Why performing an *in vitro* dermal absorption study?

Patrick DUCHENE - PhD.

Safety evaluation of cosmetic ingredients

Safety evaluations take into consideration a number of key factors including:

- **Cosmetic ingredient function**
- **Use concentration**
- **Degree of chemical purity and stability**
- **Exposure to cosmetic ingredients:**

Exposure to cosmetic ingredients occurs mainly via the skin.

In order to reach the circulation (blood and lymph vessels), cosmetic ingredients must cross a number of cell layers of the skin, where the rate-determining layer is considered being the stratum corneum (SC).

Absorbed amount:

The sum of the amounts recovered in the stratum corneum, dermis, epidermis and the receptor fluid will be considered as dermally absorbed and taken into account for further calculations.

Within the European Union and some other countries, can be excluded from calculations:

- **Test substance retained in the top few layers of the stratum corneum (i.e. contained in the first (upper) two tape strips) may be removed by desquamation and therefore may not be absorbed.**
- **This includes also substances that has not penetrated into the stratum corneum but is protected from wash-off, for example in hair follicles or sweat ducts.**

Safety evaluation of cosmetic ingredients

Systemic Exposure Dosage:

Absorbed amount

Determination of Systemic Exposure Dosage (SED)

In the absence of data, 100% dermal absorption has to be assumed to cover a "worst case" scenario.

Many regulatory Authorities will consider a reduction of the 100% default value to 10% if both the molecular weight is greater than 500 and the log Pow is either below –1 or above 4.

Margin of safety (MoS):

The SED can then be taken into consideration to calculate the margin of safety (MoS) using the No Observable Adverse Effect Level (NOAEL) of an appropriate repeated dose toxicity study with the respective ingredient when available:

MoS = NOAEL / SED

should be at least 100

Pharmaceutics: study of drug formulation

In vitro **release can be used to characterize performance characteristics of a finished topical dosage.**

Important changes in the characteristics of a drug product formula or the thermodynamic properties of the drug(s) it contains should show up as a difference in drug release

Guidance for Industry: Nonsterile Semisolid Dosage Forms Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls; In Vitro Release Testing and In Vivo Bioequivalence Documentation (SUPAC-SS CMC 7)

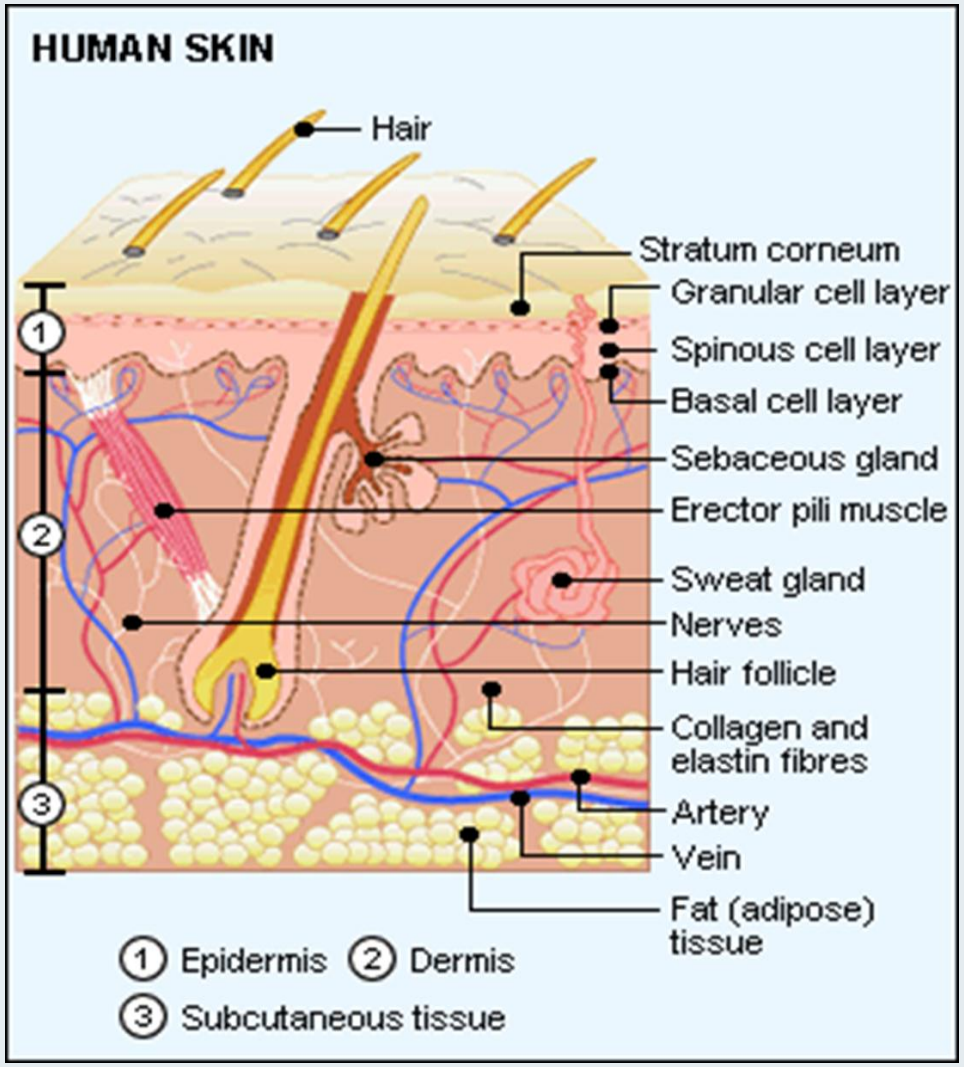
Use of synthetic membranes

In vitro **release method for topical dosage forms is based on an open chamber diffusion cell system, such as a Franz cell system fitted usually with a synthetic membrane :**

Appropriate inert and commercially available synthetic membranes, such as polysulfone, cellulose acetate/nitrate mixed ester, or Polytetrafluoroethylene 70 Fm membrane of appropriate size to fit the diffusion cell diameter.

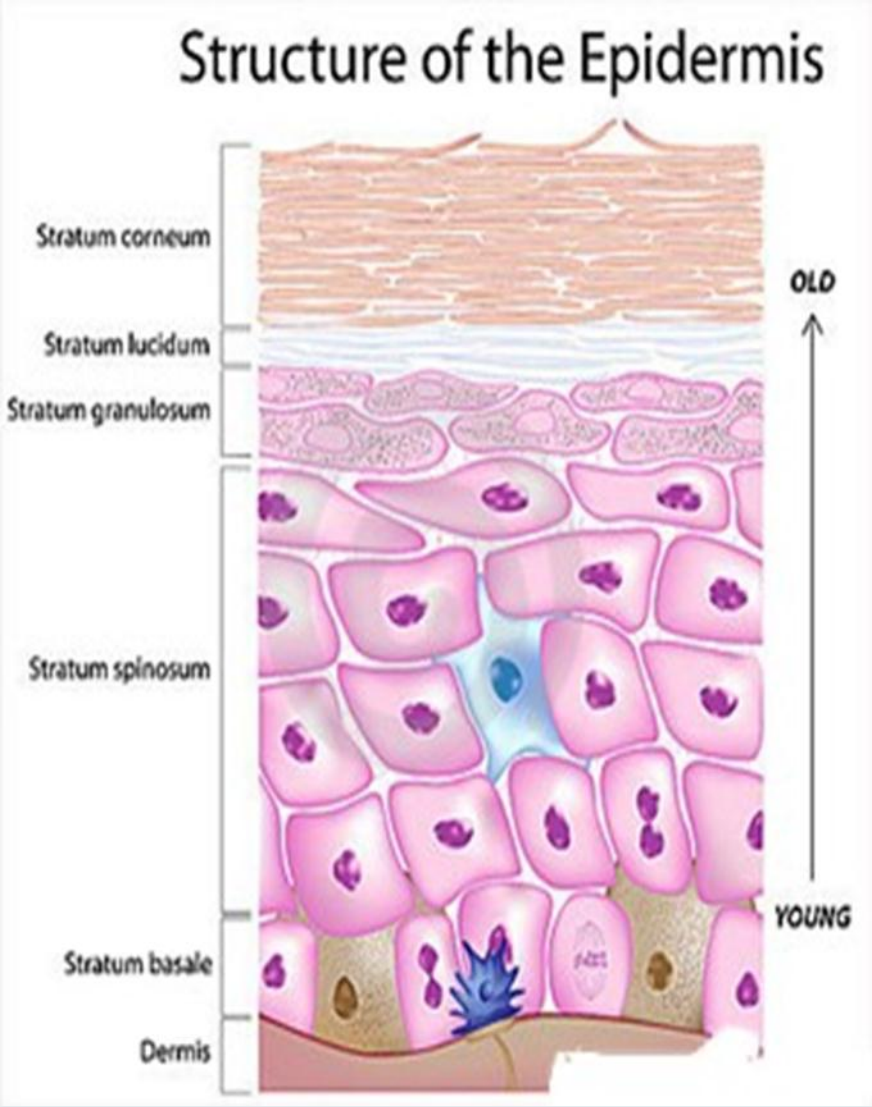
Principles of a dermal study

Use of synthetic membranes



- The skin is the human body's largest organ, and one of the more complexes.
- The layered structure of the skin and many smaller elements within these layers constitute a barrier against absorption of exogenous substances.
- The skin has two main layers:
  - the epidermis and,
  - the dermis.
- Below there is a layer of subcutaneous ("under the skin") fat.

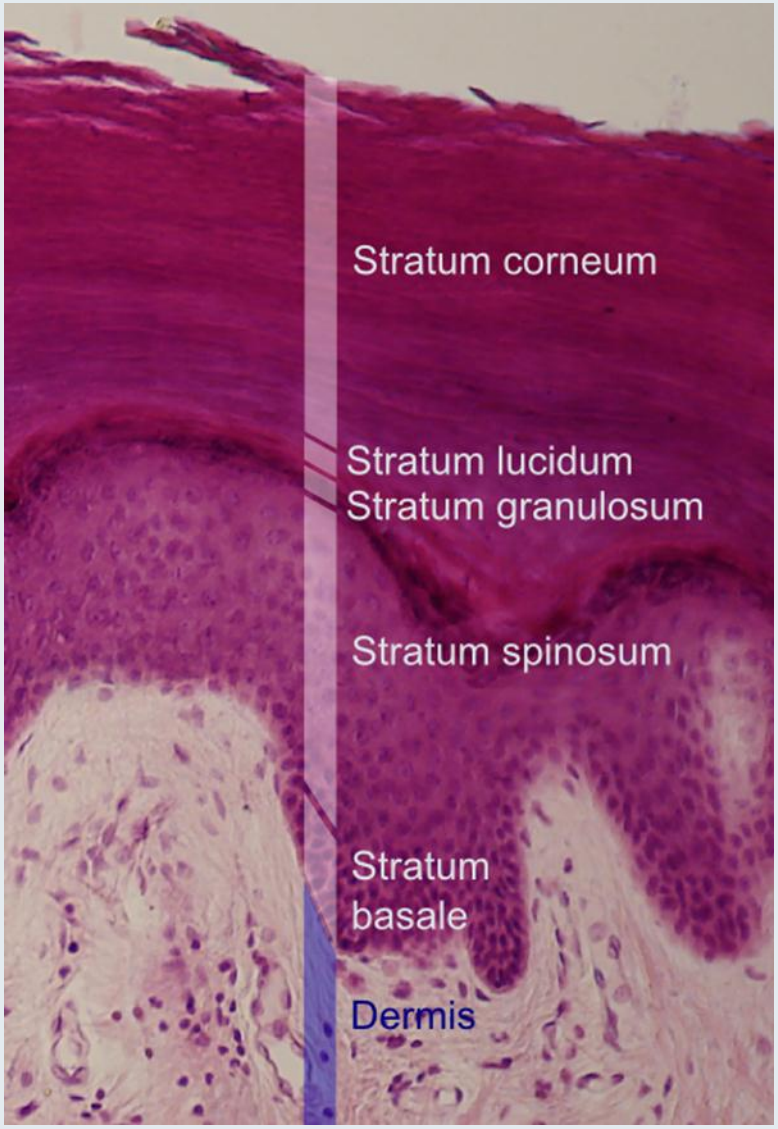
Skin is a complex matrix



The epidermis:

- Blood vessels in the dermis supply nutrients to support active growth of new skin cells which occurred in the basal cell layer.
- As the basal keratinocytes move upwards and away from their blood supply, their content and shape will change.
- Being distant from the blood supply in the dermis, the keratinocytes begin to flatten and die and accumulate keratin.

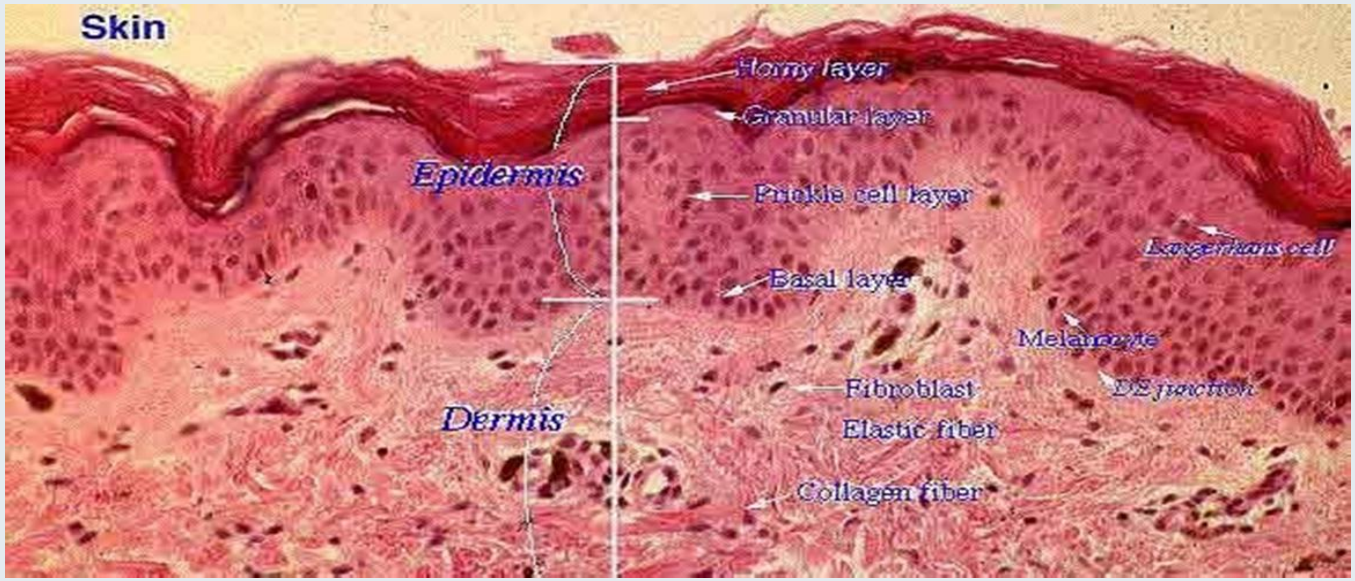
Skin is a complex matrix



The stratum corneum:

- The barrier function of the skin is located in the stratum corneum (SC):
- In the stratum corneum SC (10µm), cells are dead, contain a lot of keratin and are arranged in overlapping layers that impart a tough and waterproof character to the surface of the skin. The SC consists in corneocytes embedded in a lipid matrix.
- This lipid matrix is crucial for the lipid skin barrier function.
- Dead skin cells are continually shed from the surface of the skin.

Skin is a complex matrix

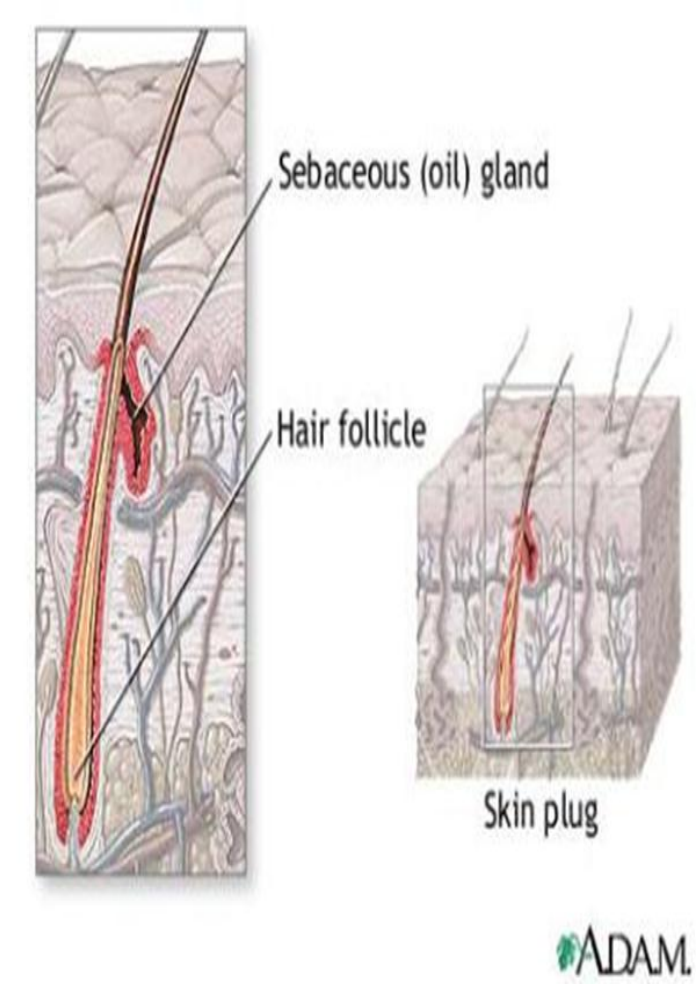


The dermis:

The dermis contains a variable amount of fat, and also collagen and elastin fibers:

- Blood vessels supply nutrients to the dividing cells in the basal layer and remove any waste products. They also help maintain body temperature.
- Specialized nerves in the dermis detect heat, cold, pain, pressure and touch and relay this information to the brain.
- Hair follicles are embedded in the dermis , each hair follicle has a layer of cells at its base that continually divides, pushing overlying cells upwards inside the follicle. These cells become keratinized and die.

Skin is a complex matrix



The dermis

- A sebaceous (“oil”) gland opens into each hair follicle and produces sebum, a lubricant for the hair and skin that helps repel water, damaging chemicals and microorganisms (“germs”).

- Sweat glands occur on all skin areas — each person has more than 2 million. When the body needs to lose heat these glands produce sweat (a mix of water, salts and some waste material such as urea). Sweat moves to the surface of the skin via the sweat duct, and evaporation of this water from the skin has a cooling effect on the body.

Skin is a complex matrix

- **The skin varies in thickness and the number of hair follicles, sebaceous glands and sweat glands in different areas of the body.**

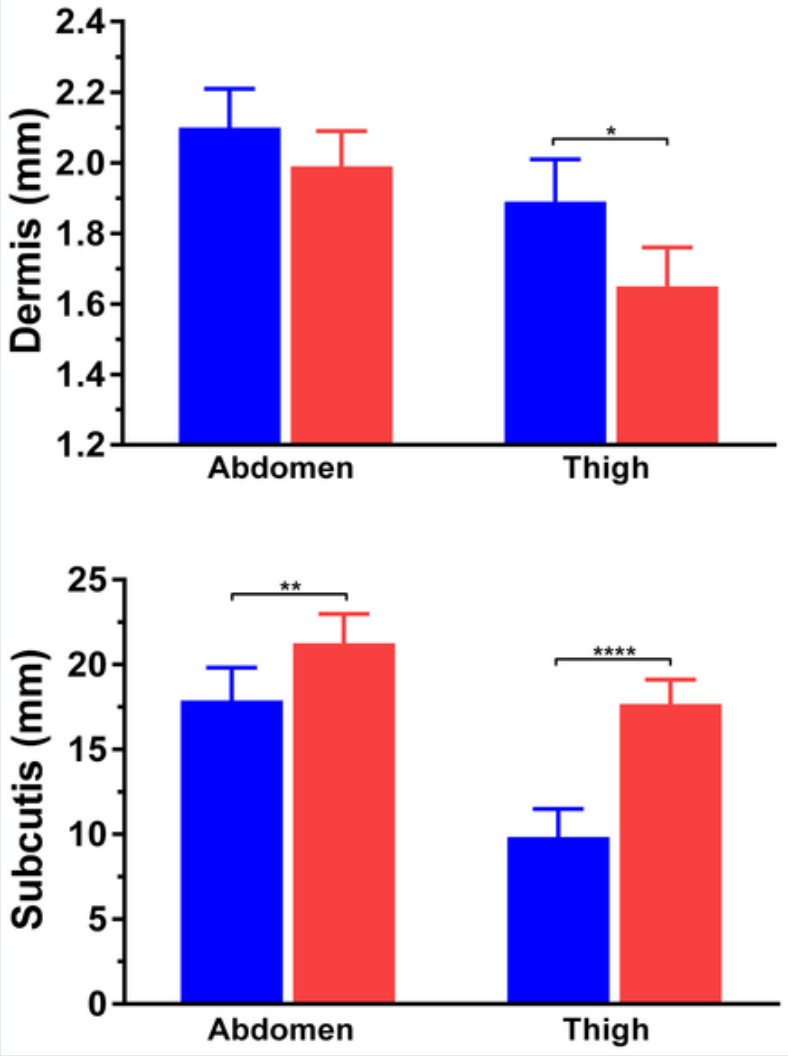
- **Skin thickness is also affected by age, pubertal status, gender, body mass index.**

- **The difference between the skin of different donors and even function of the area lead to variability which sometimes complicate the interpretation of the results.**

Skin is a complex matrix

Gender effect on thickness:

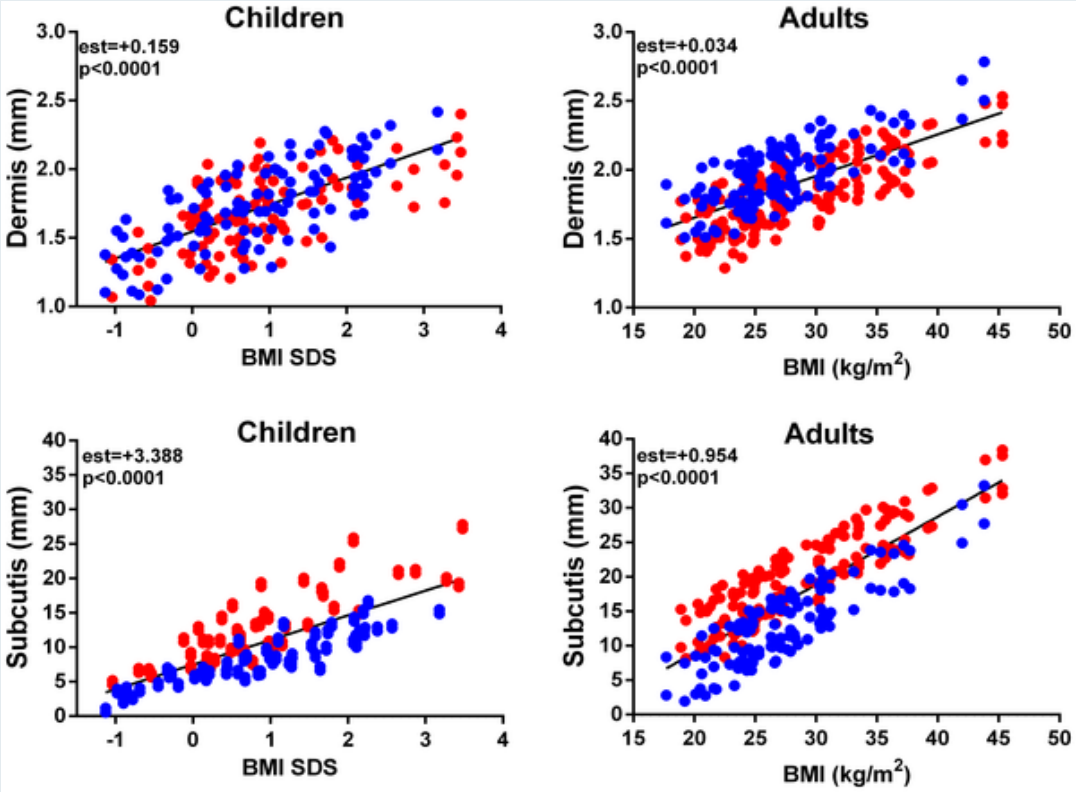
- Blue: male
- Red : female



Derraik JGB, Rademaker M, Cutfield WS, Pinto TE, Tregurtha S, et al. (2014) Effects of Age, Gender, BMI, and Anatomical Site on Skin Thickness in Children and Adults with Diabetes. PLoS ONE 9(1): e86637. doi:10.1371/journal.pone.0086637

Skin is a complex matrix

Effect of Body Mass Index on thickness:

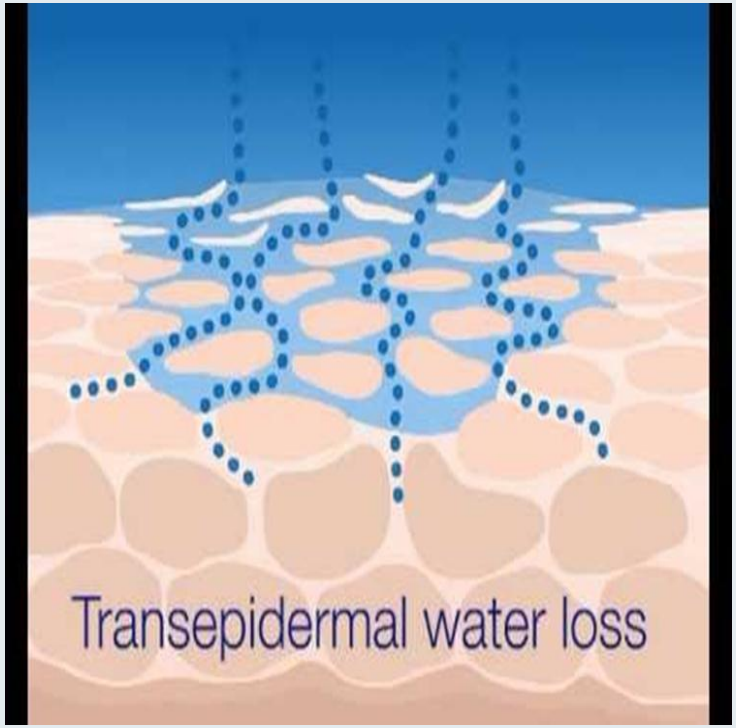


Men (blue bars) and women (red bars)
BMI was calculated for adults, and BMI standard deviation score (BMI SDS) was calculated for each child according to British 1990 standards.

Derraik JGB, Rademaker M, Cutfield WS, Pinto TE, Tregurtha S, et al. (2014) Effects of Age, Gender, BMI, and Anatomical Site on Skin Thickness in Children and Adults with Diabetes. PLoS ONE 9(1): e86637. doi:10.1371/journal.pone.0086637

Skin is a complex matrix

Skin integrity:

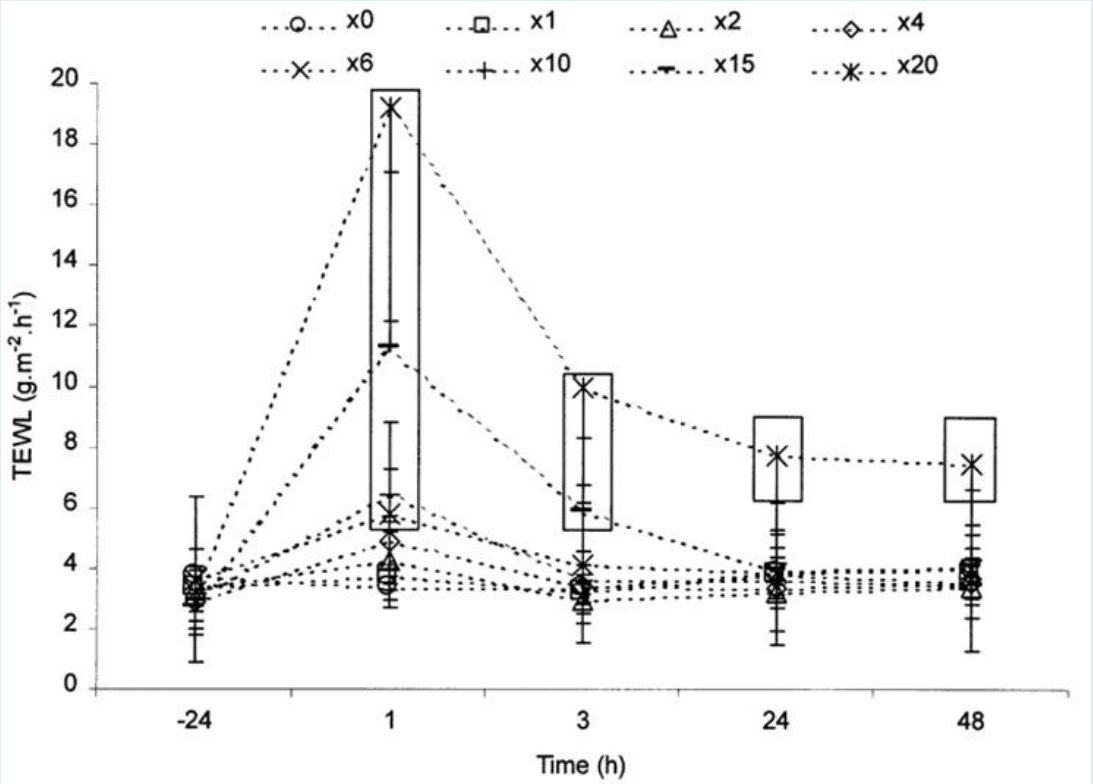


- **The measurement of transepidermal water loss (TEWL) is an important noninvasive method in order to assess the barrier function of the stratum corneum.**
- **As a consequence, TEWL has been found to be a very useful technique for studying skin irritation induced by various physical and chemical effects.**
- **Skin exposure to chemicals (detergents) and physical conditions (occlusion and stripping) can result in an increase of TEWL by a factor 5.**

Skin is a complex matrix

Skin integrity:

Variation in TEWL rates measured from full-thickness pig-back skin before (-24h) and up to 48h after tape stripping (1 to 20)



All values are mean SD of n= 6 diffusion cells containing skin from two animals.
Boxed values are significantly different ($p < 0.05$) from control (0) at 1h (4, 6, 10, 15, and 20), 3h (15 and 20), 24h (20), and 48h (20).

Transepidermal Water Loss Does Not Correlate with Skin Barrier Function In Vitro
Robert P Chilcott, Christopher H Dalton, Andrew J Emmanuel, Ceri E Allen and Simon T Bradley

Skin is a complex matrix

- **Human skin for *in vitro* studies is either taken from autopsies (cadaver skin) or obtained during cosmetic surgery.**

- **Both static (preferably with continuous stirring of the receptor fluid) and flow-through diffusion cells can be used.**

How to design a study to achieve your objectives?

Regulation for cosmetics, pharma, chemicals and agrochemicals.

- **Both *in vivo* and *in vitro* testing protocols form part of the lists of official EU and OECD test methods [EC B.44, 45; OECD 427, 428], accompanied by more detailed guidance on their performance [DG SANCO 2004, OECD 2004]. Whereas the first version of above mentioned OECD Guideline 428 was issued in 2000, the SCCNFP already adopted its first set of basic criteria for the *in vitro* assessment of dermal absorption of cosmetic ingredients in 1999 [SCCNFP/0167/99].**

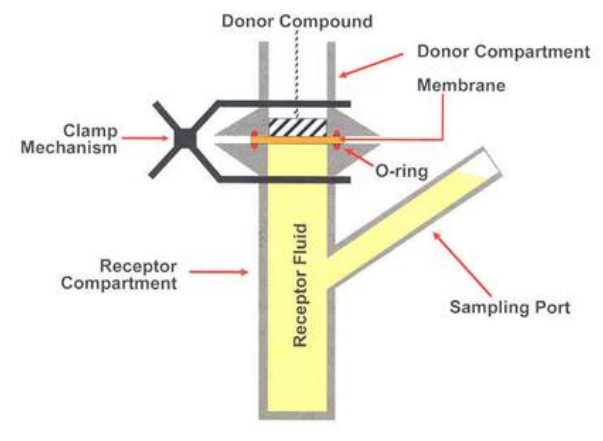
- **This opinion, most recently updated in 2010 [SCCS/1358/10], focuses on the in vitro testing of cosmetic ingredients, whereas the general EU and OECD Guidances [DG SANCO 2004, OECD 2004] address percutaneous absorption from a much broader point of view by mentioning *in vivo* methods besides *in vitro* testing and by providing specifications for agricultural products and industrial chemicals besides cosmetics.**

Study design

In these relatively complex *in vitro* studies, there are several points that require a special attention:

1) **The design of the diffusion cell (technicalities and choice between static and flow through system)**

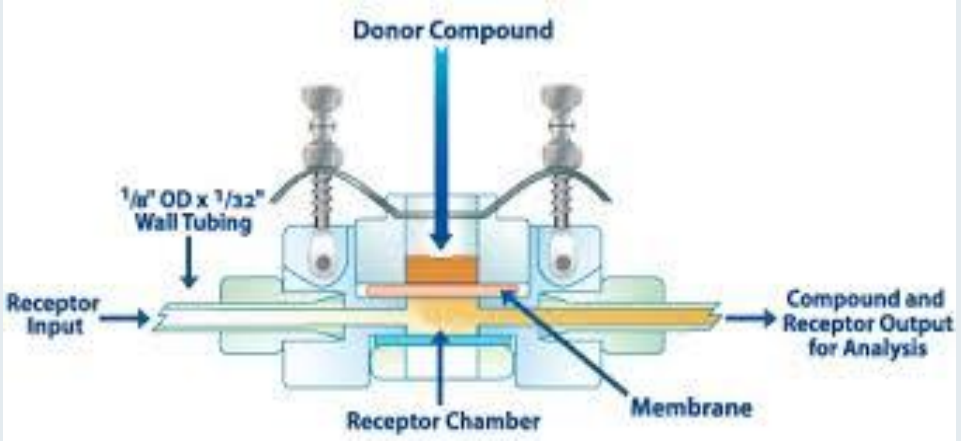
Static cells



Application area = 2 cm²

Volume of receptor fluid compartment = 7 mL

Dynamic cells



Application area = 1 cm²

Flow rate = 1mL/min

Study design

2) The choice of the receptor fluid (physiological pH, solubility and stability of chemical in receptor fluid should be demonstrated, no interference with skin/membrane integrity, analytical method, etc.).

- **The amount of penetrated substance in the receptor fluid should not exceed 10% of its saturation level at any time, in order to minimize interference with the free diffusion process that could produce an underestimation of dermal absorption.**

- **The substance should be stable in the receptor fluid for the duration of the *in vitro* test and the subsequent analysis.**

Study design

3) The skin preparations should be chosen and treated with care (human skin from an appropriate site remains the gold standard).

- **The human skin samples used will be obtained from abdominal surgery. Excess subcutaneous fat will be removed, if necessary.**
- **Information relative to the sex and age of the human donors will be specified in the final report.**
- **Skin samples will be excised and cut into pieces of 2 cm x 2 cm.**
- **Thickness will be measured using Oditest calipers according to the SOP in use in the laboratory.**

Study design

Human Full-thickness (FTS) and Dermatomed skin DMS are both applicable for dermal penetration studies *in vitro*, leading generally to results in the same order of magnitude.

However, a tendency was present that demonstrated slightly higher absorptions of test compounds in FTS than in DMS

Comparative Dermal Penetration Studies in Human Full-thickness and Dermatomed skin
K. Guth1, M. Schäfer-Korting2, E. Fabian1, B. van Ravenzwaay1, R. Landsiedel1
1BASF SE, Experimental Toxicology and Ecology, 67056 Ludwigshafen, Germany
2Freie Universität Berlin, Institute for Pharmacy, 14195 Berlin, Germany

Study design

4) Skin integrity is of key importance and should be verified:

- **Room temperature should be maintained between 20 and 25°C and the relative humidity between 30 and 70%.**
- **Around one hour after the set-up of the cells, stratum corneum integrity will be measured for each dermatomed skin sample by measuring the TEWL using evaporimeter.**
- **There should be no water on the skin and in the cell donor compartment and the measurement will be taken away from any heating source and air stream.**
- **The human skin will be included in the study if the TEWL is between 0.5 and 5 g/m2/h (1) for abdomen skin.**

Study design

5) Skin temperature has to be ascertained at normal human skin temperature:

- The passive diffusion of chemicals (and therefore their dermal absorption) is affected by temperature. The diffusion chamber and skin samples will be maintained at a constant temperature of 32°C ± 1°C.

Study design

6) The test substance has to be rigorously characterized and should correspond to the substance that is intended to be used in the finished cosmetic products

Reference Item	
Name	Ellagic acid
Supplier	Lab
Batch number	456874
Molecular weight	302
Retest date	11 April 2014
Storage conditions	Room Temperature

Study design

7) **Dose and vehicle/formulation should be representative for the in-use conditions of the intended cosmetic product. Several concentrations, including the highest concentration of the test substance in a typical formulation, should be tested.**

Batch number	LAB1	LAB2	LAB3
Compound	Ellagic acid		
Supplier	LAB		
Batch item used	456874		
Expiry date	20/05/2014		
Storage condition	Room temperature		

Study design

8) Dose, volume and contact time with the skin have to mimic in-use conditions:

- **The preparation will be applied homogeneously (without massage) on each skin sample at a rate of 5 mg/cm².**

- **The application will be performed using a spatula or positive displacement pipette and the exact amount applied will be determined by weight then recorded.**

Study design

Formulation number	1	2	3
Formulation identification	LAB1	LAB2	LAB3
Compound	Ellagic acid		
Theoretical formulation concentration	1%	1%	1%
Check of the formulation concentration	Analysis of formulation at T0 1		
Stability of the formulation	After 24h-storage at 32°C 1		
Thickness of the skin (µm)	Full thickness of skin		
Number of cell per donor	3		
Number of donor	3		
Total of cell	27		
Receptor fluid	PBS 0.05 M pH 7.4		
Sampling of Receptor Fluid	The total volume of receptor will be around 7 mL and will be sampled after 24hours		
Washing	24 hours		
Washing of the formulations	1 mL of Lauryl ether sulfate 3%		
Dismanting of the cells	24 hours		
Strips	12* strips will be performed on each skin.		
Separation Epidermis/Dermis	Yes		

Study design

- **Washing of formulation (LAV)**
  24 hours after application, the cells and the different compartments will be dismantled.

- **Strips treatment (S)**
  The strips will be taken off from the skin sample using adhesive scotch Magic 3M® by stripping. In order to standardize stripping, a weight of 150 g/cm^2 will be placed top of the Scotch tape for 10 s before taking off.

  A maximum of 12 strips will be performed on each skin and will be pooled by 5.

- **Epidermis (E) + partial dermis (D), remaining skin (RP) treatment**
  Using the scalpel blade, the epidermis (E) and partial dermis (D) corresponding to the application area will be cut and then separated.

The remaining skin (RP) will be cut in four parts. The RP vial will be analysed as the dermis, if necessary.

Study design

9) Regular sampling is required over the whole exposure period.

- **Receptor fluid (LR)**
  The receptor fluid will be taken 24hours after the application and the samples will be analysed.

Study design

10) Analysis of compartments

Appropriate analytical techniques should be used. Their validity, sensitivity and detection limits should be documented in the report.

Study design

- **Analysis of compartments**

A calibration curve will be injected before and after the samples.

<u>Acceptance criteria</u>:

- ***Deviation for the calibration standards will be ranged between ± 20.00% of the nominal value,***
- ***LLOQ and ULOQ will be included in the calibration curve, but if the LLOQ or the ULOQ will be outside the acceptance criteria, the calibration standard after the LLOQ will became the new LLOQ and the calibration standard just before the ULOQ will became the new ULOQ.***
- ***25% calibration standards can be excluded from the final calibration curve if the back calculated deviation is not comprised within ± 20.00% of the nominal concentrations.***

Study design

- **Quality control samples**

  In order to check the quality of the method during the sample assays, quality control samples will be included in the run.

  The nominal value will be used to determine the difference with the measured value of the QC.

  The quality control samples will be prepared at 2 concentration levels:
  - **6, 40 and 240 ng/mL for receptor fluid**
  - **12, 80 and 160 ng/mL for epidermis and dermis**
  - **12, 80 and 320 ng/mL for strips**
  - **30, 200 and 800 ng/mL for washing samples**

  Acceptance criteria:
  Precision (CV %) of QC samples will be calculated and will have to be ≤20.00% at least 67% of QC samples should have deviation within ±20.00%.

Study design

11) The test compound is to be determined in all relevant compartments:

- **product excess on the skin surface (dislodgeable dose),**
- **stratum corneum (e.g. adhesive tape strips),**
- **living epidermis (without stratum corneum),**
- **dermis,**
- **receptor fluid.**

Ellagic acid will be analysed in washing samples, strips, epidermis, dermis and receptor fluid according to the analytical method EURO/MOA/445.

The ellagic acid item will be used to perform the analysis.

Study design

12) Mass balance analysis and recovery data are to be provided. The overall recovery of test substance (including metabolites) should be within the range of 85-115%.

13) Variability / validity / reproducibility of the method should be discussed. The SCCS considers that for a reliable dermal absorption study, 8 skin samples from at least 4 donors should be used.

Study design

14) When dermal absorption studies are performed, often radioactive labelling of the substance under consideration is used in order to increase measuring sensitivity.

Justification should be given for the type and site of labelling chosen e.g. present or not in ring structure(s) or side chain(s), use of single or double labelling, etc.

This information is important with respect to the biotransformation and stability of the compound during the in vitro dermal absorption test.

- ⇨ **The use of 14C or 3H molecule simplifies the analysis and validation**
- ⇨ **The preparation of the radiolabelled formulation can be an issue**

Study design

15) The technical ability of the performing laboratory and the validity of the method used should be assessed at regular intervals, at least twice per year, by using reference compounds like caffeine or benzoic acid. These data should be included in the study report [OECD 2004, Van de Sandt et al. 2004].

In our laboratory, we run a ^{14}C-caffeine test twice a year.

Use

According to the objectives of the study, the protocol will be adapted to:

- **Screening test : qualified method and receptor fluid analysis only**
- **Absorption mecanism : qualified method and skin analysis only**
- **Selection of formulation/ingredient based on Kp : use of artificial membrane instead of skin**
- **Metabolism : use of fresh skin**
- **Mimic special conditions : use of altered skin**
- **Mecanical or chemical effect: same experimental conditions**
- **Hair appraisal : penetration into hair**
- **Intra-follicular : penetration through hair follicles**

Case study 1:
Influence of chemical penetration enhancers on skin permeability of ellagic acid-loaded niosomes

Distribution in all compartments of the applied dose after application of Ellagic standard formulations (in %).

Test item	Ellagic acid		
Formulation batch	LAB1	LAB2	LAB3
Skin excess	91± 6	106± 12	97 ± 7
Strips	0.08 ± 0.03	0.03 ± 0.03	0.06 ± 0.09
Epidermis	0.01 ± NC	0.01 ± 0.00482	BLQ
Dermis	BLQ	BLQ	BLQ
Receptor fluid	0.03± 0.01	0.01 ± 0.09	0.01± 0.01
E+D+RF+Strips	0.12 ± 0.03	0.05 ± 0.03	0.07 ± 0.09
Total Recovery	91 ± 6	106 ± 12	97 ± 7

Case study 1:
Influence of chemical penetration enhancers on skin permeability of ellagic acid-loaded niosomes

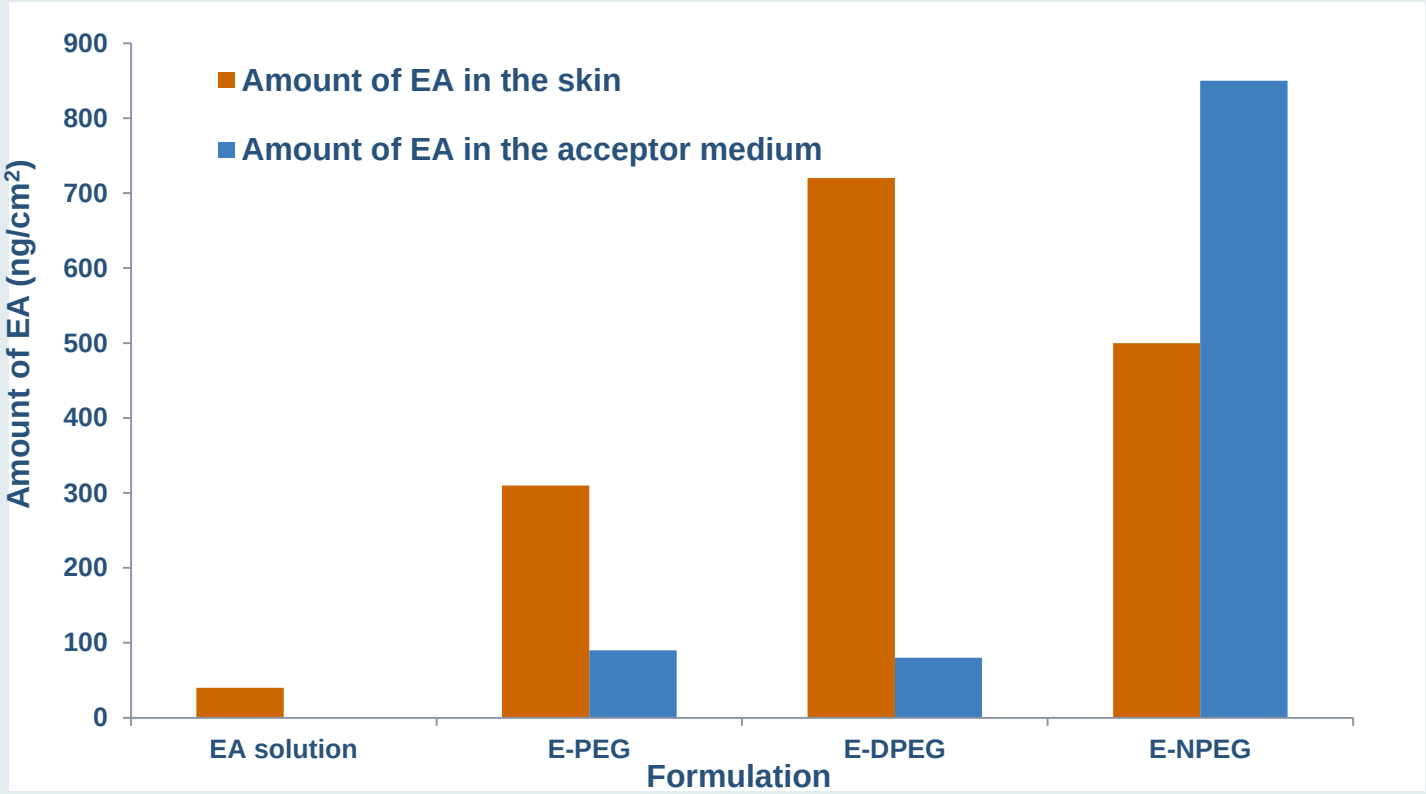
Influence of chemical penetration enhancers on skin permeability of ellagic acid-loaded niosomes,

Preparation of EA-loaded niosomes

The vesicles were prepared by reverse phase evaporation using 1:1 mole ratio of surfactants and cholesterol as vesicle forming agents. The surfactants were dissolved in diethyl ether (organic phase) with and without addition of 10% v/v DMSO or 1% v/v NMP. Polyethylene glycol 400 (PEG) was used as a solubilizer while dimethylsulfoxide (DMSO) or N-methyl- 2-pyrrolidone (NMP) was used as a skin penetration enhancer.

Varaporn Buraphacheep Junyaprasert, Asian journal of pharmaceutical sciences 8 (2013) 110-117*

Case study 1:
Influence of chemical penetration enhancers on skin permeability of ellagic acid-loaded niosomes



In vitro skin permeation study of EA solution, EA loaded niosomes containing 15% v/v PEG without chemical enhancers (E-PEG) and with 10% v/v DMSO (E-DPEG) or 1% v/v NMP (E-NPEG) after applying for 24 h

Varaporn Buraphacheep Junyaprasert, Asian journal of pharmaceutical sciences 8 (2013) 110-117*

Case study 2:
Strat-MTM membrane vs dermatomed human skin

Name	Testosterone	Benzoic acid	Sodium dodecyl sulfate
Molecular weight (g/mol)	288.4	122.1	288.4
Log Pow	3.32	1.87	1.60
Kp (µm/h)	76	22	1

Case study 2:
Strat-MTM membrane vs dermatomed human skin

Compound	Testosterone	Benzoic acid	Sodium Dodecyl Sulfate
Formulation	Ethanol/polyethylene glycol/UHQ water (5/5/90, v/v/v)		
Theoretical amount in the formulation	0,002% (w/w)	1% (w/w)	
Theoretical amount of compound applied	0.4 µg	200 µg	
Thickness of the skin (µm)	Dermatomed human skin, thickness between 300 µm and 400 µm.		
Cells detail	- 6 Strat-M™ Membrane from the same batch - 6 human skins from 3 human skin donors (n=2)		
Receptor fluid	PBS 0.01M pH 7.4 containing 6% polyoxyethylene 20 oleyl ether		PBS 0.01 M pH 7.4
Sampling of Receptor Fluid	30 min, 1, 2, 4, 6, 8 and 24 hours The total volume of receptor fluid is around 7 mL		
Washing	- 1 mL of UHQ water containing 10% of Tween 80® - 9 mL of UHQ water - dry the skin surface with three half of cotton-buds		
Washing of the formulations	24 hours after application		
Strips	2 strips were performed on each skin.		

Case study 2:
Strat-MTM membrane vs dermatomed human skin

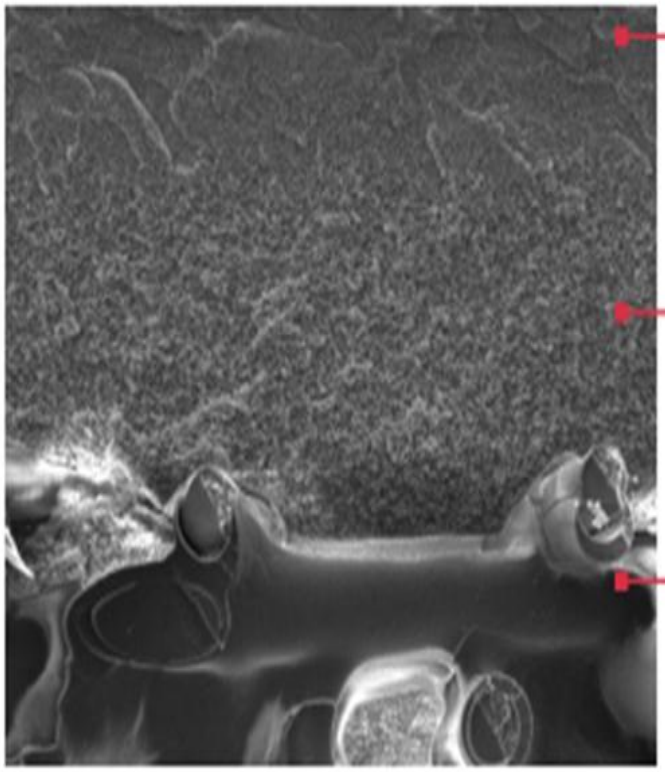
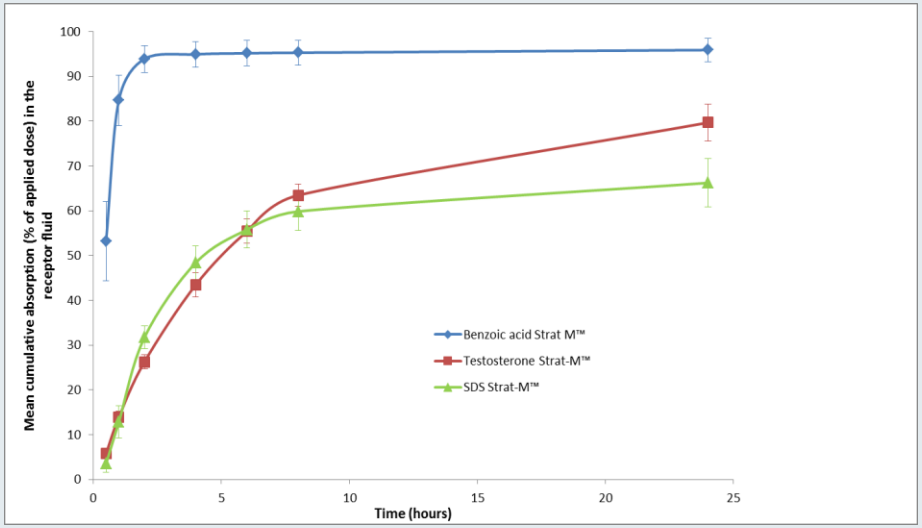


Figure 1. The multilayered structure of Strat-M™ membrane matches that of human skin.

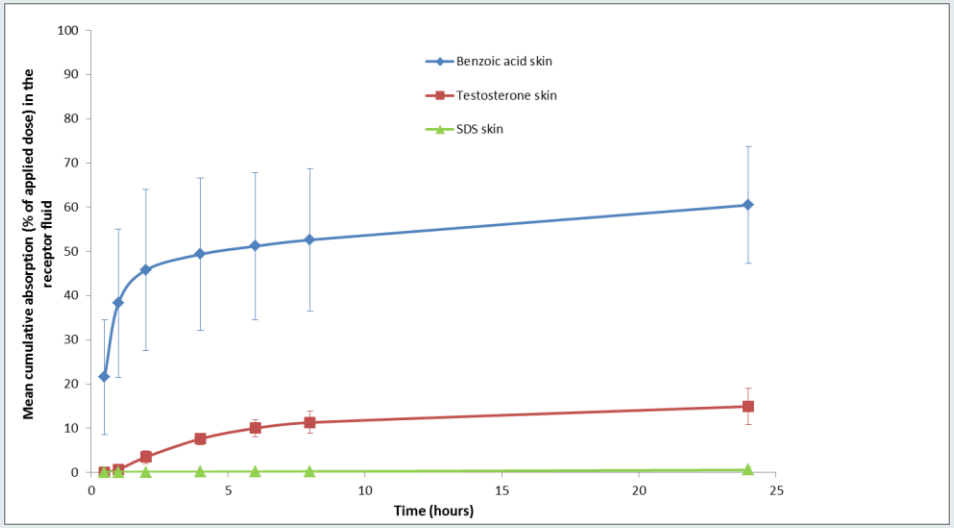
- As human skin, Strat-MTM membrane is composed of several layers. It is an artificial membrane produced by EMB Millipore.
- Two layers of polyethersulfone resistant to diffusion imitate stratum corneum and epidermis, and one layer of polyolefin is more porous, like dermis.
- Synthetic lipids are also added to the membrane to give it skin-like properties. This leads to a diffusivity and porosity gradient across the membrane.

Case study 2:
Strat-MTM membrane vs dermatomed human skin

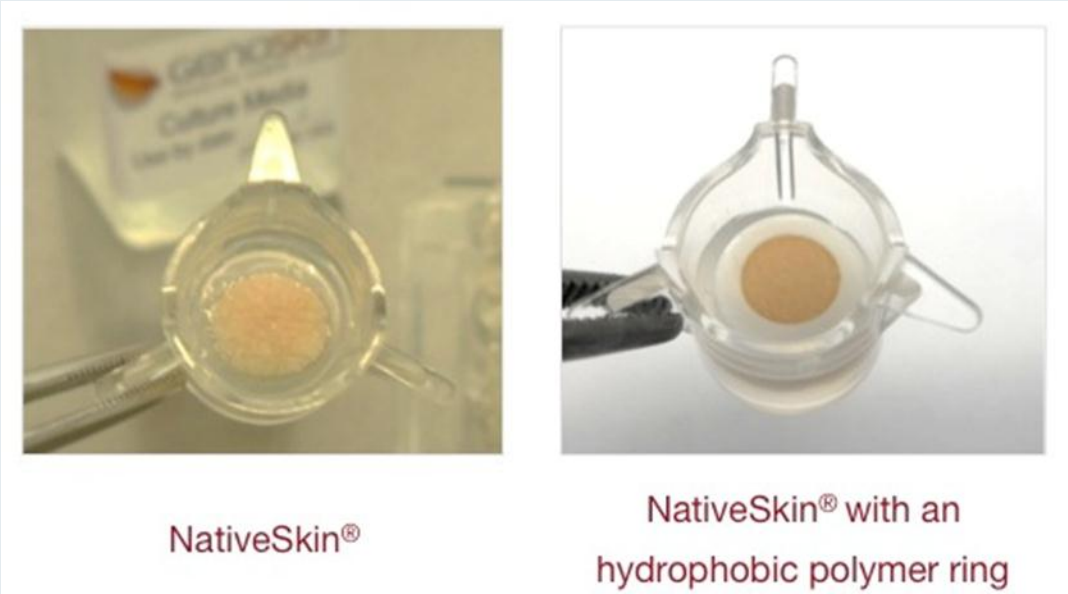
Strat-MTM membrane



Dermatomed human skin



Case study 3:
NativeSkin(Genoskin) vs fresh human skin



NativeSkin®

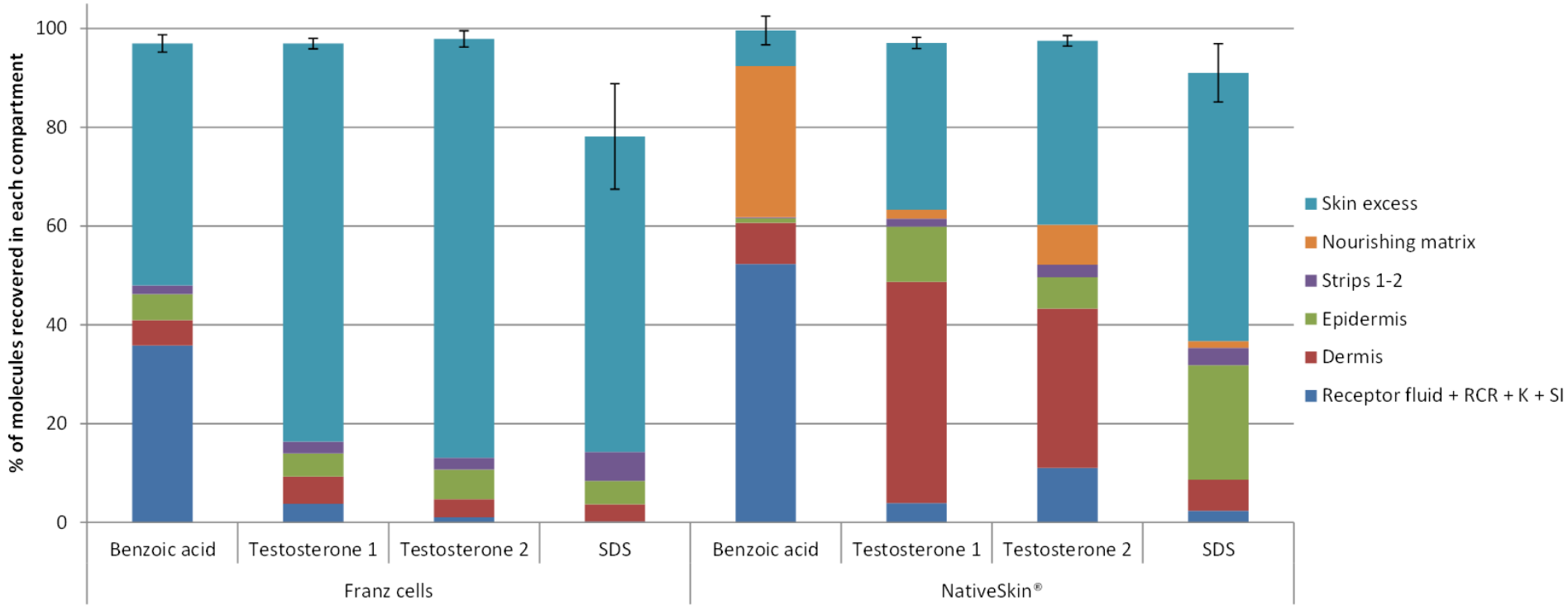
NativeSkin® with an hydrophobic polymer ring

- **NativeSkin® models are fresh human skin explant, embedded in a nourishing matrix, fixed into a cell culture insert.**
- **These inserts are then placed into 6-well plates that can be filled with culture medium or receptor fluid.**
- **Unlike commonly used skin from frozen sample, skin explants here were never frozen, and cell viability can be maintained up to 7 days at 37°C, 5% CO2 with culture medium.**

Case study 3:
NativeSkin(Genoskin) vs fresh human skin

Compound	Testosterone	Benzoic acid	Sodium Dodecyl Sulfate
Formulation	Ethanol/polyethylene glycol/UHQ water (5/5/90, v/v/v)		
Theoretical amount in the formulation	0,002% (w/w)	1% (w/w)	
Surface of skin	1 cm² for NativeSkin® 2cm² for Franz cells		
Theoretical formulation amount applied per cell	10µL/cm² : 10µL for NativeSkin®, 20µL for Franz cells		
Theoretical amount of compound applied on skin	0.4 µg	200µg	
Thickness of the skin	Full thickness skin was used (fatty tissue removed). The thickness of the skin was measured and reported.		
Number of cell per donor	3		2 for NativeSkin® 3 for Franz cells
Number of donor	4 (2 per application)	2	
Receptor fluid	PBS 0.01M pH 7.4 containing 6% polyoxyethylene 20 oleyl ether		PBS 0.01 M pH 7.4
Sampling of Receptor Fluid	30 min, 1, 2, 4, 6, 8 and 24 hours The total volume of receptor fluid is around 7mL in Franz cells and 1.5 mL for NativeSkin®		
Washing	Skin surface was: - dried with one half of cotton-bud - washed with one half of cotton-bud soaked in UHQ water with 10% of Tween 80® - rinsed with one half of cotton-bud soaked in UHQ water - dried with one half of cotton-bud		
Washing of the formulations	24 hours after the application		
Strips	2 strips were performed on each skin sample		

Case study 3:
NativeSkin(Genoskin) vs fresh human skin



Mean absorption 24 hours after application

Case study 4:
Comparison of skin models

Skin type	Permcation (µg/cm²) Mean ± sd	P_{app} (10-6cm/s)		Lag time (hours) Mean ± sd	n
		Mean ± sd	CV (%)		
Caffeine					
• Human epidermis sheets	1.12 ± 1.18	0.06 ± 0.04	62.29	1.73 ± 1.48	8
• Pig skin	0.48 ± 0.41	0.07 ± 0.05	74.82	3.92 ± 0.87	6
• Bovine udder skin	8.24 ± 3.86	0.63 ± 0.23	37;23	1.88 ± 0.42	7
• Epiderm	4.87 ± 2.67	0.24 ± 0.14	55.59	0.33 ± 0.06	5
• Skin Ethic	73.65 ± 36.58	3.63 ± 1.91	52.74	0.14 ± 0.05	6
• EPISKIN	51.25 ± 9.84	2.77 ± 0.78	24.37	1.04 ± 0.26	7
Testosterone					
• Human epidermis sheets	0.32 ± 0.27	0.42 ± 0.39	93.18	1.03 ± 2.52	8
• Pig skin	0.07 ± 0.15	0.08 ± 0.01	14.91	0.13 ± 11.92	4
• Bovine udder skin	0.14 ± 0.15	0.32 ± 0.28	89.89	1.19 ± 1.30	6
• Epiderm	2.36 ± 0.90	2.89 ± 1.09	37.82	0.00 ± 0.09	5
• Skin Ethic	4.47 ± 0.57	6.00 ± 1.17	19.55	0.14 ± 0.09	5
• EPISKIN	1.53 ± 0.47	2.11 ± 0.63	29.89	0.93 ± 0.33	7

P_{app} (apparent permeation coefficient) values, lag time and drug permeated into the acceptor medium after 6 hours for Caffeine (0.1%) and testosterone (0.004%) applied to human epidermis sheets, reconstructed epidermis and pig and bovine udder skin.
CV – coefficient of variations; sd = standard deviation; n= number of independent experiments.

Conclusion

Skin penetration model is not only used to fulfill regulatory requirement, it is also a good evaluation tool to accelerate your cosmetic development:

- Evaluation/screening of active ingredients
- Selection of formulation/application mode
- Comparaison with commercial products
- Test of apparatus

Questions