

available at www.sciencedirect.comjournal homepage: www.elsevier.com/locate/ejps

Skin penetration enhancement by a microneedle device (Dermaroller®) in vitro: Dependency on needle size and applied formulation

M.M. Badran^a, J. Kuntsche^{a,b}, A. Fahr^{a,*}

^a Friedrich-Schiller-University Jena, Department of Pharmaceutical Technology, Lessingstr. 8, 07743 Jena, Germany

^b Martin-Luther-University Halle-Wittenberg, Department of Pharmaceutics and Biopharmaceutics, Wolfgang-Langenbeck-Str. 4, 06120 Halle (Saale), Germany

ARTICLE INFO

Article history:

Received 27 August 2008

Received in revised form

4 November 2008

Accepted 7 December 2008

Published on line 25 December 2008

Keywords:

Microneedles

Invasomes

Skin morphology

Transepidermal water loss (TEWL)

Drug penetration

ABSTRACT

This study focused on the in vitro evaluation of skin perforation using a new microneedle device (Dermaroller®) with different needle lengths (150, 500 and 1500 µm). The influence of the microneedle treatment on the morphology of the skin surface (studied by light and scanning electron microscopy), on the transepidermal water loss (TEWL) and on the penetration and permeation of hydrophilic model drugs was investigated using excised human full-thickness skin. Furthermore, invasomes – highly flexible phospholipid vesicles containing terpenes and ethanol as penetration enhancer – were compared with an aqueous solution.

Elevated TEWL values were measured after Dermaroller® treatment compared to untreated human skin with a gradual increase of the TEWL over the first hour whereas afterwards the TEWL values decreased probably caused by a reduction of the pore size with time. Skin perforation with the Dermarollers® enhanced drug penetration and permeation for both formulations tested. Invasomes were more effective to deliver hydrophilic compounds into and through the skin compared to the aqueous drug solutions and the combination with skin perforation further enhanced drug penetration and permeation.

In conclusion, Dermarollers® being already commercially available for cosmetic purposes appear also promising for drug delivery purposes particularly those with medium (500 µm) and shorter (150 µm) needle lengths.

© 2008 Elsevier B.V. All rights reserved.

1. Introduction

(Trans)dermal drug delivery is an alternative pathway for achieving localized or even systemic pharmacological effects. Until now, transdermal drug delivery is possible for very few drugs, all of them having low molecular weights and high to moderate lipophilicities (Schaefer and Redelmeier, 1996). Transdermal drug delivery has impressive benefits including the avoidance of poor drug absorption or enzymatic

degradation in the gastrointestinal tract and/or the first pass effect after oral administration (Purdon et al., 2004) as well as avoidance of pain during intravenous or intramuscular injection. The challenge of transdermal drug delivery is, however, the delivery of a sufficient dose of the drugs through the skin due to the efficient barrier properties of the stratum corneum (SC), the outermost layer of skin, which acts as impermeable barrier to hydrophilic or high molecular weight drugs. Such molecules can only be delivered into or

* Corresponding author. Tel.: +49 3641 949900; fax: +49 3641 949902.

E-mail address: Alfred.fahr@uni-jena.de (A. Fahr).

URL: <http://www.uni-jena.de/lis.fahr.html> (A. Fahr).

0928-0987/\$ – see front matter © 2008 Elsevier B.V. All rights reserved.

doi:10.1016/j.ejps.2008.12.008

through the skin if the barrier function of the SC is reduced or disrupted (Gill and Prausnitz, 2007). In order to overcome the barrier properties of the SC, different approaches have been studied, chemical penetration enhancement being most widely used (Barry, 1987; Alberti et al., 2001; Williams and Barry, 2004). Penetration enhancement by physical methods includes, e.g., iontophoresis (Kalia et al., 2004), electroporation (Sen et al., 2002; Denet et al., 2004) and sonophoresis (Merino et al., 2003; Mitragotri and Kost, 2004). A further alternative presents the perforation of the SC with microneedles (Prausnitz, 2004; Kim and Lee, 2007). Microneedles disrupt the SC barrier of the skin and create pores resulting in an increased penetration (Prausnitz, 2004). As compared to subcutaneous injection, the application of microneedles allows a painless skin perforation and provides convenient delivery of therapeutic molecules into the skin (Kaushik et al., 2001). Microneedles can increase skin permeability for many compounds, ranging in size from small molecules over proteins up to drug-loaded nanoparticles that normally do not penetrate the SC (McAllister et al., 2003). In recent years microneedles have been intensively studied to improve skin permeability for molecules like calcein (Henry et al., 1998), diclofenac (Gardeniers et al., 2003), desmopressin (Cormier et al., 2004), and gene therapy vectors (Chabri et al., 2004; Pearton et al., 2008). Since the first production of microneedles by Henry et al. (1998), there has been a fast progress in the development of microperforation techniques (Prausnitz, 2004) allowing the production of hollow to compact microneedles made from different materials including glass (Martanto et al., 2006), silicon (Wilke and Morrissey, 2007), polymers (Park et al., 2005, 2007) and metal (Kim and Lee, 2007). The microneedles used in the present study are commercially available (Dermarollers®) for mainly cosmetic purposes. The needles of the Dermarollers® are made from stainless steel providing advantageous properties like mechanically strength and non-toxicity (Disegi and Eschbach, 2000). In a first evaluation of the application of Dermarollers® with respect to drug delivery, a distinctly increased deposition of a lipophilic fluorescence dye formulated in liposomes in deeper skin was found after Dermaroller® treatment (Verma, 2002). Dermarollers® with a needle length of 500 µm were recently also used for optical skin clearing purposes using glycerol (Yoon et al., 2008).

In the present study, Dermarollers® with different needle lengths were studied with respect to the efficiency of skin perforation and the penetration enhancement of hydrophilic model drugs with special focus to the quantification of the drug amount delivered to different skin layers. Additionally, the potential of the combination of skin perforation with the application of a colloidal drug carrier system (invasomes) was evaluated. Invasomes are highly flexible vesicles containing ethanol and terpenes as penetration enhancer in addition to phospholipids (Verma, 2002; Verma and Fahr, 2004; Dragicevic-Curic et al., 2008). The combination of skin perforation by microneedles with the application of invasomes may further facilitate the penetration of the vesicles deeper into the skin. The experiments focused on the influence of the treatment on the morphology of the skin surface as studied by light and scanning electron microscopy, on the transepidermal water loss (TEWL) and on the penetration and permeation of

hydrophilic model drugs (carboxyfluorescein and radiolabeled mannitol) depending on the formulation.

2. Materials and methods

2.1. Materials

Purified soybean phosphatidylcholine (SPC) dissolved in ethanol (NAT 8539, SPC:ethanol 75:25, w/w) was purchased from Phospholipid GmbH (Cologne, Germany). D-Limonene, citral and 1,8-cineole were purchased from Sigma-Aldrich (Taufkirchen, Germany) and carboxyfluorescein (CF) from Fluka (Buchs, Switzerland). Radiolabeled mannitol (in ethanol/water 9:3, 37 MBq/ml) was obtained from PerkinElmer (Boston, USA).

Phosphate buffered saline pH 7.4 was composed of 136.89 mM NaCl, 2.68 mM KCl, 1.47 mM KH₂PO₄ and 8.20 mM Na₂HPO₄·2H₂O (all from Merck, Darmstadt, Germany). Tris buffer 10 mM pH 7.4 was prepared from Trizma® pre-set crystals pH 7.4 (Sigma, St. Louis, MO, USA). The pH of the buffer solutions were adjusted to pH 7.4 with NaOH or HCl, if necessary. Purified water was prepared by successive filtration (pore size 10, 5 and 1 µm, Voigt, Wernau, Germany), deionization (Lewatit, Beyer, Leverkusen, Germany) and reverse osmosis (Milli-RX-20, Millipore, Eschborn, Germany) using an in-house water purification system. For analytics, purified water from a conventional water purification system (Millipore Direct-Q™, Millipore, Eschborn, Germany) was used. All other chemicals used in this study were of analytical grade.

The Dermarollers® were kindly provided by H. Liebl (Dermaroller S.a.r.l., Friesenheim, France). Three different models of Dermarollers® were used: C8 – needle lengths 150 µm, CIT 8 – needle lengths 500 µm and MF 8 – needle lengths 1500 µm. All types of Dermaroller® possess 24 circular arrays of 8 needles each (total 192 needles) in a cylindrical assembly (with 2 cm diameter and 2 cm length of the cylinder). A schematic presentation of a Dermaroller® is shown in Fig. 1.

2.2. Preparation of invasomes

The invasomes were composed of 1 % (w/v) terpene mixture (cineole:citral:D-limonene 45:45:10, v/v) as penetration enhancer (PE), 13.3% (w/v) ethanolic phospholipid solution (corresponding to 10% SPC) and the hydrophilic model drugs dissolved in PBS or Tris buffer (37 kBq/ml radiolabeled mannitol in PBS and 20 mM carboxyfluorescein in Tris buffer).

The terpene mixture was added to the ethanolic phospholipid solution and the mixture was vortexed for 5 min at room temperature until a clear solution was obtained. The aqueous phase (PBS or Tris buffer containing radiolabeled mannitol or CF, respectively) was slowly added to this solution using a syringe under constant vortexing (Batzri and Korn, 1973). The vortexing was continued for additional 5 min to obtain large multilamellar vesicles (MLV). Care was taken to prevent evaporation of terpenes and ethanol. Mainly unilamellar vesicles (invasomes) with a size of about 100 nm were then prepared by extrusion through polycarbonate membranes with different pore sizes (400, 200, 100 and 50 nm, Armatix, Mannheim, Germany) with the help of a Liposofast-Miniextruder (Avestin,

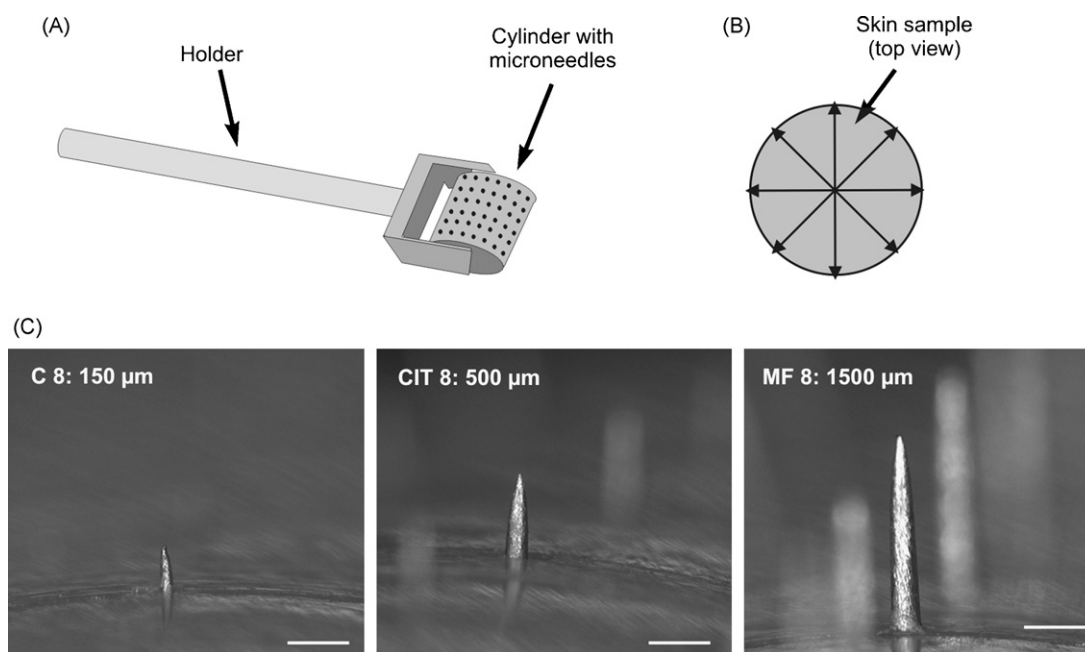


Fig. 1 – Schematic presentation of a Dermaroller® (A), of skin perforation (B) and stereo microscopic images (Leica MZ 8, Switzerland) of the needles of the different models of Dermarollers® used in this study (C). Bars represent 500 μm.

Ottawa, Canada, MacDonald et al., 1991). The invasomes were extruded through polycarbonate membrane with decreasing pore sizes from 400 to 50 nm 21 times for each membrane at room temperature.

Radiolabeled mannitol was incorporated into the invasomes in trace amounts (1 μl drug stock solution per 1 ml formulation, 37 kBq/ml). The stock solution of radiolabeled mannitol was pipetted into a Falcon tube and the solvent was allowed to evaporate. The drug was then dissolved in PBS pH 7.4. CF loaded invasomes were prepared using Tris buffer containing 20 mM CF and these solutions were also used in the penetration experiments for comparison. All invasome formulations were stored at 4 °C under light protection.

2.3. Particle size measurements

2.3.1. Photon correlation spectroscopy (PCS)

Dynamic light scattering was measured at 25 °C with a Zetasizer Nano (Malvern Instrument, Malvern, UK) or a Zeta plus instrument (Brookhaven Instruments, Brookhaven, USA). The samples were analyzed 24 h after preparation and after 1 month of storage. The invasomes were appropriately diluted with purified and filtered (0.2 μm pore size, Sterifix, B. Braun Melsungen, Melsungen, Germany) water prior to the measurements. The particle size values given are the averages of four measurements over 5 min each and are expressed as z-average and polydispersity index (PDI) determined by the cumulant analysis.

2.3.2. Laser diffraction with PIDS (LD-PIDS)

The particle size of the MLV was measured with a Coulter LS 230 (Beckman Coulter, Krefeld, Germany) combining the information from laser light diffraction and polarization intensity

differential scattering (PIDS). This method gives information on the particle size in the nanometer as well as in the micrometer range from about 40 nm up to 2000 μm (Xu, 1997). Volume distributions were calculated with the Coulter LS software using the Mie theory assuming a spherical particle shape and refractive indices of 1.45 and 1.33 for the vesicles and the dispersant, respectively. The values given are the averages of eight measurements over 90 s each.

2.4. Preparation of the skin samples

Human abdominal skin obtained after plastic surgery (Dr. L. Gruhl, Kassel, Germany) was used. The experimental protocol was approved by the ethical medical committee in Jena. The subcutaneous fat tissue was carefully removed from the skin using a scalpel and surgical scissors. After the fat tissue was completely removed, the surface of the skin was cleaned with the PBS solution and the skin was then allowed to dry at ambient air conditions for about 20 min. Care was taken to prevent any contamination of the skin surface with subcutaneous lipids. Afterwards the skin was wrapped into aluminum foil and stored at –26 °C until use. Under these conditions the skin is stable with regard to its barrier properties up to 6 months (Harrison et al., 1984; Bronaugh et al., 1986). Prior to the experiments, the skin samples were taken from the freezer and let thaw at room temperature for about 30 min. After thawing, the skin surface was carefully wiped with cotton wool balls wetted with PBS buffer.

For the SEM studies, the epidermis was isolated by heat separation (Kligman and Christophers, 1963). In brief, the full-thickness skin was heated in purified water at 60 °C for 2 min and the epidermis was then carefully peeled off from the underlying dermis using blunt forceps.

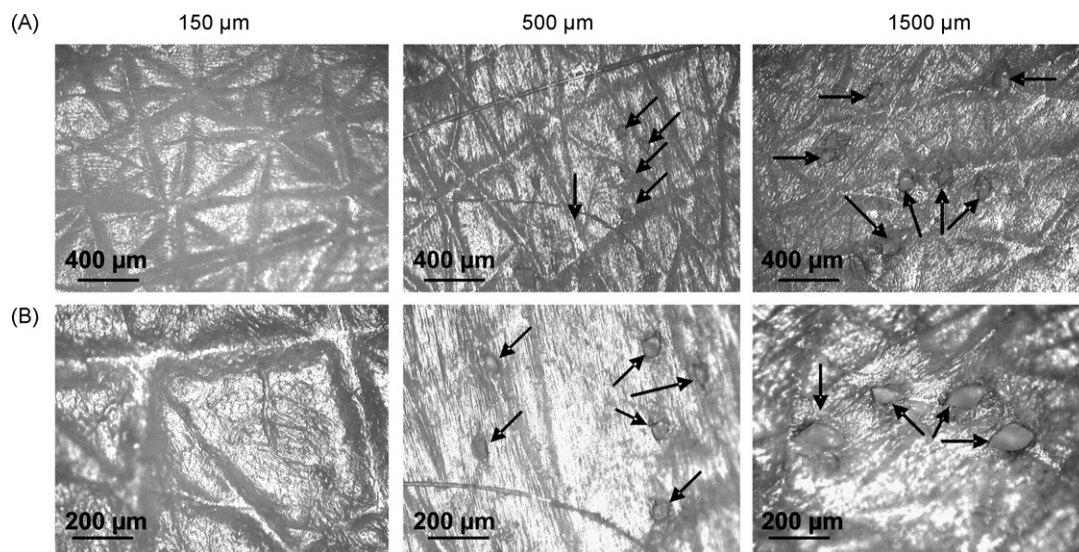


Fig. 2 – Light microscopic images of human skin treated with Dermarollers® with 50-fold (A) and 100-fold (B) magnification.

2.5. Skin perforation by Dermarollers®

Skin disks with appropriate diameters of 36 mm were punched out and placed onto filter paper soaked with PBS and supported by cork plates. As separated full-thickness skin has a strong tendency to shrink, the skin samples were stretched to about its original size and fixed with needles. Care was taken to avoid an overstretching in any direction and the skin was checked for normal surface texture by light microscopy (Leica DMRXE, Germany, Fig. 2).

The Dermarollers® differing in their needle lengths were rolled each three times in four directions (Fig. 1) over the skin surface. The microneedles were periodically checked for potential damages of the needles under a stereo microscope (Olympus SZ11).

2.6. Light microscopy

For visualization of the pores after the Dermaroller® treatment, skin samples were investigated by light microscopy (Leica DMRXE, Germany) in reflected light mode with different magnifications before and after application of the Dermarollers®. The width and length of at least five pores per skin sample was measured by the Lince analysis software version 2.31 (TU Darmstadt, Germany) and the pore size is expressed as the average of width and length of the pores.

The potential influence of epidermis separation as well as perforation of the epidermis instead of the full-thickness skin was investigated using the Dermaroller® with the longest needles (1500 µm). Separated epidermis and three full-thickness skin samples were perforated as described above and the pore sizes were determined immediately after perforation. The epidermis was then separated from perforated skin samples directly after perforation as well as after about 2 h and the pore sizes were evaluated in comparison to those of full-thickness skin again.

2.7. Scanning electron microscopy (SEM)

The epidermal membranes were placed onto filter paper soaked with PBS and supported by cork plates and fixed with needles. The epidermal membranes were then perforated similarly with the Dermarollers® as described for the full-thickness human skin. Subsequently, the epidermal membranes were fixed in formaldehyde for 10 min and dehydrated by immersion in alcoholic solutions with increasing ethanol content for each 30 min (50, 70, 96, and 100%, v/v). After dehydration, the membranes were mounted onto a metal stub with a carbon tape. The samples were coated with a gold layer (thickness around 10 nm) with a sputter coater (Edward, S150B, England) and investigated by SEM (Leica S440i, Germany) using different magnifications. To evaluate the reduction of the pore size with time after Dermaroller® treatment, the epidermis was separated about 2 h after Dermaroller® treatment and prepared for SEM as described above.

The size of each six pores per skin sample was measured as described for the light microscopic experiments to estimate the pore size.

2.8. Transepidermal water loss (TEWL)

The transepidermal water loss was measured using a VapoMeter (Delfin Technology, Kuopio, Finland) with a closed measurement chamber (Nuutinen et al., 2003).

Full-thickness human skin was mounted onto the bottom part of a glass Franz diffusion cell (FDC, PermeGear, Hellertown, USA), and the upper part of the FDC was replaced by a Teflon ring. The Teflon ring and the clamp assured that the skin was firmly fixed on the top of the FDC. The epidermal side of the skin was exposed to ambient conditions while the dermal side was in contact with the receptor fluid (PBS pH 7.4, 32 °C). Care was taken to remove all air bubbles between the underside of the skin (dermis) and the receptor solution.

If necessary, the surface of the skin was carefully dried with filter paper and the chambers were equilibrated for 30 min. The initial TEWL was then measured in triplicate. For perforation of the skin with the Dermalrollers®, the skin samples were removed from the Franz chambers and fixed on cork plates and perforated as described above. After perforation the skin samples were placed onto the Franz chambers again and the TEWL was measured in appropriate time intervals over 2 h.

2.9. *In vitro* penetration studies

Skin discs with diameters of 36 mm were punched out and mounted onto FDC with a nominal area for diffusion of 3.14 cm^2 and a receptor volume of about 15 ml. The epidermal side of the skin was exposed to ambient conditions while the dermal side was bathed with PBS pH 7.4. The receptor fluid was kept at $32 \pm 1^\circ\text{C}$ and constant stirring was maintained by magnetic stirring at 500 rpm. Care was taken to remove all air bubbles between the underside of the skin (dermis) and the receptor solution throughout the experiment. After equilibration for 30 min, the skin samples were removed from the Franz chambers and fixed in a Petri dish as described above. $10\text{ }\mu\text{l/cm}^2$ of invasomes or PBS solution containing radiolabeled mannitol were applied with an Eppendorf pipette onto a marked skin area. Directly after the application of the formulations, the skin samples were perforated with the Dermalrollers® with needle lengths of 150, 500 and $1500\text{ }\mu\text{m}$, respectively. As control, skin samples without application of Dermalrollers® were incubated with the test formulations. The skin samples were mounted onto the FDC and incubated without occlusion. Samples were taken from the receptor fluid ($700\text{ }\mu\text{l}$) every hour and the withdrawn volume was replaced by the same volume of fresh PBS buffer to maintain a constant volume. After 6 h, the formulations were removed by wiping the skin with cotton wool pads wetted with PBS buffer three to four times and the radioactivity of the used cotton wool pads was determined. The donor compartment of the FDC was wiped and analyzed together with the Eppendorf tip used for the application of the formulations. Dermalrollers® were placed in beakers filled with 20 ml ethanol (70 % v/v) for 30 min and 1 ml was taken for scintillation counting. All experiments were done in triplicate.

For determinations of the drug amount in the different skin layers, the skin was transferred onto an aluminum block, fixed onto cork plates with small pins. A Teflon mask with a central hole of 15 mm in diameter was placed on the skin sample. The SC was then subsequently removed by tape stripping. The tape strips (Cristall Klar Tesa, Beiersdorf AG, Hamburg, Germany) were slightly pressed onto the skin and a weight was lowered straining the skin with 2 kg for exactly 10 s. The tape strip was then rapidly removed and the procedure was repeated until the stratum corneum was removed completely (usually 20 tape strips). Due to analytical reasons, the stripped tapes were collected in vials according the following scheme: vial 1: strip 1, vial 2: strips 2–3, vial 3: strips 4–5, vial 4: strips 6–8, vial 5: strips 9–12, vial 6: strips 13–16, and vial 7: strips 17–20.

The remained skin (stripped skin) was frozen in liquid nitrogen and a skin disc with a diameter of 10 mm was punched out from the stripped area. The skin disc was

fixed on a pre-cooled microtome sample holder with a sufficient amount of embedding medium (Tissue-Tek, Sakura Finetek, Torrance, USA). The temperature in the cryomicrotome (Reichert-Jung 2800 Frigocut, Leica Instruments GmbH, Nussloch, Germany) was -26°C , while the temperature of the object was -15°C . The skin was cut parallel to the surface into $25\text{ }\mu\text{m}$ thick layers. The skin cuts were collected in vials according the following scheme: vial 1: initial irregular cuts, vials 2–4: each 10 cuts with a thickness of $25\text{ }\mu\text{m}$, and vial 5: the residual tissue.

The liquid samples (receptor fluid, ethanolic rinsing solution for the Dermalrollers®) were mixed with 3 ml of scintillation cocktail (Rotiszint, Carl Roth GmbH, Karlsruhe, Germany). The extraction of radiolabeled mannitol from the adhesive tapes and skin cuts was carried out after addition of 5 ml of scintillation cocktail. All samples were vortexed for at least 1 min and analyzed by liquid scintillation counting (Tri-carb 2100 TR, PerkinElmer). Blank samples were also prepared and the background was subtracted from the sample reading.

2.10. Fluorescence microscopic studies

Preparation of the skin, treatment with Dermalrollers® and incubation in FDC were carried out as described for the penetration study except that carboxyfluorescein was used as model drug instead of radiolabeled mannitol. $10\text{ }\mu\text{l/cm}^2$ invasomes, MLV or Tris buffer solution each containing 20 mM carboxyfluorescein was applied. Light protection was assured by covering the FDC loosely with aluminum foil. After incubation for 6 h, the surface of the skin was wiped with cotton wool wetted with PBS to remove any formulation left onto the skin surface until disappearance of the yellow color on the cotton. The skin samples were taken off from the FDC and immediately frozen in liquid nitrogen. The skin samples were embedded in Tissue-Tek and cross sections with a thickness of $7\text{ }\mu\text{m}$ were cut in the cryomicrotome and investigated by light (bright field) and fluorescence microscopy (excitation 470 nm, emission 520 nm, Leica DMRXP, Germany) with 100-fold magnification.

3. Results

3.1. Particle size of the invasomes and the large multilamellar vesicles (MLV)

The average size of the invasomes containing radiolabeled mannitol was $123.6 \pm 0.3\text{ nm}$ with a polydispersity index of 0.08 ± 0.01 . In the case of invasomes containing CF, the size of the vesicles was $105.5 \pm 0.2\text{ nm}$ with a polydispersity index of 0.08 ± 0.01 . The low polydispersity indices of less than 0.1 indicate a high homogeneity of the formulations and, therefore, a narrow size distribution. Only a slight increase in the vesicle size was detected after storage for 1 month in agreement with literature data showing good stability of the invasomal formulations when stored at 4°C (Dragicevic-Curic et al., 2008). The z-averages were 145.5 ± 1.3 and $115 \pm 0.2\text{ nm}$ for the invasomes loaded with radiolabeled mannitol and carboxyfluorescein, respectively, with polydispersity indices still between 0.07 and 0.11.

As expected, a broad multimodal size distribution with vesicles in the nm- up to the lower μm -range was obtained for the non-extruded MLV formulation. The main vesicle population had a size of about 330 nm (mode) and two minor fractions with maxima of about 1 and 3 μm were detected as well. The size of the cumulative volume distribution at 90% (D90-value) was about 3 μm . By PCS, a z-average of 513 ± 15 nm and a PDI of 0.27 ± 0.03 were measured.

3.2. Light microscopy

Representative microscopic images of perforated human skin are shown in Fig. 2. The pore size was about 70 and 150 μm for the Dermalrollers[®] with needle lengths of 500 and 1500 μm , respectively. No pores were visible by light microscopic inspection after perforation with the 150 μm microneedles. In reasonable agreement with the manufacturer information (250 pores/ cm^2 after 10–15 applications over the same skin area), the density of pores was approximately 170 and 180 pores/ cm^2 for the Dermalrollers[®] with a needle length of 500 and 1500 μm , respectively.

The effect of the skin preparation on the pore size was evaluated by light microscopy using the Dermalroller[®] with the longest needles (Fig. 3) because the pores created by this

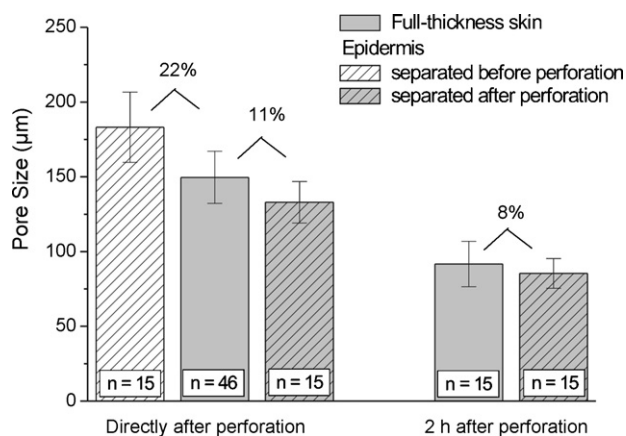


Fig. 3 – Pore sizes in dependence on the skin preparation. Human full-thickness skin and separated epidermis was treated with the 1500 μm microneedles. The epidermis of the perforated full-thickness skin was then separated directly and 2 h after Dermalroller[®] treatment. The differences (%) between the samples are given in the graphic and n refers to the number of pores measured.

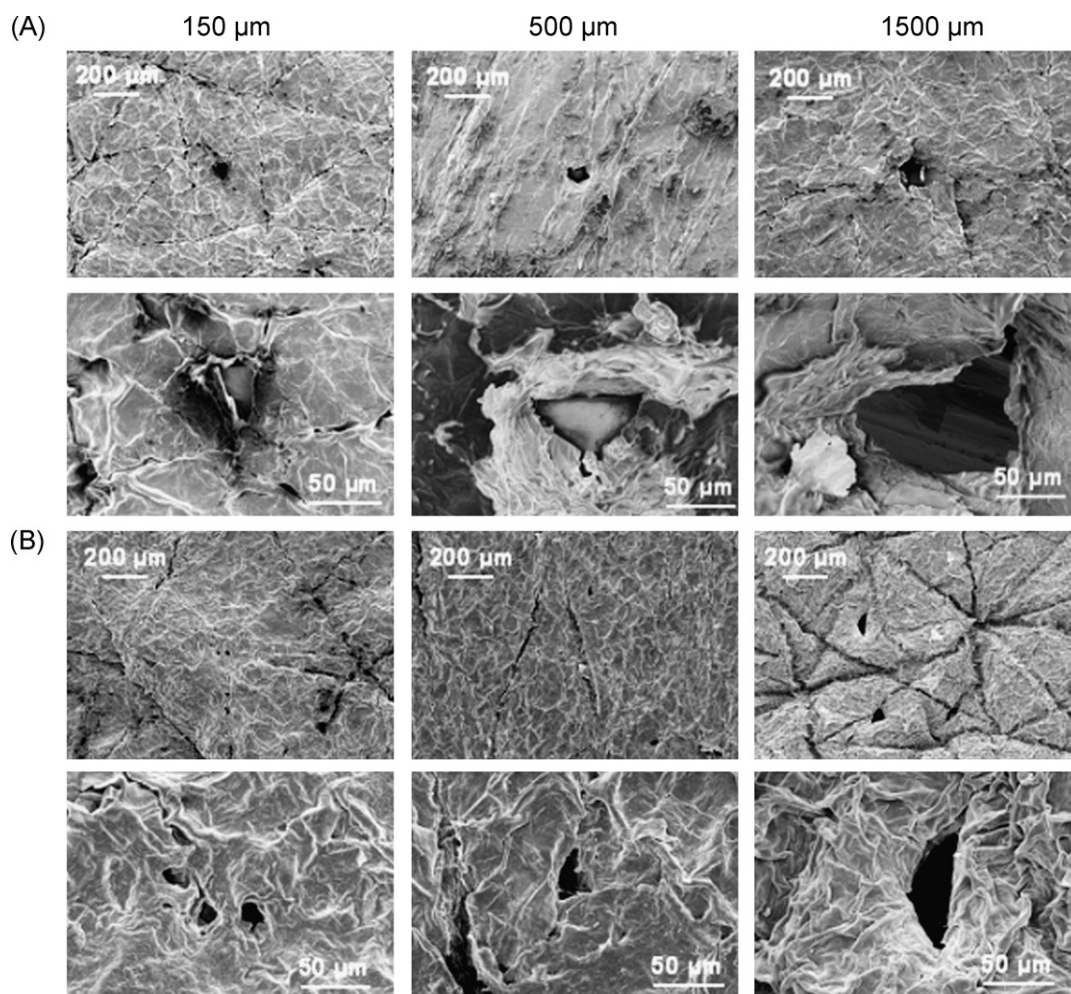


Fig. 4 – Scanning electron microscopic images of epidermal membranes treated with Dermalrollers[®] directly (A) or 2 h (B) after Dermalroller[®] treatment.

type of Dermaroller were best visible in the light microscope. The perforation of separated epidermis resulted in a larger pore size ($183 \pm 23 \mu\text{m}$, $n = 15$) compared to those obtained after perforation of the full-thickness skin ($150 \pm 17 \mu\text{m}$, $n = 46$). In the case of heat separation of the epidermis after perforation, a slightly smaller pore size was determined ($133 \pm 14 \mu\text{m}$, $n = 15$). With time, the size of the pores decreased; the pore sizes were $92 \pm 15 \mu\text{m}$ ($n = 15$) for the full-thickness skin and $85 \pm 10 \mu\text{m}$ ($n = 15$) when the epidermis was separated 2 h after perforation. Whereas the decrease in the pore size due to the separation of the epidermis was less pronounced and within the expected variations, the perforation of the epidermis leads to a larger pore size compared to perforation of the full-thickness skin.

3.3. Scanning electron microscopy (SEM)

By SEM, the morphology of the pores created by the different types of Dermaroller® could be visualized in more detail (Fig. 4). Generally, the pores often had a more or less triangular shape that has been also described in the literature (Verbaan et al., 2007; Kolli and Banga, 2008; Pearton et al., 2008). The pore size after perforation of the epidermis was about 75, 125 and $180 \mu\text{m}$ for the 150, 500 and $1500 \mu\text{m}$ microneedles, respectively (Fig. 4A). 2 h after Dermaroller® treatment, the size of the pores was distinctly decreased. The pore sizes were about 15, 30 and $75 \mu\text{m}$ for the 150, 500 and $1500 \mu\text{m}$ microneedles, respectively (Fig. 4B).

Due to the usage of the separated epidermis for perforation and determination of the pore size directly after Dermaroller® treatment, an overestimation for about 20% needs to be considered. However, even so, the pore size decreased apparently 2 h after perforation.

3.4. Transepidermal water loss (TEWL)

Measurements of the transepidermal water loss are frequently used to evaluate the integrity of the skin barrier (Roskos and Guy, 1989). Healthy skin presents an efficient barrier against water diffusion. To investigate the effect of various physical and chemical parameters on the skin barrier function, the water loss through the skin can be measured. Damage of the skin barrier normally results in an increased water loss. TEWL measurements may provide a rapid and non-invasive evaluation of the skin barrier function before penetration experiments with Franz chambers (Benech-Kieffer et al., 1997; OECD No. 28, 2004). However, there is also evidence from the literature that small damages may already lead to increased drug permeation but may not be detectable by TEWL measurements (Netzlaff et al., 2006).

To evaluate the reduction of the skin barrier by Dermaroller® treatment, the TEWL of human skin was measured before and after treatment and followed up to 2 h after treatment (Fig. 5). The TEWL of untreated skin was $5.8 \pm 0.6 \text{ g/m}^2 \text{ h}$, which agrees well with values reported in the literature (Pinnagoda et al., 1990).

The treatment with the different types of Dermarollers® resulted in an increased TEWL. The TEWL values measured directly after treatment were 11.4 ± 1.2 , 27.7 ± 1.3 and $50.6 \pm 3.7 \text{ g/m}^2 \text{ h}$ for the 150, 500 and $1500 \mu\text{m}$ micronee-

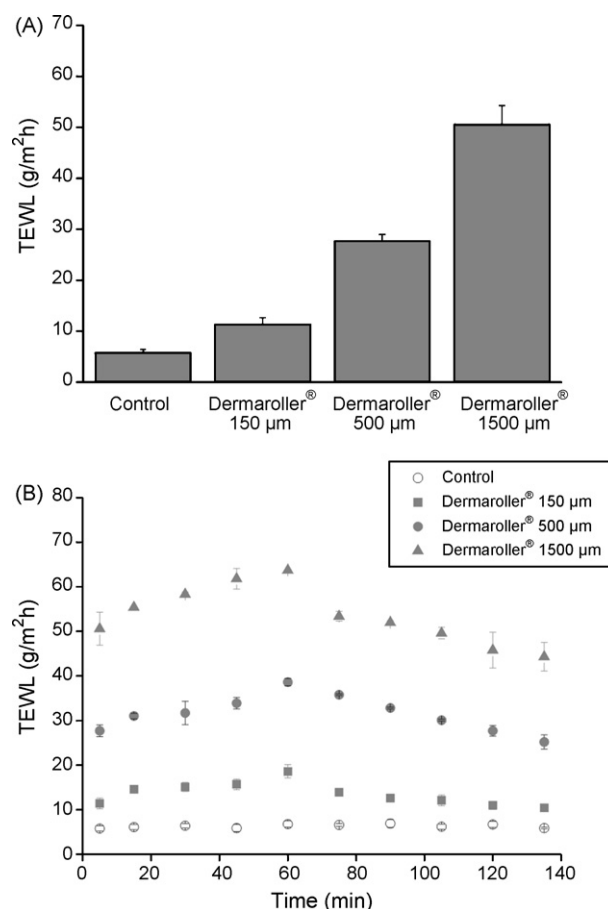


Fig. 5 – TEWL of full-thickness human skin with and without perforation with the Dermarollers®. (A) TEWL measured directly after Dermaroller® treatment and (B) TEWL in dependence on time after treatment ($n = 3$).

dles, respectively. There was a positive correlation between the TEWL and the microneedle lengths, with the $150 \mu\text{m}$ microneedles treatment resulting in the lowest and the $1500 \mu\text{m}$ microneedle treatment providing the highest TEWL value (Fig. 5A).

The TEWL was observed over 2 h after treatment with the Dermarollers®. Initially, the TEWL increased gradually reaching the maximal TEWL after about 1 h and a decrease was observed further on with TEWL values measured after 2 h even slightly lower than those measured directly after perforation (Fig. 5B).

3.5. In vitro penetration studies

In order to investigate the ability of Dermarollers® to enhance drug deposition in the skin, the penetration of radiolabeled mannitol loaded-invasomes and the respective PBS solution was studied and compared with untreated skin (no Dermaroller® treatment, Table 1, Fig. 6). Radiolabeled mannitol was used as hydrophilic label because a poor penetration into the skin by passive diffusion through the SC of such hydrophilic compound can be expected.

Table 1 – Amounts of radiolabeled mannitol (expressed as cumulative % of dose applied, $n=3$) in the different layers of human skin and the receptor fluid in dependence on the formulation (buffer and invasomes) and on the Dermaroller® treatment after 6 h of non-occlusive incubation. The controls present the incubation with the respective formulations without Dermaroller® treatment.

Treatment	Amount of dose applied (%)					Recovery (%)
	SC	Dermis	Receptor	Skin surface	Dermaroller®	
<i>Buffer solution</i>						
Control	0.24 ± 0.04	0.03 ± 0.01	Not detectable	107.30 ± 7.60	Not applicable	107.57 ± 7.64
Dermaroller 150 µm	0.55 ± 0.23	0.29 ± 0.14	3.79 ± 1.81	22.77 ± 1.65	64.02 ± 6.96	91.42 ± 3.73
Dermaroller 500 µm	0.31 ± 0.19	0.30 ± 0.05	4.12 ± 1.31	20.55 ± 4.03	65.45 ± 9.68	90.74 ± 5.84
Dermaroller 1500 µm	0.18 ± 0.13	0.14 ± 0.13	5.95 ± 0.34	18.63 ± 2.39	68.48 ± 6.62	93.38 ± 5.88
<i>Invasomes</i>						
Control	0.91 ± 0.23	0.17 ± 0.04	0.37 ± 0.04	98.02 ± 20.17	Not applicable	99.46 ± 20.35
Dermaroller 150 µm	1.31 ± 0.36	0.52 ± 0.03	5.03 ± 0.59	26.07 ± 2.17	72.09 ± 7.04	105.02 ± 19.95
Dermaroller 500 µm	0.82 ± 0.22	1.48 ± 0.37	11.20 ± 5.48	19.58 ± 9.03	63.67 ± 11.90	96.75 ± 13.75
Dermaroller 1500 µm	0.63 ± 0.25	1.11 ± 0.27	18.20 ± 1.69	16.25 ± 3.22	59.81 ± 2.38	95.99 ± 2.19

After incubation of the skin with the drug solution, only small amounts of mannitol were found in the SC and the stripped skin, and the drug was not detectable in the receptor fluid. When the skin was perforated with the Dermarollers®,

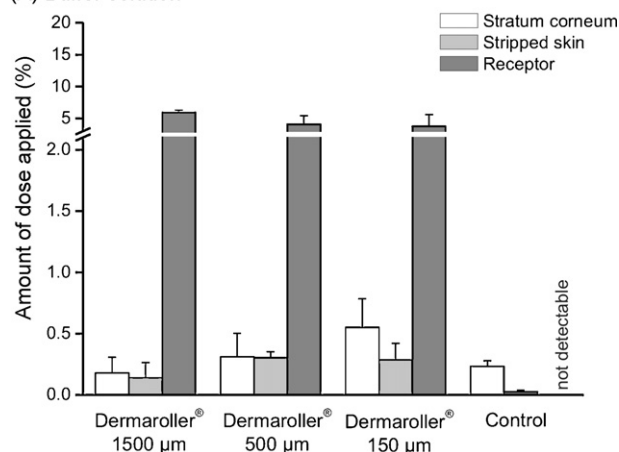
particularly the amount of mannitol pushed through the skin into the receptor fluid was increased with increasing needle lengths (Fig. 6A). However, the drug amount delivered into or through the skin was relatively low in all cases (between about 3 and 6% of the applied dose).

The application of invasomes revealed a distinctly enhanced delivery of radiolabeled mannitol into and through the skin compared to the PBS solution even without Dermaroller® treatment. The amount of radiolabeled mannitol in the SC and in the stripped skin was 3.8 and 5.7-fold higher for the invasomes compared to the buffer solution (Fig. 6A and B). Furthermore, a small amount of mannitol was even found in the receptor fluid (0.4%). This indicates that the vesicles play an important role in the delivery of mannitol into the deeper skin.

The application of Dermarollers® resulted in a further increase of the amount of the drug in the skin as well as in the receptor fluid compared to untreated skin when the invasomal formulation was applied (Fig. 6B). The combination of invasomes with the Dermaroller® with 150 µm needles showed the highest drug amount in the SC (1.4-fold) and an increased drug amount in the stripped skin (3.1-fold) as well as in the receptor fluid (13.6-fold) compared to invasomes without Dermaroller® treatment. With increasing needle length, mannitol deposition into deeper skin layers (stripped skin) and the receptor fluid was pronounced but mannitol amounts in the SC were reduced (Fig. 6B). Compared to the application of invasomes without skin perforation, the amounts of mannitol were 8.7- and 6.5-fold higher in the stripped skin and 30.3- and 49.2-fold higher in the receptor fluid (500 and 1500 µm needle lengths, respectively).

The permeation profiles are shown in Fig. 7. Interestingly, different profiles were observed after skin perforation with the Dermarollers® between the two different formulations (invasomes or buffer solution). Whereas for the invasomal formulation the drug amount in the receptor fluid continuously increased (Fig. 7A), a flattening of the permeation curve was already reached after about 2 h when mannitol was applied in PBS solution and combined with Dermaroller® treatment (Fig. 7B). As stated above, mannitol was not detectable in the receptor fluid when the skin was incubated with the buffer solution and without perforation.

(A) Buffer solution



(B) Invasomes

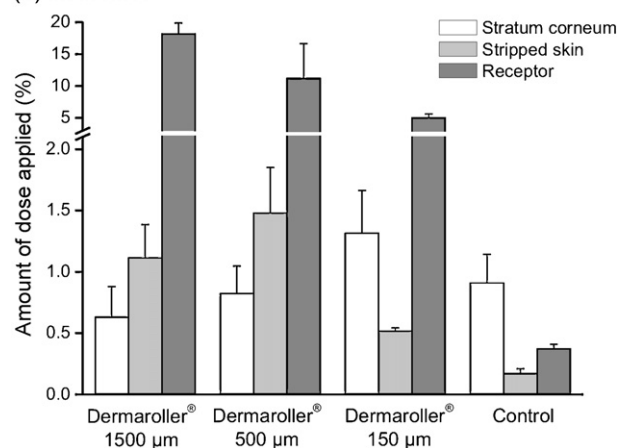


Fig. 6 – Amount of radiolabeled mannitol delivered into the SC, stripped skin and the receptor after 6 h of incubation in dependence on the formulation ((A) buffer solution; (B) invasomes) and Dermaroller® treatment ($n=3$). The controls present the skin samples without Dermaroller® treatment.

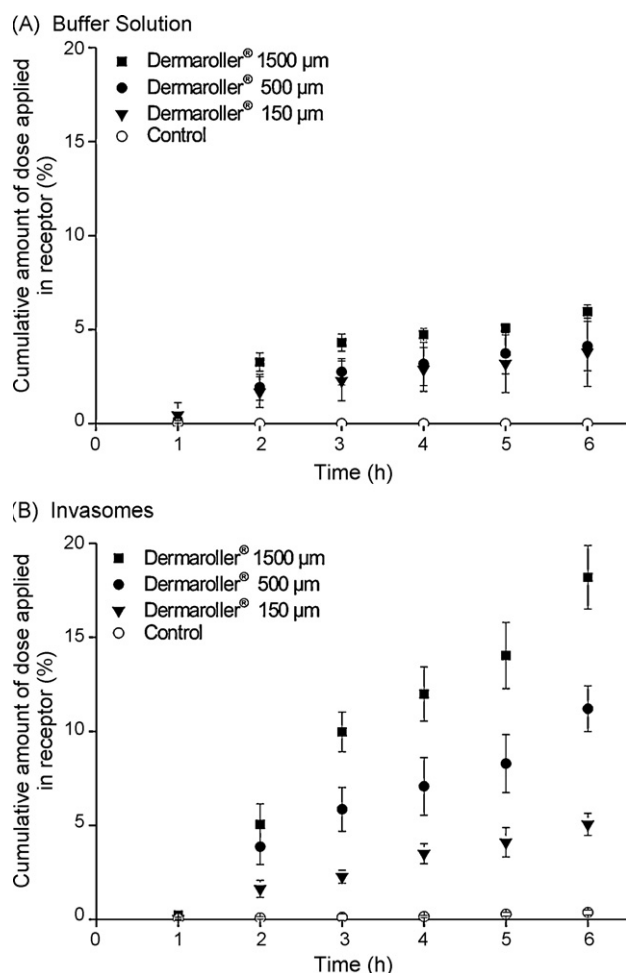


Fig. 7 – Cumulative amount of radiolabeled mannitol in the receptor compartment on the formulation ((A) buffer solution; (B) invasomes) and Dermaroller® treatment ($n = 3$). The controls present the skin samples without Dermaroller® treatment.

3.6. Fluorescence microscopic studies

Fluorescence microscopy was used to visualize the penetration depth of the fluorescent dye CF after an incubation time of 6 h. In addition to the invasomes and the buffer solution, the non-extruded invasomal formulation (MLV) was used to gain more information whether the size or the composition (e.g. ethanol and terpenes) are the main determinants for the drug penetration enhancing effect of invasomes.

In Fig. 8, representative fluorescence microscopic images of cross-sections of skin samples incubated with the invasomes (Fig. 8A), MLV (Fig. 8B) and with CF in buffer solution (Fig. 8C) with and without Dermaroller® treatment are shown. In agreement with the results of the penetration study with radiolabeled mannitol, the fluorescence microscopic images revealed that application of invasomes resulted in a deeper skin penetration of CF particularly in combination with the Dermaroller® treatment. Fluorescence intensity was distinctly increased for the perforated skin samples with the highest intensity for the skin sample treated with the 500 µm

microneedles. In the case of the Dermarollers® with microneedle lengths of 150 and 1500 µm, the fluorescence in the viable epidermis and dermis was weaker.

The application of MLV vesicles without Dermaroller® treatment resulted in a weaker (compared to invasomes) but slightly higher (compared to the buffer solution) fluorescence in the SC and no fluorescence was noticed in the viable epidermis. MLV were, therefore, not able to effectively deliver the fluorescence dye through the SC. However, in combination with the Dermarollers®, a very weak fluorescence in both the viable epidermis and dermis was observed (Fig. 8B).

When the skin was incubated with the CF solution without Dermaroller® treatment, only a very weak fluorescence was visible in the SC and no fluorescence could be detected in the viable epidermis and dermis (Fig. 8C). Skin perforation with the Dermarollers® resulted in a penetration of CF into the SC as well as deeper layers of the skin but fluorescence intensities were much lower when compared to the respective images of the skin samples incubated with invasomes. As for the invasomes, fluorescence intensity was highest when the skin was treated with the Dermaroller® with needle lengths of 500 µm.

4. Discussion

In the present study, we used commercially available microneedle devices (Dermarollers®) to overcome the SC barrier properties. Furthermore, the efficiency of microneedle treatment followed by the application of invasomes was investigated and compared with an aqueous solution of the drug with respect to a further enhancement of drug delivery into and through the skin.

The ability of the Dermarollers® to create pores through the SC could be demonstrated by the detection of pores in light and scanning electron microscopy as well as the elevated TEWL measured after skin perforation. The size range of the created pores (between about 50 and 200 µm for the Dermarollers used in this study) is in reasonable agreement with the literature data where pore sizes between a few µm up to 300 µm (Henry et al., 1998; Chabri et al., 2004; Wang et al., 2006) is described. However, as the microneedle geometry as well as the microneedle assembly (e.g. number of needles per area) has likely a great influence on the penetration depth of the microneedles as well as the size of the pores, a direct comparison of pore sizes created by different microneedle devices appears to be critical. Furthermore, it has been described that even no perforation of the SC could be achieved after manual application of shorter microneedles in a flat microneedle array (Verbaan et al., 2007). In this regard, velocity of microneedle insertion plays an important role and skin piercing was successful using microneedles with the similar lengths when the microneedle array was inserted at a defined high speed (Verbaan et al., 2008). The cylindrical assembly of the microneedle array used in this study allows skin perforation by just rolling the device over the skin surface and exerting there also a tension on the skin between adjacent needles facilitating the puncture of the needles by their respective forefront needle. This seems to be advantageous as lower forces are generally needed compared to an application of flat microneedle assemblies (Wang et al., 2006).

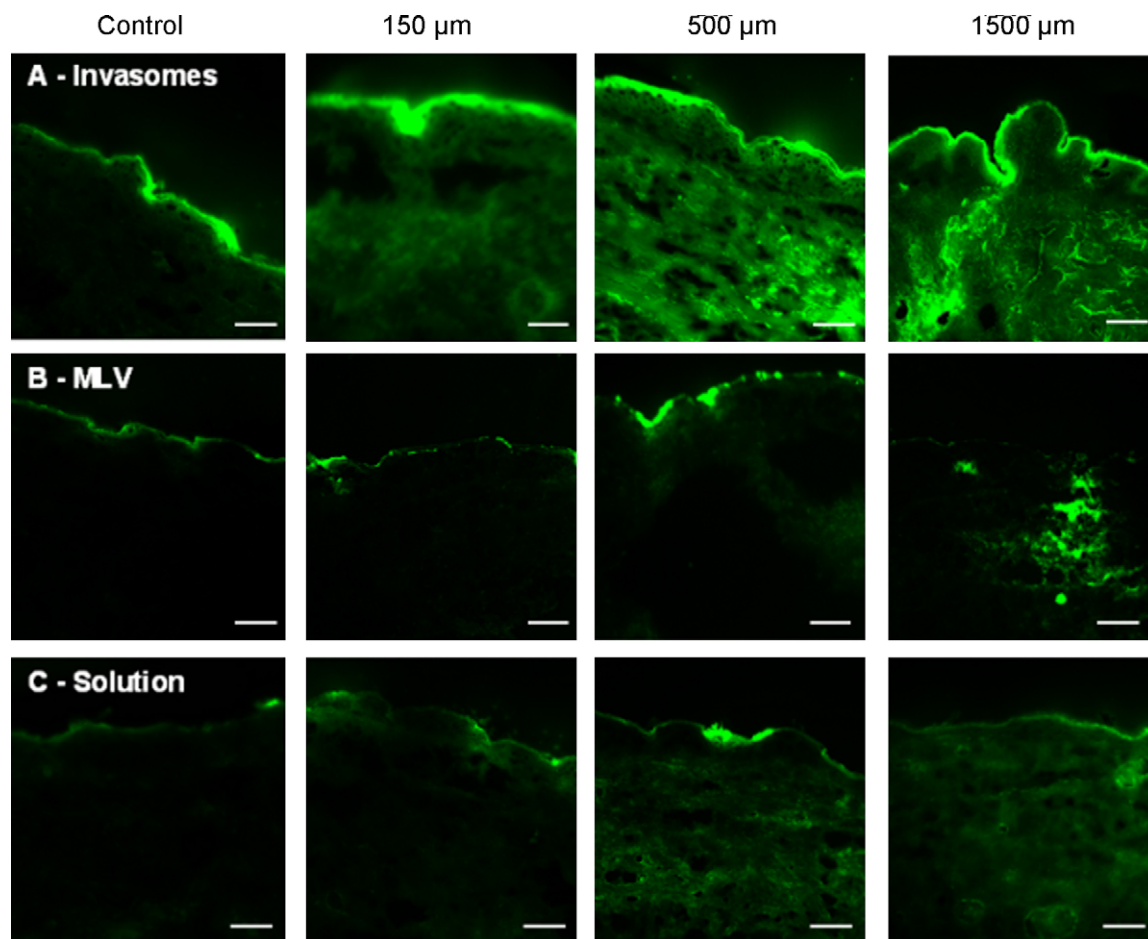


Fig. 8 – Representative fluorescence microscopic images of cross section of human abdominal skin after 6 h of incubation in dependence on the formulations ((A) invasomes, (B) MLV and (C) buffer solution) and Dermaroller® treatment. Scale bars represent 100 μm .

Recently a cylindrical assembly of biocompatible polymeric microneedles using the general geometry of the Dermaroller® was suggested but not yet tested for skin perforation (Han et al., 2007).

With increasing needle length the TEWL increased. A similar trend of the dependence of the TEWL on the needle length was found by Verbaan et al. (Verbaan et al., 2007). A possible explanation for these results may be that the Dermarollers® with longer needles penetrate the stratum corneum and enter the underlying dermal tissue resulting in an increased hydration of the SC (Caspers et al., 2000). Furthermore, the pore size was dependent on the microneedle length with the largest pore size of about 150 μm created by the Dermaroller® with the longest needles (1500 μm) and thus the highest water flux through the skin. There was a gradual increase in the TEWL over the 1st hour while afterwards the TEWL values decreased. The decline of the TEWL is probably caused by the reduction of the pore size with time. A reduced pore size was confirmed by light and SEM investigations 2 h after Dermaroller® treatment. These results are overall in good agreement with literature data although a faster decrease in the pore size might be expected in vivo due to the physiological response on skin perforation as physical stimulus. Bal et al. (2008) studied different

microneedle arrays (e.g. hollow and solid) in vivo in humans with respect to skin barrier function (TEWL), pain and skin irritation. A rapid decline of the TEWL with reaching the initial TEWL already after about 30 and 60 min was observed after skin perforation with solid microneedles with 300 and 400 μm in lengths, respectively. A rapid decrease of the pore size was also found in vivo in rats (Wang et al., 2006). The pores with initial sizes between 100 and 300 μm were not detectable anymore in the light microscope after 10–20 min. However, in a recent clinical study, a microneedle patch was evaluated for transdermal delivery of naltrexone in humans (Wermeling et al., 2008). In addition to the systemic drug delivery, the electrical resistance was measured as a function of time after skin perforation. Under these conditions (occlusion), skin resistance steadily increased but remained significantly less than the resistance of untreated skin for 30 h indicating an at least not complete closure of the pores within this time.

Finally, the influence of skin perforation on the penetration and permeation of hydrophilic model drugs was investigated with two different formulations: an aqueous solution and an invasomal formulation. Invasomes were introduced by Verma and Fahr (2004) as a new type of highly deformable vesicles for topical drug delivery. The addition of ethanol and terpenes

makes the phospholipid bilayer highly flexible and deformed vesicles could be visualized in a cryo-electron microscopic study (Dragicevic-Curic et al., 2008). Furthermore, ethanol and terpenes may also act as penetration enhancer by disturbing the SC lipid structure (Cornwell et al., 1996; Moghimi et al., 1997; Diez et al., 1998; Bhatia and Singh, 1999) and the penetration enhancing effect of invasomes has been explained by fluidizing the SC lipid structures thus disturbing the organization of the SC lipids (Verma and Fahr, 2004; Dragicevic-Curic et al., 2008). However, the results of the fluorescence microscopic experiments of the present study indicate that the small size of the vesicles presents a prerequisite for the performance of invasomes and thus not primarily the formulation components are responsible for the penetration enhancing effect. For the non-extruded formulation with vesicle sizes in the higher nm- up to the lower μm -range, fluorescence intensity was much lower compared to the invasomes with a size between 100 and 130 nm and only a little brighter compared to the aqueous solution of the fluorescence dye. In contrast, the results of the penetration and fluorescence microscopic study revealed that invasomes were most effective in delivering the hydrophilic model drugs into the SC and deeper skin layers even without skin perforation. A similar penetration enhancing of invasomes has been described for the hydrophobic photosensitizer temoporfin (Dragicevic-Curic et al., 2008). Due to the flexibility of the vesicle bilayers, penetration of invasomes into the SC may be facilitated particularly under non-occlusive conditions although the mechanism of the penetration of deformable vesicles is not yet completely understood (Cevc et al., 1995; Honeywell-Nguyen and Bouwstra, 2006). The presence of terpenes and ethanol may further enhance drug penetration by interaction with the SC lipids. Hydrophilic drug molecules are localized in the aqueous core of the vesicles and in the outer water phase. As it was shown earlier that flexible vesicles also enhanced penetration of drug dissolved in the outer water phase (Verma et al., 2003), in the present study the formulations were used as obtained after extrusion with the drug in the vesicle core and the outer water phase without further purification.

Skin perforation led to a further enhanced drug transport into and through the skin. In a recent study with lipophilic drugs, a similar trend was found when the application of deformable vesicles (containing phospholipids and a bile salt) was combined with a microneedle treatment (Qiu et al., 2008). In addition to the penetration of the invasomes through the SC that remained intact after Dermaroller® treatment and resulted in higher drug amounts in the deeper skin, it was observed in an earlier study that deformable vesicles can also trail along the hair shaft reaching the hair follicle (Verma, 2002). A similar mechanism can be expected for the pronounced penetration of the vesicles through the pores created by the Dermarollers®. An additional reason for the improved penetration could be that the invasomes, once having reached the epidermis, may fuse or aggregate with epidermal cells and are therefore removed from the equilibrium in the aqueous channels. This causes an increased invasome flow towards the epidermis from the reservoir on the skin surface. Drug deposition in the different skin layers was dependent on the needle lengths of the Dermarollers®. Skin perforation with the shortest needles led to the highest drug deposi-

tion in the SC. In contrast, when the skin was perforated with the longer microneedles, deposition of the hydrophilic model drugs in the SC was similar (500 μm needles) or even lower (1500 μm needles) as obtained after incubation of the skin with invasomes but without skin perforation. However, the Dermaroller® treatment led to higher drug amounts in the deeper skin as well as the receptor fluid for all needle lengths. As the dermis does not present a distinct barrier for hydrophilic drugs, a fast diffusion through the dermis into the receptor fluid can be assumed once the drug has passed the SC. In both the penetration and fluorescence microscopy study, drug deposition in the deeper skin layers was highest when the skin was perforated with the Dermaroller® with a needle lengths of 500 μm and this model appears therefore most promising for transdermal drug delivery purposes. The Dermaroller® with the longest needles (1500 μm) may be more critical because the depth of perforation may reach deeper regions of the underlying dermis with risk of pain and damage of small blood capillaries. The Dermaroller® with the shortest needle lengths seems to be predominant for drug delivery mainly in the SC but drug diffusion through the dermis resulting in systemic absorption needs to be considered as well.

5. Conclusion

In the present study, the ability of Dermarollers® differing in their needle lengths was investigated. The disruption of the SC barrier could be demonstrated by the presence of pores and an increased TEWL. Consequently, penetration and permeation of hydrophilic model drugs were enhanced after skin perforation with the Dermarollers® particularly when combined with the invasome formulation. The Dermaroller® with a needle length of 500 μm appears most promising for drug delivery into deeper layers or through the skin. In contrast, the treatment of the skin with the Dermaroller® with needle lengths of 150 μm led to a pronounced deposition of the model drugs in the SC. As the Dermaroller® is a commercial available device and provides an easy handling, it appears promising also for drug delivery purposes additionally to its use in cosmetics.

Acknowledgements

The authors would like to thank the Egyptian Embassy for funding the scientific studies of M. Badran at the FSU Jena; H. Liebl Co. (Friesenheim, France) for providing the Dermarollers®; Dr. L. Gruhl (Praxis für Plastische Chirurgie, Kassel) for providing human abdominal skin; Dr. J. Bossert and H. Garlipp (Institute of Material Sciences and Technology, FSU Jena) for their help in the light microscopic and scanning electron microscopic studies.

REFERENCES

- Alberti, I., Kalia, Y.N., Naik, A., Bonny, J.D., Guy, R.H., 2001. Effect of ethanol and isopropyl myristate on the availability of topical terbinafine in human stratum corneum, in vivo. *Int. J. Pharm.* 219, 11–19.

- 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.
- Barry, B.W., 1987. Mode of action of penetration enhancers in human skin. *J. Control. Rel.* 6, 85–97.
- Batzri, S., Korn, E.D., 1973. Single bilayer liposomes prepared without sonication. *BBA* 298, 1015–1019.
- Benech-Kieffer, F., Wegrich, P., Schaefer, H., 1997. Transepidermal water loss as an integrity test for skin barrier function in vitro: assay standardization. In: Brain, K.R., James, V.J., Walters, K.A. (Eds.), *Perspectives in Percutaneous Penetration*. STS, Cardiff, p. 56.
- Bhatia, K.S., Singh, J., 1999. Effect of linolenic acid/ethanol or limonene/ethanol and iontophoresis on the in vitro percutaneous absorption of LHRH and ultrastructure of human epidermis. *Int. J. Pharm.* 180, 235–250.
- Bronaugh, R.L., Stewart, R.F., Simon, M., 1986. Methods for in vitro percutaneous absorption studies. VII. Use of excised human skin. *J. Pharm. Sci.* 75, 1094–1097.
- Caspers, P.J., Lucassen, G.W., Bruining, H.A., Puppels, G.J., 2000. Automated depth-scanning confocal Raman microspectrometer for rapid in vivo determination of water concentration profiles in human skin. *J. Raman Spectrosc.* 31, 813–818.
- Cevc, G., Schätzlein, A., Blume, G., 1995. Transdermal drug carriers: basic properties, optimization and transfer efficiency in the case of epicutaneously applied peptides. *J. Control. Rel.* 36, 3–16.
- Chabri, F., Bouris, K., Jones, T., Barrow, D., Hann, A., Allender, C., Brain, K., Birchall, J., 2004. Microfabricated silicon microneedles for nonviral cutaneous gene delivery. *Br. J. Dermatol.* 150, 869–877.
- Cormier, M., Johnson, B., Ameri, M., Nyam, K., Libiran, L., Zhang, D.D., Daddona, P., 2004. Transdermal delivery of desmopressin using a coated microneedle array patch system. *J. Control. Rel.* 97, 503–511.
- Cornwell, P.A., Barry, B.W., Bouwstra, J.A., Gooris, G.S., 1996. Modes of action of terpene penetration enhancer in human skin; differential scanning calorimetry, small-angle X-ray diffraction and enhancer uptake studies. *Int. J. Pharm.* 127, 9–26.
- Denet, A.R., Vanbever, R., Preat, V., 2004. Skin electroporation for transdermal and topical delivery. *Adv. Drug Deliv. Rev.* 56, 659–674.
- Diez, I., Peraire, C., Obach, R., Domenech, J., 1998. Influence of D-limonene on the transdermal penetration of felodipine. *Eur. J. Drug Metabol. Pharmacokinet.* 23, 7–12.
- Disegi, J.A., Eschbach, L., 2000. Stainless steel in bone surgery. *Injury* 31, S-D2–S-D6.
- Dragicevic-Curic, N., Scheglmann, D., Albrecht, V., Fahr, A., 2008. Temoporfin-loaded invasomes: development, characterization and in vitro skin penetration studies. *J. Control. Rel.* 127, 59–69.
- Gardeniers, H.J.G.E., Luttge, R., Berenschot, E.J.W., Boer, M.J., Yeshurun, S.Y., Hefetz, M., van't Oever, R., van den Berg, A., 2003. Silicon micromachined hollow microneedles for transdermal liquid transport. *J. Microelectromech. Syst.* 12, 855–862.
- Gill, H.S., Prausnitz, M.R., 2007. Coated microneedles for transdermal delivery. *J. Control. Rel.* 117, 227–237.
- Han, M., Hyun, D.H., Park, H.H., Lee, S.S., Kim, C.H., Kim, C.G., 2007. A novel fabrication process for out-of-plane microneedle sheets of biocompatible polymer. *J. Micromech. Microeng.* 17, 1184–1191.
- Harrison, S.M., Barry, B.W., Dugard, P.H., 1984. Effects of freezing on human skin permeability. *J. Pharm. Pharmacol.* 36, 261–262.
- 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.
- Honeywell-Nguyen, P.L., Bouwstra, J.A., 2006. Vesicles as a tool for dermal and transdermal delivery. In: Benita, S. (Ed.), *Microencapsulation*. Marcel Dekker, New York, pp. 563–585.
- Kalia, Y.N., Naik, A., Garrison, J., Guy, R.H., 2004. Iontophoretic drug delivery. *Adv. Drug Deliv. Rev.* 56, 619–658.
- Kaushik, S., Hord, A.H., Denson, D.D., McAllister, D.V., Smitra, S., Allen, M.G., Prausnitz, M.R., 2001. Lack of pain associated with microfabricated microneedles. *Anesth. Analg.* 92, 502–504.
- Kim, K., Lee, J.B., 2007. High aspect ratio tapered hollow metallic microneedle arrays with microfluidic interconnector. *Microsyst. Technol.* 13, 231–235.
- Kligman, A.M., Christophers, E., 1963. Preparation of isolated sheets of human stratum corneum. *Arch. Dermatol.* 88, 702–705.
- Kolli, C.S., Banga, A.K., 2008. Characterization of solid maltose microneedles and their use for transdermal delivery. *Pharm. Res.* 25, 104–113.
- MacDonald, R.I., Menco, B.P., Takeshita, K., Subbarao, N.K., Hu, L.R., 1991. Small-volume extrusion apparatus for preparation of large, unilamellar vesicles. *BBA* 1061, 297–303.
- Martanto, W., Moore, J.S., Couse, T., Prausnitz, M.R., 2006. Mechanism of fluid infusion during microneedle insertion and retraction. *J. Control. Rel.* 112, 357–361.
- McAllister, D.V., Wang, P.M., Davis, S.P., Park, J.H., Canatella, P.J., Allen, M.G., Prausnitz, M.R., 2003. Microfabricated needles for transdermal delivery of macromolecules and nanoparticles: fabrication methods and transport studies. *PNAS* 100, 13755–13760.
- Merino, G., Kalia, Y.N., Guy, R.H., 2003. Ultrasound-enhanced transdermal transport. *J. Pharm. Sci.* 92, 1125–1137.
- Mitragotri, S., Kost, J., 2004. Low-frequency sonophoresis. A review. *Adv. Drug Deliv. Rev.* 56, 589–601.
- Moghim, H.R., Williams, A.C., Bary, B.W., 1997. A lamellar matrix model for stratum corneum intercellular lipids. V. Effect of terpene penetration enhancer on the structure and thermal behavior of the matrix. *Int. J. Pharm.* 146, 41–54.
- Netzlaff, F., Kostka, K.H., Lehr, C.M., Schaefer, U.F., 2006. TEWL measurements as a routine method for evaluating the integrity of epidermis sheets in static Franz diffusion cells in vitro. Limitations shown by transport data testing. *Eur. J. Pharm. Biopharm.* 63, 44–50.
- Nuutinen, J., Alanen, E., Autio, P., Lahtinen, M.R., Harvima, I., Lahtinen, T., 2003. A closed unventilated chamber for the measurement of transepidermal water loss. *Skin Res. Technol.* 9, 85–89.
- OECD, 2004. Guidance Document for the Conduct of Skin Absorption Studies, Number 28, OECD Series on Testing and Assessment.
- Park, J.H., Allen, M.G., Prausnitz, M.R., 2005. Biodegradable polymer microneedles: fabrication, mechanics and transdermal drug delivery. *J. Control. Rel.* 104, 51–66.
- Park, J.H., Yoon, Y.K., Choi, S.O., Prausnitz, M.R., Allen, M.G., 2007. Tapered conical polymer microneedles fabricated using an integrated lens technique for transdermal drug delivery. *Transact. Biomed. Eng.* 54, 903–913.
- Pearson, M., Allender, C., Brain, K., Anstey, A., Gateley, C., Wilke, N., Morrissey, A., Birchall, J., 2008. Gene delivery to the epidermal cells of human skin explants using microfabricated microneedles and hydrogel formulations. *Pharm. Res.* 25, 407–416.
- Pinnagoda, J., Tupker, R.A., Agner, T., Serup, J., 1990. Guidelines for transepidermal water loss (TEWL) measurement. A report from the standardization group of the European society of Contact Dermatitis. *Contact Dermat.* 22, 164–178.
- Prausnitz, M.R., 2004. Microneedles for transdermal drug delivery. *Adv. Drug Deliv. Rev.* 56, 581–587.
- Purdon, C.H., Azzi, C.G., Zhang, J., Smith, E.W., Maibach, H.I., 2004. Penetration enhancement of transdermal delivery—current

- permutations and limitations. *Crit. Rev. Ther. Drug Carrier Syst.* 21, 97–132.
- Qiu, Y., Gao, Y., Hu, K., Li, F., 2008. Enhancement of skin permeation of docetaxel: a novel approach combining microneedle and elastic liposomes. *J. Control. Rel.* 129, 144–150.
- Roskos, K.V., Guy, R.H., 1989. Assessment of skin barrier function using transepidermal water loss: effect of age. *Pharm. Res.* 6, 949–953.
- Schaefer, H., Redelmeier, T.E., 1996. *Skin Barrier: Principles of Percutaneous Absorption*. Karger, Basel, Switzerland, pp. 214–218 (Chapter 6).
- Sen, A., Daly, M.E., Hui, S.W., 2002. Transdermal insulin delivery using lipid enhanced electroporation. *BBA* 1564, 5–8.
- Verbaan, F.J., Bal, S.M., van den Berg, D.J., Groenink, W.H.H., Verpoorten, H., Lüttge, R., Bouwstra, J.A., 2007. Assembled microneedle arrays enhance the transport of compounds varying over a large range of molecular weight across human dermatomed skin. *J. Control. Rel.* 117, 238–245.
- Verbaan, F.J., Bal, S.M., van den Berg, D.J., Dijkman, 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. Rel.* 128, 80–88.
- Verma, D.D., 2002. *Invasomes—novel topical carriers for enhanced topical delivery: characterization and skin penetration properties*, Ph.D. Thesis, Marburg/Lahn.
- Verma, D.D., Verma, S., Blume, G., Fahr, A., 2003. Liposomes increase skin penetration of entrapped and non-entrapped hydrophilic substances into human skin: a skin penetration and confocal laser scanning microscopy study. *Eur. J. Pharm. Biopharm.* 55, 271–277.
- Verma, D.D., Fahr, A., 2004. Treatment of alopecia areata in the BEBR model using cyclosporin A lipid vesicles. *Eur. J. Dermatol.* 14, 332–338.
- Wang, P.M., Cornwell, M., Hill, J., Prausnitz, M.R., 2006. Precise microinjection into skin using hollow microneedles. *J. Invest. Dermatol.* 126, 1080–1087.
- 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. *PNAS*, 2058–2063.
- Wilke, N., Morrissey, A., 2007. Silicon microneedle formation using modified mask designs based on convex corner undercut. *J. Micromech. Microeng.* 17, 238–244.
- Williams, A.C., Barry, B.W., 2004. Penetration enhancers. *Adv. Drug Deliv. Rev.* 56, 603–618.
- Xu, R., 1997. Improvements in particle size analysis using light scattering. In: Muller, R.H., Mehnert, W. (Eds.), *Particle and Surface Characterizing Methods*. Scientific Publisher, Stuttgart, Germany, pp. 27–56.
- Yoon, J., Son, T., Choi, B., Nelson, J.S., Jung, B., 2008. Enhancement of optical skin clearing efficacy using a microneedle roller. *J. Biomed. Opt.* 13, 021103-1-5.