(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
18 October 2001 (18.10.2001)

PCT

(10) International Publication Number
WO 01/76553 A2

(51) International Patent Classification[7]: A61K 9/00

(21) International Application Number: PCT/US01/11120

(22) International Filing Date: 6 April 2001 (06.04.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/195,041 6 April 2000 (06.04.2000) US

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

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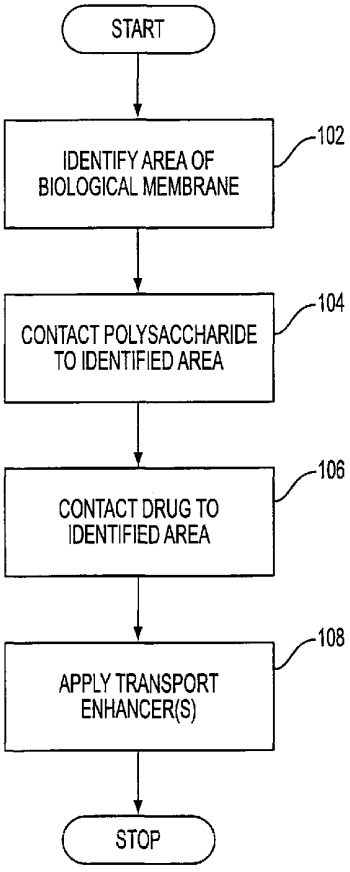
(54) Title: METHOD AND DEVICE FOR ENHANCED TRANSDERMAL DRUG DELIVERY



(57) Abstract: A method and device for enhanced transdermal drug delivery are disclosed. According to one embodiment of the present invention, the method includes the steps of (1) identifying an area of biological membrane: (2) contacting at least one high-permeability polysaccharide with the identified area of biological membrane; (3) contacting at least one drug with the identified area of biological membrane; and (4) transporting the at least one drug into or through the identified area of biological membrane. According to another embodiment of the present invention, a method for the enhanced transport of a drug into or through a biological membrane includes the steps of (1) preparing a complex of the drug with a high-permeability polysaccharide; and (2) transporting the complex into or through the biological membrane. According to another embodiment of the present invention, a device for enhanced drug delivery includes at least one high-permeability polysaccharide and at least one drug. The device may also include a contact layer that contacts a biological membrane and a medium layer containing the at least one high-permeability polysaccharide and the at least one drug .

WO 01/76553 A2

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

METHOD AND DEVICE FOR ENHANCED TRANSDERMAL DRUG DELIVERY

CROSS-REFERENCE TO RELATED APPLICATIONS

The present invention claims the benefit of U.S. Provisional Patent Application No. 60/195,041, filed April 6, 2000, the disclosure of which is hereby incorporated by reference in its entirety.

In addition, the invention is related to U.S. Patent Application No. 08/885,931, entitled "Ultrasound Enhancement of Transdermal Transport"; U.S. Patent Application No. 09/260,265, entitled "Chemical and Physical Enhancers and Ultrasound for Transdermal Drug Delivery"; and PCT International Patent Application No. PCT/US99/30067, entitled "Method and Apparatus for Enhancement of Transdermal Transport", the disclosures of which are hereby incorporated, by reference, in their entireties.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to transdermal drug delivery, and, more particularly, to a method and device for transdermal drug delivery of proteins and peptides.

2. Description of the Related Art

Transdermal drug delivery ("TDD") offers several advantages over traditional delivery methods including injections and oral delivery. When compared to oral delivery, TDD avoids gastrointestinal drug metabolism, reduces first-pass effects, and provides sustained release of drugs for up to seven days, as reported by Elias, in Percutaneous Absorption: Mechanisms-Methodology-Drug Delivery, Bronaugh, R. L. et al. (Eds), pages 1-12, Marcel Dekker, New York (1989).

The skin is a complex structure. There are at least four distinct layers of tissue: the nonviable epidermis (stratum corneum, "SC"), the viable epidermis, the viable dermis, and the subcutaneous connective tissue. Located within these layers are the skin's circulatory system, the arterial plexus, and appendages, including hair follicles, sebaceous glands, and sweat glands. The circulatory system lies in the dermis and tissues below the dermis. The capillaries do not actually enter

the epidermal tissue but come within 150 to 200 microns of the outer surface of the skin. The highly-ordered structure of the lipid bilayers confers an impermeable character to the SC (Flynn, G. L., in Percutaneous Absorption: Mechanisms-Methodology-Drug Delivery; Bronaugh, R. L. et al. (Eds.), pages 27-53, Marcel Dekker, New York, (1989). The word "transdermal" is used herein as a generic term. However, in actuality, transport of drugs occurs only across the epidermis where the drug is absorbed in the blood capillaries. In comparison to injections, TDD can reduce or eliminate the associated pain and the possibility of infection.

Several methods have been proposed to enhance transdermal drug transport, including the use of chemical enhancers, i.e., the use of chemicals to either modify the skin structure or to increase the drug concentration in a transdermal patch (Burnette, R. R., in Developmental Issues and Research Initiatives; Hadgraft J., et al. (Eds.), pages 247-288, Marcel Dekker, New York (1989); Junginger, et al. in Drug Permeation Enhancement; Hsieh, D. S., (Eds.), pages 59-90; Marcel Dekker, New York (1994)) and the use of applications of electric fields to create transient transport pathways [electroporation] or to increase the mobility of charged drugs through the skin [iontophoresis] (Prausnitz, Proc. Natl. Acad. Sci. USA 90: 10504-10508 (1993); Walters, K. A., in Transdermal Drug Delivery: Developmental Issues and Research Initiatives, Hadgraft J., Guy, R. H., (Eds.) Marcel Dekker, New York (1989)). Another approach that has been explored is the application of ultrasound.

Ultrasound has been shown to enhance transdermal transport of low-molecular weight drugs (molecular weight less than 500) across human skin, a phenomenon referred to as sonophoresis. See Levy, J. Clin. Invest. 1989, 83, 2974-2078; Kost and Langer in "Topical Drug Bioavailability, Bioequivalence, and Penetration"; pp. 91-103, Shah V. P., M. H. I., Eds. (Plenum: New York, 1993); Frideman, R. M., "Interferons: A Primer", Academic Press, New York, 1981, the disclosures of which are incorporated, by reference, in their entireties. Although a variety of ultrasound conditions have been used for sonophoresis, the most commonly used conditions correspond to therapeutic ultrasound (frequency in the range of between 1 MHz and 3 MHz, and intensity in the range of between above zero and two W/cm^2. See U.S. Pat. No. 4,767,402 to Kost, et al, the disclosure of which is incorporated, by reference, in its entirety. It is a common observation that

the typical enhancement induced by therapeutic ultrasound is less than ten-fold. In many cases, no enhancement of transdermal drug transport has been observed upon ultrasound application. U.S. Pat. Nos. 5,458,140 and 5,445,611 to Eppstein et al., the disclosures of which are incorporated, by reference, in their entireties, disclose the use of ultrasound at a frequency range of between 0.1 and 100 MHz, preferably between 3 and 30 MHz, in combination with chemical enhancers, to enhance skin permeability. The ultrasound was frequency, intensity and/or phase modulated. An increase in permeability was noted during application of the ultrasound but decreased to passive diffusion rates when ultrasound was discontinued (see Example 4 in both patents). Other examples of the use of ultrasound are provided in U.S. Patent No. 6,190,315; U.S. Patent No. 5,947,921; and U.S. Patent No. 6,002,961, the disclosures of which are incorporated, by reference, in their entireties.

U.S. Pat. No. 5,323,769 to Bommannan, the disclosure of which is incorporated by reference in its entirety, discloses ultrasound enhanced delivery of molecules into and through the skin, in combination with chemical permeation enhancers. The ultrasound is applied at frequencies above 10 MHz. The ultrasound must be applied "relatively simultaneously" with the molecules being delivered, within at least six minutes, preferably within two minutes.

Application of low frequency (between approximately 20 and 200 kHz) ultrasound can dramatically enhance transdermal transport of molecules when applied directly to the drug or at the time of collection, as described in WO 97/04832 by Massachusetts Institute of Technology. Transdermal transport enhancement induced by low-frequency ultrasound was found to be as much as 1000-fold higher than that induced by therapeutic ultrasound.

The application of a motive force before, during, and after enhancing the permeability of the skin has been disclosed in U.S. Patent No. 5,279,543, U.S. Patent No. 5,722,397, U.S. Patent No. 5,947,921, U.S. Patent No. 6,002,961, and U.S. Patent No. 6,009,343, the disclosures of which are incorporated by reference in their entireties. The purpose of using a motive force is to actively deliver drugs into or through skin. Active forces, such as pressure, sonophoresis, and electrosmotic forces, can create convective flow through the stratum corneum.

SUMMARY OF THE INVENTION

A need has arisen for method and device for transdermal drug delivery of proteins and peptides that overcomes these and other drawbacks of the related art.

According to one embodiment of the present invention, a method for transdermal delivery of proteins and peptides is disclosed. This method may also be used to deliver proteins and peptides across other biological membranes including, but not limited to, cell membranes, oral mucosa, nasal mucosa and pulmonary alveoli. The present invention uses the ability of polysaccharides to permeate biological membranes and stabilize as well as carry proteins/peptides across the membranes. The formulation can be prepared by, for example, combining the protein with polysaccharides in multiple ways including but not limited to: i) co-solvation of the drug and polysaccharide in a solvent, ii) tethering of the drug to the polysaccharide, and iii) microparticles containing the drug and the polysaccharide. Examples of polysaccharides include dextran, heparin, and hyaluronic acid.

According to one embodiment of the present invention, a method for the enhanced delivery of at least one drug across a biological membrane is provided. The method includes the steps of (1) identifying an area of biological membrane; (2) contacting at least one high-permeability polysaccharide with the identified area of biological membrane; (3) contacting at least one drug with the identified area of biological membrane; and (4) transporting the at least one drug into or through the identified area of biological membrane. Steps (2) and (3) may occur in any order, or may occur substantially simultaneously. The biological membrane may include tissue, mucous membranes, cornified tissues, oral mucosa, skin, nasal membrane, pulmonary capillary wall, cell membrane, organs, tissues, buccal, nails, and the gastro-intestinal tract. The polysaccharide may have a permeability of at least 1×10^{-5} cm/hr. Preferred polysaccharides include hyaluronic acid, heparin, dextran, chondroitin sulfate, and salts thereof.

In one embodiment, ultrasound may be applied to the biological membrane to enhance the permeability of the biological membrane. The ultrasound may be applied before, during, or after the high-permeability polysaccharide is contacted with the biological membrane.

In one embodiment, a driving force, such as an osmotic pressure gradient, a concentration gradient, iontophoresis, electroporation, magnetic fields, ultrasound, and mechanical pressure, may be applied to alter the movement of the drug through or into the biological membrane.

According to another embodiment of the present invention, a method for the enhanced transport of a drug into or through a biological membrane is disclosed. The method includes the steps of (1) preparing a complex of the drug with a high-permeability polysaccharide; and (2) transporting the complex into or through the biological membrane. The high-permeability polysaccharide may include hyaluronic acid, heparin, dextran, and chondroitin sulfate, and salts thereof. The complex may be delivered by injection, inhalation, oral ingestion, or by contact with the biological membrane. The complex may be in the form of a gel, a spray, a liquid, a powder, microdroplets, an ointment, and a cream.

According to another embodiment of the present invention, a device for enhanced drug delivery is disclosed. The device includes at least one high-permeability polysaccharide and at least one drug. The device may also include a contact layer that contacts a biological membrane and a medium layer containing the at least one high-permeability polysaccharide and the at least one drug. The device may be a wearable patch. The device may also include a source of permeabilizing force, and source of a driving force.

In one embodiment, the high-permeability polysaccharide may have a permeability of at least 1×10^{-5} cm/hr, and includes hyaluronic acid, heparin, dextran, and chondroitin sulfate, and salts thereof. The drug and the high-permeability polysaccharide may be complexed.

A technical advantage of the present invention is that a method for delivering drugs through, into, or across biological membranes involving the use of complexes of proteins/peptides with polysaccharides is disclosed. Another technical advantage of the present invention is that a method for delivering drugs across a biological membrane, including oral mucosa, nasal membrane, pulmonary capillary wall, or cell membrane is provided. Another technical advantage of the present invention is that a time for a drug to cross a biological membrane is reduced. Another technical advantage is that the high-permeability polysaccharides are very

biocompatible. Another technical advantage of the present invention is that a complex of proteins/peptides with polysaccharide is achieved by tethering of the drug to the polysaccharide through electrostatic interactions, covalent interactions, hydrogen bonding, hydrophobic interactions, van der Waals forces, or other interactions. Yet another technical advantage of the present invention is that the complex of the microparticle with polysaccharide is achieved by attaching polysaccharide on the surface of the microparticle. Another technical advantage of the present invention is that the drug may be entrapped in a polysaccharide and the release of the drug may be sustained. Still another technical advantage of the present invention is that the complex of polysaccharide with the protein/peptide can be made into various formulations, including a solution for transdermal drug delivery or injections, dry powder for inhalation, gels, sprays, microdroplets, a suspension of microparticles for oral delivery, and patches. Another technical advantage of the present invention is that additional permeabilization methods, such as the use of ultrasound, may be used to increase the permeability of a biological membrane. A technical advantage of the present invention is that additional driving forces may be applied in order to assist in the drug delivery through or into the biological membrane.

BRIEF DESCRIPTION OF THE DRAWINGS

For a more complete understanding of the present invention, the objects and advantages thereof, reference is now made to the following descriptions taken in connection with the accompanying drawings in which:

Fig. 1 is a flowchart of a method for transdermal drug delivery using polysaccharides according to one embodiment of the present invention;

Fig. 3 is a graph depicting the enhanced transdermal delivery of LHRH by hyaluronic acid according to one embodiment of the present invention;

Fig. 4 is a plot of skin permeability versus donor hyaluronic acid concentration according to one embodiment of the present invention; and

Fig. 5 is a plot of skin permeability versus donor dextran concentration according to one embodiment of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

The preferred embodiment of the present invention and its advantages are best understood by referring to **Figs. 1** through **5** of the drawings, like numerals being used for like and corresponding parts of the various drawings.

Although the present invention may be described in conjunction with human applications, veterinary applications are within the contemplation and the scope of the present invention.

In order to assist in the understanding of the present invention, the following definitions are provided:

"Ultrasound" is defined as sound at a frequency of greater than about 20 kHz. "Therapeutic ultrasound" is typically between 20 kHz and 5 MHz. "Near ultrasound" is typically about 10 kHz to about 20 kHz. It should be understood that in addition to ultrasound, near ultrasound can be used in embodiments of the present invention.

"Sonophoresis" is defined as the application of ultrasound to the biological membrane resulting in enhanced transdermal transport of molecules.

"Low frequency sonophoresis or ultrasound" is defined as sonophoresis or ultrasound at a frequency that is less than 2.5 MHz, more typically less than 1 MHz, more preferably in the range of 20 to 100 kHz.

"Biological membrane" is defined as tissue, mucous membranes, cornified tissues, oral mucosa, skin, nasal membrane, pulmonary capillary wall, cell membrane, organs, tissues, buccal, the gastro-intestinal tract, and nails, as well as other biological surfaces.

"Drug" is defined as a therapeutic, prophylactic, or diagnostic molecule or agent, and can be in a form dissolved or suspended in a liquid, a solid, or encapsulated and/or distributed in or within micro- or nano-particles, emulsions, liposomes or lipid vesicles.

"Drug delivery" is defined as the delivery of a drug into blood, lymph, interstitial fluid, a cell, tissue, or skin.

"Transdermal transport" is defined as movement of analyte into or through the biological membrane or delivery of drug into or through a biological membrane.

"Transdermal patch" is an externally-applied device for delivery or extraction of molecules into or through the biological membrane.

"Driving force" means a chemical or physical force that alters movement of a drug into or through a biological membrane.

Polysaccharides are polymers of sugar residues that have a high molecular weight ("MW"). Some polysaccharides, however, have a unique ability to cross biological membranes, such as skin, at a relatively high rate. This property of polysaccharides is especially significant because the monomers that comprise the polysaccharides permeate biological membranes at a relatively slow rate. For example, monomeric or dimeric sugar molecules, such as glucose, mannitol, and sucrose permeate skin with a permeability rate of about 1×10^{-5} cm/hr. It is well-known that hydrophilic molecules permeate biological membranes at a slow rate. This is partly due to the low partition coefficient of hydrophilic molecules in the lipid bilayers of membranes. In addition, it is also well-known that molecules having a MW of higher than about 500 Da permeate biological membrane at a slow rate due to their low diffusion coefficients.

Despite this, it has been found that some polysaccharides permeate skin with a greater permeability rate than that of their constituent monomers. For example, hyaluronic acid, or HA, having a MW of about 300,000, permeates the skin with a permeability rate of about 2×10^{-3} cm/hr from a solution containing 25 mg/ml hyaluronic acid. This permeability rate is 200 times higher than that of the monomer it is made from. Dextran, a polysaccharide having a MW of 70,000, permeates skin with a permeability rate of 4×10^{-4} cm/hr from a solution containing 400 mg/ml dextran, a value about 40 times greater than its monomer. Similarly, heparin, having a MW of between 5,000-20,000, was found to permeate with a permeability rate of 2×10^{-4} from a solution containing 300 mg/ml heparin, a value that is about 20 times higher than its monomer.

With the greater permeability rates, some polysaccharides can be used for transdermal delivery of drugs. Table 1, provided in conjunction with Example 1, below, lists the permeabilities of several sugars. For example, hyaluronic acid permeates biological membrane with a permeability rate of about 2×10^{-3} cm/hr. With this permeability, about 30 mg of hyaluronic acid can be

delivered per day from a source, such as a patch, having an 25 cm^2 area, and containing hyaluronic acid at a concentration of 25 mg/ml.

A "high-permeability polysaccharide" is a polysaccharide having a passive permeability of greater than about 1×10^{-5} cm/hr, preferably greater than about 1×10^{-4} cm/hr, and most preferably greater than about 1×10^{-3} cm/hr. Examples of high-permeability polysaccharides that are useful according to the present invention include heparin, dextran, hyaluronic acid, chondroitin sulfate, and salts thereof. One of ordinary skill in the art can readily determine the passive permeability of other high-permeability polysaccharides candidates and make additional solutions based on the foregoing criteria.

According to one embodiment of the present invention, some polysaccharides can be used in conjunction with transdermal drug delivery. Referring to **Fig. 1**, a flowchart depicting a method for transdermal drug delivery is provided. First, in step 102, an area of biological membrane into or through a drug is to be administered is identified. Preferably, the identified area is located at a site selected based on convenience to the patient as well as maximum drug penetration, as well as need. For example, the arm, thigh, and stomach represent areas of relatively thin biological membrane and high surface area, while the hands and feet are uneven and callused.

In step 104, a high-permeability polysaccharide, such as hyaluronic acid, is contacted with the identified area of biological membrane in order to increase the permeability of the biological membrane.

The area of biological membrane that is to have its permeability enhanced preferably has a size approximately equal to the size of the application device. For example, if the surface of a transdermal patch, such as the Fentanyl®, available from ALZA Corporation, Mountain View, CA, is 40 cm^2, the polysaccharide, or other enhancing forces (discussed below), are preferably administered to a surface area of 40 cm^2. Other size patches, including both smaller and larger patches, are within the contemplation of the present invention.

Additional enhancing forces can be applied to increase the permeability, or assist in the permeabilization, of the biological membrane. These forces may be applied to the biological membrane before the high-permeability

polysaccharide is contacted with the skin, in essence pre-treating the biological membrane, or they may be applied substantially simultaneously with the polysaccharide, or both. In one embodiment, ultrasound may be applied to the identified area to increase its permeability. Techniques for increasing the permeability of a biological membrane are disclosed in U.S. Patent Nos. 6,041,253 and 6,190,315, the disclosures of which is hereby incorporated by reference in their entireties. Ultrasound is preferably administered at frequencies of less than or equal to about 2.5 MHz, preferably at a frequency that is less than one MHz, more typically in the range of 20 to 100 kHz. In one embodiment, ultrasound is applied at a frequency of 50 kHz. Exposures are typically for between 20 seconds and 10 minutes, continuously, but may be shorter and/or pulsed, for example, 100 to 500 msec pulses every second for a time sufficient to permeabilize the biological membrane. The ultrasound intensity should be at a level that preferably does not raise the biological membrane temperature more than about one to two degrees Centigrade or cause permanent damage to the biological membrane, under the conditions and with the particular device to be used. This typically will be less than 20 W/cm^2, preferably less than 10 W/cm^2.

The intensity and time of application are inversely proportional, so that higher intensities should be applied for shorter periods of time, in order to avoid biological membrane damage. It should be understood that although the normal lower range of ultrasound is 20 kHz, one could achieve comparable results by varying the frequency to less than 20 kHz, that is, into the sound region down to about one kHz. The time needed is dependent upon the frequency and intensity of the ultrasound and the biological membrane condition. At 20 kHz, for example, at an intensity of 10 W/cm^2, and a duty cycle of 50%, biological membrane on a human forearm is sufficiently permeabilized in about five minutes.

Permeabilizing ultrasound can be applied for a predetermined amount of time or can be applied only until sufficient permeabilization is attained. Since biological membrane conditions can change over time, based on aging, diet, stress, and other factors, it may be preferable to measure permeability as ultrasound is applied to ensure sufficient ultrasound is applied and to minimize the risk of biological membrane damage. Several methods can be used to determine when

sufficient permeabilization has been reached. PCT International Patent Application No. PCT/US99/30067, entitled "Method and Apparatus for Enhancement of Transdermal Transport," the disclosure of which is incorporated by reference in its entirety discloses methods and devices for determining the permeabilization of skin.

One way to measure permeabilization is to measure relative biological membrane conductivity at the permeabilization site versus a reference point. These measurements are performed by applying a small AC or DC electric potential across two electrically isolated electrodes in contact with biological membrane. The electric current flowing through these electrodes is measured using an ammeter and the biological membrane resistance is measured using the values of the potential and the current.

Another way to determine when sufficient permeabilization has been reached is to measure absolute conductivity. The degree of permeability can also be monitored using a sensor that determines the concentration of the drug being delivered or analyte being extracted. As the permeability decreases, the drug concentration will decrease, and vice versa.

The biological membrane is preferably permeable for at least 30 minutes, preferably at least an hour, or two hours. Under some conditions, the biological membrane may remain permeable for up to twenty-four hours. It may be desirable to repermeabilize the biological membrane under the same, or different conditions.

Several methods may be useful to attain or maintain permeabilization for an extended period of time. Cavitation enhancers, as described more fully below can be used. The chemical and physical enhancers and driving forces described below may also act to keep the biological membrane permeable. In addition, molecules such as sodium lauryl sulfate, for example, may permeate the biological membrane and serve as spacer molecules to keep the biological membrane open.

Other forces, such as heat, a temperature, pressure, electromotive, a mechanical agitation, ultrasound, iontophoresis, electromagnetic force, a magnetic force, a photothermal, photoacoustic, microneedles, laserporation, electroporation, and combinations thereof, may also be provided to enhance the permeability of the biological membrane.

In step 106, a drug may be contacted with the identified area of biological membrane. In one embodiment, an application device, such as a patch, may be used to contact the drug to the biological membrane.

Drugs to be administered include a variety of bioactive agents, including proteins and peptides. Specific examples include insulin, erythropoietin, and interferon. Other materials include nucleic acid molecules, such as antisense, and genes encoding therapeutic proteins, synthetic organic and inorganic molecules including anti-inflammatories, antivirals, antifungals, antibiotics and local anesthetics, saccharides, polysaccharides (e.g., heparin), growth hormone, vaccines, and Leutinizing Hormone Releasing Hormone. The drug will typically be administered in an appropriate pharmaceutically acceptable carrier having an absorption coefficient similar to water, such as an aqueous gel, ointment, lotion, or suspension. Alternatively, a transdermal patch can be used as a carrier. It may be desirable to include protease inhibitors with protein and peptide drugs to minimize protease activity. Molecules for biological membrane treatment such as retinoids, dyes, and vitamin D, may also be delivered.

In one embodiment, the drug may be in the form of, or encapsulated within, a delivery device such as a liposome, lipid vesicle, emulsion or polymeric nanoparticles, microparticle, microcapsule, or microspheres (referred to collectively as microparticles unless otherwise stated). These can be formed of polymers such as polyhydroxy acids, polyorthoesters, polyanhydrides, and polyphosphazenes, or natural polymers such as collagen, polyamino acids, albumin and other proteins, alginate and other polysaccharides, and combinations thereof. The microparticles can have diameters of between 0.001 and 100 microns, although a diameter of less than 10 microns is preferred. The microparticles can be coated or formed of materials enhancing penetration, such as lipophilic materials or hydrophilic molecules, for example, polyalkylene oxide polymers, and conjugates, such as polyethylene glycol.

In another embodiment, the drug may be a complexed with a polysaccharide. This will be discussed in greater detail, below.

In step 108, a transdermal transport enhancer may be applied to enhance the transdermal transport of the drug. Transdermal transport enhancers that

can be applied before, during or after the permeabilizing include chemical enhancers and/or driving forces, physical driving forces, and cavitation producing forces.

Chemical enhancers include lipid bilayer disrupting agents and solubility enhancers. Chemical enhancers have been found to increase drug transport by different mechanisms. Chemicals that enhance permeability through lipids are known and commercially available. For example, ethanol has been found to increase the solubility of drugs up to 10,000-fold and yield a 140-fold flux increase of estradiol through the biological membrane, while unsaturated fatty acids have been shown to increase the fluidity of lipid bilayers. Examples of fatty acids that disrupt lipid bilayer include linoleic acid, capric acid, lauric acid, and neodecanoic acid, which can be in a solvent. Suitable solvents include water; diols, such as propylene glycol and glycerol; mono-alcohols, such as ethanol, propanol, and higher alcohols; DMSO; dimethylformamide; N,N-dimethylacetamide; 2-pyrrolidone; N-(2-hydroxyethyl) pyrrolidone, N-methylpyrrolidone, 1-dodecylazacycloheptan-2-one and other n-substituted-alkyl-azacycloalkyl-2-ones and other n-substituted-alkyl-azacycloalkyl-2-ones (azones).

Other chemical enhancers, not necessarily associated with binary systems, include dimethylsulfoxide (DMSO) or aqueous solutions of DMSO such as those described in U.S. Pat. No. 3,551,554 to Herschler; U.S. Pat. No. 3,711,602 to Herschler; and U.S. Pat. No. 3,711,606 to Herschler, and the azones (n-substituted-alkyl-azacycloalkyl-2-ones) such as noted in U.S. Pat. No. 4,557,943 to Coope.

Surfactants can act as solubility enhancers for some drugs as well as permeability enhancers by fluidizing the lipid bilayer. A preferred surfactant is sodium lauryl sulfate (SLS) present in an amount of about 0.25 to 5%, preferably about 1%. Other useful surfactants include fatty acids, fatty alcohols, esters of fatty acids, alkyl sulfonates, sodium salts of sulfonic acid, alkyl sulfonic acid, Tween™, Spam™, and pluronics™, typically in a concentration in the range of 0.25 to 5% weight/volume.

Driving forces include osmotic pressure gradient, concentration gradient, iontophoresis, electroporation, magnetic fields, additional ultrasound, and mechanical pressure.

Driving forces may be applied after permeabilization to enhance transport of the drug into or through the biological membrane. The driving force can be applied continuously over a period of time or at intervals during the period of permeabilization.

Application of electric current enhances transdermal transport by different mechanisms. First, application of an electric field provides an additional driving force for the transport of charged molecules across the biological membrane (electrophoresis) and second, ionic motion due to application of electric fields can induce convective flows across the biological membrane, referred to as electrosmosis. This mechanism is believed to play a dominant role in transdermal transport of neutral molecules during iontophoresis. Iontophoresis involves the application of an electrical current, preferably DC, or AC, at a current density of greater than zero up to about 1 mA/cm^2. Typically, a constant voltage is applied since resistance changes over time, usually in the range of between greater than zero and four volts.

Application of magnetic fields to the biological membrane pretreated with ultrasound can also result in a higher transport of magnetically active species across the biological membrane. For example, polymer microspheres loaded with magnetic particles could be transported across the biological membrane using sonophoresis and magnetic fields.

In one embodiment, the iontophoresis may be provided in a range of from about 10 μA to about 1000 μA. Preferably, the iontophoresis is provided at 100 μA.

Additional ultrasound can be applied at higher, lower, or the same frequency as the initial permeabilizing ultrasound. In other cases, it may be preferable to use lower frequency, "maintenance" doses of ultrasound to keep the biological membrane permeabilized.

Greater transdermal transport can be achieved by inducing cavitation either inside or outside of the biological membrane. Cavitation is the growth and oscillations of air bubbles present in fluids and air pockets present in the keratinocytes of the SC. Application of low-frequency ultrasound appears to induce cavitation inside as well as outside the biological membrane and disorganize the SC

lipid bilayers thereby enhancing transdermal transport. In addition, oscillations of cavitation bubbles can result in significant water penetration into the disordered lipid regions and can cause the formation of aqueous channels through the intercellular lipids of the SC. This allows transport of permeants across the disordered lipid domains, then across keratinocytes and the entire SC. This transport pathway can result in an enhanced transdermal transport as compared to passive transport because the diffusion coefficients of permeants through water, which is likely to primarily occupy the channels generated by ultrasound, are up to 1000-fold higher than those through the ordered lipid bilayers, and the transport path length of these aqueous channels can be much shorter (by a factor of up to 25) than that through the tortuous intercellular lipids in the case of passive transport.

Cavitation can be enhanced by providing nuclei in the form of gas bubbles, crevices, or particulate. Examples of cavitation enhancers include flourocarbons, particulate matter (for example, microspheres, silica, titanium dioxide particles, polymer particles), gases (for example, argon, air), and stabilized air bubbles.

Occurrence of cavitation on the biological membrane surface can also be enhanced by coating the biological membrane surface with a wetting agent in the entire area of application of ultrasound except for a spot. Cavitation can preferentially occur at the spot due to the difference in wetting properties of the biological membrane and the coating. The coating may be made from a polymer such as poly(methyl methacrylate) or it may be a membrane made from poly(vinyl difluoride), for example.

According to another embodiment of the present invention, the drug to be transported transdermally may be complexed with a polysaccharide, such as hyaluronic acid, and may be delivered with the polysaccharide transdermally. In one embodiment, complexation can be performed by attaching drugs to the carboxyl groups of hyaluronic acid. For example, peptide drugs can be tethered to hyaluronic acid by forming a linkage between the carboxyl group of hyaluronic acid and the amino group of the peptide. Once the drug crosses the biological membrane, this linkage can be broken by natural enzymes to release the peptide. Complexation can alternatively be performed through electrostatic interactions, covalent interactions,

hydrogen bonding, hydrophobic interactions, van der Waals forces, or other interactions between the polysaccharide and the peptide. The negative charges on the polysaccharide can be used to form a non-covalent complex with the peptide or protein.

In yet another embodiment of the present invention, polysaccharides can be used for oral drug delivery. Oral drug delivery is limited by poor stability of proteins in the gastrointestinal tract and poor transport of the proteins across oral mucosa. Complexation of proteins with polysaccharides can alleviate these limitations by increasing the stability of the proteins as well as increasing their transport across the oral mucosa. In another embodiment, complexes of polysaccharide with the protein/peptide can be made into a dry powder for inhalation. In still another embodiment, a solution containing complexes of polysaccharide with the protein/peptide can be injected. In other embodiments, the complexes may be present in gels, microdroplets, creams, sprays, etc. Other techniques for administering the complexes of polysaccharide with the protein/peptide are within the contemplation of the present invention.

In another embodiment, complexes of polysaccharides with DNA can be used for gene therapy. High-permeability of polysaccharides across membranes can enhance DNA uptake by cells. Hyaluronic acid receptors can contribute to the enhanced uptake of hyaluronic acid-DNA complex as well. Hyaluronic acid can also be attached to the particles loaded with the drug. The high permeability of hyaluronic acid across membranes can enhance trans-membrane transport of particles. The hyaluronic acid-modified particles may be taken orally, or may be injected to deliver drugs.

Polysaccharides can also be used to increase bioavailability of injectable or pulmonarily delivered drugs. The enhancement of bioavailability originates from the enhanced transdermal permeability

Referring to **Fig. 2**, a device for enhanced transdermal drug delivery is provided. Biological membrane 250 is contacted with patch 200. Patch 200 may include several layers, such as contact layer 202, medium layer 204, and membrane 206. Contact layer 202 contacts biological membrane 250. Medium layer 204 includes at least one high-permeability polysaccharide and a drug, including

complexes of polysaccharides and drugs, as discussed above. Medium layer 204 may be a hydrogel, a liquid, or other suitable medium. Membrane 206 may be a semi-permeable membrane.

In one embodiment, a source of additional permeabilization (not shown) and a source of a driving force (not shown) may be provided for patch 200. This may include, inter alia, ultrasonic transducers, electrodes, etc. as desired.

Contact layer 202 may include a transdermal adhesive (not shown) for remaining in contact with biological membrane 250. Other methods for contacting biological membrane 250 may be provided as required.

EXAMPLES

In order to better understand the present invention, a number of examples are provided. The examples do not limit the present invention in any way, and are intended to illustrate embodiments of the present invention.

Example 1 Permeabilities of Sugar Molecules

Table I summarizes the measured permeabilities of several sugar molecules including, mannitol (MW 180), inulin (MW 5,000), heparin (MW about 10,000), dextran (MW 70,000), and hyaluronic acid (MW 300,000) across pig skin measured *in vitro*. As discussed above, glucose permeates the biological membrane at a low rate, about 10^{-5} cm/hr. However, the permeability of some high molecular weight polysaccharides including heparin, dextran and hyaluronic acid is exceptionally high. Note that inulin, another polysaccharide having a molecular weight of about 5000 Da, does not permeate the biological membrane at a high rate. Thus, only some of the examined polysaccharides exhibit exceptionally high biological membrane permeability.

Table 1

No.	Sugar	Max. Measured Passive Permeability (cm/hr)
1	Mannitol	1×10^{-5}
2	Inulin	2×10^{-5}
3	Heparin	2×10^{-4}
4	Dextran	4×10^{-4}
5	Hyaluronic Acid	2×10^{-3}

The significance of the high permeability of hyaluronic acid is evident from the fact that it is comparable to the biological membrane permeability of estradiol, a low-molecular weight and hydrophobic drug for which a passive transdermal patch exists. This invention disclosure is based on the use of this exceptionally high permeability of polysaccharides for the purpose of drug delivery.

Example 2 Transport of Leutinizing Hormone Releasing Hormone

This example demonstrates the use of hyaluronic acid to enhance transdermal drug delivery. Transport of Leutinizing Hormone Releasing Hormone (LHRH), a peptide with a molecular weight of about 1100 Da, was measured. Passive biological membrane permeability to LHRH is relatively low (about 1×10^{-4} cm/hr). However, referring to **Fig. 3**, when LHRH is co-solvated with hyaluronic acid (15 mg/ml), biological membrane permeability to LHRH is increased by a factor of about 10 during the first 24 hours. The enhanced permeability of LHRH due to hyaluronic acid can be used for transdermal drug delivery. The permeability of polysaccharides across biological membrane was measured at different donor concentrations. **Fig. 4** shows the variation in the biological membrane permeability to hyaluronic acid across pig biological membrane from a solution containing hyaluronic acid at a concentration in the range of about zero to 25 mg/ml. The rate of hyaluronic acid permeation increases nearly exponentially with its concentration in the donor solution.

Note that in the case of a typical biological membrane permeating molecule, the permeability is independent of its concentration in the donor. The highest concentration of hyaluronic acid used in these experiments is limited by the viscosity of the solution. A 25 mg/ml solution of hyaluronic acid is highly viscous and limits dissolution of further hyaluronic acid. **Fig. 5** shows a similar plot in the case of dextran. Here, the biological membrane permeability increases with increasing dextran concentration up to a concentration of 200 mg/ml after which decreases with further increase in the concentration. Exceptionally high biological membrane permeability of polysaccharides is also observed in human biological membrane.

Table 2 summarizes permeability of human biological membrane to various mono- and polysaccharides under similar donor concentrations.

Table 2

No.	Sugar	Measured Passive Permeability (cm/hr) Human Biological membrane	Measured Passive Permeability (cm/hr) Pig Biological membrane
1	Mannitol	1.0×10^{-5}	1.0×10^{-5}
2	Inulin	1.0×10^{-5}	1.0×10^{-5}
3	Heparin	1.3×10^{-4}	2.2×10^{-4}
4	Dextran	9.8×10^{-4}	4.0×10^{-4}
5	Hyaluronic Acid	1.4×10^{-4}	2.9×10^{-4}

The enhanced permeation of polysaccharides is observed across human biological membrane as well. For example, biological membrane permeability to heparin, dextran and hyaluronic acid is significantly higher than to other sugar molecules. Thus, the data in **Figs. 3-5** and Tables 1-2 shows that certain polysaccharides can permeate biological membrane at exceptionally high rates and can also enhance transport of drugs that are co-solvated with them. The concept of using polysaccharides for transdermal transport enhancement is novel. Traditionally, the enhancers used in transdermal drug delivery are small and hydrophobic. These enhancers can easily penetrate biological membrane and disorder lipid bilayers, thus enhancing transdermal transport. However, polysaccharides are large in size and very hydrophilic. Still, they can permeate biological membrane and enhance transport of drugs.

Other embodiments and uses of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. All references cited herein, including all U.S. and foreign patents and patent applications, are specifically and entirely hereby incorporated herein by reference. It is intended that the specification and examples be considered exemplary only, with the true scope and spirit of the invention indicated by the following claims.

CLAIMS

1. A method for the enhanced delivery of at least one drug across a biological membrane, comprising:

identifying an area of biological membrane;

contacting at least one high-permeability polysaccharide with the identified area of biological membrane;

contacting at least one drug with the identified area of biological membrane; and

transporting the at least one drug into or through the identified area of biological membrane.

2. The method of claim 1, wherein the biological membrane is selected from the group consisting of tissue, mucous membranes, cornified tissues, oral mucosa, skin, nasal membrane, pulmonary capillary wall, cell membrane, organs, tissues, buccal, nails, and the gastro-intestinal tract.

3. The method of claim 1, wherein the step of contacting a high-permeability polysaccharide with the identified area of biological membrane comprises:

contacting a polysaccharide having a permeability of at least 1×10^{-5} cm/hr with the identified area of biological membrane.

4. The method of claim 1, wherein the step of contacting a high-permeability polysaccharide with the identified area of biological membrane comprises:

contacting a polysaccharide selected from the group consisting of heparin, dextran, chondroitin sulfate, and salts thereof, with the identified area of biological membrane.

5. The method of claim 1, wherein the step of contacting a high-permeability polysaccharide with the identified area of biological membrane comprises:

contacting at least one of hyaluronic acid and a salt thereof with the identified area of biological membrane.

6. The method of claim 1, further comprising the step of:

increasing a permeability of the area of biological membrane with at least one of heat, pressure, electromotive force, mechanical agitation, iontophoresis, electromagnetic force, magnetic force, photothermal force, photoacoustic force, a microneedle, laserporation, and electroporation.

7. The method of claim 1, further comprising the step of:

applying ultrasound to the identified area of biological membrane.

8. The method of claim 7, wherein the step of applying ultrasound to the identified area of biological membrane occurs prior to the step of contacting a high-permeability polysaccharide to the identified area of biological membrane.

9. The method of claim 7, wherein the step of applying ultrasound to the identified area of biological membrane occurs substantially simultaneously with the step of contacting a high-permeability polysaccharide to the identified area of biological membrane.

10. The method of claim 7, wherein the step of applying ultrasound to the identified area of biological membrane occurs subsequent to the step of contacting a high-permeability polysaccharide to the identified area of biological membrane.

11. The method of claim 7, wherein the step of applying ultrasound to the identified area of biological membrane comprises:

applying ultrasound having a frequency range of from about 10 kHz to about 500 kHz to the area of biological membrane.

12. The method of claim 7, wherein the step of applying ultrasound to the identified area of biological membrane comprises:

applying ultrasound having a frequency range of from about 20 kHz to about 150 kHz to the area of biological membrane.

13. The method of claim 7, wherein the step of applying ultrasound to the identified area of biological membrane comprises:

applying ultrasound having a frequency of about 50 kHz to the area of biological membrane.

14. The method of claim 1, wherein the steps of contacting at least one high-permeability polysaccharide with the identified area of biological membrane occurs prior to the step of contacting at least one drug with the identified area of biological membrane.

15. The method of claim 1, wherein the steps of contacting at least one high-permeability polysaccharide with the identified area of biological membrane occurs subsequent to the step of contacting at least one drug with the identified area of biological membrane.

16. The method of claim 1, wherein the steps of contacting at least one high-permeability polysaccharide with the identified area of biological membrane and the step of contacting at least one drug with the identified area of biological membrane occur substantially simultaneously.

17. The method of claim 1, wherein the step of contacting at least one drug with the identified area of biological membrane comprises:

contacting a complex of at least one protein with at least one high-permeability polysaccharide with the identified area of biological membrane.

18. The method of claim 17, wherein the at least one protein is tethered to the at least one high-permeability polysaccharide through at least one of electrostatic interactions, covalent interactions, hydrogen bonding, hydrophobic interactions, and van der Waals forces.

19. The method of claim 1, wherein the step of contacting at least one drug with the identified area of biological membrane comprises:

contacting a complex of at least one peptide with at least one high-permeability polysaccharide with the identified area of biological membrane.

20. The method of claim 18, wherein the at least peptide is tethered to the at least one high-permeability polysaccharide through at least one of electrostatic interactions, covalent interactions, hydrogen bonding, hydrophobic interactions, and van der Waals forces.

21. The method of claim 1, further comprising the step of:

applying a driving force to the identified area of biological membrane.

22. The method of claim 21, wherein the driving force is selected from the group consisting of an osmotic pressure gradient, a concentration gradient, iontophoresis, electroporation, magnetic fields, ultrasound, mechanical pressure, and combinations thereof.

23. The method of claim 22, wherein the driving force ultrasound is applied at a frequency of from about 100 kHz to about 5 MHz.

24. The method of claim 22, wherein the driving force iontophoresis is in a range of from about 10 μA to about 1000 μA.

25. The method of claim 1, wherein the step of contacting at least one drug with the identified area of biological membrane comprises:

contacting at least one of a protein and a peptide with the identified area of biological membrane.

26. The method of claim 1, wherein the step of contacting at least one drug with the identified area of biological membrane comprises:

contacting at least one of nucleic acid molecules, genes, synthetic organic and inorganic molecules, antivirals, antifungals, antibiotics, local anesthetics, saccharides, polysaccharides, growth hormone, vaccines, and Leutinizing Hormone Releasing Hormone with the identified area of biological membrane.

27. A method for the enhanced transport of a drug into or through a biological membrane, comprising:

preparing a complex of the drug with a high-permeability polysaccharide; and

transporting the complex into or through the biological membrane.

28. The method of claim 27, wherein the step of preparing a complex of the drug with a high-permeability polysaccharide comprises:

preparing a complex of the drug with a polysaccharide selected from the group consisting of heparin, dextran, and chondroitin sulfate, and salts thereof

29. The method of claim 27, wherein the step of preparing a complex of the drug with a high-permeability polysaccharide comprises:

preparing a complex of the drug with at least one of hyaluronic acid an a salt thereof.

30. The method of claim 27, wherein the step of transporting the complex into or through the biological membrane comprises:

injecting the complex into or through the biological membrane.

31. The method of claim 27, wherein the step of transporting the complex into or through the biological membrane comprises:

orally ingesting the complex.

32. The method of claim 27, wherein the step of transporting the complex into or through the biological membrane comprises:

inhaling the complex.

33. The method of claim 27, wherein the step of transporting the complex into or through the biological membrane comprises:

contacting the complex with the biological membrane.

34. The method of claim 27, wherein the step of preparing a complex of a drug with a high-permeability polysaccharide comprises:

preparing the complex of a drug with a high-permeability polysaccharide in a form selected from the group consisting of a gel, a spray, a liquid, a powder, microdroplets, an ointment, and a cream.

35. A device for enhanced drug delivery, comprising:

at least one high-permeability polysaccharide; and

at least one drug.

36. The device of claim 35, wherein the high-permeability polysaccharide comprises a polysaccharide having a permeability of at least 1×10^{-5} cm/hr.

37. The device of claim 35, wherein the high-permeability polysaccharide comprises a polysaccharide selected from the group consisting of heparin, dextran, and chondroitin sulfate, and salts thereof.

38. The device of claim 35, wherein the high-permeability polysaccharide comprises at least one of hyaluronic acid and a salt thereof.

39. The device of claim 35, wherein the drug and the high-permeability polysaccharide are complexed.

40. The device of claim 35, wherein the device further comprises:

a contact layer that contacts a biological membrane;

a medium layer containing the at least one high-permeability polysaccharide and the at least one drug;

wherein the device a wearable patch.

41. The device of claim 40, wherein the device further comprises:

a source of a driving force.

42. The device of claim 41, wherein the source of a driving force provides a driving force selected from the group consisting of an osmotic pressure gradient, a concentration gradient, iontophoresis, electroporation, magnetic fields, ultrasound, mechanical pressure, and combinations thereof.

43. The device of claim 40, wherein the device further comprises:

a source of a permeabilizing force.

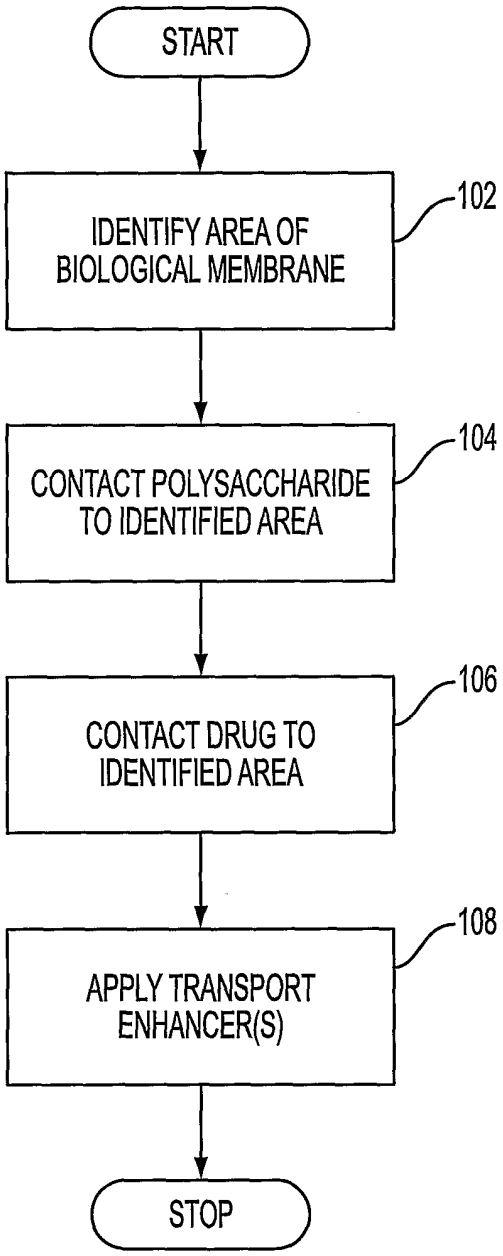

START
IDENTIFY AREA OF BIOLOGICAL MEMBRANE
102
CONTACT POLYSACCHARIDE TO IDENTIFIED AREA
104
CONTACT DRUG TO IDENTIFIED AREA
106
APPLY TRANSPORT ENHANCER(S)
108
STOP

FIG. 1

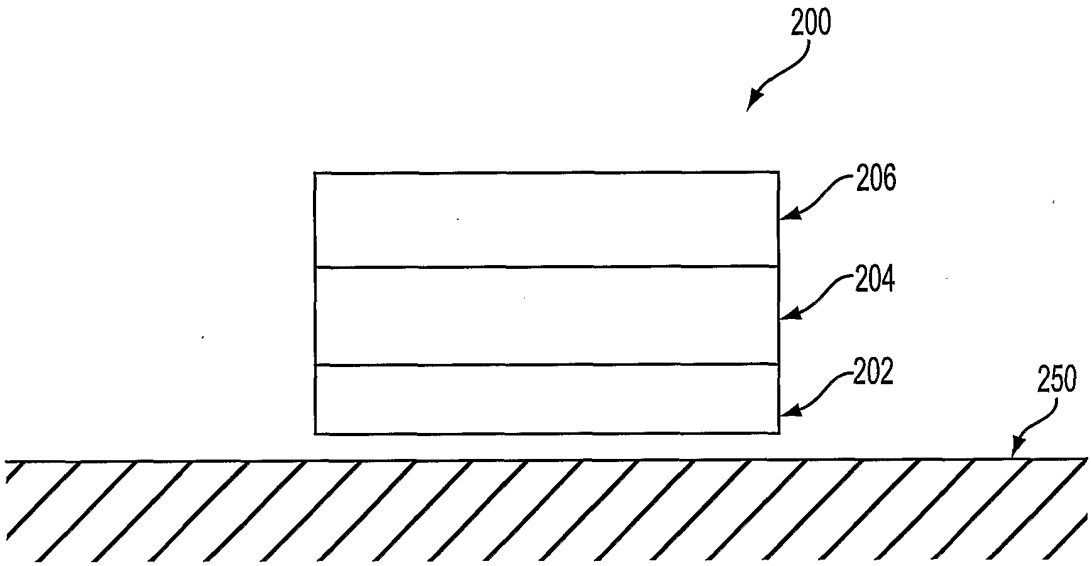


FIG. 2

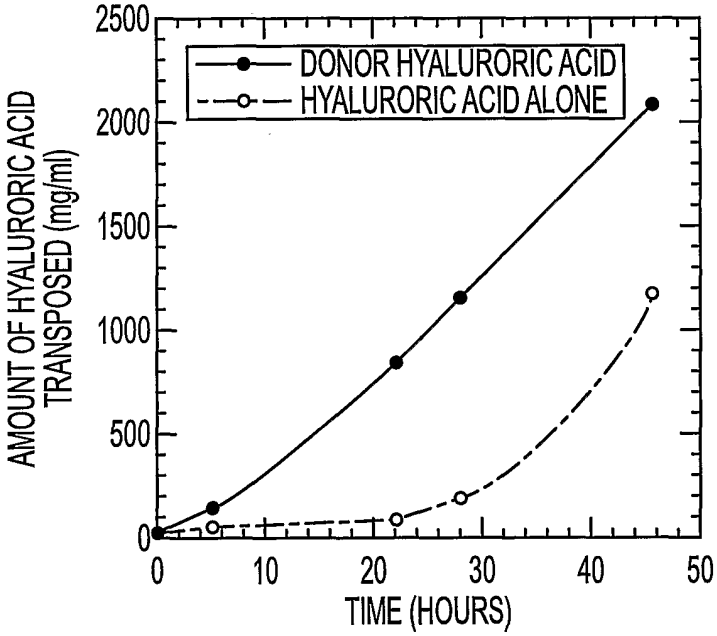

2500
2000
1500
1000
500
0
AMOUNT OF HYALURORIC ACID TRANSPOSED (mg/ml)
DONOR HYALURORIC ACID
HYALURORIC ACID ALONE
0
10
20
30
40
50
TIME (HOURS)

FIG. 3

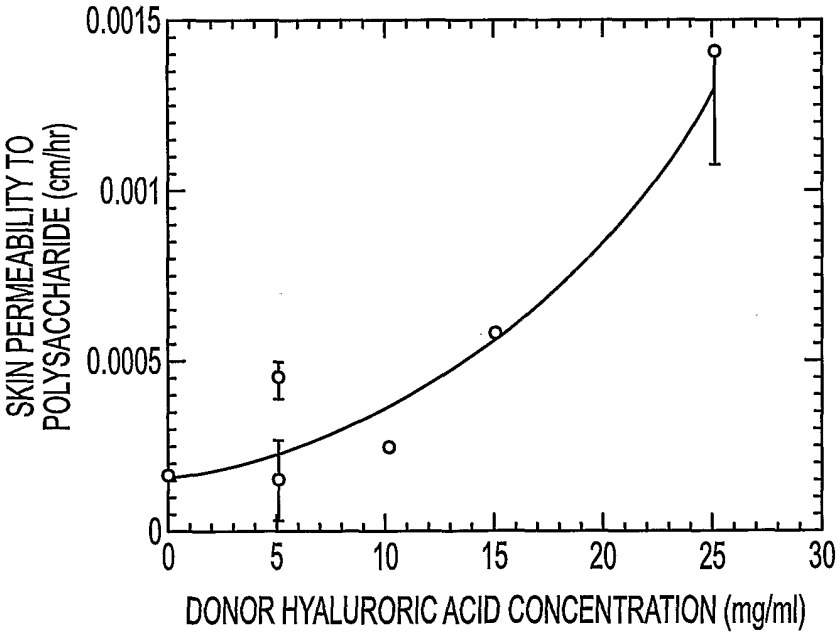

0.0015
0.001
0.0005
0
SKIN PERMEABILITY TO POLYSACCHARIDE (cm/hr)
0
5
10
15
20
25
30
DONOR HYALURORIC ACID CONCENTRATION (mg/ml)

FIG. 4

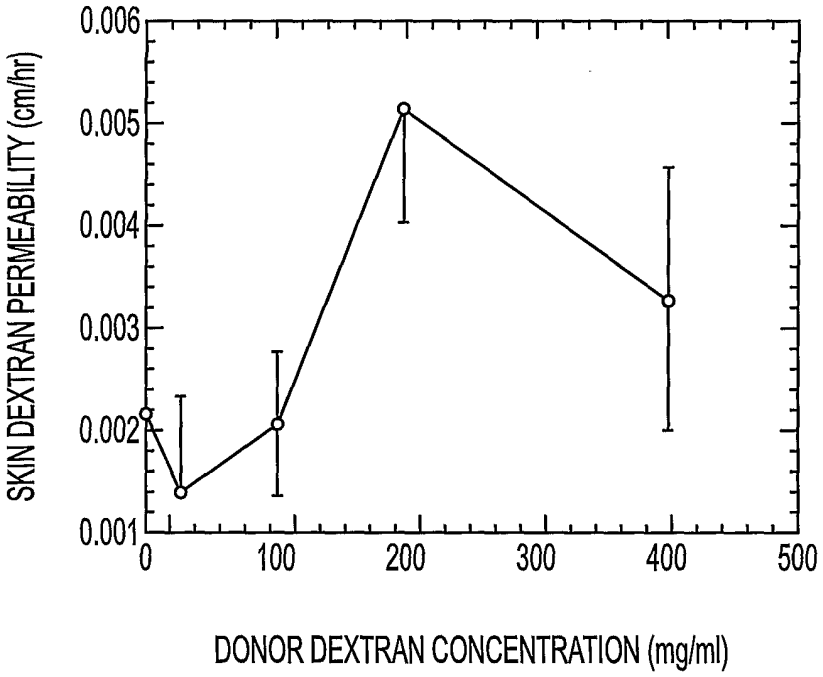

SKIN DEXTRAN PERMEABILITY (cm/hr)
0.006
0.005
0.004
0.003
0.002
0.001
0
100
200
300
400
500
DONOR DEXTRAN CONCENTRATION (mg/ml)

FIG. 5