Active enhancement methods for intra- and transdermal drug delivery: a review

Aktivne metode pospeševanja dermalnega in transdermalnega vnosa zdravilnih učinkovin

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Razširjeni izvleček

V zadnjih desetletjih se pojavlja vedno večje zanimanje za transdermalni vnos učinkovin, saj predstavlja pomemben prispevek k medicini in hkrati zanimivo alternativo drugim načinom administracije zdravil. Transdermalni vnos ima namreč v določenih primerih nekatere prednosti pred ostalimi načini vnosa. Te so predvsem izognitev prvemu prehodu jeter, boljši farmakokinetski profili, zmanjšanje neželenih stranskih učinkov in boljša sprejemljivost pri bolnikih. Uspeh transdermalnega vnosa je odvisen od sposobnosti zdravilne učinkovine, da v zadostnih količinah preide kožo, da doseže želeni farmakološki učinek. Vendar je treba poudariti, da se uporaba izraza transdermalni vnos omejuje na opis stanja, v katerem učinkovina prehaja skozi kožo v sistemski krvni obtok (npr. zdravljenje kronične bolečine s fentanilom) v nasprotju z dermalnim vnosom, ki opredeljuje usmerjanje molekule na patološka mesta v koži in ni namenjeno sistemski absorpciji.

Človeška koža je največji organ telesa, ki ima zaščitno, homeostazo vzdržujočo in senzorno vlogo. Sestavljena je iz štirih glavnih plasti: rožena plast, živi del povrhnjice, usnjica in podkožno tkivo. Obrambno sposobnost kože zagotavlja predvsem zunanja rožena plast, ki je končni produkt epidermalne diferenciacije celic. Obsega le 10–15 celičnih plasti, je okoli 10 µm debela in jo sestavljajo mrtve, sploščene in s keratinom bogate celice. Te celice, imenovane korneociti, so obdani z mešanico medceličnih lipidov (ceramidi), prostih maščobnih kislin, holesterola in holesterolnih estrov. Ti lipidi so organizirani v tako imenovane lipidne dvosloje, ki pa igrajo pomembno vlogo pri prehajanju zdravilnih učinkovin preko kože. Pri tem so ključni tudi drugi kožni vključki, kot so lasni mešički, žleze znojnice in lojnice.

Prehod molekul preko kože

Obstajajo tri glavne poti prehoda zdravilnih učinkovin preko kože: preko kožnih vključkov (transfolikularna in transglandularna pot), intercelularna in transcelularna pot. Vrsta poti je odvisna od fizikalno-kemijskih lastnosti molekule (molekulska masa, logP, stopnja ionizacije), vendar pa je prehod možen tudi s kombinacijo vseh treh poti. Transcelularna pot je pot polarnih molekul zaradi velike koncentracije hidrofilnega keratina. Po drugi strani pa lipidni medij (intercelularna pot), ki predstavlja 1 % rožene plasti, velja za pot, po kateri skozi kožo prehaja večina malih in nenabitih molekul. Relativni prispevek vsake od treh možnih poti skozi roženo plast je odvisen od narave molekule, ki jo vnašamo.

Pristopi za povečanje prehoda molekul preko kože

Zdravilne učinkovine, ki lahko pasivno prehajajo kožno pregrado, morajo imeti ustrezno lipofilnost, topnost v vodi ($10 \leq K_{o/w} \leq 1000$), molekulsko maso (≤ 500 Da), tališče (≤ 200 °C) itd. Te zahteve omejijo število učinkovin, primernih za transdermalni ali dermalni vnos. To je vzrok, da so v raziskavah razvili veliko število pasivnih in aktivnih pristopov, ki pospešujejo transdermalni vnos zdravilnih učinkovin. Pasivni pristopi vključujejo vpliv na interakcije med zdravilno učinkovino in vehiklom, optimizacijo formulacije in spremembe lastnosti rožene plasti. Eden najbolj razširjenih pasivnih pristopov za povečanje transdermalnega vnosa je uporaba kemijskih pospeševalcev vnosa. To so molekule, ki so sposobne začasno spremeniti pregradne lastnosti kože. Med te spadajo alkoholi (etanol), polialkoholi, pirolidoni, amini, amidi, maščobne kisline, sulfoksidi (DMSO), estri, terpeni, površinsko aktivne snovi, voda itd. K pasivnemu pristopu spada tudi uporaba različnih formulacij zdravilnih učinkovin. Tako se uporabljajo različne kreme,

geli, mazila in tehnologija transdermalnih obližev. Druge pasivne tehnike vključujejo še uporabo nasičenih sistemov, predzdravil, liposomov, mikroemulzij in drugih.

Aktivni pristopi vključujejo predvsem uporabo energije, ki deluje kot gonilna sila in zmanjšuje pregradni upor rožene plasti, da se izboljša transdermalni vnos zdravilnih učinkovin. V nasprotju s pasivnimi metodami lahko s pomočjo aktivnih pristopov pospešimo vnos molekul z veliko molekulsko maso (> 500 Da) in tudi hidrofilnih molekul (npr. peptidi in proteini).

Aktivne metode za pospeševanje vnosa zdravilnih učinkovin lahko razdelimo v tri glavne razrede: električne (iontoforeza, elektroporacija, radiofrekvenčna metoda, magnetoforeza) mehanske (mikroigle, abrazija kože, perforacija kože) in druge metode (lasersko sevanje, ultrazvok in termoforeza).

Iontoforeza

Iontoforeza je najbolj obširno raziskana metoda pospeševanja transdermalnega vnosa molekul in vključuje nanos neposredno na kožo v kombinaciji z dovajanjem šibkega električnega toka (0,5 mA/cm^2). Iontoforeza ne spremeni lastnosti kože, zato je v glavnem uporabna za majhne nabite molekule in makromolekule do nekaj kD. Glavna prednost iontoforeze je njena sposobnost vnosa srednjevelikih nabitih molekul, kot so peptidi in oligonukleotidi, ki običajno kože ne prehajajo niti z difuzijo kot tudi ne s kemijskimi pospeševalci vnosa. Iontoforeza ne povzroča poškodbe kože in jo bolniki dobro prenašajo.

Elektroporacija

Elektroporacija je uveljavljena metoda, ki se uporablja za povečanje permeabilnosti celičnih membran. Kratek čas trajajoči električni impulzi visoke napetosti (100 do 1000 V/cm, 10 µs do 500 ms) ustvarijo za kratek čas trajajoče hidrofilne prenosne poti v fosfolipidnih dvoslojih membran. Na tak način se zaradi prisotnosti električnega polja prepustnost membrane začasno poveča. Povečanje prepustnosti celične membrane z električnimi pulzi lahko dosežemo tako na celicah kot tudi na tkivih v *in vivo* ali *ex vivo* pogojih, kar vključuje tudi roženo plast kože. Takšno povečanje prepustnosti omogoči prehod molekulam (molekule različnih velikosti, cepiva, DNK), kar je v fizioloških pogojih nemogoče.

Ultrazvok

Ultrazvočni valovi se pri transdermalnem vnosu uporabljajo kot fizična sila za povečanje vnosa molekul skozi kožo za njihovo boljšo biorazpoložljivost. V ta namen so bile raziskane frekvence v območju od 20 kHz do 16 MHz. Od teh frekvenc se je nizkofrekvenčni ultrazvok pri transdermalnem vnosu izkazal kot najbolj učinkovit. Mehanizem delovanja pripisujejo kombinaciji termičnih in mehanskih sprememb kože, ki ga povzroča kavitacija. Uporaba ultrazvoka pri transdermalnem vnosu zajema tako majhne kot velike molekule, in sicer od analgetikov (ibuprofen), anestetikov (lidokain) do citostatikov (5-fluorouracil). Predklinične študije so bile izvedene tudi z makromolekulami, kot so inzulin, interferon in eritropoetin.

Radiofrekvenčna mikroablacija

Radiofrekvenčna (RF) mikroablacija omogoča povečanje transdermalnega vnosa z nastankom toplotno-induciranih poti skozi roženo plast zaradi uporovnega ali dielektričnega segrevanja. Učinkovitost RF mikroablacije za transdermalni vnos molekul je bila dokazana tako *in vitro* kot tudi *in vivo*. Uspešen je bil vnos tako polarnih hidrofilnih molekul z molekulsko maso nad 300 Da, kot tudi človeškega rastnega hormona (22 kDa) in molekul DNK. Hkrati pa RF mikroablacija pri kontroliranih parametrih na koži ne povzroča poškodb, rdečine ali otekline.

Laserska energija

Vključuje neposredno in nadzorovano izpostavljenost kože laserskemu žarku. Povečan prenos snovi izhaja iz mikroablacije rožene plasti brez poškodb nižjih plasti kože. Uporaba laserjev v klinični obravnavi je zelo pogosta in se najpogosteje uporablja za zdravljenje kožnih sprememb, kot so akne. Odstranitev rožene plasti s to metodo se je izkazala za učinkovito pri izboljšanju transdermalnega vnosa tako lipofilnih kot hidrofilnih molekul, tudi takih z višjo molekulsko maso (inzulin).

Mikroigle

Uporablja se več zelo kratkih igel z ostrim vrhom, ki preluknjajo zgornjo plast kože in tako na minimalno invaziven način ustvarijo fizično pot zdravilnim učinkovinam za njihov vnos v živi epidermis. Obstaja vrsta različnih oblik mikroigel, vendar jih je mogoče razdeliti v dve glavni kategoriji: trdne in votle. Mikroigle so bile za transdermalni vnos preizkušene za več različnih zdravilnih učinkovin, kot so majhne molekule (naltrekson), beljakovine (inzulin), DNA in tudi cepiva za sistemsko zdravljenje.

Transdermalni produkti v uporabi, ki vsebujejo aktivne pristope pospeševanja transdermalnega vnosa

Na trgu prevladujejo »pasivni« transdermalni obliži, vendar obstaja tudi vedno več zdravilnih učinkovin, ki so vključene v transdermalne sisteme z aktivnimi metodami pospeševanja vnosa. Najbolj zastopani na tem področju so različni iontoforetski sistemi (Phoresor™, Vyteris (Lidosite®), e-TRANS®). Eno od elektroporacijskih prototipnih naprav za transdermalni vnos so razvili v Inovio biomedical Corporation in jo testirali z različnimi spojinami za izboljšanje vnosa tako zdravilnih učinkovin kot tudi genov.

Najbolj znana naprava za transdermalni vnos z nizkofrekvenčnim ultrazvokom je naprava SonoPrep® (Sontra Medical Corporation). Od sistemov z mikroiglami pa je najpogosteje uporabljena tehnologija Macroflux®, ki ga je razvilo podjetje Zosano Pharma. Po drugi strani pa je v zadnjem času najbolj popularen napredni sistem votlih in trdnih mikroigel, tj. 3M™ mikrokanalni kožni sistem.

Abstract

Transdermal route has some advantages over other drug administration routes. These include avoidance of first pass effect (hepatic metabolism), better pharmacokinetic profile, reduction of side effects and good patient compliance. The greatest obstacle for the drugs to be delivered through the skin is overcoming the impermeable outermost layer of the skin – the stratum corneum. Quite a few enhancement techniques can be used to overcome the stratum corneum barrier and facilitate transdermal drug delivery. These include various passive (penetration enhancers, liposomes) and active approaches (electroporation, iontophoresis, microneedles), which are of prime interest for transdermal drug delivery research area.

1. Introduction

Drugs are rarely administered as pure chemical substances; mostly they are given as formulated preparations. These can vary from relatively simple solutions to complex drug delivery systems. The side effects of some drugs cannot always be eliminated; however, their undesirable behavior can also be specifically related to a particular route of administration.

In last decades, transdermal drug delivery has been actively investigated and has become a field of biomedical research with rapid development. The success of transdermal delivery depends on the ability of the drug to permeate the intact skin barrier in sufficient quantities to achieve its desired pharmacological effect. However, it needs to be emphasized that the use of the term transdermal delivery should be limited to describe a situation in which a drug diffuses through the skin into the systemic circulation for a therapeutic effect (e.g., chronic pain treatment using fentanyl). In contrast, dermal or topical delivery defines the targeting of a drug to pathological sites within the skin, not intended for systemic absorption. Dermal delivery is used for the treatments of dermatological conditions such as psoriasis, eczema and mycobacterial infections, where the disease is located in the skin.

The greatest challenge for researchers is to surmount the inherent limitations imposed by the outermost layer of the skin – the stratum corneum – to enhance the delivery of therapeutic molecules. Different approaches have been investigated to perturb skin barrier and enhance the transdermal delivery of a drug. The mostly used techniques include physical enhancers, vesicles and chemical enhancers. Recent advances in the area of topical and transdermal delivery have stemmed from application of biophysical techniques that are becoming ever more sophisticated.

The present review explores the most recent physical techniques to breach the skin barrier for drug permeation.

2. Drug administration routes

An ideal drug delivery system is user-friendly, noninvasive and able to deliver a pharmaceutically active substance to a specific site, in specific time and with required release pattern and dose. However, drugs are introduced into the body by various routes. The choice of an appropriate route depends on pharmacokinetic and pharmacodynamic properties of the drug, the dosage form in which the drug is available and also on the patient age and conditions. Therefore, drugs may be taken orally, sublingually, intravenously, intramuscularly, rectally, transdermally, etc. Each of these routes has specific advantages and disadvantages (Table 1).

Transdermal drug delivery has made an important contribution to medical practice and represents an attractive alternative to other administration routes, especially to oral and intravenous, which are the most common ones. Transdermal delivery has a lot of specific advantages, but some of the most important are noninvasiveness, prolonged delivery with transdermal drug delivery system, avoidance of liver or gastrointestinal metabolism and good acceptance by the patients.

3. Intra- and transdermal drug delivery

3.1 The skin

Human skin is the largest organ of the body (total skin surface is around 1.7 m^2) and represents more or less 10 % of the body mass of an average person. It is also a highly complex organ whose functions may be classified as protective, homeostasis-maintaining and sensory. Its barrier property allows survival in variable environment (different

Table 1: Advantages and Disadvantages of different routes of administration for systemic drug delivery

Route of administration	Advantages	Disadvantages
Sublingual	avoid first pass effect, rapid absorption, drug stability, can be administered for local effect	small dose limit, inconvenience for some patients
Oral	convenient (portable, easy, painless), economical to the patients (non-sterile, compact), variety (tablets, capsules, liquid, fast, slow release), high dose possible, high surface of absorption, good permeability of GI barrier	may be inefficient (high dose, low solubility), first pass effect (the concentration of a drug is greatly reduced before reaching the systemic circulation), food interaction, local effect (GI flora), not suitable for unconscious patients
Inhalation	bypasses liver, large surface of absorption	difficulties in regulating the exact amount of dosage, difficulties administering the drug via inhaler
Rectal	bypasses liver, useful for children or older people, drug released at slow, steady state	unpredictable absorption, not well accepted by patients
Intravenous	direct access to blood central compartment, bypasses the digestive system, does not harm the lungs or mucous membranes, rapid onset of action	increased risk of infection and overdose, risk of the peripheral vein or arterial damage, limited to highly soluble drugs, fear, trained personnel is needed, sustained/controlled action not possible
Intramuscular	depot or sustained effect is possible	unpredictable or incomplete absorption, trained personnel is needed
Subcutaneous	can be self-administered, slow, but generally complete absorption	painful, tissue damage from irritant drugs, max. 2 ml injection
Transdermal	avoidance of first pass effect, sustained and controlled delivery with transdermal drug delivery system, reduction in side effects associated with systemic toxicity, good patient acceptance and compliance, convenient and painless (non-invasive) administration, ease of dose termination	Limited selection of molecules can be delivered this way: molecular weight < 500 Da, $1 < \log P < 3$, potent drug (< 10 mg/day), absorption through diseased skin is altered, pre-systemic metabolism (enzymes in the skin), skin irritation and sensitization

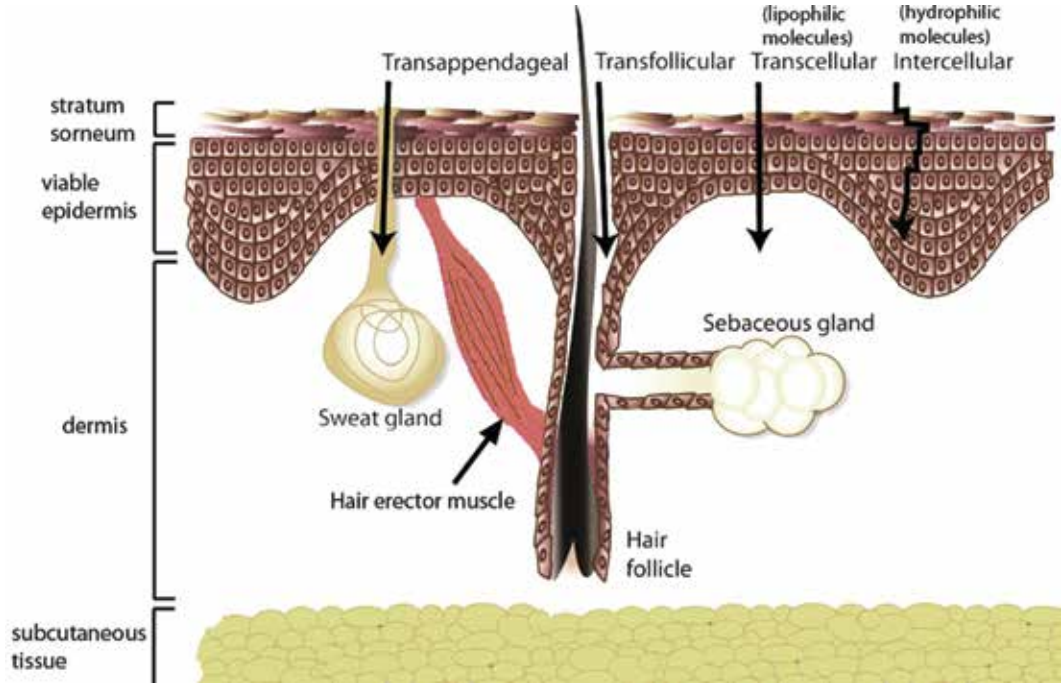


Figure 1: Cross-section of skin tissue with layers, appendages and different pathways of permeation

temperature and water content) and in the presence of environmental dangers (chemicals, bacteria, allergens, radiation). Further, it provides maintenance of the body's homeostasis, especially in terms of its composition, heat regulation, blood pressure control and excretory functions. And finally, the skin is an organ in continuous state of regeneration and repair. Essentially, the skin is made of several layers (Figure 1), the most notable ones being the stratum corneum, viable epidermis, dermis and subcutaneous tissue (hypodermis):

- Stratum corneum (SC): The outermost, nonviable layer of the epidermis is the final product of epidermal cell differentiation. It comprises only 10 to 15 cell layers and is on average around 15 µm thick (when dry, but it can swell to several times this thickness when wet), consisting of dead, flattened, anucleated, keratin-rich cells. These cells – corneocytes – are surrounded by a mixture of intercellular lipids (ceramides), free fatty acids, cholesterol and cholesterol sulfates.[1] They are structured as ordered bilayer arrays that play the key role in regulating drug flux for most permeants.
- Viable epidermis: contains no nerve cells or blood vessels and depends entirely on the underlying dermis for nutrient delivery and waste disposal. The epidermis consists primarily of keratinocytes in progressive stages of differentiation from deeper to more superficial layers. Other cells present in this layer are Langerhans dendritic cells, sensory Merkel cells and melanin-producing melanocytes. Epidermis plays an important role in pathogen surveillance and immune response.
- Dermis: This layer is responsible for the skin's structural integrity, elasticity and resilience. It also contains capillaries, depots of immune cells and a small number of nerve and muscle cells. Also, the dermis contains several skin appendages, which play an important role in the context of dermal and transdermal drug delivery: sebaceous glands, sweat glands and hair follicles.
- Subcutaneous tissue: The predominant cell type in the subcutaneous tissue are adipocytes or fat cells. Subcutaneous fat acts as a shock absorber and heat insulator, protecting underlying tissues from extreme temperatures and mechanical trauma.

3.2 Permeation pathways through the skin

There are three main pathways by which a molecule can traverse intact stratum corneum: via appendages (shunt route); trough

the intercellular lipid domains; or by a transcellular route. The type of the pathway depends on the physico-chemical properties of the drug, but most molecules pass through the stratum corneum by a combination of these routes. On the one hand the transcellular pathway represents a polar route through the membrane, especially because of the presence of predominantly highly hydrated keratin. On the other hand, the lipid domains comprise around 1 % of the stratum corneum diffusional area and this intercellular lipid route provides the principal pathway by which most small, uncharged molecules traverse the stratum corneum. It is also possible that transport occurs through the shunt routes, but their contribution to the total flux at pseudo-steady state is generally regarded as being insignificant.[2]

Permeability through the skin can be described by Fick's equation, which describes passive diffusion of molecules through the stratum corneum:

$$J = (KD_{SC}C_S) / h_{sc}$$

J transdermal flux of molecule
D_{SC} diffusion coefficient in the stratum corneum
C_s saturated solubility of the molecule in the vehicle
K partition coefficient of skin/vehicle
h_{SC} effective thickness of the stratum corneum

In accordance with the formula, we can increase the transdermal transport by increasing the diffusion and distribution of molecule in the skin and by increasing its solubility in the vehicle. The simplest approach to optimization of transdermal transport includes a selection of suitable carrier systems; that is the change in K/C_{veh}. Many innovative approaches extend this concept to creation of supersaturated systems, microemulsions or liposomes. We can also change a drug in a way to facilitate the transport by synthesizing more lipophilic derivates, while allowing release of the active substance after chemical or enzymatic degradation in the biological environment.[3,4]

3.3 Influence of permeant physicochemical properties on the route of absorption

Relative contribution of each of the three potential pathways through the stratum corneum depends on the nature of the permeant. Molecular properties of the permeant that must be taken into account are partition coefficient, molecular size and its ionization.

Partition coefficient is the factor that predicts the pathway that the permeant will choose to pass the barrier. Hydrophilic molecules will partition preferentially into the hydrated keratinocytes rather than lipid bilayers, whereas for lipophilic permeants the situation is opposite. Molecules soluble in both oil and water, however (log $P_{(octanol/water)}$ between 1 and 3), are delivered predominantly through the intercellular route. Namely, the intercellular route is not exclusively lipoidal; it also contains desmosomes and proteins associated with lipid domains as well as a thin layer of water between the polar head groups. Thus hydrophilic permeants may also partition into these polar areas, traversing the tissue via intercellular route.[5]

A second major factor determining the flux through human skin is the molecular size of the permeant. It has been shown that small molecules cross human skin faster than large molecules. Molecular weight less than 500 Da is essential to ensure ease of diffusion, since molecular diffusivity is inversely related to its size.[6]

Lastly, ionization plays an important role in the drug flux through the stratum corneum. It is widely accepted that ionisable drugs are poor transdermal permeants, because of their high solubility in water.[7]

4. Transdermal molecular transport methods of enhancement

Physicochemical properties responsible for the protective function of human skin dictate the type of permeant that can traverse the barrier. Drugs to be delivered passively via the skin need to have adequate lipophilicity and molecular size. These require-

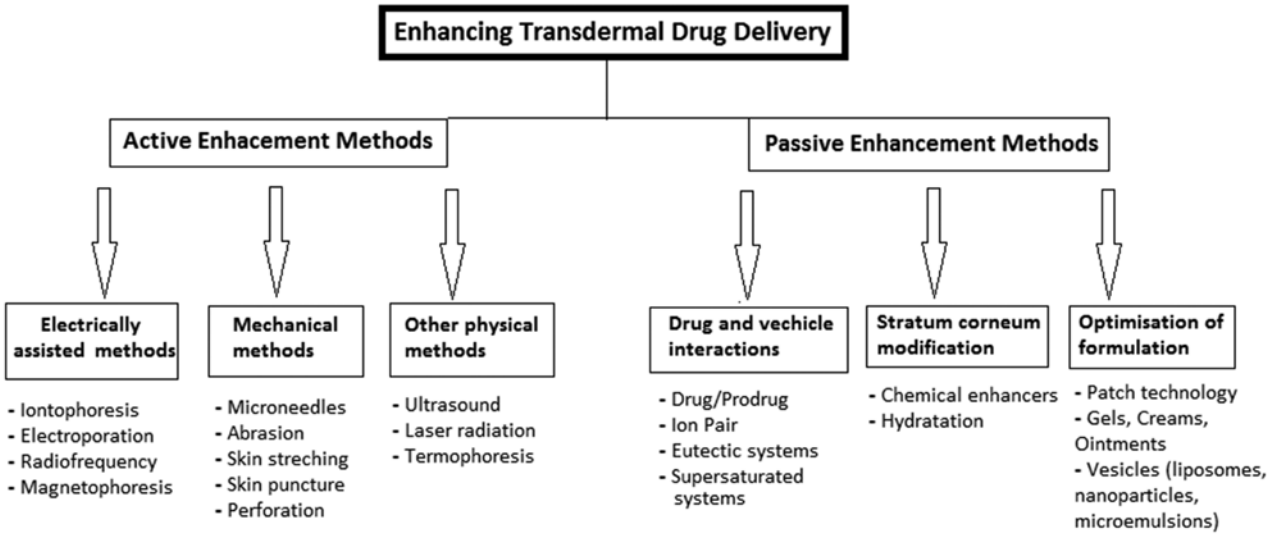


Figure 2: Transdermal drug delivery methods of enhancement[9]

ments limit the number of commercially available products based on transdermal or dermal delivery.[8] Over the past 30 years numerous studies have been performed to overcome some of the problems associated with this delivery route. Efforts to increase molecular transport have often been focused on altering lipid bilayer structure of the skin in order to make it more permeable. Modification of skin permeability has been achieved by passive and active methods of enhancement, the most important of which are illustrated in Figure 2. While passive methods are based on the formulation and chemical approaches, active use some sort of physical force to breach the skin barrier and/or provide driving force on the drug.

4.1 Passive enhancement methods

Passive approaches for transdermal enhancement include the influence on drug and vehicle interactions, optimization of formulation and stratum corneum modification. As this paper is focused on the active enhancement methods, we only briefly discuss the passive techniques.

Stratum corneum modification: One of the most widely used passive approaches for increasing transdermal transport is the use of chemical penetration enhancers. These enhancers are molecules that provide reversible reduction of the skin's barrier properties without long-term damage to the cells.[10] An ideal enhancer must be nontoxic, nonirritating, nonallergenic, pharmacologically inert and compatible with most drugs and excipients.[11] A lot of chemicals have been tested as enhancers, and examples of commonly investigated chemical enhancers include alcohols (ethanol), polyalcohols, pyrolidone, amines, amides, fatty acids, sulfoxides (dimethylsulphoxide), esters, terpenes, surfactants, water etc.[12,13] The structural diversity of chemical enhancers also makes a variety of mechanisms of action, including: a) increasing the solubility of a substance in the solvent; b) increasing the solubility of substances in the stratum corneum, which facilitates distribution of the substance from the solvent into the skin; c) reducing the diffusion barrier of the stratum corneum with perturbation into the intercellular lipids or with the impact on the intracellular keratin; d) enhancement of partitioning of the substances between the stratum corneum and viable epidermis. Potential mechanisms also include: effect on desmosome links between corneocytes or the change of metabolic activity of the skin. Although many chemicals have been evaluated as passive penetration enhancers, so far none has proven to be ideal.

Drug and vechicle interactions: This includes the use of prodrugs, ion pairing, eutectic or supersaturated systems. Prodrugs are usually designed with lipophilic moi-

eties attached to the parent compound. The lipophilicity facilitates partitioning of the molecule into the stratum corneum. Once in the tissue, the prodrug may be cleaved in the skin to liberate the active compound or can pass intact through to the systemic circulation for activation. With the use of ion pairing we are able to enhance passive transport of charged molecules. We can simply form an ion pair by combining an oppositely charged species with the charged permeant. Usually we do that by forming a salt of the active molecule (sulfate, hydrochloride…).

It was shown that also the melting point of a molecule can influence transdermal transport and one method by which the melting point of a drug delivery system can be reduced is by eutectic formation. Eutectic system is a mixture of two components that do not interact to form a new chemical compound but which, at a certain ratio, inhibit the crystallization process of one another. This results in a system that possesses a lower melting point than either of the two components. There is a clear relationship between melting point and solubility, and there are several theoretical models available that predict solubility from melting point data. Solubility is correlated to permeability coefficient, which is important for molecules to permeate the skin barrier.

To achieve optimal transdermal molecular flux, we can also use a saturated solution, or supersaturated systems to further increase thermodynamic activity.[2]

Formulation modification: Skin permeability can be increased by optimization of formulation or drug carrying vehicle. There are different vehicles such as creams, gels, ointments and patch technology in use. The most promising vesicles in transdermal research field have shown to be liposomes, nanoparticles and microemulsions.[14,15]

Unfortunately, passive methods do not greatly improve the permeation of drugs and the amount of the delivered drug is still limited.

4.2 Active enhancement methods

Active enhancement methods involve the use of external energy to act as a driving force and/or to reduce the barrier nature of the stratum corneum with the aim of enhancing drug permeation. In contrast to passive methods, they are capable of delivering therapeutically active, large molecular weight (> 500 Da) and also hydrophilic molecules (e. g., peptides and proteins) into and through the skin.

Active enhancement methods can be divided into three major classes: electrical methods (iontophoresis, electroporation, radiofrequency, magnetophoresis), mechanical methods (microneedles, abrasion, skin stretching, skin puncture and perforation) and other physical methods (laser radiation, ultrasound, termophoresis).[16] In continuation, we will focus on the most popular of these techniques.

4.2.1 Iontophoresis

Mechanism of action: Iontophoresis is the most extensively used physical enhancement technique and involves topical application of a drug either directly to the skin or indirectly via dosage forms, combined with the application of low-level electric current (0.5 mA/cm^2).[17] An increase in drug permeation as a result of this method can be attributed to two principal mechanisms: electrophoresis and electroosmosis. Charged molecules are moved through the skin via electrophoresis (also termed electromigration), which represents the dominant contribution to molecular transport. The basic principle involves the application of a charged drug to the skin under the electrode of the same charge. When current is applied, the drug will migrate to the electrode of the opposite polarity, placed either under the skin or at a short distance away on the skin surface (Figure 3). On the other hand, neutral molecules are moved by convective flow as a result of electro-osmotic forces upon application of electric current.[18] This occurs when endogenous cations, such as sodium, migrate to the cathode, and simultaneously carry water molecules in the same direction. This convection can pull even uncharged drug molecules in the same direction.[19]

Uses: Iontophoresis does not change the skin barrier itself, which is why it is mostly applicable to small charged molecules and

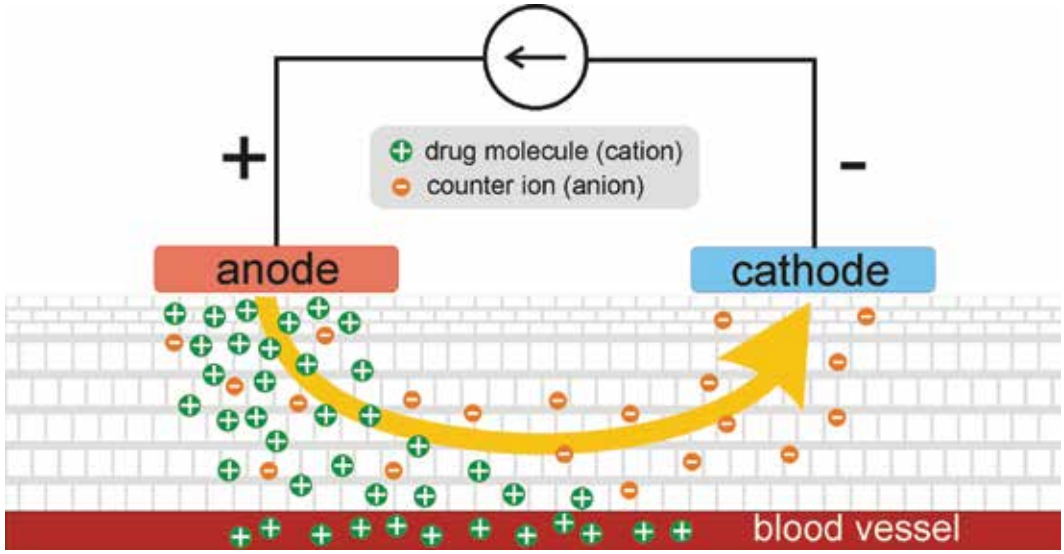


Figure 3: Iontophoresis: A charged drug is placed in a reservoir under the electrode of the same charge – the occurrence of electrophoresis or electromigration. The principle of electroosmosis is omitted from the figure, for clarity.

some macromolecules up to a few kD. A number of transdermal iontophoretic drug products have been made available so far. Currently, iontophoresis is clinically used to rapidly deliver lidocaine for local anesthesia,[20] dexamethasone sodium phosphate as the treatment of musculoskeletal inflammation,[21,22] pilocarpine to induce sweating as a part of cystic fibrosis diagnostic test,[23] tap water to treat hyperhydrosis (i.e., excessive sweating),[24] fentanyl for the management of acute postoperative pain,[25] as well as to extract glucose from the skin for glucose monitoring.[26-28] Parameters that need to be taken into account when designing iontophoretic skin delivery systems include the type of electrode, charge, molecular mass and other physico-chemical properties of the drug, plus the electrical cycle and formulation factors (pH of the system, presence of competing ions).[29,30]

Advantages and limitations: The principal advantage of iontophoresis is its ability to deliver compounds that are charged and have medium molecular weights, such as peptides and oligonucleotides. These macromolecules would normally not permeate the skin via passive diffusion mechanisms even with the assistance of chemical penetration enhancers. Also, this method provides a fast onset of action and controlled drug delivery depending on the amount of electric current, independent of the level of skin permeability. It is also well accepted by patients and causes no skin damage. The limitation of iontophoretic systems, however, is the amount of current that can be used in humans to avoid pain and the irreversible damage such currents could do to the barrier properties of the skin.[31]

4.2.2. Electroporation

Mechanism of action: Electroporation is a well-established method used to transiently permeabilize cell membranes. Electric pulses of high voltage and short duration are applied to cells or tissues in order to create transient aqueous pores in phospholipid bilayers of cell membrane.[32,33] Such increase in cell membrane permeability facilitates cellular uptake of small and larger molecules, such as various drugs or DNA, which, in normal conditions, are unable to traverse cell membrane.[34] Further, the potential of electroporation to create aqueous pathways across the skin's outermost layer, the stratum corneum, to enhance transdermal drug delivery has also been demonstrated (Figure 4). Because of its composition, the stratum corneum resistance is by orders of magnitude higher than that of deeper tissues, and the high electric field resulting from the application of electric pulses is present mostly in the stratum corneum. However, as a result of electroporation, the resistance of stratum corneum rapidly decreases and high electric field distributes into deeper tissues/layers.[34-37]

Numerous treatment parameters such as the amplitude, length and number of electric pulses influence the success of drug

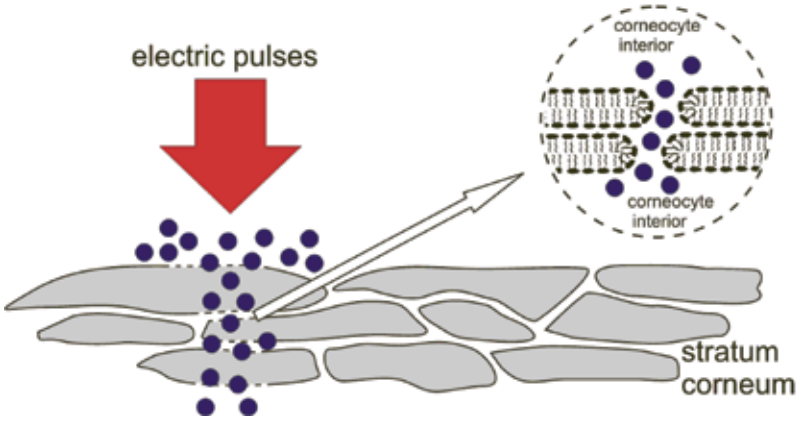


Figure 4: Electroporation of the stratum corneum: pathways are created through lipid bilayers of corneocytes in the stratum corneum – transcellular route.

delivery. Different forms of electric pulses can be used, but the most common are exponentially decaying pulses or square-wave pulses. In general, an increase in the amplitude, length and number of pulses results in increased permeability of the stratum corneum. Consequently, molecular flux is enhanced until maximum value is reached beyond which increasing pulse parameters has negligible effect on the flux.[38]

Molecular transport through transiently permeabilized skin by electroporation arises at different times and from different mechanisms. Enhanced diffusion, during and after pulses, and electrophoretic movement with very slight electroosmosis during the pulse are the main mechanisms of molecular transport.[39]

Uses: Electroporation has attracted great interest for its potential to deliver large molecules to the skin. Furthermore, electroporation has successfully been used to enhance skin permeability for molecules with differing lipophilicity and size (small molecules, proteins, peptides and oligonucleotides), including biopharmaceuticals with molecular weight greater than 7 kDa.[32] In addition, electroporation can also be used for applications where, after intradermal injection, the therapeutic molecules need to be inserted in the viable skin cells, such as for gene transfection. Transdermal gene delivery by electroporation has been tried by topically applying DNA on the skin before electroporation.[40] Unfortunately, due to the barrier function of the skin, the transfection is rather low and restricted to the epidermis. However, by using intradermal injection in combination with electric pulses, the impermeable stratum corneum is surpassed and electroporation is only used for successful permeabilization of target cells for gene transfection.[41]

Advantages and limitations: In principle, there is no limit of a size and charge of molecules which can be successfully delivered through or into the skin, however the efficacy for high-molecular weight compounds is low. Further, as mentioned above, electroporation can be used for applications such as gene delivery, in which the target place of action is the cell interior. However, if the pulses are not carefully selected (their amplitude is too high or duration is too long) this can cause skin damage and/or irritation. Also, molecular transport into and out of the skin during the time of increased permeability is relatively nonspecific, which makes dose control more difficult.

4.2.3 Ultrasound (Sonophoresis)

Mechanism of action: Ultrasound is sound waves at a frequency above upper limit of human hearing capacity (around 20 kHz). Ultrasound is typically classified as high-frequency or diagnostic ultrasound (above 3 MHz), medium-frequency or therapeutic ultrasound (1–3 MHz) and low-frequency or power ultrasound (20–100 kHz). The term used especially in transdermal drug delivery is sonophoresis, a technique used to increase drug permeation through or into the skin with enhanced bioavailability by application of ultrasound waves as physical force. Frequencies in the range of 20 kHz to 16 MHz have been investigated for this purpose.[42,43] Of these sound frequencies low-frequency ultrasound is being recognized as the most efficient for transdermal applications. The parameters of ultrasound waves to be used and duration of exposure depend on the nature of the drug, especially molecular weight and lipophilicity.[44,45]

Mechanistically, sonophoresis is considered to enhance drug delivery through a combination of thermal and mechanical alterations within the skin tissue. It is reported that sonophoresis induces the formation of small gaseous pockets in the coupling medium that, upon their collapse, emits shock

waves, thus disrupting the stratum corneum (cavitation). They further cause an increase in pore size and alteration in the stratum corneum lipid architecture.[46,47] Cavitation is considered to be the predominant mechanism of low-frequency sonophoresis and probably accounts for the enhanced transport of polar macromolecules such as insulin, interferon-γ and erythropoietin across human skin.[48]

Uses: Application of ultrasound in drug delivery into or through the skin includes small and large molecules. Some of drug classes, which were investigated, include analgesics (ibuprofen), corticosteroids (hydrocortisone),[49] anesthetics (lidocaine), antibiotics and anticancer agents (5-fluorouracil). Several preclinical studies reported successful delivery of the macromolecules, such as insulin, interferon and erythropoietin across human skin.[50] Clinical studies were mainly oriented to elicit local effects. Investigations of therapeutic effects of ultrasound with ketoprofen,[51] diclofenac,[52] ibuprofen[53] and also lidocaine[54] in humans were performed.

Advantages and limitations: Sonophoresis is an enhancement technique which is mostly used for hydrophilic molecules. In an earlier study that utilized drug molecules with varying octanol/water partition coefficient, flux of lipophilic drugs across rat skin after applying ultrasound was the same as before its application. In contrast, the flux of hydrophilic molecules was increased more than sevenfold after applying ultrasound.[55] By using ultrasound we also need to pay attention to the skin damage that this method can cause at higher intensities.

4.2.4 Radiofrequency (RF) Microablation

Mechanism of action: Cell ablation using alternating current at radiofrequencies has been used in medical procedures for decades. At the most basic level, electrical energy is used to create heat, resulting in removal of unwanted tissue, such as tumor.[56,57] Recently, it gained momentum as a method to enhance transdermal drug delivery through thermally-induced pathways in the stratum corneum. There are two principal mechanisms by which the temperature of a material is increased in electromagnetic field: i) resistive heating: current flow in conductive material induced by the electric field generates heat due to resistive losses in the material (tissue); ii) dielectric heating: molecular rotation occurs in materials containing polar molecules, which will align themselves in the field by rotating. Alternating field continuously rotates the molecules, or causes vibrations, thereby heating the material (tissue).

Uses: The efficacy of RF microablation for transdermal drug delivery has been shown both in vitro and in vivo. The delivery of polar hydrophilic molecules of molecular weight over 300 Da, and even of human growth protein (22 kDa), which poorly permeates the lipophilic stratum corneum barrier was significantly enhanced through excised porcine ear skin and through rat skin in vivo by using radiofrequency microelectrodes.[58,59] Also, using RF microablation to facilitate the delivery of naked DNA into the skin has been shown to greatly enhance gene expression.[60] TransPharma and Altea have modified and implemented the use of RF ablation and developed the RF-MicroChannel technology to create passages through the skin that allow a novel approach for transdermal drug delivery. Clinical trials for delivering human growth hormone (hGH) have been successfully completed, in contrast with human parathyroid hormone (hPTH), where clinical trials are still in progress.

Advantages and limitations: It has been reported that RF microablation produces no skin damage, erythema or edema[58,59] due to the relatively low depth of the microchannels formed by RF microablation and the fact that only a small portion of the treated skin area is covered by electrodes (less than 0.2 % of the area). However, treatment parameters need to be carefully selected, as excessive heating can otherwise cause thermal damage to the underlying viable tissues.

4.2.5 Laser energy

Mechanism of action: This method involves direct and controlled exposure of the skin to a laser beam that results in microablation of the stratum corneum without damaging the underlying viable skin. Removal

of the stratum corneum by this method has been shown to enhance the delivery of both lipophilic and hydrophilic drugs.[61,62]

The use of lasers in clinical therapies is quite common and their effects on biological membranes are well documented. Most widespread is the use of lasers for the treatment of dermatological conditions such as acne.

The extent of skin barrier disruption by laser energy is controlled by parameters such as wavelength, pulse length, pulse energy, pulse number and pulse repetition frequency.[63]

Uses: Lasers are quite successful in enhancing the permeation through the stratum corneum even of large molecular weight solutes with the application of high intensity laser beam.[64] An example of a molecule that can successfully be delivered through the skin (in vivo) with laser energy enhancement technique is insulin.[65,66]

Advantages and limitations: The use of lasers for transdermal delivery offers advantages such as controlled removal of the stratum corneum, short treatment time, a painless intra- or transdermal delivery method and mild adverse effects. In contrast, the safety and clinical efficacy of this technique still need to be resolved.[61]

4.2.6 Microneedles

Mechanism of action: Microneedles represent a unique technological approach to enhance drug permeation across the stratum corneum. Microneedle-based delivery involves an array of needles with sharp tip, which are used to pierce upper skin layers in a minimally invasive manner creating a physical pathway for therapeutic molecules to diffuse into and/or through the skin.[67,68] This strategy typically employs between 1 and 400 needles (100 µm to 1 mm apart), which may vary from 150 µm to 1000 µm in length having a diameter from 50 µm to 80 µm. The microneedle array is applied to the skin surface so that microneedles penetrate the stratum corneum to bypass it and deliver the drug directly into the viable epidermis. As the epidermis does not contain nociceptors and the microneedles do not penetrate to the dermis, pain sensation is not elicited. The only similar sensation is to wearing a plaster.

There is a range of different geometries being developed, but they can be divided into two main categories: solid and hollow.[69] Solid microneedles can be applied to the skin and then removed to form pores before a drug formulation is applied onto the region. Another approach uses solid microneedles coated with a drug then inserted into the skin. Solid microneedles are usually made from silicon or metal. Hollow microneedles, on the other hand, offer the possibility of transporting drugs through the needle interiors by diffusion or by pressure driven flow, for more rapid drug delivery from a drug reservoir.[70,71] (Figure 5) The reservoir may contain a drug, solution of a drug, gel or solid particles, and also the membrane to separate the drug from the skin and achieve a controlled release of the drug from its reservoir. In addition to solid and hollow microneedles other designs have been created, such as polymer microneedles that dissolve upon application.[72,73]

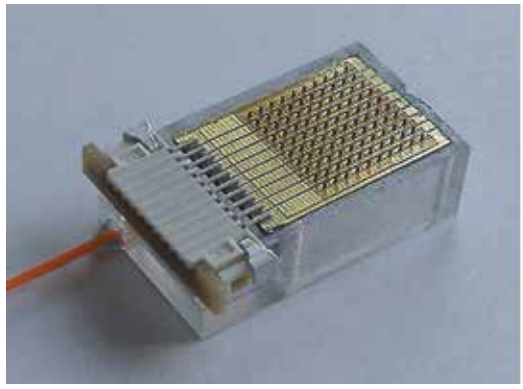
Uses: Microneedles have been used to enable transdermal delivery of several drugs, such as small molecules (naltrexone),[70] proteins (insulin),[74,75] DNA[76] and also vaccines for systemic action.[77]

Advantages and limitations: Microneedles can be used for all kinds of molecules, including nanoparticles, and there is practically no size limit to the molecules that can be delivered, which is the biggest advantage of microneedle-based technologies. Limitations of this delivery method, however, can be insufficient needle penetration or breaking of the delicate microneedles.

4.3 Choosing the optimal method for specific application

When selecting optimal enhancement method for transdermal delivery of different types of molecules, both benefits and limitations of each method should be taken into account.

The upper limit of molecular size of the substances that can be delivered transdermally with iontophoresis is generally believed to be around 10 to 12 kDa. Candidate



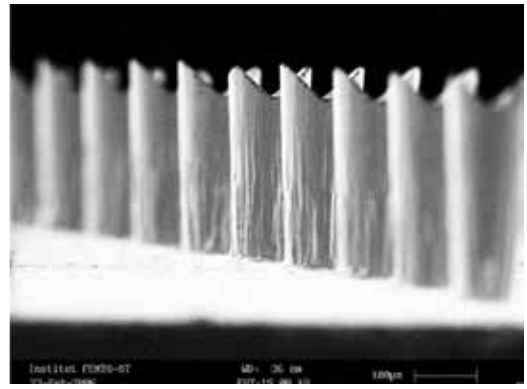


Figure 5: Microneedle-microfluidics system for dermal gene delivery developed in the framework of FP6 project Angioskin. Plasmid is delivered via hollow microneedles that serve as electrodes, delivering electroporation pulses to facilitate cellular uptake of the plasmid.[71]

molecules also need to have some aqueous solubility and ideally should be charged. Iontophoresis also allows modulated delivery by controlling the current.

In contrast to iontophoresis, electroporation shows a great potential to deliver large molecules such as various drugs or DNA, which are in normal conditions unable to traverse the skin.

When using electroporation, we have to be careful when choosing a voltage, to avoid irritation of the skin and unpleasant sensations caused by electric pulses.

Ultrasound is a method of choice for transdermal delivery of hydrophilic molecules. It can also be used in combination with some sort of skin pretreatment, such as laser energy.

Further, the microchannels created in the skin by microneedles, laser or radiofrequency microablation are several microns in diameter and therefore pose no size limits for the molecules that can be delivered through them. In fact, even small particles can be delivered via these microchannels, such as nanoparticles. Appropriate candidates for delivery by microneedles are vaccines, for which drug-coated microneedles are usually used, offering the advantage of drug being placed directly into the skin upon insertion.[78]

Several studies have been made describing two or more enhancement methods used in combination, showing even more efficacy in the context of transdermal drug delivery. However, they represent more complex technology which makes regulatory approval more difficult.[79]

5. Transdermal products using active enhancement methods on the market

Transdermal market is dominated by "passive" transdermal patches but there are also more and more drugs incorporated into transdermal systems using active enhancement techniques (Table 2). The most represented in this area are various iontophoretic systems. The Phoresor™ device (Iomed Inc.) was the first iontophoretic device on the market, which was approved by the FDA in the late 1970s. Well known is also the Vyteris lidocaine delivery system for local dermal anesthesia and E-TRANS® technology (Alza Corporation) with fentanyl for the treatment of acute pain, which was approved and then withdrawn because of technical reasons.[16]

One of the electroporation transdermal prototype devices had been developed by Inovio Biomedical Corporation and they tested it with various compounds to improve drug delivery and also to achieve gene delivery.[80] There had been also other electroporation transdermal system proposed by other groups, such as Pliquett et al. with the patent of apparatus and method for electroporation of tissue,[81] Zhang et al. with electrically assisted transdermal method and apparatus for the treatment of erectile dysfunction[82] and Wong et al. with electroporation with a new needle-free microelectrode array.[83]

The best known low-frequency ultrasound device for transdermal drug delivery is SonoPrep®, a device made by Sontra Medical Corporation. SonoPrep®, which is able to reduce the time before the onset of action associated with the delivery of local anesthetic. Similar quick onset of action af-

ter topically applied anesthetic (lidocaine), can be observed when using a laser device made by Norwood Abbey Ltd.

The microneedle technology most used in research and clinical trials is the Macroflux® made by Zosano Pharma. This patch can be used either in combination with a drug reservoir or by coating the drug on the microneedle array.[80] Most advanced hollow and solid microneedle systems now are made by 3M Drug delivery systems. They developed a microneedle-based drug delivery platform, collectively referred to as MTS (Microstructured Transdermal Systems, advertised as 3M™ Microchannel Skin System).

Conclusion

Transdermal drug delivery has a big potential for delivering drugs that are not suitable for administration through other routes, such as intravenous or oral. Skin tissue represents a convenient, readily accessible as well as large surface area for drug entry. It is more patient-friendly and can overcome many of the disadvantages of some other delivery routes. Furthermore, a successful immune response can be elicited due to a large number of antigen presenting cells in the skin. Unfortunately, transdermal flux is often too low, which requires temporary reduction of the skin barrier properties to ensure therapeutically signifi-

Table 2: Examples of transdermal products based on active enhancement techniques

Drug	Delivery system	Developmental status	Company (Trade name of the product)
Lidocaine HCl and Epinephrine	Iontophoresis	On market	Vyteris (Lidosite®)
Cosmeceuticals/antiwrinkle patch	Iontophoresis	On market	Isis Biopolymer (Isis Patch)
Sumatriptan	Iontophoresis	Phase 3 completed	Nupathe (Zelrix)
Zolmitriptan	Iontophoresis	Phase 1 completed	Vyteris (Vyteris active patch)
Lidocaine	Iontophoresis	On market	Iomed Inc. (Phoresor®)
Fentanyl	Iontophoresis	On market then withdrawn	Alza Corporation (E-TRANS® technology) & Janssen Pharmaceutica (IONSYS™)
DNA vaccine	Microneedles + electroporation	Phase 1	CytoPulse Sciences (Easy Vax™)
Lidocaine	Sonophoresis	On market	Sontra Medical Corporation (SonoPrep®)
hGH and other drugs	RF microchannels	Phase 1 completed	TransPharma Medical (ViaDerm) & Altea Therapeutics
Lidocaine	Laser microablation	On market	Norwood Abbey (Epiture Easytouch™)
Influenza Vaccine hGH, lidocaine	Microneedles Microneedles Microneedles	On market Phase 2 Phase 1 completed	Sanofi Pasteur Ltd. (Intanza®/IDflu®) Zosano Pharma (Macroflux®) 3M Drug delivery systems (3M™ Microchannel Skin System)

cant delivery. This is achieved by different enhancement methods, both passive and active, that are constantly being developed and improved. Passive enhancement methods such as chemical enhancers, although extensively investigated, have achieved limited enhancement of transdermal transport of small molecules, and have failed to deliver larger molecules at therapeutic rates. On the other hand, active enhancement methods involving some sort of external energy to either reduce the stratum corneum barrier property or provide a driving force for the drug, have shown more success for delivery of both small molecules as well as macromolecules. Their disadvantage, however, is their reliance on enhancement devices, which reduces easiness of application and increases cost. Also, the use of energy sources for causing mechanical, thermal or electrical disruption of the skin raises more safety issues. Despite the hurdles, the advantages of avoiding gastrointestinal tract, controlling the drug release kinetics, the ability to adjust the dose and patient compliance, make transdermal route worth investing further research efforts. By using different combinations of the described enhancement methods, further optimized for each application, transdermal delivery can be advanced even further to translate its potential into clinical outcome.

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