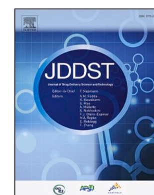
Journal of Drug Delivery Science and Technology 69 (2022) 103161

Contents lists available at ScienceDirect

Journal of Drug Delivery Science and Technology

journal homepage: www.elsevier.com/locate/jddst



Opinion paper

The effect of non-invasive dermal electroporation on skin barrier function and skin permeation in combination with different dermal formulations

Nikolett Kis [a], Anita Kovács [a], Mária Budai-Szűcs [a], Gábor Erős [b], Erzsébet Csányi [a], Szilvia Berkó [a,*]

[a] Institute of Pharmaceutical Technology and Regulatory Affairs, Faculty of Pharmacy, University of Szeged, Szeged, Hungary
[b] Department of Dermatology and Allergology, University of Szeged, Szeged, Hungary

ARTICLE INFO

Keywords:
Electroporation
FITC-dextran
Film-forming system
Skin permeation
Fluorescence microscopy

ABSTRACT

Purpose: The aim of this research was to investigate the effect of non-invasive dermal electroporation (EP) on the barrier function of the skin and on the permeation of a model macromolecule in combination with different dermal formulations.
Methods: Skin samples were treated with non-invasive dermal EP treatment for 2 min. Firstly, the effect of EP on the barrier function of the skin was examined on mouse skin *in vivo* by measuring transepidermal water loss (TEWL). Then, the effect of EP on human skin permeation was investigated *ex vivo* under a fluorescence microscope in combination with different dermal formulations. The human skin was treated with a solution, a hydrogel, and a film-forming system (FFS) containing 4 kDa fluorescein isothiocyanate-dextran (FITC-dextran) with or without EP. The increased fluorescence intensity shows the presence of FITC-dextran in the skin layers.
Results: The results showed, that the TEWL values increased rapidly after the treatment, and it took approximately 5 min to be restored. The results of permeation experiments showed that just slight permeation of FITC-dextran could be noticed from any formulation without EP; however, the permeation from the solution and the FFS increased highly in combination with EP.
Conclusion: The EP decreased the barrier function of the skin reversibly and the structure of SC was restored in a short time after the treatment. FITC-dextran, as a macromolecule, can just slightly permeate into the skin with passive diffusion. EP could increase the permeation rate of FITC-dextran remarkably compared to the control treatments; however, the composition of the formulations has a great influence on the permeation.

1. Introduction

The skin protects our body from the external environment and foreign materials and sets a barrier against the loss of water. The stratum corneum (SC) forms the outermost layer of the skin, which is 15–20 μm thick and provides an effective barrier against drug permeation [1,2]. Thereby, the permeation of drugs through the SC is one of the greatest challenges for pharmaceutical technology. Nevertheless, dermal drug delivery is still an attractive administration route of drugs due to its advantages. It is a non-invasive and painless route, thereby improving patient adherence. Furthermore, the first-pass effect and the gastrointestinal system can be avoided, so it is a good alternative, for example, in the case of gastric irritation [3–5].

In the case of dermal drug delivery, different layers of the skin can be targeted. On the one hand, drugs can be applied in transdermal formulations, which penetrate through the skin and be absorbed into the systematic circulation [6]. On the other hand, drugs can also permeate into different skin layers (epidermis or dermis) and affect locally; for instance, in acne therapy, the upper layers of the skin are exposed, while in local anesthesia, the sensory nerve endings should be reached. The depth of the permeation depends on the applied therapy [7].

The bioavailability of dermal formulations depends on the capability of drugs to transport through the SC [8]. From a pharmaceutical technology perspective, there are three possible dermal routes through which the drug transport can be provided: transcellular, intercellular, and follicular permeation pathways via appendages [9,10]. Physicochemical properties of drugs have a high influence on the type of the pathway; however, most molecules go through the combination of these

* Corresponding author.
E-mail addresses: kis.nikolett@szte.hu (N. Kis), gasparne.kovacs.anita@szte.hu (A. Kovács), budai-szucs.maria@szte.hu (M. Budai-Szűcs), eros.gabor.1979@gmail.com (G. Erős), sooscsanyi@gmail.com (E. Csányi), berko.szilvia@szte.hu (S. Berkó).

https://doi.org/10.1016/j.jddst.2022.103161
Received 19 October 2021; Received in revised form 20 January 2022; Accepted 4 February 2022
Available online 5 February 2022
1773-2247/© 2022 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

administration routes [11]. Partition coefficient, ionization, and the molecular size of chemicals should be considered during the examination of drug permeation. For instance, up to 500 Da, the molecules can cross the SC by passive diffusion; however the smaller molecules have a higher capability to go through the SC [12,13].

Different permeation enhancer techniques have been studied to widen the range of drugs for dermal delivery, and new techniques are currently under development. They can be categorized into passive and active methods based on increasing drug permeation with or without providing the driving force [14]. Passive approaches can increase the skin permeation by increasing the solubility of drugs in the SC, for instance, by increasing skin hydration or using chemical enhancers [15,16]. Furthermore, using different types of formulations, such as vesicles (e.g., nanoparticles, microemulsion), can enhance the drug diffusivity in the SC [17]. Finally, increasing the drug concentration in the formulation, for instance, with a supersaturated system, also leads to improved drug permeation, as in the case of film-forming systems (FFS) [18]. In contrast, active approaches use physical forces to decrease the skin barrier and provide the driving force even for the permeation of hydrophilic substances and macromolecules [12]. The active methods are distinguished based on the applied energy. Electroporation and iontophoresis use electrical energy to increase the permeation [19,20]. Mechanical methods involve microneedles, abrasion, and stretching techniques, while thermal methods such as radiofrequency, laser, and photomechanical waves, use warmth as a driving force [21].

Electroporation (EP) is an active approach, which promotes drug permeation by high voltage and short duration pulses. Due to the electric pulses, the structure of lipid bilayers changes, aqueous pores are formed, which allow macromolecules such as DNA, antibodies, and proteins to transport through them [22–24]. Different electrodes can be used to deliver pulses: needle, plate, needle-free microelectrode array, and multi-electrode array electrodes. The drug delivery can be controlled by amplitude, duration, and the number of pulses [25]. On the one hand, during EP treatment cellular membrane permeability can be accelerated so cellular uptake can be increased. Due to this widely used method, called electrochemotherapy, antitumor agents are delivered intracellularly to treat tumors located in the skin [26–29]. On the other hand, needle-free non-invasive dermal EP has high relevance in medical treatments because it can be used on the skin surface to accelerate both transdermal and topical drug administration [25,30–33]. During the EP treatment, the resistance of SC decreases, the electric field reaches the deeper layer of the skin, and drugs can permeate more effectively [12]. Dermal EP can be applied alone or in combination with other permeation enhancement methods (e.g., with a specialized drug delivery system) because their combined effect could further improve the drug permeation [34]. This method could be an appropriate alternative in painful and chronic medical conditions and could also be beneficial in enhancing patient compliance [35,36]. Furthermore, non-invasive dermal EP is also preferred in the cosmetic industry as a part of mesotherapy [37–39]. This treatment may decrease the unpleasant sign of stretch marks and wrinkles and can help regain skin firmness. The active ingredients are rubbed in with a circular motion by using a special treating handpiece, thereby the clients can feel comfortable due to its relaxing effect [35,36].

EP is frequently applied in combination with other techniques to increase permeation because the combined use with different formulations could be favorable both during clinical or cosmetic treatments [3]. However, further investigations are required for the effective application of non-invasive dermal EP. In this research, we set the goal to investigate the permeation enhancer property of EP with the following aims:

- Investigate the effect of non-invasive dermal EP on mouse skin by measuring transepidermal water loss (TEWL) to examine the change in the barrier function of SC.
- Develop a routine examination method using fluorescence microscopy to investigate the changes in permeation of a model macromolecule caused by EP.
- Investigate the permeation enhancing effect of non-invasive dermal EP in combination with different dermal formulations on a model macromolecule.

2. Material and methods

2.1. Materials

Fluorescein isothiocyanate-dextran (FITC-dextran) at an average molecular weight of 4000 Da was supplied by Sigma Aldrich (Budapest, Hungary). Poly (vinyl alcohol) (87–90% hydrolyzed, average mol wt. 30,000–70,000) was provided by Sigma-Aldrich (Budapest, Hungary). Ethanol (96 per centum, Ph. Eur. 9.) was obtained from Molar Chemicals Ltd. (Budapest, Hungary). Xantural ® 180 Xanthan Gum was from CP Kelco A Huber Company. EMAL® 270D (70% sodium laureth sulfate (SLES)) was from Kao Chemicals Europe S.L. (Barcelona, Spain). Purified and deionized water was used (Milli-Q system, Millipore, Milford, MA, USA).

2.2. Protocol of EP treatment

Non-invasive EP treatment (Fig. 1) was applied *in vivo* on the dorsal surface of mouse skin and *ex vivo* on full-thickness human abdominal skin with a Mezoforte Duo Mez 120905-D instrument (Dr Derm Equipment Ltd., Budapest, Hungary). The device is equipped with a polypropylene-covered treating handpiece, which has a plate electrode (25 mm in diameter) contacting indirectly to the treated surface. Modulation involves 900 V with a duration of 5 ms, followed by a 20 ms break. The EP treatment lasts for 2 min (2400 periods/min) [33,40,41].

2.3. Examination of the effect of EP on skin barrier function in vivo

In order to determine the *in vivo* effect of EP on the skin barrier function, 3-4-month-old male SKH-1 hairless mice were used for the examination. EP was applied on the dorsal skin surface of hairless mice. The animals were anesthetized with a mixture of ketamine (90 mg/kg bodyweight) and xylazine (25 mg/kg bodyweight) before the treatment intraperitoneally. After the experiments, an overdose of ketamine (300 mg/kg) was added to euthanize them. A thermoneutral environment with a 12-h light-dark cycle was ensured for the mice. They were kept in plastic cages, and they had access to standard laboratory chow and water. All experiments were in full accordance with the NIH guidelines, and the interventions were approved by the Ethical Committee for the Protection of Animals in Scientific Research at the University of Szeged (license number: V./145/2013) [42]. Transepidermal water loss (TEWL) was measured by using a Tewameter TM 300 attached to a Multi Probe Adapter MPA 5 (Courage und Khazaka, 6 Germany). The measurement was started immediately after the EP treatment, and it was carried out for 7 min continuously. The device measured TEWL every second. Three parallel measurements were performed on three different mice. The results are shown in percentage compared to the TEWL value of the untreated mouse skin ±standard deviation. The following correlation was used at each point during the evaluation [30,42,43].

$$\text{TEWL}\ (\%) = (\text{TEWL}_{\text{treated}}/\text{TEWL}_{\text{untreated}})\ \text{x}\ 100\text{–}100 \quad (1)$$

2.4. Examination of the permeation enhancer effect of EP ex vivo

Excised human skin was provided by a routine plastic surgery procedure from a Caucasian female patient in the Department of Dermatology and Allergology, University of Szeged. Ethical permission is not

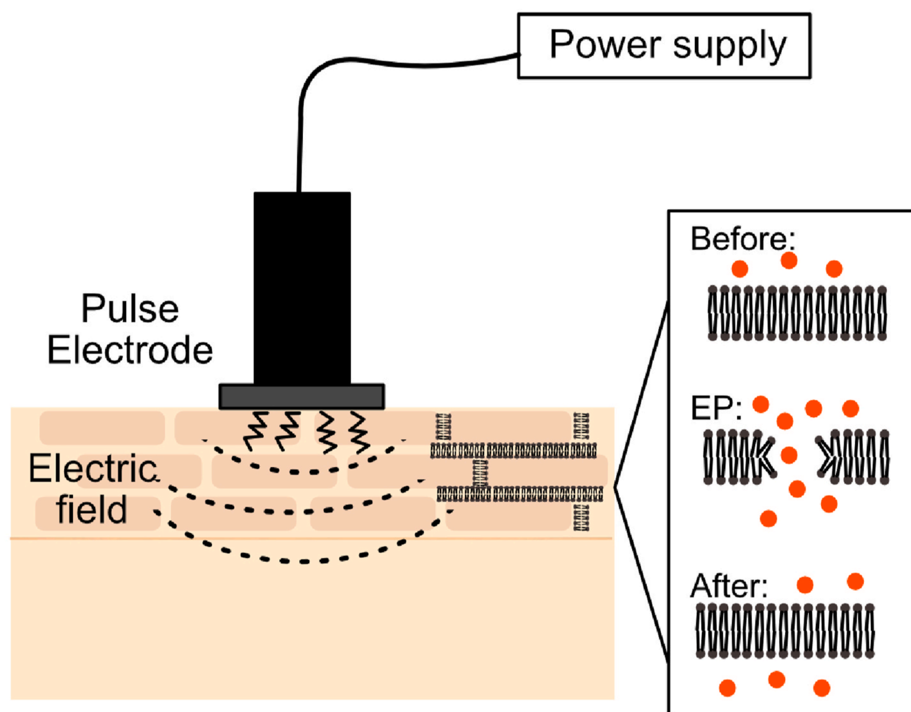


Fig. 1. Mechanism of non-invasive electroporation treatment.

needed to examine skin permeation *ex vivo* because tissue samples to be destroyed are hazardous waste in Hungary (Act CLIV of 1997 on Health, Section 210/A, Hungary). The *ex vivo* skin permeation studies (Notification document number: 83/2008) were announced to the local ethical committee (Regional Ethics Committee for Human Biomedical Research, University of Szeged)). After the plastic surgery, the skin surface was gently cleaned with cotton swabs and stored at −21 °C for up to 6 months before the usage [44–46].

The effect of EP was detected *ex vivo* under a fluorescence microscope. 4 kDa FITC-conjugated dextran was applied as a model substance in the formulations. FITC-dextran is water-soluble and has a high molecular weight, thereby its passive permeation through the skin with diffusion is limited [47]. Full-thickness subcutaneous fat-free human abdominal skin was used in the experiment at room temperature. The skin sections were defrosted and stored on filter papers covered in phosphate-buffered saline solution to maintain their hydration.

In the first experiment, the passive permeation of FITC-dextran from different dermal formulations was detected without EP. 0.2 g of each formulation was applied on the skin surface with 5, 10, and 30 min of observation time to ensure the permeation of FITC-dextran. After the treatment, the residual formulation was wiped. In the second experiment, the effect of EP was examined in combination with the different formulations. In this case, a 2-min-long EP treatment was administered on the skin surface then the formulation was applied on the treated skin for 5, 10, and 30 min. After the treatment, the residual formulation was wiped.

After the treatments, a piece of the treated skin was frozen and sectioned with Leica CM1950 Cryostat (Leica Biosystems GmbH, Wetzlar, Germany). 10-μm-thick cross-sections were placed on slides and analyzed with a light microscope (LEICA DM6 B, Leica Microsystems GmbH, Wetzlar, Germany) at room temperature under a red fluorescence filter (580–660 nm) to avoid the autofluorescence of the skin [48, 49]. FITC-dextran has an emission maximum at 520 nm. 200× magnification was used during the examination. The exposition time was 1.00 s, and the gain value was 5. Pictures about the untreated skin were also recorded to provide negative control, and skin treated with sodium laureth sulfate formulation (SLES) was used as a positive control. Pictures about the treatments were recorded and compared to the controls and to each other visually. ImageJ software was used to evaluate the color intensity of the pictures and the relative standard deviation (RSD, %), which refers to the distribution of color intensity in a picture. The increase in intensity is shown in percentage compared to the negative control (untreated skin). The increase in intensity can be evaluated as a relevant change only if it is higher than 50% and the RSD is low because this evaluation method is not sensitive enough to distinguish small changes.

2.5. Preparation of the formulations

The SLES solution was used to make a positive control. During the formulation, 0.1% FITC-dextran was dissolved in 10% purified water and dispersed in EMAL® 270D to 100% [50].

Furthermore, three different formulations were made: solution, hydrogel, and FFS. In all formulations, 0.1% FITC-dextran was used to detect its permeation under the fluorescence microscope. In the case of the solution and the hydrogel, FITC-dextran was dissolved in purified water. Additionally, 3% xanthan gum was added to the hydrogel to provide gel consistency [51].

During the formulation of the FFS, 10% PVA was dissolved in water at 80 °C under continuous mixing, and 0.1% FITC-dextran and 10% ethanol were added after it cooled down [52]. In this formulation, PVA and Xanthan gum were used as film-forming excipients, while ethanol is the volatile component. During the application, the evaporation of volatile component ensures the formation of a supersaturated system [53,54]. Fig. 2 shows the composition of the positive control and the examined formulations.

3. Results

3.1. Examination of the effect of EP on skin barrier function in vivo

This experiment aimed to detect the effect of EP treatment *in vivo* on mouse skin. It is described in the literature that the lipid bilayer structure of the SC changes after the EP treatment and microchannels open,

A, Positive control
SLES formulation
• 4 kDa FITC-dextran • EMAL® 270D (70% SLES) • Purified water

B, Formulations		
Solution	Hydrogel	FFS
• 4 kDa FITC-dextran • Purified water	• 4 kDa FITC-dextran • Xanthan gum • Purified water	• 4 kDa FITC-dextran • PVA • Ethanol 96% • Xanthan gum • Purified water

Fig. 2. Composition of the positive control (A) and the examined formulations (B).

allowing the permeation of the substances through the skin [55,56]. However, it is also essential to restore the original structure, so the effect of EP should be reversible. We aimed to examine the changes in the skin barrier function caused by EP and obtain information about how long it takes to get the ordered structure under the applied conditions measuring by TEWL values [57,58].

The results show that after EP treatment, the TEWL values increased by more than 500% abruptly. This increase is enormous compared to the normal TEWL, which can prove the effect of EP on the skin barrier. As the examination progressed, TEWL decreased gradually. After 5 min, TEWL was restored almost to the original condition (around 20%), and only a slight fluctuation could be observed, which is a natural property of the skin [58]. Fig. 3 shows the effect of EP on the TEWL values. The changes in TEWL are shown in percentage compared to the untreated mouse skin.

3.2. Examination of the permeation enhancer effect of EP ex vivo

3.2.1. Control experiments

Two control experiments were carried out during the examination: the negative and the positive control. In the case of negative control, the untreated skin was examined to determine the appearance of the skin under fluorescence microscope. In the recorded pictures, the SC appeared with high fluorescence intensity (Fig. 4A). This attribute of untreated skin has been previously observed in the literature, suggesting a physiological appearance of SC structure [59]. However, the lower epidermal and dermal skin layers have only slight intensity under the fluorescence filter (Fig. 4B), so we can detect the permeated FITC-dextran in these skin layers without the autofluorescence of the skin interfering with the evaluation.

In the case of positive control, pictures about the SLES treatment were taken without EP, where sodium laureth sulfate was used as a permeation enhancer to provide permeation. From this formulation, a high permeation rate could be expected because alkyl sulfates can break down the barrier structure and allow all substances to pass through the SC [60]. The increase in intensity (%) compared to the untreated skin (negative control) ± relative standard deviation (RSD (%)) is shown below the pictures. Relevant increase, where the fluorescence intensity increases at least by 50% and the RSD is low, is highlighted in orange. The results show (Fig. 5) that the light intensity of the skin is strongly increased after the SLES treatment, which means that SLES decreased the barrier function of the SC, and FITC-dextran could penetrate into the lower skin layers, as was expected. The intensity already increased after 5 min of observation time (83%), but the highest intensity could be observed after 10 min (177%). In 30 min, only slight fluorescence intensity could be seen (48%), suggesting a lower presence of FITC-dextran in these skin layers. Based on the results, the pictures of the SLES treatment are appropriate for use as a positive control during the evaluation of further treatments.

3.2.2. Investigation of different formulations with and without EP

Firstly, the solution containing FITC-dextran was applied on the skin surface to examine its permeation (Fig. 6A). It can be noticed that fluorescence intensity increased slightly with increasing observation time. However, there are no remarkable differences between the pictures, so significant permeation could not be observed. This result was

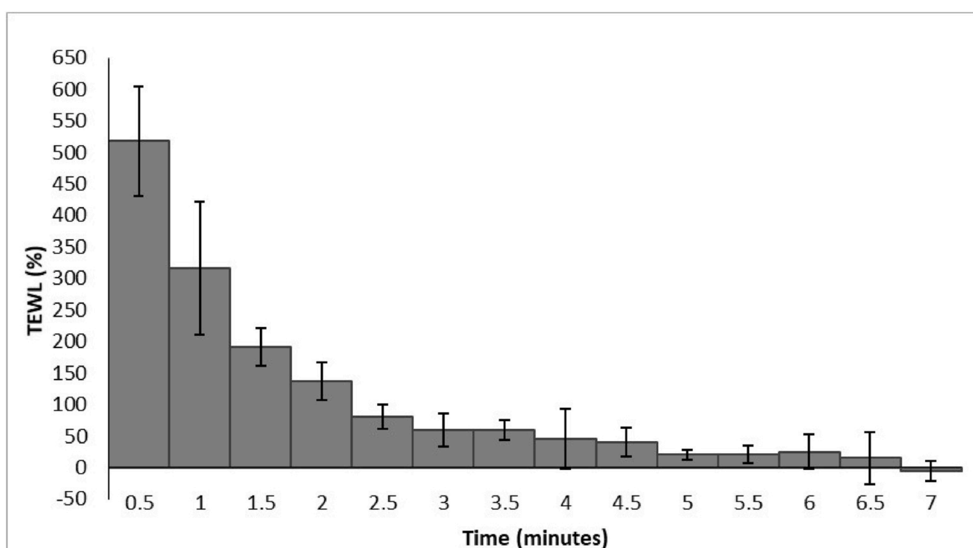


Fig. 3. Effect of electroporation on the skin barrier function by measuring transepidermal water loss (TEWL).
Notes: TEWL values measured after EP treatment. The measurements were carried out continuously for 7 min. The changes in the TEWL values are shown in percentage (%) compared to the untreated mouse skin ±SD. Abbreviations: SD, standard deviation of the mean.

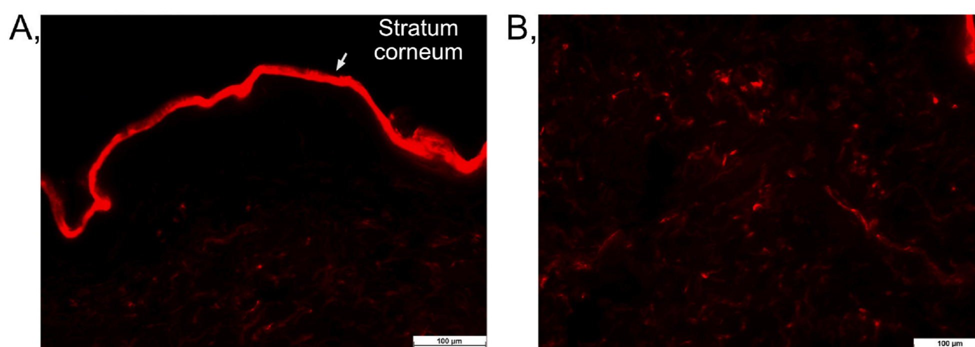


Fig. 4. Examination of untreated skin with fluorescence microscopy: The upper skin layers with the stratum corneum (A) and the lower skin layers (B).

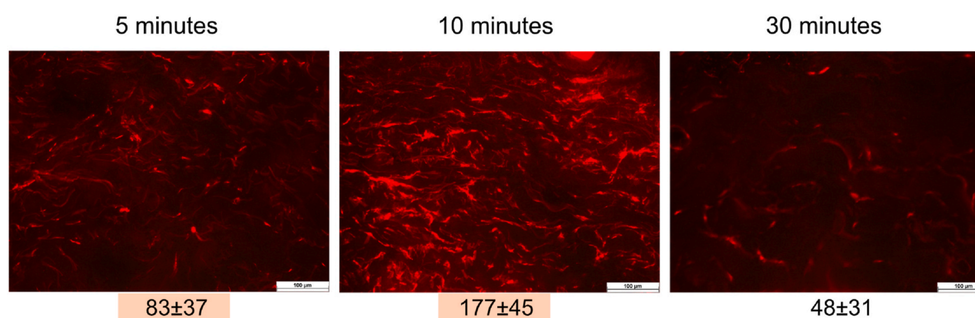


Fig. 5. Skin treated with the SLES with 5-, 10-, and 30-min observation time. The increase in intensity (%) ± RSD (%) compared to the untreated skin (negative control) is shown below the picture. Relevant increases are highlighted in orange. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

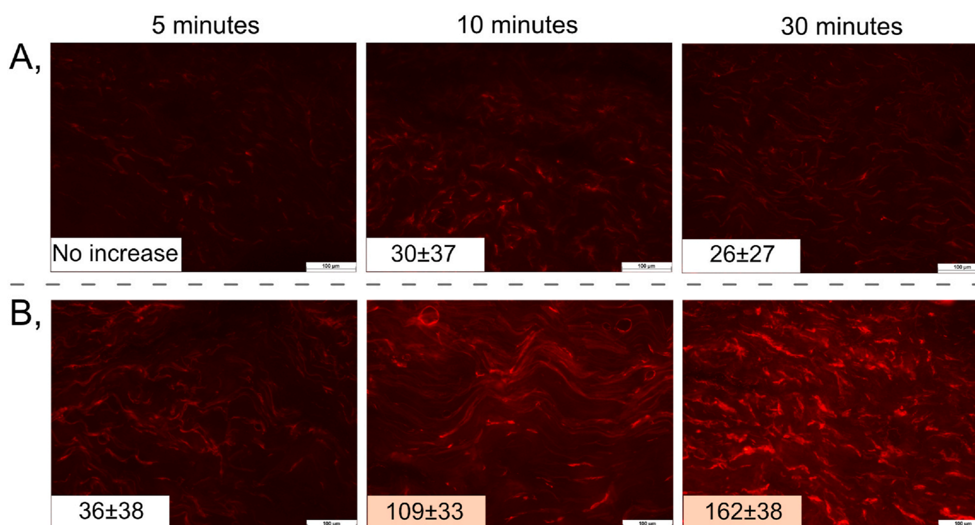


Fig. 6. Skin treated with the solution containing FITC-dextran (A) and in combination with EP (B) with 5-, 10-, and 30-min observation time. The increase in intensity (%) ± RSD (%) compared to the untreated skin (negative control) is shown below the picture. Relevant increases are highlighted in orange. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

expected because the high-molecular-weight dextran cannot permeate into the skin by passive diffusion [13]. Then, the combination of EP and solution treatment was investigated (Fig. 6B). With higher observation times, increased fluorescence intensity can be seen. This intensity is remarkable compared to the controls and much higher compared to the treatment without EP. After 10 min, a 109% permeation increase could be noticed, but the highest permeation was after 30 min of observation time (162%).

Secondly, hydrogel containing FITC-dextran was examined (Fig. 7A). The pictures do not show any changes in intensity, or only slight intensity could be observed in the lower skin layers compared to the negative control, thus FITC-dextran could not be detected in the skin after 5, 10, or 30 min. Thereafter, the combined effect of EP and hydrogel treatment was investigated (Fig. 7B). Interestingly, only the treatment with 5-min observation time showed a significant increase in intensity compared to the negative control; however, the RSD value was also high. In the other cases, the fluorescence intensity was just slightly higher than in the hydrogel treatment without EP. Permeation cannot be

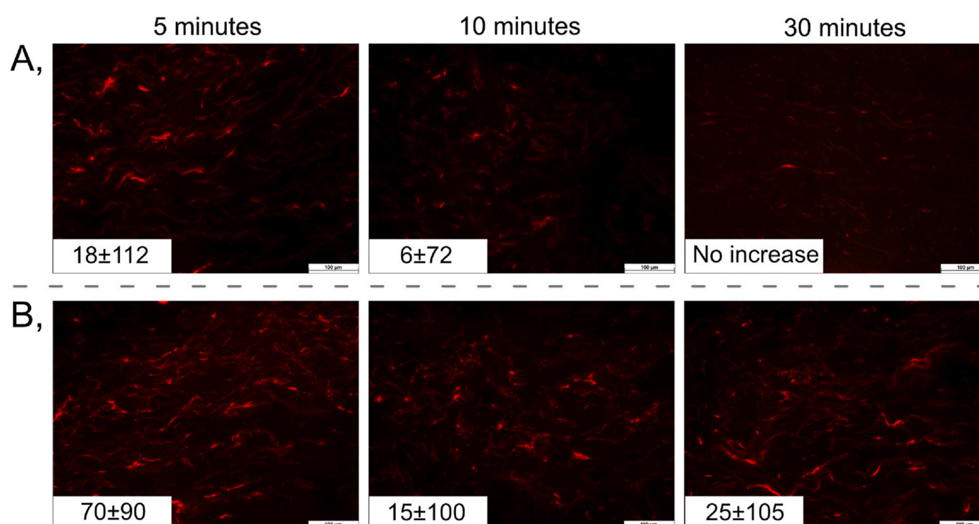


Fig. 7. Skin treated with the hydrogel containing FITC-dextran (A) and in combination with EP (B) with 5-, 10-, and 30-min observation time. The increase in intensity (%) ± RSD (%) compared to the untreated skin (negative control) is shown below the picture.

assumed from the calculated intensity due to the high RSD values.

Finally, FFS containing FITC-dextran was investigated. The results show (Fig. 8A) that there is just a slight increase in the intensity after 30 min of FFS treatment. However, this change was not significant compared to the negative control. However, the combination of FFS and EP treatment (Fig. 8B) caused a significant increase in fluorescence intensity after 30 min of observation time, suggesting a higher presence of FITC-dextran in these skin layers.

4. Discussion

4.1. Examination of the effect of EP on skin barrier function in vivo

The findings of TEWL values after non-invasive EP treatment indicate that EP makes a relevant change in the barrier function of the SC. The exposure time of EP was 5 min under the present conditions on mouse skin. During this period, microchannels are open for permeation through the SC, and the defense system of the skin is reduced. However, the skin barrier function is restored in a short time, which proves that EP has a reversible effect. This property is essential for the safe applicability both in clinical treatment and in cosmetic use; furthermore, it allows EP to be performed as a routine treatment and reduces the possibility of the permeation of external stressors after the treatment. During the evaluation, differences between mouse skin and human skin need to be considered; for instance, mouse skin is thinner than human adult skin [61]. Although the exposure time of non-invasive EP on mouse skin cannot be directly translated to the human skin, it can be concluded that the skin structure is restored in a short time, and this can be presumed in human skin, too. Based on these results, 5, 10, and 30 min of observation times were applied in the *ex vivo* experiments to provide enough time for the permeation of the model substance into the lower skin layers.

4.2. Examination of the permeation enhancer effect of EP ex vivo

The control experiments showed that the untreated SC has high fluorescence intensity, but the lower skin layers appear dark in the pictures. Thereby, the intensity from penetrated FITC-dextran can be seen in the skin, thus the untreated skin is appropriate to provide

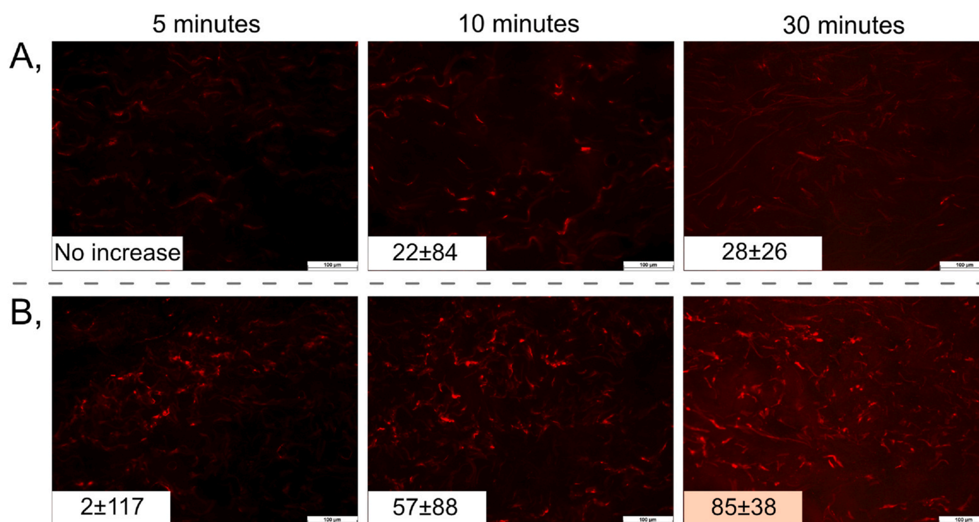


Fig. 8. Skin treated with the FFS containing FITC-dextran (A) and in combination with EP (B) with 5-, 10-, and 30-min observation time. The increase in intensity (%) ± RSD (%) compared to the untreated skin (negative control) is shown below the picture. The relevant increase is highlighted in orange. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

negative control for the evaluation. In the case of positive control, SLES treatment was performed to obtain a picture where FITC-dextran undoubtedly permeated into the skin. During the SLES treatment, sodium laureth sulfate as a permeation enhancer decreases the barrier function of the skin and ensures the high permeation of FITC-dextran. SLES treatment with 10 min of observation time showed outstandingly high fluorescence intensity in the examined skin layers, so this picture can be used as a positive control.

In the following, different dermal formulations (solution, hydrogel, and FFS) containing FITC-dextran were used in combination with EP treatment to evaluate their skin permeation enhancer effect. Based on the results, it can be assumed that only a low amount of FITC-dextran (4000 Da) can permeate from all formulations without EP because the SC forms an effective barrier against chemicals with molecular weights higher than 500 Da [13].

In the case of the solution containing FITC-dextran, the EP treatment could improve the permeation remarkably. Based on this, EP decreased the barrier function of SC effectively and provided a permeation route for FITC-dextran. Interestingly, the fluorescence intensity showed only a slight increase due to the EP and hydrogel treatment, so just a small FITC-dextran permeation can be assumed during the examined time interval. This can be explained by the composition of the preparation. The hydrogel contained xanthan gum as a viscosity enhancer excipient in the formulation to provide the appropriate gel consistency. However, xanthan gum is also a macromolecule, thereby it can interfere with the skin permeation of other macromolecules by retaining FITC-dextran in the formulation and delaying the permeation of the model substance [51,62]. In the applied concentration, the effect of xanthan gum was highly remarkable, so not even the EP treatment could ensure the permeation of FITC-dextran. Thus, the viscosity enhancer excipient needs to be replaced in the hydrogel formulation for efficient therapy treated with a macromolecule and EP. In the case of FFS treatment, EP had a favorable effect on the permeation of FITC-dextran. This formulation also contained xanthan gum, but its concentration was a third of the concentration in the hydrogel, so the composition of FFS allows the release of FITC-dextran, and the permeation enhancer effect of EP could prevail. Based on these results, the composition of the formulation greatly influences the permeation enhancing effect of EP. From the examined compositions, the solution and FFS were appropriate for co-administration with EP.

When evaluating the permeation with different observation times, it can be concluded that the permeation of FITC-dextran increased with the observation period. A 5-min observation time after EP was enough to detect the permeation; however, a 10 min of observation time was required for a significant increase under the applied conditions. Based on this, a higher exposure time of EP could be assumed for human skin treatment than observed in the *in vivo* experiments on mouse skin.

5. Conclusion

During this research work, the effect of non-invasive dermal EP was investigated. EP is a commonly used technique in health care and in the cosmetic industry; however, the information about its safety and applicability is required to improve. In the first part of the experiment, the effect of EP on the skin barrier function was examined *in vivo* on mouse skin, and the results showed that EP modified the skin structure reversibly, so the SC barrier was restored in a short time after the treatment. During the experiments, a routine examination method was successfully developed to detect the permeation enhancing effect of EP on a model macromolecule (4 kDa FITC-dextran) with fluorescence microscopy. Three different dermal formulations (solution, hydrogel, and FFS) were examined, and the results showed that FITC-dextran permeated slightly from these formulations passively. EP could increase the permeation rate of FITC-dextran remarkably compared to the control treatments; however, the composition of the formulation has a great influence on the permeation.

Funding

This research was funded by the Gedeon Richter's Talentum Foundation (19-21 Gyömrői Street, Budapest, Hungary, 1103) and by project EFOP 3.6.3-VEKOP-16-2017-00009. The APC was funded by The University of Szeged Open Access Fund (FundRef, Grant No. 5536).

Author statement

Nikolett Kis: Conceptualization, Investigation, Visualization, Writing- Original draft preparation, Anita Kovács: Conceptualization, Writing- Reviewing and Editing, Mária Budai-Szűcs: Methodology, Gábor Erős: Methodology, Investigation, Erzsébet Csányi: Supervision, Szilvia Berkó: Conceptualization, Supervision.

Declaration of competing interest

The author reports no conflicts of interest in this work.

References

[1] J. Hadgraft, Skin, the final frontier, Int. J. Pharm. 224 (2001) 1–18, https://doi.org/10.1016/S0378-5173(01)00731-1.
[2] 5.1 Layers of the Skin - Anatomy and Physiology | OpenStax, (n.d.). https://openstax.org/books/anatomy-and-physiology/pages/5-1-layers-of-the-skin (accessed June 18, 2021).
[3] K. Ita, Perspectives on transdermal electroporation, Pharmaceutics 8 (2016) 9, https://doi.org/10.3390/pharmaceutics8010009.
[4] T. Tanner, R. Marks, Delivering drugs by the transdermal route: review and comment, Skin Res. Technol. 14 (2008) 249–260, https://doi.org/10.1111/j.1600-0846.2008.00316.x.
[5] M.B. Brown, G.P. Martin, S.A. Jones, F.K. Akomeah, Dermal and transdermal drug delivery systems: current and future prospects, Drug Deliv. 13 (2006) 175–187, https://doi.org/10.1080/10717540500455975.
[6] A.L.M. Ruela, A.G. Perissinato, M.E. de S. Lino, P.S. Mudrik, G.R. Pereira, Evaluation of skin absorption of drugs from topical and transdermal formulations, Braz. J. Pharmaceut. Sci. 52 (2016) 527–544, https://doi.org/10.1590/s1984-82502016000300018.
[7] J.N. Mayba, M.J. Gooderham, A guide to topical vehicle formulations, J. Cutan. Med. Surg. 22 (2018) 207–212, https://doi.org/10.1177/1203475417743234.
[8] K.W. Ng, Penetration enhancement of topical formulations, Pharmaceutics 10 (2018) 51, https://doi.org/10.3390/pharmaceutics10020051.
[9] W. Badri, F. Mohamed, M. Affendi, Ae Asbahani, K. Miladi, Q. Nazari, C. Viennet, S. Robin, H. Fessi, A. Elaissari, Topical co-delivery of indomethacin and nigella sativa l. essential oil in poly- ε-caprolactone nanoparticles: in vivo study of anti-inflammatory activity, Int. J. Adv. Res. 6 (2018) 801–816, https://doi.org/10.21474/IJAR01/6916.
[10] V. Gupta, P. Trivedi, In vitro and in vivo characterization of pharmaceutical topical nanocarriers containing anticancer drugs for skin cancer treatment, in: Lipid Nanocarriers for Drug Targeting, Elsevier, 2018, pp. 563–627, https://doi.org/10.1016/B978-0-12-813687-4.00015-3.
[11] S. Jain, N. Patel, M.K. Shah, P. Khatri, N. Vora, Recent advances in lipid-based vesicles and particulate carriers for topical and transdermal application, J. Pharm. Sci. 106 (2017) 423–445, https://doi.org/10.1016/j.xphs.2016.10.001.
[12] B. Zorec, N. Pavselj, Active enhancement methods for intra- and transdermal drug delivery: a review, Zdravniški Vestnik. 82 (2013) 339–356.
[13] J. Bos, M. Meinardi, The 500 Dalton rule for the skin penetration of chemical compounds and drugs, Exp. Dermatol. 9 (2000) 165–169, https://doi.org/10.1034/j.1600-0625.2000.009003165.x.
[14] A. Alexander, S. Dwivedi, Ajazuddin, T.K. Giri, S. Saraf, S. Saraf, D.K. Tripathi, Approaches for breaking the barriers of drug permeation through transdermal drug delivery, J. Contr. Release 164 (2012) 26–40, https://doi.org/10.1016/j.jconrel.2012.09.017.
[15] E.H. Mojumdar, Q.D. Pham, D. Topgaard, E. Sparr, Skin hydration: interplay between molecular dynamics, structure and water uptake in the stratum corneum, Sci. Rep. 7 (2017) 15712, https://doi.org/10.1038/s41598-017-15921-5.
[16] N. Dragicevic, J.P. Atkinson, H.I. Maibach, Chemical penetration enhancers: classification and mode of action, in: N. Dragicevic, H.I. Maibach (Eds.), Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement: Modification of the Stratum Corneum, Springer, Berlin, Heidelberg, 2015, pp. 11–27, https://doi.org/10.1007/978-3-662-47039-8_2.
[17] H.V. Ngo, P.H.L. Tran, B.-J. Lee, T.T.D. Tran, Development of film-forming gel containing nanoparticles for transdermal drug delivery, Nanotechnology 30 (2019) 415102, https://doi.org/10.1088/1361-6528/ab2e29.
[18] F.F.D. de Oliveira, L.R. de Menezes, M.I.B. Tavares, Film-forming systems in topically administered pharmaceutical formulations, MSA 11 (2020) 576–590, https://doi.org/10.4236/msa.2020.118038.
[19] R. Rai, C.R. Srinivas, Iontophoresis in dermatology, Indian J. Dermatol. Venereol. Leprol. 71 (2005) 236–241, https://doi.org/10.4103/0378-6323.16613.

[20] T.-W. Wong, S.-F. Ko, S.-W. Hui, Enhancing transdermal drug delivery with electroporation, Recent Pat. Drug Deliv. Formulation 2 (2008) 51–57, https://doi.org/10.2174/187221108783331393.

[21] R. Wang, Q. Bian, Y. Xu, D. Xu, J. Gao, Recent advances in mechanical force-assisted transdermal delivery of macromolecular drugs, Int. J. Pharm. 602 (2021) 120598, https://doi.org/10.1016/j.ijpharm.2021.120598.

[22] M. Bharkatiya, R. Nema, Skin penetration enhancement techniques, J. Young Pharm. 1 (2009) 110, https://doi.org/10.4103/0975-1483.55741.

[23] M. Bakonyi, S. Berkó, G. Erős, G. Varju, C.A. Dehelean, M. Budai-Szűcs, E. Csányi, A review of electroporation-based antitumor skin therapies and investigation of betulinic acid-loaded ointment, Anti Cancer Agents Med. Chem. 18 (2018) 693–701, https://doi.org/10.2174/1871520617666171113120255.

[24] J.C. Weaver, Electroporation: a general phenomenon for manipulating cells and tissues: Electroporation, J. Cell. Biochem. 51 (1993) 426–435, https://doi.org/10.1002/jcb.2400510407.

[25] T.-W. Wong, C.-H. Chen, C.-C. Huang, C.-D. Lin, S.-W. Hui, Painless electroporation with a new needle-free microelectrode array to enhance transdermal drug delivery, J. Contr. Release 110 (2006) 557–565, https://doi.org/10.1016/j.jconrel.2005.11.003.

[26] B. Mali, T. Jarm, M. Snoj, G. Sersa, D. Miklavcic, Antitumor effectiveness of electrochemotherapy: a systematic review and meta-analysis, Eur. J. Surg. Oncol. 39 (2013) 4–16, https://doi.org/10.1016/j.ejso.2012.08.016.

[27] L.F. Glass, M. Jaroszeski, R. Gilbert, D.S. Reintgen, R. Heller, Intralesional bleomycin-mediated electrochemotherapy in 20 patients with basal cell carcinoma, J. Am. Acad. Dermatol. 37 (1997) 596–599, https://doi.org/10.1016/S0190-9622(97)70178-6.

[28] J. Gehl, P.F. Geertsen, Efficient palliation of haemorrhaging malignant melanoma skin metastases by electrochemotherapy, Melanoma Res. 10 (2000) 585–589, https://doi.org/10.1097/00008390-200012000-00011.

[29] L.M. Mir, L.F. Glass, G. Sersa, J. Teissié, C. Domenge, D. Miklavcic, M.J. Jaroszeski, S. Orlowski, D.S. Reintgen, Z. Rudolf, M. Belehradek, R. Gilbert, M.P. Rols, J. Belehradek, J.M. Bachaud, R. DeConti, B. Stabuc, M. Cemazar, P. Coninx, R. Heller, Effective treatment of cutaneous and subcutaneous malignant tumours by electrochemotherapy, Br. J. Cancer 77 (1998) 2336–2342.

[30] A.-R. Denet, R. Vanbever, V. Préat, Skin electroporation for transdermal and topical delivery, Adv. Drug Deliv. Rev. 56 (2004) 659–674, https://doi.org/10.1016/j.addr.2003.10.027.

[31] D. Ramadon, M.T.C. McCrudden, A.J. Courtenay, R.F. Donnelly, Enhancement strategies for transdermal drug delivery systems: current trends and applications, Drug Deliv. Transl. Res. 1–34 (2021), https://doi.org/10.1007/s13346-021-00909-6.

[32] A. Hussain, G.M. Khan, A. Wahab, M. Akhlaq, S. Rahman, H. Altaf, N. Akhtar, M. Qayyum, Potential enhancers for transdermal drug delivery: a review, Int. J. Basic Med. Sci. Pharm. 4 (2014) 19–22.

[33] P. Hartmann, E. Butt, Á. Fehér, Á.L. Szilágyi, K.D. Jász, B. Balázs, M. Bakonyi, S. Berkó, G. Erős, M. Boros, G. Horváth, E. Varga, E. Csányi, Electroporation-enhanced transdermal diclofenac sodium delivery into the knee joint in a rat model of acute arthritis, Drug Des. Dev. Ther. 12 (2018) 1917–1930, https://doi.org/10.2147/DDDT.S161703.

[34] W. Liu, X. Yang, Y. Zhu, H. Chen, H. Xu, Nanostructured lipid carriers as vehicles for transdermal iontophoretic drug delivery, Conf. Proc. IEEE Eng. Med. Biol. Soc. (2005) 1236–1239, https://doi.org/10.1109/IEMBS.2005.1616648, 2005.

[35] Mesotherapy, AAOMED. (n.d.). https://www.aaomed.org/default.aspx?bid=70 (accessed June 18, 2021).

[36] Mesoskinline - Non-Invasive Mesotherapy | Needle/Injection Free Mesotherapy Treatment, (n.d.). https://mesoskinline.com/(accessed June 18, 2021).

[37] D. Konda, D. Thappa, Mesotherapy: what is new? Indian J. Dermatol., Venereol. Leprol. 79 (2013) 127–134, https://doi.org/10.4103/0378-6323.104689.

[38] G. Sivagnanam, Mesotherapy – the French connection, J. Pharmacol. Pharmacother. 1 (2010) 4–8, https://doi.org/10.4103/0976-500X.64529.

[39] L. Zhang, S. Lerner, W.V. Rustrum, G.A. Hofmann, Electroporation-mediated topical delivery of vitamin C for cosmetic applications, Bioelectrochem. Bioenerg. 48 (1999) 453–461, https://doi.org/10.1016/s0302-4598(99)00026-4.

[40] S. Berkó, K.F. Szűcs, B. Balázs, E. Csányi, G. Varju, A. Sztojkov-Ivanov, M. Budai-Szűcs, J. Bóta, R. Gáspár, Electroporation-delivered transdermal neostigmine in rats: equivalent action to intravenous administration, DDDT 10 (2016) 1695–1701, https://doi.org/10.2147/DDDT.S102959.

[41] C. Danciu, S. Berkó, G. Varju, B. Balázs, L. Kemény, I.B. Németh, A. Cioca, A. Petruș, C. Dehelean, C.I. Cosmin, E. Amaricai, C.C. Toma, The effect of electroporation of a lyotroic liquid crystal genistein-based formulation in the recovery of murine melanoma lesions, Int. J. Mol. Sci. 16 (2015) 15425–15441, https://doi.org/10.3390/ijms160715425.

[42] M. Bakonyi, S. Berkó, A. Kovács, M. Budai-Szűcs, N. Kis, G. Erős, I. Csóka, E. Csányi, Application of quality by design principles in the development and evaluation of semisolid drug carrier systems for the transdermal delivery of lidocaine, J. Drug Deliv. Sci. Technol. 44 (2018) 136–145, https://doi.org/10.1016/j.jddst.2017.12.001.

[43] Y. Tokudome, K. Sugibayashi, Mechanism of the synergic effects of calcium chloride and electroporation on the in vitro enhanced skin permeation of drugs, J. Contr. Release 95 (2004) 267–274, https://doi.org/10.1016/j.jconrel.2003.12.014.

[44] OECD (Organisation for Economic Co-operation and Development), OECD, in: Guidance Document for the Conduct of Skin Absorption Studies, vol. 28, OECD Environmental Health and Safety Publications. Testing and Assessment, Paris, 2004. n.d. https://www.oecd.org/chemicalsafety/testing/48532204.pdf. (Accessed 1 October 2021).

[45] IPCS, Dermal Absorption. Environmental Health Criteria 235. Geneva, WHO, 2006 (n.d.), http://www.who.int/ipcs/publications/ehc/ehc235.pdf?ua=1. (Accessed 1 October 2021).

[46] A.M. Barbero, H.F. Frasch, Effect of frozen human epidermis storage duration and cryoprotectant on barrier function using two model compounds, SPP 29 (2016) 31–40, https://doi.org/10.1159/000441038.

[47] Fluorescein isothiocyanate–dextran average mol wt 4,000, (FITC:Glucose = 1:250) | Sigma-Aldrich, (n.d.). http://www.sigmaaldrich.com/(accessed June 18, 2021).

[48] A.K. Murray, T.L. Moore, J.B. Manning, C.E.M. Griffiths, A.L. Herrick, Noninvasive measurement of skin autofluorescence is increased in patients with systemic sclerosis: an indicator of increased advanced glycation endproducts? J. Rheumatol. 39 (2012) 1654–1658, https://doi.org/10.3899/jrheum.111359.

[49] H.L. Zhao, C.P. Zhang, H. Zhu, Y.F. Jiang, X.B. Fu, Autofluorescence of collagen fibres in scar, Skin Res. Technol. 23 (2017) 588–592, https://doi.org/10.1111/srt.12375.

[50] Copyright © Kao Corporation, EMAL 270D | Kao Chemicals, EMAL 270D. (n.d.). https://chemical.kao.com/en/products/B0001816_en/(accessed August 24, 2021).

[51] A. Shalviri, Q. Liu, M. Abdekhodaie, X.Y. Wu, Novel modified starch–xanthan gum hydrogels for controlled drug delivery: synthesis and characterization, Carbohydr. Polym. 79 (2010) 898–907, https://doi.org/10.1016/j.carbpol.2009.10.016.

[52] A. Kovács, N. Kis, M. Budai-Szűcs, A. Gácsi, E. Csányi, I. Csóka, S. Berkó, QbD-based investigation of dermal semisolid in situ film-forming systems for local anaesthesia, Drug Des. Dev. Ther. 14 (2020) 5059–5076, https://doi.org/10.2147/DDDT.S279727.

[53] N. Kis, A. Kovács, M. Budai-Szűcs, A. Gácsi, E. Csányi, I. Csóka, S. Berkó, Investigation of silicone-containing semisolid in situ film-forming systems using QbD tools, Pharmaceutics 11 (2019) 660, https://doi.org/10.3390/pharmaceutics11120660.

[54] K. Kathe, H. Kathpalia, Film forming systems for topical and transdermal drug delivery, Asian J. Pharm. Sci. 12 (2017) 487–497.

[55] S. Feng, L. Zhu, Z. Huang, H. Wang, H. Li, H. Zhou, L. Lu, Y. Wang, Z. Liu, L. Liu, Controlled release of optimized electroporation enhances the transdermal efficiency of sinomenine hydrochloride for treating arthritis in vitro and in clinic, Drug Des. Dev. Ther. 11 (2017) 1737–1752, https://doi.org/10.2147/DDDT.S136313.

[56] S. Cross, M. Roberts, Physical enhancement of transdermal drug application: is delivery technology keeping up with pharmaceutical development? Curr. Drug Deliv. 1 (2004) 81–92, https://doi.org/10.2174/1567201043480045.

[57] M. Akdeniz, S. Gabriel, A. Lichterfeld-Kottner, U. Blume-Peytavi, J. Kottner, Transepidermal water loss in healthy adults: a systematic review and meta-analysis update, Br. J. Dermatol. 179 (2018) 1049–1055, https://doi.org/10.1111/bjd.17025.

[58] S.J. van Rensburg, A. Franken, J.L.D. Plessis, Measurement of transepidermal water loss, stratum corneum hydration and skin surface pH in occupational settings: a review, Skin Res. Technol. 25 (2019) 595–605, https://doi.org/10.1111/srt.12711.

[59] M.A. Ashtikar, D.D. Verma, A. Fahr, Confocal microscopy for visualization of skin penetration, in: N. Dragicevic, H.I. Maibach (Eds.), Percutaneous Penetration Enhancers Drug Penetration Into/Through the Skin: Methodology and General Considerations, Springer, Berlin, Heidelberg, 2017, pp. 255–281, https://doi.org/10.1007/978-3-662-53270-6_15.

[60] C. Jongh, M. Verberk, C. Withagen, J. Jacobs, T. Rustemeyer, S. Kezic, Stratum corneum cytokines and skin irritation response to sodium lauryl sulfate, Contact Dermatitis 54 (2006) 325–333, https://doi.org/10.1111/j.0105-1873.2006.00848.x.

[61] L.S. Hansen, J.E. Coggle, J. Wells, M.W. Charles, The influence of the hair cycle on the thickness of mouse skin, Anat. Rec. 210 (1984) 569–573, https://doi.org/10.1002/ar.1092100404.

[62] F. Alvarez-Manceñido, K. Braeckmans, S.C. De Smedt, J. Demeester, M. Landin, R. Martínez-Pacheco, Characterization of diffusion of macromolecules in konjac glucomannan solutions and gels by fluorescence recovery after photobleaching technique, Int. J. Pharm. 316 (2006) 37–46, https://doi.org/10.1016/j.ijpharm.2006.02.029.