www.eurocosmetics-magazine.com

B 50962

EURO COSMETICS

THE INTERNATIONAL MAGAZ
FOR COSMETICS AND FRAGRANCES

3

CFDA listed

Restores urban and sensitive skin by reinforcing its physical barrier and achieving a microbiota similar to that of our ancestors living in closer contact with nature.

Increases **microbia diversity**

Aids in **preventing dehydration**

Offers **healthy** and **protected skin**

in-cosmetics global
RAI Amsterdam • 17-19 April 2018
VISIT US ON STAND C30

Lipotec
A Lubrizol Company
www.lipotec.com

COVER STORY
LIPOTEC'S
EYESERYL® *peptide*

ARTIFICIAL INTELLIGENCE

INTERVIEW
IRENA JAMES
Vice President YG Laboratories

"SCROOPINESS"
Understanding the Phenomenon

ERIC PEETERS
Global Business Director
Dow Home & Personal Care
about new DOWSIL™
product brand name

EVONIK
new interactive tool
"Sensory Kaleidoscope"
p. 11

English/German

March 2018
VOLUME NO. 26

Testing the Future

By Marta Cavo, Maurizio Aiello, Silvia Scaglione *

Introduction

Every day, our body interacts with hundreds of substances of all kinds: the air we breathe, the medicines we take, the snacks we eat. Our skin does the same.

Skin is the largest and fastest-growing organ of human body[1]; it is composed of two main layers: the epidermis (outer) and the dermis (inner). Cells in the epidermis are continually replaced: this process slows as we age. The dermis contains nerve endings, hair follicles, sweat glands and blood vessels. Together, these two layers provide us with protection and comfort.

Though it may seem to be no more than a covering for tissues, skin performs an excessively important role, protecting us from the external world: it provides a protection against mechanical, chemical, thermal and microbial factors, it helps us stay warm when it's cold and it keeps stuff out, including germs and water.

Like any other organ it may be subject to **disease,** and its afflictions take many forms. Skin conditions can result from an endless variety of causes ranging from heredity, allergy and fungal growth to bacterial, viral and parasitic infection; these may come from agents which reach the skin from outside, from the blood, or from an inherent instability of the epidermal cells. Moreover, skin is overexposed to risk factors, such as the **ultraviolet (UV) radiation** arriving to earth surface and the **environmental pollution,** both inducing skin disorders, predominantly cutaneous malignancies, immunosuppression and aging.

Last but not least, skin defines much of our standard for **beauty.** A bright and youthful skin means health and vitality. Like every part of our body, skin responds to care and attention: proper treatments can revitalize skin and slow the aging process, keeping skin healthier and longer.

For all these reasons, nowadays the market calls for modern and more responsible skincare products, whose testing represents a crucial step for the evaluation of product safety and efficacy. Rigorous material selection and testing are crucial so that products perform as expected and consumers are protected. Depending on the specific type of product, manufacturers should conduce specific testing to ensure the safety and usefulness of their cosmetic products. They may also perform additional tests to meet specific quality or performance requirements of consumers and buyers.

* React4life S.r.l., Genova (Italy)

What *in vitro* solutions does the community offer?

The test industry is undergoing radical modifications: animal tests of cosmetic products and ingredients are now banished at European level. This restriction has been a significant driver to the ***in vitro* testing sector** in recent years. As a result, the industry has seen technological advancements and innovations to make tests both more useful and cost effective.

In the cosmetics industry, *in vitro* testing is typically used to confirm the lack of certain toxic properties in cosmetic and personal care products, finally achieving regulatory approval. *In vitro* **toxicology** testing offers a non-animal alternative to the cosmetics and personal care industry, allowing the effective evaluation of the potential of end products and ingredients to cause skin irritation, skin corrosion and other adverse side effects when consumers use them. The main test methods are **2D cell-based and biochemical assays,** performed to assess acute toxicity, photo-toxicity and mutagenicity. In particular, **the most** diffused **tests are the** cytotoxicity (according to UNI EN ISO 10993-10), the skin irritation (according to OECD 439), the determination of phototoxic potential (according to OECD 432), the determination of mutagenic potential (according to OECD 471), the determination of corrosive potential (according to OECD 435) and the cell viability assay (MTT and/or NRU) tests.

In vitro tests can also be used to test the **efficacy** of products, such as the absorption of a substance applied to skin. According to OECD guidelines, the absorption of a product through skin can be evaluated *in vitro* using a skin sample separating two chambers of a **diffusion cell.** In this context, two main instruments have been using for *in vitro* diffusion tests: 3D skin equivalents and Franz cells [2, 3].

3D skin equivalents are easy-to-use highly differentiated *in vitro* human skins typically generated from primary human keratinocytes on a substrate containing human dermal fibroblasts. Skin equivalents are used to assess the efficacy and mode of action of novel agents. They are grown at the air-liquid interface, which allows full epidermal stratification and epidermal-dermal interactions to occur. 3D skin equivalents may be removed from the insert by carefully cutting around the membrane edges with a scalpel. The skin is typically fragile and requires careful handling during removal.

Franz Cell chambers are *in vitro* permeation assays frequently used in formulation development. Their main use is related to the measurement of drug release. They can be used with a synthetic membrane, a tissue construct or (more commonly) a biological sample that separates the donor compartment containing the test product from the receiver compartment filled with collection medium. The lower compartment is maintained in agitation by a magnetic bar. In the cosmetics field, the test product is applied to

the membrane via the top chamber. The bottom chamber contains fluid from which samples are taken at regular intervals for analysis. This testing determines the amount of active that has permeated the membrane at each time point.

Although both 3D skin equivalents and Franz cells have been widely adopted in the cosmetic field as devices for testing, these systems have some limitations mainly related to their conformation. First, these systems limit the hosting of different skin tissues: skin biopsies and equivalents may in fact differ in terms of thickness, shape and size, and the tissue chamber should take into consideration it. Moreover, the fluid present in 3D skin equivalents and Franz cells is static in the upper compartments, while in Franz cells it is agitated in the lower compartment by a magnetic stirrer, thus avoiding the possibility to properly set the fluid velocities as the physiological ones. 3D skin equivalents systems typically exist in different pre-established dimensions (e.g. 6 or 24 well-plates), reducing their versatility; both in 3D skin equivalents and in Franz cells, fluid volumes can't be adjusted.

Until now the main instruments adopted for the in vitro tests allow to mimic only a segment of the skin organ environment (e.g. full thickness skin or a chemical diffusion across a membrane); therefore at the moment it is not available a combined, realistic and fully compliant platform where cosmetic tests may be carried out according to the different end-user claims.

The revolution: MOOD by React4life

Trying to fill these gaps, the **MOOD (Multi-Organ On Device)** technology was born (see Figure 1).

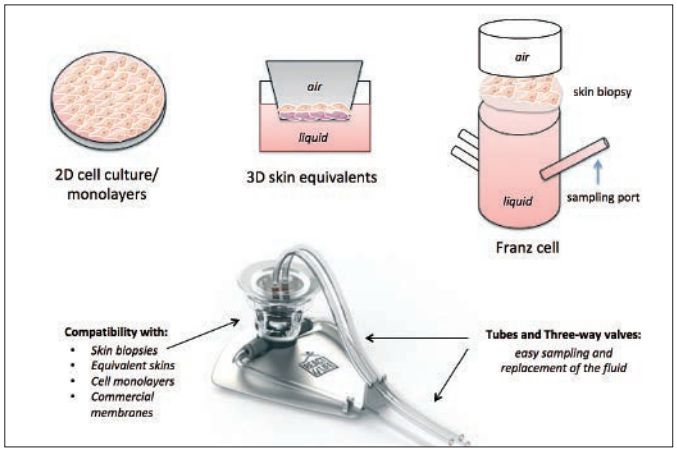

Fig. 1 – MOOD overcomes the current technologies used for cosmetic testing, providing a dynamic, versatile and more similar to the in vivo context.

MOOD is an under patent device which supports the hosting and culture of tissues in a physiological and dynamic environment. Thanks to its conformation, MOOD can host cell monolayers (e.g. keratinocytes of fibroblasts seeded on a porous membrane), commercial filters or membranes, equivalent skins or skin biopsies. MOOD is compatible with the most used skin equivalents in the cosmetics field, such as for example EpiSkin by SkinEthic, EpiDerm by MatTek, Epidermal Skin Test by CellSystems (see Figure 2).

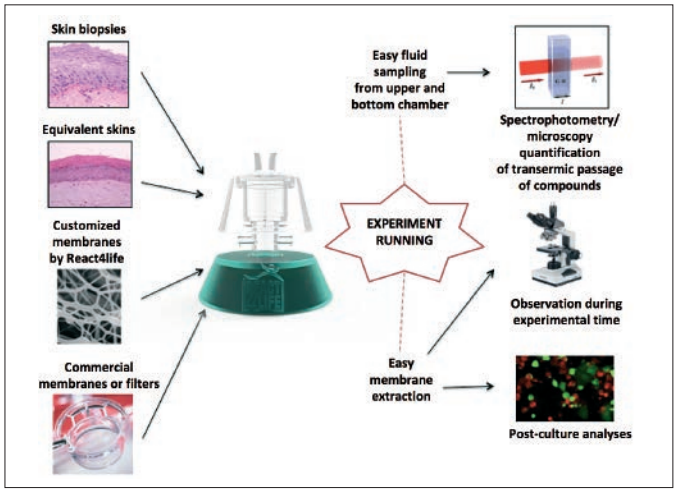

Fig. 2 – The versatility of MOOD by React4life allows hosting skin biopsies from patients, equivalent skins, customized membranes or commercial membranes and filters. During the experiments, fluid can be sampled and analyzed (e.g. through spectrophotometric technique) and membrane can be observed by moving the donor chamber under an inverted optical microscope. At the end of the experiments, membranes can be fixed and stained to be observed with standard optical microscope, confocal microscope, scanning electron microscope, etc.

Within MOOD, fluids are dynamic, and their velocity can be easily chosen and adjusted in order to reproduce the physiological speeds, such as the capillary blood velocity below the skin, or the polluted air above, or seawater turbulence to test the water resistance of sun protective products (SPF creams).

As regards transdermal tests, the winning feature of MOOD is the possibility to carry out both biological/structural analyses on the membrane (such as MTT proliferation tests, viability tests, microscopy analyses, compactness of the skin) and quantitative transdermal tests (such as to quantify the amount of compound/pollution/radiation that has passed the skin).

The value proposition of the MOOD technology is:
– **Versatility,** since MOOD can host equivalent skins, customized or commercialized membranes without size restrictions, as well as 3D clinically relevant biopsies;
– **Dynamicity,** being equipped with fluidic circuits that maintain a physiological velocity of the fluid (air, liquid);
– **Modularity,** since it can be connected with other MOODs to mimic cross-talk between diverse organs.

In the cosmetics field, specific in vitro tests can be provided with MOOD, such as the transdermal, the antipollution and the anti-UV tests.

Transdermal tests (see Figure 3A)
New wellness compounds, drug formulations or other composites may be applied over the skin tissue cultured within MOOD and their passage through skin may be monitored and quantified over

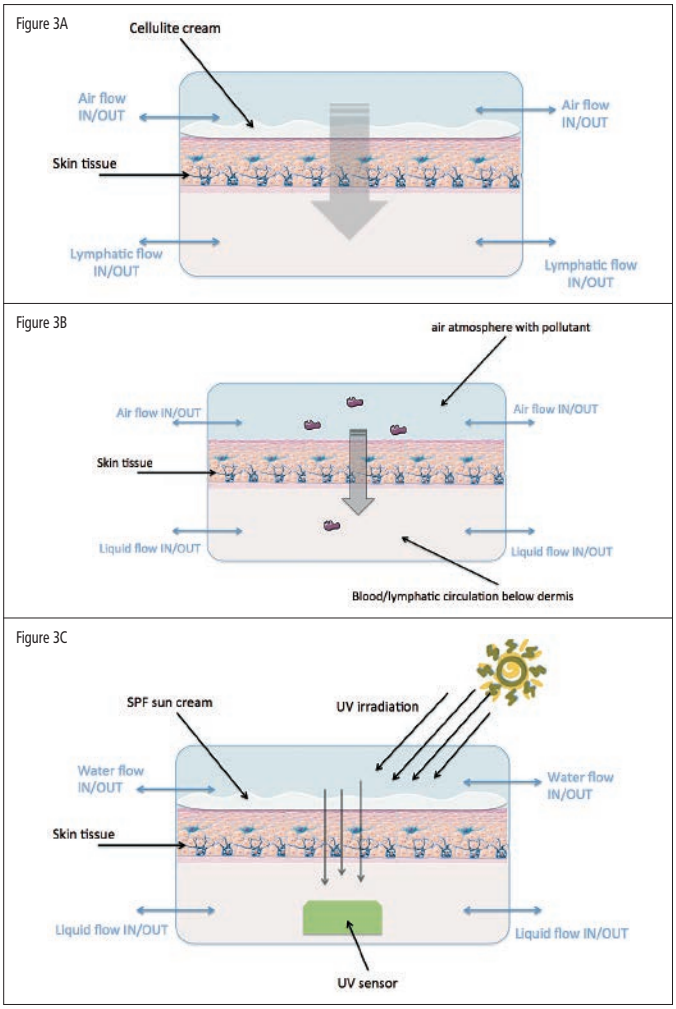

Fig. 3 – Some in vitro tests performed within MOOD technology: trans-dermal, anti-pollution and anti-UV.

time. In many cases, in fact, it is mandatory to evaluate if the cosmetic formulation (i.e. cellulite cream) is able to fully pass through the epidermis and dermis tissue, finally reaching the lymphatic circuit.

Advantages of MOOD with respect to standard techniques:
1. Possibility to host both human biopsies and artificial tissues of different size and thickness;
2. Possibility to mimic the blood capillary velocity or the lymphatic capillary velocity in the bottom chamber.
3. Possibility to evaluate and quantify over time the drug/compound passage across the skin.

Anti-pollution tests (see Figure 3B)

MOOD allows hosting skin tissues and evaluating in a quantitative way the quantity of heavy metals or other atmosphere pollutants crossing the skin tissue, with and without the application of antipollution cosmetic products. The culture media placed below the skin is sampled over time and analyzed to quantify the percentage of pollution passed and to evaluate the percentage blocked by the treatment.

Advantages of MOOD with respect to standard techniques:
1. Possibility to host both human biopsies and artificial tissues of different size and thickness;
2. Possibility to mimic the blood capillary velocity in the bottom chamber;
3. Possibility to have a dynamic environment in the upper chamber, useful for pollution particles to not settle after a few seconds.

Anti-UV tests of sun creams

This is an innovative test, laboratory validated and soon available for the entire market.

By using a specific UV-ray sensor embedded in the MOOD technology, the quantity of UV radiation passed through the skin after application of a sun cream may be quantified over time. In order to test the efficacy of waterproof products, the system may be used both in dry (air/liquid interface) and in wet (liquid/liquid interface) conditions.

Advantages of MOOD with respect to standard techniques:
1. Possibility to host both human biopsies and artificial tissues of different size and thickness;
2. Possibility to mimic the blood capillary velocity in the bottom chamber;
3. Possibility to work both in air/liquid and liquid/liquid interface for the assessment of waterproof sun creams.

How does MOOD work?

MOOD consists of a main chamber representing the **receiving compartment,** inside which a second chamber, the donor compartment, can be stuck. The **donor compartment** can be directly represented by a 3D skin equivalent (EpiSkin by SkinEthic, EpiDerm by MatTek, Epidermal Skin Test by CellSystems, etc.) or by a provided support hosting a biopsy or an engineered membrane. MOOD has a cylindrical shape, recalling shape of a Petri dish, ensuring good maneuverability of the materials within.

MOOD is produced with a **biocompatible USP class VI** material, suitable for working with biological and cellular parts.

MOOD is powered by a **peristaltic pump,** which allows setting the flow rate and the consequent fluid velocity inside the chambers. MOOD can be filled with liquids or air depending on the test you are interested in. For example, to evaluate the effect of anti-pollution or sunscreen creams on skin, you can cultivate the skin in a classic **air-liquid interface** that allows the cream to be exposed to air and the skin to receive the necessary nutrients from the underlying culture medium. Contrariwise, to evaluate the resistance of waterproof creams, a **liquid-liquid interface** consisting of water in the upper chamber and culture medium in the lower chamber can be used.

MOOD is connected to the peristaltic pump through oxygen-permeable tubes and three-way valves providing an easy replacement and sampling of the culture medium and good mixing of the receptor solution in contact with the underside of the skin.

If necessary, MOOD can work into a standard humidified incubator at controlled temperature and CO2.

***During the experiments,* fluid can be sampled over time and analyzed** (e.g. through spectrophotometric technique or microscopy techniques); **membrane can be also observed** by moving the donor chamber under an inverted optical microscope (see Figure 4).

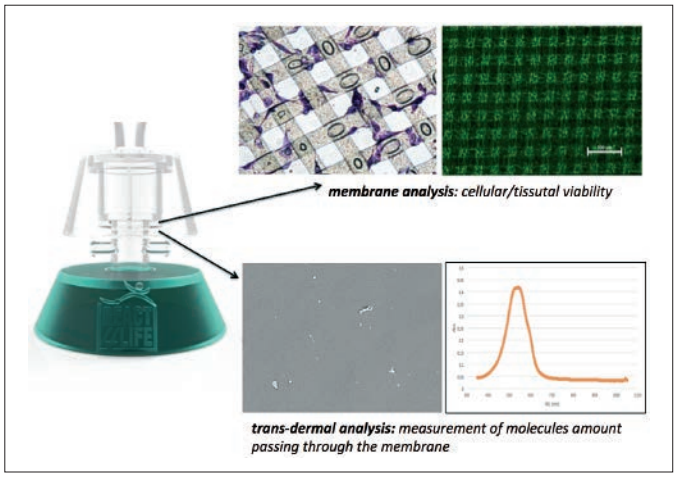

Fig. 4 – the winning feature of MOOD is the possibility to carry out both biological/structural analyses on the membrane (such as MTT proliferation tests, viability tests, microscopy analyses, compactness of the skin) and quantitative transdermal tests (such as to quantify the amount of compound/pollution/radiation that has passed the skin).

***At the end of the experiments,* membranes can be fixed and stained** to be observed with standard optical microscope, confocal microscope, scanning electron microscope, etc.

In the next future, MOOD may be used with **different fluid configurations:**

– Tangential flow configuration: the upper and the lower chambers are perfused by two different and independent flows; the upper chamber receives a tangential fluid flow, mimicking the air-skin interaction.

– Horizontal flow configuration: the upper and the lower chambers are perfused by two different and independent flows; the upper chamber receives a horizontal fluid flow, mimicking the fluid-skin interaction (e.g. water flow induced shear stress).

– Perfusion flow configuration, suitable for 3D cells construct perfusion: the flow crosses the cells layer in vertical direction.

References

1 *Kezic, Sanja, et al. "Skin barrier in atopic dermatitis." Frontiers in bioscience (Landmark edition) 19 (2014): 542-556.*

2 *Wagner, Ilka, 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 13.18 (2013): 3538-3547.*

3 *Lee, Pil H., Robert Conradi, and Veerababu Shanmugasundaram. "Development of an in silico model for human skin permeation based on a Franz cell skin permeability assay." Bioorganic & medicinal chemistry letters20.1 (2010): 69-73.*