, "dermal" includes skin and mucosa, which includes oral, buccal, nasal, rectal and vaginal mucosa. In some embodiments, the term also refers to delivery and transport of an agent through a cell wall (e.g. red blood cell wall) into the cell.

The term "transdermal formulation" refers to a composition or formulation of an agent that upon application to the skin or mucosa delivers the agent or a derivative (e.g. a S-nitrosothiol-containing molecule derived from a thiol-

containing molecule) of the agent across the skin or mucosa (or any other surface noted above). A transdermal formulation may be in the form of a solution, suspension, gel, ointment, cream, emulsion, microemulsion, nanoemulsion, paste, balm, magma, lotion, mousse, wax, or liposome. A kit or transdermal delivery system incorporating the transdermal formulation may be in the form of, for example, a patch, a swab, a nebulizer, a sprayer, a sponge or a pouch.

Transdermal Formulation

An aspect of this disclosure provides a transdermal formulation, which delivers a therapeutically effective amount of an agent transdermally. The formulation generally includes

(a) an effective amount of an active agent such as an sirtuin-1 (SIRT1) activating agent, an NO booster and/or an NO precursor, wherein the NO booster increases systemic production of NO, and wherein the NO precursor comprises an NO releasing agent or derives a NO releasing agent,

(b) a solvent in an amount sufficient to dissolve the effective amount of the NO booster or the NO precursor; and optionally

(c) a fatty acid,

wherein upon administration, the effective amount of active agent (e.g. the sirtuin-1 (SIRT1) activating agent), the NO booster and/or the NO releasing agent) is delivered transdermally. In some embodiments, the active agent is delivered into blood vessels for the active agent to enter systemic circulation from arteries or veins. In some embodiments, the active agent is delivered into deep layers of the skin (e.g. the upper epidermal layers or the lower epidermal layers below the stratum corneum).

The level of NO in a subject can be measured using known technology as disclosed in for example, U.S. Pat. Nos. 9,044,182 and 8,425,428. In some embodiments, an effective amount is an amount sufficient to have a measurable effect on a disease including for example hypertension, inflammation, osteoarthritis, endothelial dysfunction, dermatological condition, ophthalmological condition, bacterial infection, viral infection, ischemia reperfusion injury, hypoxia reoxygenation injury, cytokine storm phenomena, cerebral malaria, Chagas disease, and hemoglobinopathies such as Sickle Cell Disease and HbE/betaThalassemia, type 2 diabetes, and Lupus. In some embodiments, an effective amount is an amount sufficient to have a measurable positive effect on blood flow and/or vasodilation, and/or a measurable negative effect on blood pressure. In some embodiments, the effect on blood flow and/or vasodilation is observed local to the site of topical application. In some embodiments, an effective amount is an amount sufficient to have a measurable effect on an inflammatory disease, e.g., an inflammatory dermatosis, inflammatory bowel disease and inflammation of the general vasculature including the blood brain barrier due to chemotherapy as evidenced by an appropriate clinical parameter, e.g., an improvement in Physician's Global Assessment after treatment with the formulation. In some embodiments, an effective amount is an amount sufficient to obtain a systemic or local level of nitric oxide to have a desired effect, e.g., have a measurable positive effect on blood flow and/or vasodilation, have a measurable negative effect on blood pressure, and/or have a measurable effect on an inflammatory dermatosis disease, e.g., an inflammatory dermatosis as evidenced by an appropriate clinical parameter.

Non-limiting examples of the SIRT1 activating agent the active agent include polyphenol, flavonoid, stilbenoid, secosteroid, and other phytochemicals or natural products which promote formation of NO.

After the NO booster or NO precursor is delivered transdermally, it leads to generation of NO in the body of a subject. The NO booster or NO precursor is in an amount effective to increase NO systemically or locally to a level high enough to achieve the objective of treating a disease or condition. In some embodiments, the NO booster comprises polyphenol, flavonoid, stilbenoid, secosteroid, or natural products that promote NO production. In some embodiments, the NO precursor comprises a S-nitrosothiol-containing molecule, or a thiol-containing molecule and a nitrite source. In some embodiments, the NO booster contains one or more of curcuminoids, flavonoids, berberine, resveratrol, vitamin D source and their pharmaceutically acceptable salts and derivatives. Curcuminoids are linear diarylheptanoids and include for example curcumin, demethoxycurcumin, and bisdemethoxycurcumin. Flavonoids have a 3-hydroxyflavone backbone and include for example 3-hydroxyflavone, azaleatin, fisetin, galangin, gossypetin, kaempferide, kaempferol, isorhamnetin, morin, myricetin, natsudaidain, pachypodol, quercetin, rhamnazin, and rhamnetin. Nonlimiting examples of vitamin D source includes vitamin D2 and vitamin D3, and any precursors to vitamin D. Nonlimiting examples of polyphenols including plant extracts, brazilin, and theaflavins (e.g. theaflavin (TF-1), theaflavin-3-gallate (TF-2a), theaflavin-3'-gallate (TF-2b), and theaflavin-3, 3'-digallate (TF-3)).

In some embodiments, the NO booster consists essentially of curcumin, demethoxycurcumin, bisdemethoxycurcumin, quercetin, berberine, resveratrol, vitamin D source and any combination thereof.

In some embodiments, the formulation contains both a NO booster and a NO precursor. For instance, the combination of curcumin and an NO releasing agent (e.g. S-nitrosothiol-containing molecule or thiol-containing agent) in the polyol/fatty acid system can be a potent formulation for treating localized inflammation and infections and at the same time provide the systemic benefits of curcumin to control systemic inflammation.

Additional examples of curcuminoids include methylcurcumin, demethoxy curcumin, bisdemethoxycurcumin, sodium curcuminate, dibenzoylmethane, acetylcurcumin, feruloyl methane, tetrahydrocurcumin, 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione (curcumin1), 1,7-bis(piperonyl)-1,6-heptadiene-3,5-dione (piperonyl curcumin) 1,7-bis(2-hydroxy naphthyl)-1,6-heptadiene-2,5-dione (2-hydroxyl naphthyl curcumin) and 1,1-bis(phenyl)-1,3,8,10 undecatetraene-5,7-dione. In some embodiments the NO booster is curcumin, or a synthetic curcumin that is 80%, 85%, 90% or 98% pure diferuloylmethane.

In some embodiments, the transdermal formulation includes, as an active ingredient for treating a disease or condition, one or more curcuminoids and optionally one or more of polyphenol, flavonoid, stilbenoid, secosteroid, or natural products that promote NO production. In some embodiments, the transdermal formulation includes one or both of curcumin and quercetin, and optionally one or more of one or more of polyphenol, flavonoid, stilbenoid, and secosteroid.

The NO precursor is either S-nitrosothiol-containing molecule or a mixture containing a thiol-containing molecule and a nitrite source. When in contact with an acid source, the nitrite source gives rise to nitrous acid, which can then nitrosate the reactive thiol of the thiol-containing molecule. The S-nitrosothiol-containing molecule releases NO to the subject in need thereof.

Various thiol-containing molecules can be used as a precursor. Examples include glutathione, N-acetyl cysteine

(NAC), N-acetylpenicillamine, cysteine, and their derivatives. The amino group of either cysteine or NAC can be acetylated with acetyl or other carbonyl of different carbon length (e.g. COC_{2-30} alkyl). By adjusting the length of the carbon chain, the solubility and lipophilicity of the molecule can be modified. Similarly, the carboxylic group of cysteine can be converted into an ester (e.g., ethyl ester, or other substituted or unsubstituted C_{3-30} alkyl ester) or an amide having NR_2 moiety (wherein each R is independently H or other substituted or unsubstituted C_{3-30} alkyl). Variation of the carbon chain allows for modulation of the properties of the molecule.

Various inorganic compounds can serve as a nitrite source. Nonlimiting examples of the nitrite source include an alkali metal nitrite, an alkaline earth metal nitrite, a transition metal nitrite and an ammonium nitrite. In some embodiments, the nitrite source is potassium nitrite, sodium nitrite, rubidium nitrite, strontium nitrite, barium nitrite, calcium nitrite, copper nitrite, zinc nitrite, or mixtures thereof. The nitrite can also comprise a natural source such as extracts of lettuce and spinach. In some embodiments, the nitrite source is saturated in the polyol solvent. The nitrite source can also be comprised of nitrite loaded nanoparticles.

Nitrite loaded nanoparticles can be prepared by techniques known in the art including for example procedures report in U.S. Pat. No. 8,333,997, the entire disclosure of which is hereby incorporated by reference. To limit NO release from the nanoparticles during production, the medium should maintain a pH above 7.5 or so throughout the preparation. The nitrite loaded nanoparticles can then be mixed with solvent system of polyol and fatty acid (e.g. PEG400 and myristic acid) and remain stable until exposed to an aqueous environment. In the presence of thiol containing molecules, the nitrite loaded nanoparticles will allow for the formation of S-nitrosothiols when the mixture is exposed to an acid source or slightly acid aqueous environment on the skin.

The use of the nitrite loaded nanoparticles allows for the use of high concentrations of nitrite under hydrophobic conditions. The combination of the nitrite loaded nanoparticles with the solvent system of polyol and fatty acid (e.g. PEG400 and myristic acid) regardless of the other included deliverables, allows for a stable mixture that will both release NO and S-nitrosate thiols when exposed to an aqueous environment. There is no release of nitrite or production of NO in the viscous solvent until water or an acid source is introduced.

The acid source may be separately packed from the mixture containing a thiol-containing molecule and a nitrite source and mixed with the nitrite prior to administration. For instance, the nitrite source and the acid source can be separately enclosed in permeable or frangible pouches. The amount and concentration of the acid can be adjusted depending on the amounts of other agents and the nature of the acid. Nonlimiting examples of the acid include acetic acid, oxalic acid, and citric acid.

Nonlimiting examples of S-nitrosothiol-containing molecules include S-nitroso-Glutathione (GSNO), S-nitroso-N-acetylcysteine (SNAC), S-Nitroso-N-acetylpenicillamine (SNAP), and S-nitroso-human serum albumin (SNO-HAS). Similar to a thiol-containing molecule, these S-nitrosothiol-containing molecules can be modified by varying the carbon chain in the respective ester, amide, or N-acyl moiety to fine tune their properties.

A solvent such as a polyol suitable for the delivery system allows for high concentrations of poorly soluble agents. In addition, it should be biocompatible with a profile as safe for biomedical applications. Moreover, it will ideally facilitate skin and mucosal permeation to allow for transdermal delivery. Nonlimiting examples of polyols include polyethylene glycol, polypropylene glycol, ethylene glycol, propylene glycol, and glycerol. In some embodiments, the polyol is polyethylene glycol (PEG). In some embodiments, the PEG has a molecular weight ranging from about 100 to about 2000, from about 100 to about 1000, from about 100 to about 800, from about 100 to about 600, from about 200 to about 600, or from about 200 to about 400 daltons. In some embodiments, the formulation is substantially free from water.

In some embodiments, the solvent of the formulation consists essentially of the polyol. In some embodiments, the formulation may include one or more additional solvents. Nonlimiting examples include mineral oil, petrolatum, castor oil, essential oils such as eugenol, menthol, cineole, or rose oil, n-methyl pyrrolidone, vegetable oils, oleyl alcohol, dipropylene glycol, polyoxyethylene derivative of sorbitan esters, saturated polyglycolyzed C_{8-10} glycerides, polyoxyethylated fatty acid glycerides, oleic acid, dimethylsulfoxide (DMSO), fatty alcohol, isopropyl myristate (IPM), triacetin, ethyl oleate, isostearic acid, medium chain fatty acid and other fatty acids, and mixtures thereof. In addition to dissolving the agent, these solvents can also serve as plasticizer so that the formulation can be flexible, stretchable, moldable and/or otherwise skin friendly.

In some embodiments, the polyol solvent is low molecular weight polyethylene glycol (PEG) with a molecular weight ranging from about 50 to about 2000, from about 50 to about 1000, from about 100 to about 1000, from about 100 to about 800, from about 100 to about 700, from about 100 to about 600, from about 200 to about 800, from about 200 to about 600, or from about 200 to about 400 daltons. Nonlimiting examples of the molecular weight of the polyol solvent include about 100, include about 200, include about 300, include about 400, include about 500, include about 600, include about 800, and include about 1000. Short chain PEG molecules such as PEG200 and PEG400 that are liquid at ambient temperatures are particularly useful.

The fatty acid serves as a permeation enhancer. Nonlimiting examples of fatty acids include myristoleic acid, palmitoleic acid, sapienic acid, oleic acid, elaidic acid, vaccenic acid, linoleic acid, linoelaidic acid, α-linolenic acid, arachidonic acid, eicosapentaenoic acid, erucic acid, docosahexaenoic acid, caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, stearic acid, arachidic acid, behenic acid, lignoceric acid, cerotic acid, myristic acid and any combinations thereof. In some embodiments, the fatty acid is myristic acid. Mid-sized fatty acids such myristic acid and/or other fatty acids of comparable size/molecular weight are particularly useful. In some embodiments, the formulation does not contain fatty acid or contains only a trace or an insignificant amount of fatty acid.

In some embodiments, the permeation enhancer consists essentially of a fatty acid or ester thereof. In some embodiments, the formulation may contain one or more additional permeation enhancers. Non-limiting examples include surfactants, alcohols, fatty alcohols and glycol, esters, fatty acid esters and fatty alcohol esters, esters of long chain fatty acids with methyl, ethyl, isopropyl alcohols, esters of fatty alcohols with acetic acid, lactic acid as well as oleic acid, diethanolamine, essential oils, terpene and terpenoids, amides, urea, polyoxyethylene fatty alcohol ethers, polyoxyethylene fatty acid esters, sulfoxide, ether alcohol, pyrrolidones, transcarbam, capsaicin derivatives, dimethylamino acid esters, peptides, iminosulfuranes, dicarboxylic

acid esters, nanocarriers, triglycerides, hydrocarbons, phospholipids either alone or in combinations thereof.

By adjusting the amount and ratio of the polyol, the fatty acid, and the NO booster or the NO precursor, the solubility of the fatty acid and the agent in the NO booster or the NO precursor as well as the physical state (e.g. liquid or gell or semisolid) of the formulation and the release profile of the active agent can be controlled. The ratio between the polyol and the fatty acid impacts the form of the solution and generally ranges from about 5:1 to about 500:1, from about 5:1 to about 100:1, from about 1:1 to about 100:1, from about 20:1 to about 100:1, from about 30:1 to about 100:1, from about 20:1 to about 80:1, from about 20:1 to about 60:1, from about 50:1 to about 10:1, or from about 30:1 to about 50:1 from about 40:1 to about 10:1, from about 40:1 to about 10:1, from about 20:1 to about 60:1, from about 20:1 to about 15:1, from about 18:1 to about 12:1 by weight. In some embodiments, the concentration of the fatty acid in the polyol ranges from about 0.01 M to about 1 M, from about 0.01 M to about 0.8 M, from about 0.01 M to about 0.6 M, from about 0.01 M to about 0.4 M, from about 0.01 M to about 0.2 M, from about 0.01 M to about 0.15 M, from about 0.01 M to about 0.1 M, from about 0.02 M to about 0.2 M, from about 0.02 M to about 0.1 M, from about 0.04 M to about 0.08 M, or from about 0.06 M to about 0.1 M. In further exemplary embodiments, the the concentration of the fatty acid in the polyol is about 0.01 M, about 0.02 M, about 0.03 M, about 0.04 M, about 0.06 M, about 0.08 M, about 0.1 M or about 0.12 M. In some embodiments, the fatty acid is saturated in the polyol. In some embodiments, the polyol is PEG. In some embodiments, the fatty acid is myristic acid.

The scope and amount of polyol and fatty acid are as described above and can be modified by one of ordinary skill in the art in view of the practical needs without undue experiments. In some embodiments, the fatty acid is selected from myristoleic acid, palmitoleic acid, sapienic acid, oleic acid, elaidic acid, vaccenic acid, linoleic acid, linoelaidic acid, α-linolenic acid, arachidonic acid, eicosapentaenoic acid, erucic acid, docosahexaenoic acid, caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, stearic acid, arachidic acid, behenic acid, lignoceric acid, cerotic acid, and any combinations thereof. In some embodiments, the polyol is selected from the group consisting of polyethylene glycol, polypropylene glycol, ethylene glycol, propylene glycol, glycerol, and combinations thereof. In some embodiments, the polyol is polyethylene glycol having a molecular weight ranging from 200 to about 600. In some embodiments, the polyol and the fatty acid are in a ratio ranging from about 5:1 to about 100:1 by weight.

The fatty acid may range from about from about 0.1% to about 30%, from about 0.5% to about 20%, from about 1% to about 15%, from about 1% to about 10%, from about 1% to about 5%, from about 2% to about 8%, or from about 4% to about 8% of the total weight of the NO booster or NO precursor, the polyol and the fatty acid (if present) or the total weight of the formulation. Nonlimiting examples of the amount of the fatty acid include about 1%, about 3%, about 5%, about 7%, about 8%, or about 10% by weight.

Water may cause aggregation and microparticle formation. In some embodiments, the transdermal formulation is anhydrous or substantially free from water. Minimizing or eliminating water from the formulation can help with maintaining uniform distribution of the fatty acid and/or the active ingredient (e.g. NO booster or NO precursor) in the polyol (e.g. PEG). In some embodiments, water in the transdermal formulation is less than 5%, less than 2%, less than 1%, less than 0.5%, less than 0.1%, or less than 0.01% by weight.

In some embodiments, the transdermal formulation includes at least one water repelling agent, also referred to as a water repellant. Examples of water repelling agents include silicones, such as cyclomethicone, dimethicone, simethicone, C_{26}-28 alkyl dimethicone, C_{26}-28 alkyl methicone, polyphenylsisquioxane, trimethylsiloxysilicate and crosspolymers of cyclopentasiloxane and dimethicone/vinyltrimethylsiloxysilicate, and blends thereof. The water repelling agent may be particularly useful in embodiments where the topical vehicle is used with a water-reactive agent, such as a nitric oxide-releasing agent whereby the nitric oxide is released in the presence of water (e.g., a diazeniumdialate or sodium nitrite). In other cases, such as when the active agent is not water sensitive, a water repelling agent may or may not be included.

Depending on the therapeutic goal of the formulation, the NO booster or NO precursor or mixtures thereof ranges from about 0.05% to about 80%, from about 0.05% to about 50%, from about 0.05% to about 35%, from about 0.05% to about 30%, from about 0.05% to about 20%, from about 0.05% to about 10%, from about 0.1% to about 20%, from about 0.1% to about 10%, from about 0.1% to about 5%, from about 0.5% to about 20%, from about 0.5% to about 10%, from about 0.5% to about 5%, from about 1% to about 20%, from about 1% to about 10%, or from about 1% to about 5% of the total weight of the NO booster or NO precursor, the polyol and the fatty acid (if present) or the total weight of the formulation. Nonlimiting examples of the amount of the NO booster or NO precursor or mixtures thereof in the formulation include about 1%, about 3%, about 5%, about 7%, about 8%, about 10%, about 12%, and about 15% by weight in the formulation. In some embodiments, the amount of the the NO booster or NO precursor or mixtures thereof in the formulation or in a dosage unit of the formulation ranges from about 0.001 mg to about 20 g, about 0.002 mg to about 20 g, about 0.004 mg to about 20 g, about 0.006 mg to about 20 g, about 0.008 mg to about 20 g, about 0.01 mg to about 20 g, from about 0.05 mg to about 20 g, from about 0.1 mg to about 20 g, from about 0.1 mg to about 5 g, from about 0.1 mg to about 2 g, from about 0.1 mg to about 1 g, from about 1 mg to about 5 g, from about 1 mg to about 1 g, from about 10 mg to about 100 mg, from about 5 mg to about 50 mg, or from about 10 mg to about 30 mg in a dosage unit of the formulation. A dosage unit can be a physically distinct package form (e.g. a capsule, a patch, a vial). A dosage unit can also be a predetermined portion of the formulation for each individual administration. For instance, a suitable amount as a dosage unit of the formulation can be taken out of a container for direct topical application or for loading onto a patch or any suitable carrier before being applied topically. The amount or size of the dosage unit can be readily adjusted depending on the intended use and the area of application. Nonlimiting examples of the amount of the NO booster or NO precursor in a dosage unit include about 0.001 mg, about 0.002 mg, about 0.004 mg, about 0.006 mg, about 0.008 mg, about 0.01 mg, about 0.02 mg, about 0.04 mg, about 0.06 mg, about 0.08 mg, about 0.1 mg, about 0.2 mg, about 0.4 mg, about 0.06 mg, about 0.08 mg, about 1 mg, about 2 mg, about 5 mg, about 10 mg, about 20 mg, about 30 mg, about 40 mg, about 50 mg, about 60 mg, about 80 mg, about 100 mg, about 200 mg, about 300 mg, about 400 mg, about 500 mg, about 600 mg, about 800 mg, about 1 g, about 2 g, about 5 g, about 10 g, about 15 g, about 20 g, about 25 g, about 30 g, about 35 g, about 40 g, about 50

g, about 60 g, about 80 g, and about 100 g. In some embodiments, the NO booster in a dosage unit ranges from about 0.01 to about 1 mg, from about 0.01 to about 0.5 mg, or from about 0.1 to about 0.5 mg. In some embodiments, the NO booster is curcuminoids, which is selected from one, two or three of curcumin, demethoxycurcumin, and bisdemethoxycurcumin. The dosage unit can be administered once, twice, three times a day or as needed. In some embodiments, the dosage unit is administered once every day, every two days, every three days, every four days, every five days, every six days, every seven days, or every ten days.

In some embodiments, the ratio between the NO booster or NO precursor and the polyol ranges from about 1:5 to about 1:100, from about 1:5 to about 1:50, from about 1:5 to about 1:30, from about 1:5 to about 1:20, from about 1:8 to about 1:15, or from about 1:10 to about 1:15 by weight. In some embodiments, the polyol is PEG. In some embodiments, the fatty acid is myristic acid. In some embodiments, the formulation is substantially free from piperine. Depending on the disease or condition to be treated and the location of administration, the ratio and amount of the PEG, fatty acid and active agent may be selected so that the resulting formulation is a liquid, a gel or other suitable form. Additional agents may be added to control the physical state of the formulation.

In some embodiments, the transdermal formulation contains PEG, myristic acid and/or other fatty acids of comparable size/molecular weight, and an NO booster or NO precursor. In some embodiments, the transdermal formulation contains PEG, myristic acid and/or other fatty acids of comparable size/molecular weight, an NO booster or NO precursor and a secondary agent. In some embodiments, the transdermal formulation contains PEG, myristic acid, and an NO booster selected from at least one of curcumin, demethoxycurcumin, bisdemethoxycurcumin, quercetin, berberine, resveratrol and vitamin D. In some embodiments, the transdermal formulation contains PEG, myristic acid, and one or more NO booster selected from at least one of curcumin, demethoxycurcumin, bisdemethoxycurcumin, quercetin, berberine, resveratrol and vitamin D, and an NO precursor or secondary agent. In some embodiments, the transdermal formulation contains PEG having a molecular weight ranging from about 200 to about 500 (e.g. PEG 200, PEG300, PEG 400, or PEG 500). The ratio between the PEG and the myristic acid (and/or other fatty acids of comparable size/molecular weight) ranges from about 5:1 to about 100:1 (e.g. 6:1, 8:1, 10:1, 12:1, 15:1, 18:1, 20:1, 25:1, 30:1, 40:1, 50:1, 60:1, or 80:1) by weight. The ratio between the PEG and the NO booster ranges from about 5:1 to about 100:1 by weight (e.g. 6:1, 8:1, 10:1, 12:1, 15:1, 18:1, 20:1, 25:1, 30:1, 40:1, 50:1, 60:1, or 80:1). In some embodiments, the formulation contains curcumin. In some embodiments, the formulation contains curcumin, demethoxycurcumin, bisdemethoxycurcumin, or any combination thereof. In some embodiments, the formulation contains vitamin D. In some embodiments, the concentration of an individual active ingredient in the formulation ranges from about 0.01 M to about 1 M, from about 0.05 M to about 0.5 M, from about 0.05 M to about 0.3 M, or from about 0.1 M to about 0.2 M. Nonlimiting examples of the concentration of an active ingredient (e.g. curcumin) in the polyol (e.g. PEG) include about 0.06 M, about 0.08 M, about 0.1 M, about 0.12 M, about 0.14 M, about 0.16 M, about 0.18 M, about 0.20 M, about 0.25 M, about 0.30 M, about 0.40 M, about 0.60 M, and about 0.80 M.

The transdermal formula has an extended shelf life with minimum amount of decomposition of the active ingredient (s). In some embodiments, the active ingredient(s) of the formulation remain stable by more than 95% or more than 99% for a period of a least 1 month, at least 3 months, at least 6 months, or at least 12 months. In some embodiments, the transdermal formulation includes one or more curcuminoids, myristic acid and PEG. The one or more curcuminoid ranges from about 2% to about 10%, from about 3% to about 8% or from about 4% to about 6% in the formulation by weight. Myristic acid ranges from about 1% to about 10%, from about 1% to about 8%, from about 2% to about 8% or from about 4% to about 6% in the formulation by weight. PEG ranges from about 60% to about 95%, from about 70% to about 90%, from about 80% to about 90% or from about 95% to about 90% in the formulation by weight. In some embodiments, the PEG is PEG400.

In some embodiments, the formulation further includes a gelling agent or thickener which keeps it in a semi-solid form or solid form. Nonlimiting examples of gelling agents or thickeners include carbomers, methyl cellulose, hydroxypropylmethyl cellulose, poloxamers, polyacrylic acid, alginate, chitosan, xanthan gum, gellan gum, xyloglucan, paraffins, silicone, petroleum jelly, cocoa butter and polyalkylene glycol of high molecular weight. Additional examples include polyethylene oxide, ammonia methacrylate, carrageenan, cellulose acetate phthalate aqueous such as CAPNF from Eastman, carboxy methyl cellulose Na, carboxy polymethylene, cellulose, cellulose acetate (microcrystalline), cellulose polymers, divinyl benzene styrene, ethyl cellulose, ethylene vinyl acetate, silicone, polyisobutylene, shellac (FMC BioPolymer), guar gum, guar rosin, cellulose derivatives such as hydroxy ethyl cellulose, hydroxy methyl cellulose, hydroxy propyl cellulose, hydroxypropyl methyl cellulose, carboxymethyl cellulose, and methyl cellulose, hypromellose phthalate (hydroxypropyl methylcellulose phthalate), methyl acrylate, microcrystalline wax, polyvinyl alcohol, polyvinyl acetate, polyvinyl acetate phthalate such as Suretic from Colorcon, PVP ethyl cellulose, polyvinylpyrrolidone (PVP), acrylate, PEG/PVP, trimethyl siloxysilicate, maleic acid/anhydride copolymersl, polacrilin, poloxamer, poly glactic acid/poly-l-lactic acid, terpene resin, locust bean gum, prolamine (Zein), acrylic copolymers, polyurethane dispersions, gelatin (both type A and type B from various sources such as pig, cattle, and fish), dextrin, starch, polyvinyl alcohol-polyethylene glycol copolymers, methacrylic acid-ethyl acrylate copolymers such as BASF's Kollicoat polymers, methacrylic acid and methacrylate based polymers such as poly(methacrylic acid) copolymers and methylmethacrylate copolymers, including Rohm and Haas' Eudragit polymers (Eudragit (E, L, NE, RL, RS, S100)), Esters of polyvinylmethylether/maleic anhydride copolymer such as Gantrez ES-425, Gantrez ES-225 available from ISP, and mixtures thereof. Nonlimiting examples of polyalkylene glycol of high molecular weight include PEG and polypropylene glycol (PPG). The polyalkylene glycol may have a molecular weight of more than 1k, more than 2k, more than 3k, more than 4k, more than 6k, more than 8k, more than 10k, more than 15k, more than 20k, more than 25k, or more than 30k Daltons. Without limiting in scope the semisolid formulation can be in the dosage form of an ointment, gel, cream, emulsion, paste, lotion or liposome.

In some embodiments, the formulation includes a combination of small and large polyalkylene glycols, which have a MW difference ranging from 500 to 5000, from 1000 to 3000, from 1000 to 2000, or from 1500 to 2000 Daltons. By

adjusting the ratio between the two or more polyalkylene glycols, viscosity and rate/extent of both skin penetration and uptake by the circulation can be controlled. For instance, the combination may include one or both of PEG and PPG each having a MW ranging from from 100 to 2000, from from 200 to 2000, from from 400 to 1000, or from from 500 to 800 Daltons. The combination may also include one or both of PEG and PPG each having a higher MW ranging from from 800 to 5000, from from 1000 to 3000, or from from 1000 to 2000 Daltons. In further exemplary embodiments, one of polyalkylene glycols has MW of 100, 200, 400, 600, or 800, and another of the polyalkylene glycols has MW of 1000, 1500, 2000, 2500, or 3000. In some embodiments, the combination include a PEG of 400 and a PEG of 2000 Daltons. In some embodiments, the ratio between the low MW polyalkylene glycol and the high MW polyalkylene glycol ranges from about 10:1 to about 1:10, from about 5:1 to about 1:5, from about 2:1 to about 1:2 by weight. Further exemplary ratios between the low MW polyalkylene glycol (e.g. PEG and/or PPG) and the high MW polyalkylene glycol (e.g. PEG and/or PPG) include 10:1, 8:1, 6:1, 4:1, 2:1, 1:1, 1:2, 1:4, 1:6, 1:8 and 1:10.

In some embodiments, the formulation does not include additional therapeutic agent other than the NO booster or NO precursor. In some embodiments, the active agent in the formulation consists essentially of the NO booster and/or NO precursor described herein. In some embodiments, the formulation may include an additional therapeutic agent including for example an antioxidant, an antibiotic, antiviral and/or antifungal agent.

The formulation may include other components including, for example, solubilizers, skin immersion enhancers, surfactants, cosolvents, thickener or viscosifying agents, preservatives, isotonizing agents, isoosmotizing agents, absorption enhancers of the agent, mucoadhesive polymers, non-mucoadhesive polymers, chelants, stabilizers, antioxidants, and mixtures thereof.

In some embodiments, the thickener is selected from one or more of carbomers, methyl cellulose, hydroxypropylmethyl cellulose, poloxamers, polyacrylic acid, alginate, chitosan, xanthan gum, gellan gum, xyloglucan, paraffins, silicone, petroleum jelly, and cocoa butter.

Nonlimiting examples of solubilizers include, but are not limited to, diethylene glycol monoethyl ether (ethoxydiglycol, commercially available as Transcutol®) and diethylene glycol monoethyl ether oleate (Soficutol®). Commercially available under the tradename of Poly™); polyethylene castor oil derivatives such as polyoxy 35 castor oil, polyoxy 40 hydrogenated castor oil; polyethylene glycol, especially low molecular weight polyethylene glycols; Polyethylene glycol derivatives such as caprylic/capric acid glycerides (commercially available as Labrasol®); alkylmethyl sulfoxides such as DMSO; pyrrolidones such as 2-pyrrolidone and N-methyl-2-pyrrolidone; and DMA. Many solubilizers can also function as absorption enhancers. A single solubilizer can be incorporated into the formulation, or a mixture of solubilizers can be incorporated into the formulation.

Nonlimiting examples of skin immersion enhancers help to facilitate the passage of therapeutic levels of active agents to pass through reasonably sized areas of unbroken skin. Suitable enhancers are well known in the art and include, for example, lower alcohols such as methanol, ethanol and 2-propanol; alkylmethyls such as dimethylsulfoxide (DMSO), decylmethylsulfoxide (C 10 MSO) and tetradecylmethylsulfoxide. sulfoxides; urea; 2-pyrrolidone, N-methyl-2-pyrrolidone and N-pyrrolidone such as -(hydroxyethyl) pyrrolidone N, N-diethyl-m-toluamide; C 2-C 6 alkane diols; dimethylformamide (DMF), N,N-dimethylacetamide (DMA) and miscellaneous solvents such as tetrahydrofurfuryl alcohol; and 1-substituted azacycloheptan-2-ones, especially 1-n-dodecylazacycloheptan-2-one (laurocapram. Available from Whitby Research Incorporated, Richmond, Va. under the tradename Azone®).

Among surfactants there may be mentioned, for example, polyethoxylated glycerides, polysorbates, poloxamers, sodium lauryl sulphate, phospholipids, such as phosphatidylcholine or phosphatidylglycerol and their derivatives, polyoxyethylenated hydrogenated castor oil, polyoxyethylenated fatty acids, mixtures of mono-, di-, and triglycerides of fatty acids optionally polyoxyethylenated, and mixtures thereof.

Among preservatives there may be mentioned, for example, benzalkonium chloride, boric acid, benzoic acid, C1-4 alkyl esters of p-hydroxybenzoic acid, chlorobutanol, benzyl alcohol, phenylethyl alcohol, organometallic derivatives of mercury, polyquaternium such as polyquaternium 1, and mixtures thereof.

Among isotonizing and isoosmotizing agents there may be mentioned, for example, inorganic salts such as sodium chloride, dextrose, trehalose, mannitol, amino acids, and mixtures thereof.

Among mucoadhesive polymers there may be mentioned, for example, hyaluronic acid, polygalacturonic acid, polyacrylic acid, carboxymethyl amylose, carboxymethyl chitin, chondroitin sulphate, methyl cellulose, gelatin, hydroxymethyl cellulose, hydroxypropyl cellulose, hydroxypropylmethyl cellulose, sodium carboxymethyl cellulose, xanthan gum, chitosan, carbopol, polycarbophil, gellan gum, carrageenan, alginates, pectin, poloxamer, and mixtures thereof. Among non-mucoadhesive polymers there may be mentioned, for example, polyvinyl alcohol. Among chelants there may be mentioned, for example, disodium edetate, and disodium cromoglycate. Among antioxidants there may be mentioned, for example, sodium metabisulfite, sodium bisulfate, acetylcysteine, ascorbic acid, and mixtures thereof.

By adjusting the amount and ratio of the polyol, the fatty acid, and the NO booster or the NO precursor, the solubility of the fatty acid and the active agent as well as the physical state of the formulation and the release profile of the active agent can be controlled. In some embodiments, the polyol, the fatty acid, the active ingredient and other necessary components are configured in a ratio such that the formulation provides a rapid onset of action within about 5 minutes, within about 10 minutes, within about 15 minutes, or within about 30 minutes.

The transdermal formulation disclosed herein can provide extended or continued release of the agent (e.g. NO booster or the S-nitrosothiol-containing molecule). In some embodiments, the formulation provides extended release of the agent (transdermal delivery into blood circulation) for a period of 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours, 10 hours, 12 hours, 15 hours, 18 hours, 24 hours, about 2 days, about 3 days, about 5 days, or about 7 days. By selecting the polyol solvent and the fatty acid in a suitable ratio, the rate of the release can also be controlled. In some embodiments, one, two or three of the following parameters can be achieved for the formulation:

(a) less than 15%, less than 20%, less than 25%, less than 30%, or less than 35% of the agent is delivered into blood circulation within about 30 minutes, within about 1 hour, about 2 hours, about 3 hours, about 4 hours, or about 5 hours;

(b) from about 25% to about 90%, from about 30% to about 85%, from about 35% to about 70%, from about 40% to about 70%, from about 50% to about 60%, from about 35% to about 50%, from about 40% to about 60% or from about 35% to about 80% of the agent is delivered into blood circulation within about 6 hours, about 8 hours, about 10 hours, about 12 hours, or about 14 hours; and

(c) more than 60%, more than 70%, or more than 80% of the agent is delivered into blood circulation in about 16 hours, about 18 hours, about 20 hours, about 22 hours, about 24 hours, about 36 hours, or about 48 hours.

In some embodiments, the active agent and the carriers (e.g. polyol or fatty acid) and their amounts in the formulation are selected so that a window of therapeutic effect is maintained for about 30 minutes, about 1 hour, about 2 hours, about 4 hours, about 6 hours, about 8 hours, about 10 hours, about 12 hours, about 14 hours, about 24 hours, about 2 days, about 3 days, about 5 days, or about 7 days, wherein the plasma concentration of the active agent varies by less than 5%, less than 10%, less than 15%, less than 20%, less than 25%, less than 30% or less than 40% during such a window. In some embodiments, the window starts within about 10 minutes, within about 20 minutes, within about 30 minutes, within about 1 hour, or within about 2 hours after the formulation is administered.

The formulation may include a secondary agent. Examples of secondary agents include hypertension agents, antimicrobial agents, anti-inflammatory agents, analgesic agents, anesthetic agents, antihistamine agents, antiseptic agents, immunosuppressants, antihemorrhagic agents, vasodilators, wound healing agents, anti-biofilm agents and mixtures thereof. Alternatively, the secondary agents can be in a separate formulation and/or is separately administered from the transdermal formulation described herein.

Examples of anti-inflammatory agents include nonsteroidal anti-inflammatory agents (NSAIDs); propionic acid derivatives such as ibuprofen and naproxen; acetic acid derivatives such as indomethacin; enolic acid derivatives such as meloxicam, acetaminophen; methyl salicylate; monoglycol salicylate; aspirin; mefenamic acid; flufenamic acid; indomethacin; diclofenac; alclofenac; diclofenac sodium; ibuprofen; ketoprofen; naproxen; pranoprofen; fenoprofen; sulindac; fenclofenac; clidanac; flurbiprofen; fentiazac; bufexamac; piroxicam; phenylbutazone; oxyphenbutazone; clofezone; pentazocine; mepirizole; tiaramide hydrochloride; steroids such as clobetasol propionate, bethamethasone dipropionate, halbetasol proprionate, diflorasone diacetate, fluocinonide, halcinonide, amcinonide, desoximetasone, triamcinolone acetonide, mometasone furoate, fluticasone proprionate, betamethasone diproprionate, triamcinolone acetonide, fluticasone propionate, desonide, fluocinolone acetonide, hydrocortisone vlaerate, prednicarbate, triamcinolone acetonide, fluocinolone acetonide, hydrocortisone and others known in the art, predonisolone, dexamethasone, fluocinolone acetonide, hydrocortisone acetate, predonisolone acetate, methylpredonisolone, dexamethasone acetate, betamethasone, betamethasone valerate, flumetasone, fluorometholone, beclomethasone diproprionate, fluocinonide, topical corticosteroids, and may be one of the lower potency corticosteroids such as hydrocortisone, hydrocortisone-21-monoesters (e.g., hydrocortisone-21-acetate, hydrocortisone-21-butyrate, hydrocortisone-21-propionate, hydrocortisone-21-valerate, etc.), hydrocortisone-17,21-diesters (e.g., hydrocortisone-17,21-diacetate, hydrocortisone-17-acetate-21-butyrate, hydrocortisone-17,21-dibutyrate, etc.), alclometasone, dexamethasone, flumethasone, prednisolone, or methylprednisolone, or may be a higher potency corticosteroid such as clobetasol propionate, betamethasone benzoate, betamethasone dipropionate, diflorasone diacetate, fluocinonide, mometasone furoate, triamcinolone acetonide.

In some embodiments, the formulation contains an anti-viral agent such as acyclovir, trifluridine, idoxuridine, penciclovir, famciclovir, cidofovir, gancyclovir, valacyclovir, podofilox, podophyllotoxin, ribavirin, abacavir, delavirdine, didanosine, efavirenz, lamivudine, nevirapine, stavudine, zalcitabine, zidovudine, amprenavir, indinavir, nelfinavir, ritonavir, saquinavir, amantadine, interferon, oseltamivir, ribavirin, rimantadine, zanamivir, and combinations thereof. Anti-viral treatment may be used to treat both localized and systemic viral infections, such as Covid-19, cold sores or genital herpes.

Examples of antimicrobial agents include penicillins and related drugs, carbapenems, cephalosporins and related drugs, erythromycin, aminoglycosides, bacitracin, gramicidin, mupirocin, chloramphenicol, thiamphenicol, fusidate sodium, lincomycin, clindamycin, macrolides, novobiocin, polymyxins, rifamycins, spectinomysin, tetracyclines, vanomycin, teicoplanin, streptogramins, anti-folate agents including sulfonamides, trimethoprim and its combinations and pyrimethamine, synthetic antibacterials including nitrofurans, methenamine mandelate and methenamine hippurate, nitroimidazoles, quinolones, fluoroquinolones, isoniazid, ethambutol, pyrazinamide, para-aminosalicylic acid (PAS), cycloserine, capreomycin, ethionamide, prothionamide, thiacetazone, viomycin, eveminomycin, glycopeptide, glyclyclycline, ketolides, oxazolidinone; imipenen, amikacin, netilmicin, fosfomycin, gentamycin, ceftriaxone, Ziracin, Linezolid, Synercid, Aztreonam, and Metronidazole, Epiroprim, Sanfetrinem sodium, Biapenem, Dynemicin, Cefluprenam, Cefoselis, Sanfetrinem celexetil, Cefpirome, Mersacidin, Rifalazil, Kosan, Lenapenem, Veneprim, Sulopenem, ritipenam acoxyl, Cyclothialidine, micacocidin A, carumonam, Cefozopran and Cefetamet pivoxil.

Examples of antihistamine agents include diphenhydramine hydrochloride, diphenhydramine salicylate, diphenhydramine, chlorpheniramine hydrochloride, chlorpheniramine maleate isothipendyl hydrochloride, tripelennamine hydrochloride, promethazine hydrochloride, methdilazine hydrochloride, and the like. Examples of local anesthetic agents include dibucaine hydrochloride, dibucaine, lidocaine hydrochloride, lidocaine, benzocaine, p-butylaminobenzoic acid 2-(die-ethylamino) ethyl ester hydrochloride, procaine hydrochloride, tetracaine, tetracaine hydrochloride, chloroprocaine hydrochloride, oxyprocaine hydrochloride, mepivacaine, cocaine hydrochloride, piperocaine hydrochloride, dyclonine and dyclonine hydrochloride.

Examples of antiseptic agents include alcohols, quaternary ammonium compounds, boric acid, chlorhexidine and chlorhexidine derivatives, iodine, phenols, terpenes, bactericides, disinfectants including thimerosal, phenol, thymol, benzalkonium chloride, benzethonium chloride, chlorhexidine, povidone iode, cetylpyridinium chloride, eugenol and trimethylammonium bromide.

Examples of analgesic agents include alfentanil, benzocaine, buprenorphine, butorphanol, butamben, capsaicin, clonidine, codeine, dibucaine, enkephalin, fentanyl, hydrocodone, hydromorphone, indomethacin, lidocaine, levor-

phanol, meperidine, methadone, morphine, nicomorphine, opium, oxybuprocaine, oxycodone, oxymorphone, pentazocine, pramoxine, proparacaine, propoxyphene, proxymetacaine, sufentanil, tetracaine and tramadol.

Examples of anesthetic agents include alcohols such as phenol; benzyl benzoate; calamine; chloroxylenol; dyclonine; ketamine; menthol; pramoxine; resorcinol; troclosan; procaine drugs such as benzocaine, bupivacaine, chloroprocaine; cinchocaine; cocaine; dexivacaine; diamocaine; dibucaine; etidocaine; hexylcaine; levobupivacaine; lidocaine; mepivacaine; oxethazaine; prilocaine; procaine; proparacaine; propoxycaine; pyrrocaine; risocaine; rodocaine; ropivacaine; tetracaine; and derivatives, such as pharmaceutically acceptable salts and esters including bupivacaine HCl, chloroprocaine HCl, diamocaine cyclamate, dibucaine HCl, dyclonine HCl, etidocaine HCl, levobupivacaine HCl, lidocaine HCl, mepivacaine HCl, pramoxine HCl, prilocaine HCl, procaine HCl, proparacaine HCl, propoxycaine HCl, ropivacaine HCl, and tetracaine HCl.

Examples of antihemorrhagic agents include thrombin, phytonadione, protamine sulfate, aminocaproic acid, tranexamic acid, carbazochrome, carbaxochrome sodium sulfanate, rutin and hesperidin. Additional examples of secondary or additional agents include chemotherapeutics (tyrosine kinase inhibitor, immune checkpoint inhibitor, VEGF inhibitor, etc) and glucose lowering medications (e.g. Metformin).

Additional examples of secondary agents include daunorubicin, daunomycin, dactinomycin, doxorubicin, epirubicin, idarubicin, esorubicin, bleomycin, maphosphamide, ifosfamide, cytosine arabinoside, bischloroethyl-nitrosourea, Busulfan, mitomycin C, actinomycin D, mithramycin, prednisone, hydroxypregesterone, testosterone, tamoxifen, dacarbacine, procarbazine, hexamethylmelamine, pentamethylmelamine, mitoxantrone, amsacrine, chlorambucil, methylcyclohexylnitrosourea, nitrogen mustard, Melphalan, cyclophosphamide, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-azacytidine, hydroxyurea, deoxycoformycin, 4-hydroxyperoxycyclo-phosphoramide, 5-fluorouracil (5-FU), 5-Fluorodeoxyuridine (5-FUdR), methotrexate (MTX), colchicine, taxol, vincristine, vinblastine, etoposide (VP-16), trimetrexate, irinotecan, topotecan, gemcitabine, teniposide, cisplatin and diethylstilbestrol. In some embodiments, secondary agents that can be used in combination with the formulation disclosed herein or incorporated into the same transdermal formulation include anti-inflammatory agents, analgesics, antibacterial agents, antifungal agents, antibiotics, vitamins, and antioxidants. In some embodiments, the secondary agent is selected from piperine, anthranilic acid, benzophenone, camphor derivatives, cinnamic acid esters (for example, octyl methoxycinnamate), dibenzoylmethane (for example, butylmethoxydibenzoylmethane), p-aminobenzoic acid (PABA) and its derivatives, salicylic acid esters, and PDES inhibitors (e.g. sildenafil (Viagra), tadalafil (Cialis), vardenafil (Levitra), and avanafil (Stendra)).

Any of the secondary agents described herein can be incorporated in the same transdermal formulation. Alternatively, in some embodiments of any method disclosed herein, a secondary agent can be administered separately from the transdermal formulation via any suitable route including oral, transdermal and parenteral routes.

In some embodiments, a combination of active agents in the transdermal formulation produces a synergistic therapeutic effect. For example, curcumin and a PDES inhibitor, when incorporated in the same transdermal formulation or administered sequentially in conjunction with each other can lead to early symptomatic recovery (fever, cough, sore throat, and breathlessness), less deterioration, fewer red flag signs in patients with viral infections (e.g. COVID-19).

Transdermal Delivery System

A kit or a transdermal delivery system may contain, in an amount sufficient for at least one agent, any combination of the components described herein, and may further include instructions recorded in a tangible form for use of the components. In some applications, one or more components may be provided in pre-measured single use amounts in individual, typically disposable, patches, tubes or equivalent containers.

The formulation disclosed herein can be incorporated into a transdermal delivery system or kit and used as a patch, swab, aerosol, cream, sponge, sprayer, nebulizer or via other suitable means. The transdermal delivery system may also include an instruction manual on the administration of the formulation and one or more of the treatment methods disclosed in this patent document. A liquid or semisolid formulation can be directly applied to the skin for example with a swab or a sponge. Alternatively, a transdermal delivery system may include a layer coated or impregnated with the liquid, semisolid, or solid transdermal formulation. For instance, a patch may have a layer impregnated with the liquid formulation or coated with a semisolid or solid formulation. A transdermal delivery system may also include an adhesive member for attaching it to the skin.

The transdermal delivery system or kit can be in any suitable shape for applying to a subject in need thereof. For example, a sponge loaded with the formulation disclosed herein can be shaped as a circular, cylindrical, cone, planar, tubular, and other symmetrical or asymmetrical shape) for inserting in to a body cavity or attaching or applying to a target location, and may include an applicator or applicator portion. The sponge can be made from a material which absorbs liquid through capillary action. Alternatively, the material may be hydrophilic or hygroscopic or coated with a hydrophilic or hygroscopic layer that exhibits affinity for aqueous solution, especially water moisture for example from the site or body cavity where the reservoir is placed. A sponge with absorbent characteristics can be made from natural or synthetic materials, which include for example polyester, polyurethane, and vegetal cellulose.

In some embodiments, the formulation is incorporated into a liquid reservoir. The reservoir can be used independently, or it can be attached to or enclosed partially or completely in a sponge. Alternatively, the content of the liquid reservoir, after necessary processing or mixing with an additional agent, can be loaded to a sponge for application. In formulations involving a nitrite source that requires an acid to generate nitrous acid and NO, the acid can be added to the reservoir containing the nitrite prior to administration. Alternatively, a dual liquid reservoir system can be employed. For instance, a pouch contains thiol-containing molecule and nitrite in polyol solvent system and a separate pouch contains the acid source. Of course, additional pouches can be used to enclose the thiol-containing molecule or nitrite or fatty acid separately. Prior to administration or upon contact with the skin, the contents of the pouches are mixed to initiate the reaction between the acid and nitrite and subsequent nitration of the thiol-containing molecule. The pouches for enclosing the NO precursor or the acid source are generally frangible or permeable containers, which do not contact each other or are separated by a non-permeable and removable barrier prior to administration of the formulation. Upon administration, the acid and the NO precursor can permeate out of their respective

pouches to mix with each other under pressing from the user after removal of the barrier. The acid and the NO precursor can also be mixed simply by breaking the pouches upon or prior to administration. In a further exemplary embodiment, the acid and the NO precursor are mixed in a container prior to administration. The resulting mixture is soaked up with a swab, a sponge, or an absorbing patch and then applied to the skin.

In some embodiments, the pouch has a permeable or semi-permeable membrane surface that is optionally coated with an adhesive for affixing the membrane to the skin. Instead of adhesive coating, the pouch can also be affixed to the skin by holding the pouch to the skin and then covering the pouch with an adhesive patch or enclosing sheet. Commercially available pouchstock material such as DuPont's SURLYN® can be also used for liquid reservoir. Additional examples include coextruded ethylene acrylic acid/low-density polyethylene (EAA/LDPE) material, or BAREX® from INEOS (acrylonitrile-methyl acrylate).

In some embodiments where the NO precursor is a mixture of thio-containing molecule and nitrite, the formulation can be incorporated into a patch. A layer of the patch is impregnated with the the NO precursor in the polyol and fatty acid solvent system, whereas the acid source is disposed in a separate layer. The two layers do not come into contact with each other until attachment of the patch to skin or before administration. By applying pressure to the patch, contents of the different layers can be mixed. Alternatively, the patch includes a non-permeable barrier between the two layers and removal of the barrier before administration allows for mixing the nitrite and acid.

In some embodiments, the formulation is a solid which contains a thickener or solidifying material such as cocoa butter. In some embodiments, the formulation is a solid or semisolid which contains petroleum jelly. The solid or semisolid formulation can be applied to the skin or melt when rubbed with pressure on to the skin.

In some embodiments, the formulation is filled into a nebulizer or sprayer, which delivers the agent in aerosol form to the nose, mouth, or lung of a subject in need. Carbon dioxide or other suitable gas can be used as a propellant.

The systems or kits can comprise any number of additional reagents or substances that are useful for practicing a method of the invention. The kits or systems of the invention can be provided at any temperature. For example, for storage of kits including certain S-nitrosothiol-containing molecules in a liquid or gel, they may be provided and maintained at a suitable temperature, or around 0° C.

The kits or systems can also include instruction manuals and packaging materials for holding the container or combination of containers. Instructions, such as written directions or videotaped demonstrations detailing the use of the transdermal formulation for treating target diseases and conditions, can be included with the kit or systems. Typical packaging materials for such kits and systems include solid matrices (e.g., glass, plastic, paper, foil, and the like) that hold the components in any of a variety of configurations (e.g., in a pouch, tube, and the like).

Such kits or systems may also include information, such as scientific literature references, package insert materials, clinical trial results, and/or summaries of these and the like, which indicate or establish the activities and/or advantages of the composition, and/or which describe dosing, administration, side effects, drug interactions, or other information useful to the health care provider. Such information may be based on the results of various studies, for example, studies using experimental animals involving in vivo models and studies based on human clinical trials. Kits or systems described herein can be provided, marketed and/or promoted to health providers, including physicians, nurses, pharmacists, formulary officials, and the like. Kits may also, in some embodiments, be marketed directly to the consumer.

Method of Use

Because the transdermal formulation introduces high levels of curcuminoids and/or other potent anti-inflammatory and/or anti-oxidant agents locally and/or systemically, it provides a rapid intervention for various diseases and conditions.

The transdermal formulation can be administered in any suitable route to deliver the active ingredient across the skin, mucosa, or membrane of a subject's body. Nonlimiting examples of suitable routes include for example topical (e.g. instillation and mucosal path including vaginal and rectal delivery), pulmonary (e.g. by inhalation or insufflation of powders or aerosols including by nebulizer), intratracheal, intranasal, and epithelial route. In some embodiments, the transdermal formulation includes one or both of curcumin and quercetin, and optionally one or more of one or more of polyphenol, flavonoid, stilbenoid, and secosteroid.

Without being limited to any particular theory, it is postulated that the transdermal formulation disclosed herein delivers an active agent transdermally to a subject and enhances the systemic or local NO level in the subject. In some embodiments, the formulation contains an effective amount of an NO booster to increase systemic or local NO level in the subject. In some embodiments, the formulation contains an effective amount of an NO precursor, wherein the method converts the NO precursor to for example the S-nitrosothiol-containing molecule, which releases NO transdermally to the subject. In some embodiments, the formulation may contain both an NO booster and an NO precursor. In some embodiments the formulation may also contain a secondary agent as defined above.

The transdermal formulation achieves NO enhancement through pathways including up-regulation of endothelial nitric oxide synthase (eNOS), enhancement of the activity of eNOS, and reduction of the levels of ROS. For example, ROS scavenges NO, causes eNOS decoupling resulting in the cessation of eNOS associated NO synthase and instead further production of ROS by eNOS. Meanwhile, loss of the endothelium results in: i) loss the flow mediated mechanos-transduction mechanism for controlling NO production from eNOS; and in the loss of the cell free zone next to the endothelium which prevents NO scavenging by hemoglobin in the red blood cells. The transdermal formulation of this patent document provides an effective amount of the active agent the can enhances NO level in the endothelial lining of blood vessels by inhibiting ROS scavenges NO and limiting the degradation of the glycocalyx lining of the endothelium.

The transdermal formulation can be applied to the body surface or body cavity of a subject. For example, the method of enhancing systemic or local NO level or treating a disease or condition may involve inserting, between cheek and gum, a sponge loaded with the formulation disclosed herein.

Various diseases or conditions can be treated with the transdermal formulation disclosed herein. Nonlimiting examples of the diseases and conditions include hypertension, inflammation, endothelial dysfunction, dermatological conditions, ophthalmological conditions, bacterial infection, viral infection, ischemia reperfusion injury, hypoxia reoxygenation injury, cytokine storm phenomena, sickle cell disease, inflammatory consequences of an acute sickle cell crisis and other hemoglobinopathies including HbE/betaThalassemia, Chagas disease, type 2 diabetes, Lupus, and

transient inflammatory conditions including "brain fog" due to chemotherapy and leaky gut syndrome.

In further exemplary embodiments, the transdermal formulation and system can be used as a transdermal therapy for preventing, managing and reversing the clinical consequences of inflammatory diseases including diabetes, COVID-19 infection and sickle cell disease, providing topical treatment of hypertension or topical treatment of osteoarthritis, reversing acute inflammatory cascades (cytokine storm), improving the safety and efficacy of transfused stored red blood cells, treating cerebral malaria or Chagas disease, or treating other early stage acute inflammatory diseases.

Phytochemicals (e.g. curcuminoids) have been shown to have antiviral activity. For instance, it has recently been shown that the glycocalyx can prevent access of viruses to the ACE2 binding receptor on endothelial cells thus limiting uptake and replication. Underlying endothelial dysfunction degrades the glycocalyx thus increasing access of the virus to the ACE2 binding site. Curcumin and many of these other phytochemicals protect and preserve the glycocalyx by reducing ROS production and enhancing endothelial NO production (vide infra). These phytochemicals also reduce the pro-inflammatory insults due to diet and obesity by normalizing lipid and glucose metabolism including insulin production and utilization. For treating toxic chemical and metal triggered inflammation, curcumin and other phytochemicals can chelate and eliminate the toxics from the blood. It also limits the inflammatory response to inhaled toxic agents thus lowering the propensity for the progression towards ARDS. The phytochemicals stabilize red blood cells thus minimizing toxic agent-induced hemolysis which is a potent trigger of inflammation.

The formulations disclosed herein are capable of addressing pro-inflammatory insults including acute inflammatory insults triggered by certain viral infection (e.g. SARS CoV2, Dengue fever, and influenza), obesity and glucose induced inflammatory triggers, and inflammation triggered by exposure to toxic metals and chemicals. For example, in patients with long COVID, side effects attributed to COVID that become manifest well after the seeming recovery from the primary infection include brain fog, fatigue, achiness, clotting issues, myocarditis, edema and more. Most of these long COVID symptoms can be attributed to a continued imbalance between pro-inflammatory and anti-inflammatory factors that favor development of and persistence of endothelial dysfunction. The formulations and methods disclosed herein can be applied to the treatment of these clinical manifestations of long COVID.

In some embodiments of the treatment methods, the transdermal formulation disclosed herein is applied to treating diseases or conditions commonly associated with a "cytokine storm" including but not limited to: COVID-19 infection, sepsis, systemic inflammatory response syndrome (SIRS), cachexia, septic shock syndrome, traumatic brain injury (e.g., cerebral cytokine storm), graft versus host disease (GVHD), or the result of treatment with activated immune cells, e.g., IL-2 activated T cells, T cells activated with anti-CD19 Chimeric Antigen Receptor (CAR) T cells. Besides the impact on endothelial function, active agents such as curcumin at sufficient concentrations acts to efficiently block the binding of the spike protein on SARS CoV 2 to the ACE2 binding site on endothelial cells and pulmonary epithelial cells thus inhibiting viral replication in vulnerable subjects.

In some embodiments of the treatment methods disclosed herein, the transdermal formulation is administered to treat vascular leakage caused by a disease or condition. Nonlimiting exemplary diseases or conditions include vascular leak syndrome, infectious disease, inflammatory diseases, inter alia, sepsis, lupus, irritable bowel disease, inflammatory bowel disease and inflammation of the general vasculature including the blood brain barrier due to chemotherapy. Vascular leakage is characterized by hypotension, peripheral edema, and hypoalbuminemia. Vascular leakage can also be associated with diseases due to pathogens, inter alia, viruses and bacteria.

In some embodiments of the treatment methods disclosed herein, the transdermal formulation is administered to treat or reduce the risk of a cardiovascular disease associated with endothelial dysfunction. Endothelial cells are important constituents of blood vessels that play critical roles in cardiovascular homeostasis by regulating blood fluidity and fibrinolysis, vascular tone, angiogenesis, monocyte/leukocyte adhesion, and platelet aggregation. The occurrence of endothelial dysfunction disrupts the endothelial barrier permeability that is a part of inflammatory response in the development of cardiovascular diseases. Nonlimiting examples of cardiovascular diseases include coronary artery diseases (CAD) such as angina and myocardial infarction (commonly known as a heart attack), stroke, heart failure, hypertensive heart disease, rheumatic heart disease, cardiomyopathy, abnormal heart rhythms, congenital heart disease, valvular heart disease, carditis, aortic aneurysms, peripheral artery disease, thromboembolic disease, and venous thrombosis.

In some embodiments, the amount/dosage of the active agent(s) are selected and/or the administration schedule are configures so that the method increases or decreases the level of a biomarker associated with a cardiovascular disease in the subject by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, or at least about 60% in comparison with a control (without treatment with the transdermal formulation) or the level prior to the treatment with the transdermal formulation disclosed herein. Nonlimiting examples of the biomarkers associated with cardiovascular diseases include white blood cell count (WBC), erythrocyte sedimentation rate (ESR), serum C-reactive protein (CRP), cardiac troponin, Creatinine kinase (CK), CK-MB and myoglobin. In some embodiments, the subject prior to the treatment has an abnormal level of one or more biomarkers associated with a cardiovascular disease, wherein the abnormal level of the one or more biomarkers is higher or lower than a normal level or the level of a healthy subject by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, or at least about 60%.

Further examples of infectious diseases commonly associated with a "cytokine storm" or vascular leakage include but are not limited to, Coronaviruses (COV's including CoVid-19/(SARS-CoV-2) coronavirus infection), malaria, avian influenza, smallpox, pandemic influenza, adult respiratory distress syndrome (ARDS), severe acute respiratory syndrome (SARS). Certain specific infectious agents include but are not limited to Ebola, Marburg, Crimean-Congo hemorrhagic fever (CCHF), South American hemorrhagic fever, dengue, yellow fever, Rift Valley fever, Omsk hemorrhagic fever virus, Kyasanur Forest, Junin, Machupo, Sabia, Guanarito, Garissa, Ilesha, or Lassa fever viruses. In some embodiments, the infectious disease is caused by virus, bacteria, fungus, helminths, protozoan, or hemorrhagic infectious agents. In some embodiments, the infectious disease is caused by coronaviruses (cow's including

covid-19) arenaviridae, filoviridae, bunyaviridae, flaviviridae, or rhabdoviridae virus. In some embodiments, the transdermal formulation and the methods described herein can be applied to the treatment of septic shock syndrome, a chronic inflammatory response to the infectious disease.

The methods disclosed herein can also be applied to the treatment of various types of pain including for example, neuropathic pain, surgery related pain, trauma, periodontal or other dental procedure related pain, and orthopedic or arthritic pain. For instance, pain related with periodontal or other dental procedure can be treated with sponge inserted into a subject's mouth at a suitable location such as between cheek and gum. The transdermal formulation can be administered before or after the onset of pain. For example, the formulation can be administered to a subject prior to a surgical procedure as a prophylactic method to mitigate the pain.

Conventional medications for neuropathic pain have various levels of side effects. The transdermal formulations disclosed herein can be used alone or in combination with conventional drugs, including for example gabapentinoids, tricyclic antidepressants, and/or selective serotonin-norepinephrine reuptake inhibitors as the first-line drugs, lidocaine, capsaicin, and/or tramadol as second-line drugs, and morphine, oxycodone, botulinum toxin-A, and other opioids as third-line treatments. As a result, the transdermal formulations provide the benefit of reduced reliance on conventional pain medication and minimized side effects.

The transdermal formulations can also be applied to postoperative pain management. For individuals undergoing surgeries, including for example, bypass, and thoracic surgery, coronary, groin hernia repair, and leg amputation, the transdermal formulations can serve as alternative therapeutics to treat these aforementioned pain conditions.

As described above, the formulation can be adminstered in any suitable form for the methods disclosed herein. In some embodiments of any formulation or method disclosed herein, the formulation is in a semisolid form or solid form and is rubbed or rolled on the skin or mucosal surface of the subject. In some embodiments, a patch is coated or impregnated with the formulation in a liquid, semisolid or solid form. In some embodiments, one or more of the NO booster, NO precursor and acid source are in a liquid reservoir prior to administration. In some embodiments, the formulation is administered via a sprayer or nebulizer. In some embodiments, the subject is a human. In some embodiments, the presence of symptoms, signs, and/or risk factors of a disease or condition to be treated is determined before beginning administration of the formulation.

The transdermal formulation of this patent document can be administered to activate NAD-dependent deacetylase sirtuin-1 (SIRT1) in a subject. Accordingly, various diseases or conditions associated with dysfunctional SIRT1 can be treated. The sirtuins are a class of NAD+-dependent protein deacetylase enzymes that regulate a wide variety of cellular activities that promote cell survival and extend lifespan in response to environmental stress. Sirtuins exert their effect by removing acetyl groups from certain target proteins in the presence of oxidized nicotinamide adenine dinucleotide (NAD+). For example, the yeast sirtuin enzyme Sir2 (silent information regulator 2), originally identified for its role in silencing transcription of DNA, has also been shown to promote cell survival in response to caloric restriction. Similarly, in *C. elegans*, the sirtuin enzyme SIR-2.1 has been shown to extend lifespan. In mammalian cells, the sirtuin enzyme SIRT1 (a homolog of the yeast Sir2 and *C. elegans* SIR-2.1 enzymes) deacetylates the tumor suppressor p53 to promote cell survival. SIRT1 has been reported to regulate various pathways, including for example, restoring angiogenic function and the secretion of proangiogenic factors in endothelial progenitor cell. Seminal papers have demonstrated that SIRT1 is involved in the protection against excessive inflammation and oxidative stress by deacetylating NFκB and Forkhead box 0 transcription factors. Furthermore, SIRT1 inhibits cellular senescence, promotes keratinocyte differentiation, and protects against UV-induced DNA damage. Several studies have also demonstrated that downregulated or dysfunctional SIRT1 is associated with various diseases such as in a diabetic milieu and that SIRT1 overexpression improves glucose intolerance and insulin sensitivity and protects against diabetes. Sirtuins therefore appear to be activated as part of a beneficial cellular response to stress, resulting in cell survival and extended lifespan.

Activators of sirtuins may therefore be beneficial in effecting fundamental cellular processes that protect cells from stress and prevent or treat various diseases or conditions, and lengthen healthy life.

Transdermal delivery of NO enhancing and SIRT1 activating therapeutics allows for facile combination with oral treatments that target other relevant disease pathways not effectively addressed through the transdermally delivered agents. The method includes administering to the subject in need thereof a transdermal formulation disclosed herein. In some embodiments, the formulation includes (a) a therapeutically effective amount of a SIRT1 activating agent; (b) a polyol solvent in an amount sufficient to dissolve the SIRT1 activating agent; and (c) a fatty acid. The SIRT1 activating agent can be one or more NO boosters described above. In some embodiments, the SIRT1 activating agent includes one or more of curcuminoids, berberine, quercetin, resveratrol, and fisetin. The amount of the activating agent can be adjusted depending on the nature of the agent and the disease or condition to be treated. In some embodiments, the activating agent ranges from about 0.05% to about 40% by weight in the formulation. In some embodiments, the formulation provides continued release of the activating agent over a period of about 15 hours.

Treatment of acute and chronic diseases or other conditions can benefit from enhanced systemic nitric oxide levels in the endothelium and/or activation of the SIRT1 and NRF2 signaling pathways. Non-limiting diseases or conditions include sickle cell disease, HbE/betaThalassemia and other thalassemias, diabetic retinopathies, glaucoma, dry eye syndrome, and surgery triggered inflammation.

In some embodiments, the method enhances SIRT1 activity in a subject. The scope and composition of the formulation are as described above. In some embodiments, the formulation includes (a) a therapeutically effective amount of a SIRT1 activating agent; (b) a polyol solvent in an amount sufficient to dissolve the SIRT1 activating agent; and optionally (c) a fatty acid. The SIRT1 activating agent can be one or more NO boosters described above. In some embodiments, the SIRT1 activating agent includes one or more of curcuminoids, berberine, quercetin, resveratrol, and fisetin. The amount of the activating agent can be adjusted depending on the nature of the agent and the disease or condition to be treated. In some embodiments, the activating agent ranges from about 0.05% to about 40% by weight in the formulation. In some embodiments, the formulation provides continued release of the activating agent over a period of about 1, about 2, about 4, about 8, about 10, about 15 or about 24 hours. In some embodiments, the disease or condition is selected from aging, chronic and acute inflam-

matory condition, chemically induced vascular inflammation, viral infection, bacterial infection, and fungal infection. In some embodiments, the subject the transdermal formulation, diagnosing the subject as having endothelial dysfunction or a disease or condition associated with endothelial dysfunction. In some embodiments, the subject has been diagnosed as having a disease or condition selected from the group consisting of neurodegenerative disease, diabetic kidney disease, diabetes, cardiovascular disease, endothelial dysfunction, muscular dystrophy, pain, neuroinflammatory condition, abnormal vascular homeostasis, and lupus.

The transdermal formulation of this patent document can be administered to promote the therapeutic effect or reduce adverse events of another therapy. In some embodiments of any method disclosed herein, the transdermal formulation of this patent document can be administered prior to, simultaneously with, or subsequent to another therapy, which includes for example orally administered medication, intravenous infusion, intramuscular infusion, topical medical treatment, and/or surgery. In some embodiments, the transdermal formulation is administered prior to an additional therapy for the disease or condition. For instance, topical pretreatment with the formulation disclosed herein before transfusions can maximize tissue perfusion and minimize transfusion associated inflammation. Topical pretreatment with the formulation or concomitant administration with another therapy can also reduce adverse events associated with the therapy (e.g. side effect associated with glucose lowering medications such as Metformin, skin rash, oral cavity mucositis/stomatitis associated with chemotherapeutics).

The transdermal formulation can also enhance endothelial function in a subject. Accordingly, various diseases or conditions associated with dysfunctional or imbalanced endothelial function can be treated. The endothelium has two interrelated major elements that are essential for vascular homeostasis: the glycocalyx and endothelial nitric oxide synthase (eNOS). The hair-like projections from the endothelium called the glycocalyx are responsible for: i) maintaining vascular integrity and thus limiting vascular leakage and access of macrophages and lipids into the deeper layers of the vascular wall (triggers for plaque formation); ii) controlling excessive production of reactive oxygen species (ROS) by acting as depot for superoxide dismutase (SOD) a potent antioxidant; iii) modulation of blood flow in response to physiological demands through sheer stress controlled production of nitric oxide by eNOS; iv) limiting access to and binding to the endothelium of blood borne cells (RBC's monocytes, leukocytes), platelets, and infectious agents; v) limit activation of platelets; vi) prevention of blood flow stagnation; vii) insuring continued NO production by eNOS by preventing eNOS decoupling as a result of excess ROS. In the decoupled state eNOS no longer generates NO but instead produces more inflammation generating ROS; viii) maintaining a cell free zone along the endothelial layer and thus preventing scavenging endothelial generated NO by RBC's in close proximity to the endothelial layer. Meanwhile, nitric oxide generated by endothelial nitric oxide synthase (eNOS) is essential for vascular homeostasis. The critical function of eNO include: i) maintain tissue perfusion/oxygenation; ii) prevent blood flow stagnation; iii) prevent a pro-coagulopathy environment; iv) repolarize activated macrophages and thus promote tissue repair and limit tissue damage; v) regulate pro and anti-inflammatory processes (balance between pro-inflammatory iNOS activity that generates damaging peroxynitrite and anti-inflammatory eNOS activity that produces eNO, activation of SIRT-1); vi) prevent inflammatory damage due to ischemia reperfusion and hypoxia reoxygenation; vii) prevent ROS induced damage including lipid peroxidation and glycocalyx degradation; viii) creates a depot of stored nitrosothiols within the endothelium and surrounding vascular layers that can rapidly provide NO under conditions requiring enhanced levels of NO as in the case of extreme muscular activity.

Endothelial dysfunction is a physiological dysfunction of normal biochemical processes carried out by the endothelium, the cells lining the inner surface of blood vessels. A hallmark of endothelial dysfunction is impaired endothelium-dependent vasodilation, which is mediated by nitric oxide (NO) produced by endothelial nitric oxide synthase (eNOS), a constitutive form of NOS that is principally expressed in endothelial cells. In healthy vasculature, NO produced by the endothelium diffuses to vascular smooth muscle cells (VSMC), where it activates guanylate cyclase and stimulates production of cyclic guanosine monophosphate (cGMP), thereby promoting relaxation of the VSMC and, consequently, vasodilation. Other functions of the endothelium (e.g., inhibition of platelet aggregation, inhibition of leukocyte adherence, and inhibition of VSMC proliferation) are also mediated by NO. In dysfunctional endothelium, NO production is impaired. Endothelial dysfunction can be detected clinically for example by elevations in the number of circulating endothelial cells (CECs).

Endothelial dysfunction is associated with various diseases including for example hypertension, coronary artery disease, heart failure, stroke, peripheral artery disease, diabetes, chronic renal failure, abnormal vascular smooth muscle cell proliferation and other cardiovascular diseases, Type 2 diabetes, insulin resistance and other metabolic syndrome, Lupus, HIV, inflammation resulting from radiation and drug treatments (e.g. chemotherapies), Hemoglobinopathies (Sickle cell disease, HbE/beta Thalassemia, Cytokine storm associated conditions induced by viral diseases (e.g. SARS CoV 2, Dengue fever, influenza, hemorrhagic shock, hemorrhagic fevers), erectile dysfunction secondary to surgery induced inflammation, and inflammation associated with increased populations of senescent cells typically occurring with age. Moreover, endothelial dysfunction is thought to be a key event in the development of atherosclerosis and predates clinically obvious vascular pathology by many years. Endothelial dysfunction has also been shown to be of prognostic significance in predicting vascular events including stroke and myocardial infarctions. In addition, endothelial dysfunction was shown to be implicated in inflammation, infection, immune system dysfunction, sleep apnea, sepsis, chronic obstructive pulmonary disease, exposure to pro-inflammatory agents.

The methods disclosed herein are applicable to the treatment of acute consequences as well as chronic consequences of endothelial dysfunction. Examples of chronic consequences of endothelial dysfunction include diseases and conditions described above. In some embodiments, the method is applicable to treating acute consequences including, for example, cytokine storm and associated, physical activity or diet triggered hypoxic/ischemic organ damage (e.g. heart attack due to insufficient tissue perfusion/oxygenation), stroke, micro and macro emboli, pulmonary embolus, ischemia reperfusion injury, hypoxia reoxygenation injury, and long covid which is a consequence of ongoing chronic inflammation/endothelial dysfunction. In some embodiments, the method is applicable to treating chronic consequences including, for example, Cardiovascular disease (CVD), coronary artery disease (CAD), renal

failure, cognitive decline and enhanced predisposition to dementia, hypertension, sexual dysfunction, slow healing wounds, accelerated stent failure/closure, coronary artery bypass failure, slow healing wounds, reduced tolerance for physical activity due to mitochondrial dysfunction, accelerated age related conditions, osteoarthritis, transient ischemic events, diabetic retinopathy, decreased insulin production due to inflammation initiated damage to pancreatic beta cells, HIV induced CVD, CVS and CAD secondary to ongoing periodic episodes of either sleep apnea and/or blood flow stagnation (e.g. sickle cell disease). Additional applications of the method include transfusions that include either RBC's or hemoglobin based oxygen carriers (HBOCs) and kidney dialysis.

The transdermal formulation can be administered to treat various local conditions associated endothelial dysfunction. For example, local conditions such as slow healing leg ulcers and erectile dysfunction are associated with underlying and often severe endothelial dysfunction which limits blood flow the damaged tissues. A method for treating slow healing leg ulcers includes sustained local delivery of nitric oxide to eliminate biofilms and infection that prevent therapeutic efficacy of agents that are designed to accelerate wound closure. In parallel transdermal delivery of an active agent such as curcumin will normalize the systemic vasculature thus promoting tissue oxygenation and allowing stem cell migration and development. The transdermal formulation can be administered in combination with an antibiotic agent or any suitable wound healing agent. For erectile dysfunction, the transdermal delivery of an agent such as curcumin can be used alone or in combination with topical nitric oxide and/or oral PD5 inhibitors to restore systemic vascular health and reduce systemic inflammation. Given that systemic NO booster (e.g. curcumin) enhances NO production in the endothelium and that oral supplementation with PD5 inhibitors extends the duration of action of NO, the combination of transdermally delivered active agent with oral PD5 inhibitors will accelerate recovery of the endothelium for patients with endothelial dysfunction including long covid and cytokine storm.

The transdermal formulation disclosed herein can also be administered to a subject in need thereof for decreasing ROS production, peroxynitrite production (via deactivation of iNOS activity) and/or increasing eNO production in the endothelium. Without being limited to any particular theory, it is postulated that the formulation provides pleiotropic effect of upregulation and/or activation of multiple anti-inflammatory and antioxidant enzymes and signaling pathways including for example Sirtuin 1 (SIRT1) and other inflammation modulating sirtuins, PPAR(gamma) (Peroxisome Proliferator Activated Receptor-Gamma), peroxisome proliferator-activated receptor-gamma coactivator (PGC)-1alpha is a member of a family of transcription coactivators that plays a central role in the regulation of cellular energy metabolism. AMP-activated protein kinase (AMPK) is a phylogenetically conserved fuel-sensing enzyme that is present in all mammalian cells. When activated AMPK stimulates energy generating processes such as glucose uptake and fatty acid oxidation and decreases energy consuming processes such as protein and lipid synthesis. The transcription factor Nrf2 (nuclear factor erythroid 2-related factor 2), a major regulator of antioxidant and cellular protective genes, is primarily activated in response to oxidative stress. SIRT1/PGC-1α/PPAR-γ pathway, eNOS mediated enhancement of eNO production, PPARP's, Nrf2, Heme oxygenase, AMPK, and ACE2 (angiotensin-converting enzyme 2, or ACE2 "receptor," the protein provides the entry point for the coronavirus to hook into and infect a wide range of human cells. The formulation can also be applied to down regulation or inhibition of (toll like receptor 4 part of the triggering mechanism for inflammation) TLR4, NADPH oxidases (NADPH oxidase (nicotinamide adenine dinucleotide phosphate oxidase) is a membrane-bound enzyme complex that faces the extracellular space and generates reactive oxygen species), and ACE (ACE (Angiotensin I Converting Enzyme).

The transdermal formulation described herein can be applied to the treatment and management of acute pro-inflammatory insults as well as the chronic consequences of many pro-inflammatory conditions that can promote or relate to endothelial dysfunction (ED). Nonlimiting examples of diseases or conditions associated with endothelial dysfunction (ED) include cardiovascular disease, renal failure, cognitive decline, slow healing wounds, hypertension, stroke, microemboli, edema, sexual dysfunction, retinopathy, neuropathy, and neuropathic pain.

For acute diseases or conditions, the formulation is capable of rapidly initiating global anti-inflammatory and antioxidant activity to short circuit and limit the progression leading to the severe consequences of extreme ED. For example, a suitable patch or sponge loaded with the formulation provides extremely high concentration of NO stimulating agents such as curcumin and insert between the gums and cheeks for a to be determine time to insure rapid and sustained delivery of therapeutic levels of curcuminoids or other anti-inflammatory, anti-oxidant and NO stimulating agents. This safe approach eliminates concern over overdosing with NO directly and additionally activates the full repertoire of host based anti-inflammatory and antioxidant pathways. The use of transdermally delivered systemically or locally active agents such as curcuminoids can stimulate NO production in the vasculature and/or reducing the overproduction of reactive oxygen species (ROS). This combination of enhancing endothelial generated NO and shutting down ROS production is designed to prevent, limit and reverse ED and its consequences.

The transdermal formulation of this patent document can be administered to a subject in need thereof for treating diabetes and associated inflammation and other conditions. Inflammation in adipose tissue promotes insulin resistance and hyperglycemia, both of which cause and extend endothelial dysfunction. In addition, chronic untreated endothelial dysfunction generated and enhanced by hyperglycemia, excess ROS production and other diabetes associated factors is the common pathway through which type 2 diabetes and other pro-inflammatory triggers the end stage clinical symptoms including for example cardiovascular disease, renal failure, hypertension, stroke and microemboli, slow healing wounds, sexual and bladder dysfunction. neuropathic pain, and cognitive decline. By reducing blood glucose, reversing insulin resistance, reducing elevated blood glucose level or procedure-induced exaggerated and sustained rise in blood glucose, reducing levels of ROS, enhancing NO levels in the endothelium and restoring vascular homeostasis, the transdermal formulation of this patent document minimizes the negative and/or undesirable outcomes associated with surgery, transfusions, stent implants, dialysis and any other invasive procedure that can promote systemic inflammation. The formulation can upregulate Nrf2 and the associated antioxidant enzymes including the potent antioxidant heme oxygenase (HO-1) and reduces oxidative stress (thereby allowing for recovery of damaged endothelium, treating endothelial dysfunction and preventing the onset or the progression of endothelial

dysfunction). Further, it limits the diabetes enhanced glucose response post-surgery and shortens the recovery time of the elevated glucose levels. Further, it can limit the extended and exaggerated stress-induced glucose spike in stressed (e.g. injuries, surgery and blood transfusion) diabetics, and the inflammatory consequences of the stress including urogenital dysfunction post prostatectomy.

The transdermal formulation can thus be administered to limit the negative clinical consequences associated with inflammation triggers in subjects with preexisting conditions or at risk of developing conditions that promote hyperglycemia and the ensuing endothelial dysfunction. In some embodiments, the formulation is administered prophylactically to a subject at risk of developing hyperglycemia. In some embodiments, the subject has been determined to have hyperglycemia.

In some embodiments, the amount/dosage of the active agent(s) are selected and/or the administration schedule are configures so that the method restores insulin sensitivity and/or reduces elevated blood glucose by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, or at least about 60% in comparison with a control (without treatment with the transdermal formulation) or the level prior to the treatment with the transdermal formulation disclosed herein. In some embodiments, the subject has been diagnosed to have diabetes (e.g. type 2 diabetes), undergone invasive procedures such as surgery, blood transfusion, stent implants, and dialysis, or suffered injury. In some embodiments, the subject prior to the treatment has an abnormal level of blood glucose, wherein the abnormal level is higher or lower than a normal level or the level of a healthy subject by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least 80% or at least 100%.

The transdermal formulations disclosed herein are capable of reducing the level of pro-inflamatory cytokines. In some embodiments of the methods disclosed herein, the amount/dosage of the active agent(s) are selected and/or the administration schedule are configures so that one or more of biomarkers are reduced or modified by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60% or at least about 100% in comparison with a control or the level prior to the treatment with the transdermal formulation. Nonlimiting examples of the markers that can be reduced or modified by the transdermal formulations disclosed herein include TNF-α, TGFβ, MCP-1, IL-1α, IL-1β, IL-6, IL-10, IL-1, IL-18, MIF, TNF-β, MMP9, HIF-1, GLUT1, Hemox, PDK1, VEGF, CD11, EMR1, CXCR4, CCR5, IL-8, receptor for advanced glycation end products (RAGE), hsCRP, Total Antioxidant Capacity (TAC), Prostaglandins, leukotrienes, substance P, Phosphatidylserine surface presentation on RBCs, Selectins, lamins and Cahedrins, Immunoglobulin receptors, chondroitin sulfate, syndecan-1, IL-1a/b, TNF-a, IL-6, D-dimer and other markers that reflect propensity to abnormal blood clotting, emboli formation, thrombosis, C-reactive protein (CRP), Nrf2, NFkappa B, glutathione peroxidase (GPx), superoxide dismutase (SOD), Syndecan-1, HMW-hyaluronic acid (1,000-6,000 kDa), A disintegrin and metalloprotease with thrombospondin type 1 repeats-13, Protein C, Von Willebrand factor, Chondroitin sulfate, and sP-selectin. Additional examples include markers associated with blood pressure, vasodilation, blood flow dynamics, vascular leakage/edema, M1/M2 Macrophage polarization, Soluble platelet selectin, Heparan sulfate, and cell and tissue oxygenation. In some embodiments, the subject prior to the treatment has an abnormal level of one or more biomarkers or pro-inflamatory cytokines, wherein the abnormal level of the biomarkers or one or more cytokines is higher or lower than a normal level or the level of a healthy subject by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60% or at least about 100%.

In some embodiments of the methods disclosed herein, the subject has been diagnosed as having a disease or condition selected from the group consisting of aging, chronic and acute inflammatory condition, chemically induced vascular and/or pulmonary inflammation, viral infection, bacterial infection, and fungal infection.

Further examples of disease or condition that can be treated with methods disclosed herein include neurodegenerative disease, diabetic kidney disease, diabetes, cardiovascular disease, endothelial dysfunction, muscular dystrophy, pain, neuroinflammatory condition, abnormal vascular homeostasis, lupus, Parkinson's disease, Alzheimer's disease, Huntington's disease, amyotrophic lateral sclerosis, neurodegenerative consequences of traumatic brain injury or cerebral hemorrhage, hypertension, inflammation, osteoarthritis, rheumatoid arthritis, endothelial dysfunction, dermatological condition, ophthalmological condition, bacterial infection, viral infection, ischemia reperfusion injury, hypoxia reoxygenation injury, cytokine storm phenomena, cerebral malaria, Chagas disease, hemoglobinopathies, type 2 diabetes, coronavirus, skin/dermatological conditions, acne, inflammatory skin conditions, raynaud's disease, post herpetic lesions, shingles, skin infections, wounds, burns, leg ulcers, sickle cell, diabetic, onychomycosis, peripheral vascular disease, infected and/or inflamed mucosal tissues, erectile dysfunction, female sexual dysfunction, vaginal infections/inflammation, catheter associated urinary tract infection, sinusitis, cystic fibrosis, acute respiratory distress syndrome, pulmonary fibrosis, chronic obstructive pulmonary disease (COPD), bronchiectasis, pulmonary infections including tuberculosis, pulmonary hypertension, and burns and other open wounds, inner ear infection, outer ear infection, gastric and intestinal diseases, and acute vascular inflammatory conditions. Further examples of diseases treatable with methods described herein include infectious disease is selected from the group consisting of Coronaviruses (including SARS-CoV-2), Ebola, Dengue fever, hemorrhagic shock, endotoxic shock, acellular hemoglobin toxicity due to hemolysis and/or the use of acellular hemoglobin based blood substitutes (HBOC's), Rift valley fever, Marburg, Crimean-Congo hemorrhagic fever (CCHF), South American hemorrhagic fever, dengue, yellow fever, Omsk hemorrhagic fever virus, Kyasanur Forest, Junin, Machupo, Sabia, Guanarito, Garissa, Ilesha, and Lassa fever viruses.

Further examples of diseases or conditions treatable with the formulations disclosed herein include neurodegenerative diseases or disorders (e.g. Alzheimer's disease (AD), Huntington's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS), multiple sclerosis and disorders caused by polyglutamine aggregation); skeletal muscle disease (e.g. Duchenne muscular dystrophy, skeletal muscle atrophy, Becker muscular dystrophy or myotonic Dystrophy); metabolic disorders (e.g. insulin resistance, diabetes, obesity, impaired glucose tolerance, high blood cholesterol, hyperglycemia, dyslipidemia and hyperlipidemia); adult-onset diabetes, diabetic nephropathy, neuropathy (e.g. sensory neuropathy, autonomic neuropathy, motor neuropathy, retinopathy); bone disease (e.g. osteoporosis), blood disease

(e.g. leukemia); liver disease (e.g. due to alcohol abuse or hepatitis); Obesity; bone resorption, macular degeneration aging, AIDS-related dementia, ALS, Bell's palsy, atherosclerosis, heart disease (for example, arrhythmia, chronic congestive heart failure, ischemic stroke, coronary artery disease and cardiomyopathy), Chronic degenerative disease (e.g., myocardial disease), chronic renal failure, type 2 diabetes, ulcer, cataract, presbyopia, glomerulonephritis, Guillain-Barre syndrome, hemorrhagic stroke, rheumatoid arthritis, inflammatory bowel disease, SLE, Crohn's disease, Diseases or disorders associated with osteoarthritis, osteoporosis, chronic obstructive pulmonary disease (COPD), pneumonia, skin aging, urinary incontinence, mitochondrial dysfunction (e.g. mitochondrial myopathy, encephalopathy, Leber's disease, Lee encephalopathy, Pearson Disease, lactate acidosis, "mitochondrial encephalopathy, lactate acidosis and stroke-like symptoms" (MELAS), muscular diseases, including neuromuscular diseases, such as muscular dystrophy and myopathy, and diseases or disorders associated with neuronal death, aging, or other conditions characterized by unwanted cell loss. In some embodiments, the disease or condition is selected from Parkinson's disease, Alzheimer's disease, Huntington's disease, amyotrophic lateral sclerosis, and neurodegenerative consequences of traumatic brain injury or cerebral hemorrhage, sickle cell disease, thalassemias (e.g. HbE/beta Thalassemia), diabetic retinopathies, glaucoma, dry eye syndrome, and inflammation triggered by surgery, aging, chronic and acute inflammatory condition, chemically induced vascular and/or pulmonary inflammation, viral infection, bacterial infection, fungal infection, diabetic kidney disease, diabetes, cardiovascular disease, endothelial dysfunction, muscular dystrophy, pain, neuroinflammatory condition, abnormal vascular homeostasis, lupus, retinopathies including diabetic retinopathy, macular degeneration, peripheral vascular disease, long term systemic consequences of chemo and radiation therapy, brain fog, rheumatoid arthritis, soft tissue injuries (muscle, tendons and ligaments), surgery induced inflammatory sequelae (urogenital dysfunction), transfusion induced inflammation, inhibition of stent restenosis, limit inflammatory consequences of dialysis, post dental procedure inflammation and pain, neuropathic pain from any proinflammatory insult including peripheral neuropathy, spinal neuropathy, arthritic pain, cognitive dysfunction in children due to cerebral vascular damage due to sickle cell disease, cytokine storm due to corona virus, dengue fever, ebola, rift valley fever and influenza, cerebral malaria, metastatic spread of tumors via dysfunctional blood vessels, systemic consequences of psoriasis, dementias including Alzheimer's disease and Pick's disease, post traumatic brain injury and hemorrhagic shock.

Autoimmune and immune related disorders and diseases can also be treated or prevented with methods described herein. Exemplary autoimmune diseases and immune related disorder include systemic lupus erythematosis, rheumatoid arthritis, osteoarthritis, juvenile chronic arthritis, a spondyloarthropathy, systemic sclerosis, an idiopathic inflammatory myopathy, Sjogren's syndrome, systemic vasculitis, sarcoidosis, autoimmune hemolytic anemia, autoimmune thrombocytopenia, thyroiditis, diabetes mellitus, immune-mediated renal disease, a demyelinating disease of the central or peripheral nervous system, idiopathic demyelinating polyneuropathy, Guillain-Barr syndrome, a chronic inflammatory demyelinating polyneuropathy, a hepatobiliary disease, infectious or autoimmune chronic active hepatitis, primary biliary cirrhosis, granulomatous hepatitis, sclerosing cholangitis, inflammatory bowel disease, gluten-sensitive enteropathy, Whipple's disease, an autoimmune or immune-mediated skin disease, a bullous skin disease, erythema multiforme, contact dermatitis, psoriasis, an allergic disease, asthma, allergic rhinitis, atopic dermatitis, food hypersensitivity, urticaria, an immunologic disease of the lung, eosinophilic pneumonias, idiopathic pulmonary fibrosis, hypersensitivity pneumonitis, systemic lupus erythematosus, scleroderma, and arthritis.

Non-limiting examples of neurological diseases that can be treated or the progression of which can be limited with methods of this patent document include neurodegenerative disorders include stroke, Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS; Lou Gehrig's disease), diffuse Lewy body disease, chorea-acanthocytosis, primary lateral sclerosis, Multiple Sclerosis (MS), and Friedreich's ataxia, Periventricular leukomalacia (PVL), ALS-Parkinson's-Dementia complex of Guam, Wilson's disease, cerebral palsy, progressive supranuclear palsy (Steel-Richardson syndrome), bulbar and pseudobulbar palsy, diabetic retinopathy, multi-infarct dementia, macular degeneration, Pick's disease, diffuse Lewy body disease, prion diseases such as Creutzfeldt-Jakob, Gerstmann-Straussler-Scheinker disease, Kuru and fatal familial insomnia, primary lateral sclerosis, degenerative ataxias, Machado-Joseph disease/spinocerebellar ataxia type 3 and olivopontocerebellar degenerations, spinal and spinobulbar muscular atrophy (Kennedy's disease), familial spastic paraplegia, Wohlfart-Kugelberg-Welander disease, Tay-Sach's disease, multisystem degeneration (Shy-Drager syndrome), Gilles De La Tourette's disease, familial dysautonomia (Riley-Day syndrome), Kugelberg-Welander disease, subacute sclerosing panencephalitis, Werdnig-Hoffmann disease, synucleinopathies (including multiple system atrophy), Sandhoff disease, cortical basal degeneration, spastic paraparesis, primary progressive aphasia, progressive multifocal leukoencephalopathy, striatonigral degeneration, familial spastic disease, chronic epileptic conditions associated with neurodegeneration, Binswanger's disease, and dementia (including all underlying etiologies of dementia).

Insulin resistance disorders treatable with methods of this patent document include any disease or condition that is caused by or contributed to by insulin resistance. Examples include: diabetes, obesity, metabolic syndrome, insulin-resistance syndromes, syndrome X, insulin resistance, high blood pressure, hypertension, high blood cholesterol, dyslipidemia, hyperlipidemia, dyslipidemia, atherosclerotic disease including stroke, coronary artery disease or myocardial infarction, hyperglycemia, hyperinsulinemia and/or hyperproinsulinemia, impaired glucose tolerance, delayed insulin release, diabetic complications, including coronary heart disease, angina pectoris, congestive heart failure, stroke, cognitive functions in dementia, retinopathy, peripheral neuropathy, nephropathy, glomerulonephriti s, glomerulosclerosi s, nephrotic syndrome, hypertensive nephrosclerosis some types of cancer (such as endometrial, breast, prostate, and colon), complications of pregnancy, poor female reproductive health (such as menstrual irregularities, infertility, irregular ovulation, polycystic ovarian syndrome (PCOS)), lipodystrophy, cholesterol related disorders, such as gallstones, cholescystitis and cholelithiasis, gout, obstructive sleep apnea and respiratory problems, osteoarthritis, and prevention and treatment of bone loss, e.g. osteoporosis. Further application of methods of of this patent document include promoting wound healing such as can also be used to promote wound healing and diabetes-impaired wound healing.

In some embodiments of any method disclosed herein, there also includes a step of determining a subject as having downregulated or dysfunctional SIRT1 in comparison with a normal standard or reference. In some embodiments, the methods disclosed herein further include, prior to administering to the subject the transdermal formulation, diagnosing the subject as having endothelial dysfunction or a disease or condition associated with endothelial dysfunction.

In some embodiments of the methods disclosed herein, there is included a step of determining the subject as having a systemic NO level or plasma nitrite and/or nitrate level lower than a normal level or a standard of heathy references by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 40%, at least 50%, or at least 60%.

In any of the methods disclosed herein, the treatment regimen can be administered after a symptom is observed clinically or a clinical manifestation of a disease or condition has taken place. Alternatively, the method can be used prophylactically before the onset or observation of any clinical symptom. For instance, the methods disclosed herein are capable of addressing pro-inflammatory insults including acute inflammatory insults triggered by certain viral infection (e.g. SARS CoV2, Dengue fever, and influenza), obesity and glucose induced inflammatory triggers, and inflammation triggered by exposure to toxic metals and chemicals. The treatment can be administered when a symptom has been identified or prior to the onset or observation of any symptom. In further examples, the transdermal formulation can be administered prophylactically to prevent inflammatory consequences of surgery including post surgical inflammation induced pain or erectile dysfunction.

The methods disclosed herein can increase NO level systemically or locally. In some embodiments of any transdermal formulation or method of this patent document, the amounts of active ingredients in the transdermal formulation (e.g. the NO booster and/or the NO precursor, the polyol, the optional fatty acid, and/or other components) are selected so that it increases a subject's systemic or local NO level or plasma nitrite and/or nitrate level by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 40%, at least 50%, at least 60% or more in comparison with a control or the NO level or plasma nitrite and/or nitrate level prior to administering the formulation. In some embodiments, the desired increase or change can be achieved within about 1 hour, within about 2 hours, within about 3 hours, within about 5 hours, or within about 8 hours. In some embodiments, the increase or change can be maintained for a period of about 1 day, about 3 days, about 5 days, about 7 days, about 10 days, about 15 days, about 30 days or more. Various methods can be used for measuring the NO level or plasma nitrite and/or nitrate level, including for example the colorimetric method using the Griess reagent and the chemiluminescence method.

In some embodiments of any transdermal formulation or method of this patent document, the amounts of the active ingredients in the transdermal formulation (e.g. the NO booster and/or the NO precursor, the polyol, the optional fatty acid, and/or other components) are selected so that the systolic pressure and/or diastolic pressure and/or mean arterial pressure of the subject is reduced by at least 2, at least 4, at least 6, at least 8, at least 10, at least 12, at least 14, about 18, at least 20, at least 25, at least 30, at least 35, at least 40 or more mm Hg over the above period of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more days in comparison with a control or the NO level or plasma nitrite and/or nitrate level prior to administering the formulation.

In some embodiments of any method disclosed herein, the subject has been diagnosed to have hypertension or is at risk of developing hypertension. The transdermal formulation disclosed herein can be administered once, twice, three time or as needed over a period of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more days. In some embodiments, the formulation in the form of for example a patch, cream, or gel is administered once every 1, 3, 5, 7, or 10 days.

Another aspect of this disclosure provides a method of incorporating an agent into a cell by contacting the cell with the formulation disclosed herein. As a result of enhanced NO level, the method can stabilize the cells, improve storage properties and reverse storage lesions. In some embodiments, the cells are red blood cells. In some embodiments, the formulation is in a liquid form. In some embodiments, the formulation includes S-nitrosothiol-containing molecule.

Treatment of cells (e.g. red blood cells) with the formulation described herein can delivery NO booster (e.g. curcumin) and optionally other antiinflammatory/antioxidant agents into the lipid membrane or beyond cytosol should stabilize the cells for storage, and convert the transfused cells into long lasting (circulating) anti-inflammatory agents that can slowly deliver these agents to endothelial linings in the capillaries and other small diameter vessels. Similarly, treatment of the cells with a formulation containing high concentrations of a NO precursor such as lipophilic S-nitrosothiol and the S—NO derivative of alkyl ester derivatives of NAC should be effective in transnitrosating thiols in and on the cells. Nitrosation of key thiols on and in the red blood cells (including beta 93 on Hb and the thiols of the Band 3 protein) has been shown to stabilize red blood cells against oxidative damage, microparticle formation and hemolysis. Such red blood cells can thus be used as a vehicle to deliver NO to thiols on the endothelium as well.

Another aspect provides a method of improving the the safety and efficacy of transfusion by contacting the cells or fluid to be transfused with the formulation prior to or during the transfusion procedure. For instance, the formulation containing NO booster or NO precursor can be administered prior to or during blood transfusion to protect the red blood cells and enhance the therapeutic effect. In some embodiments, the method includes contacting the formulation with the cells or fluid 10 minutes, 15 minutes, 30 minutes, 1 hour, 2 hours, or more than 4 hours before the transfusion takes place.

A related aspect provides a method of delivering an active agent transdermally to a subject in need thereof. The method includes administering to the subject a transdermal formulation disclosed herein. By delivering an effective amount of the active agent locally or systemically, a rapid and extended therapeutic effect can be achieved. The scope of the target diseases or conditions is as described above. In some embodiments, the transdermal formulation includes, as an active ingredient for treating the disease or condition, one or more curcuminoids and optionally one or more of polyphenol, flavonoid, stilbenoid, secosteroid, or natural products that promote NO production. In some embodiments, the transdermal formulation includes one or both of curcumin and quercetin, and optionally one or more of one or more of polyphenol, flavonoid, stilbenoid, and secosteroid.

Method of Manufacturing

Another aspect of the present disclosure provides a method of manufacturing the formulation disclosed herein. The method generally includes preparing a solution of the polyol solvent and the fatty acid permeation enhancer, followed by addition of the agent (NO booster or NO

precursor). If necessary, the solution can be heated to a suitable temperature to dissolve fatty acid and/or the agent. In some embodiments, the active agent (e.g. NO booster or NO precursor or the nitrite source) is saturated in the solution. After cooling down the solution, the formulation may turn into a gel and the precipitated excess active agent can be easily removed. Variations in condition or sequence of mixing or addition of different ingredients/components are also feasible as long as the NO booster or NO precursor is suitably distributed in the formulation to achieve desirable therapeutic effects.

In some exemplary embodiments, the agent dissolved in the solution is filled into a container (e.g., a sprayer or nebulizer; a permeable or frangible pouch as described above) or soaked into a dispensing vehicle (e.g., swab, sponge or absorbing layer of a patch). When the NO precursor mixture contains a nitrite source, an acid source can be stored for example in a separate pouch or a separate layer of a patch. The NO precursor mixture and the acid source can also be separated by a removable barrier disposed between two different compartments of a container or between two layers of a patch.

In some embodiments, the formulation is in the form of a gel or semisolid. A gelling agent or thickener can be added to adjust the form of the formulation. The formulation is then filled into a suitable container and then dispensed as gels, ointments, creams, emulsions, microemulsions, nanoemulsions, pastes, balms, or other suitable forms. The semisolid formulation can also be coated on a backing member (e.g., a support layer of a patch).

Depending on the amount and nature of the thickener, the formulation can also be prepared into a solid form. For example, a solution of the NO booster (e.g. curcumin, demethoxycurcumin, bisdemethoxycurcumin, quercetin, berberine) can be mixed with melted pure cocoa butter and then cooled. The resulting solid formulation melts when rubbed with pressure on to human skin.

General procedures for mixing the reagents and handling the manufacturing process are available through the common knowledge or pharmaceutical technology handbooks well known to a person skilled in the art, for example, Remington: The Science and Practice of Pharmacy, 20th edition, Lippincott, Williams & Wilkins, Philadelphia, 2000, or in the review article Souza et al, Topical ocular delivery of therapeutics: carrier systems and physical methods, J. Pharm. Pharmacol., 2013, 66, 507-530.

Administration Regimen

The effective amount of the agent (NO booster or NO precursor) in the formulation or kit described herein for effectively enhancing systemic NO level will depend on the route of administration, the type of subject, including human, being treated, and the physical characteristics of the specific subject under consideration. The dose or amount can be tailored to achieve a desired effect, but will depend on such factors as weight, diet, concurrent medication and other factors which those skilled in the medical arts will recognize. More specifically, an effective amount or therapeutically effective amount means an amount of the agent effective to increase the systemic NO to a level to prevent, alleviate or ameliorate symptoms of disease or prolong the survival of the subject being treated. The administration of the formulation may be adjusted to provide the optimal therapeutic response or prolonged beneficial effects. For example, the formulation may be topically administered more than two or more than three times a day. Alternatively, the amount or administration frequency may be reduced if necessary. Determination of an effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

In non-human animal studies, applications of potential products are commenced at higher dosage levels, with dosage being decreased until the desired effect is no longer achieved or adverse side effects disappear. The dosage may range broadly, depending upon the desired effects and the therapeutic indication. Typically, the effective amount of the agent or dosage in the formulation may be about 10 microgram/kg to about 100 mg/kg body weight, preferably about 100 microgram/kg to about 10 mg/kg body weight. Alternatively, dosages may be based and calculated upon the surface area of the patient, as understood by those of skill in the art.

In exemplary embodiments, the formulation is administered once a day, twice a day, three times a day, once in two days, once in three days, once in a week, one every two weeks or once a month.

The exact formulation, route of administration and dosage for the pharmaceutical compositions can be chosen by the individual physician in view of the patient's condition. (see e.g., Fingl et al. 1975, in "The Pharmacological Basis of Therapeutics", which is hereby incorporated herein by reference in its entirety, with particular reference to Ch. 1, p. 1).

It should be noted that the attending physician would know how to and when to terminate, interrupt, or adjust administration due to toxicity or organ dysfunctions. Conversely, the attending physician would also know to adjust treatment to higher levels if the clinical response were not adequate (precluding toxicity). The magnitude of an administered dose in the management of the disorder of interest will vary with the severity of the condition to be treated and to the route of administration. The severity of the condition may, for example, be evaluated, in part, by standard prognostic evaluation methods. Further, the dose and perhaps dose frequency will also vary according to the age, body weight, and response of the individual patient. A program comparable to that discussed above may be used in veterinary medicine.

The formulation disclosed herein can be evaluated for efficacy and toxicity using known methods. For example, the toxicology of the formulation may be established by determining in vitro toxicity towards a cell line, such as a mammalian, and preferably human, cell line. The results of such studies are often predictive of toxicity in animals, such as mammals, or more specifically, humans. Alternatively, the toxicity in an animal model, such as mice, rats, rabbits, or monkeys, may be determined using known methods. The efficacy of a particular compound may be established using several recognized methods, such as in vitro methods, animal models, or human clinical trials. Recognized in vitro models exist for nearly every class of condition. Similarly, acceptable animal models may be used to establish efficacy of chemicals to treat such conditions. When selecting a model to determine efficacy, the skilled artisan can be guided by the state of the art to choose an appropriate model, dose, and route of administration, and regime. Of course, human clinical trials can also be used to determine the efficacy of the active agents of the transdermal fin humans.

The formulation or kit described herein may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the formulated agent. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. The pack or dispenser may also be accompanied with a notice associated with the container in form prescribed by a govern-

mental agency regulating the manufacture, use, or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the drug for human or veterinary administration. Such notice, for example, may be the labeling approved by the U.S. Food and Drug Administration for prescription drugs, or the approved product insert.

All references cited herein are incorporated herein by reference in their entireties.

EXAMPLES

Example 1

Preparation of Transdermal Formulation

Vehicles for loading the active ingredient were prepared as illustrated in samples 1-4. Myristic acid (solid flakes) was dissolved in PEG400 upon warming the mixture in a warm water bath. Shaking accelerated the formation of the resulting clear solution. The following four concentrations of myristic acid (MA) in PEG400 were prepared and evaluated. Samples 1, 2, 3 and 4 were all clear solutions when the samples were allowed to attain ambient temperatures.

a. 30 ml of PEG400+1.5 grams of MA (Sample 1)
b. 30 ml of PEG400+3 grams of MA (Sample 2)
c. 30 ml of PEG400+0.75 grams of MA (Sample 3)
d. 30 ml of PEG400+2.25 grams of MA (Sample 4)

The samples as vehicles were loaded with NO enhancing agent. The sample carrier formulations [or solvent systems] were loaded with 95% pure curcumin (Curcumin 95) as the NO boosting agent to test loading potential. 1.5 grams of Curcumin 95 was added to Samples 1, 2, 3. The resulting Curcumin 95 loaded samples were warmed and shaken until what appeared to be the maximum amount of dissolving of the curcumin occurred, and then were allowed to cool back to ambient temperature. All three samples allowed for the dissolving of almost all of the added curcumin resulting in an intensely colored dark solution. Sample 3 provided the higher solubility and remained liquid for weeks. Samples 1 and 2 were initially liquid but formed a solid uniform gel over a period of several hours. Sample 3 was prepared by dissolving 0.75 grams of MA in 30 ml's of PEG400 and heating in a water bath (~60-80 C) for ~15 minutes. Sample 4 was prepared by dissolving 2.25 grams of MA in 30 mls of PEG400 and heating in a water bath (~60-80 C) for ~15 minutes. Both sample 3 and sample 4 were uniform solutions when the heating cycle was finished. Upon cooling sample 3 remained liquid whereas sample 4 formed a uniform gel.

Once loading potential was established, sample transdermal formulations containing curcugen as the NO enhancing agent were made. Curcugen is a product containing curcumin, demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC). The three curcuminoids account for about 50% by weight in curcugen. Sample formulations V3.3 and V4.3 were made by adding curcugen to Sample 3 and sample 4 vehicles, respectively (3 grams of curcugen in 30 mls of either solvent). The resulting mixtures were heated and shaken for ~15 minutes, after which both appeared as dark maroon uniform solutions. Upon cooling V3.3 (3 grams curcugen per 30 ml Sample 3 vehicle) remained liquid; whereas V4.3 (3 grams curcugen per 30 ml Sample 4 vehicle) became a uniform gel having the same dark maroon color (same color as V3.3 when heated).

No loss of color was observed over a period of at least three months for samples stored at ambient temperature. In marked contrast, the same volume of water with the same amount of added curcumin showed almost no color in the liquid phase and a substantial amount of undissolved material.

V4.3 was loaded into a syringe when warm and hence in the liquid state. The liquid in the syringe gelled when cooled to ambient temperatures but could be squeezed out of the syringe as a gel that slowly melted on the skin. The applied gel was easily be covered and trapped in a water proof and leakproof transparent bandage (e.g. Mepitel transparent film dressing). The dressing prevented loss of the applied formulation and could remain in place for several days without loss of formulation. When the Mepitel dressing was removed after several days there was no adhered formulation on the dressing and all the formulation was in the skin as reflected in an absence of staining on tissue that was aggressively wiped over the colored skin that was below the dressing. The color was fully lost after 7-8 days.

The addition of water to the PEG400/MA based formulation can create a nonuniform emulsion that poses difficulties for topical use. The presence of water limits solubility of curcumin, curcugen, quercetin and berberine in PEG. As a comparison, the same amount of curcumin was mixed with the same volume of water (i.e. 3 g. curcumin in 30 ml water). The resulting mixture showed almost no color in the liquid phase and contained a substantial amount of undissolved material.

The use of higher concentration MA solutions with PEG as the base solvent allowed for easy preparation of concentrated saturated solutions with low solubility NO enhancing actives such as curcumin, curcugen, quercetin, berberine and related molecules by simply adding an excess of the active agent to the heated high MA PEG/MA solvent system (e.g. V4.3) and allowing the mixture to cool. The resulting uniform gel was fully saturated with the active agent, while the undissolved excess remained as a solid at the bottom of the tube. The uniform saturated gel was thereafter easily removed and separated from the undissolved material.

The Sample 3 solvent system was also tested as a vehicle for loading potential with the following low solubility reagents using the above method: quercetin, berberine, N-acetyl cysteine amide (NACA) and N-acetyl cysteine ethyl ester (NAC-ethylester). In all cases, the solvent system allowed for substantial dissolution of these hard to solubilize agents. A comparison between an aqueous solution and Sample 3 solution loaded with quercetin and berberine revealed minimal dissolved material in the aqueous sample and a high degree of solubilization in Sample 3.

Similar results were obtained for quercetin in sample 3 and sample 4 as vehicle but the solubility of the quercetin in these solvents was less than for curcugen (~200-300 mg quercetin in 30 ml of either sample 3 or sample 4). As with curcumin and curcugen, quercetin loaded sample 3 and sample 4 do not and do form a gel at ambient temperatures respectively.

The PEG400/MA solutions were combined with other delivery vehicles (e.g. petroleum jelly). Sample 3 with curcumin was mixed with petroleum jelly resulting in a uniformly colored gel that remained stable (no loss of color) over a period of over two months. Sample 3 with the dissolved SNO derivative of NAC amide and of NAC was easily combined with petroleum jelly to produce a stable pink jelly. Stability is likely derived from the low water activity and high viscosity which inhibit the loss of NO from the thiol group. Different sized aliquots of Sample 3 (both with curcumin and with curcugen) were mixed with melted pure cocoa butter and then cooled. Optimized mixtures yielded solid uniformly yellow/orange colored blocks/tubes

of cocoa butter that remained solid at ambient temperature. The solid material melted when rubbed with pressure onto human skin. Similar results were obtained with coconut oil but the lower melting point of coconut oil made it difficult to apply to human skin without smearing and dripping. Combinations of the two oils were also tested. The use of PEG400/MA with cocoa butter showed most promising properties as a topical delivery vehicle suitable for cosmetic and dermatological applications due to the more suitable hardness for the resulting curcumin loaded cocoa butter. Increasing the amount of added PEG400/MA solvent to the cocoa butter eventually resulted in a gel like substance that remained stable color wise.

The solutions of the two lipophilic NAC derivatives were prepared using sodium nitrite saturated PEG400, resulting in a clear solution. The lipophilic derivatives of NAC readily dissolved under conditions where NAC had limited solubility. NAC is water soluble and the two derivatives are marginally soluble in water. The two solutions were then treated with several drops of acetic acid to trigger the formation of nitrous acid from the nitrite which can then nitrosate the reactive thiol on both of the NAC derivatives. The solutions turned pink indicative of S-nitrosothiol formation. The solutions remained pink for several days. The corresponding aqueous solutions lost color within hours. The use of nitrite saturated PEG400 allows for formulations that can be used to generate NO and S-nitrosothiols through mixing with reagents that acidify the mixture. A double frangible pouch incorporated into a patch may be used for a sustained NO delivery vehicle suitable for topical and transdermal applications.

Example 2

Effects on blood pressure (BP) on rats of V2.3 formulation (2 g curcugen per 30 ml Sample 3 vehicle) were examined. The formulation was topically applied to the shaved abdomen of 5 sprague dawley rats. A Q tip saturated with V2.3 was rubbed onto the shaved rat belly. In all instances (N=5) the application of V2.3 produced a 20% drop in systemic blood pressure within 15 to 20 minutes of application. The reduced blood pressure was maintained for the three hour observation window with no indication of recovery. Results (N=6) from a second laboratory in which 6 rats were similarly tested showed a similar result (20% drop in blood pressure). Similar results with V3.3 were consistently obtained on a single human subject upon application of V3.3 to the forearm (~0.05 ml). A comparable drop in BP occurred within 15 to 20 minutes of application and the lowered BP persisted for many hours, only gradually returning to the higher initial values after 12 to 24 hours. Similar results on the same human subject were obtained using V4.3. The cocoa butter doped with Sample 3 (V1.3) also exhibited similar physiological consequences when applied to the single human test subject. The control Sample 3 without one or more curcuminoids or curcugen elicited no physiological consequences when applied to either rat or human.

V3.3 was applied to a skin flap with an optical window on healthy hamsters. The optical window allowed for the monitoring of blood vessels in the dermal layer below the site of administration of the V3.3. A dose dependent increase in vessel diameter at the site of the topical application was observed within several minutes of application.

Effects on NO Plasma levels. Plasma levels of NO degradation products (nitrite/nitrate) were measured subsequent to topical application of V3.3 in 4 rats. Plasma levels of NO degradation products (nitrite/nitrate) in two of the tested rats were shown to increase by 15% consistent with the drop in blood pressure originating from enhanced production of nitric oxide due to the known ability of curcumin to upregulate endothelial nitric oxide synthase (eNOS) and hence NO production. Two control animals showed no such increase under the same conditions. The results indicate that transdermal curcuminoids can elevate systemic NO levels.

The drop in blood pressure within 15 to 20 minutes after topical application of a curcumin containing sample indicates that the curcumin is being delivered transdermally and that therapeutically effective levels are present within that short time period. The drop in blood pressure and increase in nitrite/nitrate plasma levels are consistent with the known effect of curcumin with respect to upregulation of nitric oxide production in the endothelium by eNOS. The physiological response is persistent over many hours consistent with topically delivered curcumin/curcugen is being delivered into the circulation in a sustained manner. In contrast, curcumin delivered into the circulation either via oral route or IV has a circulation time of only 2 hours due to the liver rapidly converting curcumin to an inactive agent. Even large dosing of oral curcumin has not been observed to produce this pronounced and prolonged physiological response.

Physiological response transdermal curcuminoid formulation in animals was also examined for V3.3 (Curcugen 9 g, myristic acid 2.25 g, PEG 400 90 ml) and V4.3 (Curcugen 9 g, myristic acid 6.75 g, PEG 400 90 ml).

Blood pressure was measure in rodents subsequent to topical application of transdermal curcuminoid formulation. Both rats and mice show up to a 20% reduction in BP with V3.3 being more effective than V4.3 both in terms of time of onset and extent of BP drop.

It was also observed that topical application of either V3.3 or V4.3 resulted in increased levels of plasma nitrite and nitrate of the three hour monitoring window subsequent to a single topical dose. Over the same time period the BP underwent a sustained decrease (over the three hour window). A sustained build up in the detectable concentration of plasma curcuminoids was observed over a three hour window subsequent to topical application of V3.3 in rats (N=3). Oral curcumin plasma levels peak in an hour and drop to near undetectable levels within three hours.

Example 3

Control on Systemic Inflammation Via Transdermal Delivery of NO Booster or NO Precursor.

Inhibition on development of severe vascular leakage in an acute inflammation rat model was examined with topically applied Vascarta formulation V3.3. The study used the following lipopolysaccharide (LPS) induced cytokine storm protocol:

a. LPS (10 mg/kg) was IP infused every 24 hours to initiate and sustain an acute inflammatory response.
b. The LPS treated rats were subjected to topical application (0.1 ml) of Vascarta formulation V3.3 starting at the time of the first LPS treatment and repeated every 24 hours for three days
c. Physiological parameters derived from drawn blood were measured every 24 hours
d. After three days the animals were anesthetized and surgically opened to allow for the intravital fluorescence imaging of the macro and micro vasculature.
    i. Formulations of fluorescent labeled albumin and dextran (500 kDa) were IV infused and fluorescence derived images of the vasculature and surrounding

tissue were used to determine the rate of extravasation out of the vasculature and into the surrounding tissues for both albumin and dextran.

Topical application of the vehicle (PEG400/MA) on the LPS treated rats revealed a pattern of rapid and extreme leakage for both albumin and the much larger dextran consistent with what was observed under conditions of severe acute inflammation. However, topical daily application of V3.3 dramatically reduced the amount of leakage for albumin as shown in FIG. **1**. The observed low level of leakage is approximately the same as what was observed for a control animal. Similar results were observed for dextran as shown in FIG. **2**. Extensive vascular leakage is a potentially lethal consequence of the cytokine storm independent of cause (COVID-19, Ebola, Dengue fever, hemorrhagic shock, endotoxic shock, Rift valley fever etc). The transdermal formulation provided a dramatic positive intervention outcome with far reaching clinical implications.

Example 4

This study evaluated potential therapeutic efficacy of a transdermally delivered transdermal formulation according to the invention (V4.3: curcugen 9 g, myristic acid 6.75 g, PEG 400 90 ml) in an acute vascular inflammation mouse model. Three cohorts each with three subjects were studied and compared. In Cohort 1, the subjects were infused with LPS but without no topical treatments (untreated). In Cohort 2, the subjects were infused with LPS topical treatment (0.1 ml of V4.3). In Cohort 3, LPS infusion was followed by topical treatment (0.1 ml of V4.3) four hours after LPS infusion. Endotoxemia was induced by infusion of 10 mg/kg of LPS (Lipopolysaccharides from *E. coli* serotype 0128: B12, Sigma Aldrich St. Louis, Mo.). The procedures were the same as those described in earlier published studies (Williams A T, Muller C R, Govender K, Navati M S, Friedman A J, Friedman J M, Cabrales P. Control of systemic inflammation through early nitric oxide supplementation with nitric oxide releasing nanoparticles. Free Radic Biol Med. 2020; 161:15-22. Epub 2020/10/05. doi: 10.1016/jfreeradbiomed.2020.09.025. PubMed PMID: 33011274; PMCID: PMC7529593 and references therein).

It was observed that topically applied V4.3 (curcuminoids dissolved in a PEG400/myristic acid mix) acted both as a prophylactic and interventional treatment for lipopolysaccharide (LPS) induced cytokine storm. The results include the vascular consequences and cytokine profile as a function of time for three different groups of LPS treated mice: i) no topical treatment with V4.3; ii) pretreatment with topical V4.3 prior to the LPS treatment and iii) topical treatment with V4.3 after the onset of the LPS initiated cytokine storm. In terms of the response of the microvasculature to LPS treatment in the three groups, treatment with topical V4.3 both limited arterial dilation, which is indicative of shock induced vascular collapse, and maintained arterial blood flow. It was also observed that topical V4.3 prevented the steep drop in functional capillary density (FCD) that occurs with LPS induced endotoxemia. As shown in FIG. **3**, V4.3 was effective both as a prophylactic and as an interventional treatment in preventive steep decline in FCD relative to baseline (BL). FCD correlates with survival in that it reflects the ability to maintain tissue perfusion and deliver oxygen to tissues. Further, it was discovered that both pre-treatment and interventional treatment with topically applied V4.3 limited the production of pro-inflammatory cytokines. These results confirmed the efficacy of the transdermal formulation in limiting the inflammatory consequences when administered topically as a prophylactic or as an active therapeutic subsequent to onset of the inflammation.

Example 5

This study evaluated prevention of LPS induced vascular leakage via topically administered curcuminoids using a transdermal formulation according to the present invention in an LPS endotoxemia rat model. LPS (*E coli O*26:B6) was inoculated to rat model (10 mg/kg/day). A transdermal curcumin formulation (V3.3: curcugen 9 g, myristic acid 2.25 g, PEG 400 90 ml) or vehicle control was applied daily for three days (0.1 ml/dose). On day 3, the animals were surgically prepared for intravital and fluorescence microscopy. It was observed that treatment with the transdermal curcumin formulation prevented the LPS induced early phase leakage (first 4 hours). Vascular leakage is dramatically reduced subsequent to topical application of V3.3 in an LPS induced inflammation mode. Meanwhile, topical application of V4.3 (curcugen 9 g, myristic Acid 6.75 g, PEG 400 90 ml) both prior to and subsequent to LPS induced inflammation in mice (N=3) reduced the LPS induced increase in pro-inflammatory cytokine levels.

This experiment, combined with results from the earlier studies, indicates that topical transdermal curcumin formulation of this patent document can limit inflammation induced vascular leakage which is a characteristic of the cytokine storm and other inflammation inducing conditions.

Example 6

Skin permeation of transdermal formulations of the present invention was studied. Skin permeation of the following transdermal formulations according to the present invention were studied using confocal microscopy. The formulations differed only in the amount of myristic acid:

a. Lot 43: V4.3 Curcugen 9 g, Myristic Acid 6.75 g, PEG 400 90 ml
b. Lot 39: V3.3 Curcugen 9 g, Myristic Acid 2.25 g, PEG 400 90 ml
c. Lot 38: V0.3 Curcugen 9 g, PEG 400 90 ml

The formulations solidified as temperatures approached zero centigrade. The viscosity was visibly reduced in all samples as the temperature was raised above ambient. Visocosity of the formulations and their ingredients are as follows:

Water 0.9 cP; PEG400 (100%) 99 cP; PEG400/water 90%, 80 cP; V3.3: 149.8 cP; PEG400+myristic acid, V3.3: 149.8 cP; PEG400+myristic acid, V4.3: 4220 cP.

Curcuminoids have a broad absorbance spectrum with a maximum absorbance at 425 nm. All human skin sample integrity was measured using TEWL data and all samples used had good integrity of the skin barrier. A DAPI filter was used to visualize the curcuminoid penetration. All images were taken at the same settings.

All three formulations showed penetration of the curcuminoids into the skin. At the earliest time point of one hour penetration of the curcuminoids into the stratum corneum with all three formulations was observed.

In the formulation of lot 38, in both donors, broadening of fluorescent band occurred from 3 to 6 hours. Broadening of band was not seen in the formulation of lot 39 and 43 until the 24th hour. The broadening suggests the permeation of the curcuminoids into the upper epidermal layers just below the stratum corneum. In the formulation of lot 39, in both donors, the amount of curcuminoids between 3 to 6 hours decreased in both donors. However, a decrease in the

intensity for curcuminoids in the formulation of lot 38 and 43 was observed after 6 hours. In the formulation of lot 38, fluorescent spots were seen below the stratum corneum at the early time points. However, no fluorescent spots were seen above the stratum corneum with the formulation of lot 39 and 43. Transepidermal water loss (TEWL) values are comparable for all formulations and all time points suggesting that all human skin sample barrier integrity was the same.

The decrease in fluorescence over time in the three formulations is an indication of penetration of the curcuminoids into deeper layers of the skin. (Low amounts of penetration of the actives may have not be visualized and/or quantified due to the high autofluorescence of the untreated skin). Based on this, the formulation of lot 39 shows earliest penetration of the curcuminoids into the skin. The depth of fluorescence was also determined. This was measured at values between 5 microns to 25 microns. The formulation of lot 38 showed the fastest broadening of the fluorescent band suggesting faster skin penetration of the curcuminoids.

Example 7

The effect of the transdermal formulation of this patent on limiting or preventing the onset of cardiovascular inducing inflammation in a diabetic rat model was examined in ZDSD diabetic rat model. The 3 rats were on a normal diet for 60 days without symptoms of diabetes. Starting on ~day 65 the rats were started on a high fat diet that resulted in a slow increase in blood glucose. The animals were treated topically with formulation V4.3 every two days for the entire test period out to 80 days. Cytokine profile showed the onset of inflammation at ~day 75. The treatment with with formulation V4.3 limited the increase in pro-inflammatory cytokines seen in the shams at day 75. In particular, the profile for IL-18 showed clear evidence of the formulation in preventing the increase in IL-18 which is a marker for propensity of developing cardiovascular consequences in diabetics.

Example 7

The effect of treatment of mice with severe advanced endothelial dysfunction was examined with Formulation V4.3. A total of 24 male C57BL/6J mice aged 6-8 weeks were used in the study. Mice were housed in an animal facility under a 12/12-h day/night cycle and access to food and water ad libitum. The study was carried out over a period of 4 weeks. Week 1 was used for adaptation as the animals were divided into experimental groups. Two groups received L-NAME (50 mg/kg) in their drinking water for two weeks (week 2 and 3) to chronically induce nitric oxide synthase (NOS) inhibition. One L-NAME treated group received topical formulation V4.3 at a dosing of 0.1 ml daily for the last 10 days before characterization. The L-NAME untreated group was used as Sham group.

Topical treatment was initiated after the onset of the condition. The results showed direct evidence of the treatment, which restores elements of normal endothelial function (decreased oxidative stress in plasma and RBCs, decreased leukocyte adhesion to the endothelium indicative of recovery of the glycocalyx, and improved functional capillary density). In comparison with the Sham group, the group treated with the transdermal formulation exhibited a lower level of TNF-α, TGFβ, MCP-1, IL-1α, IL-1β, IL-6, IL-10, and IL-10. Better results from the treated group were also observed in terms of microhemodynamic changes, cell adhesion, vascular response of isolated aortic vessels, changes in erythrocyte and plasma antioxidants, hypoxia, reoxygenation, and systemic hemodynamic changes.

TABLE 1

Changes in body weight, relative tissue weights and water intake at the end of the study.

	BW, g	Kidney relative to BW	Heart relative to BW	Daily Water intake, mL
Control	25 ± 4	1.44 ± 0.07	1.16 ± 0.08	4.3 ± 0.6
Curcumin	24 ± 3	1.32 ± 0.08	1.08 ± 0.07	4.7 ± 0.8
Sham	26 ± 4	0.82 ± 0.04	0.87 ± 0.06	3.9 ± 0.4

TABLE 2

Changes in Erythrocyte and plasma antioxidants at the end of the study

	Erytrocyte SOD, unit/g_{Hb}	Erytrocyte Catalase, unit/g_{Hb}	Erytrocyte GSH, μmole/g_{Hb}	Plasma GSH, μM
Control	3.4 ± 0.2	23.1 ± 3.0	4.2 ± 0.6	2.1 ± 0.4
Curcumin	4.0 ± 0.4	28.3 ± 2.7	5.1 ± 0.6	2.7 ± 0.6
Sham	5.1 ± 0.5	36.9 ± 2.4	6.0 ± 0.7	3.4 ± 0.5

It will be appreciated by persons skilled in the art that the inventions described herein are not limited to what has been particularly shown and described. Rather, the scope of the invention is defined by the claims which follow. It should further be understood that the above description is only representative of illustrative examples of embodiments. The description has not attempted to exhaustively enumerate all possible variations. The alternate embodiments may not have been presented for a specific component of a composition, or a step of the method, and may result from a different combination of described constituents, or that other undescribed PEG alternate embodiments may be available for a formulation, kit or method, is not to be considered a disclaimer of those alternate embodiments. It will be appreciated that many of those undescribed embodiments are within the literal scope of the following claims, and others are equivalent.

The invention claimed is:

1. A method of treating a disease or condition in a subject, comprising administering to the subject a transdermal formulation, wherein the transdermal formulation comprises:

(a) an effective amount of an NO booster and optionally an NO precursor, wherein the NO booster comprises one or more agents selected from the group consisting of curcumin, demethoxycurcumin, bisdemethoxycurcumin, quercetin, berberine, resveratrol and vitamin D, and wherein the NO precursor comprises a S-nitrosothiol-containing molecule or a thiol-containing molecule and a nitrite source;

(b) a polyol, wherein the polyol and the NO booster are in a ratio ranging from about 8:1 to about 12:1, and the NO booster is dissolved in the polyol; and optionally

(c) a fatty acid, wherein the polyol and the fatty acid are in a ratio ranging from about 10:1 to about 50:1 by weight,

wherein the amounts of the polyol and the fatty acid are selected so that upon administration the effective amount of the NO booster is delivered transdermally;

wherein the disease or condition is selected from the group consisting of hypertension, inflammation, osteoarthritis, rheumatoid arthritis, endothelial dys-

function, dermatological condition, ophthalmological condition, bacterial infection, viral infection, ischemia reperfusion injury, hypoxia reoxygenation injury, cytokine storm phenomena, cerebral malaria, Chagas disease, hemoglobinopathies, type 2 diabetes, neurodegenerative disease, Lupus, long Covid and vascular leakage.

2. The method of claim **1**, wherein the disease or condition is cytokine storm phenomena caused by an infectious disease.

3. The method of claim **2**, wherein the infectious disease is selected from the group consisting of Coronaviruses, Ebola, Dengue fever, hemorrhagic shock, endotoxic shock, Rift valley fever, Marburg, Crimean-Congo hemorrhagic fever (CCHF), South American hemorrhagic fever, dengue, yellow fever, Omsk hemorrhagic fever virus, Kyasanur Forest, Junin, Machupo, Sabia, Guanarito, Garissa, Ilesha, and Lassa fever viruses.

4. The method of claim **1**, wherein the disease or condition is cytokine storm phenomena caused by COVID-19 infection or systemic inflammatory response syndrome (SIRS).

5. The method of claim **1**, wherein the inflammation is a pulmonary inflammation disease selected from the group consisting of cystic fibrosis, acute respiratory distress syndrome, pulmonary fibrosis, chronic obstructive pulmonary disease (COPD), bronchiectasis, pulmonary infections, and pulmonary hypertension.

6. The method of claim **1**, wherein the disease or condition is vascular leakage.

7. The method of claim **6**, wherein the vascular leakage is caused by a disease is selected from vascular leak syndrome, infectious disease, inflammatory diseases, inter alia, sepsis, lupus, irritable bowel disease, inflammatory bowel disease and inflammation of general vasculature.

8. The method of claim **1**, wherein the disease or condition is neurodegenerative disease selected from the group consisting of Parkinson's disease, Alzheimer's disease, Huntington's disease, amyotrophic lateral sclerosis, and neurodegenerative consequences of traumatic brain injury or cerebral hemorrhage.

9. The method of claim **1**, wherein the NO booster and its amount are selected to reduce one or more inflammatory makers by at least about 10%, wherein the one or more inflamatory makers are selected from the group consisting of TNF-α, TGFβ, MCP-1, IL-1α, IL-1β, IL-6, IL-10, MIF, TNFβ, MMP9, HIF-1, GLUT1, Hemox, PDK1, VEGF, CD11, and EMR1.

10. The method of claim **1**, wherein the disease or condition is COVID-19 infection, clinical manifestations of long Covid, sepsis, or systemic inflammatory response syndrome (SIRS).

11. The method of claim **10**, wherein the transdermal formulation comprises

(a) an effective amount of one or more agents selected from the group consisting of curcumin, demethoxycurcumin, bisdemethoxycurcumin, and quercetin;

(b) PEG having a molecular weight ranging from about 200 to about 600 in an amount sufficient to dissolve the effective amount of the one or more agents, wherein the PEG and the one or more agents are in a ratio ranging from about 8:1 to about 12:1; and

(c) myristic acid, wherein the PEG and the myristic acid are in a ratio ranging from about 10:1 to about 50:1 by weight.

12. The method of claim **11**, wherein the one or more agents comprise curcumin.

13. The method of claim **11**, wherein the one or more agents and their amount are selected so that it increases a subject's systemic NO level or plasma nitrite level by at least 10%.

14. The method of claim **11**, wherein the disease or condition is COVID-19 infection.

15. The method of claim **11**, wherein the transdermal formulation is administered prophylactically.

16. The method of claim **1**, wherein the NO booster is curcumin.

17. The method of claim **1**, wherein the polyol is PEG.

18. The method of claim **1**, wherein the transdermal formulation comprises the fatty acid.

19. The method of claim **18**, wherein the fatty acid is myristic acid.

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