Microporation in Penetration Enhancement

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17.1 Introduction

The important requisite for an efficient drug delivery therapy is to deliver the drug at the site of action at optimal concentrations and for optimal time periods. However, in most instances, these drug molecules require to cross one or more biological barriers in the body in order to reach its target. The body contains many biological barriers that serve to protect its interior from a variety of external invaders, including therapeutic molecules. Several potential therapeutic agents have been limited by their inability to reach systemic circulation, due to the excellent barrier properties of the biological membranes, such as *stratum corneum* (SC) of the skin or sclera/cornea of the eye and others. In addition, the selective permeation of the therapeutic agents is limited by its specific physicochemical properties.

Of the many biological barriers, the researchers remained most attractive to the human skin, the largest single organ of the body, for local or systemic drug delivery. This route of delivery offers many advantages over oral drug delivery, which includes avoidance of gastrointestinal tract and liver, first-pass effects, controlled and continuous drug delivery, easy removal of the dosage form and importantly good patient compliance. However, as stated above the outermost layer of the skin, the SC, remains the key for the skin barrier function to xenobiotics ensuring a difficultly in passage of most drugs both into and through

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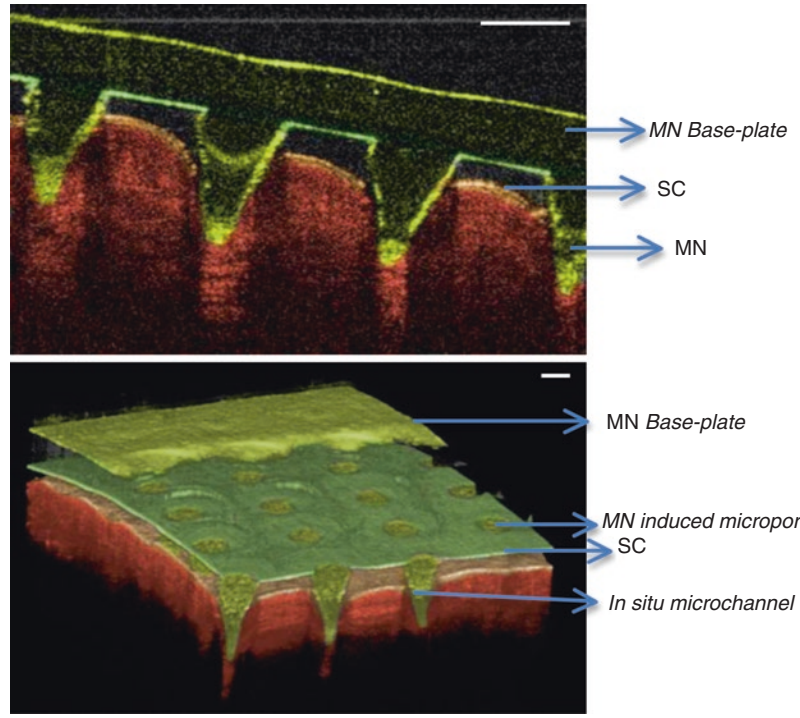

Fig. 17.1 Optical coherence topography (OCT) images showing microporation of human skin by using polymeric MN array (with height 600 μm, width at base 300 μm, spacing 300 μm) in (**a**) 2D and (**b**) 3D. *Scale bar* is 300 μm in each case (Reprinted with permission from Elsevier, Donnelly et al. 2010)

the skin (Barry 2001). Like the other biological barriers, the SC has highly organised lipid matrix, which have a crystalline, gel or liquid crystalline character, and their arrangement provides great resistance to molecular penetration of the membrane (White et al. 1988; Bouwstra et al. 1994).

In transdermal drug delivery, two types of technologies are used (i.e. passive and/or active) to overcome the barrier function of SC and to enhance the permeation of therapeutic molecules. Passive methods include the use of chemical enhancers, emulsions and lipid assemblies. However, these methods may have a lag time of up to several hours and cannot be easily adapted for rapid onset or modulated delivery timing. Furthermore, the advent of new advances in the field of biotechnological drug products, such as peptidomimetics, peptides, proteins and oligonucleotides, has generated new challenges for the need of cutting-edge drug delivery devices, which otherwise have limited permeation across the biological membrane (Kalia et al. 2004). These limitations of passive method compelled researchers from the academic and industrial researchers to make use of active methods for permeation enhancement across biological membrane (Prausnitz et al. 1993). A number of approaches to enhance the transdermal drug delivery have been discussed elsewhere in the book. However, this chapter discusses about various techniques principally assisting in skin microporation, to desired depths, that gain importance and are growing at a faster rate than before.

Microporation of skin enhances the permeability of drug molecules, and it is based on the principle of creating micron-sized channels/pores, of defined dimensions, in the skin, which can then allow the transport of water-soluble molecules and macromolecules. Figure 17.1 shows a typical microporated skin sample following application of microneedles (MNs). Microporation-based drug delivery devices have been described as one of the few third-generation enhancement strategies, which have been indicated to have a significant impact on medicine (Prausnitz and Langer 2008). A number of techniques, based on either mechanical or external energy sources, have been studied to create micropores in the skin (Prausnitz et al. 1993); such techniques include

laser microporation, thermal microporation, electroporation (Moatti-Sirat et al. 1992), radio frequency (Schmidtke et al. 1998), microneedle (MN) arrays (Donnelly et al. 2010), ultrasound/phonophoresis or sonophoresis (Tamada et al. 1995) and high-pressure gas/powder or liquid microporation (Mitragotri et al. 1995). In addition to passage of drugs into the body, microporation devices have successfully demonstrated the collection of biological fluid samples from the body or for certain medical or surgical procedures (Anubhav et al. 2008). This chapter specifically discusses the mechanism of microporation by different techniques, pore closure phenomenon following microporation and safety of the microporated skin, and it also addresses a series of microporation-based products that are presently in preclinical or clinical phase of development.

17.2 Mechanism of Creating Micropores by Different Microporation Devices

Microporation devices are considered to be noninvasive or minimally invasive in nature and therefore its application is painless. Drugs delivered using these minimally invasive or noninvasive microporation devices are generally absorbed into the body as quickly as the drugs administered by conventional subcutaneous needle injection (Brearley et al. 2007; Harris et al. 2006). Different microporation techniques work on different principles, but all techniques have one common goal, which is to disturb the principle barrier of the skin, i.e. SC by creating micropores (Fig. 17.2). Once created these micropores or pathways are orders of magnitude bigger than molecular dimension and therefore should readily permit transport of macromolecules as well as possibly supramolecular complexes and microparticles (Bouwstra et al. 1994). For drug delivery applications, microporation of the skin by different devices can be divided into two categories, i.e. mechanical method or active method. Mechanical method-dependent microporation technique involves the use of the MN-based devices. In contrast, thermal, radio frequency, ultrasound/phonophoresis, high-pressure jet and electroporation are based on active microporation techniques. The following sections detail the mechanism of microporation by different devices; however, readers are requested to refer relevant chapters in this book for more detailed studies on enhanced drug delivery achieved by using these devices.

17.2.1 Active Microporation Techniques

Active microporation of the skin can be achieved by using external source of energy. Therefore, these methods require much more complicated devices than the mechanical-based microporation devices, which need physical application to create micropores or microchannels, as shown in Fig. 17.2.

17.2.1.1 High-Pressure Gas or Liquid Microporation

Invented in the 1940s, the high-pressure jet injectors are perhaps the oldest microporation device intended to eliminate the directly mechanical piercing of the skin by hypodermic needles and/or syringes. Jet injections employ a high-speed jet to puncture the skin and deliver drugs without the use of a needle (Baxter and Mitragotri 2005). Injectors can be broadly classified as either multi-use nozzle jet injectors (MUNJIs) or disposable-cartridge jet injectors (DCJIs) (Baxter and Mitragotri 2005; Mitragotri 2006). These devices basically consist of a power source, usually a compressed gas and spring or piezoelectric actuator which upon actuation pushes a piston, which in turn impacts on a drug-loaded compartment, causing a surge in pressure and release of the drug-containing vehicle through a nozzle as a jet at a speed of between 100 and 200 ms^{-1} (Mitragotri 2006). The jet creates micropores upon impinging on the skin and delivers the drug at a depth dependent, either directly into muscles or subcutaneous or intradermal layers, on the characteristics of the jet, namely, orifice diameter, jet exit veloc-

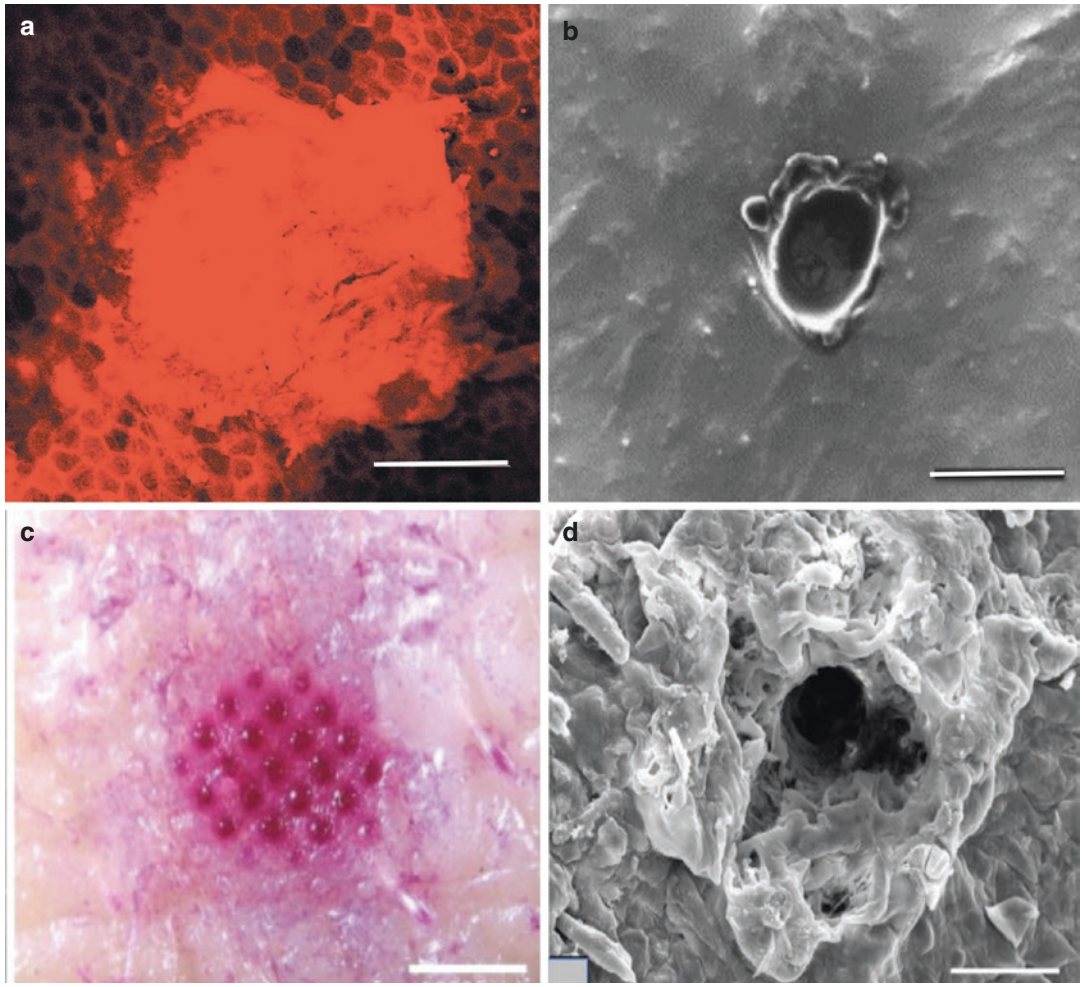

Fig. 17.2 (**a**) Image showing penetration of microjets into the human skin in vitro. It also shows the intact structure of corneocytes around the injection site. The image was taken 15–30 min postinjection. *Scale bar* is 200 μm (Arora et al. 2007). (**b**) Scanning electron microscopy of radio frequency (RF)-microchannels in heat-separated epidermal membrane following two applications of ViaDerm™, high magnification showing dimensions of microchannels; *scale bar* is 100 μm (Birchall et al. 2006). (**c**) Image of micropore arrays on pig cadaver skin after 30-min delivery of sulforhodamine to thermally ablated skin. *Scale bar* is 1000 μm (Lee et al. 2011). (**d**) Scanning electron microscopy image of a single pore following laser microporation in mouse skin, generated by delivery of eight pulses at 0.76 J/cm^2 per pulse. *Scale bar* is 50 μm (Reprinted with permission from Elsevier, Weiss et al. 2012)

ity and distance travelled. Micropore diameter is comparable to the jet diameter, which increases with increase in the distance travelled. The skin erosion and fracture are the root causes of micropore formation, as shown in Fig. 17.2a (Baxter and Mitragotri 2005). Several patents are in place for this method of drug delivery, in addition to the multitude of delivery devices already available, some of which are listed below under their trademark names: PenJet®, J-Tip®, Cross-Ject®, PowderJect®, MediJect®, Injex 30®, MHI-500®, LectraJet® and Impla-Ject®.

Literature review indicates enhancement of numerous drug molecules has been achieved by utilising this technique. However, in addition to drug delivery, jet injectors have also been proposed for the delivery of anti-ageing cosmetic products. For example, have proposed needle-free injector kits and quantities of dermal filling material for use in soft tissue augmentation. More

specifically, the needle-free injectors allow injection of viscous materials, such as collagen, hyaluronic acid and other polymers that are useful as dermal fillers to fill undesired lines, wrinkles and folds.

17.2.1.2 Radio Frequency Microporation

Percutaneous penetration can also be facilitated by ablation of the outer layers of the skin by using alternating electrical current at radio frequency (RF) of 100–500 kHz. The passage of this current through cells in the upper skin strata, via an array of microelectrodes placed on the skin, propagates ionic vibrations through skin cells resulting in local heating, liquid evaporation and removal of cells. As a result, transient aqueous microchannels are created across SC and epidermis, called RF-microchannels (Fig. 17.2b), which enable or augment effective movement of water-soluble substances through the skin. Compared to other electrically assisted drug delivery techniques, such as electroporation and iontophoresis, microchannels formed using RF ablation are relatively large which enables transport of high-molecular weight compounds without the need for ionising or polarising the molecules. Furthermore, the formed microchannels do not reach underlying nerve endings and blood vessels; therefore, skin trauma and neural stimulations are minimised like with other microporation techniques discussed here (Sintov et al. 2003; Levin et al. 2005). Additionally, combination of RF-microchannel generation in conjunction with iontophoresis has also been studied (Levin et al. 2007a, b). For example, pretreatment of the human skin in vivo using ViaDerm™, a RF-microporation-based device, followed by iontophoretic patch application was reported to facilitate insulin delivery by a factor of 2.5 compared to ViaDerm™ treatment alone (Levin et al. 2007a, b).

17.2.1.3 Thermal Microporation

It has been well documented that the flux of drugs through the skin is temperature sensitive and this factor has been utilised in transdermal and other types of delivery systems in recent times. This began with patented transdermal patch devices, which used heat to formulate the patch, rather than to increase the flux of drug across the skin (Konno et al. 1987; Kuratomi and Miyauchi 1988; Stewart 1989). In contrast, microporation of biological membrane was used to enhance drug delivery. Thermal microporation of skin involves application of rapid and controlled pulses of thermal energy by means of tiny resistive elements to a defined site on the skin surface to create micropores. The thermal energy will be passed through the array of tiny elements for few milliseconds, which causes flash vaporisation of SC cells in an area about the width of a human hair to create micropores (Fig. 17.2c) (Banga 2006).

Devices that directly used heat to increase the flux of drug across the skin became more prevalent in the 1990s. In the last 20 years, many new devices and methods have been established that utilise the microporation effects that are achieved when thermal energy is focussed on the skin and other biological membranes. In recent years more advanced devices have been developed, with some reaching full clinical trial and mass-scale production such as Altea Passport™ system. Furthermore, a combination of thermal microporation with other techniques has demonstrated much-improved enhancement than use of thermal microporation alone.

17.2.1.4 Laser Microporation

Ablation of the skin can also be achieved by using a laser emitted at a defined wavelength, which is directly absorbed by the tissue to form micropores, where irradiation of laser energy causes instant tissue vaporisation due to flash evaporation of water within in the irritated area following microexplosion that results in tissue ablation (Fig. 17.2d) (Nelson et al. 1991). It is this rapid energy loss from the ablated site, which protects the surrounding tissue from heat-induced damage. The two optimal wavelengths at which skin ablation can be achieved are short-wavelength ultraviolet and mid-infrared, which is absorbed by tissue proteins and tissue water, respectively. The amount of SC removal can be efficiently controlled by

controlling the level of energy imparted on the skin, especially when applied at lower energy levels (Nelson et al. 1991).

17.2.1.5 Ultrasound (Phonophoresis, Sonophoresis) Microporation

Ultrasound is defined as sound with frequency ranging from 0.02 to 10.0 MHz and an intensity range of 0.0–3.0 W/cm^2 (Mitragotri et al. 2000). Sonophoresis, also known as phonophoresis, describes the effects of ultrasound on the movement of drugs through intact skin and into soft tissues (Ng and Lui 2002). Ultrasound-enhanced drug delivery has several important advantages in that it is noninvasive, can be carefully controlled and can penetrate to desired depths into the body. The early use of ultrasound as a physical enhancer in transdermal drug delivery is developed nearly 50 years ago, a method referred to as sonophoresis or phonophoresis (Ng and Lui 2002). Exposure of a biological membrane to ultrasound causes sonoporation, which is the temporary, non-destructive perforation of the cell membrane. This transient state enhances permeability of therapeutic agents into cells and tissues (Harvey et al. 2002). However, the preceding is not intended to digress from the major principal of the review; however, before we emphasise the ultrasound-patented drug delivery systems, it is important to situate these systems in their contextual background.

Ultrasound is produced by a transducer composed of a piezoelectric crystal, which defines the frequency of emitted waves and converts electric energy into mechanical energy in the form of oscillations, generating acoustic waves. During the propagation of these acoustic waves through a given medium, a wave is partially scattered and absorbed by the medium, resulting in attenuation of the emitted wave with the lost energy being converted into heat ultrasound which can be emitted either continuously (continuous mode) or in a sequential mode (pulsed mode) (Machet and Boucaud 2002). The mechanism of ultrasound effects on the skin in drug delivery is not clearly understood; however, various different mechanisms were proposed (Lavon and Kost 2004; Tachibana and Tachibana 1999; Joshi and Raje 2002), as follows:

(i) Cavitation: Ultrasound generates gaseous cavities in a medium, and their subsequent collapse causes release of shock wave, which then causes structural alterations in the surrounding tissue. Cavitation leads to disordering of the lipid bilayers and formation of aqueous channels in the skin through which drugs can permeate and therefore increases the bioavailability of the drugs.
(ii) Thermal effects (increasing of temperature).
(iii) Induction of convective transport.
(iv) Mechanical effects (stress occurred because of pressure which is induced by ultrasound).

The experimental findings suggest that among all the ultrasound-related phenomena, evaluated cavitation has the dominant role in sonophoresis, which suggests that application of low-frequency ultrasound should enhance transdermal transport more effectively (Machet and Boucaud 2002).

Applications of ultrasound differ depending upon the frequency range of ultrasound used. For example, high-frequency (3–10 MHz) ultrasound is used for diagnostic conditions in clinical imaging, medium-frequency (0.7–3.0 MHz) ultrasound for therapeutic physical therapy and low-frequency (18–100 kHz) ultrasound for lithotripsy, cataract emulsification, liposuction, cancer therapy, dental descaling and ultrasonic scalpels (Gustavo et al. 2003).

There are innumerable applicators in the market that use sonophoresis technology. For instance, the ultrasonic teeth cleaning devices used by dentists have a frequency range of 25–40 KHz. Moreover, portable, pocket-size sonicators with rechargeable batteries for drug injection and analyte monitoring characterised with sensors are also available commercially (Mitragotri et al. 2000). In future applications, ultrasound technology seems to show promise for immunisation with vaccines and topical gene therapy. Some current applications consist of

drug delivery, administration of targeted therapeutic and diagnostic agents, detection and determination of analyte, termination of cancer tissues, fatty tissues or kidney and gall bladder stones (Mitragotri et al. 1995).

It can be seen from various literature reports that ultrasound was used in an attempt to enhance the absorption of different molecules through the human skin. More recently, it was demonstrated that low-frequency ultrasound (<100 kHz), which causes cavitational disordering of SC, has been used to provide enhanced transdermal transport of low-molecular weight drugs, as well as high-molecular weight proteins (insulin, γ-interferon and erythropoietin) across the human skin (Mitragotri et al. 1995). There is good evidence for reversible effect on SC and potential usefulness of ultrasound within clinical settings (Mitragotri et al. 1996). But the commercial availability of ultrasound devices, especially for transdermal drug delivery, is very limited, even though many patents were filed. This represents that there is a need for more sophisticated devices, particularly addressing the advantages of low-frequency ultrasound for its non-thermal bioeffects, mostly by cavitations. Apart from the drug delivery applications, the novel noninvasive ultrasonic devices can conceivably be used in the extraction of clinically important analytes from the interstitial fluids of the skin. However, the successful application of these novel devices in drug delivery or monitoring needs to demonstrate successful preclinical or clinical studies before commercialisation.

17.2.1.6 Electroporation Microporation

Electroporation or electropermeabilisation is the temporary perturbation of structural lipid bilayer of biological membranes by the application of high-voltage pulses (≥50 V) and allows DNA or other macromolecules to enter the cells (Banga and Prausnitz 1998). When a short pulse is applied to generate a supra-breakdown potential across the membrane, the membrane will breakdown, leading to the creation of microchannels.

17.2.2 Physical Microporation Technique

17.2.2.1 MN Microporation

Apart from the various different techniques mentioned above, the microporation of the biological membrane, to desired depths, can also be achieved by the use of MNs (Fig. 17.1a). Even though, ALZA Corporation appears to be the first to use MN described in the late 1976 patent (Gerstel and Place 1976), the first paper to demonstrate MNs for transdermal delivery was not published until 1998 (Henry et al. 1998). MNs consist of plurality of microprojection arrays, generally ranging from 50 to 2000 μm in height, of different shapes and sizes. These MNs are attached to a base support, and simple physical application of such MN arrays to the skin surface can create transport pathways of micron dimensions in the biological membrane (Fig. 17.1b), unlike the above microporation devices that need external source of energy. MN can be solid or have a hollow bore and can be made from metal, polymers, elemental silicon or glass. For a detailed review on MN types, manufacturing technology and application in transdermal drug delivery in clinical studies, the reader is requested to refer relevant chapters in volume four of this book. Importantly, there has been a substantial increase in the attention that MN technology has received over the last 5 years, with a number of publications concerning MN evaluation more than tripling since 2005.

17.3 Safety Associated with the Use of Microporation Devices

Microporation of the skin results in breaching the skin's SC barrier, thereby enhancing delivery of drugs of different physicochemical properties. However, application of microporation devices could also be associated with sensation of pain, erythema or both. Furthermore, by creating microchannels across the skin's SC, its barrier property is compromised, whereby increasing the risk of invasion of exogenous materials (e.g.

microorganisms), which depended upon the micropore dimensions. Additionally, multiple application of same microporation device can also pose the risk of contamination and could easily cause cross contamination between individuals. Furthermore, the nature of material used in fabrication of microporation device could also cause safety concerns, particularly if the material remains within the skin tissue due to improper use or accidental breakage during application. All the above concerns are integral component of these types of delivery systems and therefore need careful consideration. For example, in the past high-pressure jet devices have been employed for mass delivery of vaccination through the skin but fell from favour when a link to hepatitis B spread was established after vaccination with multi-dose injectors, which was caused by cross contamination between patients following jet application. This issue, in combination with the variability in patient response, such as occasional pain, discomfort and local reactions, inconvenience of use compared with injections and cost are potential barriers for the development and commercialisation of this drug delivery method (Hingson et al. 1963). Despite more than 50 years of clinical use and hundreds of patents, these jet injectors have not reached their full potential, in terms of replacing the routine needle-based delivery. However, recent demands of effective delivery of macromolecules including DNA (deoxyribonucleic acid), insulin, growth hormones, vaccines and other biotechnological products (Mitragotri 2006), in addition to other therapeutic drug molecules, have resulted in the improvement on the existing technologies of jet injectors. Especially, microjet injectors with low volumes, smaller nozzle diameters, and pulsed small injection volumes are considerably beneficial in reduction of occasional pain and consistence delivery. In addition, a better understanding of the jet injections on the skin at a cellular level, variability in jet penetration depending on skin properties, mechanisms of jet injections and mechanical properties of the skin need a special attention for effective performance. Likewise, RF-microchannelling devices and electroporation, which have shown, enhanced the delivery of both low- and high-molecular weight therapeutic molecules which are another promising technology. However, wider usage of such devices can only demonstrate the effectiveness of the technology and the suitability of devices.

On the other hand, the limited number of studies has demonstrated the use of laser-based microporation devices for the microporation of biological membrane; the possibility of portable laser devices will definitely improve the market potential transdermal drug delivery. However, more studies are required to demonstrate the clinical safety of using high-powered laser, the cost and its application for both drug delivery and minimally invasive monitoring.

The practicality of ultrasound microporation in health sciences as a biomedical applicator, as well as a therapeutic agent, is increasing. The use of ultrasound as an aid for increasing skin permeability depends upon the non-thermal bioeffects of its cavitation. In essence, attention should be paid to the issue of ultrasound technology's effects on the structure of the skin to develop a useful tool that takes accounts for safety issues.

For all the microporation devices, a database of information on the microchannel closure rates should be provided, and factors affecting this should be thoroughly investigated, since the open micropore could jeopardise the skin's barrier property.

17.3.1 Micropore Closure and Recovery Following Microporation Devices

Microporation devices can create transient aqueous microchannels, which enable transmembrane delivery of a wide range of molecules. But, creation of micron-sized pores or microchannels will also disrupt the SC barrier function of the skin. The degree of disruption is dependent upon the micropore dimensions, for example, in MN-based microporation technique, the needle of longer length causes greater

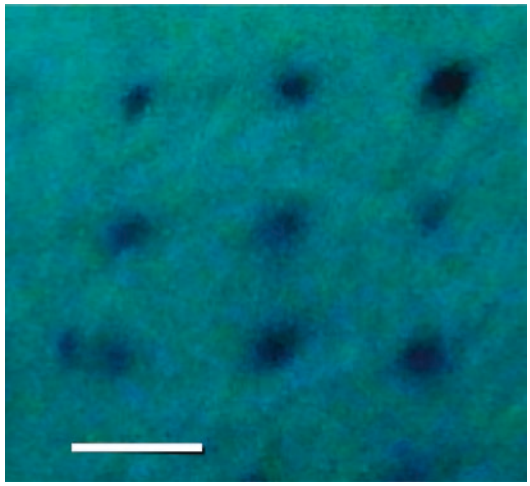

Fig. 17.3 A digital image of MN penetration followed by methylene blue staining that shows a 100 % penetration into the *stratum corneum* of neonatal porcine skin in vitro, following an insertion forces of 0.03 N per needle or greater

barrier disruption than a needle of shorter length. However, irrespective of type of microporation method, the microchannels reseal over defined duration of time due to the skin's natural repair mechanisms (Menon et al. 1992). Though significant the number of studies has been shown to improve drug delivery across microporated skin, very few studies have shown the characterisation of microchannels dimensions and its closure rates. In doing so, numerous instrumental methods have been reported to measure the rate of the skin's resealing behaviour following application of microporation devices, such as dye staining, transepidermal water loss (TEWL) measurements, confocal microscopy, electrical impedance spectroscopy, histological staining and OCT. Noninvasive techniques such as TEWL measurements and digital imaging, following methylene blue staining (Fig. 17.3) of the microporated skin, are used as traditional techniques to determine the pore recovery or pore closure. However, more reliable and real-time in situ imaging techniques, such as OCT, have been demonstrated to give detailed information about the depth of penetration, dissolution (in the case of soluble MNs) and also skin recovery on a patient-to-patient basis (Fig. 17.4).

Banga's research group demonstrated, using maltose MNs, that the microchannels created on hairless rat skin recover their barrier function within 3–4 h, and microchannels closed within 15 h after poration when exposed to the environment. However, in occluded conditions, the microchannels remained open for up to 72 h in vivo (Kalluri and Banga 2011). Recently, Prausnitz's research group performed first human experiments to analyse the resealing of the skin's barrier properties after insertion of MNs using electrical impedance spectroscopy. In this study different MN geometries were investigated. Results indicated that in the absence of occlusion, all MN-treated sites recovered barrier properties within 2 h, whilst it took nearly 3–40 h for resealing of microchannels depending on MN geometry (Gupta et al. 2011). Brogden et al. (2012) demonstrate that a one-time MN treatment, in first human study, and daily topical application of diclofenac sodium can prolong the lifetime of micropores, when measured by impedance spectroscopy, suggesting the involvement of subclinical inflammation in micropore healing. A methylene blue staining study by Brichall's group showed that the MN-induced micropores close at 8–24 h after the removal of MNs immediately (Haq et al. 2009).

Generally, medical device-related infections do occur due to contaminated needles. Whilst there is no doubt that alcohol wipes are used before vaccination, injections given by patients to themselves in their own homes, such as subcutaneous enoxaparin and insulin, are not typically preceded by skin cleansing. In any case, it is well established that the use of alcohol to decontaminate the skin is not particularly effective, with significant bioburdens remaining (Hoffman 2001). Importantly, regular use of alcoholic gels can lead to irritant and allergic dermatitis, which alter the composition of the resident flora (Elsner 2006). On the other hand, if microporation-based drug delivery systems are to realise their undoubted potential, then they must be able to be used safely and routinely by patients in their own homes. Because microporation devices create micropores and its degree of pore closure varies with different dimensions of microporating

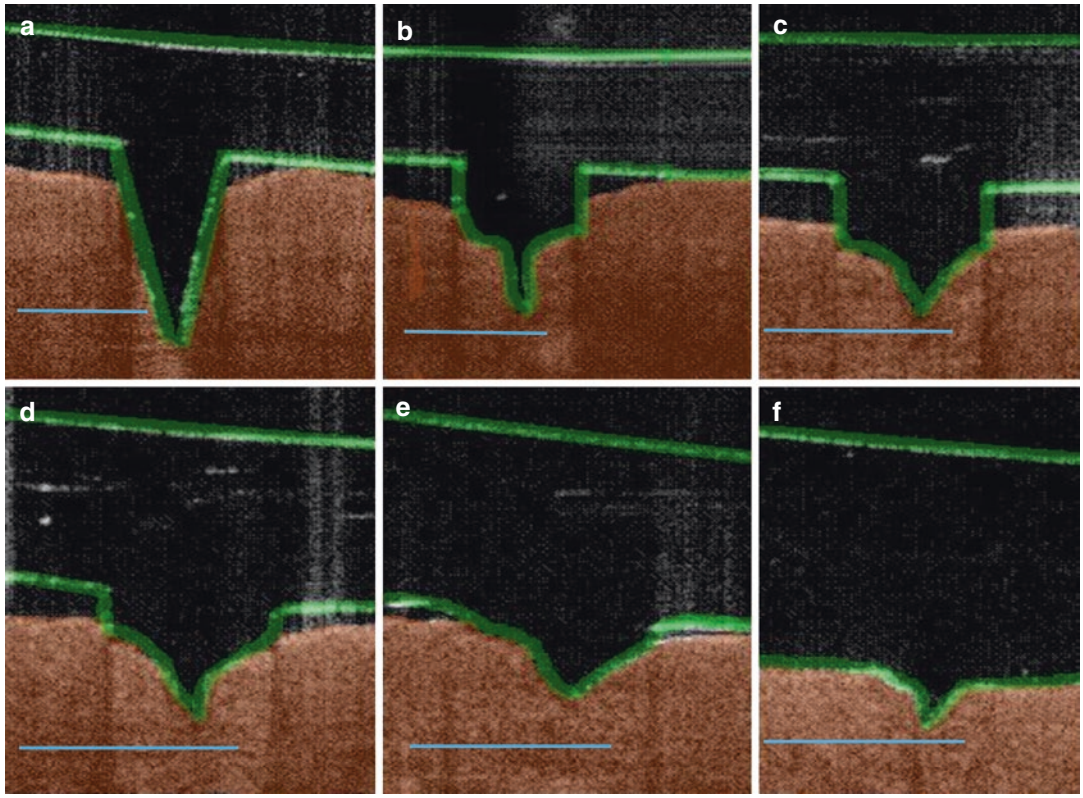

Fig. 17.4 OCT images of in vitro dissolution profile of MNs (with height 600 μm, width at base 300 μm, spacing 300 μm) in porcine skin over a 3-h period (**a** = time 0; **b** = time 15 min, **c** = time 30 min, **d** = 60 min, **e** = 120 min, **f** = 180 min). (*Scale bar* represents length of 300 μm). It can been seen that in this microporation technique, the MN dissolves and at the same time the skin starts the recovery process, which is dependent upon the type of MN and its dissolution rate. On the other hand, this technique can be used for real-time in situ monitoring for both MN dissolution/swelling and skin recovery (Reprinted with permission from Elsevier, Donnelly et al. 2010)

devices, it is important that no contamination of sterile epidermis will occur. Such as microbial ingress into viable epidermis that may occur during the closure of the pores, therefore, safety due to this needs consideration.

We have recently demonstrated that the microorganisms (i.e. *Candida albicans*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*) could traverse through the MN-induced holes in the SC. It has been clearly shown, using two different models, that representative Gram-positive (*S. epidermidis*) and Gram-negative (*P. aeruginosa*) bacteria and fungi (*C. albicans*) can indeed traverse MN-induced holes. Hypodermic needle puncture led to significantly greater microbial penetration across an in vitro membrane (i.e. Silescol®) than MN puncture in each case. This was despite the cross-sectional area of the needle employed (0.5 mm^2) being less than the combined cross-sectional area of the 30 MN of the array (1.5 mm^2). This study suggested that the infection risk associated with skin application of MNs is very minimal and it is likely to be less than that associated with hypodermic needles. Safety can be enhanced by aseptic or sterile manufacture and by fabricating MNs from self-disabling materials (e.g. dissolving or biodegradable polymers) to prevent inappropriate or accidental reuse (Donnelly et al. 2009). Similar to our study, Li and colleagues (Li et al. 2010) have also studied potential infection through microchannels in in vivo rat model. In this study, super-short silicon MNs of 70–80 μm heights and macroneedle of 1500 μm in height or

abrasion using sterile equipment were tested, following which a solution of *Staphylococcus aureus* culture was applied to the treated sites. Blood samples were collected and analysed for white blood cell, leukomonocyte and neutrophil granulocyte count. Results indicated that the super-short MNs were similar to that of control group (rats without any treatment). In contrast, macroneedle treatment and abrasion lead to higher cell counts. Thus, MN treatment appears to be safe but should be used within certain dimensions if not it may result infection, which also applies to other microporation techniques. Therefore, it is important for researchers to demonstrate that the pores created on the skin following microporation application will reseal within specified time. Furthermore, infection risk associated with skin application of MNs is likely to be less than that associated with hypodermic needles. However, in supporting widespread clinical use of MN-based delivery systems, more animal studies are needed to conclusively demonstrate its in vivo safety. Similar studies are imperative to be conducted by all the other range of microporating devices, as each work on different principles and could cause different rates of skin resealing kinetics.

17.4 Microporating Devices in Clinical Trial

The US Food and Drug Administration (FDA) approved the first transdermal patch products in 1981. Now, the transdermal drug delivery market, worth $12.7 billion dollars in 2005, is expected to reach $32 billion in 2015 (Research Facts Ltd 2009). Importantly, the microporation devices that significantly enhance the delivery of wide range of molecules, including biotechnological products, across the skin can have marked effect on the market size of transdermal drug delivery. Accordingly, many pharmaceutical companies are actively involved in the evaluation of their microporation devices for clinical studies.

TransPharma Medical's implementation of its innovative RF-MicroChannel Technology is the self-applied ViaDor™ drug delivery system. TransPharma recently replaced the name of its drug delivery system from ViaDerm to ViaDor™. Pretreatment of rats with ViaDerm™ device and following administration of medical patch containing testosterone-sulfobutyl ether-ß-cyclodextrin solution resulted in significantly higher serum concentration of testosterone compared to control rats treated with patch only (Levin et al. 2005). Similarly, ViaDerm™ enhancement of transdermal delivery of granisetron hydrochloride from granisetron patches in vivo (Levin and Dorit 2005) as well as delivery of cosmetic agents such as salicylic acid and caffeine from solutions or commercial products in vitro was also demonstrated. Further, ViaDerm™ was reported to facilitate transdermal immunisation with ovalbumin and trivalent influenza vaccine in male guinea pigs. Comparison between well-established and widely used vaccination routes (subcutaneous and intramascular) and transdermal vaccination using the above-mentioned apparatus indicated that ViaDerm™ treatment can shorten the time for appearance of titres of IgG and IgA antibodies proving usefulness of the device as a potential vaccine administration system (Levin et al. 2007a, b; Levin 2007).

In addition, TransPharma currently has three drug products in clinical trials, the ViaDor-hPTH (1–34), a novel transdermal hPTH (1–34) (human parathyroid hormone) product for the treatment of osteoporosis developed in collaboration with Eli Lilly and currently in Phase 2b clinical studies; ViaDor-GLP1 (glucagon-like peptide-1) agonist for the treatment of type II diabetes that has completed Phase 1b clinical study; and the ViaDor-Calcitonin that has completed a Phase 1 clinical trial (TransPharma 2012). Recently, Syneron Medical Ltd., the leading global aesthetic device company, announced that it has signed a definitive agreement to acquire substantially all the assets of TransPharma Medical Ltd.

Altea's PassPort® system is comprised of a single-use disposable PassPort® patch and a reuseable handheld applicator. The former consists of a conventional transdermal patch attached

to an array of metallic filaments ('porator'). Pressing the activation button of the applicator releases a single pulse of electrical energy to the porator, where it is converted into thermal energy. The rapid conduction of this thermal energy into the surface of the skin painlessly ablates the SC under each filament to create microchannels. When the applicator is removed, a simple fold-over design aligns the transdermal patch with the newly formed microchannels. Indeed, at the beginning of April 2009, Altea Therapeutics announced that it has entered into an agreement with Eli Lilly and Company and Amylin Pharmaceuticals, Inc. to develop and commercialise a novel daily transdermal patch delivering sustained levels of exenatide utilising the Altea Therapeutics proprietary PassPort® transdermal delivery system. Currently, Altea is developing on four clinical-stage products using PassPort® technology: enoxaparin sodium, exenatide, basal insulin and fentanyl citrate. Altea Therapeutics, supported by Eli Lilly and Company and Amylin, recently completed an initial Phase 1 clinical study of the exenatide transdermal patch in people with type 2 diabetes (TechConnect 2012). Altea has also completed Phase I clinical studies of transdermal patches delivering basal levels of insulin in managing type 1 and 2 diabetes. A Phase I clinical trial is underway for fentanyl citrate (Dubin 2011). Now, Japanese material manufacturer Nitto Denko has acquired all of Altea's patents, trademarks and lab equipment, planning to use the company's transdermal technology to launch the patch on its own.

The limited number of companies has demonstrated the use of electroporation devices for commercial use, for example, Inovio established the use of electroporation to deliver and enhance the potency of DNA-based immunotherapies and vaccines, which consist of electrical pulse generators and needle-electrode applicators (Genetronics 2012). Inovio Pharmaceuticals or its partners and collaborators are conducting multiple clinical trials for DNA vaccines to prevent and/or treat cancers and infectious diseases.

In addition to the above, Pantec Biosolutions AG has patented many other patents (Bragagna et al. 2005; Bragagna et al. 2006a, b, c), and currently this technology called P.L.E.A.S.E.® (Painless Laser Epidermal System) is in clinical studies to deliver small- and large-molecular weight therapeutics. Development of transdermal patch systems, optimised for use with the P.L.E.A.S.E.® technology, is ongoing. In addition, the company has completed investigations with diclofenac and lidocaine. Using the P.L.E.A.S.E.® device, the company is currently in clinical trials for the delivery of in vitro fertilisation (IVF) hormone, with an estimated market value of US$ 1.5–2 billion. In December 2008 the company has received CE marking for P.L.E.A.S.E.® (Pantec Biosolutions 2012). At the end of 2010, Pantec Biosolutions announced that a FSH (follicle-stimulating hormone of 32 kDa) patch used in conjunction with the company's novel P.L.E.A.S.E.® technology has successfully achieved proof of concept in an exploratory clinical trial (Dubin 2011). On the other hand, Norwood Abbey, a biotechnology company, has developed Epiture Easytouch™. The laser-based Epiture Easytouch™ is claimed to be a revolutionary new drug delivery device that uses a laser to painlessly remove the SC and helps the skin to more rapidly absorb topical anaesthesia. It was shown, in human studies, that the dermal anaesthesia of 4% lidocaine cream reduced the onset time from 60 to 5 min (Epiture Easytouch 2012). The US and Australian regulatory bodies have approved the Norwood Abbey system for the administration of a topically applied anaesthetic.

Echo Therapeutics, Inc. (formerly Sontra Medical) is a developing Prelude® SkinPrep System, a sonoporation-based platform technology, to allow for significantly enhanced and painless skin permeation that will enable two important applications: analyte extraction, with the Symphony® tCGM System for needle-free, continuous glucose monitoring of hospital patients as the first application. And, needle-free delivery of topical lidocaine, as the first application, is ongoing (a 510(k) premarket notification has been submitted to the US FDA). Additional applications for painless, needle-free delivery of drugs are planned.

Under current investigations, MN-based systems are perhaps the most promising micropora-

tion systems. Several of such systems, following patent publication, are moving towards commercial availability. In addition to the systems described below, several other companies/research centres are also actively involved in investigation of novel MN devices. These include 3 M, Therajet, Norwood Abbey, NanoPass Technologies Ltd., Apogee Technology (PyraDerm™), Integrated Sensing Systems, Inc. (ISSYS), Animas Corporation-Debiotech, Imtek, Kumetrix, Micronit Microfluidics B.V., Nano Device, Silex Microsystems AB, SpectRx, Valeritas, Zeopane and Elegaphy.

Becton, Dickinson and Company (BD) has patented MN devices primarily for vaccine delivery (Paul 2002). The vaccines division of Sanofi-Aventis received the marketing authorisation from the European Commission for the first intradermal (ID) influenza vaccine Intanza® or IDflu®, using the BD Soluvia™ microinjection system (BD 2012). Independently, BD conducted clinical trials involving more than 700 subjects and 3500 injections with BD Soluvia™ and demonstrated that the system is barely perceptible, safe and easy to use and showed reproducible injections to the dermal layer, irrespective of the subject's gender, age, ethnicity and body mass. Zosano Pharma™ is currently preparing to enter a pivotal Phase III clinical trial using the Macroflux® technology developed by ALZA. A solid drug-coated MN patch system (MN height 190 μm) for the delivery of parathyroid hormone in the treatment of severe postmenopausal osteoporosis will be used. There appears to be a high likelihood of positive outcomes, based upon extremely encouraging and relatively large-scale Phase II results. Importantly, Zosano Pharma™ has incorporated an applicator system as an essential component of this delivery system. This applicator applies a consistent, pain-free force and has been optimised for easy use by elderly patients. Furthermore, focus study groups (288 postmenopausal women with osteoporosis aged 60–85 years) were conducted to evaluate patient perception of this technology, with positive outcomes noted. It was highlighted that 93 % of patients liked the patch concept 'extremely well', whilst 90 % rated it as easy to use, as exemplified by the fact that 82 % of patients were capable of applying the patch correctly the first time without any help. Indeed, it appears that incorporation of the applicator device lead to enhanced patient acceptability and faith in the device as a drug delivery system. NanoPass Technologies Ltd. has conducted a number of clinical trials demonstrating effective, safe and painless intradermal delivery of local anaesthetics, insulin and influenza vaccine via their MicronJet® technology (hollow MN device). Similarly, 3 M's microstructured transdermal system (MTS), having either solid or hollow MNs, has shown promising results in several preclinical studies investigating delivery of proteins, peptides and vaccines.

Conclusion

Conventional needles and syringes that directly pierce the skin surface remain the main invasive drug delivering method. Needles are the most efficient and cost-effective devices to administer medicaments to local skin compartments or to the systemic circulation. This direct administration of medicaments is the simplest way of by passing SC barrier. Despite this, the needle-based therapy suffers many disadvantages, such as needle-stick injuries associated among the patients and the healthcare workers, painful administration process, poor patient compliance and extra cost in disposing them. In addition, needle-transferred infections such as HIV-1 and hepatitis B or C infections also remain a major problem in the developing world. On the other hand, the barrier property of SC limits successful transdermal drug delivery by traditional patch-based drug delivery systems. But microporation techniques have the potential to overcome the above problems and can achieve successful delivery of macromolecules and biopharmaceuticals across biological barriers. Given the potential of such agents as next-generation therapeutics, it is hardly surprising that a significant number of firms are actively involved in fabrication and evaluation of different microporation devices. Indeed, it has been shown that microporation techniques, and the combination thereof, can enable the successful delivery of a wide range

of drug molecules. A number of companies, as detailed above, have demonstrated clinical efficacy of such devices, and some are in the process of being launched onto the market in the near future. However, before these microporation devices find widespread use, researchers must perfect the devices and techniques for optimal usage. Furthermore, comprehensive additional clinical studies are necessary to show that creation of pores in biological membranes is safe and reversible.

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