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Evaluation needle length and density of microneedle arrays in the pretreatment of skin for transdermal drug delivery

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ABSTRACT

Solid silicon microneedle arrays with different needle lengths (ranging from 100 to 1100 μm) and needle densities (ranging from 400 to 11,900 needles/cm^2) were used to penetrate epidermal membrane of human cadaver skin. After this pretreatment, the electrical resistance of the skin and the flux of acyclovir across the skin were monitored. A linear correlation between the acyclovir flux and the inverse of the skin electric resistance was observed. Microneedle arrays with longer needles (>600 μm) were more effective in creating pathways across skin and enhancing drug flux, and microneedle arrays with lower needle densities (<2000 needles/cm^2) were more effective in enhancing drug flux if the microneedles with long enough needle length (>600 μm). In addition, the microneedle arrays were used to penetrate hairless rat skin in vivo, and the trans-epidermal water loss (TEWL) of the rat skin was measured before and after the pretreatment. Treating rat skin with microneedle arrays of lower needle density and longer needle length was more effective in increasing TEWL. Integrity of the stratum corneum barrier of the penetrated rat skin as measured by TEWL recovered back to its base line level within 24 h after the microneedle pretreatment.

1. Introduction

Delivery of drugs into and through the skin via dermal and transdermal systems have been studied for decades. Advantages associated with this route of drug delivery include (Guy, 1996): improved bioavailability due to avoidance of first pass hepatic and gastrointestinal metabolism, increased patient compliance due to reduction of multiple dosing regimes, and side effect reduction due to optimization of blood concentration versus time profile. However, only a handful of transdermal drug products have been developed (Prausnitz et al., 2004) in part because the skin acts as a barrier that prevents most drugs or therapeutic agents to penetrate into the body at a therapeutic rate. The rate limiting barrier in the skin, called the stratum corneum, is a superficial layer composed of densely packed dead corneocytes and intercellular lipids. Several approaches have been investigated to alter the barrier of the stratum corneum to enhance the drug transport across skin, some of these approaches include chemical enhancers (Williams and Barry, 2004), heat (Sawyer et al., 2009), electroporation (Prausnitz et al., 1993), iontophoresis (Yan et al., 2005), sonophoresis (Tachibana, 1992), laser ablation (Lee et al., 2002), and microneedles (Prausnitz, 2004).

The application of microneedles for transdermal drug delivery was initiated in the 1970s (Gerstel and Place, 1976). Gerstel and Place proposed using multiple needle shaped projections for percutaneous drug delivery with needle length of 5–100 μm in order to penetrate the stratum corneum without penetrating interior layers of the skin or contacting the nerves of the skin. In the 1990s more researchers began to investigate the application of microneedles for transdermal drug delivery, in part because micro-fabrication technologies made it possible for the manufacturing microneedles. Solid silicon microneedle arrays with length of 150 μm was investigated and it was shown that the pretreatment of human skin with the microneedle arrays significantly increased calcein permeability (Henry et al., 1998). In addition, Kim et al., showed that 100 μm length microneedle arrays fabricated with silicon dioxide demonstrated adequate robustness for transdermal applications (Kim et al., 2006). Polycarbonate microneedles with length of 200 and 500 μm and density of 45–154 needles/cm^2 had also been fabricated to study their effects on transdermal permeability (Oh et al., 2008). It had been demonstrated the possibility of using hollow glass microneedles for micro-infusion (Martanto et al., 2006a,b). In addition, some researchers fabricated microneedles with polymers (Park et al., 2006) or carbohydrates (Kolli and Banga, 2008) for transdermal drug delivery.

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Table 1
Acyclovir flux and standard deviation corresponding to a microneedle array dimension and density.

Microneedle length (μm)	Needle density (needles/cm^2)	Microneedle base width (μm)	Flux (μg/cm^2/h)	Flux standard deviation
100	900	133	0.506	0.032
100	2000	77	0.315	0.277
100	5625	60	0.198	0.055
100	11,900	46	0.176	0.146
200	900	167	0.443	0.400
200	4000	70	0.571	0.141
200	5625	80	0.304	0.277
200	11,900	50	0.473	0.319
300	11,900	52	0.387	0.315
400	2000	92	3.351	0.766
400	5625	80	0.053	0.051
650	400	250	3.686	1.812
650	900	167	4.536[a]	3.837[a]
650	2000	112	5.026	2.535
850	400	306	3.902	2.226
850	2000	124	3.672	2.321
1100	400	250	10.697[a]	7.541[a]
1100	900	167	6.901[a]	4.877[a]
Control (0)	Control (0)	Control (0)	0.088[b]	0.074[b]

Note: a—experiments were conducted with 6 HEMs; b—experiments were conducted with 9 HEMs; all other experiments were conducted with 3 HEMs.

Although there are many studies utilizing microneedle arrays for transdermal drug delivery, there has been no systematic investigation into the interplay of microneedle length and microneedle density of microneedle arrays. This study was to investigate the microneedle length effect and the microneedle density effect for skin pretreatment with microneedle arrays comprising a broad range of microneedle lengths and densities, with the purpose to find a microneedle array configuration with optimal needle length and needle density for pretreatment of skin for transdermal drug delivery.

2. Materials and methods

2.1. Materials

Solid silicon microneedle arrays with the needle lengths ranged from 100 to 1100 μm, and needle densities ranged from 400 to 11,900 needles/cm^2 were fabricated using a deep reactive ion etching technique by Dr. Bruce Gale in the Department of Mechanical Engineering at the University of Utah (Salt Lake City, UT). The detailed configurations of the microneedle arrays used in this study are listed in Table 1. Fig. 1 shows a representative scanning electron microscopy image of a microneedle array with 200 μm needle length and 4000 needles/cm^2 needle density. All the microneedle arrays were in 1 cm^2 square shape size. Each microneedle array was adhered to the flat surface of a 10 ml plastic syringe plunger (purchased from VWR) by using double sided adhesive tape. Human cadaver skin was purchased from the New York Fire Fighters Skin Bank. Human epidermal membrane (HEM), the epidermal layer of human cadaver skin was separated by a heat separation method (Peck et al., 1995). Female hairless rats older than 8 weeks were purchased from Charles River Laboratories. Phosphate buffer saline solution (PBS) 0.15 M at pH 7.4 was prepared from PBS tablets purchased from Spectrum Chemicals. Acyclovir (USP grade) was purchased from Spectrum Chemicals.

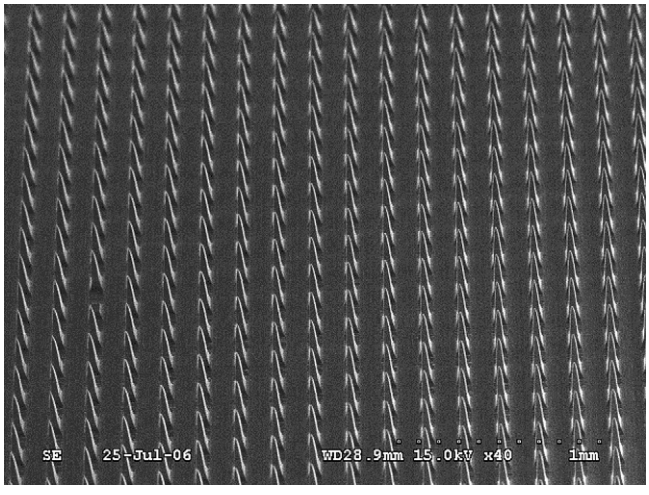

Fig. 1. Scanning electron microscopy image of a microneedle array with a needle length of 200 μm and array density of 4000 needles/cm^2.

2.2. In vitro HEM penetrated with microneedle arrays

Before HEM was used for in vitro studies the membrane integrity was verified. Specifically, HEM was sandwiched between the donor and receiver chambers of a Franz cell, then both chambers were filled with PBS solution. A silver/silver chloride electrode was placed in each chamber with the electrodes positioned close to the HEM. A small DC voltage (200 mV) was applied across the HEM by BK Precision DC regulated Power supply (Model # 1623A), and the electric current across skin was monitored by a multimeter to obtain the HEM resistance by applying the Ohm's law. Only HEM with electric resistance higher than 15 kΩ/cm^2 was used. The HEM was removed from the Franz cell and placed on a soft sponge pad with the stratum corneum layer facing up and penetrated with a microneedle array (mounted on the syringe plunger) with a force of 44.5 N for 10 s. The penetration force was controlled by a Mecmesin compact force gauge (purchased from VWR). Then the HEM was remounted in the Franz diffusion cell with the penetrated skin site aligned with the diffusion area of the Franz cell. After equilibrating with the PBS solution for 15 min, the HEM resistance after microneedle puncture pretreatment was measured again and monitored during the experiment period. Acyclovir saturated solution (in pH 7.4 PBS) was prepared freshly a day earlier before the flux experiment and equilibrated under shaking condition at room temperature for overnight. The donor chamber was filled with 0.5 ml of the acyclovir saturated PBS solution with some solid drug particles present and the receiver chamber was filled with 5 ml of PBS solution. Acyclovir flux across HEM with or without microneedle array pretreatment (control condition) was monitored. Samples of 0.8 ml were taken from the receiver chamber at 2, 4, 6, and 8 h, and 0.8 ml of fresh PBS solution was added back to the receiver

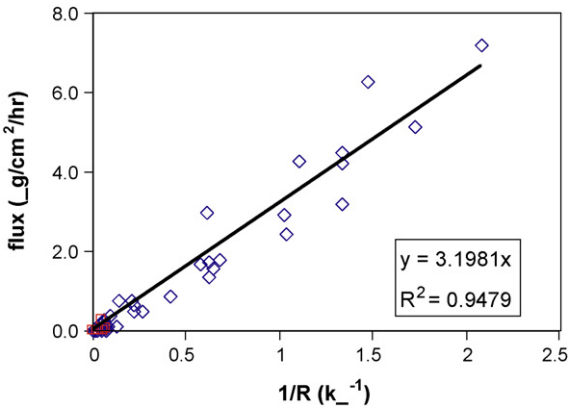

Fig. 2. Relationship between the drug flux and the inverse electric resistance of HEMs after microneedle pretreatment. Data points (open squares) from the control condition were also included.

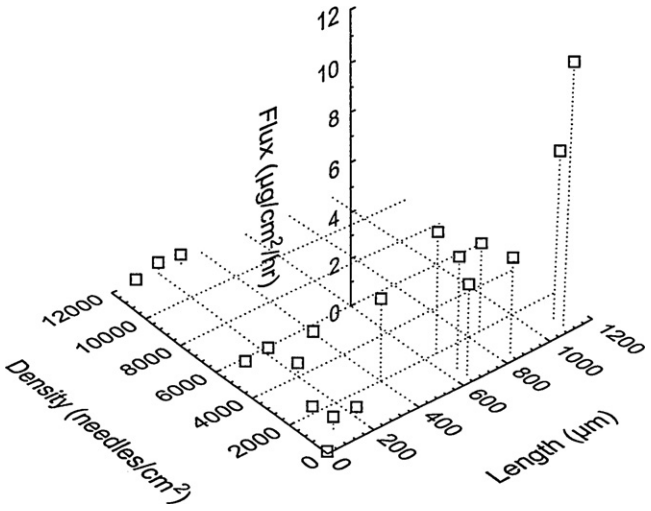

Fig. 3. Relationship between the drug flux (μg/cm²/h) at the *z* axis and corresponding microneedle arrays with the microneedle length (μm) at the *x* axis and microneedle density (needles/cm²) at the *y* axis applied during the HEM pretreatment.

chamber. Samples were analyzed by HPLC and the steady state flux values were calculated by linear regression of the receiver chamber accumulative drug amounts per surface area versus time plot.

2.3. In vivo hairless rat skin penetrated with microneedle arrays

The hairless rats were kept under anesthetized condition with an isoflurane vaporizer (Vapomatic mode 2, purchased from Summit Anesthesia Solutions). On the central back skin of the rats was marked by a cross to divide the back skin into 4 different sections, in each section a circle was drawn on the skin with the area of the circle just large enough to contain a square with the area of 1 cm². Before the microneedle penetration, trans-epidermal water loss (TEWL) of the circled skin area was measured by a Delfin Vapometer. Then the circled skin area was pulled to the side from the rat and laid flat on the experiment platform, and then it was penetrated by a microneedle array with a force of 44.5 N for 10 s. The microneedle arrays with the configurations of 650 × 400, 650 × 900, 650 × 2000, 850 × 400, 850 × 2000, and 1100 × 900 (length, μm) × (density, needles/cm²) were used to penetrate the rat skin. Triplicate experiments were conducted for each microneedle configuration. Immediately after the microneedle treatment, usually within 1–2 min, the TEWL was measured again. Then the TEWL of the penetrated skin area was measured at 2 h, 6 h, and 24 h after the microneedle penetration. A Student's *t*-test analysis was conducted to look into whether there was a significant difference in the TEWL values of rat skin punctured with different microneedle arrays.

3. Results

3.1. In vitro HEM penetrated with microneedle arrays

HEM resistance was monitored after the microneedle pretreatment until the end of the 8 h flux study. The HEM resistances remained relatively unchanged during the experiment period (data not shown). This indicated there was no skin recovery for human cadaver skin after the microneedle pretreatment. Transport of water soluble molecules, such as acyclovir, across human skin, is mainly through the aqueous pathway as evidenced by a linear correlation between the drug permeability coefficient and the inverse of the skin resistance (if the sizes of the electrolytes in solution are close to the size of the hydrate drug molecular size) (Yan et al., 2004). Fig. 2 presents the data showing the linear correlation between the inverse of the electrical resistance of HEM and the flux of acyclovir across the HEM. The data points of the control condition (without microneedle pretreatment) were also included. A good linear correlation was observed, which suggested the drug transported mainly through the aqueous pathways in the HEM created by the microneedle arrays. Further, measuring the electrical resistance of HEM may be a good indicator to screen the effectiveness of microneedle pretreatment on skin. Using HEM instead of full thickness skin was preferred here because transport across the stratum corneum for water soluble drugs was the rate limiting step, and inclusion of the dermis layer in the experiments could compromise the experiment results.

The average acyclovir flux and the corresponding standard deviation through the HEMs after pretreated with each set of microneedle arrays or under the control condition were listed in Table 1. In addition, the average acyclovir flux across the HEMs after being penetrated with each set of microneedle arrays was plotted against its needle length and needle density in Fig. 3. The drug flux data of the control condition which showed as zero needle density and zero needle length was also included in Fig. 3. For those microneedle arrays with lengths in the range of 100, 200, and 300 μm, the acyclovir fluxes across HEM after being penetrated with those microneedle arrays were low, and they were less than 10 times of the acyclovir flux across HEM without microneedle pretreatment. Much higher acyclovir fluxes were observed when HEM was pretreated with microneedle arrays with microneedle lengths above 600 μm. Highest acyclovir fluxes were observed for HEM pretreated with the longest microneedle arrays (1100 μm). For the microneedle arrays with needle length of 400 μm, high acyclovir flux was observed for the microneedle array with needle density of 2000 needles/cm², and low acyclovir flux was observed for microneedle array with needle density of 5625 needle/cm². In addition, we also observed some difference in acyclovir fluxes for microneedle arrays with needle length of 1100 μm but two different needle densities of 400 and 900 needles/cm², and the lower density one showed higher fluxes.

3.2. In vivo hairless rat skin penetrated with microneedle arrays

Microneedle arrays with needle length above 600 μm were studied further on rat skin in vivo to see how the two factors, needle length and needle density impact the effectiveness of pretreatment in vivo. We measured the trans-epidermal water loss (TEWL) of the rat skin since it had been used as an indicator of

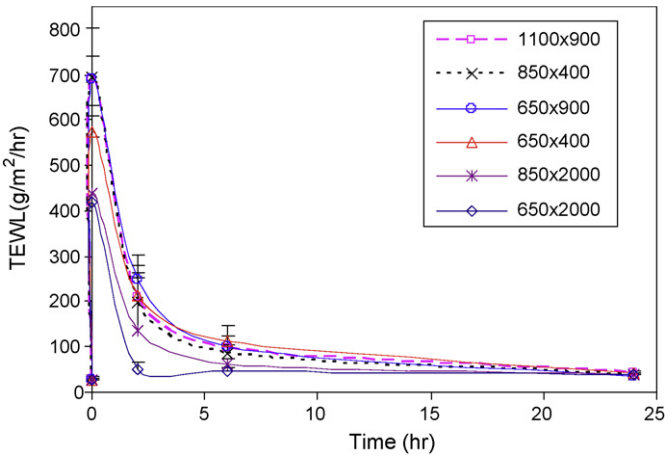

Fig. 4. The TEWL ($g/m^2/h$) of rat skin before and after the pretreatment with microneedle arrays with the configurations of 650×400, 650×900, 650×2000, 850×400, 850×2000, and 1100×900 (length, μm) × (density, needles/cm^2).

the skin integrity (Teo et al., 2005; Bal et al., 2008). Fig. 4 shows the TEWL of the hairless rat skin before and after being penetrated with a variety of microneedle arrays with different needle lengths (650, 850 or 1100 μm) and needle densities (400, 900, or 2000 needles/cm^2). The TEWL of the rat skin increased dramatically right after the microneedle puncture, with 10–25 fold increase for all the rat skin penetrated by microneedle arrays. After the microneedle pretreatment, the TEWL of the penetrated rat skin rapidly decreased in the first several hours, and the TEWL of the rat skin at 24 h after the pretreatment almost reached the TEWL of the rat skin before the pretreatment. These results indicate that rat skin recovers immediately after the skin is penetrated by the microneedle array and recovers within 24 h after the pretreatment. There was some difference in the TEWL profiles of the rat skin penetrated with different microneedle arrays. The TEWL of the rat skin immediately after pretreatment with microneedle array of 2000 needles/cm^2 needle density was lower than the others and it also decreased faster than TEWL of rat skin that was penetrated by lower needle density microneedle arrays. Especially for the microneedle array of 650×2000, the TEWL values at 2 and 6 h points were significantly ($P < 0.05$) lower than the TEWL values of other microneedle configurations (except the configuration 850×2000, which showed no significance). The TEWL profiles of the rat skin penetrated with microneedle arrays of different needle lengths (650, 850, and 1100 μm) but with lower needle densities (400 and 900 needle/cm^2) did not show much difference.

4. Discussion

The stratum corneum layer of the skin is the major barrier for transdermal drug delivery. It is about 15 μm thick when dry and about 48 μm thick when fully hydrated (Gerstel and Place, 1976). The ideal microneedle length would be just long enough to penetrate across the stratum corneum layer to create pathways for drug transport without disturbing the nerve endings in the dermis layer to cause any pain sensation during the application. Based on this idea, Gerstel et al. proposed in their patent that the length of the projections of their drug delivery device to be 5–100 μm (Gerstel and Place, 1976). Henry et al. demonstrated that the using of microneedle arrays with length of 150 μm to penetrate human epidermis in vitro could dramatically increase the permeability of calcein up to 25,000 fold (Henry et al., 1998). Wu et al also reported the applying of octagonal shape microneedle arrays with length of 150 μm on skin in vitro can improve calcein permeability by 10^4–10^5 fold (Wu et al., 2008).

In this study, we observed some in vitro acyclovir flux enhancement after HEM pretreated with microneedle array with length from 100 to 300 μm as shown in Fig. 2, but the enhancement induced by pretreatment with those microneedle arrays was not as dramatic as observed by other researchers with orders of magnitude increase. This could be because we studied a much smaller molecule, acyclovir with molecular weight around 225, compared to calcein they investigated with a molecular weight of 623. Larger molecules tend to show a bigger flux increase compared to the control condition based on the hindrance factor for drug transport across skin (Li et al., 2001). Other researchers also reported that there was not much enhancement with short needle microneedle arrays. For example, Teo et al. only observed a rough 10-fold enhancement of the transdermal transport in vitro after rat skin penetrated by microneedle array with length of 150 μm and they did not observe any enhancement in vivo (Teo et al., 2005); Oh et al. observed a 5.5-fold increase of calcein permeability in vitro for their microneedle arrays with 500 μm in length and 3.5-fold increase for the microneedle arrays with 200 μm in length (Oh et al., 2008). They indicated that the shape of the microneedle tip may not be sharp enough to facilitate an effective puncture.

However, we did observed a significant flux increase when microneedle arrays with needle length longer than 600 μm were used to penetrate the HEM. All the microneedle arrays used in this study were fabricated in the same method, and it did not appear that the tip of those longer microneedle arrays were sharper than that of the shorter microneedle arrays. However, a soft sponge pad was used to have the HEM laid on it during the application of microneedle arrays to puncture the HEM. This was used to mimic the soft tissue underneath the human epidermis and prevent the fracture of the microneedles during puncture. However, this elasticity property of the skin could allow the skin to fold around the microneedles during the puncture and consequently lead to an ineffective puncture. This phenomenon is more pronounced for short microneedles with length less than 300 μm (Martanto et al., 2006b; Verbaan et al., 2008). Some researchers tried to improve the application method to improve the puncture efficiency of the short length microneedle arrays. Verbaan et al. (2008) tried to improve the piercing of short microneedle arrays with an impact insertion method; Yang and Zahn (2004) applied vibratory actuation to control the microneedle insertion forces and the piercing for short microneedles. In our study, we only controlled the applied force during the microneedle application and the microneedle arrays were held steady during the application. Therefore, our application method would not prevent the skin wrapping effect which leads to the ineffectiveness of the short microneedle arrays in our study. On the other hand, if the needle length of the microneedle array is long enough, then the skin would not be able to effectively wrap around the needles, which is in agreement with results of higher fluxes with longer needle as shown in Fig. 3.

In addition, we observed significant enhancement in acyclovir flux across HEM pretreated with microneedle arrays with 400 μm in needle length and 2000 needles/cm^2 in needle density, but a lower enhancement of drug flux was observed for the microneedles with same needle length but a higher needle density of 5625 needle/cm^2. In our study, the applying force on the microneedle arrays were the same, but the force to an individual needle would be smaller for microneedle arrays with higher needle density. This phenomenon is similar to the "nail bed" effect, in which there are a sufficient number of sharp pointing nails on a bed such that the weight distributed among the nails is not sufficient to exert the pressure needed by each nail to break the skin. Therefore, lower density microneedle array would produce a more effective puncture into skin. It could be possible that the application method of applying a constant force on all the microneedle arrays could benefit the low density microneedles to some extent because there

was more force applied to each single needle for the low density microneedles. It would be worthwhile in future studies to look into the needle density effect by applying force on the microneedle arrays proportional to the number of the needles in the arrays.

It was puzzling that for microneedle arrays with needle length of 650 or 850 μm and needle density of 400, 900, or 2000 needles/cm^2, that the acyclovir flux across HEM after pretreatment were very close, or there was not much needle density effect. The TEWL results from in vivo rat skin pretreatment study with these microneedle arrays were generally in agreement with the in vitro flux results. The TEWL profiles after the pretreatment of rat skin with microneedle arrays with needle densities of 400 or 900 needles/cm^2 showed no difference: the peak values were very close and the skin recovery rates were also very close. The only difference was that the TEWL profiles after the pretreatment of rat skin with microneedles with density of 2000 needles/cm^2 was lower than those of the other two lower densities microneedles, where for the in vitro pretreatment study there was no difference for all three needle density levels. Nevertheless, the in vivo microneedle pretreatment study confirmed that there was a good correlation between the in vivo skin pretreatment and in vitro skin pretreatment with the microneedle arrays. From both studies it was observed that if the needle length of the microneedles were long enough (between 650 and 1100 μm), and at needle densities between 400 and 900 needles/cm^2, similar effective skin pretreatment results can be obtained.

A recent article reported that a 3-fold increase in needle length from 480 to 1450 μm lead to a 7-fold increase of pain in human volunteers and a 10 fold increase in needle density from 5 to 50 needles lead to a 2 fold increase in pain (Gill et al., 2008). Another study evaluated the pain score of human subjects after pretreatment with microneedles, and reported no significant differences in pain caused by microneedles with difference length from 200 to 550 μm (Bal et al., 2008). It was also found that by applying microneedle arrays with length of 620 μm for transdermal delivery of naltrxone to humans, most subjects tolerated the microneedles very well (Wermeling et al., 2008). Based on these reports, the microneedle array with needle length of 650 μm and needle density of 400 needles/cm^2 would cause some tolerable discomfort to a patient but would provide effective skin pretreatment for transdermal drug delivery compared to the other configurations investigated. There may be some other microneedle configurations with shorter needle lengths and less needle densities that may show effective skin pretreatment. For example, the in vitro HEM pretreatment with the 400 μm in length 2000 needles/cm^2 microneedle array showed reasonably good acyclovir flux. Microneedle arrays with configurations of 400 μm in length and lower needle densities were not fabricated for this study, but these may show good skin pretreatment for transdermal drug delivery based from the results of other microneedle array configurations.

In the in vivo rat skin pretreatment study, a more than 10-fold increase of the TEWL was observed immediately after the microneedle pretreatment, and the TEWL at 24 h decreased back to the level of the skin before pretreatment, indicating that the rat skin recovers quickly after microneedle pretreatment. Haq et al. evaluated the TEWL on human subject after the microneedle pretreatment with length of 180 and 280 μm, from which they only observed a modest increase of the TEWL from 5.1 to 8.8 g/m^2/h immediately after the pretreatment, and they also observed that the TEWL of human skin recovered back to the base line level after 24 h (Haq et al., 2009). The results could also indicate that longer needles may be needed for more effective skin pretreatment. In addition, microneedle pretreatment on skin in vivo was different from the pretreatment on the human cadaver skin in vitro, where the skin resistance remained constant after the pretreatment, indicating no skin recovery in human cadaver skin. However, microneedle pretreatment would not be feasible for transdermal drug delivery if the skin recovers its barrier properties too quickly, resulting in a narrow window for drug delivery. A narrow window of delivery in the case of acyclovir would limit the application of microneedles to products requiring a bolus delivery, such as transdermal vaccination.

5. Conclusions

Skin pretreatment by microneedle arrays with different needle lengths and needle densities for transdermal drug delivery was investigated by using acyclovir as a model drug. Microneedle arrays with sufficient needle length (>600 μm) resulted in higher acyclovir flux across skin compared to the microneedle arrays with short needle length (<300 μm). Further increase of needle length beyond the sufficient length (600 μm) did not show a significant increase in drug flux. In addition, lower needle density arrays were more effective in enhancing drug flux. It was also demonstrated that microneedle arrays with lower needle density were more effective in increasing TEWL after the pretreatment of rat skin in vivo.

References

Bal, S.M., Caussin, J., Pavel, S., Bouwstra, J.A., 2008. In vivo assessment of safety of microneedle arrays in human skin. Eur. J. Pharm. Sci. 35, 193–202.

Gerstel, M.S., Place, V.A., 1976. Drug delivery device. United States Patent, 3,964,482.

Gill, H.S., Denson, D.D., Burris, B.A., Prausnitz, M.R., 2008. Effect of microneedle design on pain in human volunteers. Clin. J. Pain 24, 585–594.

Guy, R.H., 1996. Current status and future prospects of transdermal drug delivery. Pharm. Res. 13, 1765–1769.

Haq, M.I., Smith, E., John, D.N., Kalavala, M., Edwards, C., Anstey, A., Morrissey, A., Birchall, J.C., 2009. Clinical administration of microneedles: skin puncture, pain and sensation. Biomed. Microdevices 11, 35–47.

Henry, S., McAllister, D.V., Allen, M.G., Prausnitz, M.R., 1998. Microfabricated microneedles: a novel approach to transdermal drug delivery. J. Pharm. Sci. 87, 922–925.

Kim, S., Shetty, S., Price, D., Bhansali, S., 2006. Skin penetration of silicon dioxide microneedle arrays. Conf. Proc. IEEE Eng. Med. Biol. Soc. 1, 4088–4091.

Kolli, C.S., Banga, A.K., 2008. Characterization of solid maltose microneedles and their use for transdermal delivery. Pharm. Res. 25, 104–113.

Lee, W.R., Shen, S.C., Wang, K.H., Hu, C.H., Fang, J.Y., 2002. The effect of laser treatment on skin to enhance and control transdermal delivery of 5-fluorouracil. J. Pharm. Sci. 91, 1613–1626.

Li, S.K., Ghanem, A.H., Teng, C.L., Hardee, G.E., Higuchi, W.I., 2001. Iontophoretic transport of oligonucleotides across human epidermal membrane: a study of the Nernst–Planck model. J. Pharm. Sci. 90, 915–931.

Martanto, W., Moore, J.S., Couse, T., Prausnitz, M.R., 2006a. Mechanism of fluid infusion during microneedle insertion and retraction. J. Control. Release 112, 357–361.

Martanto, W., Moore, J.S., Kashlan, O., Kamath, R., Wang, P.M., O'Neal, J.M., Prausnitz, M.R., 2006b. Microinfusion using hollow microneedles. Pharm. Res. 23, 104–113.

Oh, J.H., Park, H.H., Do, K.Y., Han, M., Hyun, D.H., Kim, C.G., Kim, C.H., Lee, S.S., Hwang, S.J., Shin, S.C., Cho, C.W., 2008. Influence of the delivery systems using a microneedle array on the permeation of a hydrophilic molecule, calcein. Eur. J. Pharm. Biopharm. 69, 1040–1045.

Park, J.H., Allen, M.G., Prausnitz, M.R., 2006. Polymer microneedles for controlled-release drug delivery. Pharm. Res. 23, 1008–1019.

Peck, K.D., Ghanem, A.H., Higuchi, W.I., 1995. The effect of temperature upon the permeation of polar and ionic solutes through human epidermal membrane. J. Pharm. Sci. 84, 975–982.

Prausnitz, M.R., 2004. Microneedles for transdermal drug delivery. Adv. Drug Deliv. Rev. 56, 581–587.

Prausnitz, M.R., Bose, V.G., Langer, R., Weaver, J.C., 1993. Electroporation of mammalian skin: a mechanism to enhance transdermal drug delivery. Proc. Natl. Acad. Sci. U.S.A. 90, 10504–10508.

Prausnitz, M.R., Mitragotri, S., Langer, R., 2004. Current status and future potential of transdermal drug delivery. Nat. Rev. Drug Discov. 3, 115–124.

Sawyer, J., Febbraro, S., Masud, S., Ashburn, M.A., Campbell, J.C., 2009. Heated lidocaine/tetracaine patch (Synera, Rapydan) compared with lidocaine/prilocaine cream (EMLA) for topical anaesthesia before vascular access. Br. J. Anaesth. 102, 210–215.

Tachibana, K., 1992. Transdermal delivery of insulin to alloxan-diabetic rabbits by ultrasound exposure. Pharm. Res. 9, 952–954.

Teo, M.A., Shearwood, C., Ng, K.C., Lu, J., Moochhala, S., 2005. In vitro and in vivo characterization of MEMS microneedles. Biomed. Microdevices 7, 47–52.

Verbaan, F.J., Bal, S.M., van den Berg, D.J., Dijksman, J.A., van Hecke, M., Verpoorten, H., van den Berg, A., Luttge, R., Bouwstra, J.A., 2008. Improved piercing of

microneedle arrays in dermatomed human skin by an impact insertion method. J. Control. Release 128, 80–88.
Wermeling, D.P., Banks, S.L., Hudson, D.A., Gill, H.S., Gupta, J., Prausnitz, M.R., Stinchcomb, A.L., 2008. Microneedles permit transdermal delivery of a skin-impermeant medication to humans. Proc. Natl. Acad. Sci. U.S.A. 105, 2058–2063.
Williams, A.C., Barry, B.W., 2004. Penetration enhancers. Adv. Drug Deliv. Rev. 56, 603–618.
Wu, Y., Qiu, Y., Zhang, S., Qin, G., Gao, Y., 2008. Microneedle-based drug delivery: studies on delivery parameters and biocompatibility. Biomed. Microdevices 10, 601–610.
Yan, G., Li, S.K., Peck, K.D., Zhu, H., Higuchi, W.I., 2004. Quantitative study of electrophoretic and electroosmotic enhancement during alternating current iontophoresis across synthetic membranes. J. Pharm. Sci. 93, 2895–2908.
Yan, G., Peck, K.D., Zhu, H., Higuchi, W.I., Li, S.K., 2005. Effects of electrophoresis and electroosmosis during alternating current iontophoresis across human epidermal membrane. J. Pharm. Sci. 94, 547–558.
Yang, M., Zahn, J.D., 2004. Microneedle insertion force reduction using vibratory actuation. Biomed. Microdevices 6, 177–182.