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FELLOWSHIP FINAL REPORT

Knowledge transfer on RAMAN spectroscopy and skin-on-a-chip technology to study transdermal drug delivery

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ABSTRACT

Several ex vivo and in vitro skin models are available in the toolbox of dermatological and cosmetic research. Some of them are widely used in drug penetration testing. The excised skins show higher variability, while the in vitro skins provide more reproducible data. The aim of the current study was to compare the chemical composition of different skin models (excised rat skin, human skin and human reconstructed epidermis) by measurement of ceramides, cholesterol, lactate, urea, protein and water at different dephts of the tissues. The second goal was to compile a testing system which includes a skin-on-a-chip diffusion setup and a confocal Raman spectroscopy for testing drug diffusion across the skin barrier and accumulation in the tissue models. A hydrophylic drug caffeine and the P-glycoprotein substrate quinidine were used in the study as a topical cream formulation. The results indicate that although the transdermal diffusion of quinidine is lower, the skin accumulation was similar for the two drugs. The different skin models allowed comparable permeability for both compounds, but chemical composition differed. The human skin was abundant in ceramides and cholesterol, while the reconstructed skin contained less water and more urea and protein. Based on these results it can be concluded that skin-chip and confocal Raman microspectroscopy are suitable for monitoring drug penetration and distribution in different skin layers during and at the end of exposure. Furthermore, the human skin obtained from obese patients is not the most relevant model for skin absorption testing in pharmaceutical research.

1- Introduction

The aim of the current experiments was to study the chemical composition of excised rat skins (subjected to mechanical pretreatment), the human reconstructed epidermis (HRE) (MatTek EpiDerm) and excised human skins from plastic surgery, by confocal Raman spectroscopy, and also to investigate their permeability and penetration capacity for two model drugs. The effect of the diffusion study itself on the ex vivo and in vitro skins (barrier function and epidermis composition) was evaluated at the end of skin-on-a-chip experiments as well. Caffeine was used as a hydrophylic model drug (logP=-0.07, Brown et al 2009) and quinidine is a well-known P-glycoprotein (P-gp) substrate compound (Sziráki et al, 2011; Sziráki et al, 2013) with low water solubility (logP=3.44, Pyka-Pajak et al, 2006). P-glycoprotein was found to be expressed in different cell types of the skin (keratinocytes, melanocytes, endothelial cells) (Ito et al, 2008; Fujita et al, 2017, Li et al, 2006) and its absorptive orientation has been reported by different research groups (Hashimoto et al, 2017, Bajza et al, 2020). Based on these data a three-step study protocol had been working out. First a composition analysis was performed in the intact skin models, then the subjects were mounted in a skin-on-a-chip diffusion chamber and treated topically with the cream formulation of the model drugs and

subsequently a 5-hours diffusion study has been conducted. Thereafter the tissues have been analysed again by confocal Raman spectroscopy and the skin accumulation of the model drugs was determined. The transepidermal water loss (TEWL) as an indicator of epidermal barrier function has also been measured before and after the diffusion experiments.

2- Experimental details

2.1. Excised skin preparation

Male Wistar rats (ToxiCoop, Budapest, Hungary) with 250–350 g bodyweight (2.5–3.5 months old) were used for skin preparation. The animals had free access to food and water before the study. The experiments were performed in compliance with the guidelines of the Association for Assessment and Accreditation of Laboratory Animal Care International, and were in accordance with the spirit of the license issued by the Directorate for the Safety of the Food Chain and Animal Health, Budapest and Pest Agricultural Administrative Authority, Hungary (PE/EA/4122-7/2016). Rat skins were used for the skin composition and skin diffusion experiments. The animals were kept at least 5 days in animal facility (22± 3oC, 50± 20% humidity and 12 hours light-dark cycle) before the excision of the skin. Then the rats were deeply anaesthetised with 400 mg/kg i.p. chlorale-hyrate. The abdominal skin was shaved and epilated with a commercially available epilatory cream (ISANA® cream from Rossmann, Burgwedel, Germany). After 5 minute expostion time the cream was removed by a wipe and the skin surface was gently washed with tap water. After drying, the skins were either tape stripped 10 times (10TS), or left intact (0TS controls). The proper size of the tissue was then excised and the samples were placed on -80 oC deep freezer until the diffusion experiments.

Human abdominal skin tissues obtained from plastic sugery clinics (Révész Plasztika, Budapest, Hungary) (permission number: 6501- 6 /2019/EKU, Budapest, Hungary) were used only for skin composition analysis experiments. The skins were kept in -80oC until the Raman spectroscopy. On the afternoon, before the day of the diffusion or Raman experiments, the tissues were taken from the freezer and transported to 4-8 oC cold room where they were defrost overnight. Next day the tissues were subjected to Raman analysis in the RiverD system (GEN-2, Rotterdam, The Netherlands) and then the rat skins were mounted in the skin-on-a-chip device, where the subcutaneus surface faced to the peripheral perfusion fluid (PPF) and the startum corneum was exposed to the cream treatment. The composition of PPF is described at 2.3..

2.2. Human reconstructed epidermis (HRE) preparation

EpiDerm human reconstructed in vitro epidermal tissues were purchased from MatTek Life Sciences, Bratislava, Slovakia. The histological structure of the HRE is shown in Figure 1. The 3D skin models were used within three days after delivery and in the meantime the tissues were kept on 4-8oC in a cold room. The afternoon, before the day of the diffusion experiment, the tissues together with their transwell inserts were transported into a six-well plate (provided by the manufacturer) and placed into 0.9 mL medium (provided by the manufacturer) at 37oC . The tissues were then incubated overnight (37 oC, 5% CO2). Next morning after 30 minute hydration in Dulbeco-Phosphate-Buffered Saline (D-PBS) (provided by MatTek) on 32oC, the inserts were placed into the skin-on-a-chip device and the perfusion was started at 4 µL/min flow rate, on 32oC. The test formulation was pipetted on the surface of stratum corneum using MicroMan gel pipette (Gilson, Middleton, WI, United States), and the sample collection started immediately after it. Thereafter the perfusate samples were taken in every 30 min through 5 hours.

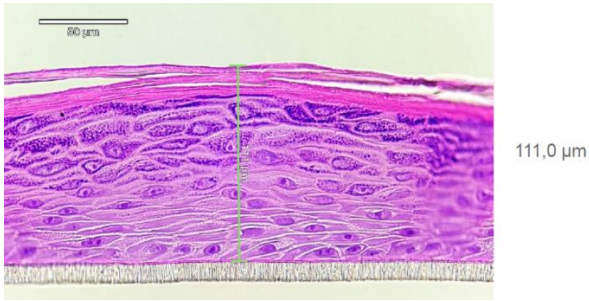

Figure 1. Histology report on EpiDerm tissue (HRE, EPI-200; LotNo:36125)) after hematoxilin-eosin staining

provided by MatTek Life Sciences. Well differentiated epidermis consisting of basal, spinous, granular layer of keratinocytes and stratum corneum. At least 4 viable cell layers are present. The tissue thickness is 70-130 µm (average 111.0 µm).

2.3. Solutions

In the skin-on-a-chip microfluidic device, peripheral perfusion fluid (PPF) was used as an extracellular fluid substitute acceptor solution in excised skin studies. It is composed of the following components: 147 mM NaCl, 4 mM KCl, and 2.3 mM CaCl2·2 H2O. All substances were acquired from Sigma-Hungary Kft, Budapest, Hungary. For human reconstructed skin substitutes D-PBS was used as provided by MatTek Life Sciences (Bratislava, Slovakia) in the EpiDerm (Epi-212) kit.

2.4. Model drugs and formulations

Caffeine was used as a hydrophylic model drug and quinidine was used as P-glycoportein (P-gp) substrate model drug. Both drugs were purchased from Sigma Hungary Kft, Budapest, Hungary, and applied as a suspension cream. 2 g of caffeine/quinidine were dispersed for cream formulation with 4.1 g of liquid paraffin in a mortar with pestle. Then 47 g of white soft paraffin cream (containing polysorbate60 4 %, white soft paraffin 26 %, liquid paraffin 8 m/m%, propylene glycol 10 %, cetostearyl alcohol 12 m/m%, and purified water 40 %), 10 g propylene glycol, and 36.9 g of 0.21 % citric acid aqueous solution were added.

2.5. Skin-on-a-chip diffusion studies

Similarly to the traditional Franz-diffusion cell system, the polydimethylsiloxane-based microfluidic chip is also composed of three functional elements: on top was a donor compartment where the examined formulation was placed, the bottom was the receptor compartment, and in middle an integrated skin sample was placed, as described in details in our previous papers [13–15].

The diffusion surface of the skin was 0.50 cm2 and it was treated with 0.7-0.8 g of the different formulations (caffeine or quinidine creams, 2% of each) by a positive displacement piston gel pipette.

Contrary to static Franz-diffusion system, the microfluidic diffusion chamber is a dynamic system, where the flow is continuous below the treated skin at the subcutaneous area. The PPF or D-PBS solution were loaded into a 5 mL syringe. Then a tube was connected to the microfluidic chip, the air bubbles were removed from the syringe, the connected Teflon tubing and from the microchannel of the chip. Flow rate was kept at 4µL/min during the experiments, generated by a programmable syringe pump (NE-1000, New Era, Farmingdale, NY, USA). The whole setup was placed into a dry thermostate incubator on 32oC for 6 hrs. The PPF/D-PBS solution was running through the chip, filling the receptor chamber reservoir and the microfluidic channel and leaving the device at the outlet into the collection vials. The perfusates were analysed by HPLC for caffeine or quinidine content immediately after the diffusion experiment.

2.6. Determination of caffeine and quinidine in the perfusion samples (HPLC)

High pressure liquid chromatography (HPLC) analysis was performed using an Ultimate 3000 (Thermo Fisher Scientific, Voisins-le-Bretonneux, France) piloted with Chromeleon 7.1 software. A C18 column with the particle size of 5 µm and length of 4.6×150mm (Interchim, Montluçon, France) was used and adjusted on 25 °C. The software used was Chromeleon 7.1 (Thermo Scientific, chromatography data system software).

For caffeine quantification: the samples were analysed to quantify the concentration of caffeine and establish the time-dependent permeation kinetics using a DAD UV ultimate 3000 detector. Detection wavelength for caffeine was 272 nm. The mobile phase used in isocratic mode was methanol 50%: water 50% (v/v) + 10 mM of phosphoric acid. The injection volume of samples

was 10 µL. The duration of the analysis of each sample was 6 min at a flow rate of 1 ml min-1. A standard calibration curve was prepared prior to analysis. Standards were obtained in triplicates by dissolving caffeine in PBS at concentrations of 1, 5, 10, 100 and 500 µg mL-1. The observed retention time was 2.621 ± 0.008 min.

For quinidine quantification: the samples were analysed using a DAD UV Ultimate 3000 detector. Absorbance was recorded at 250 nm. The analysis was performed in isocratic mode using a acetonitrile / water (10% / 90%) mobile phase supplemented with 3 g of hexylamine, the pH of the mobile phase was adjusted to 2.8 by adding phosphoric acid, according to the European pharmacopeia method. The injection volume of samples was 10 µL. The duration of the analysis of each sample was 5 min at a flow rate of 1 ml min-1. A standard calibration curve was prepared prior to analysis. Standards were obtained in triplicates by dissolving quinidine in the mobile phase at concentrations of 0.1, 0.5, 1, 5 and 10 µg mL-1. The observed retention time was 3.033 ± 0.005 min.

2.7. Confocal RAMAN spectroscopy

Confocal Raman Spectroscopy was performed for analysis of skin spectra, using a confocal Raman microspectrometer (GEN2 Skin composition Analyzer, River Diagnostics, Rotterdam, The Netherlands) equipped with two incorporated lasers, operating at different wavelengths. The first laser wavelenght was 785 nm and used for the analysis of the skin fingerprint region (FP) (400–1800 cm-1), with a 25 mW power on the skin. The second laser, at 671 nm was used for the analysis of the high wave number (HWN) region (2500–4000 cm-1), and its power was 20mW on the skin.

For each skin sampels, 15 areas were recored using depth profiling (z-profiling) for both FP and HWN regions. The FP z-profilings were carried out with a 3 s exposure time and 4 µm steps up to 28 µm. For the HWN measurements, 1 s exposure time and 4µm steps were used for z-profiling up 40 µm as a final depth. For each skin samples, 15 locations were recorded in both FP and HWN region.

The instrument is daily calibrated using a integrated Neon-Argon lampe which features peaks at precisely known emission wavelenghts. The wavelenghts shift is calibrated using a polymethyl methacrylate (PlexiglasTM) standard.

Skin components profiles, namely ceramides, fatty acids, cholesterol, lactate (pH=4), proteins, urea and water content were calculated using the SkinTools 3 analysis software (RiverD International B.V., version 3.3.201202, Rotterdam, The Netherlands) according to the pervious work of Caspers et al. [16].

The quantification of caffeine and quinidine were calculated using the SkinTools 3 analysis software (RiverD International B.V., version 3.3.201202, Rotterdam, The Netherlands) according to quantification method described by Caspers et al. [17].

The device is regularly used for in vivo and ex vivo analysis of both human and porcine skin [18,19] and is able to monitor treatment-induced changes in skin parameters.

2.8. Transepidermal water loss (TEWL)

The TEWL is an important parameter that is representative of the skin's barrier function in vivo. In ex vivo experiments, it can serve as an indicator for the integrity of the skin barrier. TEWL measurements were performed with the condenser-chamber device Vapometer® (Delfin Technologies Ltd., Kuopio, Finnland). With its closed-chamber principle the Vapometer® creates a microclimate within the measurement compartment. Thus, measurements remain unaffected by external air turbulences. The device measures water evaporating from the skin in g/m2/h. Increased TEWL indicates skin barrier damage [20–22].

2.9. Data analysis

Data analysis were perfomed using SkinTools 3 (RiverD International B.V., version 3.3.201202, Rotterdam, The Netherlands). The spectra were cut between 400 and 1800 cm-1 for the FP region. For the HWN region the spectra were cut between

2500 and 3600 cm-1. A baseline subtraction was applied, using a 3rd degree polymer function.

3- Results and discussion

The skin composition of human and rat skin and also the human reconstructed epidermis was analysed by testing 6 different indicators: ceramides and fatty acids, cholesterol, lactate (pH4), water, urea and proteins. Ceramides are a family of waxy lipids which are composed of sphingosine and a fatty acid. Ceramide is the main component of the stratum corneum of the epidermis in human skin [23,24]. Ceramides, cholesterol and saturated fatty acids create a water-impermeable, protective structure to prevent water loss through the skin due to evaporation, as well as they make a barrier against the entry of microorganisms and external chemicals [24]. The stratum corneum is composed of 50% ceramides, 25% cholesterol, and 15% free fatty acids [25]. Cholesterol synthesis is mainly essential for skin barrier homeostasis [26–28], but a recent research has speculated that the stratum corneum cholesterol domains may have a more complex role in the skin, other than a barrier limiting water loss and the entry of chemicals [29]. Numerous studies have shown that skin cholesterol content is associated with deposition of cholesterol in the coronary arteries and aorta.

Lactate is a component of natural moisturizing factor (NMF) and has a complex role in the skin. It stabilizes the pH, has moisturizing effect (together with proteins, urea, lactic acid, sugar and inorganic ions such as potassium and magnesium ions), anti-aging and keratolytic action [30]. Due to these functions sodium lactate is frequently used ingredient in various cosmetics. Proteins and urea are also belonging to the NMF family and have moisturizing, rehydrating and anti-aging effects. They are used both in dermatological products and in cosmetics.

The water content e.g. the hydration is a key aspect of the skin that influences its physical and mechanical properties. Hydration leads to changes in the molecular arrangement of the peptides in the keratin filaments as well as dynamics of C-H bond reorientation of amino acids in the protruding terminals of keratin protein within the stratum corneum. The changes in molecular structure and dynamics occur at a threshold hydration (ca. 85% relative humidity) [31].

The ceramide/fatty acid composition of rat skin, HRE and human skin are presented in Fig 2A. The composition profile characteristics were similar in rat and human excised skins (the highest values can be seen in the skin surface and there is a monotonous decrease with the depths. The HRE showed a moderately increasing tendency with the depth. Cholesterol (Fig 2B) was also parallel in excised skins (human and rat), but different in EpiDerm (HRE). In the reconstructed tissue an increase was followed by reduction of cholesterol levels in the deeper layers. Lactate profile (Fig 2C,) was comparable in rat skin and HRE, but different in human tissue, showing a higher lactate level in the outermost layer of the startum corneum, and decreasing values at deeper z-profiles. The water content (Fig 2D) peaked at about 12-16 μm depths in the border zone of dead and viable epidermis in rat and human skins, while in HRE the highest hydration can be observed in the surface.

The proteins (Fig 2E) and urea (Fig 2F) showed similar characteristics in human and rat skins with a maximum concentration at 12 and 8 μm depths for urea and at 4 μm for proteins, respectively. On the contrary, HRE has the maximum urea content on the outermost surface of startum corneum and the protein levels were increasing to 8 μm depths and then showed a plateau level in the whole thickness studied (until 28 μm depths).

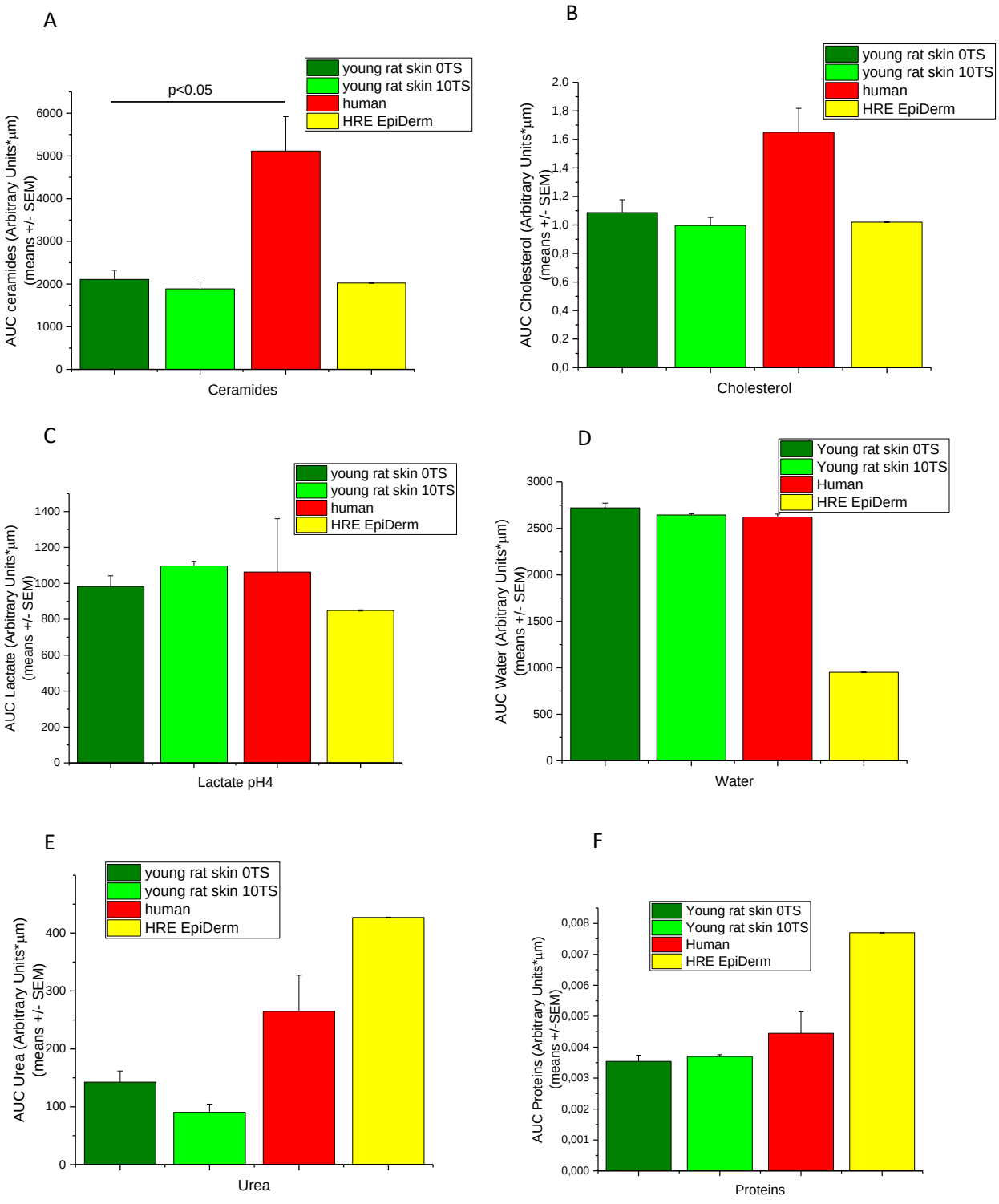

Figure 2. Area under the skin depths-Arbitrary Units curves (AUC) for intact rat skins (0TS), mechanically pretreated rat skins (10TS), intact human skins and EpiDerm human reconstructed epidermis (HRE). Measurements were taken on the epidermis at 8-10 different locations for 0-40 □m depths for water, and for 0-28 □m depths for all the other skin components. (Number of subjects=3-2, with N= 8-10 locations for each).

3.4. Transdermal absorption of the model drugs (caffeine & quinidine)

Caffeine penetration from cream formulation through the rat skin (10TS) and HRE was investigated in skin-on-chip diffusion chamber. The concentration time-profiles of the two different diffusion subjects were similar. The cumulative amounts reached 25 and 28 ng/cm2 values after 5 h dinamic perfusion with PPF (Fig 3A,B).

After the skin-chip study the accumulated caffeine content was also analyzed at 8 different skin depths from 0-28 μm) in the subjects (rat skin 10TS and HRE) (Fig 4A,B). As it can be seen in the Raman spectra, the full thickness excised skin accumulated approximately twice more caffeine than the HRE. These results can be explained by the higher water content of the rat skin than the HRE as it is shown in Fig 2D.

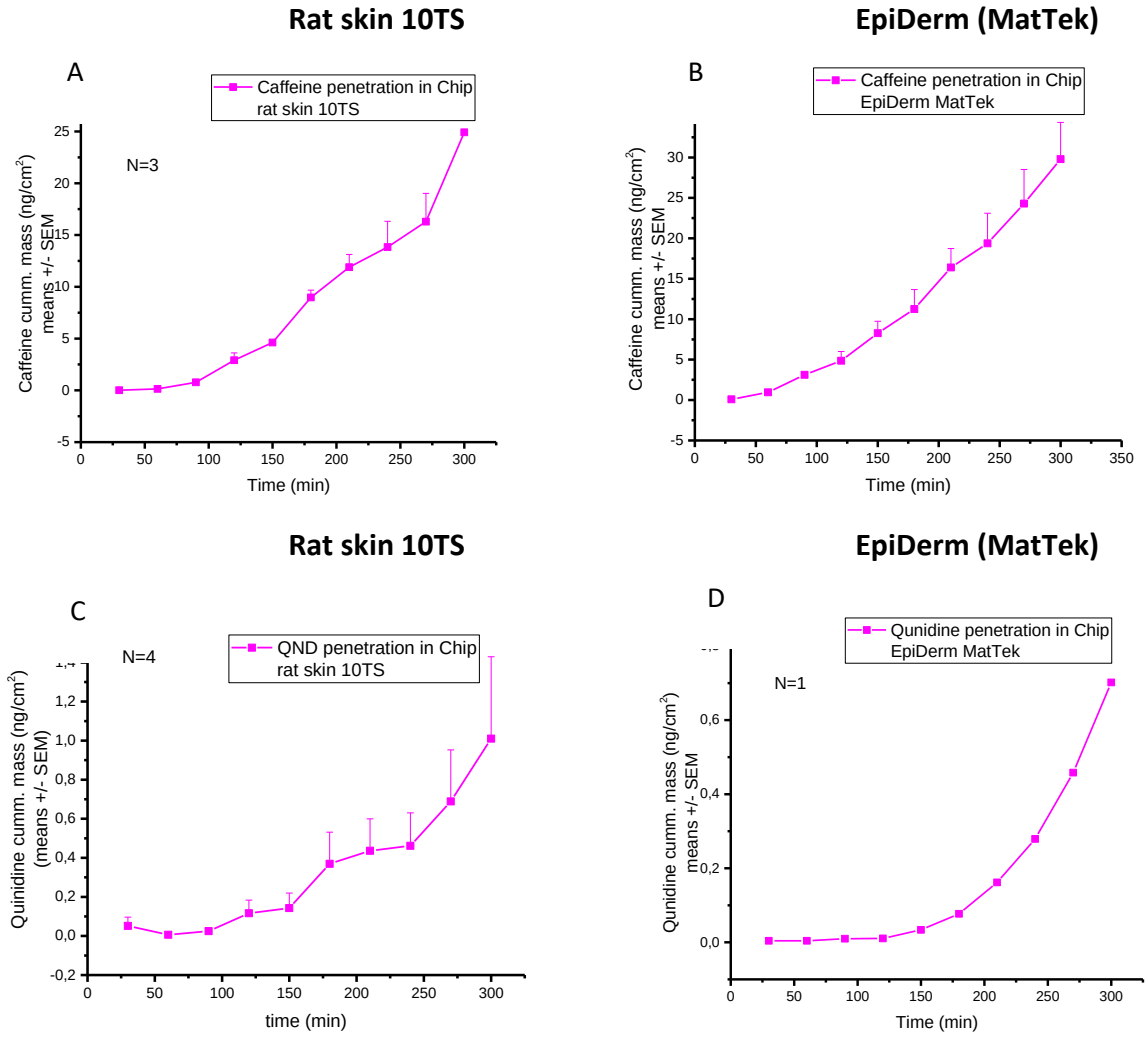

Figure 3. Transdermal diffusion (cumulative mass ng/cm^2) of 1) caffeine, in mechanically pretreated rat skins (A) and EpiDerm HRE tissues (MatTek) (B), and 2) quinidine in mechanically pretreated rat skins (C) and EpiDerm HRE tissues (MatTek) (D). The investigations were performed in skin-on-a-chip microfluidic device. N=3-4 for rat skins and N=2-1 for EpiDerm human reconstructed epidermis model.

3.5. Tissue penetration of the model drugs (caffeine & quinidine)

Quinidine penetration from cream formulation through the rat skin (10TS) and HRE was also investigated in skin-on-chip diffusion chamber. The concentration time-profiles of the two different diffusion subjects were similar in shape, but the transepidermal diffusion was moderately higher in the excised skins than in the reconstructed tissue. The maximum cumulative amounts of quinidine reached were 0.7 ng/cm2 in HRE and 1 ng/cm2 in rat skins after 5 h dinamic perfusion with PPF (Fig 3C,D).

After the skin-chip study the accumulated quinidine content was also analysed at 8 different skin depths from 0-28 μm in the two subjects (Fig 4C,D). As it can be seen in the Raman spectroscopic spectra, the full thickness excised skin accumulated less quinidine than the HRE. As quinidine is a relatively lipophilic and non water soluble compound (logP=3.4), the low hydration of

the reconstructed epidermis might be better chemical environment than the skin for the accumulation for this substance.

Comparing caffeine and quinidine diffusion (Fig 3A,B vs Fig 3C,D), the degree of absorption across the skin or skin substitutes is much higher in case of caffeine indicating that caffeine is more soluble both in the cream formulation, and in the extracellular matrix of the skin. In excised tissues caffeine is able to penetrate across the barrier through the transappendageal route and easily diffuse into the dermis and subcutis. On the other hand, quinidine is the substrate of several efflux transporters which are expressed in the various skin cells. As indicated by Raman spectroscopy, the accumulation of quinidine is similar or even higher in the subjects than that of the caffeine, which can be a consequence of absorptive orientation of the efflux pumps for quinidine

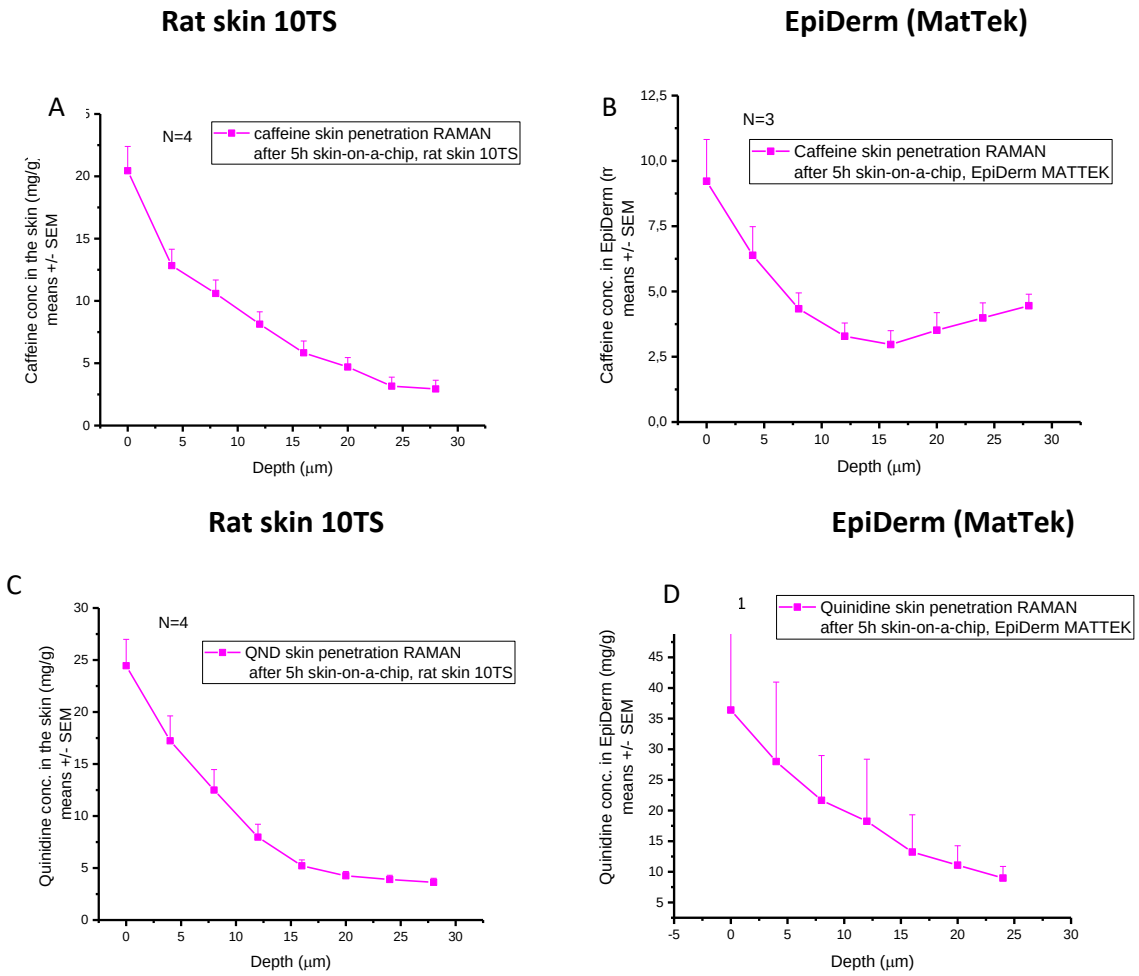

Figure 4. Accumulation of the two model drugs (caffeine and quinidine) in the skins and skin substitutes determined by Confocal Raman Spectroscopy. Caffeine penetration in mechanically pretreated rat skins (A) and in EpiDerm HRE tissues (MatTek) (B). Quinidine penetration in mechanically pretreated rat skins (C) and in EpiDerm RHE tissues (MatTek) (D). 4 rat skins and 3-1 EpiDerm human reconstructed epidermis were studied. N=7-10 different z-profiling positions were applied for each individual sample.

3.6. Epidermal barrier function

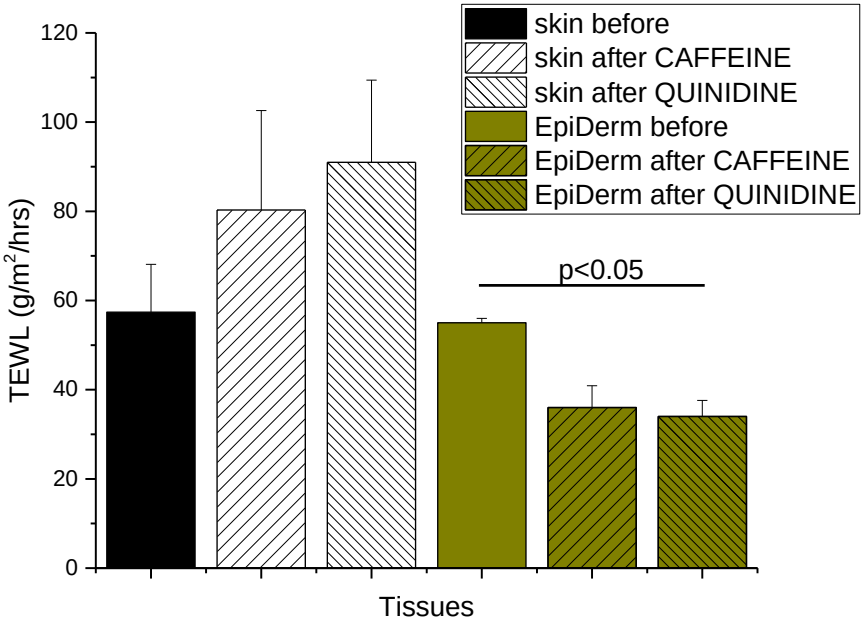

Figure 5. Transepidermal water loss (TEWL) as the indicator of dermal barrier function, determined in 1) rat skin samples after 10 tape strippings before any kind of treatment or after 5h exposition to 2% caffeine or quinidine cream in skin-on-a-chip device; 2) in EpiDerm HRE tissues before any kind of treatment or after 5h exposition to 2% caffeine or quinidine cream in skin-on-a-chip device. N=11 for the skin before, and N=3-4 for the other groups. Data are expressed as means ± SEM.

The thickened startum corneum (10TS) of excised rat skins showed similar barrier function to HRE as indicated by the TEWL values (57.4±10.7 and 56.0±1.0 g/m2/h, respectively). However, after 5h exposition to drug containing creams (caffeine or quinidine) and continuous perfusion in skin-chip, an increasing permeability was demonstrated in rat skins (80.3±22.3 and 91.0±18.4 g/m2/h), and stronger barrier function could be seen in HRE (36.0±4.9 and 34.0±3.6 g/m2/h). The difference between the TEWL values of control HRE and HRE after quinidine exposure was statistically significant (p=0.0356 by Student-t test). The standard error (SEM) values and therefore the variability of the model was much lower in reconstructed epidermis, than in rodent skins.

4- Discussion and conclusion

Reconstructed human epidermis was used for the first time in this study for analyzing the transepidermal diffusion and accumulation of small molecule model drugs in skin-on-a-chip and by confocal Raman spectroscopy. The current setup seems to be feasible for applying transwell inserts directly on the chips. The dynamics, induced by dermal microcirculation was also considered using continuous perfusion of the device across the microfluidic channel with physiological solutions. Some more complex systems were recently reported, where the tissue flexibility, the capillary endothelial network and the immunological cell types (e.g. HL-60 leukocytes) are also included to the microfluidic system [32,33]. The future research should focus on the creation of more physiological in vitro systems with own vascularity and immunological components to provide more appropriate structure and composition of the artificial tissue. These investigations can be regarded as an early stage

discovery research, which makes possible a relatively rapid screening of the test drugs for barrier penetration and tissue accumulation. As the main components of the skin barrier are localized in the stratum corneum and granular epidermis (tight junctions) HRE also includes the most important elements of this barrier.

The basic questions of the current study were whether 1) the 5-hour diffusion cell study has an impact on the properties of the skins/skin equivalents, 2) is there any difference between the composition of human skin, rat skin and HRE, 3) can the transdermal diffusion be measured in parallel with the skin accumulation of the test drugs (caffeine and quinidine), 4) can the human skins be replaced with rat skins or with HRE in skin-on-a-chip diffusion experiments?

Based on the results the following answers can be given to the above questions: 1) In preliminary experiments the rat skins were tested in skin-on-a-chip device without topical treatment and with 5-hour topical treatment with blank cream (data are not shown). No difference was observed in the skin composition before and after the study. In the second series of the experiments 5-hour topical treatment with caffeine/quinidine creams was applied and no difference found in ceramide, cholesterol, lactate, water, urea and protein contents. The barrier function of the skins was also checked before and after the study, and impaired permeability was found in animal skin, but better barrier function was seen in HRE. These results indicate that HRE is more resistant to the shear stress occurring in the skin-chip, and also the placement of transwell inserts containing HRE to the chip is non-invasive, contrary to the tension which is applied on the border of skin tissues during fixation into the chip (first generation device). Also high increase in the hydration could be observed in rat skins after the diffusion experiments 2) The first difference in the skin composition can be detected in ceramide, fatty acids and cholesterol contents. The level of these factors are much higher in the human skin tissues which can be explained by the obesity of the donors. This observation indicates that not all human skin sample is good for pharmaceutical or dermatological studies. Sometimes the donors from plastic surgery are not well-comparable with the healthy individuals, and a normal animal skin, or HRE can be more relevant or more predictive to the physiological conditions. The next difference between the subjects tested is, that the HRE contains less water but more elements of natural moisturizing factor (NMF), like urea or proteins, than the skin tissues. 3) This study provided evidence that it is possible to measure the transdermal/transepidermal diffusion in skin and skin equivalent and afterward the tissue models can be transferred to measurement window of confocal Raman spectroscopy and the drug accumulation could also be detected layer-by-layer. This finding can be important in dermatological drug developments to predict the pharmacokinetic profile of the medicaments, and also the localize the main portion of the drug within the skin as a function of time. 4) As it is shown in Fig 3, the diffusion study provided comparable results in rat skin and in HRE both in case of caffeine and in quinidine. Furthermore, the data are more even, and the variability is much lower in the artificial tissues.

In summary, the findings of current research confirm that HRE can be a good substitute of human or animal tissues in diffusion studies also in skin-on-a-chip, although there are some differences in the composition of the structural elements between HRE and skins. Furthermore, the pathological human obese skin tissues can be less relevant models in pharmaceutical studies than HRE for prediction of drug penetration and accumulation in physiological or dermatological conditions (like psoriasis, atopic dermatitis and inflamed or dry scaly skin). For testing drug absorption in these skin disorders appropriate preclinical models are available [34–36] and these can be used. To analyze the skin composition in various dermatological conditions further studies are needed.

5- Perspectives of future collaborations with the host laboratory

The current results will be further analyzed and published in an international journal together with the host laboratory. Some site visits and student exchange are also planned for the next years.

6- Articles published in the framework of the fellowship

The fellowship program was only three months which is quite short period for preparing articles. But it was a good incipience for a larger collaboration. The technology transfer was successfully accomplished, and some data has already been presented in the Le STUDIUM Thursday in Tours (March 03, 2022) and the Le STUDIUM virtual Conference (Skin Models in Cosmetic Science: Bridging Established Methods and Novel Technologies, 2nd meeting, April 7-8 2022).

7- Acknowledgements

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