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**Boger R., et al, "Restoring vascular nitric oxide formation by L-arginine improves the symptoms of intermittent claudication in patients with peripheral arterial occlusive disease", JACC, 1998, vol 32, no 5, pp 1336-1344**

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(54) Title: BENEFICIAL EFFECTS OF INCREASING LOCAL BLOOD FLOW

(57) Abstract: The present invention generally relates to the improvement of tissue health by increasing local blood flow. In some aspects of the invention, increased local blood flow is effected by the transdermal delivery of the nitric oxide precursor L-arginine and/or its derivatives alone, or optionally in conjunction with an adjunct such as theophylline. The transdermal delivery is effected, in certain embodiments through the means of a hostile biophysical environment, such as that created by a high ionic strength environment. Various pathological states caused by, or occurring in conjunction with, insufficient blood flow, can be treated using the systems and methods of the invention as described herein. In other embodiments, increased blood flow using the systems and methods of the invention may result in enhanced healing, for example, through greater availability of the constituents of the blood. Examples of conditions which may benefit from increased blood flow include, but are not limited to, erectile dysfunction, hair loss, female sexual dissatisfaction, sagging facial or other body tissue, peripheral vascular disease including claudication, neuropathy, skin ulcers, bone healing, wound healing, viral and bacterial infection, and skin grafting.

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**BENEFICIAL EFFECTS OF INCREASING LOCAL BLOOD FLOW****RELATED APPLICATIONS**

This application claims the benefit of U.S. Provisional Patent Application Serial  
5 No. 60/563,563, filed April 19, 2004, entitled "Use of Transdermal L-Arginine and  
Adjuncts to Cause Beneficial Effects by Increasing Local Blood Flow," by E.T. Fossel;  
U.S. Provisional Patent Application Serial No. 60/563,551, filed April 19, 2004, entitled  
"Use of a Transdermal L-Arginine Preparation and Adjuncts to Improve Outcome in  
Transplant and Plastic Surgery," by E.T. Fossel; U.S. Provisional Patent Application  
10 Serial No. 60/563,557, filed April 19, 2004, entitled "A Transdermal Preparation of L-  
Arginine to Improve Flow in Peripheral Artery Disease and Prevent Claudication," by  
E.T. Fossel; U.S. Provisional Patent Application Serial No. 60/563,556, filed April 19,  
2004, entitled "Transdermal Delivery of L-Arginine Preparation to Regress Neuropathy  
and Heal and Prevent Ulcers," by E.T. Fossel; U.S. Provisional Patent Application Serial  
15 No. 60/563,553, filed April 19, 2004, entitled "Use of A Transdermal L-Arginine and  
Adjuncts to Improve Bone Healing," by E.T. Fossel; U.S. Provisional Patent Application  
Serial No. 60/563,554, filed April 19, 2004, entitled "Use of a Transdermal Preparation  
of L-Arginine and Adjuncts to Effect Wound Healing," by E.T. Fossel; U.S. Provisional  
Patent Application Serial No. 60/563,555, filed April 19, 2004, entitled "Use of a  
20 Transdermal Preparation of L-Arginine and Adjuncts to Facilitate Healing of Infection,"  
by E.T. Fossel; and U.S. Provisional Patent Application Serial No. 60/563,565, filed  
April 19, 2004, entitled "Use of Arginine and Arginine Derivatives and Adjuncts to  
Improve Grafting of Real and Artificial Skin," by E.T. Fossel.

This application also claims the benefit of U.S. Provisional Patent Application  
25 Serial No. 60/563,558, filed April 19, 2004, entitled "Flow Assisted Topical Transdermal  
Methods of Drug Delivery," by E.T. Fossel; U.S. Provisional Patent Application Serial  
No. 60/563,559, filed April 19, 2004, entitled "Transdermal Delivery of Pharmaceutical  
Agents Effected by a Hostile Biophysical Environment," by E.T. Fossel; U.S.  
Provisional Patent Application Serial No. 60/563,560, filed April 19, 2004, entitled  
30 "Transdermal Drug Delivery by Means of a High Ionic Strength Environment," by E.T.  
Fossel; U.S. Provisional Patent Application Serial No. 60/563,561, filed April 19, 2004,  
entitled "Use of a Silicon Based Matrix for Transdermal Delivery of L-Arginine and  
Adjuncts to Cause Beneficial Effects," by E.T. Fossel; U.S. Provisional Patent

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Application Serial No. 60/563,562, filed April 19, 2004, entitled "Transdermal Preparation of Ibuprofen to Reduce Pain and Inflammation," by E.T. Fossel; U.S. Provisional Patent Application Serial No. 60/563,572, filed April 19, 2004, entitled "An Augmented Flow Assisted Preparation to Increase Muscle Size and Performance," by E.T. Fossel; U.S. Provisional Patent Application Serial No. 60/563,564, filed April 19, 2004, entitled "A Flow Assisted Preparation to Increase Muscle Size and Performance," by E.T. Fossel; U.S. Provisional Patent Application Serial No. 60/563,566, filed April 19, 2004, entitled "Transdermal Delivery of L-Arginine for the Purpose of Enhancing the Appearance of the Female Breast," by E.T. Fossel; U.S. Provisional Patent Application Serial No. 60/563,567, filed April 19, 2004, entitled "A Transdermal Augmented L-Arginine Preparation for Treatment of Headache," by E.T. Fossel; U.S. Provisional Patent Application Serial No. 60/563,552, filed April 19, 2004, entitled "Flow Assisted Topical Transdermal Method of Drug Delivery of Drugs with Systemic Toxicity," by E.T. Fossel; U.S. Provisional Patent Application Serial No. 60/563,569, filed April 19, 2004, entitled "Transdermal Delivery of Systematically Toxic Pharmaceutical Agents Effected by a Hostile Biophysical Environment," by E.T. Fossel; U.S. Provisional Patent Application Serial No. 60/563,570, filed April 19, 2004, entitled "Transdermal Preparations to Improve Muscle Function and Size," by E.T. Fossel; U.S. Provisional Patent Application Serial No. 60/563,571, filed April 19, 2004, entitled "Transdermal Flow Assisted Localized Delivery of Chemotherapeutic Agents," by E.T. Fossel; U.S. Provisional Patent Application Serial No. 60/563,573, filed April 19, 2004, entitled "Flow Assisted Transdermal Preparations of Ephedra and Ephedra Components to Avoid Adverse Effects," by E.T. Fossel; U.S. Provisional Patent Application Serial No. 60/563,574, filed April 19, 2004, entitled "Augmented Flow Assisted Transdermal Delivery of Anabolic Steroids," by E.T. Fossel; U.S. Provisional Patent Application Serial No. 60/563,575, filed April 19, 2004, entitled "Flow Assisted Transdermal Delivery of Anabolic Steroids," by E.T. Fossel; and U.S. Provisional Patent Application Serial No. 60/563,576, filed April 19, 2004, entitled "Transdermal Delivery of Ephedra and Ephedra Components by Use of A Hostile Biophysical Environment," by E.T. Fossel.

Each of the above applications is incorporated herein by reference.

#### **FIELD OF INVENTION**

The present invention generally relates to the improvement of tissue health.

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## BACKGROUND

Poor circulation in the extremities is a major problem in people with diabetes and others. There is nothing available to treat this problem. Symptoms are managed through use of special socks and shoes and moisturizing creams. In addition, effective healing  
5 and functionality following plastic and transplant surgery is often less than optimal. Healing often takes much too long. Effective healing and functionality is greatly enhanced by increased blood flow which results in improved oxygenation and tissue nutrition.

Local transdermal delivery of drugs, while desirable, is limited by current  
10 technologies. Few pharmaceutical entities have successfully been delivered transdermally in effective dosages. For example, a limited number of drugs, such as steroids, nicotine and nitroglycerine, which are non-charged and do not form hydrogen bonds, have been successfully delivered by passive diffusion, relying on the concentration gradient between outside and inside the skin to deliver the agent in  
15 accordance with Fick's first law of diffusion. The amount of pharmaceutical agent that can be delivered through simple diffusion is also limited. For instance, once the concentration inside the stratum corneum becomes equal to that outside, flow of pharmaceutical agent stops.

As these examples illustrate, what is needed are systems and methods for  
20 increasing local blood flow in tissues.

## SUMMARY OF THE INVENTION

The present invention generally relates to the improvement of tissue health by increasing local blood flow. The subject matter of the present invention involves, in some cases, interrelated products, alternative solutions to a particular problem, and/or a  
25 plurality of different uses of one or more systems and/or articles.

In some cases, various beneficial substances which function at local sites, rather than systemically, may be more efficaciously administered by transdermal rather than systemic administration. If the beneficial substances is delivered through the skin, a higher dose in the tissue to be treated may be achieved. In addition, a substantially lower  
30 total body dose may be achieved in some cases. This can be understood if one considers the non-limiting example of treating pain in finger joints with a NSAID (a nonsteroidal anti-inflammatory drug). If the pain is to be treated systemically, e.g., by oral administration, the whole body, including the finger joints, is dosed with the NSAID.

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The concentration of NSAID is approximately the same throughout the body, including the finger joints. If, on the other hand, one were to apply the NSAID transdermally to the finger joints, the rest of the body would not be dosed (or would be dosed to a significantly lesser extent). Thus, the total dose of transdermal NSAID would only be a  
5 fraction of the dosage required for systemic administration.

One aspect of the invention is directed to a method comprising an act of administering, to a subject, a delivery vehicle comprising a nitric oxide donor contained within a hostile biophysical environment. Another aspect of the invention is directed to an article comprising a cream containing a nitric oxide donor in a hostile biophysical  
10 environment.

This invention relates, in one aspect, to the field of localized transdermal delivery of substances which have a beneficial effect, for example, to the transdermal delivery of herbs, vitamins, minerals, pharmaceutical agents, drugs, peptides, dietary supplements, or other substances effected by a hostile biophysical environment. The hostile  
15 biophysical environment may comprise a high ionic strength vehicle in some embodiments, and delivery may be enhanced, in certain cases, by various techniques to increase local blood flow at the delivery site. For instance, substances with localized action may avoid systemic toxicity if delivered locally and transdermally.

In some embodiments, the beneficial substances delivered transdermally may  
20 improve health, improve body function, or treat a variety of disease states.

In one set of embodiments, the invention relates to the field of headache treatment, and in some cases, to use of arginine and/or arginine derivatives or adjuncts to provide effective headache relief. This invention also relates, in another set of embodiments, to the field of relief of pain and/or inflammation and in some cases, to a  
25 transdermal preparation of ibuprofen to reduce pain and/or inflammation. In certain instances, the ibuprofen is delivered from a vehicle into the tissue through the use of a hostile biophysical environment.

This invention also relates, in yet another set of embodiments, to systems and methods for improving uptake of the muscle improving agents, for example, by  
30 increasing local blood flow by delivering a nitric oxide donor such as L-arginine, either alone or with an adjunct such as theophylline. This invention also relates, in still another set of embodiments, to topical methods of administering anabolic steroids, for example,

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steroids that exhibit unacceptable systemic toxicity. The steroids may also promote improved muscle size and function through the use of enhanced blood flow.

This invention relates, in one set of embodiments, to topical methods of administer chemotherapeutic or antiviral agents, for instance, to promote healing or  
5 recovery, or to prevent recurrence of a localized cancer or viral infections.

In yet another set of embodiments, this invention relates to the field of enhanced sexual function. In some cases, arginine and/or arginine derivatives and adjuncts may be applied to increase genital blood flow, which may increase sexual function.

In one aspect, the invention is a method. The method includes, in one set of  
10 embodiments, an act of applying, to a portion of the skin of a subject, a delivery vehicle comprising a pharmaceutical agent in a hostile biophysical environment.

In another aspect, the invention includes a delivery vehicle. In one set of embodiments, the delivery vehicle includes a nitric oxide donor, and a pharmaceutical agent at a dosage effective to treat a localized medical condition, wherein the dosage is  
15 lower than the effective dosage of the pharmaceutical agent when taken orally. In another set of embodiments, the delivery vehicle includes a nitric oxide donor, and a pharmaceutical agent able to treat one or more medical conditions selected from the group consisting of cramps, pain, migraine, arthritis, swelling, sexual dysfunction, hair loss, skin ulcers, and migraine.

20 Several methods are disclosed herein of administering a subject (which may be human or a non-human animal) with a composition for prevention or treatment of a particular condition. It is to be understood that in each such aspect of the invention, the invention specifically includes, also, the composition for use in the treatment or prevention of that particular condition, as well as use of the composition for the  
25 manufacture of a medicament for the treatment or prevention of that particular condition.

The present invention, in another aspect, is directed to a method of making one or more of the embodiments described herein. In yet another aspect, the present invention is directed to a method of using one or more of the embodiments described herein. In still another aspect, the present invention is directed to a method of promoting one or  
30 more of the embodiments described herein.

Other advantages and novel features of the present invention will become apparent from the following detailed description of various non-limiting embodiments of the invention. In cases where the present specification and a document incorporated by



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reference include conflicting and/or inconsistent disclosure, the present specification shall control. If two or more documents incorporated by reference include conflicting and/or inconsistent disclosure with respect to each other, then the document having the later effective date shall control.

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## DETAILED DESCRIPTION

The present invention generally relates to the improvement of tissue health by increasing local blood flow. In some aspects of the invention, increased local blood flow is effected by the transdermal delivery of the nitric oxide precursor L-arginine and/or its derivatives alone, or optionally in conjunction with an adjunct such as theophylline. The transdermal delivery is effected, in certain embodiments through the means of a hostile biophysical environment, such as that created by a high ionic strength environment. Various pathological states caused by, or occurring in conjunction with, insufficient blood flow, can be treated using the systems and methods of the invention as described herein. In other embodiments, increased blood flow using the systems and methods of the invention may result in enhanced healing, for example, through greater availability of the constituents of the blood. Examples of conditions which may benefit from increased blood flow include, but are not limited to, erectile dysfunction, hair loss, female sexual dissatisfaction, sagging facial or other body tissue, peripheral vascular disease including claudication, neuropathy, skin ulcers, bone healing, wound healing, viral and bacterial infection, and skin grafting.

The following documents are incorporated herein by reference: U.S. Provisional Patent Application Serial Nos. 60/563,563, 60/563,558, 60/563,559, 60/563,560, 60/563,561, 60/563,562, 60/563,572, 60/563,564, 60/563,565, 60/563,566, 60/563,567, 60/563,553, 60/563,554, 60/563,555, 60/563,556, 60/563,557, 60/563,551, 60/563,552, 60/563,569, 60/563,570, 60/563,571, 60/563,573, 60/563,574, 60/563,575, and 60/563,576, each filed April 19, 2004, by E.T. Fossel; U.S. Provisional Patent Application Serial No. 60/546,214, filed February 23, 2004, entitled "Topical Delivery of a Nitric Oxide Donor to Improve Body and Skin Appearance," by E.T. Fossel; U.S. Patent Application Serial No. 08/932,227, filed September 17, 1997, entitled "Topical Delivery of Arginine of Cause Beneficial Effects," by E.T. Fossel, published as 2002/0041903 on April 11, 2002; U.S. Patent Application Serial No. 10/201,635, filed July 22, 2002, entitled "Topical Delivery of L-Arginine to Cause Beneficial Effects," by E.T. Fossel, published as 2003/0028169 on February 6, 2003; U.S. Patent Application

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Serial No. 10/213,286, filed August 5, 2002, entitled "Topical and Oral Arginine to Cause Beneficial Effects," by E.T. Fossel, published as 2003/0018076 on January 23, 2003; International Patent Application No. PCT/US98/19429, filed September 17, 1998, entitled "A Delivery of Arginine to Cause Beneficial Effects," by E.T. Fossel, published  
5 as WO 99/13717 on March 25, 1999; U.S. Patent No. 5,895,658, issued April 20, 1999, entitled "Topical Delivery of L-Arginine to Cause Tissue Warming," by E.T. Fossel; U.S. Patent No. 5,922,332, issued July 13, 1999, entitled "Topical Delivery of Arginine to Overcome Pain," by E.T. Fossel; U.S. Patent No. 6,207,713, issued March 27, 2001, entitled "Topical and Oral Delivery of Arginine to Cause Beneficial Effects," by E.T.  
10 Fossel; U.S. Patent No. 6,458,841, issued October 1, 2002, entitled "Topical and Oral Delivery of Arginine to Cause Beneficial Effects," by E.T. Fossel; International Patent Application No. PCT/US2005/005726, filed February 23, 2005, entitled "Topical Delivery of a Nitric Oxide Donor to Improve Body and Skin Appearance," by E.T. Fossel; and an international patent application filed on even date herewith, entitled  
15 "Transdermal Delivery of Beneficial Substances Effected by a Hostile Biophysical Environment," by E.T. Fossel.

Detailed descriptions of the various embodiments are provided herein. It is to be understood, however, that the present invention may be embodied in various forms. Therefore, specific details disclosed herein are not to be interpreted as limiting, but rather  
20 as a basis for the claims and as a representative basis for teaching one skilled in the art to employ the present invention in virtually any appropriately detailed system, structure, or manner.

The present invention, in one aspect, provides various systems and techniques for increasing local blood flow. For example, increased blood flow may be used to  
25 introduce pharmaceutical agents (e.g., drugs, biological compounds, etc.) to aid in treatment of medical conditions or diseases and the symptoms associated thereof (for example, to treat a subject diagnosed with a medical condition or disease, as described herein), and/or the increased blood flow may be used to provide effective treatment of medical conditions or diseases and/or ailments with the minimum amount of  
30 pharmaceutical agents possible to provide effective levels of medication to an effected area topically while limiting side effects. In one set of embodiments, a nitric oxide donor such as L-arginine and/or L-arginine hydrochloride in an effective concentration may be used to increase localized blood flow, which may enhance delivery of a pharmaceutical

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agent or other beneficial substance, e.g., to locally afflicted tissue. Nitric oxide may relax the blood vessels, allowing for increased blood flow. In some cases, one or more nitric oxide donors (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, etc. nitric oxide donors) may be combined with one or more beneficial substances (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, etc. beneficial substances) in a suitable hostile biophysical environment, as described herein.

One set of embodiments provides for increased blood flow to the genitals, for example, using a nitric oxide donor such as L-arginine, optionally in combination with a silicon-based transdermal preparation and/or an adjunct such as theophylline. Adequate local genital blood flow is important for optimal sexual function and satisfaction in both men and women. In men it is important to achieve and maintain an erection. In women it is important for nerve sensitivity which is required to attain satisfying orgasms. In some cases, the preparation may be contained within a condom, optionally with other sexual-enhancing agents, such as lubricants.

A non-limiting example of such a preparation includes a silicon-based vehicle (e.g., a vehicle that contains a silicon-containing substance) with properties of excellent absorption into the skin which also contains L-arginine hydrochloride (7.5% w/v), theophylline (5% w/v) and a mixture of high molecular weight polydimethylsiloxane and a low viscosity cyclotetrasiloxane (commercially known as Dow Corning 1411 fluid), and water prepared as an emulsion. The silicon emulsion provides a hostile biophysical environment in this example. The emulsion is applied to the genitals (e.g., the penis, or the clitoris and/or the vagina) and rubbed in until absorbed. The emulsion may facilitate enhanced blood flow to the genitals, bringing oxygen and other nutrients and blood to that tissue. In addition, the silicon may act as a lubricant for improved enjoyment of sexual function. Additional preparations are discussed in more detail herein. Other examples of silicon-containing substances include polydimethylsiloxane, cyclopentasiloxane, dimethicol, or dimethicon. For example, a preparation of the invention may be a cream containing water (20-80%), a polydimethylsiloxane/cyclopentasiloxane mixture (20-90% w/v) and TWEEN 20 (1-10%), and the pH may be between about 3 and about 11.

Besides L-arginine and L-arginine hydrochloride, other non-limiting examples of nitric oxide donors include D,L-arginine, D-arginine, or alkyl (e.g., ethyl, methyl, propyl, isopropyl, butyl, isobutyl, *tert*-butyl, etc.) esters of L-arginine and/or D-arginine (e.g., a methyl ester, an ethyl ester, a propyl ester, a butyl ester, etc.) and/or salts thereof, as well

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as other derivatives of arginine and other nitric oxide donors. For instance, non-limiting examples of pharmaceutically acceptable salts include hydrochloride, glutamate, butyrate, or glycolate (e.g., resulting in L-arginine glutamate, L-arginine butyrate, L-arginine glycolate, D-arginine hydrochloride, D-arginine glutamate, etc.). Other  
5 examples of nitric oxide donors include L-arginine-based compounds such as, but not limited to, L-homoarginine, N-hydroxy-L-arginine, nitrosylated L-arginine, nitrosylated L-arginine, nitrosylated N-hydroxy-L-arginine, nitrosylated N-hydroxy-L-arginine, citrulline, omithine, linsidomine, nipride, glutamine, etc., and salts thereof (e.g., hydrochloride, glutamate, butyrate, glycolate, etc.). Still other non-limiting examples of  
10 nitric oxide donors include S-nitrosothiols, nitrites, 2-hydroxy-2-nitrosohydrazines, or substrates of various forms of nitric oxide synthase. In some cases, the nitric oxide may be a compound that stimulates endogenous production of nitric oxide *in vivo*. Examples of such compounds include, but are not limited to, L-arginine, substrates of various forms of nitric oxide synthase, certain cytokines, adenosine, bradykinin, calreticulin, bisacodyl, phenolphthalein, OH-arginine, or endothelein.  
15

It should be understood that, in any of the embodiments described herein that describe L-arginine, other nitric oxide donors may also be used instead, or in combination with, L-arginine, in other embodiments of the invention.

Without wishing to be bound to any theory, it is generally believed that the flow  
20 of the pharmaceutical agent or other beneficial substance across the skin may slow as it builds up within the tissue. Fick's first law of diffusion suggests that when the concentration inside becomes substantially equal to that outside, passive flow stops. The increased local blood flow may prevent or at least decrease the stoppage of the flow of the pharmaceutical agent or other beneficial substance. Thus, when the vehicle  
25 containing the pharmaceutical agent or other beneficial substance and a nitric oxide donor, such as L-arginine, is applied to the skin, the pharmaceutical agent or other beneficial substance exits the vehicle into the tissue more readily, as the pharmaceutical agent is dispersed by flow and does not build up in concentration in the tissue. Thus, in certain embodiments, pharmaceutical agents or other beneficial substances may be  
30 introduced into the skin, for example, ibuprofen, anabolic steroids, or other agents or substances described herein.

A "nitric oxide donor," as used herein, is a compound that contains therein a nitric oxide (NO) moiety, where the compound is able to release nitric oxide and/or

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chemically transfer the nitric oxide moiety to another molecule, directly or indirectly, for example, through a biological process. The nitric oxide donor may release nitric oxide into the skin, and/or tissues such as muscles and/or elements of the circulatory system in close proximity to the surface of the skin. Non-limiting examples of nitric oxide donors  
5 include arginine (e.g., L-arginine and/or D-arginine), arginine derivatives (e.g., L-arginine hydrochloride and/or D-arginine hydrochloride), nitroglycerin, polysaccharide-bound nitric oxide-nucleophile adducts, N-nitroso-N-substituted hydroxylamines, 1,3-(nitrooxymethyl)phenyl-2-hydroxybenzoate, etc., as described in more detail herein. In some cases, the concentration of nitric oxide and/or the nitric oxide donor may be  
10 tailored to have a duration of effective treatment of at least about 3 hours, at least about 5 hours, or at least about 8 hours or more in certain instances. The duration may also be controlled, for instance, by controlling the concentration of a penetrating agent used in conjunction with nitric oxide and/or the nitric oxide donor. The actual concentration for a particular application can be determined by those of ordinary skill in the art using no  
15 more than routine experimentation, for example, by measuring the amount of transport of nitric oxide and/or the nitric oxide donor as a function of concentration *in vitro* across cadaver skin or suitable animal models, skin grafts, synthetic model membranes, or the like.

As a particular non-limiting example, in one embodiment, nitric oxide is provided  
20 using L-arginine, for example, at a concentration of at least about 0.5% by weight (wt% or w/v) of L-arginine (optionally with one or more penetrating agents as discussed herein, for example, a penetrating agent able to create a hostile biophysical environment), at least about 0.75 wt%, at least about 1 wt%, at least about 2 wt%, at least about 3 wt%, at least about 5 wt%, at least about 7 wt%, at least about 10 wt%, or at least  
25 about 15 wt%. The L-arginine may be present in a suitable delivery vehicle, such as a cream or a lotion. L-arginine may be particularly useful in some cases due to its low toxicity, its high solubility, or its low cost. Other examples of nitric oxide donors are discussed in International Patent Application No. PCT/US2005/005726, filed February 23, 2005, entitled "Topical Delivery of a Nitric Oxide Donor to Improve Body and Skin  
30 Appearance," by E.T. Fossel, incorporated herein by reference.

One aspect of the invention provides for the delivery of nitric oxide and/or nitric oxide donors into the body, as further described below, and such treatments may be systemic or localized, e.g., directed to a specific location of the body, such as the head,

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arms, legs, feet, etc., depending on the specific application. The nitric oxide and/or nitric oxide donor may increase local blood flow, thereby enhancing tissue health. Increased blood flow may also assist in the healing process, e.g., where injury or surgery has occurred.

5           In one set of embodiments, nitric oxide and/or a nitric oxide donor (e.g., arginine and/or an arginine derivative), optionally including an adjunct such as theophylline, may be applied to a subject to improve the outcome of various medical conditions, such as surgical treatments (e.g., at a site of surgery). Non limiting examples include transplant and plastic surgery, graft sites of real or artificial skin, or other surgically treated areas.

10       In some embodiments of the invention, a treatment of the invention may be applied to improve flow in peripheral artery disease and/or prevent claudication, to improve the circulation in the feet of people with diabetes and others with impaired circulation, to regress neuropathy, to heal or prevent ulcers, to improve bone healing, to treat infection (e.g., bacterial infections, viral infections, fungal infections, etc.), , and/or to improve  
15       wound healing.

          In another set of embodiments, nitric oxide and/or a nitric oxide donor (e.g., arginine and/or an arginine derivative), optionally including an adjunct such as theophylline, may be applied to a subject having peripheral artery disease (PAD), for example, in subjects treated invasively or non-invasively. For instance, arteries are often  
20       reopened by use of angioplasty, arthectomy or bypass surgery, or through the use of intravenous drug treatments, such as Corlapam, Flolan, or Primacor. Left untreated or unsuccessfully treated, PAD can lead to claudication, which can be incapacitating, resulting not only in great pain but loss of the ability to carry on a normal life. Various systems and methods of the present invention can be used in some cases, as a  
25       replacement for and/or in conjunction with such methods of treatment.

          Yet another set of embodiments provides for the enhancement of bone healing by increasing local blood flow. Bone healing is a slow and complex process, and it is enhanced by a variety of proteins and cells in the blood. An increase in blood flow rate may thus enhance bone healing. In some cases, the application of a nitric oxide and/or a  
30       nitric oxide donor (e.g., arginine and/or an arginine derivative), optionally including an adjunct such as theophylline, may increase blood flow to the bone. Thus, for example, a fractured bone (including a broken bone) may be treated in certain embodiments of the invention.

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In still another set of embodiments, an infection may be treated by increasing local blood flow. The body fights infection using cells and cell derived materials found in the blood. Increasing blood flow to the site of an infection can enhance the body's mechanisms for fighting infection. Thus, the application of a nitric oxide and/or a nitric  
5 oxide donor (e.g., arginine and/or an arginine derivative), optionally including an adjunct such as theophylline, may increase blood flow to the site of infection, which may promote healing.

Another set of embodiments is generally directed to the treatment of blood flow in persons with diabetes, e.g., in the hands and/or feet. The application of a nitric oxide  
10 and/or a nitric oxide donor (e.g., arginine and/or an arginine derivative), optionally including an adjunct such as theophylline, may be used to treat such conditions, thereby increasing blood flow within the hands and/or feet. In some cases, long lasting improvement in blood flow, and/or regression of diabetic neuropathy may be achieved. For example, local blood flow may be increased by at least about 20% or at least about  
15 30%. Still another set of embodiments of the invention are directed to the prevention or treatment of diabetic skin ulcers, e.g., by increasing blood flow, as previously described.

In still another set of embodiments, nitric oxide and/or a nitric oxide donor (e.g., arginine and/or an arginine derivative), optionally including an adjunct such as  
20 theophylline, may be applied to the skin, for example, to a skin graft and/or graft material of a skin graft, to a wound in the skin, etc. Often, skin grafts do not have sufficient blood flow, which may lead to graft failure. By enhancing the blood flow to the skin graft, e.g., using the nitric oxide and/or a nitric oxide donor, graft failure may be reduced. In some cases, the nitric oxide and/or nitric oxide donor may be applied to  
25 tissues proximate the skin graft, and/or the nitric oxide and/or nitric oxide donor may be induced to migrate to tissues adjacent the skin graft.

Another set of embodiments of the invention is directed to the enhancement of the transdermal delivery of a pharmaceutical agent or other beneficial substance through the use of a nitric oxide and/or a nitric oxide donor (e.g., arginine and/or an arginine  
30 derivative), optionally including an adjunct such as theophylline, to increase blood flow at the site of molecular transport. Such embodiments may be relatively simple, inexpensive, and/or non-irritating, and in many cases, no physical or mechanical devices

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are required. Such transport may be further increased, for example, in combination with penetrating agents or the like, as described herein.

Non-limiting examples of pharmaceutical agents include small molecules (e.g., having a molecular weight of less than about 2,000 Da, less than about 1,500 Da, or less than about 1,000 Da), peptides (e.g., having less than about 10, less than about 15, less than about 20, or less than about 25 amino acids), proteins (typically larger than peptides), hormones, vitamins, nucleic acids, or the like. Additional examples of suitable pharmaceutical agents for use with the present invention include, but are not limited to, NSAIDs (nonsteroidal anti-inflammatory drugs) such as acetylsalicylsalicylic acid, naproxen, celecoxib, refecoxib, etc.; pharmaceutical agents with narcotic action such as morphine, codeine, propoxyphene, oxycodone, hydrocodon, or other similar narcotics; pharmaceutical agents for erectile or sexual dysfunction such as yohimbie, alprostadil, sildenafil, cialis, uprima, vardenafil, or the like; pharmaceutical agents for migraine such as dihydroergotamine and its salts, ergotamine and its salts, sumatriptan and its salts, rizatriptan and its salts, zolmitriptan and its salts, etc.; pharmaceutical agents for hair treatment such as finasteride, efloornithine, minoxidil, or the like; or other pharmaceutical agents such as niacin, lidocaine, benzocaine, ibuprofen, etc. Additional examples includes muscle improving agents, for example, creatine or creatine precursors (e.g., creatine phosphate), arginine and/or other nitric oxide donors, and/or ATP precursors such as, inosine, adenosine, inosine, adenine, hypoxanthine, ribose, phosphate (e.g., monosodium phosphate), etc., and/or anabolic steroid agents, such as androstene, DHEA, androstenediol, androstenedione, or the like. Another example is ephedra or its components, such as ephedrine and pseudoephedrine. Yet another example are chemotherapeutic agents or agents for treating cancer and/or viral infections, for example, but not limited to tamoxifen (e.g., for breast cancer treatment), *cis*-platin, carboplatin and related molecules, cyclophosphamide and related molecules, vinca alkaloids, epipodophyllotoxins including taxol, acyclovir, or the like. For example, the cancer and/or viral infections may be skin cancer, breast cancer, penile cancer, testicular cancer, or other localized cancers, or viral infections, such as herpes.

As a particular, non-limiting example, ibuprofen is an effective agent against pain when orally administered. However, it is irritating to the lining of the stomach, and people with a tendency to develop ulcers or have an irritated upper gastrointestinal track are typically warned to avoid the use of ibuprofen. The present invention thus allows the



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topical application of ibuprofen to the site of inflammation or pain, while avoiding the rest of the body, especially the stomach.

As another particular, non-limiting example, while growth hormones, steroids, supplements, and other such agents have been administered orally and by injection to  
5 improve muscle size and function, these muscle improving agents are often distributed throughout the body, resulting in only a small portion of the agent acting at the muscle area being used and developed. Muscle requires both creatine phosphate (CrP) and adenosine triphosphate (ATP) to function. Often muscle has insufficient amounts of these substances and their precursors to maintain high level function. Administration of  
10 these substances and their precursors have been attempted but in low dose that is ineffective and in high dose is both very expensive and produces side effects such as gastrointestinal distress. Use of topical transdermal delivery to the desired muscle or muscles of a muscle improving agent, according to various embodiments, may localize the dose to the desired area, and potentially results in a higher concentration of the agent  
15 at the desired area.

Thus, another aspect of the invention provides for the delivery of beneficial substances such as pharmaceutical agents (e.g., drugs, biological compounds, etc.) into the body, and such treatments may be systemic or localized, e.g., directed to a specific location of the body, such as the head, one or more specific muscles, the genitals, etc.,  
20 depending on the specific application.

In one set of embodiments, pharmaceutical agents are introduced to aid in treatment of medical conditions or diseases, and the symptoms associated thereof. In some embodiments, the invention provides for the treatment of medical conditions or diseases and/or ailments using pharmaceutical agents (for example, to treat a subject  
25 diagnosed with a medical condition or disease, as described herein), and in some cases, the invention provides for the delivery of a minimum amount of pharmaceutical agents to provide effective levels of medication to an effected area topically while limiting side effects. In some cases, the effective dosage of the pharmaceutical agent may be lower than the effective dosage of the pharmaceutical agent when taken orally. Other  
30 embodiments of the invention provides methods for treating cancer, viral infections, erectile dysfunction, sexual dysfunction, an ulcer, swelling, or arthritis. Still another embodiment of the invention provides methods for treating pain, for example, pain from migraine, other headaches, joint pain, muscle pain and other types of pain, Yet another

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embodiment of the present invention provides methods for restoring hair growth, for example, on a portion of the scalp, which may be scarce in hair.

A variety of methods for effecting or improving absorption of beneficial substances (including pharmaceutical agents) are also included in various aspects of the invention. In some cases, a hostile biophysical environment may be used. In a hostile biophysical environment, the environment surrounding the beneficial substance may be such that the beneficial substance is a chemically/energetically unfavorable environment, relative to the skin (e.g., the chemical potential and/or the free energy of the beneficial substance within the hostile biophysical environment is significantly greater than the chemical potential and/or the free energy of the beneficial substance within the skin, thus energetically favoring transport into the skin), especially the stratum corneum. The hostile biophysical environment which raises the chemical potential and/or the free energy of the beneficial substance can be comprised of a high ionic strength, a high concentration of osmotic agents such as ureas, sugars, or carbohydrates, a high pH environment (e.g., greater than about 9, greater than about 10, greater than about 11, greater than about 12, or greater than about 13), a low pH environment (less than about 5, less than about 4, less than about 3 or less than about 2), highly hydrophobic components, or highly hydrophilic components or other substances that cause an increase in the chemical potential and/or free energy of the beneficial substance. A hydrophobic component may have an octanol-water partition coefficient of at least about 100, at least about 1000, at least about  $10^4$ , at least about  $10^5$ , or more in some cases. Similarly, a hydrophilic component may have an octanol-water partition coefficient of less than about 0.01, less than about  $10^{-3}$ , less than about  $10^{-4}$ , or less than about  $10^{-5}$  in some cases.

In some cases, the delivery vehicle defines the biophysical hostile environment. In other cases, the beneficial substance may be packaged in such a way that it is carried into tissue and/or its charge is neutralized by derivitization and/or by forming a neutral salt. Examples of biophysically hostile environments include, but are not limited to, high ionic strength environments (e.g., by the addition of ureas, sugars, carbohydrates, and/or ionic salts such as lithium chloride, sodium chloride, potassium chloride, calcium chloride, magnesium chloride, choline chloride, sodium fluoride, lithium bromide, etc., as well as combinations of these and/or other agents, for instance at high ionic strengths (for example, greater than about 0.25 M, greater than about 1 M, greater than about 2 M, greater than about 3 M, greater than about 5 M, greater than about 10 M, greater than

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about 15 M, greater than about 20 M, greater than about 25 M, etc., or in some cases, between about 0.25 M and about 15 M, between about 5 M and about 15 M, between about 10 M and about 15 M, etc.); high or low pH environments (e.g., by adding pharmaceutically acceptable acids or bases, for example, such that the pH is between  
5 about 3 and about 7, between about 3 and about 6, between about 3 and about 5, between about 7 and about 11, between about 8 and about 11, between about 9 and about 11, etc.); or highly hydrophobic environments (e.g., by decreasing water content and increasing lipid, oil and/or wax content of the environment). Other highly charged molecules such as polylysine, polyglutamine, polyaspartate, etc., or copolymers of such  
10 highly charged amino acids may also be used in certain embodiments to create the hostile biophysical environment. Non-limiting examples of packaging which would be carried into tissue includes liposomes or emulsions of collagen, collagen peptides or other components of skin or basement membrane. Non-limiting examples of neutralization of charge include delivery of the beneficial substance in the form or an  
15 ester or salt which is electronically neutral. In some embodiments, the hostile biophysical environment may include any two or more of these conditions. For instance, the hostile biophysical environment may include high ionic strength and a high pH or a low pH, a highly hydrophobic environment and a high pH or a low pH, a highly hydrophobic environment that includes liposomes, or the like.

20 A hostile biophysical environment may also be created in some embodiments by placing a nitric oxide and/or nitric oxide donor that is relatively highly charged into a hydrophobic, oily environment such as in an oil-based cream or lotion containing little or no water. Absorption may further be aided by combining the use of hostile biophysical environments with the use of penetrating agents, as further described below.

25 It should be noted that a hostile biophysical environment optimized for one beneficial substance may not necessarily be optimal for another beneficial substance. For example, an optimal hostile biophysical environment for a beneficial substance that is non-charged and does not form hydrogen bonds, in one embodiment of the invention, may not necessarily be optimal for other embodiments of the invention, in which a  
30 beneficial substance is charged, and/or in embodiments in which the beneficial substance is able to form hydrogen bonds. Thus, different hostile biophysical environment(s) may be prepared or optimized for different application(s) including different beneficial substance(s) being delivered using the hostile biophysical environment(s).

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In certain aspects of the invention, a pharmaceutical agent or other beneficial substance may be combined with a penetrating agent, i.e., an agent that increases transport of the pharmaceutical agent or other beneficial substance into the skin, relative to transport in the absence of the pharmaceutical agent or other beneficial substance. In  
5 some embodiments, the penetrating agent may be combined with a hostile biophysical environment. Examples of penetrating agents include oleoresin capsicum or its constituents, or certain molecules containing heterocyclic rings to which are attached hydrocarbon chains.

Non-limiting examples of penetrating agents include, but are not limited to,  
10 cationic, anionic, or nonionic surfactants (e.g., sodium dodecyl sulfate, polyoxamers, etc.); fatty acids and alcohols (e.g., ethanol, oleic acid, lauric acid, liposomes, etc.); anticholinergic agents (e.g., benzilonium bromide, oxyphenonium bromide); alkanones (e.g., n-heptane); amides (e.g., urea, N,N-dimethyl-m-toluamide); fatty acid esters (e.g., n-butyrate); organic acids (e.g., citric acid); polyols (e.g., ethylene glycol, glycerol);  
15 sulfoxides (e.g., dimethylsulfoxide); terpenes (e.g., cyclohexene); ureas; sugars; carbohydrates or other agents. In one embodiment, the penetrating agent includes a salt, e.g., as previously described.

In some aspects of the invention, a nitric oxide and/or nitric oxide donor, and/or a pharmaceutical agent or other beneficial substance, may be administered using a delivery  
20 vehicle such as a cream, gel, liquid, lotion, spray, aerosol, or transdermal patch. Examples of delivery vehicles are discussed below. The delivery vehicle may promote transfer into the skin of an effective concentration of the nitric oxide and/or nitric oxide donor, and/or a pharmaceutical agent or other beneficial substance, directly or indirectly. For instance, the delivery vehicle may include one or more penetrating agents, as further  
25 described herein. In some embodiments, the delivery vehicle may include a hostile biophysical environment, e.g., using a penetrating agent, etc., as described herein. Those of ordinary skill in the art will know of systems and techniques for incorporating a nitric oxide and/or nitric oxide donor, and/or a pharmaceutical agent or other beneficial substance within delivery vehicles such as a cream, gel, liquid, lotion, spray, aerosol, or  
30 transdermal patch. In some cases, the concentration of the nitric oxide and/or nitric oxide donor, and/or a pharmaceutical agent or other beneficial substance in the delivery vehicle can be reduced with the inclusion of a greater amount or concentration of penetrating agent, or increased to lengthen the beneficial effect. In one set of

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embodiments, the nitric oxide and/or nitric oxide donor, and/or a pharmaceutical agent or other beneficial substance may be used in conjunction with an adjunct, such as theophylline (for example, at 10% weight by volume).

In some embodiments, the cream may include a nitric oxide and/or nitric oxide donor, and one or more ionic salts at a concentration at least sufficient to produce a hostile biophysical environment with respect to the beneficial substance. Other materials may be present within the delivery vehicle, for example, buffers, preservatives, surfactants, etc. For instance, the cream may include one or more of water, mineral oil, glyceryl stearate, squalene, propylene glycol stearate, wheat germ oil, glyceryl stearate, isopropyl myristate, steryl stearate, polysorbate 60, propylene glycol, oleic acid, tocopherol acetate, collagen, sorbitan stearate, vitamin A and D, triethanolamine, methylparaben, aloe vera extract, imidazolidinyl urea, propylparaben, PND, or BHA.

As specific non-limiting examples, the cream may have one or more of (w/v): water (20-80%), white oil (3-18%), glyceryl stearate (0.25-12%), squalene (0.25-12%), cetyl alcohol (0.1-11%), propylene glycol stearate (0.1-11%), wheat germ oil (0.1-6%), polysorbate 60 (0.1-5%), propylene glycol (0.05-5%), collagen (0.05-5%), sorbitan stearate (0.05-5%), vitamin A (0.02-4%), vitamin D (0.02-4%), vitamin E (0.02-4%), triethanolamine (0.01-4%), methylparaben (0.01-4%), aloe vera extract (0.01-4%), imidazolidinyl urea (0.01-4%), propylparaben (0.01-4%), BHA (0.01-4%), L-arginine Hydrochloride (0.25-25%), sodium chloride (0.25-25%), magnesium chloride (0.25-25%), and/or choline chloride (0.25-25%). The percentages of each compound can vary (or the compound may be absent in some cases), for example, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 20%, etc.

In another embodiment, the cream may include a beneficial substance, such as ibuprofen, and one or more of the following: water (20-80%), L-arginine hydrochloride (0-25%), sodium chloride (0-25%), potassium chloride (0-25%), glyceryl stearate (0-15%), cetyl alcohol (0-15%), squalene (0-15%), isopropyl myristate (0-15%), oleic acid (0-15%), Tween 20 (0-10%), and/or butanediol (0-10%). The percentages of each compound can vary (or the compound may be absent in some cases), for example, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 20%, etc.

In some embodiments, the cream may include a beneficial substance, and one or more ionic salts at a concentration at least sufficient to produce a hostile biophysical

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environment with respect to the beneficial substance. For example, the cream may include one or more of (w/v): a charged and/or hydrogen bonding beneficial substance with systemic toxicity (0.001-30%), choline chloride (1-30%), sodium chloride (2-30%), and/or magnesium chloride (1-20% w/v). In another example, the cream may include  
5 one or more of (w/v): L-arginine hydrochloride (2.5-25%), choline chloride (10-30%), sodium chloride (5-20%), and/or magnesium chloride (5-20%). In still another example, the cream may include one or more of (w/v): creatine (0.001-30%), inosine (0.001-30%), choline chloride (1-30%), sodium chloride (2-30%), magnesium chloride (1-20%), L-arginine (0.1-25%), and/or theophylline (0.1-20%). In some cases, the cream may also  
10 contain L-arginine hydrochloride (0-12.5% w/v) and/or theophylline (0-10% w/v). The percentages of each compound can vary (or the compound may be absent in some cases), for example, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 20%, etc. In these examples, choline chloride, sodium chloride and magnesium chloride can provide a high ionic strength environment.

15 In certain aspects of the invention, multiple treatments of the delivery vehicle may increase the duration of the effects of the nitric oxide and/or the nitric oxide donor, for example two, three, four, five, or more treatments may be applied, depending on the particular application. For example, with repeated administrations, the beneficial effects of each treatment may be extended up to ten or twenty hours after treatment, or more in  
20 some cases. Such treatments may be given at any suitable frequency, depending on the particular application, for example, every 4 hours, every 8 hours, every 12 hours, every 18 hours, every 1 day, every 2 days, every 3 days, every week, etc. For instance, the treatment may be provided between about 2 and about 30 times within a time period of about 30 days. In some cases, the first treatment may be given at a higher level or  
25 concentration than subsequent treatments.

The following examples are intended to illustrate certain embodiments of the present invention, but do not exemplify the full scope of the invention.

#### EXAMPLE 1

A 58 year old man suffering from claudication of the lower leg applied a cream  
30 comprising L-arginine hydrochloride (12.5% w/v), choline chloride (10% w/v), magnesium chloride (5% w/v), and sodium chloride (5% w/v) in a penetrating base to his legs nightly. After using it daily for three days, the cramps from claudication never recurred as long as he continued daily use of the cream.

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### EXAMPLE 2

A 72 year old man with a twelve year history of PAD and claudication severely incapacitating him was treated in this example. He began daily use of a cream containing L-arginine hydrochloride (12.5% w/v), choline chloride (10% w/v),  
5 magnesium chloride (5% w/v), and sodium chloride (5% w/v) in a penetrating base to his lower legs. After three days of use the frequency of the attacks were markedly reduced, and after ten days the attacks had ceased. Continued daily use of the cream continued to prevent attacks.

### EXAMPLE 3

10 Circulatory impairment and its sequelae have long been known to be a major complication of diabetes. For instance, it has been shown that, in diabetes, the functionality of the endothelial nitric oxide (NO)/nitric oxide synthase (eNOS) system is impaired. NO is generated in the endothelium through the oxidation of the amino acid, L-arginine by the enzyme eNOS. NO causes vascular smooth muscle to relax resulting  
15 in increased blood flow. In addition to being a substrate of eNOS, L-Arginine facilitates the dimerization of two identical subunits of eNOS, forming a homodimer. The enzyme is only active in the dimeric form. Under proper conditions, dimerization occurs rapidly, on a timescale of minutes. Once formed the dimer is generally stable.

Subjects with diabetes may have abnormally low levels of L-Arginine and  
20 elevated levels of the eNOS inhibitor, asymmetric dimethylarginine (ADME) in their plasma. Though the value of increasing L-Arginine levels in cases of impaired circulation is now recognized, practical schemes for therapeutic use of L-Arginine have been illusive. In this example, it was determined whether supplying L-Arginine transdermally would improve vascular function of the feet in patients with diabetes, as  
25 indicated by flow and temperature.

The example was designed as a double-blind vehicle-controlled two-period crossover protocol, with washout periods of one week. Sixteen subjects were enrolled and thirteen completed the study (age 56 +/- 8 yr). After analyzing the data it was shown that the effect of L-arginine persisted throughout the washout periods (Tables 1 and 2,  
30 AU standing for Arbitrary Units). Because of this, except for the initial exposure of L-arginine on virgin feet, the analysis was altered to determine the effect from cumulative exposure to L-arginine throughout the protocol. Blood flow was measured at the metatarsal and Achilles area using a Doppler flow meter, and temperature was measured

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at the metatarsal and big toe areas using an infrared thermometer. The active cream was a water-based moisturizing vehicle containing 12.5% L-arginine hydrochloride in a hostile biophysical environment comprising a high concentrations of choline chloride, sodium chloride and magnesium chloride. The control vehicle was identical, except that  
5 the L-arginine was omitted.

At the first visit, after baseline measurements were made each subject rubbed active cream (4 mg of L-arginine/cm<sup>2</sup>) into one foot and vehicle into the other. After thirty minutes measurements were made again. A one week wash out period followed. Patients returned after the wash out period and flow and temperature measurements were  
10 made. They were then randomly given either active or placebo cream and told to rub it into their feet in the morning and evening every day for two weeks. At the end of two weeks subjects returned and again measurements were made. A second one week wash out period followed that third visit. At the end of that period subjects returned and measurements were made. They were given the cross over product and told again to rub  
15 it into their feet morning and evening for two weeks. The subjects returned for final flow and temperature measurements at the end of that period.

At the first visit flow was increased at the Achilles in the foot with active cream from 8.1 +/- 3.3 to 11.5 +/- 5.5 (p=0.05) thirty minutes after application. In the foot that received placebo cream flow failed to increase (8.1 +/-1.4 vs. 8.3 +/- 2.2). Further, at the  
20 last visit the temperature at the metatarsal area had risen from the initial value of 82.0 +/- 2.3 to 86.9 +/-2.4 (p<0.0001) and the temperature of the big toe had risen from the initial visit value of 74.4 +/- 4.2 to 82.4 +/-4.8 (p< 0.0001). At the last visit the flow at the metatarsal area had risen from 8.7 +/-4.3 to 11.6 +/- 5.5 (p<0.0001) and flow at the Achilles area had risen from 8.4 +/- 2.5 to 11.4 +/- 5.5 (p=0.02). While the failure of the  
25 L-arginine effect to wash out removed the opportunity for placebo control, the improvement in temperature and flow were substantial and highly statistically significant. Though puzzling, one explanation of the persistence of the L-arginine effect is that the local tissue concentration of L-arginine becomes high enough to cause inactive monomers of eNOS to form active dimers.

30 Thus, in the patients studied in this example with diabetes, treatment of their feet with a transdermal preparation of L-arginine improved both flow and temperature, and this effect was surprisingly long lasting. Such improvement of compromised local blood flow would be beneficial and could reduce the complications of the disease.



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**Table 1. Effect of Transdermal L-Arginine Cream on Temperature**

	<u>Metatarsal (°F)</u>	<u>p vs. Visit 1</u>	<u>Big Toe (°F)</u>	<u>p vs Visit 1</u>
Visit 1	82.0 +/- 2.3		74.4 +/- 4.2	
5 Visit 2	84.1 +/- 3.4	0.004	77.7 +/- 5.3	0.01
Visit 3	87.0 +/- 2.4	<0.0001	83.6 +/- 4.9	<0.0001
Visit 4	86.1 +/- 2.4	<0.0001	80.6 +/- 5.4	<0.0001
Visit 5	86.9 +/- 2.4	<0.0001	82.4 +/- 4.8	<0.0001

**Table 2. Effect of Transdermal L-Arginine Cream on Flow**

	<u>Metatarsal (AU)</u>	<u>p vs. Visit 1</u>	<u>Achilles (AU)</u>	<u>p vs. Visit 1</u>
Visit 1	8.7 +/- 4.3		8.4 +/- 2.5	
Visit 2	10.8 +/- 5.9	NS	8.5 +/- 3.9	NS
Visit 3	10.8 +/- 4.8	0.05	9.2 +/- 3.9	NS
15 Visit 4	11.6 +/- 8.3	NS	10.0 +/- 4.2	0.06
Visit 5	11.6 +/- 5.5	<0.0001	11.4 +/- 5.5	0.02

**EXAMPLE 4**

In this example, a 57 year old woman with severe arthritis in her hands and fingers applied a cream comprising a hostile biophysical environment, along with 10% w/v ibuprofen and 12.5% w/v L-arginine, to her hands. She rubbed the cream into the skin of her hands and fingers until completely absorbed. Within 10 minutes she noticed substantial relief from the pain. Within 30 minutes the pain was completely gone. Pain relief persisted for several hours.

**EXAMPLE 5**

In this example, a 37 year old man with shoulder pain applied a cream comprising a hostile biophysical environment, along with 10% w/v ibuprofen and 12.5% w/v L-arginine, to the painful shoulder. He rubbed the cream in until it was completely absorbed. Within 30 minutes the pain was completely gone. The pain never returned.

**EXAMPLE 6**

A 54 year old woman with a severe headache in her right temple applied a cream comprising a hostile biophysical environment, 10% w/v ibuprofen, and 12.5% w/v L-arginine to the painful temple. She rubbed the cream in until completely absorbed.

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Within 10 minutes substantial relief of the headache was achieved. Within 20 minutes the pain was gone. The pain never returned.

#### EXAMPLE 7

A 33 year old woman with a history of genital herpes infection was treated with a topical transdermal preparation of acyclovir. Herpes is characterized by outbreaks which start as a red, sometimes itching area and progress to open sores. The acyclovir preparation included a hostile biophysical environment, 2.5% w/v acyclovir, and 12.5 % w/v L-Arginine. This preparation was applied as soon as the red and sometimes itching areas appeared. This treatment resulted in regression of the insipient herpes outbreak, returning the area to normal within two days and preventing the open sores from developing.

#### EXAMPLE 8

This example illustrates one method of preparing a transdermal formula of the invention including ibuprofen. The final composition is shown in Table 3. Of course, those of ordinary skill in the art will understand that percentages other than the ones listed below are also possible, according to other embodiments of the invention.

**Table 3. Example of a Transdermal Preparation**

20	Water	49%
	L-Arginine Hydrochloride	7.5%
	Ibuprofen (sodium salt)	7.5%
	Sodium Chloride	10%
	Potassium Chloride	5%
25	Glyceryl Stearate (SE)	7%
	Cetyl Alcohol	7%
	Squalene	2%
	Isopropyl Myristate	1%
	Oleic Acid	1%
30	Tween 20	2%
	Butanediol	1%

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To prepare the formulation in this example, sodium chloride, potassium chloride, L-arginine and ibuprofen were mixed in water, then heated to 74 degrees C with rapid mixing. In a separate container, the remaining ingredients were mixed together and heated to 74 degrees C. The other ingredients were then added to the water phase at 74  
5 degrees C with rapid mixing. The mixture was then cooled to room temperature with continued mixing. At this point, an emulsion formed with a relatively thin consistency. The emulsion was then homogenized at high speed at room temperature to thicken the consistency.

10 According to aspects of the invention described and illustrated herein, beneficial substance(s) (e.g., pharmaceutical agent(s)) may be provided (e.g., in a delivery vehicle) at a concentration of between about 0.1% and about 25% (for example at a concentration of 0.1%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%,  
15 15%, 20%, etc.). However, higher (e.g., above 25%, 30%, 40%, 50% or higher) or lower (e.g., below 0.1%, 0.05% or lower) concentrations of beneficial substance(s) may be used. As used herein (for a beneficial substance or any other compound described herein) a concentration % may be a % by weight, a % by volume, or a % weight by volume. As used herein, a beneficial substance may be, for example, a charged  
20 beneficial substance, a non-charged beneficial substance, a beneficial substance that forms hydrogen bonds, a beneficial substance that does not form hydrogen bonds, etc.

While several embodiments of the present invention have been described and illustrated herein, those of ordinary skill in the art will readily envision a variety of other means and/or structures for performing the functions and/or obtaining the results and/or one or more of the advantages described herein, and each of such variations and/or  
25 modifications is deemed to be within the scope of the present invention. More generally, those skilled in the art will readily appreciate that all parameters, dimensions, materials, and configurations described herein are meant to be exemplary and that the actual parameters, dimensions, materials, and/or configurations will depend upon the specific application or applications for which the teachings of the present invention is/are used.  
30 Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. It is, therefore, to be understood that the foregoing embodiments are presented by way of example only and that, within the scope of the appended claims and

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equivalents thereto, the invention may be practiced otherwise than as specifically described and claimed. The present invention is directed to each individual feature, system, article, material, kit, and/or method described herein. In addition, any combination of two or more such features, systems, articles, materials, kits, and/or methods, if such features, systems, articles, materials, kits, and/or methods are not mutually inconsistent, is included within the scope of the present invention.

All definitions, as defined and used herein, should be understood to control over dictionary definitions, definitions in documents incorporated by reference, and/or ordinary meanings of the defined terms.

The indefinite articles “a” and “an,” as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean “at least one.”

The phrase “and/or,” as used herein in the specification and in the claims, should be understood to mean “either or both” of the elements so conjoined, i.e., elements that are conjunctively present in some cases and disjunctively present in other cases. Multiple elements listed with “and/or” should be construed in the same fashion, i.e., “one or more” of the elements so conjoined. Other elements may optionally be present other than the elements specifically identified by the “and/or” clause, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, a reference to “A and/or B”, when used in conjunction with open-ended language such as “comprising” can refer, in one embodiment, to A only (optionally including elements other than B); in another embodiment, to B only (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); etc.

As used herein in the specification and in the claims, “or” should be understood to have the same meaning as “and/or” as defined above. For example, when separating items in a list, “or” or “and/or” shall be interpreted as being inclusive, i.e., the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally, additional unlisted items. Only terms clearly indicated to the contrary, such as “only one of” or “exactly one of,” or, when used in the claims, “consisting of,” will refer to the inclusion of exactly one element of a number or list of elements. In general, the term “or” as used herein shall only be interpreted as indicating exclusive alternatives (i.e. “one or the other but not both”) when preceded by terms of exclusivity, such as

“either,” “one of,” “only one of,” or “exactly one of.” “Consisting essentially of,” when used in the claims, shall have its ordinary meaning as used in the field of patent law.

As used herein in the specification and in the claims, the phrase “at least one,” in reference to a list of one or more elements, should be understood to mean at least one element selected from any one or more of the elements in the list of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements and not excluding any combinations of elements in the list of elements. This definition also allows that elements may optionally be present other than the elements specifically identified within the list of elements to which the phrase “at least one” refers, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, “at least one of A and B” (or, equivalently, “at least one of A or B,” or, equivalently “at least one of A and/or B”) can refer, in one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another embodiment, to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another embodiment, to at least one, optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); etc.

It should also be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one step or act, the order of the steps or acts of the method is not necessarily limited to the order in which the steps or acts of the method are recited.

In the claims, as well as in the specification above, all transitional phrases such as “comprising,” “including,” “carrying,” “having,” “containing,” “involving,” “holding,” “composed of,” and the like are to be understood to be open-ended, i.e., to mean including but not limited to. Only the transitional phrases “consisting of” and “consisting essentially of” shall be closed or semi-closed transitional phrases, respectively, as set forth in the United States Patent Office Manual of Patent Examining Procedures, Section 2111.03.

The reference to any prior art in this specification is not, and should not be taken as, an acknowledgement or any form of suggestion that the prior art forms part of the common general knowledge in Australia.

What is claimed is:

**CLAIMS**

1. A method, comprising:  
administering a delivery vehicle to a subject having or at risk of claudication, wherein the delivery vehicle comprises a nitric oxide donor and a hostile biophysical environment having an ionic strength of at least about 0.25 M.
2. The method of claim 1, comprising administering the delivery vehicle to a leg of the subject.
3. The method of claim 1, comprising administering the delivery vehicle to a foot of the subject.
4. The method of any preceding claim, wherein the delivery vehicle is a cream.
5. The method of any preceding claim, wherein the nitric oxide donor is L-arginine, L-arginine hydrochloride, and/or a derivative of L-arginine.
6. The method of claim 5, wherein the nitric oxide donor derivative is L-arginine methyl ester.
7. The method of claim 5, wherein the nitric oxide donor derivative is L-arginine butyl ester.
8. The method of any preceding claim, wherein the nitric oxide donor is present at a concentration of between about 0.05% and about 25% by weight.
9. The method of any preceding claim, wherein the hostile biophysical environment has an ionic strength of at least about 1 M.

10. The method of any preceding claim, wherein the hostile biophysical environment comprises one or more of sodium chloride, choline chloride, magnesium chloride or calcium chloride.
11. The method of any preceding claim, wherein the hostile biophysical environment comprises a urea.
12. The method of any preceding claim, wherein the hostile biophysical environment comprises a carbohydrate.
13. The method of any preceding claim, wherein the hostile biophysical environment comprises a component having an octanol-water partition coefficient of at least about 1000.
14. The method of claim 13, wherein the octanol-water partition coefficient is less than about  $10^{-3}$ .
15. The method of any preceding claim, wherein the hostile biophysical environment comprises one or more of sodium chloride, potassium chloride, choline chloride, magnesium chloride and calcium chloride.
16. The method of any preceding claim, wherein the hostile biophysical environment has a pH of at least 9.
17. The method of any one of claims 1-15, wherein the hostile biophysical environment has a pH of less than about 5.
18. The method of any one of claims 1-15, wherein the hostile biophysical environment has a pH of between about 3 and about 11.

19. The method of any preceding claim, wherein the nitric oxide donor has a concentration of between about 0.05% and about 25% by weight.
20. The method of any preceding claim, wherein the delivery vehicle further comprises one or more of water, mineral oil, glyceryl stearate, squalene, propylene glycol stearate, wheat germ oil, glyceryl stearate, isopropyl myristate, steryl stearate, polysorbate 60, propylene glycol, oleic acid, tocopherol acetate, collagen, sorbitan stearate, vitamin A, vitamin D, triethanolamine, methylparaben, aloe vera extract, imidazolidinyl urea, or propylparaben.
21. The method of any preceding claim, wherein the delivery vehicle further comprises an NSAID.
22. The method of claim 21, wherein the NSAID comprises ibuprofen.
23. The method of any preceding claim, wherein the delivery vehicle further comprises one or more of naproxen, celecoxib, refecoxib, morphine, propoxyphene, oxycodone or hydrocodon.