Chapter 10

Measurement and Simulation of Permeation and Diffusion in Native and Cultivated Tissue Constructs

Hao-Hsiang Hsu, Katharina Schimek, Uwe Marx and Ralf Pörtner

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.72904

Abstract

Characterization of native skin or cultured 3D skin models with respect to permeability plays an important role for the development and testing of pharmaceuticals and cosmetics. Extensive efforts have been dedicated to determining the key parameters describing permeability and diffusion. Whereas respective methods are well established for native skin biopsies, only few are available for 3D skin models, as these have usually much lower dimensions. In this chapter, some fundamentals about permeation and diffusion as well as state of the art of measurement methods used for skin biopsies are summarized. An alternative method for the determination of the permeation in a membrane insert system and the use of a modular simulation to support permeability studies is presented and discussed.

Keywords: skin models, permeation, diffusion, membrane insert system

1. Introduction

Permeability studies are indispensable to characterize the transport of substances through the skin, either natural skin or cultivated 3D skin models. This is evident for dermal drug delivery systems, where drugs can be applied in the form of creams or patches on the skin. Furthermore, permeability studies play an important role in toxicity tests applied in drug development as well as substance testing. In this respect, human three-dimensional skin models have become an interesting tool.

Animal experiments are still a common method of testing drugs and also for skin, which is ethically controversial, cost-intensive and time-consuming. The fact is that drug testing on

human skin is more efficient in comparison to animal skin just like rat, mouse and guinea pig [1, 2]. Furthermore, in 2013 the European Regulation (EC) No 1223/2009 entered into force, which prohibits animal experiments for cosmetics products. Artificial skin models based on human cells are intended to replace animal experiments. Especially the barrier function between artificial and human skin can differ, so permeation and diffusion investigation in this area is necessary.

Most methods for determination of diffusion and permeability parameters have been developed for large biopsies and can hardly be applied for small-scale 3D skin tissue cultures. But this is indispensable, if multi-well test systems with several samples run in parallel or multi-organ-chips are applied. Therefore, this chapter will first give a brief overview of standard methods used to investigate permeation and diffusion on the skin. Then an alternative method based on skin tissue cultures in a membrane insert system is introduced. By this, the permeation coefficient of substances through a skin-tissue barrier can be determined. The diffusion coefficient is estimated via parameter optimization performed in COMSOL Multiphysics. This software tool helps to describe the physical effects of the experimental set-ups more precisely and can further be used to reduce the required amount of experiments significantly.

2. Penetration, diffusion and permeation through the skin

Penetration describes the entering of a substance into the skin. The entering process and depth of substance penetration through the skin depends on the physical and chemical character of the substance and the skin. The permeation of the skin is the pathway of a substance from the surface to the blood vessel. From a scientific point of view, it is the permeation of a substance through the skin layers. Diffusion is the physical process of randomized particle movement. If a concentration gradient exists, the particles move in the direction of lower concentration.

The human skin consists of three layers, the epidermis, dermis and subcutis. The stratum corneum (horny layer) is the upper layer of the epidermis and forms the main barrier of the skin. Substances such as drugs and chemicals penetrate through the skin barrier in three possible routes: the transcellular, the intercellular and the appendageal route (see **Figure 1**) [3–5].

The transcellular route leads the permeating substances directly through the cells. Here, the substances have to pass alternating lipophilic and hydrophilic layers. This is probably the

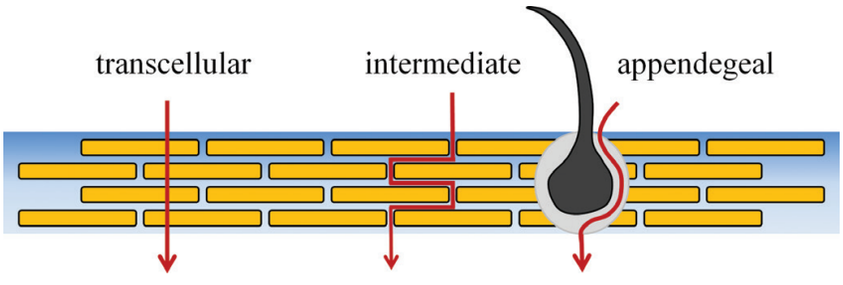

Figure 1. Schematic illustration of the three possible pathways of a permeating substance through the stratum corneum.

most difficult way for substances, because they should have lipophilic and hydrophilic properties. Until now, it is not clear if hydrophilic substances choose this pathway [6].

Alternatively, there is a way through the intercellular spaces between the cells. This is called the intercellular route. The intermediate space consists of cholesterols, ceramides and free fatty acids [7]. Because of the fatty acids, lipophilic substances can pass easier through the intercellular route in contrast to lipophobic substances [8]. Another barrier in this intercellular space is the tight junctions [9]. These are networks of strands which are formed by membrane proteins connecting cells. They are located between the keratinocytes in the stratum granulosum of the epidermis. An important function of the tight junctions, which are formed during the differentiation of keratinocytes, is to protect the skin from water loss [10].

The appendageal route describes the penetration of a substance through skin appendages like hair follicles and glands. Since hair follicles and glands build only a small part of the human skin, their relevance for skin permeation was neglected for a long time. Its importance was shown recently by a researcher as permeation is better in a skin area containing hair follicles and glands in comparison to an area without them. A specific characteristic of the hair follicle is it reservoir function. In follicles, substances can be stored up to 10 days and can penetrate gradually into the skin. This aspect is interesting for drug delivery over the skin. For example, alcohol prefers the appendageal penetration route, as it would otherwise evaporate quite fast on the surface of the skin. [11–14]

So far the penetration and permeation in the skin was described. To get more in detail, the physical aspect of diffusion and permeation will be explained. Diffusion is a transport process where molecules move via Brownian motion in a volume or area. It is driven by the concentration gradient in the direction from higher to lower concentration [15]. Adolf Fick (1829–1901) verified the coherence between heat transfer and diffusion, which led to the Fick's first law of diffusion:

$$F = -D\frac{\partial C}{\partial x} \tag{1}$$

According to Eq. (1), the flux F in the one partial direction x is proportional to the gradient of concentration C. D is the diffusion coefficient or diffusivity. [16, 17]

Permeation is an aspect of diffusion. Whereas diffusion is related to the movement of molecules in a system, permeation describes how fast molecules move through a system. An example is the permeation of a substance within a volume V_A and a donor concentration c_D through a membrane with a surface A. The acceptor concentration c_A of the permeating substance on the other side can be detected over the time t. Eq. (2) for the permeation coefficient P can be derived from the Fick's law of diffusion [18, 19]:

$$\frac{dc_A}{dt} = P \cdot A\frac{c_D}{V_A} \tag{2}$$

This equation can only be used for $c_D \gg c_A$. With respect to skin, the permeation coefficient is the preferred parameter, as it is easier to measure compared to the diffusion coefficient. Due to the different layers of the skin, permeation and diffusion coefficient changes all the time from layer to layer.

3. State of the art for investigation of penetration, diffusion and permeation within the skin

To understand and investigate diffusion and permeation of the skin biopsies, several methods have been established. Some are summarized in the following.

The Franz diffusion cell is a well-known device to measure the permeation of a substance through a skin biopsy. This device consists of two chambers where the skin biopsy (or any other barrier) is fixed in between. The test substance can be applied to the top chamber (donor) of the skin; it permeates through the barrier into the bottom chamber (acceptor). The fluid in the bottom chamber is mixed by means of a magnetic stirrer. On this side, samples can be taken and the concentration can be analyzed. The concentration of the acceptor is plotted over the time and the permeation coefficient can be calculated according to Eq. (2). The whole system can be temperature-controlled. The usual size (height) of a Franz diffusion cell is in the range of 19–179 mm. Besides permeation investigation, this system is also used to test the quality of skin models and the effects of pharmaceutical substances on the skin. [18, 20–26]

Fluorescence recovery after photobleaching (FRAP) is a method to measure molecular diffusion in tissues or gels, mainly for high molecular weight compounds. For this, the substance to be analyzed must be labeled with a fluorochrome. Mostly fluorescence-labeled proteins or FITC-dextranes (fluorescein isothiocyanate-dextranes) with different molecular sizes are used. The tissues or gels have to be soaked with this substance. This can be realized by storing the material in the fluorescence substance for some days or in case of a gel, to directly polymerize in the fluorescent substance. Then, a confocal laser is used to bleach out a certain area (mostly a line or a point) in the material. Because of diffusion, bleached molecules will move and change their position with fluorescent particles and the fluorescence recovers. After the bleaching process, the area will be scanned several times. The recovery time of fluorescence intensity is used to determine the diffusion coefficient of the substance in the material. For this, software for image analysis is used. [27–30]

Further examples for imaging methods for the determination of diffusion of molecules in skin are Fourier-transform-infrared (FTIR) spectroscopy [31, 32], two-photon fluorescence correlation spectroscopy in combination with fluorescence correlation spectroscopy (FCS) [33, 34] and optical coherence tomography [35]. These methods are noninvasive and nondestructive. Furthermore, some of them can detect molecules without fluorescence labeling. One big disadvantage is the equipment. For these imaging methods, special microscopes or also cost-intensive tomographs are needed.

A method to investigate the penetration process of substances into the skin is tape stripping. After treatment of the skin with the substance of interest the stratum corneum is ripped of layer by layer with an adhesive film. Then, the amount of the substance can be analyzed. For this, there are different methods to determine the concentration of the substance. One method is to detect the substances directly on the film, for example titanium dioxide can be analyzed with X-ray fluorescent measurement and fluorescent-labeled substances can be detected via laser scanning microscopy. Another possibility is to remove the skin layer from the film and apply standardized analytical methods to determine the substance concentration. With tape stripping it is possible to observe where the substance of interest is localized and how deep

they can penetrate into the skin. It is minimal invasive and possible to investigate the penetration directly on human skin. A disadvantage of this method is the undefined thicknesses of the stripped layer. It varies from experiment to experiment and differs with the skin model or skin type. The thickness can be estimated by weighing. [36–39]

As mentioned before, the above methods all together provide a detailed characterization of diffusion and permeation effects within the skin. But most of them can hardly be adapted to skin tissue models used in drug and substance testing. Here usually small culture devices, e.g. 12- or 96-well plates are preferred, as they allow for handling of a large number of samples in parallel. Furthermore, most methods require treatment of the sample in one or the other way. Therefore, it is quite difficult to determine the time-depending changes of diffusion and permeation.

4. Skin tissue models

The need to evaluate skin permeation, test cosmetic products and toxicologically screen topically applied compounds is evident. Historically, several millions of animal experiments have been performed worldwide to address this purpose [40]. Since animal experiments are under massive debate, ethical and regulatory issues, but also severe differences between animal and human data pushed the development and commercialization of diverse *in vitro* skin models [41, 42]. Human skin equivalents (HSEs) can be categorized into two main groups: the epidermis-only and full-thickness models. For both, the differentiation of keratinocytes and hence development of the various layers of the epidermis is important to model actual skin barrier properties more closely. In this context, the direct exposure to air as well as the culture media that supply nutrients for cell growth and differentiation from below has been found to be beneficial [43]. Growing cells on a porous membrane is one of the most commonly used ways to accomplish this air-liquid interface culture. According to the Organization for Economic Co-operation and Development (OECD) test guideline 431 (skin corrosion) and 439 (skin irritation), currently validated skin models include EpiSkin™ (L'oreal, France), EpiDerm™ SIT (MatTek Corporation, USA), SkinEthic™ RHE (SkinEthic laboratories, France), EpiCS® (CellSystems, Germany) and LabCyte EPI-MODEL24 SIT (Japan Tissue Engineering Co., Japan). These 3D skin models are all composed of one cell type only, the keratinocytes, mimicking the epidermis of native human skin and are especially advantageous with respect to high reproducibility [44]. However still not validated, there are also commercially available full thickness skin models composed of an additional dermal layer (e.g., GraftSkin®, EpiDermFT®, and Pheninon®). The models described are nowadays widely used for animal-free tests in drug development as well as, the chemical and cosmetics industries.

5. Determination of permeation and diffusion coefficients in membrane insert systems via measurement and simulation

A common method for *in vitro* cultivation of skin models is the use of a membrane insert system. This system consists of a plastic vessel with a permeable membrane at the bottom. It enables the cultivation of skin tissue models in airlift on the membrane and guarantees the

supply of nutrients from below. As the membrane insert system has two separate chambers, it can be used for permeation studies similar to the Franz diffusion cell. In the following, an experimental procedure for the determination of permeation coefficients in Transwell® systems (12 and 96 well) and simulations with COMSOL Multiphysics for estimation of diffusion coefficients will be discussed. Details can be found in [53].

5.1. Measurement and simulation.

The scheme of a permeation experiment in a membrane insert system is shown in **Figure 2**. The tissue barrier is established on the membrane. It is composed of agarose gel or 3D tissue to validate the method. The 3D tissues consist of a collagen matrix with human fibroblasts within and HaCaT cells on the top of the matrix. On top of the barrier, the donor is applied. The donor contains the substance to be analyzed, which permeates through the barrier. The acceptor, which collects the permeating substance, is located on the other side of the membrane in the receiver vessel. Temperature, humidity and mixing are parameters that influence the permeation and should be kept constant. It is recommended to perform the experiment on a shaker in an incubator with 37°C and ≥90% humidity (conditions for human cell culture). To avoid hydrodynamic pressure, the fluid surface in the insert (donor) and in the receiver vessel (acceptor) should be on the same level. The used volume for the experiment in 12 and 96 Transwell® systems is shown in **Table 1**. Sampling, like in the Franz diffusion cell, is difficult because of the small volume in the acceptor. A solution is the use of fluorescence-labeled substances, by which the permeate concentration can be detected via fluorescence measurement in the receiver vessel. A more elaborate possibility is to run several permeation experiments in parallel and take a sample from one vessel per time point. Then, the concentration of the substance can be measured analytically.

The permeation experiment was simulated with COMSOL Multiphysics. This program is based on the finite element method and offers different physic simulation modules. This

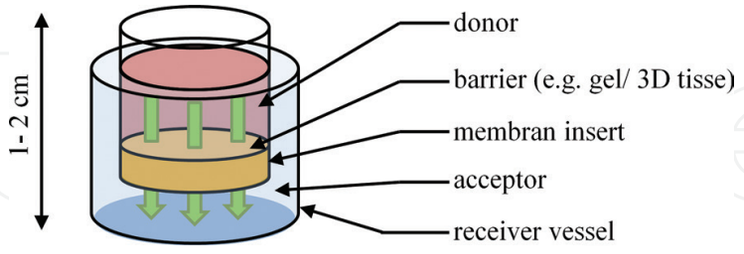

Figure 2. Schematic sketch of a membrane insert system.

Transwell System	Volume in acceptor	Volume in donor
96	75 µl	300 µl
12	590 µl	1845 µl

Table 1. Liquid volume in the acceptor and the donor in different membrane insert systems with a barrier of 2 mm thickness.

structure enables the computation of different physical problems in one simulation. The permeation experiment was simulated with the module "transport diluted species", which uses the Fick's law to simulate diffusion processes. In order to determine the diffusion coefficient, a parameter optimization was performed with the "optimization module". Some simplifications have to be done in order to simulate the experiment. In the experiment, the permeating substance passes through a barrier and a membrane. For the simulation, these two phases were resumed as one homogenous material. It is not possible to resolve the different phases, as investigations that are more detailed would be necessary. The diffusion coefficient of the permeating substance in the liquid phases (in donor and acceptor) was determined in preliminary mixing tests and was found to be 1×10^{-9} m^2/s. This parameter represents the molecular distribution in the mixing process. Furthermore, the geometry of the membrane insert system was simplified. In reality, the system is slightly conical. The simplified geometry is a cylinder. All boundaries of the geometry were set as "no slip". The concave surface of the agarose gel was approximated with a spherical shape. In **Figure 3**, the geometry and the mesh of the 96- and 12-well Transwell® systems are shown.

5.2. Influence of different settings and validation of the system.

Different membrane insert sizes influence the permeation within the system. Investigations with fluorescein sodium salt and 2% agarose gel in 96- and 12-well membrane insert systems are shown in **Figure 4a**. The time course of the acceptor concentration in the 12-well system was steeper in comparison to the 96-well system. Therefore, the fluorescein sodium salt permeates faster through the barrier in the 12-well system compared to the 96-well system. A reason for this is the concentration gradient in the gap between the membrane and the bottom of the receiver vessel. Because of the ratio "volume to permeation surface" and the gap size, the concentration will be balanced faster in case of the 12-well system. The gradient can be reduced by increasing the mixing frequency and amplitude. However, this is limited due to spillover of the liquid. By simulating these experiments, the different concentration distributions below the membrane can be visualized. In **Figure 4c** and **d** the concentration at different time points is plotted over the length below the membrane from the middle point to the edge of the receiver vessel (see red line on **Figure 4b**). The concentration difference between the middle and edge in 12-well systems is higher than that in 96-well systems. This indicates a better and faster concentration balance in the larger system, which explains the accelerated permeation.

The reproducibility of the suggested method is an important aspect. The permeation coefficient determined in different permeation experiments with fluorescein sodium salt through 2% agarose gel differed up to 40.9%. Although the value seems to be quite high, it is still within the range of deviation of permeability experiments with Franz diffusion cells reported in the literature [24]. It was found that small concentration variations during the preparation of the donor substance can cause large variation. By using one stock solution for different experiments, the deviation can be reduced to 29%. Further reasons for the deviation can also be small pipetting errors and variations of the barrier, for example, variation of the gel concentration or volume.

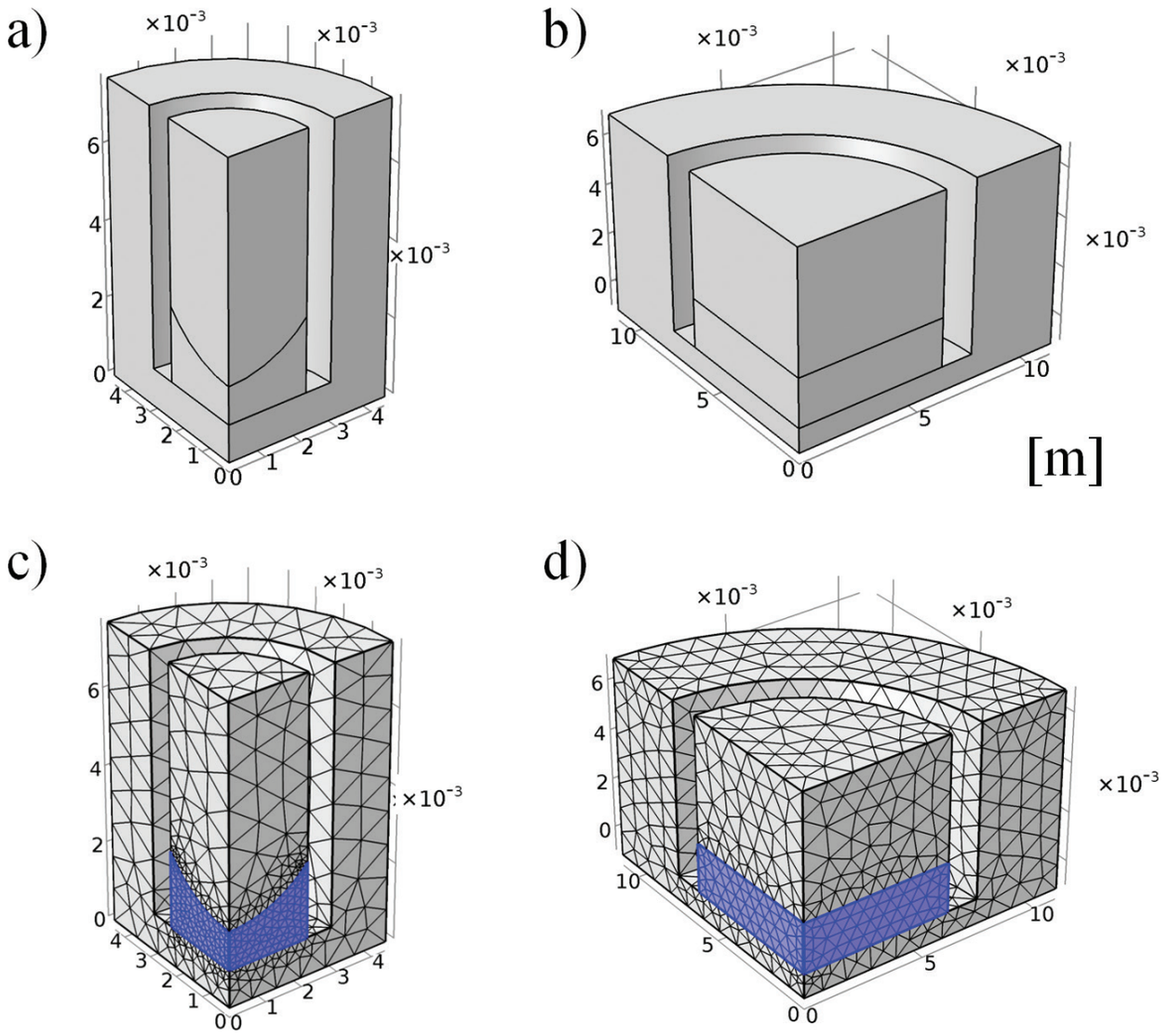

Figure 3. Geometry of the 96 a) and 12 b) Transwell® system and the used mesh c) and d) implemented in COMSOL **Multiphysics**.

The membrane itself also has an influence on the permeation. Same experiments as described above were carried out with different membranes in a 12 Transwell® system. Membranes consisting of polycarbonate (PC) with 0.4 and 3.0 μm pore size showed a quite similar permeation coefficient of 8.03 and 8.1 × 10^{-8} m/s. For polyethylene (PE) membranes with 0.4 μm pore size the mean value of the permeation coefficient was 5.94 × 10^{-8} m/s and for PE with 3.0 μm pore size it was 8.59 × 10^{-8} m/s (see **Figure 6a**). Except for the PE membrane with pore size of 3.0 μm there is no significant difference. The reason for this is the pore density of the membrane. In total, the pore surface of PE membranes with 0.4 μm pore size is 0.25 mm^2 per 1 cm^2 and for the other membranes 6.3–7.05 mm^2 per 1 cm^2. The material of the membrane seems not to influence the permeation.

The suggested method is sensitive enough to determine a cover layer of HaCaT cells of 3D skin tissue. To prove this, in a permeation experiment different 3D tissue model was tested in

http://dx.doi.org/10.5772/intechopen.72904

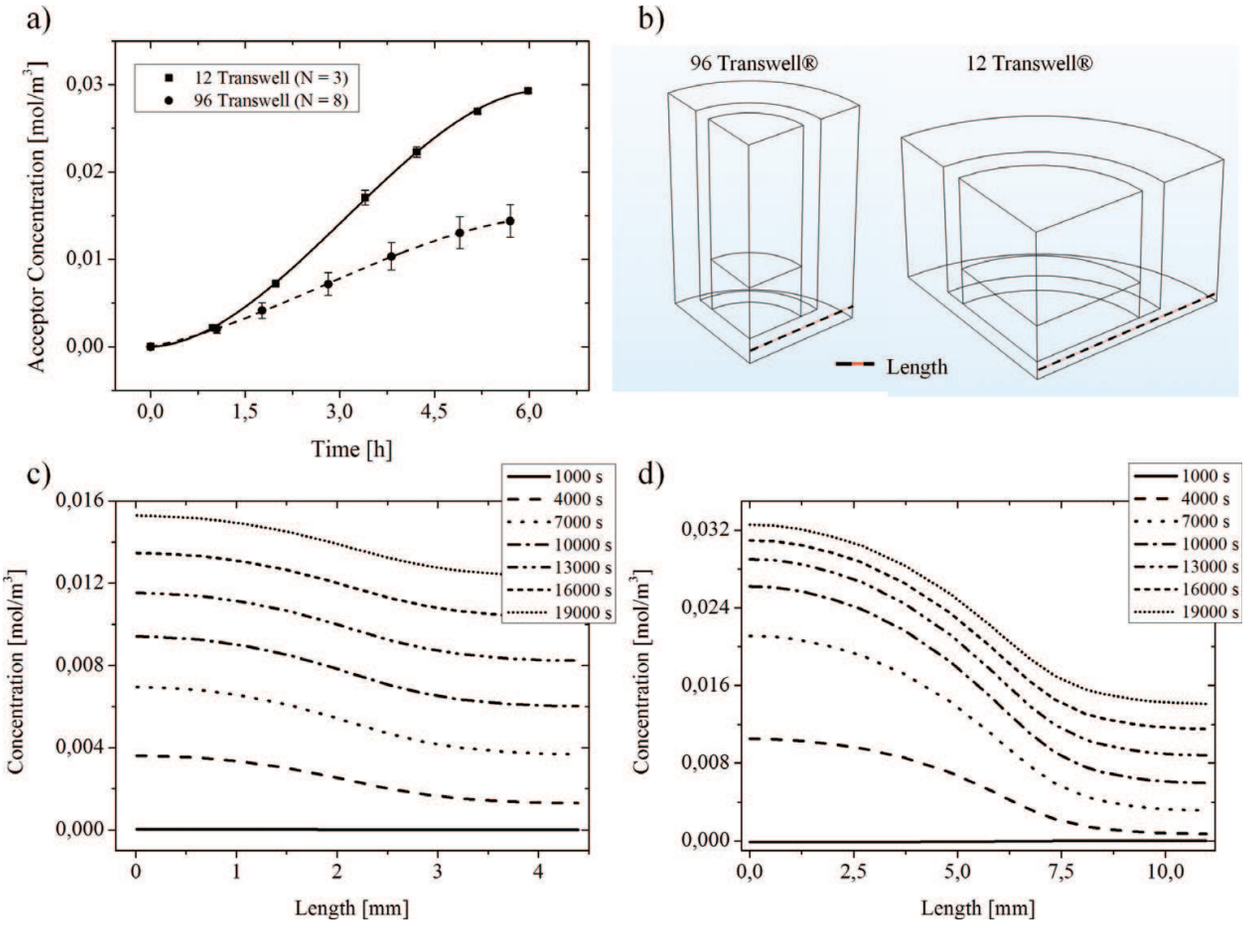

Figure 4. Permeation in 12- and 96-well membrane insert systems. (a) Experimental results of permeation of fluorescein sodium salt through 2% agarose. (b) Geometry of the 12 and 96 Transwell® system for the simulation and the position of the concentration measurement. (c) and (d) the concentration distribution at different time over the length at the red lined position on (b) ((c) 96 and (d) 12 Transwell® system).

a 12 Transwell® system. The 3D tissues consisted of a collagen matrix with different constellations of cell layers. Human primary fibroblasts were integrated into the collagen matrix and HaCaT cells were seeded on the top. A representative example of the 3D tissue is shown in **Figure 5**.

The results show that the permeation coefficient decreases when additional cell layers are added in the tissue model. The permeation coefficient of fluorescein sodium salt through collagen matrix (without cells) is 2.18×10^{-8} m/s, 1.85×10^{-8} m/s in tissue models with fibroblast and 1.67×10^{-8} m/s in models with HaCaT cells (see **Figure 6b)**. These results represent very well the barrier function of keratinocytes of the skin [3].

The particle size influences the permeation behavior through gels and biomaterials. It is well known that smaller molecules permeate faster through a matrix mesh than larger particles. This was already observed for permeation experiments through sclera [19], human epidermal membrane [45], human skin [33] and rat skin [3]. Fluorescein sodium salt and fluorescein isothiocyanate-dextrans (FITC-dextran) were used to vary the molecular size from 376 g/mol up

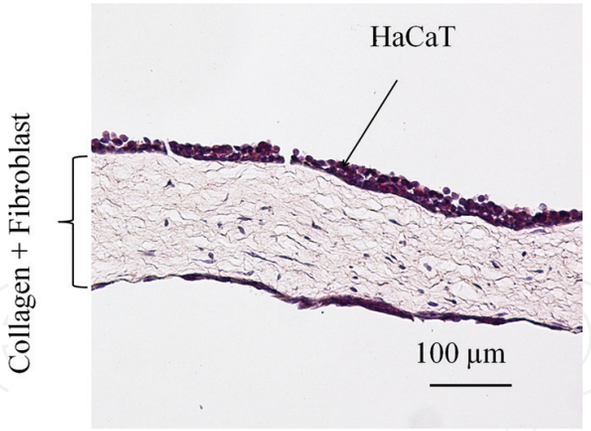

Figure 5. Hematoxylin and eosin stain of 3D tissue model consisting of collagen matrix with fibroblast and HaCaT seeded on the top.

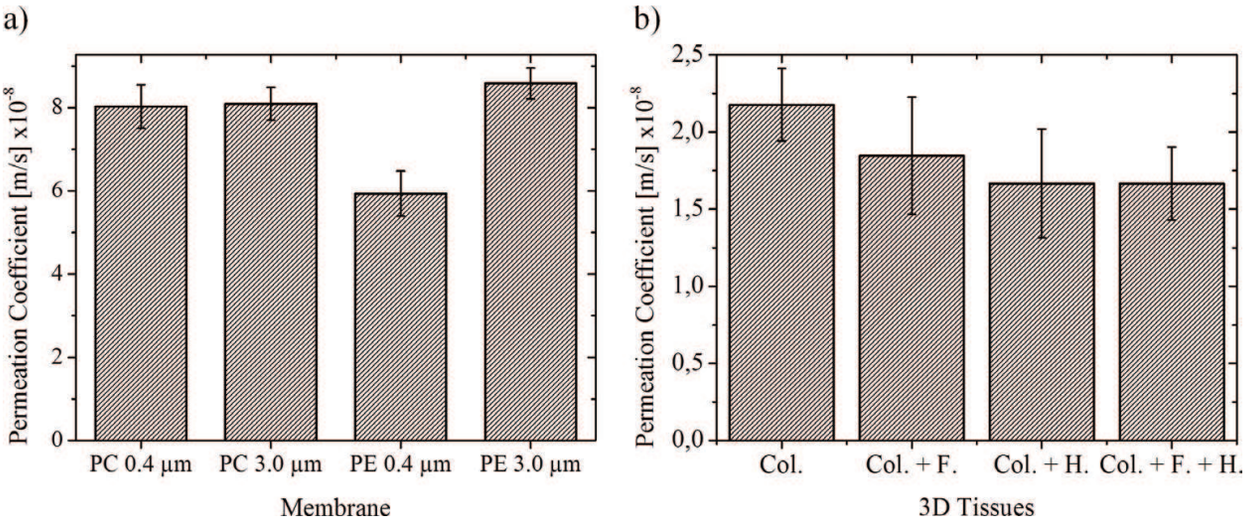

Figure 6. Permeation through different membranes and 3D skin tissues. (a) Results of permeation experiment with fluorescein sodium salt through 2% agarose gel on different membranes (n = 3). The 12 Transwell® system was used and membranes consisting of polycarbonate (PC) and polyethylene (PE) with pore sizes of 3.0 and 0.4 µm were tested. (b) Results of permeation experiments with fluorescein sodium salt through different 3D tissues in a 12 Transwell® system (n = 6). The 3D skin tissue consisted of collagen (Col.), collagen with primary human fibroblast (Col. + F.), collagen with HaCaTs (Col. + H.) and collagen with primary human fibroblast and HaCaTs (Col. F. + H.).

to 40,000 g/mol for permeation experiments in a 96 Transwell® system. The results show similar correlations between permeation coefficient and molecular size as the studies mentioned above. There is almost a linear relationship between these two factors, which is well described by the Navier–Stokes equation (see **Figure 7**). An exception is FITC-dextran 40,000 g/mol, which deviates from the linearity.

These experiments were simulated with COMSOL Multiphysics, where the diffusion coefficient is fitted on the experimental data. The simulation based on Fick's law is quite accurate for the permeation of substances with a small molecular size from 376 g/mol up to 4000 g/mol. The simulation shows good agreement with the experiment which is exemplary shown

http://dx.doi.org/10.5772/intechopen.72904

in **Figure 8a**. In the case of larger molecular size, the simulation is different from experimental results. A closer look shows that the experimental data increased and flattened faster compared to the simulation (see **Figure 8b)**. A possible reason for this effect could be the presence of particle size distribution in the substance. The migration of smaller particles reduces the lag time in the beginning of the permeation process, where large particles can increase the friction and slows down the diffusion. Especially the second effect leads to abnormal diffusion [46, 47]. This cannot be simulated with equations based on Fick's law.

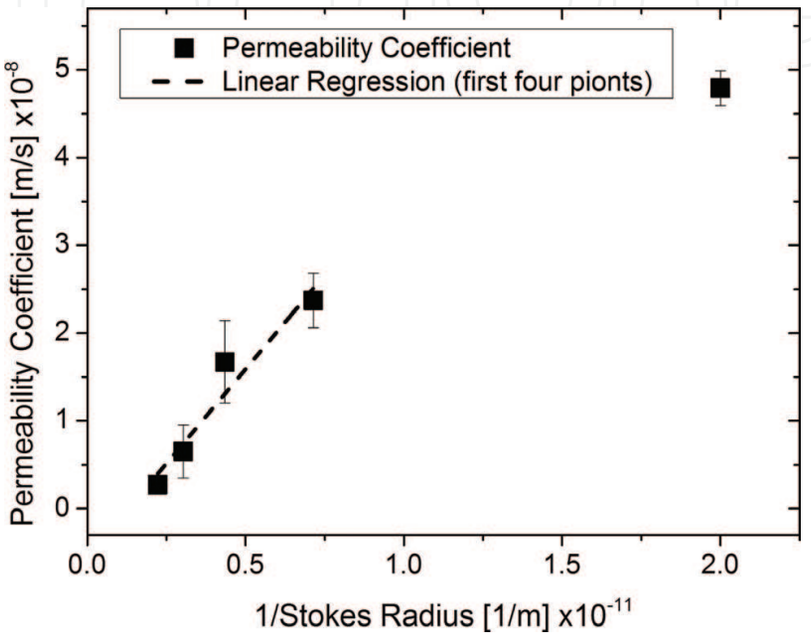

Figure 7. Permeability coefficient plotted over stokes radius. Results of permeation experiment in 96 Transwell® system with fluorescein sodium salt and FITC-dextran through 2% agarose gel.

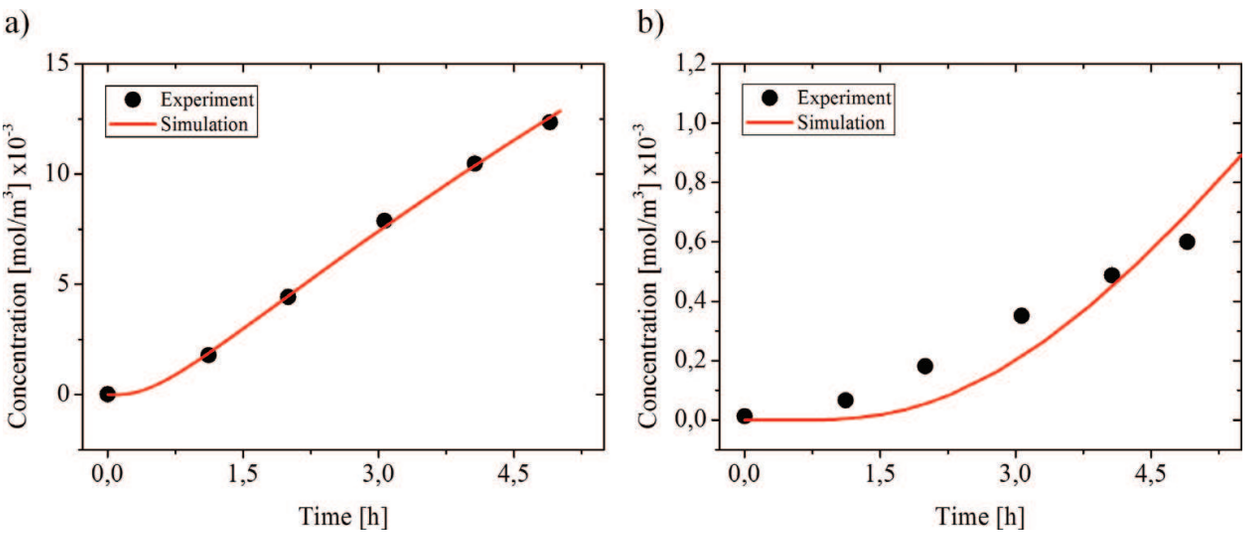

Figure 8. Simulation of permeation experiment with different molecular sizes. Results of permeation experiments and simulations with COMSOL **Multiphysics** of (a) fluorescein sodium salt and (b) FITC dextran 10,000 mg/mol in 96 Transwell systems through 2% agarose gel.

6. Conclusion and prospective

The studies have shown that the membrane insert system is a possible alternative for permeation studies. An advantage of the system is the small size. The membrane insert system of 96-well plates from CORNING has a cultivation surface of 0.143 cm^2 and a height of 2 cm. This reduces significantly the number of cells, materials and substances needed for the cultivation. In comparison to the Franz diffusion cell, the handling of such a system is easier and one experiment can be run with a large specimen number. A time-intensive mounting process of the samples (skin) is no longer required and the experiment can directly execute in the system. The sensitivity of this system is good enough to differ between 3D tissues as well as different cell layers and to detect different molecular sizes of the substance.

It should be considered that the permeation is detected through a membrane and the size of the system influences the permeation properties. The specimen has to cover up the whole membrane, otherwise the substance will pass by. Unlike the imaging and stripping method, it is not possible to measure the diffusion and penetration inside the membrane insert system. Alternatively, the diffusion can be calculated or estimated by simulation. Furthermore, this method can be used to investigate changes in the permeation behavior of the skin model during the cultivation or it can also be adapted for other systems, which use membrane insert systems. An example is the Two-Organ-on-a-Chip, a variant of TissUse's Multi-Organ-Chip platform [48–52]. This device enables the integration of skin models in a membrane insert system. Therefore this method can be used to investigate the permeation process into an organ-on-a-chip system in order to understand the substance distribution.

With the help of the simulation in COMSOL Multiphysics, it is possible to calculate the diffusion process in the membrane insert system. It is limited to small particle sizes and normal diffusion described by Fick's law. Otherwise, it is possible to optimize the simulation by integration of abnormal diffusion. Furthermore, the simulation is an attractive tool to support the experiments. On the one hand, it can be used to understand physical phenomena and to reduce experimental effort. On the other hand, it is modular and can be integrated into a more complex system to support permeation studies.

Acknowledgements

This work was created with financial support from Deutsche Forschungsgemeinschaft (DFG) under grant No. PO413/12-1 and LA 1028/7-1.

Appendices and nomenclatures

EC	European regulation
FITC dextran	fluorescein isothiocyanate-dextrans

FRAP	fluorescence recovery after photobleaching
FTIR	Fourier-transform-infrared
HaCaT	human adult low calcium high temperature
HSE	human skin equivalents
OECD	organization for economic co-operation and development

Author details

Hao-Hsiang Hsu[1], Katharina Schimek[2], Uwe Marx[2] and Ralf Pörtner[1]*

*Address all correspondence to: poertner@tuhh.de

1 Institute of Bioprocess and Biosystems Engineering, Hamburg University of Technology, Hamburg, Germany

2 TissUse GmbH, Berlin, Germany

References

[1] Ackermann K, Lombardi Borgia S, Korting H, Mewes K, Schäfer-Korting M. The Phenion® full-thickness skin model for percutaneous absorption testing. Skin Pharmacology and Physiology. 2010;**23**(2):105-112. DOI: 10.1159/000265681

[2] Asbill C. Evaluation of a human bio-engineered skin equivalent for drug permeation studies. Pharmaceutical Research. 2000;**17**(9):1092-1097. DOI: 10.1023/A:1026405712870

[3] Hadgraft J. Skin, the final frontier. International Journal of Pharmaceutics. 2001;**224**(1-2): 1-18. DOI: 10.1016/S0378-5173(01)00731-1

[4] Barry BW. Mode of action of penetration enhancers in human skin. Journal of Controlled Release. 1987;**6**(1):85-97. DOI: 10.1016/0168-3659(87)90066-6

[5] Valenzuela P, Simon JA. Nanoparticle delivery for transdermal HRT. Nanomedicine: Nanotechnology, Biology and Medicine. 2012;**8**:83-89. DOI: 10.1016/j.nano.2012.05.008

[6] Barbero AM, Frasch HF. Transcellular route of diffusion through stratum corneum: Results from finite element models. Journal of Pharmaceutical Sciences. 2006;**95**(10):2186-2194. DOI: 10.1002/jps.20695

[7] Bouwstra J, Pilgram G, Gooris G, Koerten H, Ponec M. New aspects of the skin barrier organization. Skin Pharmacology and Applied Skin Physiology. 2001;**14**:52-62. PubMed ID: 11509908

[8] Wertz PW. Lipids and barrier function of the skin. Acta Dermato-Venereologica. 2000; **208**:7-11. PubMed ID: 10884933

[9] Tsukita S, Furuse M, Itoh M. Multifunctional strands in tight junctions. Nature Reviews. Molecular Cell Biology. 2001;**2**(4):285-293. DOI: 10.1038/35067088

[10] Tsuruta D, Green KJ, Getsios S, Jonathan CRJ. The barrier function of skin: how to keep a tight lid on water loss. Trends in Cell Biology. 2002;**12**(8):355-357. PubMed ID: 12191905

[11] Knorr F et al. Follicular transport route – Research progress and future perspectives. European Journal of Pharmaceutics and Biopharmaceutics. 2009;**71**(2):173-180. DOI: 10.1016/j.ejpb.2008.11.001

[12] Schaefer H, Lademann J. The role of follicular penetration. Skin Pharmacology and Applied Skin Physiology. 2001;**14**:23-27. DOI: 10.1159/000056386

[13] Lademann J et al. Hair follicles—An efficient storage and penetration pathway for topically applied substances. Skin Pharmacology and Physiology. 2008;**21**(3):150-155. DOI: 10.1159/000131079

[14] Otberg N et al. The role of hair follicles in the percutaneous absorption of caffeine. British Journal of Clinical Pharmacology. 2008;**65**(4):488-492. DOI: 10.1111/j.1365-2125.2007.03065.x

[15] Wilkinson DS. Mass transport in solids and fluids. Cambridge University Press. 2000. ISBN: 9780521624947

[16] Cussler EL. Diffusion: Mass Transfer in Fluid Systems. 2nd ed. Cambridge University Press; 1997. ISBN: 0-521-45078-0

[17] Crank J. The Mathematics of diffusion. 2nd ed. Oxford, Clarendon Press; 1975. ISBN: 0-19-853411-6

[18] Netzlaff F et al. Permeability of the reconstructed human epidermis model Episkin® in comparison to various human skin preparations. European Journal of Pharmaceutics and Biopharmaceutics. 2007;**66**(1):127-134. DOI: 10.1016/j.ejpb.2006.08.012

[19] Jayakrishna A et al. Diffusion of high molecular weight compounds trough Sclera. IOVS. 2000;**41**(5):1181-1185. DOI: b, 19.02.2015

[20] Ackermann K, Lombardi Borgia S, Korting H, Mewes K, Schäfer-Korting M. The Phenion® full-thickness skin model for percutaneous absorption testing. Skin Pharmacology and Physiology. 2010;**23**(2):105-112. DOI: 10.1159/000265681

[21] Bran B et al. A new discriminative criterion for the development of Franz diffusion tests for transdermal pharmaceuticals. Journal of Pharmaceutical Sciences. 2010;**13**(2):218-230. DOI: 10.18433/J3WS33

[22] Pineau A, Guillard O, Favreau F, Marrauld A, Fauconneau B. In vitro study of percutaneous absorption of aluminum from antiperspirants through human skin in the Franz diffusion cell. Journal of Inorganic Biochemistry. 2012;**110**:21-26. DOI: 10.1016/j.jinorgbio.2012.02.013

[23] Larese Filon F et al. In vitro percutaneous absorption of cobalt. International Archives of Occupational and Environmental Health. 2004;(2):85-89. DOI: 10.1007/s00420-003-0455-4

[24] Ng S-F, Rouse JJ, Sanderson FD, Meidan V, Eccleston GM. Validation of a static Franz diffusion cell system for in vitro permeation studies. AAPS PharmSciTech. 2010;**11**(3):1432-1441. DOI: 10.1208/s12249-010-9522-9

[25] Bonferoni MC, Rossi S, Ferrari F, Caramella C. A modified Franz diffusion cell for simultaneous assessment of drug release and Washability of Mucoadhesive gels. Pharmaceutical Development and Technology. 1999;**4**(1):45-53. DOI: 10.1080/10837459908984223

[26] Bartosova L, Bajgar J. Transdermal drug delivery in vitro using diffusion cells. CMC. 2012;**19**(27):4671-4677. DOI: 10.2174/092986712803306358

[27] Pluen A, Netti PA, Jain RK, David A, Berk DA. Diffusion of macromolecules in agarose gels: Comparison of linear and globular configurations. Biophysical Journal. 1999;**77**(1):542-552. DOI: 10.1016/S0006-3495(99)76911-0

[28] Cornelissen LH, Bronneberg D, Oomens CWJ, Baaijens FPT. Diffusion measurements in epidermal tissues with fluorescent recovery after photobleaching. ISBS. 2008;**14**(4):462-467. DOI: 10.1111/j.1600-0846.2008.00313.x

[29] Leddy HA, Guilak F. Site-specific molecular diffusion in articular cartilage measured using fluorescence recovery after Photobleaching. Annals of Biomedical Engineering. 2003;**31**(7):753-760. DOI: 10.1114/1.1581879

[30] Seiffert S, Oppermann W. Systematic evaluation of FRAP experiments performed in a confocal laser scanning microscope. Journal of Microscopy. 2005;**220**(1):20-30. DOI: 10.1111/j.1365-2818.2005.01512.x

[31] Pirot F, Kalia YN, Stinchcomb AL, Keating G, Bunge A, Guy RH. Characterization of the permeability barrier of human skin in vivo. Proceedings of National Academy of Sciences USA. 1997;**94**:1562-1567. 0027-8424/97/941562-6$2.00/0

[32] Tetteh J et al. Local examination of skin diffusion using FTIR spectroscopic imaging and multivariate target factor analysis. Analytica Chimica Acta. 2009;**642**(1-2):246-256. DOI: 10.1016/j.aca.2009.03.002

[33] Guldbrand S et al. Two-photon fluorescence correlation spectroscopy as a tool for measuring molecular diffusion within human skin. European Journal of Pharmaceutics and Biopharmaceutics. 2013;**84**(2):430-436. DOI: 10.1016/j.ejpb.2012.10.001

[34] Berland KM, So PT, Gratton E. Two-photon fluorescence correlation spectroscopy: Method and application to the intracellular environment. Biophysical Journal. 1995;**68**(2):694-701. DOI: 10.1016/S0006-3495(95)80230-4

[35] Ghosn MG et al. Monitoring of glucose permeability in monkey skin in vivo using optical coherence tomography. Journal of Biophotonics. 2010;**3**(1-2):25-33. DOI: 10.1002/jbio.200910075

[36] Teichmann A et al. Differential stripping: Determination of the amount of topically applied substances penetrated into the hair follicles. The Journal of Investigative Dermatology. 2005;**125**(2):264-269. DOI: 10.1111/j.0022-202X.2005.23779.x

[37] Surber C, Schwarb FP, Smith EW. Tape-stripping technique. Cutaneous and Ocular Toxicology. 2002;**20**(4):461-474. DOI: 10.1081/CUS-120001870

[38] Weigmann H-J, Lademann MH, Schaefer H, Sterry W. Determination of the horny layer profile by tape stripping in combination with optical spectroscopy in the visible range as a prerequisite to quantify percutaneous absorption. Skin Pharmacology and Physiology. 1999;**12**(1-2):34-45. DOI: 10.1159/000029844

[39] Jacobi U, Weigmann H-J, Ulrich J, Sterry W, Lademann J. Estimation of the relative stratum corneum amount removed by tape stripping. ISBS. 2005;**11**(2):91-96. DOI: 10.1111/j.1600-0846.2005.00094.x

[40] Macfarlane M et al. A tiered approach to the use of alternatives to animal testing for the safety assessment of cosmetics: Skin irritation. Regulatory Toxicology and Pharmacology. 2009;**54**(2):188-196. DOI: 10.1016/j.yrtph.2009.04.003

[41] Basketter D, Jírova D, Kandárová H. Review of skin irritation/corrosion hazards on the basis of human data: A regulatory perspective. Interdisciplinary Toxicology. 2012;**5**(2):98-104. DOI: 10.2478/v10102-012-0017-2

[42] York M, Griffiths HA, Whittle E, Basketter DA. Evaluation of a human patch test for the identification and classification of skin irritation potential. Contact Dermatitis. 1996; **34**(3):204-212

[43] Prunieras M, Regnier M, Woodley D. Methods for cultivation of keratinocytes with an air-liquid Interface. The Journal of Investigative Dermatology. 1983;**81**:28-33. DOI: 10.1111/1523-1747.ep12540324

[44] Mathes SH, Ruffner H, Graf-Hausner U. The use of skin models in drug development. Advanced Drug Delivery Reviews. 2014;**69-70**:81-102. DOI: 10.1016/j.addr.2013.12.006

[45] Peck KD, Ghanem A, Higuchi WI. Hindered diffusion of polar molecules through and effective pore radii estimates of intact and ethanol treated human epidermal membrane. Pharmaceutical Research. 1994;**11**(9):1306-1314. DOI: 10.1023/A:1018998529283

[46] Laurent TC, Sundelöf L-O, Wik KO, Wärmegard B. Diffusion of dextran in concentrated solutions. European Journal of Biochemistry. 1976;**68**:95-102. DOI: 10.1111/j.1432-1033.1976.tb10767.x

[47] Metzler R, Klafter J. The random walk's guide to anomalous diffusion: A fractional dynamics approach. Physics Reports. 2000;**339**(1):1-77. DOI: 10.1016/S0370-1573(00)00070-3

[48] Ataç B et al. Skin and hair on-a-chip: In vitro skin models versus ex vivo tissue maintenance with dynamic perfusion. Lab on a Chip. 2013;**13**(18):3555-3561. DOI: 10.1039/c3lc50227a

[49] Maschmeyer I et al. Chip-based human liver–intestine and liver–skin co-cultures – A first step toward systemic repeated dose substance testing in vitro. European Journal of Pharmaceutics and Biopharmaceutics. 2015;**95**:77-87. DOI: 10.1016/j.ejpb.2015.03.002

[50] Materne E-M et al. The multi-organ Chip - a microfluidic platform for long-term multi-tissue coculture. JoVE. 2015;**98**:1-11. DOI: 10.3791/525264

[51] Schimek K et al. Integrating biological vasculature into a multi-organ-chip microsystem. Lab on a Chip. 2013;**13**(18):3588. DOI: 10.1039/c3lc50217a

[52] Wagner I et al. A dynamic multi-organ-chip for long-term cultivation and substance testing proven by 3D human liver and skin tissue co-culture. Lab on a Chip. 2013;**13**(18):3538. DOI: 10.1039/c3lc50234a

[53] Hsu H et al. A method for determination and simulation of permeability and diffusion in a 3D tissue model in a membrane insert system for multi-well plates. JOVE. 2017 https://www.jove.com/video/56412/a-method-for-determination-simulation-permeability-diffusion-3d

IntechOpen