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"The first adjustable TEWL Biomimicry Models: Bridging In Vitro and In Vivo Assessments for Advanced Cosmetic Testing"

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1. Introduction

Transepidermal Water Loss (TEWL) is a critical indicator of skin barrier integrity and plays a central role in evaluating the performance of cosmetic formulations. While its relevance is well established in the skincare field – particularly for moisturizers, barrier repair products, and protective treatments – it is also increasingly used in the assessment of makeup products, where barrier interaction and skin physiology must be carefully considered [1].

Traditionally, TEWL has been assessed using *in vivo* methods, which, while scientifically validated, present several limitations [2]. These include inter-individual variability, time-consuming protocols, ethical considerations, and limited suitability for high-throughput or early-stage screening. Moreover, *in vivo* TEWL measurements rely on highly sensitive instruments whose readings can be significantly affected by external factors such as stress, temperature shifts, behavior changes in volunteers, and operator-dependent variability [2]. These fluctuations introduce considerable variability, complicating both interpretation and reproducibility.

As the cosmetic industry increasingly embraces data-driven development and predictive modeling, the demand for standardized, scalable, and reproducible testing platforms has grown substantially. To address these challenges, the present study introduces a novel biomimetic *in vitro* TEWL system designed to replicate the water loss dynamics of human skin under controlled laboratory conditions.

This system consists of two key components. First, a microfluidic consumable that functions as a skin model, featuring a microporous membrane and a skin-like surface that mimics TEWL in a stable and repeatable manner. Second, a dedicated instrument into which the consumable is inserted. This device controls both the environmental and fluidic features, and it is equipped

with two integrated TEWL sensors. These sensors are mechanically and automatically positioned over each consumable and enable continuous, real-time TEWL measurements with customizable TEWL and minimal operator intervention.

The primary objective of this study was to evaluate the performance and predictive relevance of this *in vitro* model by comparing its results to traditional *in vivo* TEWL measurements. Three formulations – three oil-in-water emulsions (products A, B and C) – were tested using both methods. The aim was to determine the extent of correlation between *in vivo* and *in vitro* data and assess the potential of the model as a robust, early-stage screening tool for TEWL evaluation.

2. Materials and Methods

2.1 In vitro biomimetic model and instrumentation

The *in vitro* experiments were conducted using a TEWL biomimetic system consisting of (i) a microfluidic skin model designed to mimic human transepidermal water loss under stable and controlled conditions, and (ii) a dedicated instrument for environmental regulation and automated measurement.

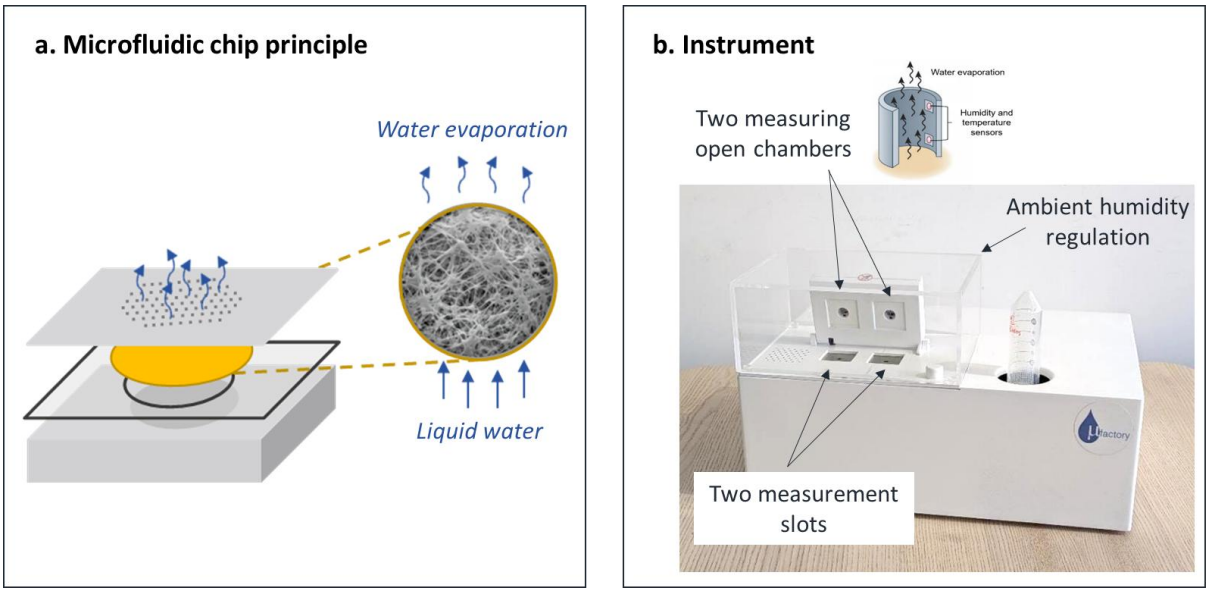


Figure 1. Overview of the *in vitro* TEWL biomimetic system

The microfluidic model is a multi-layered polymeric chip (Figure 1a). It integrates:

- A PDMS layer embedding a reservoir filled with liquid milliQ water,
- A hydrophobic intermediate membrane allowing only water vapor transfer,
- A PU-based upper membrane featuring a roughness similar to human skin and patterned with 60 µm pores to replicate the structure of human epidermis.

This assembly allows continuous water evaporation mimicking human TEWL under precisely regulated surface temperature and humidity conditions. The assembled chips are inserted into a dedicated instrument (Figure 1b) that:

- Maintains a constant ambient humidity level (60%) inside a closed chamber,
- Actively controls surface temperature of the microfluidic model to regulate the simulated TEWL value (from 5 to 23 g/m²/h) (Figure 2a),
- Integrates dual open-chamber humidity sensors to quantify water vapor gradients,
- Automates all setting and data acquisition through a dedicated software.

As a first validation of the performance and relevance of the *in vitro* skin model, a control experiment was conducted using Petroleum jelly, a well-known film-forming agent recognized for its high efficacy in reducing TEWL *in vivo* [3,4]. As expected, a strong and stable reduction in TEWL was observed throughout the measurement period, confirming the model's ability to detect and quantify the occlusive properties of topical formulations (Figure 2b).

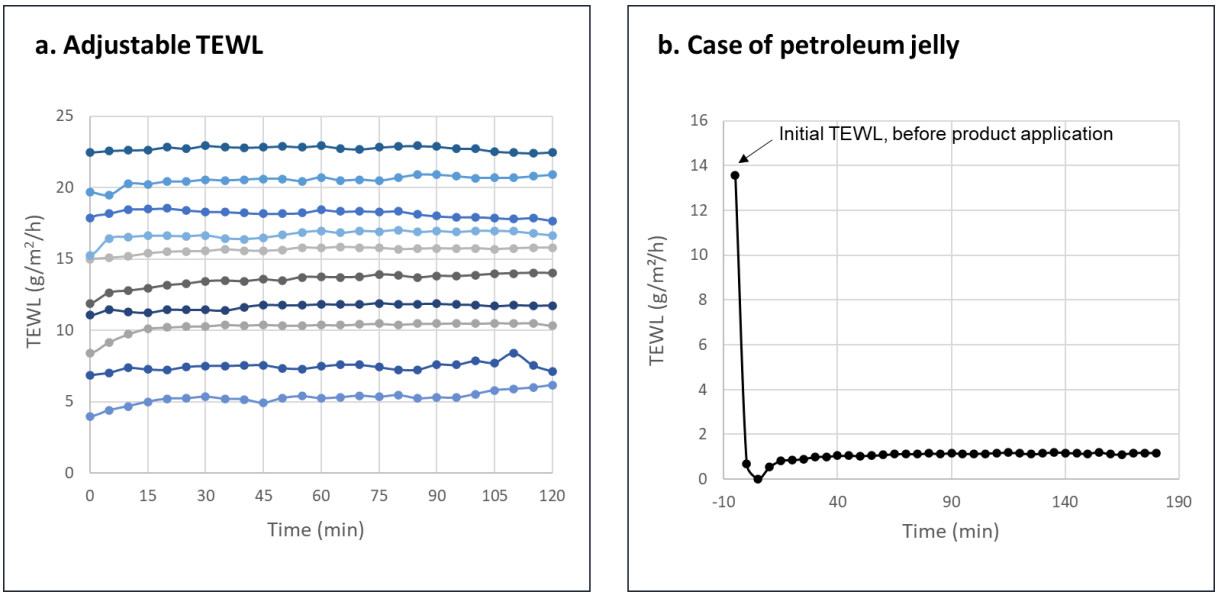


Figure 2. a. Range of TEWL levels reproducible with the *in vitro* microfluidic skin model. The system allows precise and stable reproduction of a wide range of TEWL values over a 2-hour period. Each curve represents a different TEWL setpoint, demonstrating the model's capacity to simulate various physiological and pathological conditions [5]. **2. b.** Effect of Petroleum jelly application on the *in vitro* skin model, initial TEWL of 14 g/m²/h, with a stable reduction of ~12 g/m²/h over 3 hours.

2.2 In vivo protocol

The *in vivo* study involved 12 healthy volunteers (women between 18 and 60 years old) with baseline facial TEWL ranging from 11 to 25 g/m²/h participated in this study. Volunteers must

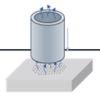
not apply any skin care products to the face the day before and the morning of the test, must not wear make-up on the day of the test, and must stop drinking hot beverages and smoking 1 hour before the beginning of the test. The volunteers remained in the laboratory under stable climatic conditions throughout the test. Test areas were marked on both cheeks (left and right zygomatic zones) to ensure reproducible positioning. One area was left untreated as control, while the other received 2 mg/cm² of the tested product. The assignment of control and treated zones was randomized to minimize lateral bias. The product was applied to the designated zone using a finger coat to ensure consistent spreading by hand.

After 30 minutes of acclimatization in a controlled environment laboratory (50±10% humidity, 21±2°C temperature), TEWL measurements were taken using a Tewameter® TM300 (Courage + Khazaka) at three time points:

- Baseline (T0), before product application, on both zones,
- 30 minutes post-application (T30min), on both zones,
- 2 hours post-application (T2h), on both zones.

The same procedure was applied to each volunteer and for each tested product. The testing was conducted over three different days separated of at least 24 hours, with only one product tested per day (Table 1).

Data were analyzed on product performance at T30 minutes and T2 hours, considering the control area variability, Product (Tx-T0) - Control (Tx-T0). Data distribution is analyzed for normality using the Shapiro Wilk test. Comparison of product is performed using the Friedman test at T30min and T2H.



	in vivo	*in vitro*
Tested products	A, B, C	A, B, C
Measurement time points	T0, T30min, T2h	T0, and continuous over 2h
Quantity of product applied	2 mg/cm²	2.6 mg/cm²
Initial TEWL range	11-25 g/m²/h	14-16 g/m²/h
Replicates / subjects	12 women (23 – 51 years old)	Triplicates on skin model
Zones	1 cheek treated, and 1 cheek for control	1 chip mimicking 14 g/m²/h of TEWL, and 1 chip mimicking 0 g/m²/h of TEWL
Ambient humidity	43.5 %	60 %
Ambient temperature	21.7 °C	23.6 °C
Substrate temperature	Skin temperature	23 °C
Total study duration	4 weeks	1 week

Table 1. Experimental conditions summary

2.3 In vitro protocol

In vitro testing was carried out on T-Skin® (Microfactory) (Table 1), biomimetic skin models, under controlled conditions (60% ambient humidity, 22°C substrate temperature), adjusted to simulate TEWL values between 14 and 16 g/m²/h. Three replicates were performed for each tested formulation. Two models were simultaneously placed in the instrument:

- One producing a baseline TEWL between 14 -16 g/m²/h,
- One adjusted to simulate zero TEWL, acting as a reference for solvent evaporation measurement.

Each model was initialized for 5 minutes to establish a baseline reference, with data collected every 10 seconds. Subsequently, 2.6 mg/cm² of product was applied to both surfaces. TEWL measurements were then recorded continuously for 2 hours, maintaining a data acquisition interval of 10 seconds

The signal from the reference model (no TEWL) allows subtraction of solvent-related evaporation from the active model signal, yielding a precise quantification of the formulation's impact on TEWL.

3. Results

3.1 Comparison of TEWL measurement instruments: Tewameter® TM300 vs T-Skin®

To ensure the robustness of the *in vitro – in vivo* correlation established in this study, it was essential to verify that the measurement system used *in vitro* (T-Skin®, Microfactory) provided results comparable to those obtained with the standard instrument (Tewameter® TM300, Courage + Khazaka) [6]. To this end, TEWL values were measured directly on the microfluidic skin model using both devices, under similar experimental conditions.

A series of experiments was conducted across a range of controlled TEWL levels produced by the biomimetic model, from low (10 g/m²/h) to high (30 g/m²/h) evaporation rates. Each TEWL level was measured alternately with the Tewameter® and T-Skin® probe to ensure minimal variation due to temporal drift or environmental fluctuation.

The resulting data were plotted as a scatter plot (Figure 3), where each point represents a pair of measurements taken with the two instruments on the same microfluidic model. The results show a linear correlation between the two measurement systems across the full range of TEWL values, with no systematic bias observed. These findings confirm the compatibility and equivalence of both instruments in measuring TEWL on the microfluidic model.

This validation step is critical to the interpretation of subsequent *in vitro* results, and ensures that any observed *in vitro* – *in vivo* correlation is not confounded by instrument variability.

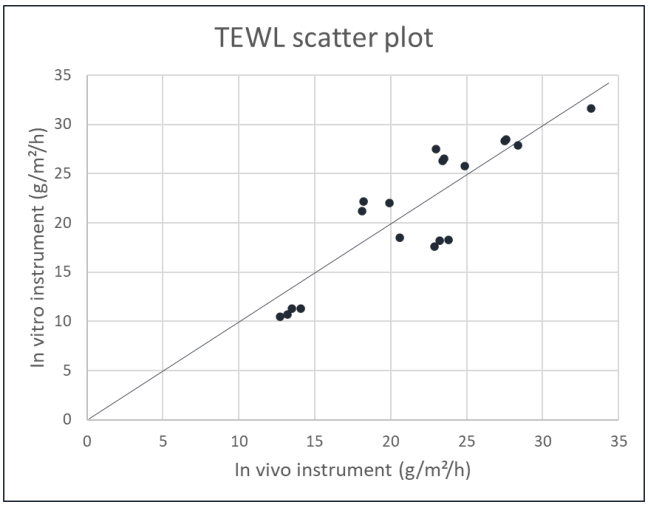


Figure 3. Correlation between the two instruments on a microfluidic skin model (R^2= 0.78), validating their equivalence for in vitro measurements.

3.2 In vivo results

Regarding product performance, at 30 minutes, Product A increased TEWL by +2.3 g/m²/h, Product B decreased TEWL by -0.13 g/m²/h, and Product C decreased TEWL by -1.87 g/m²/h. After 2 hours of application, Product A increased TEWL by 0.7 g/m²/h, Product B decreased TEWL by -0.41 g/m²/h, and Product C decreased TEWL by -1.3 g/m²/h.
Comparison of product performance allowed significant differences after 30 minutes of application. Product A has been significantly different from Products B and C. Products B and C showed no significant difference.
After 2 hours of application, this comparison between the three products showed no significant difference (Figure 4).

In summary, at T30 minutes and T2 hours, products B and C reduced TEWL and product A increased it. Comparison of products showed that products B and C decreased significantly the TEWL at T30 minutes versus product A. However, this difference disappeared at T2 hours.

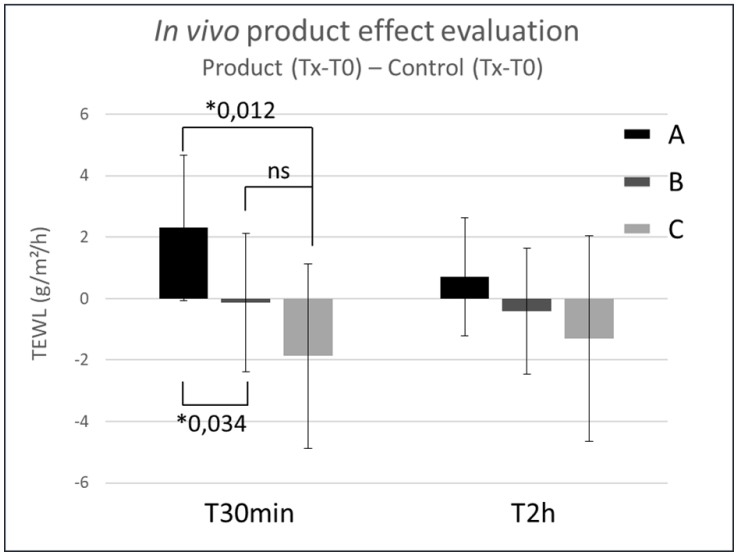


Figure 4. *In vivo* assessment of the products effect on TEWL. * significant difference (p value < 0,05)

3.3 In vitro results

The three products were evaluated under controlled conditions (60% ambient humidity, 22°C substrate temperature) using the *in vitro* microfluidic skin model, set to produce a stable initial TEWL of approximately 14 g/m²/h (to correspond to the mean TEWL of the volunteers of the *in vivo* study). Upon application, all products showed an immediate effect in reducing TEWL, confirming their film-forming properties during the first 30 minutes (Figure 5a). However, noticeable differences were observed in both the magnitude and duration of the effect between formulations.

Product A induced a rapid but modest decrease in TEWL within the first 30 minutes, with its efficacy diminishing progressively over time. In contrast, products B and C demonstrated a more pronounced and sustained reduction in water loss. At 60 minutes, their effects were comparable, but product C maintained a slightly higher efficacy over the 80-minute period (Figure 5).

The presented TEWL values have been corrected to exclude the contribution of solvent evaporation, isolating the impact of the formulations on the TEWL. These results underline the model's sensitivity in detecting differences in product performance and its capacity to mimic physiological TEWL dynamics over time.

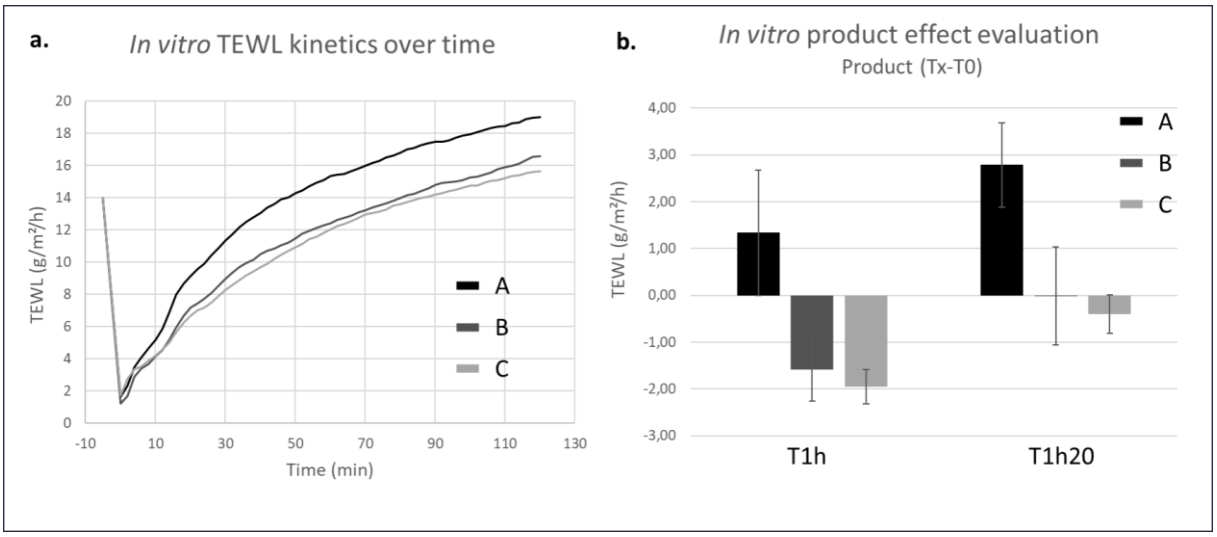


Figure 5. *In vitro* assessment of the products effect on TEWL. TEWL values have been corrected to exclude the contribution of solvent evaporation. **a.** TEWL kinetics in the assessment of 3 products over 2h*;* initial TEWL, before products application, of 14 g/m²/h. **b.** Comparison of the products efficacy after 1h and 1h20.

The TEWL reduction measured at T60 minutes *in vitro* aligns closely with the effects observed at T30 minutes post-application *in vivo*, while the 80-minute *in vitro* values correspond to the 2-hour *in vivo* measurements. The shift in timing can be explained by intrinsic differences between the models: *in vivo* measurements are influenced by the initial evaporation of the product solvent, while *in vitro* solvent evaporation may take longer due to the model not being maintained at skin temperature. Differences in physicochemical properties between the model and the skin may also explain this shift.

4. Discussion

This study aimed to evaluate the predictive relevance of a novel biomimetic *in vitro* model replicating transepidermal water loss (TEWL), by comparing its performance to classical *in vivo* measurements. The findings demonstrate a strong alignment between the two approaches, supporting the capacity of the *in vitro* system to reproduce the dynamic behavior of human skin regarding water loss modulation after cosmetic formulation application.

Nevertheless, several physiological differences between the *in vitro* model and human skin must be acknowledged. A key distinction lies in the surface temperature control: while human skin surface temperature typically ranges from 32°C to 34°C, the *in vitro model* was operated

at a surface temperature of 22°C. This lower temperature influences evaporation kinetics and leads to slightly reduced baseline TEWL values compared to those observed *in vivo* [7].

Despite these differences, the *in vitro* model successfully mirrored the ranking and relative efficacy of the tested formulations, reflecting similar temporal trends to those measured on human volunteers. This outcome suggests that while absolute TEWL values differ, the model remains highly reliable for comparative evaluations. In addition, TEWL measurements performed *in vitro* displayed significantly lower variability than those obtained *in vivo*, highlighting the superior reproducibility and standardization of the model. The controlled experimental environment also minimizes external factors such as environmental fluctuations or emotional stress, which often introduce variability in *in vivo* studies.

These results are consistent with previous efforts to develop *in vitro* skin barrier models, thus the present system represents a significant advancement by enabling the adjustment of TEWL values to match different physiological or pathological conditions. Future developments could focus on integrating temperature regulation closer to physiological skin values or establishing correction factors to improve quantitative correlations with *in vivo* data.

Additionally, expanding the evaluation to a broader range of cosmetic formulations and performing film-forming long-term assessments would further validate the robustness and versatility of the model.

5. Conclusion

This study presents a novel biomimetic i*n vitro* model capable of replicating transepidermal water loss (TEWL) under controlled and reproducible conditions. The system demonstrated strong predictive relevance when compared to conventional *in vivo* TEWL measurements, despite physiological differences such as a lower surface temperature.

The model accurately reflected the ranking and dynamics of cosmetic formulation efficacy, highlighting its value as an early-stage screening tool for skin barrier function evaluation. By reducing environmental and operator-dependent variability, this approach provides a standardized, scalable alternative to traditional *in vivo* testing methods, meeting the increasing demand for ethical, efficient, and reproducible cosmetic evaluation platforms.

Future improvements should focus on refining physiological parameters, such as surface temperature adjustment, and broadening the range of tested products, thereby strengthening the predictive power and practical applications of this *in vitro* technology.

6. References

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