Combined Use of Iontophoresis and Other Physical Methods

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22.1 Introduction

The skin protects the internal environment in our body and has two unique barriers. The outermost layer of the skin, the stratum corneum, is a lipophilic, physical barrier, and the second barrier is biochemical—the viable epidermis and dermis underneath the stratum corneum (viable skin). The stratum corneum's function as a barrier by both diffusion and partition makes the transdermal delivery of drugs difficult. Thus, the first phase in the past development of transdermal therapeutic systems was focused on identifying potent drugs that would be well absorbed in the skin. However, the transdermal route allowed the transport of only a limited amount of drugs (Wester and Maibach 1983), and it was difficult to increase the drug absorption because of a limiting size of transdermal patches ($\leq$ approximately 40 cm^2) (Pfister 1997). Thus, the focus of the second phase was to increase the amount of drug that penetrated transdermally. Researchers created chemical penetration enhancers (Williams and Barry 2004) against the stratum corneum's barrier function, and this succeeded for low molecular weight drugs. The third phase was to enhance and control the skin penetration flux by physical enhancement methods (Brown et al. 2006). The physical enhancers also brought a pulsed control of the flux. The more recent progress in transdermal drug delivery has been achieved based on not only medicine and pharmacology but also engineering science and bioinformatics.

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In this chapter, we summarize the fundamental knowledge about skin penetration and physical or active enhancing methods, in particular iontophoresis, electroporation, sonophoresis (ultrasound), and microneedle methods. We discuss the advantages and disadvantages of the combined use of physical enhancers, which is the next stage of transdermal drug delivery.

22.2 General Principles of Skin Penetration

Skin penetration flux follows Fick's second law of diffusion. Because the skin is a bilayered structure, the following diffusion equations are given for each layer:

Stratum corneum

$$\frac{\partial C_{sc}}{\partial t} = D_{sc}\frac{\partial^2 C_{sc}}{\partial x^2}, 0 \le x \le h \tag{22.1}$$

Viable skin

$$\frac{\partial C_{vs}}{\partial t} = D_{vs}\frac{\partial^2 C_{vs}}{\partial x^2} - kC_{vs},\ h < x < H \tag{22.2}$$

where C is the drug concentration, D is the diffusion coefficient, h is the thickness of the stratum corneum, H is the thickness of the skin, t is time, and k is the first-order enzymatic reaction rate. The subscripts *sc* and *vs* stand for stratum corneum and viable skin, respectively. We can solve the diffusion phenomena through skin using Eqs. (22.1) and (22.2) to obtain the following initial and boundary conditions:

Initial condition

$$t = 0; C_{sc} = C_{vs} = 0,\ 0 \le x \le H$$

Boundary conditions

$$x = 0\left(stratum\ corneum\ surface\right); C_{sc} = K_{sc}C_d$$

$$x = h\left(stratum\ corneum\ and\ viable\ skin\ boundary\right); C_{sc} = K_{sc/vs}C_{vs}$$

$$x = H\left(viable\ skin\ surface\right); C_{vs} = 0\left(sink\ condition\right)$$

where C_d is the concentration in the drug formulation, K_{sc} is the partition coefficient between the skin surface and the drug formulation, and $K_{sc/vs}$ is the partition coefficient between the stratum corneum and the viable skin.

We simplify the skin penetration as a single-layered skin model because the skin penetration flux of drugs is controlled by the stratum corneum's barrier function. Under the conditions of steady-state penetration with perfect sink condition, Eq. (22.1) can be replaced by the following equation:

$$\frac{dQ}{dt} = \frac{D_{sc}\Delta C K_{sc}}{h} \tag{22.3}$$

where Q is the cumulative amount of drug and ΔC is the concentration gradient between both ends of the skin.

22.3 Physical Enhancement

In the past two decades, the progress in biotechnology and bioinformatics has led to novel and potent drugs (i.e., peptides, antibodies, oligonucleotides, and small interfering RNAs (siRNAs)) and the possibility to establish safer and more effective drug delivery strategies. Transdermal delivery would be advantageous for these new drugs because they are metabolized easily in the gastrointestinal tract by oral delivery. However, these drugs can hardly penetrate through the skin because of their high molecular weight and hydrophilic properties. Because the skin penetration flux is simplified as Eq. (22.3), the flux is increased by the improvement of D_{sc}, ΔC, K_{sc}, and h individually and at the same time. Three effective enhancement methods are (1) to

increase the diffusion coefficient in the stratum corneum, (2) to increase the concentration in the drug formulation and in the stratum corneum, and (3) to decrease the thickness of the stratum corneum or to create new pathways across the stratum corneum. The physical enhancements are achieved by the use of external energy to add a driving force for the skin penetration and mechanical force to reduce the barrier function of the stratum corneum.

22.3.1 Iontophoresis

Iontophoresis using a low current density (the limitation is ≤ 0.5 mA/cm^2 for human skin) is a superior method for the percutaneous absorption of drugs with a molecular weight between 200 and 300 Da (Yoshida and Roberts 1992). This enhancement method is based on electrorepulsion and/or electroosmosis. Electroosmosis is a convective flow that goes across the stratum corneum from the anode to the cathode when the pH value of the skin surface is more than 4.0 (Kim et al. 1993). Thus, the flux of nonionized drugs is also increased by electroosmosis. The diffusion equation with no enzymatic reaction across the stratum corneum under iontophoresis becomes the following:

$$\frac{\partial C_{sc}}{\partial t} = D_{sc}\frac{\partial^2 C_{sc}}{\partial x^2} + \frac{zFED_{sc}}{RTh}\frac{\partial C_{sc}}{\partial x} - \mu_{sc}\frac{\partial C_{sc}}{\partial x} \quad (22.4)$$

where z is the charge number of the ionized drug, F is the Faraday constant, E is the electric field, R is the gas constant, T is the absolute temperature, and u is the velocity of the convective flow.

Because the electrorepulsion and electroosmosis occur only under an electric field and are affected by the current density, it becomes possible to achieve a continuous, pulsed, and reversed delivery of drugs, to terminate the application immediately, and to reduce inter- and intraindividual variation. The advantages of iontophoresis bring possibilities for transdermal therapeutic systems. Iontophoresis has been studied for systematic and topical delivery, and some devices using electric fields such as the E-TRANS® (Alza/Ortho-McNeil Pharmaceutical Co.) for fentanyl

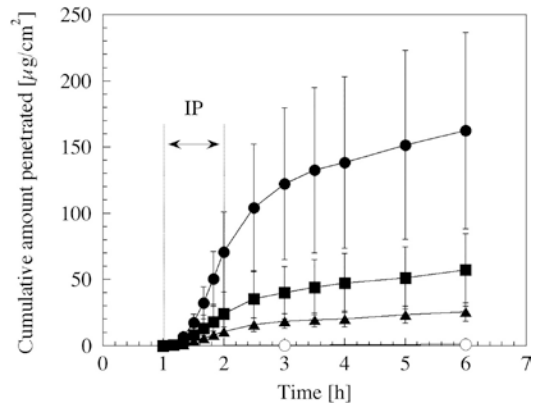

Fig. 22.1 Effect of current density on the skin penetration of vitamin B_{12}. The electric field was applied for 1 h after 1 h of passive transport. *Closed circles*, 0.6 mA/cm^2; *closed squares*, 0.3 mA/cm^2; *closed triangles*, 0.15 mA/cm^2; *open circles*, control experiment (0 mA/cm^2). *IP* iontophoresis (Reproduced with permission from The Society of Chemical Engineers, Japan)

(Gupta et al. 1999), the LidoSite® (Vyteris Inc.) for lidocaine (Pasero 2006), the IontoPatch® (Travanti Pharma Inc.) for dexamethasone (Chaturvedula et al. 2005), and the GlucoWatch® (Cygnus Inc.) as a glucose monitor (Garg et al. 1999) have actually come onto the market.

It must be noted that there are several reports in which iontophoresis caused irreversible damages to the skin (Burnette and Ongpipattanakul 1988; Inada et al. 1994; Wang et al. 1993). Miyagi et al. (2006) reported about the effect of molecular weight of drugs on the iontophoretic enhancement of flux. The flux of vitamin B_{12} (MW = 1355) increased appreciably in parallel with the constant current density (~0.6 mA/cm^2) and reached a plateau after the current was turned off (Fig. 22.1). In contrast, the flux of fluorescein isothiocyanate (FITC)-dextran (FD-4, average MW = 4400; FD-10, average MW = 11,000; FD-20, average MW = 19,000) increased continuously after a lag time (0.5 h for FD-4 and 1.5 h for FD-10 and FD-20) (Fig. 22.2). Kanikkannan (2002) also reported that iontophoresis might not be a suitable method for the transdermal delivery of peptides (>7000 Da). These results indicate that iontophoresis might be able to enhance the flux of drugs with high molecular weight (≥5 kDa), but it is impossible to control the flux.

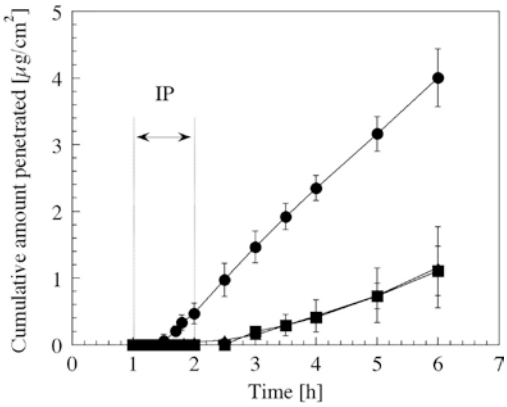

Fig. 22.2 Effect of iontophoresis (0.3 mA/cm^2 for 1 h) on the flux of FITC-dextran (*circles*, FD-4; *triangles*, FD-10; *squares*, FD-20) (Reproduced with permission from The Society of Chemical Engineers, Japan)

22.3.2 Electroporation

Electroporation is also electro-assisted enhancement methods as well as iontophoresis and was originally used as a transfection method entering deoxyribonucleic acid (DNA) into the cell; high-voltage pulse applications for very short durations of time make transient pores in the cell membrane (Zerbib et al. 1985). Prausnitz et al. (1993) first reported the use of electroporation in transdermal delivery research. They achieved the enhancement of the transdermal flux of calcein (MW = 623, −4 charge) at in vitro and in vivo experiments. After this report, the skin penetration enhancement for macromolecules has been reported (Lombry et al. 2000; Riviere et al. 1995; Vanbever et al. 1998; Zhang et al. 2002; Zhao et al. 2006). Electrical studies have shown that the enhancement mechanism of electroporation and the factors of voltage, pulse length, and pulse rate affect the drug penetration flux (Banga et al. 1999; Denet et al. 2004; Sharma et al. 2000; Vanbever et al. 1996). Skin resistance dramatically decreases on a time scale of milliseconds by high-voltage pulses (Prausnitz 1996). The fast decrease of skin resistance causes the creation and expansion of pores in the stratum corneum (Pliquett et al. 1995), and the slow decrease may involve the change of the stratum corneum structure by thermal effects (Pliquett and Gusbeth 2000). The pathways created by electrical pulse immediately close after cutting off the pulse, and, however, the skin resistance does not completely recover when electrical stimulus was too strong (Pliquett et al. 1995). Riviere et al. (1995) observed skin irritation after application of electroporation (a single exponential voltage pulse for 5 ms, $\leq$ 1000 V). An electroporation pulse had a transient erythema and no adverse irritation. Therefore, the advantages of electroporation are: (1) to cause insignificant skin damage, (2) to show the enhancement effect quickly, and (3) to increase the skin flux of macromolecules with a molecular weight greater than 7000 Da which limit for iontophoresis (Denet et al. 2004; Kanikkannan 2002).

22.3.3 Ultrasound

The enhancement of drug penetration is determined by ultrasound parameters (i.e., frequency, intensity, duty cycle, and duration of application) (Shirouzu et al. 2008). This is because physicochemical phenomena, rising temperature, acoustic streaming, the generation of convective flow, and the cavitation (Barnett et al. 1994; Liu et al. 1998; Mitragotri et al. 2000) caused by sonophoresis are influenced by: each ultrasound parameter, skin models, physicochemical properties of drugs, and experimental conditions. The flux of lidocaine hydrochloride (MW = 270, Fig. 22.3a) and that of vitamin B_{12} (Fig. 22.3b) were influenced by the ultrasound frequency (2 MHz and 300 kHz, 410 J/cm^2). The vitamin B_{12} flux was also affected by the energy flux (intensity × treatment time × duty cycle) (Fig. 22.4) (Shirouzu et al. 2008). The enhancement mechanism involves mechanical, thermal, and physiological changes of the skin, in particular the imploding cavitation bubbles that disrupt the structure of the lipid bilayers in the stratum corneum (Tezel and Mitragotri 2003).

Sonophoresis is divided into three categories based on frequency: low-frequency (~kHz), therapeutic-frequency (1–3 MHz), and high-frequency (3–16 MHz) ultrasound. Bommannan et al. (1992) reported that high-frequency

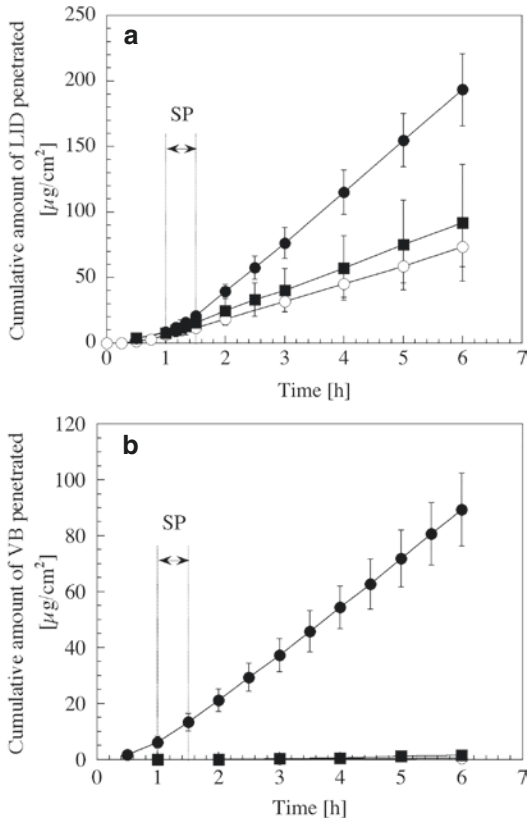

Fig. 22.3 Enhancement of the skin penetration of lidocaine hydrochloride (LID, (**a**)) and vitamin B_{12} (VB, (**b**)) by sonophoresis (SP) for 30 min. The energy flux of ultrasound was controlled at 410 J/cm^2. *Closed circles*, low-frequency ultrasound (300 kHz); *closed squares*, therapeutic-frequency ultrasound (2 MHz); *open circles*, control experiment without sonophoresis

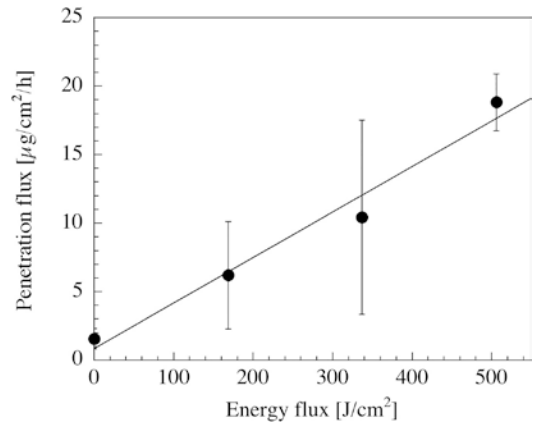

Fig. 22.4 Influence of energy flux (intensity × treatment time × duty cycle) of ultrasound on the penetration flux of vitamin B_{12}. The ultrasound frequency was 300 kHz. The flux increased in proportion to energy flux (Reproduced with permission from The Society of Chemical Engineers, Japan)

ultrasound increased the flux of salicylic acid and, at the same time, resulted in the structural alteration of skin tissue by the application of heat for 20 min. Therapeutic-frequency ultrasound is widely used in treatment, diagnosis, and physiotherapy. Therapeutic-frequency ultrasound at the intensity within 0–2 W/cm^2 can induce reversible changes in the skin barrier and can enhance the flux of low molecular weight drugs (Mitragotri et al. 1995; Yamashita et al. 1996). However, it hardly improves the flux of high molecular weight drugs. The sonic waves of low-frequency ultrasound can deeply penetrate into the skin tissue, and, moreover, low-frequency ultrasound generates cavitation bubbles at lower intensity than therapeutic-frequency ultrasound. Low-frequency sonophoresis (20 kHz, 7 W/cm^2, 50 % duty cycle) is used for the enhancement of high molecular weight drugs, insulin (MW = 5805 Da), heparin (12 k–15 kDa), and interferon-gamma (IFN-γ) (15 k–25 kDa) (Mitragotri et al. 1996; Mitragotri and Kost 2001). The US Food and Drug Administration (FDA) recently approved the SonoPrep® system (55 kHz, 15 W/cm^2, Santra Medical Co.) as a transdermal delivery system for lidocaine. This system, using low-frequency ultrasound, is expected to be used as a needle-free blood glucose monitor.

Sonophoresis is an excellent method for transdermal drug delivery, but some researchers have reported skin tissue damage caused by sonophoresis under the higher intensity. Low-frequency ultrasound (20 kHz) at intensities lower than 2.5 W/cm^2 did not affect skin tissues, whereas intensities of 5.2 W/cm^2 caused irreversible changes in skin tissue (Boucaud et al. 2001). The skin is a water-rich tissue, and, thus, enzyme deactivation may certainly occur in the skin tissue by cavitation. The effect of the intensity and duration of ultrasound application (1 MHz, 4.3 W/cm^2) on the bioconversion of an ester drug was investigated using a hairless mouse skin in vitro (Fig. 22.5) (Hikima et al. 1998). Enzyme deactivation may be partly responsible for free

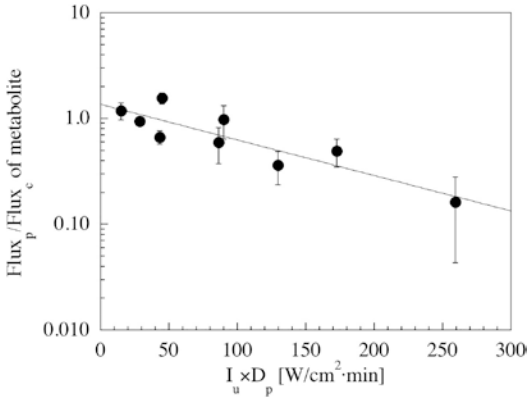

Fig. 22.5 Ratio of the flux of metabolite in skin treated by ultrasound ($Flux_p$) to the flux of metabolite in untreated skin (the control experiment, $Flux_c$) as a function of the product of intensity (I_u) and treatment time (D_p) (Reproduced with permission from Springer)

radicals generated in the reservoir solution and tissue fluid during an ultrasound pretreatment of the skin. More research is needed to identify the interactions among ultrasound parameters and to establish the safe use of drugs for transdermal therapeutic systems.

22.3.4 Microneedles

Microfabrication technology for drug delivery has been used in oral delivery (Verma et al. 2000), dermal delivery (Prausnitz et al. 2003), and implantable delivery (LaVan et al. 2003; Staples et al. 2006). Microneedles have been fabricated using microelectromechanical systems (MEMS) and have been developed to enhance the flux without the pain of piercing, as microneedles do not reach the nerve endings at the upper dermis. The drug application by microneedles was classified according to the material and design: (1) solid microneedles made from silicon, metal, and polymer that pierce the stratum corneum before drug application (Martanto et al. 2004; Hikima et al. 2012), (2) solid microneedles from metal and polymer coated with drug (Gill and Prausnitz 2007), (3) solid microneedles from biodegradable polymer coated with drug and contained the drug (Park et al. 2006), and (4) hollow microneedles from metal and polymer for drug solutions (Häfeli et al. 2009).

The Macroflux® transdermal microprojection delivery system with titanium microneedles coated with drug and the microstructured transdermal system (MTS) microneedle patch were developed by ALZA Co. and 3 M, respectively. Intracutaneous immunization with Macroflux® had similar immunoglobulin G (IgG) titers compared to intramuscular, subcutaneous, and intradermal injections (Matriano et al. 2002). Low-dose influenza vaccines with NanoPass® comprised of hollow silicon microneedles resulted in immunogenic reactions similar to the full-dose intramuscular vaccination (Damme et al. 2009). Microneedles may be suitable for the intracutaneous delivery of high molecular weight drugs, but there have been some reports that the insertion depth of a microneedle into the stratum corneum is influenced by the shape of the microneedle (Bal et al. 2010), the force of insertion (Davis et al. 2004), and the skin compaction during microneedle insertion (Martanto et al. 2006). Therefore, further detailed investigations into the mechanisms of drug transport by microneedles through the stratum corneum and the mechanical properties of the stratum corneum are necessary.

22.4 Combination of Iontophoresis with Other Physical Enhancement Methods

Chemical and physical enhancers have been studied for their use with transdermal therapeutic systems, to achieve active transport and to control the penetration flux of drugs. However, researchers reported that the strength of an undesirable stimulus, dermatitis, and irreversible skin damage increases in proportion to the enhancement effect of enhancers on the penetration flux of drugs (Boucaud et al. 2001; Ledger 1992). Thus, the application of enhancers in humans is limited due to their undesirable side effects. Protein and peptide drugs with high molecular weights do not penetrate across the skin easily (Kanikkannan 2002), and the

penetration flux continuously increases after the electric current is removed (Miyagi et al. 2006). Therefore, researchers have been investigating the combined use of enhancers for reasons of safety, economy, and efficacy (Fang et al. 2002; Mitragotri 2000; Wang et al. 2005). Moreover, the combination of enhancers leads to the synergistic enhancement of transdermal drug delivery. There are many reports about the synergistic enhancement by the combined use of enhancers, such as the combinations of iontophoresis with chemical enhancers (Pillai et al. 2004; Rastogi and Singh 2005), iontophoresis with electroporation (Chang et al. 2000), iontophoresis with sonophoresis (Fang et al. 2002; Le et al. 2000; Shirouzu et al. 2008), iontophoresis with microneedles (Katikaneni et al. 2009; Lin et al. 2001), as well as sonophoresis with chemical enhancers (Johnson et al. 1996; Lavon et al. 2005), sonophoresis with electroporation (Kost et al. 1996), laser radiation with microdermabrasion (Fang et al. 2004), and others.

However, some researchers reported also a lower penetration flux when a combination of enhancers was used compared to the use of a single enhancement method. Denet et al. (2003) indicated that the electroosmotic flux during iontophoresis (0.25 mA/cm^2 for 3 h or 0.5 mA/cm^2 for 9 h) was decreased by the accumulation of a positively charged drug, timolol maleate, in the stratum corneum by electroporation pretreatment (400 V, 10 msec, 10 pulses). Singh and Jayaswal (2008) reported that the chemical enhancer Azone® inhibited the effect of an electric current (0.45 mA/cm^2, 6 h) on 5-FU transport because it interacted with the components of the stratum corneum. X-ray, attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR), and differential scanning calorimetry (DSC) studies revealed that the pretreatment with the chemical enhancer hexadecyltrimethylammonium bromide changed the electrical and structural properties of the stratum corneum, with the result that the skin penetration flux of propranolol hydrochloride decreased (Chesnoy et al. 1999). Therefore, the mechanisms underlying the penetration enhancement by each enhancer must be further investigated so that the appropriate combination of enhancers can be identified.

22.4.1 Combination of Iontophoresis and Electroporation

While iontophoresis directly acts on the drug molecule and the movement of water through the stratum corneum, electroporation causes the change of stratum corneum structure. These methods have a different penetration mechanism, thereby the combined use of iontophoresis and electroporation could synergistically enhance the skin penetration flux of drug. Examples of the combined use of iontophoresis and electroporation are summarized in Table 22.1. Although electrical pulses of lower voltage less than 100 V followed by iontophoresis did not increase the flux of salmon calcitonin (MW = 3600), the flux was enhanced synergistically by the combined use of iontophoresis and electric pulse of 120 V (Chang et al. 2000). Ching et al. (2012) studied whether three different molecular weight biomarkers, urea (MW = 60 Da), osteopontin (MW = 33 kDa), and prostate-specific antigen (MW = 34 kDa), can be extracted from the skin by the combination of iontophoresis and electroporation in vitro. This technique is well known as reverse iontophoresis that is used as a diagnostic method by extracting molecules through the skin (Garg et al. 1999; Mize et al. 1997). Ching et al. (2012) concluded that transdermal extraction of prostate-specific antigen and osteopontin was possible only when applying reverse iontophoresis in combination with a high-voltage ($\geq$ 296 V/cm) electroporation.

Iontophoresis can control the skin penetration flux of drugs by switching current on/off, and, on the other hand, electroporation can enhance the flux of macromolecules. The combined use of iontophoresis and electroporation may be possible to control the flux of macromolecules; however, it may be difficult for resealing pathways created by electrical pulse immediately. Therefore, further detailed investigations are

Table 22.1 Examples of the combined use of iontophoresis and electroporation

Drugs/chemicals	Applied conditions	Skin	Ref.
Luteinizing hormone-releasing hormone	EP: 1 pulse of 1000 V IP: 0.5 mA/cm^2, 30 min	Human, in vitro	Bommannan et al. (1994)
Salmon calcitonin	EP: 6 pulses of 60, 100, and 120 V, 10 ms each IP: 0.5 mA/cm^2, 4 h	Human, in vitro	Chang et al. (2000)
Buprenorphine HCl	EP: 20 pulses of 500 V, 10 ms IP: 0.5 mA/cm^2, 4 h	Human and pig, in vitro	Bose et al (2001)
Dextran sulfate	EP: 6 pulses of 100, 250, and 500 V, 10 ms IP: 0.5 mA/cm^2, 6 h	Human and pig, in vitro	Badkar and Banga (2002)
Human parathyroid hormone (1–34)	EP: 100, 200 and 300 V, 20 pulses of 100 ms pulse length with 1 s interval between each pulse IP: 0.2 mA/cm^2, 12 h	Porcine, in vitro	Medi and Singh (2003)
Insulin	EP: 10 pulses of 150 or 300 V, 10 ms IP: 0.4 mA/cm^2, 1 h	Rat, in vivo	Tokumoto et al. (2006)
Ferric pyrophosphate	EP: 100 pulses of 120 V, 10 ms at 5 Hz IP: constant voltage (0.5, 2, 4 V), 30 min	Porcine, in vitro	Vaka et al. (2011)
Urea, prostate-specific antigen, osteopontin	EP: 10 pulses/s of 74, 148, 296, and 592 V/cm, 1 ms IP: 0.3 mA/cm^2	Porcine, in vitro	Ching et al. (2012)

EP electroporation, *IP* iontophoresis

needed for the practical application of the combined use of iontophoresis and electroporation.

22.4.2 Combination of Iontophoresis and Sonophoresis

The combined use of iontophoresis and sonophoresis is a practical approach to enhance the flux synergistically, because the electric properties of skin tissue are not affected by ultrasound treatment at all. Table 22.2 summarizes the literature regarding the combined use of iontophoresis and sonophoresis. The frequency of the ultrasound was an important factor for the synergistic enhancement. The skin was pretreated by ultrasound (300 kHz and 2 MHz) for 30 min, and then electric field (0.35 mA/cm^2, 1 h) was applied to the skin (Fig. 22.6). The combination of therapeutic-frequency ultrasound and electric field did not cause the synergistic enhancement of vitamin B_{12}. Hikima et al. (2009) reported the effect of the application time of sonophoresis (Fig. 22.7). The flux of vitamin B_{12} increased to 48 times compared to the control flux when 30 min ultrasound and 1 h iontophoresis were applied simultaneously. On the other hand, a 177-fold synergistic enhancement of the drug flux was achieved when the electric field applied after the ultrasound pretreatment. These results may indicate that sonophoresis changes the stratum corneum structure by cavitation and iontophoresis produced the additional forces of electroosmosis.

Skin anesthesia using a topical anesthetic (lidocaine hydrochloride) usually requires 30–60 min. Iontophoresis (0.79 mA/cm^2) enhanced the skin penetration of lidocaine, but it required at least 10 min. The combination of ultrasound pretreatment and 0.20 mA/cm^2 electrocurrent for 2 min gave the same anesthetic effect as 10 min iontophoresis (0.79 mA/cm^2) (Spierings et al. 2008). Ultrasound pretreatment can shorten the required intensity and duration of current intensity of electric field. For example, Shirouzu et al. (2008) reported that a combination of sonophoresis and iontophoresis synergistically increased and temporally controlled the penetration flux of vitamin

Table 22.2 The combined use of iontophoresis and sonophoresis

Drugs/chemicals	Applied conditions	Skin	Ref.
Heparin	U: 20 kHz, 7.4 W/cm^2, pulsed mode, ≥ 10 min IP: 0.45 mA/cm^2, 1 h	Pig, in vitro	Le et al. (2000)
Sodium nonivamide acetate	U: 20 kHz, 0.5 W/cm^2, 2 h pretreatment IP: 0.5 mA/cm^2, 6 h	Nude mouse, in vitro	Fang et al. (2002)
Lidocaine hydrochloride	U: 55 kHz, 15 W/cm^2 (SonoPrep®) IP: 1 mA, 2 min	Human, in vivo	Spierings et al. (2008)
Vitamin B_{12}	U: 300 kHz, 5.2 W/cm^2, pulsed mode, 30 min IP: 0.3 mA/cm^2, 1 h	Hairless mouse, in vitro	Shirouzu et al. (2008)
Benzoic acid, lidocaine hydrochloride, indomethacin, hydrocortisone, timolol maleate, vitamin B_{12}, vancomycin hydrochloride	U: 300 kHz, 5.2 W/cm^2, pulsed mode, 30 min IP: 0.3 mA/cm^2, 1 h	Hairless mouse, in vitro	Hikima et al. (2009)

U ultrasound, *IP* iontophoresis

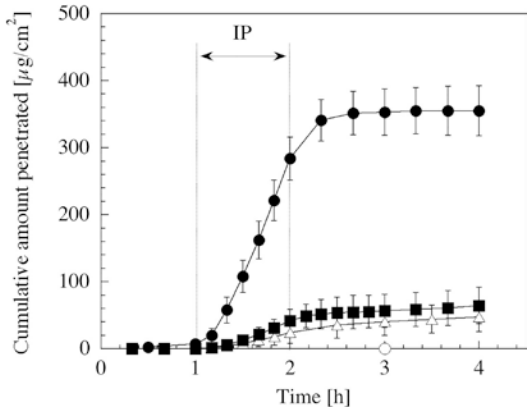

Fig. 22.6 Effect of ultrasound frequency on the synergistic enhancement of skin penetration of vitamin B_{12}. The skin was pretreated by ultrasound for 30 min before starting the experiment. *Closed circles*, low frequency (300 kHz); *closed squares*, therapeutic frequency (2 MHz); *open triangles*, iontophoresis without ultrasound pretreatment; *open circles*, control experiment

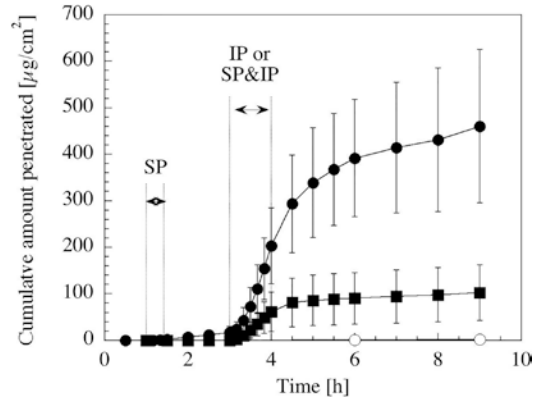

Fig. 22.7 Differences in the enhancement effects of vitamin B_{12} according to treatment time by sonophoresis and iontophoresis. *Closed circles*, pretreatment with sonophoresis for 30 min and then iontophoresis applied for 1 h; *closed squares*, sonophoresis for 30 min and iontophoresis for 1 h simultaneously applied; *open circles*, control experiment

B_{12} in vitro (Fig. 22.8). The ratio of the drug flux compared to the control flux was synergistically increased to 217 times by the combined use of iontophoresis and sonophoresis. The flux of heparin (average MW = 10,000) (Le et al. 2000) and that of sodium nonivamide acetate (MW = 375) (Fang et al. 2002) were synergistically enhanced by the application of iontophoresis and sonophoresis. Hikima et al. (2009) investigated the mechanism of the synergistic effects of sonophoresis and iontophoresis on skin penetration. They performed in vitro skin penetration experiments using seven model chemicals with different electric charges and molecular weights. The synergistic effects were observed in nonionized and high molecular weight (approximately 1500 Da) drugs (Fig. 22.9a–d). Hikima et al. (2009) concluded that the electroosmotic flow was the key factor

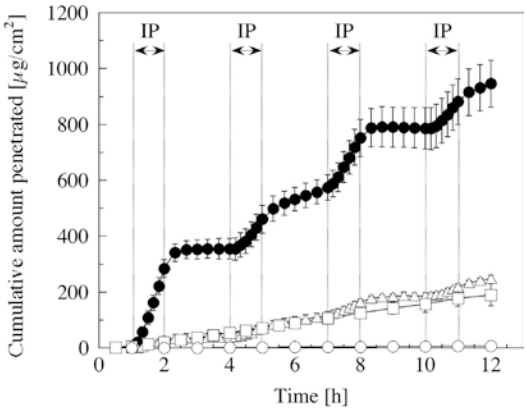

Fig. 22.8 Synergistic enhancement of vitamin B_{12} by the combined use of sonophoresis and iontophoresis. The skin was pretreated by ultrasound (300 kHz) before starting the experiment, and the electric field was applied four times during the experiment. *Closed circles*, combination of sonophoresis and iontophoresis; *open triangles*, iontophoresis; *open squares*, sonophoresis; *open circles*, control experiment (Reproduced with permission from The Society of Chemical Engineers, Japan)

in the synergistic penetration enhancement of drugs by the combined use of iontophoresis and sonophoresis.

22.4.3 Combination of Iontophoresis and Microneedles

Table 22.3 summarizes the literature about the combined uses of iontophoresis and microneedles. Although there are various types of microneedles, regarding the used material, shape, length, and density of needles, their combination with iontophoresis provided synergistic enhancement of the drug flux (Table 22.3). The mechanism of skin penetration enhancement by microneedles is to create new transport pathways across the stratum corneum, with the result that high molecular weight and highly hydrophilic drugs are able to transport across the stratum corneum. The combined use of iontophoresis and microneedles can be expected to provide a synergistic enhancement of the flux because a microneedle makes only pores in the stratum corneum mechanically, while iontophoresis improves the movement of drugs in these pores. Thus, there are many reports that iontophoresis was applied to the skin pretreated by microneedles. For example, Chen et al. (2009) reported that insulin using insulin-loaded nanovesicles with various charge and size was delivered into the skin by a combination of microneedles and iontophoresis. Positively charged nanovesicles with the average diameter of 107 nm were withdrawn on the skin pretreated by microneedles and then iontophoresis (0.2 mA/cm^2, on/off ratio of 1:1, and frequency of 100 Hz) applied continuously for 3 h, and the result decreased the blood glucose levels comparable to the levels achieved by subcutaneous injection. On the other hand, Garland et al. (2012) indicated that a synergistic effect on the increase of FITC-bovine serum albumin (BSA) flux was produced by the simultaneous application of iontophoresis and microneedle. They succeeded biodegradable polymeric microneedles and iontophoresis in a one-step application.

As aforementioned in this chapter by Eq. (22.4), the enhancement mechanism of iontophoresis involves electrorepulsion and electroosmosis. Katikaneni et al. (2009) studied the effect of pretreatment with microneedles on the skin penetration of acetaminophen, as a marker of electroosmosis in vitro. Iontophoresis enhanced the penetration flux of acetaminophen by seven times across the pretreated skin, suggesting that electroosmotic flow through microchannels made by the microneedles persisted. They also reported that the flux of a large molecular weight drug, daniplestim (MW = 12.76 kDa, $pI = 6.2$), was increased by electroosmosis under the combination of iontophoresis and microneedles. Using a mathematical simulation, Tojo (2005) discussed the importance of electroosmotic flow caused by an electric field for the synergistic effect of iontophoresis and microneedle combination. The blood concentration of human growth hormone (MW = 22 kDa, $pI = 5.0$) in hairless guinea pig is shown in Fig. 22.10. The lines and plots in Fig. 22.10 express the simulation data and experimental data from Cormier and Daddona (2003), respectively. Cormier and Daddona (2003) applied human growth hormone to the skin pretreated by Macroflux® (Zosano Pharm™, Inc.)

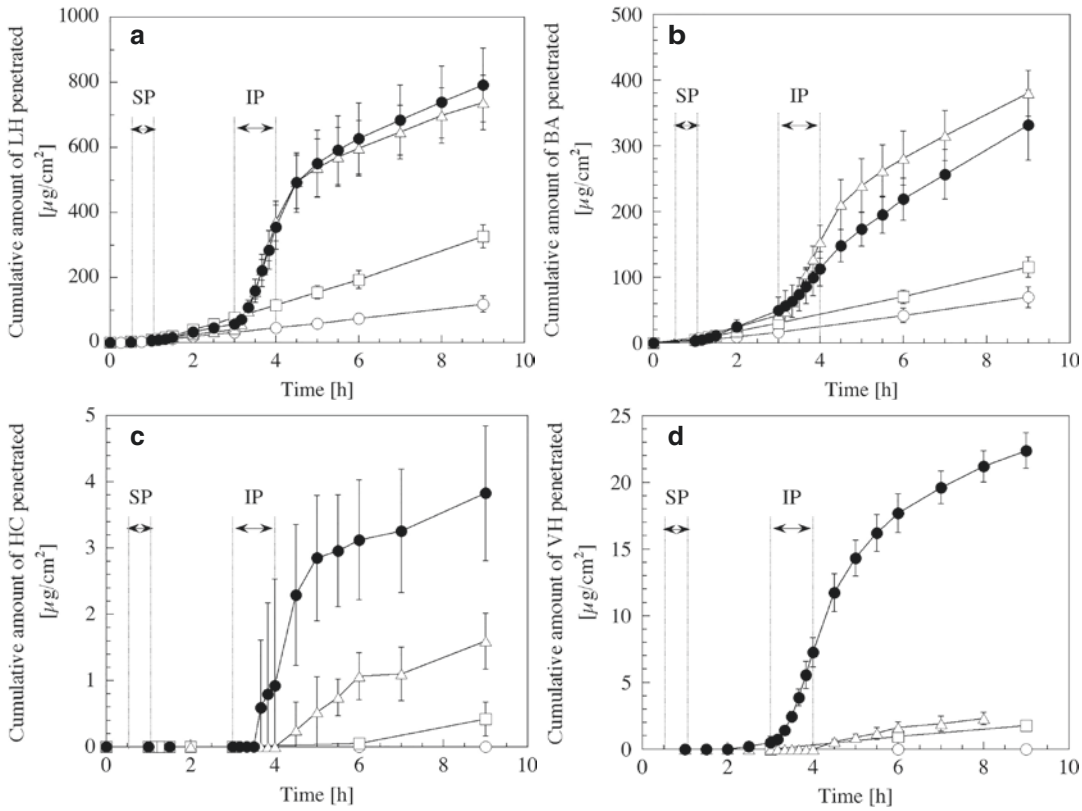

Fig. 22.9 Penetration enhancement of lidocaine hydrochloride (LH, a positive charged molecule, (**a**)), benzoic acid (BA, a negative charged molecule, (**b**)), hydrocortisone (HC, a nonionized and low molecular weight molecule, (**c**)), and vancomycin hydrochrolide (VH, a positive charged and high molecular weight molecule, (**d**)). *Closed circles*, combination of sonophoresis and iontophoresis; *open triangles*, iontophoresis; *open squares*, sonophoresis; *open circles*, control experiment (Reproduced with permission from Pharmaceutical Society of Japan)

Table 22.3 The combined use of iontophoresis and microneedles

Drugs/chemicals	Applied conditions	Skin	Ref.
ISIS2303 (oligodeoxynucleotide)	MN: Macroflux®, pressed by finger force IP: 0.1 mA/cm^2, 4 h	Hairless guinea pig, in vivo	Lin et al. (2001)
D_2O, FITC-dextrans (FD-4, FD-10, FD-40, FD-70, FD-2000)	MN: stainless, nine needles, 400 μm length, pressed at 1.6 kg/cm^2 for 10 s IP: 0.3 mA/cm^2, 5 h	Hairless rat, in vitro	Wu et al. (2007)
Insulin-loaded nanovesicle	MN: solid stainless, 296/2 cm^2, 800 μm length, pressed at 9.0 N for 2 min IP: 0.2 mA/cm^2 with on/off ratio of 1:1, 5 h	SD rat, in vivo	Chen et al. (2009)
Daniplestim	MN: maltose, 162 needles, 500 μm length, pressed at 0.625 kg/cm^2 for 60 s IP: 0.5 mA/cm^2, 6 h	Hairless rat, in vitro	Katikaneni et al. (2009)
Salmon calcitonin	MN: maltose, 81 needles, 500 μm length IP: 0.2 mA/cm^2, 1 h	Hairless rat, in vivo	Vemulapalli et al. (2012)
Theophylline, methylene blue, fluorescein sodium, insulin, FITC-BSA	MN: PMVE/MA loaded with drug, 361 needles, 600 μm length, pressed at 11 N and loaded 3.5 g weight on MN IP: 0.5 mA, 6 h	Porcine, in vitro	Garland et al. (2012)

MN microneedle, *IP* iontophoresis

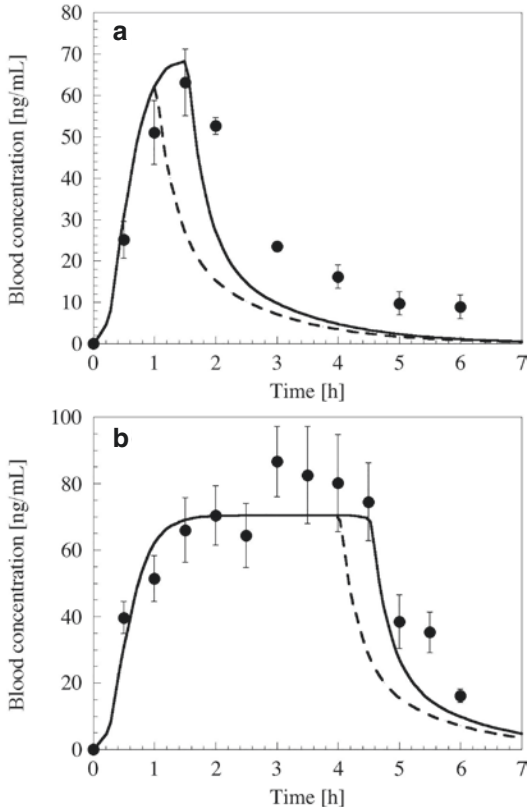

Fig. 22.10 Transdermal delivery of human growth hormone by the combination of iontophoresis and microneedles. Iontophoresis was applied for 1 h (**a**) and 4 h (**b**) to the skin pretreated by Macroflux®. *Closed plot*, in vivo experimental data from (Cormier and Daddona 2003); *solid line*, electroosmotic flow remained active for 30 min; *dashed line*, the electroosmotic flow vanished immediately after the current was turned off

and turned electric current off at 1 h (Fig. 22.10a) and at 4 h (Fig. 22.10b). Although the concentration of human growth hormone was under detection level in passive and iontophoresis-only application (data not shown), turning the current on/off under the combination of iontophoresis and microneedles controlled the time course of the plasma concentration. Each dashed and solid line in Fig. 22.10 in the study performed by Tojo (2005) was calculated on using a bilayer skin model assuming that the thickness of the stratum corneum by microneedles was reduced. The solid lines assumed that the electroosmotic flow across the skin continued for 30 min after iontophoresis was shut off. The solid lines approximately satisfied the transient profiles following the shutoff of the current after 1 h (Fig. 22.10a) and 4 h (Fig. 22.10b). This finding suggested that the electroosmotic flow caused the synergistic enhancement, and it did not stop immediately when the iontophoresis was terminated. Therefore, the electroosmotic flow is an important factor of the combined use of iontophoresis and microneedle for the synergistic penetration enhancement of macromolecules.

Conclusions

This chapter has overviewed the combined use of iontophoresis and other physical enhancers, electroporation, sonophoresis, and microneedle. While each physical enhancer has the advantage to increase the skin penetration flux of macromolecules, the application of physical enhancer is limited due to the undesirable and irreversible skin damages. However, the combination of physical enhancers brings us the possibility to enhance and control the skin penetration flux of proteins (i.e., peptides, antibodies, and interferons) and nucleic acids (i.e., oligonucleotides, micro-RNAs (miRNAs), and siRNAs). When the combined use of enhancers is investigated, we must pay attention to the enhancement mechanism of each physical enhancer and identify the appropriate combination. A practical approach to enhance the flux synergistically with iontophoresis is to use an enhancer that increases the drug flux without changing the electric properties of the skin tissue. Further detailed investigations are needed for controlling the flux of macromolecules by combining iontophoresis with other physica l enhancers.

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