REVIEW ARTICLE

Percutaneous penetration enhancement in transdermal drug delivery

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The transdermal route has numerous advantages over the more traditional drug delivery routes. These include high bioavailability, absence of first pass hepatic metabolism, steady drug plasma concentrations, and the fact that therapy is non-invasive. The main obstacle to permeating drug molecules is the outermost layer of the skin, the stratum corneum. Consequently, research into enhancing transdermal drug delivery (TDD) by overcoming this layer, is an area of prime interest. This review article is written to provide a coverage commentary of the recent advancements in TDD enhancement techniques.

Key words: *Biological enhancement, chemical enhancement, physical enhancement, drug formulation, drug modification, percutaneous penetration, transdermal drug delivery*

INTRODUCTION

Optimal therapeutic outcomes require not only a potent drug, but also an effective drug delivery system. Transdermal drug delivery (TDD) is the utilization of the skin as the drug entry point, to achieve systemic drug delivery. TDD offers several advantages over other more conventional drug delivery routes, such as, the oral route. These advantages include the avoidance of hepatic first pass and gastrointestinal metabolism. The transdermal route also avoids the peaks and troughs in the drug plasma concentration that are usually associated with the oral route. These fluctuations in drug plasma concentration can lead to side effects or treatment failure. TDD also presents a straightforward dosage regimen, and by avoiding the oral route, abdominal discomfort and irritation that is caused by some drugs is eliminated. Furthermore, the improved compliance associated with the transdermal route can be particularly beneficial when dealing with vulnerable patient groups such as children and the mentally ill. The usefulness of the transdermal route is limited by the fact that the skin forms an excellent barrier to exogenous chemicals. The key to successful TDD relies on the ability to find a strategy that can temporarily remove or reduce the inherent barrier properties of the skin. Over the last 40 years much research in this field has been focused on developing different strategies to overcome this barrier. Here, transdermal enhancement strategies have been divided into five groups: drug formulation factors, drug modification, chemical enhancement, physical enhancement, and biological enhancement techniques.

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DRUG FORMULATION-BASED ENHANCEMENT APPROACHES

Formulation-based enhancement techniques are often based upon our understanding of Equation 1, which is derived from Fick's first law of diffusion. Adolf Fick modified the equation so that it could be used to model steady state diffusion across a membrane of thickness, h.

$$J = PDC_v / h \quad (1)$$

Where J = flux, C_v = permeant concentration in the formulation, D = permeant diffusion coefficient, and P = permeant partition coefficient between the formulation and the membrane.

It is clear that transdermal drug flux can be improved by increasing the permeant partition co-efficient or the concentration of the drug in the vehicle. There are a variety of means by which these factors can be altered, with the result being an increase in permeation flux. However, the practical application of Equation 1 is limited, as where skin is considered, the length of the diffusion pathway varies depending upon the

route across the stratum corneum (SC) that the permeating molecule takes. This is overcome by defining the permeability coefficient (k_p), which is given by Equation 2.

$$k_p = DP/h \quad (2)$$

$$J = k_p C_v \quad (3)$$

Consequently, Equation 1, the modified version of Fick's first law, can now be simplified and written as Equation 3.

pH regulation

According to the pH partition theory, a permeant, which is weakly acidic or basic, may have a low apparent partition coefficient, which can reduce the likelihood of the drug partitioning into the skin. To circumvent this problem a buffered vehicle can be employed where the drug will be largely unionized and thus have a more favorable apparent partition coefficient. The general rule is: pH < pKa for weakly acidic drugs to be unionized and pKa < pH for weakly basic drugs to be unionized. However, the extremes of pH that may be required to prevent drug ionization could result in skin irritation. For example, lidocaine, which has a pKa value of 8, would require a buffered vehicle of pH 9 to ensure that the drug is 90% unionized. Moreover, there is the possibility that pH extremes may also denature some endogenous enzymes localized in the skin. An alternative approach to altering the pH of the formulation is to introduce an ion pair for a charged drug such as the hydrochloride salt of lidocaine or the sulfate of terbutaline.[1,2] It has been demonstrated that ion paring mechanisms can be employed to enhance the permeation of ionized drugs, as the ionized drugs interact with the counter ion agents that will then permeate the biological membrane more readily.[3] In one study, the permeation of the drug on dansteron was paired with oleic acid, which is also known to act as a permeation enhancer.[4] In another experiment the researcher investigated the effect of ion pairing on meloxicam, with six organic bases and four normal permeation enhancers, which include oleic acid, menthol, azone, and N-methyl-2-pyrrolidone.[5] The cumulative permeation is markedly increased in the presence of either a counter ion or chemical enhancers, and the results clearly suggest that the degree of enhancement depends only on the structure and hydrophilicity of the counter ions. However, overall only the limited permeation enhancement ratio is achieved with ion pairing techniques. Further detailed review of ion pair techniques for drug delivery across the biological membrane is available in Neubert R.[6]

Supersaturation

Equations 1 and 2 illustrate how the flux of a drug across a membrane is directly proportional to its concentration in the vehicle. This is because drug concentration in the delivery vehicle is directly related to the thermodynamic activity of the drug in the vehicle. Thermodynamic activity is the driving force behind the diffusion of the drug down a concentration gradient.[7] For this reason, researchers often conduct diffusion experiments with the drug in a vehicle concentration at the saturation solubility level, thereby maximizing the thermodynamic activity to 1. This will theoretically elicit the maximum flux and it will also allow direct comparisons between different formulations or chemical enhancement techniques. Consequently, a given increase in the maximum solubility of the drug can result in a higher flux, and this concept is supported by many studies employing supersaturated systems.[8,9] Supersaturated systems can be obtained by a variety of techniques such as solvent evaporation, back cooling, and the addition of a co-solvent. Supersaturation by solvent evaporation can occur when a volatile solvent such as ethanol is used to dissolve a drug in a non-occluded or open system. The solvent evaporates leaving an increasingly concentrated drug solution with a thermodynamic activity in excess of 1. However, because the supersaturated state is inherently unstable, the drug will often crystallize rapidly, thus reducing the thermodynamic activity.[10] This problem can sometimes be prevented or delayed by the use of anti-nucleating agents such as hydroxypropyl-methylcellulose (HPMC) and poly-vinylpyrrolidone (PVP).[8,11] The addition of PVP to oestradiol solutions is seen to produce an 18-fold increase in drug saturated solubility, due to inhibition of drug crystallization by PVP.[8] The co-solvent approach involves the dissolution of the drug in two solvents, one in which the drug has a high solubility and the second in which the drug has a much lower solubility. The drug is first dissolved in the better solvent to its maximum solubility and this solution is subsequently diluted with the second solvent. The resultant mixture will initially have a higher drug solubility compared to if the drug was directly dissolved in the binary mixture of this composition. Again, the addition of an anti-nucleating agent will prevent or delay the crystallization of the drug. Supersaturated formulations prepared by the co-solvent method with an anti-nucleating agent have been shown to be stable for an excess of two months, whereas, without an anti-nucleating agent the same formulation lasts for less than an hour in the supersaturated state.[11] A potential commercial weakness of this enhancement technique is that increased quantities of drug are required, which may not be cost-effective.

PERMEANT MODIFICATION

It is widely acknowledged that for a compound to permeate the skin in significant quantities it should possess a Log P of approximately 1 – 3.[12] If Log P of a permeant is outside of this range it may not easily partition from the formulation into the skin, thus according to Equation 1, it will exhibit a low transdermal flux. However, a simple chemical modification of the drug could often be carried out to alter its Log P to a more favorable value and optimize partitioning it into the skin. Thus the prodrug approach, use of enantiomers, and melting point depression are examples of drug modification-based enhancement in TDD.

Prodrug approach

A prodrug can be defined as a compound that usually has little or no pharmacological activity itself, but becomes active after bioconversion in the body. The expression was first termed in 1958, by Albert, and in the intervening half-century, prodrugs have been administered via all possible routes, including transdermally.[13-15] The main objective of synthesizing a prodrug for transdermal delivery is to create a compound of optimal Log P (1 – 3), which may result in improved transdermal flux. However, the active moiety must be subsequently liberated for the parent moiety to exert its therapeutic effect. A considerable amount of research has been carried out in the area of transdermal prodrugs, and a number of researchers have found that prodrugs can exhibit improved percutaneous flux compared to the parent compound.[16,17]

In a permeation study using cadaver skin, the transdermal flux of piperazinyl derivatives of naproxen was shown to be up to nine-fold that of the parent drug. In another study two alkyl esters of morphine, morphine propionate and morphine enanthate, were synthesized as potential prodrugs for transdermal delivery, and it was found that both these prodrugs showed two-to-five-fold enhancement, respectively, compared to the parent drug morphine. Conversely, the flux of haloperidol across guinea pig skin was found to exceed that of any of its *O*-acyl derivatives.[18] It was postulated that the lower flux exhibited by the prodrugs was due to a combination of poorer aqueous solubility and increased molecular weight. Prodrugs have also been synthesized by coupling drugs to known chemical penetration enhancers. In one study, using rodent skin, it was found that the flux of the prodrug, 4,5-dihydroisoxazol-3-yl, synthesized from cycloserine and fatty acids, was 20-fold that of the parent drug.[19]

Melting point depression

The effect of the melting point on transdermal flux can be clearly observed when permeation of enantiomers from a racemic mixture is assessed. In one such experiment, the enantiomers with the lowest melting point, from a selection of three chiral beta-blocker molecules, namely, atenolol, alprenolol, and propranolol have been shown to exhibit a heightened flux compared to the other two racemic mixtures.[20] This clearly demonstrates that a lower melting point can increase drug flux. However, the choice of the enantiomer is sometimes limited by therapeutic activity. Similarly, a eutectic system can be prepared to induce lowering in the melting point of a drug. The interaction between a drug and a second compound in a eutectic mixture can lower the melting point of the binary mixture to levels that are lower than the melting points of the individual compounds. Eutectic systems have also been developed by selecting a drug and a second compound that is a potent chemical enhancer.[21,22] One system consisting of ibuprofen : thymol (40 : 60) gave a flux that was 12 times greater than the flux of ibuprofen, from a saturated aqueous solution. Kang also demonstrated similar findings, by developing a eutectic system consisting of lidocaine and 1-menthol.[22] The permeation of lidocaine from this system was significantly higher than its permeation from an aqueous solution in the presence and absence of propylene glycol across a snake skin membrane. The use of eutectic systems to effect melting point depression appear to be a valuable tool in TDD and can be employed in combination with other techniques to enhance transdermal flux.

CHEMICAL ENHANCEMENT

Chemical penetration enhancers are substances that aid the percutaneous penetration of a drug by partitioning into the SC and temporarily altering the lipid-protein arrangement. This change induces a temporary and reversible decrease in the SC barrier properties.[23] There are numerous chemicals that are able to exert these effects: Sulfoxides, azone, pyrrolidones, fatty acids, alcohols, glycols, surfactants, urea, and terpenes, have all been shown to act as transdermal penetration enhancers. Additionally, lipid-based vesicles such as liposomes and variants thereof have also been shown to enhance TDD. It has been proposed that any chemical that is to be used in the facilitation of TDD should ideally possess the following general requirements:[24]

1. It should be non-toxic and not irritating to the skin.
2. When removed from the skin, the barrier properties should reform fully and rapidly.
3. It should be cosmetically acceptable and give an appropriate skin feel.
4. It should be compatible with a wide range of excipients and transdermal drugs.
5. Its action should be rapid and the duration of effect should be both predictable and reproducible.
6. It should have no pharmacological activity within the body.
7. It should work in one direction only, such that it solely facilitates the absorbance of drugs while not enhancing the egress of any substance from the body.

No one chemical has yet been discovered that possesses all of the characteristics outlined above, however, some chemicals do demonstrate several of these attributes.

Water

Water has been well-characterized as an agent that can increase drug flux across the skin.[25] The water content of the SC in humans is typically 15 to 20% under normal physiological conditions. However, when fully hydrated, such as may occur when the skin is occluded, the highest water content can increase the weight of the SC by up to 400%.[24] The hydration of the occluded area increases because little or no water is lost through evaporation. Topical formulations that employ hydrophobic bases as vehicles can also achieve this effect as oily bases limit transepidermal water loss

(TEWL). It has been suggested that hydration causes swelling of the corneocytes, which in turn affects the arrangement of the SC lipid bilayers. These disruptions cause a merging of the interrupted polar and continuous non-polar intercellular routes to form a continuous combined polar and non-polar route across the SC.[26] However, it has been argued that there is actually no major modification in the SC lipid packing except for water pools in the SC lipid bilayer.[27] It seems certain that SC hydration can increase drug flux, but more research is needed to clearly address the exact mechanism of TDD enhancement.

Sulfoxides

One of the most potent chemical penetration enhancers is the keratolytic aprotic solvent, dimethylsulfoxide (DMSO). This substance enhances the movement of both lipophilic and hydrophilic drugs across the skin, thus, it can be described as a non-specific permeation enhancer. DMSO is concentration-dependent and works well when it is at concentrations above 90%.[24] Although a potent enhancer, DMSO suffers from being both toxic and an irritant. Erythema, wheals, and contact urticaria have all been reported as a result of topical DMSO application.[28] This report of adverse effects has driven researchers to investigate other structurally related substances, such as, dimethylacetamide (DMAC), dimethylformamide (DMF), and the alkyl derivative decylmethylsulfoxide (DCMS). These compounds have all demonstrated percutaneous penetration enhancement, but there is no demonstrable improvement in the toxicity or irritancy profiles.[29] The mode of action of these agents is complex. They are believed to damage the keratin present in the corneocytes and desmosomes, as well as, interact with the distorting the SC lipid bilayers.[24]

Azone

Azone has been specifically synthesized as a transdermal penetration enhancer.[24] It consists of a polar cyclic amide group attached to a non-polar alkyl group. It is highly lipophilic with a Log $P_{(oct/water)}$ of 6.2; consequently, it is soluble in many organic solvents, but not in water. Similar to DMSO, it is described as being a non-selective penetration enhancer, because it has been shown to enhance the permeation of both lipophilic and hydrophilic drugs. Its effects are, however, more pronounced with hydrophilic permeants.[30] The toxicity and irritancy of Azone is very low even when applied undiluted making it more suitable from a regulatory point of view. Its action is concentration-dependent, with maximum penetration enhancement being observed at working concentrations of < 5% and no significant enhancement being reported when the concentration exceeds 15%.[30] The mechanism of action of Azone has been attributed to its 'soup-spoon' shape, which may disrupt the ordered SC lipid packing, thus improving drug flux across the skin.[31] Azone has been shown to possess some anti-viral activity, which in itself need not necessarily be problematic, however, it does indicate that this molecule could possess pharmacological activity that exhibits some effect on human physiological processes.[32] The fate and effects of Azone and its metabolites are still under investigation.

Pyrrolidones

Pyrrolidones are aprotic organic compounds consisting of a five-membered lactam ring. Pyrrolidones are typically colorless liquids and they are miscible with a wide variety of solvents including water, ethanol, diethyl ether, chloroform, benzene, ethyl acetate, and carbon disulfide. 2-Pyrrolidone is an intermediate in the manufacture of polymers, such as, polyvinylpyrrolidone and polypyrrolidone. 2-Pyrrolidone has been shown to enhance the permeation of both lipophilic and hydrophilic compounds across the skin and is marketed as the transdermal enhancer, Soluphor® P, by BASF Corporation. The mode of action of 2-pyrrolidone is believed to be due to its effect on drug partitioning, however, these effects are more prominent with hydrophilic permeants.[24] 2-Pyrrolidone diffuses into the epidermis and is thought to fill any void within the epidermal layers thus modifying the chemical composition of the epidermis and consequently altering the partitioning behavior of the permeant between the formulation and skin compartments.

Fatty acids

Many fatty acids have been investigated for their potential transdermal enhancing activity.[33] Fatty acids can be grouped into two categories based on the presence or absence of double bonds in their alkyl chains. Among the saturated fatty acids, lauric acid (C_{12}) is thought to be the most potent enhancer, while oleic acid (C_{18}), which possesses one cis-configuration double bond at carbon 9, is one of the leading unsaturated fatty acid enhancers.[24] Oleic acid is a non-selective enhancer and optimal enhancement occurs when it is present at concentrations below 10%. Spectroscopic studies suggest that its mode of action can be attributed to its effect on SC lipid packing.[34] It has been suggested that linoleic acid and linolenic acid, which both possess a C_{18} chain with two and three cis double bonds, respectively, may possess superior transdermal penetration enhancement activity to oleic acid.[35]

Fatty acid esters

Many fatty acid esters have shown promise as chemicals capable of enhancing TDD. These include isopropyl myristate (IPM), isopropyl palmitate (IPP), ethyl ethanoate (EE), methyl ethanoate (ME), butyl ethanoate (BE), and ethyl oleate (OE). This list is not exhaustive and many other fatty acid esters have been investigated for transdermal applications. One widely used example in both the cosmetic and pharmaceutical industries is IPM. This compound has been shown to increase the partitioning of drugs into the SC and also increased the fluidity of the SC lipid bilayers.[36] The effects of IPM on the permeation of a wide range of drugs have been investigated and it is included in a number of marketed transdermal formulations.[26] The simpler short-chain fatty acid esters such

as EE have also been shown to have a positive effect on drug flux and the mechanism of action is thought to be associated with an increase in SC lipid fluidity.[37]

Alcohols

Ethanol is the most notable of the alcohols to be investigated as a transdermal enhancer and it has been employed as an excipient in many transdermal devices.[26] Its enhancing effect is concentration-dependant, being more effective at low concentrations when it is used in largely aqueous systems.[38] The mechanism of action of ethanol and the other simple straight-chain aliphatic alcohols has been attributed to an increase in the partitioning of the drug into the SC as well as a possible supersaturation effect caused by its evaporation.[39] Propane-1,2-diol, more widely known as propylene glycol, has been shown to increase the permeation of drugs at a modest rate by itself, however, a synergistic effect when combined with other enhancers such as oleic acid and ethanol has been noted.[24] Propane-1,2-diol holds the advantage of being a non-irritant and non-toxic, as such, its use is widespread in cosmetic and pharmaceutical products. The mechanism of action is attributed to an increase in the partitioning of the drug from the formulation into the SC in addition to some SC lipid disturbance.[40]

Surfactants

Surface active agents or surfactants are molecules that possess both polar and non-polar regions within one molecule. They are widely used in the detergents and in cosmetic and pharmaceutical industries to solubilize poorly soluble molecules in the desired vehicle. The surfactants can be divided into four major groups: cationic surfactants, anionic surfactants, zwitterionic surfactants, and non-ionic surfactants. The cationic surfactants such as cetyl-trimethyl-ammonium bromide (CTAB), cetylpyridinium chloride, and benzalkonium chloride have been demonstrated to significantly increase the transdermal flux of some drugs, however, the irritation and damage that they cause to the skin means that this class has only limited use as a chemical enhancer.[41] Similarly, the zwitterionic surfactants also have limited use as transdermal enhancers, as they also can cause skin irritation.[42] Consequently, most research in this area is carried out using the anionic and non-ionic surfactants that tend cause less skin irritation. The anionic surfactant class, which includes sodium laurate and sodium lauryl sulfate, disrupts the packing of SC lipids leading to improved drug penetration across the skin. The non-ionic surfactants such as Polysorbates, Spans, and Tweens tend to cause less irritation and less damage to the skin, but the enhancement factor tends to be lower than the anionic class.

Urea

Urea is a strong humectant that has been used to treat skin scaling and hyper-keratolytic disorders.[43] Studies have shown that urea can increase drug transdermal penetration, but the improvement is relatively modest.[44] Urea has, however, had a considerable impact on transdermal enhancement as the search for analogs of urea has led to the discovery of 1-alkyl-4-imidazolin-2-one, which has comparable enhancement activity to that of Azone.[45]

Terpenes

The terpenes are secondary metabolites that are mostly synthesized in plants. Terpenes consist of a five carbon isoprene unit, which undergoes a condensation reaction to form a much larger (10 to 40 carbon) terpene framework. This larger terpene structure can then be subjected to further biochemical processing, which produces the final range of terpenes. Thousands of different terpenes have been identified to date and many have been investigated as transdermal enhancers. The terpenes have a good safety profile and are non-irritant and non-toxic to the skin. Furthermore, they also possess attributes such as low MW, low melting point, and moderate lipophilicity, which can promote rapid SC penetration and a consequent onset of enhancement activity.[46] The common terpenes that have been evaluated for transdermal use include limonene, pinene, carvol, carvonemethone, methol, thymol, and cineole. These compounds have been shown to enhance the penetration of both lipophilic and hydrophilic molecules across the human SC membrane.[47] The smaller terpenes tend to be more effective than the larger ones; furthermore, the hydrocarbon terpenes have tended to improve penetration of lipophilic drugs, whereas, the more polar terpenes improve the permeation of hydrophilic drugs.[24] Based on this observation, the mode of action could be due to the terpene penetrating the SC and modifying the polarity of this region, which in turn, improves the partitioning of the drug into the SC.

Liposomes, niosomes, transfersomes and ethosomes

Lipid-based encapsulation techniques have been used in drug delivery via several routes for many years. These particulates can be characterized as lipid or lipid and surfactant bilayer spheres. Hydrophilic drugs can be incorporated within the aqueous core of these complex structures, whereas, hydrophobic drugs may be incorporated within the bilayer. This strategy has been successfully developed and applied to many parenteral and oral formulations, especially for tissue-specific drug delivery. Liposome and niosome formulations have also been utilized in topical drug delivery where improved drug retention within the SC has been accompanied by minimal transdermal flux.[48,49] Liposomes for topical delivery are usually constructed using phospholipids or ceramides, whereas, the niosome (a liposome variant) has an additional non-ionic surfactant included in its formulation. Although showing promise in the area of topical drug delivery, the use of conventional liposomes (including niosomes) is unlikely to lead to any advances in transdermal drug delivery. This topical drug delivery by conventional liposomes is thought to be linked to the large size of the liposomes and the deformation that takes place within the SC. Interestingly, a liposome derivative, the

transfersome, has been shown to permeate the skin into the systemic circulation. Transfersomes consist of phospholipids, surfactants, and a small amount of ethanol, which is added in the final preparative stage. Transfersomes are ultra-flexible, thus allowing them to squeeze through narrow intercellular spaces within the skin.[50] The driving force of transfersome permeation is claimed to be generated by xerophobia, a condition where an entity moves from a low water potential area to a high water potential area, to remain hydrated and avoid dryness.[51] This force is manifested *in vivo* (where the water gradient increases as one moves away from the skin surface) to facilitate transfer of some movements to the deeper skin layers, until it is taken up by the systemic circulation. Further research into these highly deformable vehicles is needed to assess whether they are likely to be capable of delivering drugs at clinically efficacious level. A further variant of the liposome is the ethosome, which is a soft vesicle consisting of phospholipids and a high amount of ethanol. This variant is also highly deformable and has also been shown to be capable of delivering a variety of drugs, including testosterone, trihexyphenidyl hydrochloride, and insulin across the skin.[52] The mode of action of these flexible liposomes may be associated with the presence of ethanol, which can enhance drug percutaneous penetration via an, as yet, unknown mechanism.

PHYSICAL ENHANCEMENT

In recent years a number of physical techniques have been employed to enhance TDD. The strategies that are utilized typically bypass, alter or force the drug through the SC. Physical enhancement methods include techniques such as drug particulate bombardment, laser-based ablation of the SC, microneedles, ultrasound, electroporation, and iontophoresis. These approaches have demonstrated significant enhancement of TDD, even when the permeant molecules are large macromolecules such as peptides and polypeptides.

Drug particulate bombardment

This technique typically uses a high speed jet of helium gas to drive solid drug particles across the SC. Other approaches have used a mechanical hammer-like plunger to exert a driving force, which delivers a liquid drug formulation across the skin.[40] Drug particulate bombardment is suitable for delivering a set amount of drug, that is, finite transdermal dosing rather than the zero-order infinite delivery associated with passive diffusion. The main advantage of this technique is that it overcomes the SC by mechanical force and directly deposits the drug into the viable epidermis. In many ways this technique is similar to a subcutaneous injection, but it has the added advantage of avoiding the use of a needle, which may have a positive impact upon patient compliance. Disadvantages include lower dosage accuracy and the requirement that the device is operated by a healthcare professional. The lower confidence in dosage accuracy is thought to be because there is variation in inter-individual SC thickness and the fact that the SC can swell or shrink depending on the surrounding relative humidity. Incorrect handling of the device can lead to drug particles being delivered to an unintended location or the SC being damaged and its barrier function being temporarily compromised. These devices are mostly still in the development and optimization stage, however, this technique has shown great potential in delivering DNA-based vaccines to the skin in order to have *in situ* and a lasting immunological effect.[53]

Laser ablation of SC

The SC is the principal layer that retards percutaneous drug permeation. Consequently, much work has been concentrated on the ablation of SC in order to diminish this barrier. Tape-stripping, where the SC is removed by successive application and removal of adhesive cellophane tape, has received less attention as a clinical application, as it is imprecise and rather impractical due to body hair snatching when the tape is removed. However, laser ablation has been seen to be a more practical technique, as it is very precise in the thickness of SC that can be removed.[54] The thickness and area of the SC that is to be removed can be controlled by manipulating the laser light intensity, wavelength, and spot size coverage.[54] The measurement of damage induced to the underlying viable tissue after the procedure has been conducted *in vitro* on a pig, and it has been found to be acceptable compared to the chemical methods of ablation.[55] However, the main disadvantage of this technique and that of tape-stripping is that the removal of the SC can cause complications, as, before the SC has been fully replaced the patient may be exposed to noxious chemicals and / or pathogenic microbes. Furthermore, laser ablation devices are currently very expensive, and consequently, this technique is likely to have only a niche market.

Micro-fabricated needles

One of the more recent physical strategies for facilitating the transdermal delivery of drugs is the use of micro-fabricated needles (microneedles). This strategy typically employs between 1 and 400 miniature needles (100 μm apart), which may vary in height from 150 μm to 1000 μm and typically have a diameter of around 50 μm to 80 μm at the base.[56,57] It has been demonstrated that the use of microneedles for this purpose does not induce pain, irritation, swelling or noticeable skin damage.[58] The absence of pain is a result of the microneedles being of insufficient length to reach the dermis, which is rich in nerve fibres associated with pain receptors. Microneedle arrays are typically prepared from silicon, stainless steel or titanium.[57] The use of microneedles for this application may be divided into three approaches. The first is to puncture the SC with a microneedle array followed by the subsequent application of the drug formulation, typically in the form of a patch. The second approach employs drug-coated microneedles that release the drug *in situ* immediately after puncturing the skin. The final

approach sees the drug being delivered passively or with the assistance of an electrical pump, through an array of hollow microneedles. The second method may be appropriate as a finite TDD system, whereas, the third approach would be more suitable for infinite dosing. It has been demonstrated that the use of microneedles can increase drug flux up to 25000-fold for calcein, which at 623 Daltons, is a relatively low molecular weight permeant.[40] A four-fold increase in flux has been reported for very large proteins such as bovine serum albumin, which has a molecular weight of approximately 66,000 Daltons.[59] One risk in the use of microneedles is that these devices may be damaged during their application and removal. Little is known about the biological fate of small fragments of the array, which may become detached during use. However, it has been demonstrated that a force of 10 N used for placing and removing a patch containing an array of 400 silicon microneedles did not show any appreciable damage to the needles after application.[56] An additional hazard is that following the termination of treatment and device removal, the patient is subsequently left with a biohazard requiring disposal.

Ultrasound

Ultrasound is defined as sound waves that are at a frequency above the upper of limit of human hearing capacity, which is about 20 kHz for most individuals. It can be classified as a power ultrasound, which ranges from 20 – 100 kHz, therapeutic ultrasound is in the range of 1 – 3 MHz, and finally diagnostic ultrasound, which has a frequency exceeding 3 MHz. Of these sound frequencies, power ultrasound waves have been shown to enhance transdermal flux. Mitragotri *et al.* (1995), found that the flux of estradiol was increased 13-fold when using 1 MHz ultrasound, however, when the same drug was subjected to 3 MHz ultrasound the flux was only about 1.5 times greater than the control. It was demonstrated that power ultrasound was the most effective in increasing the TDD flux for both small and large molecules.[60] The same authors also demonstrated that the distance between the SC and the ultrasound device is inversely proportional to the enhancement of drug flux. It was also found that the barrier properties of the SC diminished over a 12-hour period following ultrasound treatment and then slowly returned to the initial levels between 12 and 24 hours after cessation of the treatment. The mechanism of action of ultrasound transdermal enhancement is believed to be related to the formation of cavities within the SC lipid region, which allows drugs to permeate more readily.[61] This technique has a potential to be applied to transdermal applications, however, the main obstacle will probably be the cost of future devices. Furthermore, the long duration of exposure to ultrasound that is required for successful drug administration may have a negative impact upon patient compliance.

Iontophoresis

Iontophoresis can be used to enhance the transdermal delivery of common, ionic compounds by employing an electrical potential difference. The basic principle involves the application of a charged drug to the skin in close proximity to an electrode of the same charge. When current is applied, the drug will migrate to the oppositely charged electrode, which would be either under the skin or a short distance away on the skin surface. Neutral molecules can also be delivered by iontophoresis due to electro-osmotic convection.[62] 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. The efficiency of iontophoresis depends upon the type of electrode, charge, and molecular mass of the drug, plus the electrical cycle and formulation factors.[63] Drug flux across the skin is directly proportional to the current applied, but it should be borne in mind that electric current higher than 0.5 mA cm^{-2} may cause damage to the skin.[40] Some inert electrodes such as platinum have been reported to decrease the skin pH from 5.6 to 2.6 after six hours of iontophoresis, and this is due to the discharge of hydroxide ions at the anode, leading to increased hydrogen ions left within the formulation.[64] Parameters such as drug charge density (monovalent or divalent) and molecular weight are inversely related to iontophoresis-aided drug flux.[64,65] The principal advantage of iontophoresis is its ability to deliver compounds that are charged and have high molecular weights, such as proteins, peptides, and oligonucleotides. These macromolecules would not normally be expected to permeate the skin via passive diffusion mechanisms even with the assistance of chemical penetration enhancers. Moreover, iontophoresis reduces intra-individual and inter-individual variation as the force driving drug delivery can be controlled. Additionally, the time taken for an efficacious dose to be delivered transdermally with the assistance of iontophoresis can be much shorter than passive diffusion.[66] Although iontophoresis has been able to achieve significant increases in the transdermal absorption of many drugs, it has not yet been used to successfully deliver larger peptides such as insulin.[67] As a result, studies have employed various chemical penetration enhancers in tandem with iontophoresis.[67] Such combination approaches have been found to significantly improve the absorption of insulin and many other drugs that could not be delivered effectively using iontophoresis alone. A potential drawback to using iontophoresis is that the hair follicle, the pathway of least resistance through the skin, may be irreversibly damaged by the electric current.[68] Furthermore, the prototype iontophoresis devices that have been developed for transdermal use tend to be expensive and also rather bulky.

Electroporation

Electroporation involves the application of a large electrical current ranging from 10 – 1000 V/cm^{-1} for a very short period of time, typically microseconds to milliseconds, in order to create transitory pores in the lipids of the SC.[69] These pores then permit the drug molecule to permeate the SC more readily, with a consequence of higher transdermal

flux. Parameters such as the type of electrode used, voltage applied, time of exposure and the total number of pulses delivered can be optimized to increase drug flux. This technique was initially developed for intracellular delivery of macromolecules and it has proven to be very effective in gene delivery across the bacterial cytoplasmic membrane. The approach has since been applied to TDD, and it has been demonstrated that the administration of vaccines and drugs across human skin can be enhanced.[70] A number of studies have shown that electroporation is a superior technique to iontophoresis for facilitating the transdermal delivery of lidocaine, tetracaine, and fentanyl.[71-73] However, similar to iontophoresis, the skin can be damaged by the application of high electrical current during electroporation. In order to avoid such damage, electroporation has been coupled with other approaches, such as the use of chemical enhancers, in order to enhance transdermal diffusion without the need for such high electrical current.[74] When electroporation was used in combination with the penetration enhancer Azone it was found that a very low current of 0.025 mA/cm^2 provided enhancement that was comparable to that of a much higher current (0.5 mA/cm^2) in the absence of Azone. Although this technique has been in use for many years, further studies are required in order optimize its use for transdermal applications.[75]

BIOLOGICAL ENHANCEMENT TECHNIQUES

Biological enhancement in TDD can be achieved by disrupting the final cell and tissue differentiation process that occurs in the epidermis. This process is critical to the body's ability to develop an efficient barrier against exogenous substances. One of the key processes is the formation of intercellular lipid within the stratum lucidum (SL) and SC layers. The ordered nature of these intercellular lipids is the primary factor that restricts drug permeation across the skin.[76] These lipids are synthesized *in situ* in the stratum basale (SB), stratum spinosum (SP), and stratum granulosum (SG), where they can be viewed as lamellar bodies (LB). The lipids present in these LB include sphingomyelins, glucosylceramides, phospholipids, and cholesterol-sulfate, however, the lipids found in the intercellular regions of the SC comprise largely of cholesterol, free fatty acids, and notably, ceramides. Glucosylceramides are converted to ceramides with the aid of β-glucocerebrosidase; the failure to form ceramides from glucosylceramides results in severe barrier abnormality and delayed barrier recovery after acute pertubations.[77,78] Likewise, the sphingomyelins are also converted by acid sphingomylinase to ceramides (type 2 and 5). The phospholipids are degraded by the phopholipase A_2 enzyme to give free fatty acids, which are also the required key to barrier integrity. Recent studies have demonstrated that the fluid intercellular lipid in the SC consists of only cholesterol and ceramides, where the presence of free fatty acid in the fluid cholesterol and ceramide structure yields the crystalline lipid structure.[79] An interesting observation of the epidermal tissue differentiation process is the conversion of cholesterol to cholesterol sulfate from the SB to the SC. Cholesterol sulfate is also thought to play an important role in SC barrier formation. Many inhibitors have been used to alter these biosynthetic processes with the aim of enhancing TDD. The transdermal flux of lidocaine increased by almost an order of magnitude when hairless mouse skin was given the fatty acid synthesis inhibitor 5-(tetradecyloxy)-2-furancarboxylic acid and the cholesterol synthesis inhibitor fluvastatin.[80]

CONCLUSION

Transdermal drug delivery has enormous potential as a means of delivering drugs that cannot be administered via the oral route, if the inherent weakness can be properly curbed. It is necessary to temporarily reduce the barrier properties of the skin in order to ensure that a clinically efficacious dose be delivered. Thus, successful TDD relies on techniques, such as, manipulating drug formulation, drug modification, chemical enhancement, physical enhancement, and biological enhancement or a combination thereof.

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