UNIVERSITÀ DELLA CALABRIA
DIPARTIMENTO DI
FARMACIA E SCIENZE
DELLA SALUTE
E DELLA NUTRIZIONE

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REPORT

IN VITRO DETERMINATION OF THE BARRIER EFFECT AND FILMOGEN ON HUMAN ORAL EPITHELIUM RECONSTITUTED IN VITRO

SAMPLE	**FTP 82 GEL MUCOSITI LOTTO H2543M**

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REPORT N° EFFBA 0903F FTP 82 GEL MUCOSITI H2543 M

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INTRODUCTION

EpiSkin® is an "in vitro" reconstituted human epidermis model formed by normal human epidermal cells and fibroblasts cultured on a collagen matrix at the air-liquid interface.

This model is histologically similar to human skin in vivo. EpiSkin® is produced in conformity with the reference quality standard ISO 9001, guaranteeing traceability and reproducibility of epidermal tissues

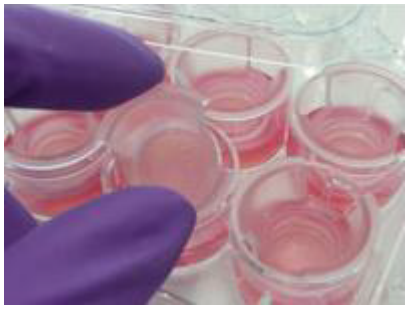

Fig. 1, (Fonte, SkinEthic)

These in vitro reconstituted human skin models are biologically relevant and represent a highly predictive and reproducible tool for preclinical evaluations. Thanks to their histomorphological structure, it is possible to evaluate the potential barrier effect of a product directly on the tissue models, through topical applications of the product under examination and experimental conditions that mimic the physiological conditions.

The introduction of these experimental models constitutes a promising scientific innovation in areas where animal testing is highly regulated (chemicals and active ingredients) or completely banned, while the development, validation and implementation of alternative methods are strongly promoted.

DETERMINATION OF THE BARRIER AND FILMOGEN EFFECT

The test intends to evaluate the possible barrier and film-forming effect exerted by the products under examination on a model of human oral mucosa reconstructed in vitro according to validated protocols.

The in vitro model of oral epithelium developed by SkinEthic Laboratories consists of a three-dimensional multilayer culture of TR146 placed on a polycarbonate insert [2]. This model is very similar in terms of morphology, biochemical and physiological properties to human tissues and today represents an important alternative to animal tests. The increased biological relevance and predictivity of these models derives from the presence of an organized tissue with different cell layers that allow to evaluate the topically applied products under realistic experimental conditions. Therefore, 3D tissue models can be used as a tool to assess the safety of finished products, and determine the biocompatibility and mechanism of action of medical devices.

The filmogen effect of the following product:

- **FTP 82 GEL MUCOSITI H2543 M**

It has been studied on a Reconstituted Human Oral Epithelium model to evaluate its potential barrier effect.

The film-forming properties were determined by a multiparametric approach in the evaluation, which includes the measurement of transepithelial electrical resistance (TEER) and the determination of tissue permeability (passage of caffeine and the lucifer yellow assay).

The test product was applied topically on the surface of the epithelium 3 hours before the start of the experiment and compared with a negative control, consisting of a saline solution (NaCl 0.9%) which has no action on the tissues, and a positive control, consisting of petroleum jelly.

Experimental part

START TEST: 21-02-2019

END TEST: 23-02-2019

- ***Oral Human Epithelium reconstituted in vitro***

received EPISKIN kits, the tissues were removed from the culture tool and transferred to a 12-wells plate previously filled with a holding tool (1 mL / well pre-warmed to 37°C) and incubated at 37 ° C, 5% CO2.

Name and Batch	**Reconstructed Human Oral Epithelium 19-HOE -008**
Manufactured	Episkin
Thickness	80 µm
Histolog at Day 5 (layers >4)	6

HOLDING TOOL	Batch	19 SGM 019

➢ *TEER measurement and passage of caffeine determination*

The measurement of transepithelial electrical resistance is a technique that is used to measure the integrity of the membranes [3]. Before the start of the experiment, the TEER was measured using the Millicell-ERS instrument. The results were reported in figure 3.

For the in vitro study on percutaneous adsorption, the ODECD 428 guidelines recommend the use of caffeine as a reference compound. In fact, in the present study, caffeine, thanks to its ability to overcome the epithelial barrier even in the absence of damage, was used as an irritant to evaluate the propensity of a product to form a protective film on the skin, thus determining the potential barrier effect of the product under investigation. The reduction of the passage of caffeine through the 3D model of human epidermis reconstituted in vitro is therefore considered as an index of the protective efficacy of the product under examination.

The experiment was conducted using 30 µL of product, carefully positioned on the human oral epithelium reconstituted in vitro. The treated epidermis was left for 3 hours at room temperature, to avoid problems in the formation of the film. Thereafter, the tissues were transferred to a 12-well plate, previously filled with 1 mL / well of saline (recipient compartment). Subsequently, 0.1 mL of a 1% (w / v) caffeine solution was applied to the epidermis (donor compartment) and the tissues were incubated at 37 ° C, 5% CO2. After 3 hours the receptor fluid was withdrawn from the receiving compartment and the samples were analyzed by HPLC-UV (JASCO BIP-I; JASCO UV DEC.100.V DETECTOR). The results were expressed as a reduction in the passage of caffeine (%) compared to the control (table 1 and figure 2).

Subsequently, the excess sample was removed by washing and the TEER was measured again (T = 6).

➢ *Lucifer yellow assay*

Following the determination of the passage of caffeine, the excess sample removal and TEER measurement (T = 6 h), the tissue permeability was determined after Lucifer Yellow Assay [4].

Lucifer yellow is a fluorescent dye used for tissue permeability studies. When the membranes are intact, this dye has a poor permeability, while in the case of damaged structures, Lucifer yellow shows a high permeability. Therefore this test is used in order to determine the integrity of the membranes in the presence of substances to be examined.

For this purpose, 0.5 mL of Lucifer yellow was placed in the apical compartment (AL), while 1 mL of saline was placed inside the basolateral compartment (BL). After an incubation period of 1 h at 37 ° C, the passage of the Lucifer yellow dye was determined by spectrofluorimetric analysis (Synergy H1 - Hybrid Reader - BioTek) at 428 nm (excitation) and at 535 nm (emission). The flow was calculated using the following formula:

LY Flux% = (RFU BL / RFU AP t = 0) x 100

Where is it:

RFU = fluorescence measurement

RESULTS

The results obtained, reported on Table 1 and Figure 2, suggest barrier and film-forming properties in vitro for the tested sample.

	FILMOGEN EFFECT	BARRIER EFFECT 6h	
Sample	***Passage of cafeine reduction (%)***	***TEER***	***Lucifer yellow***
FTP 82 GEL MUCOSITI H2543 M	**30,1%**	COMPLIANT	COMPLIANT

Table 1.

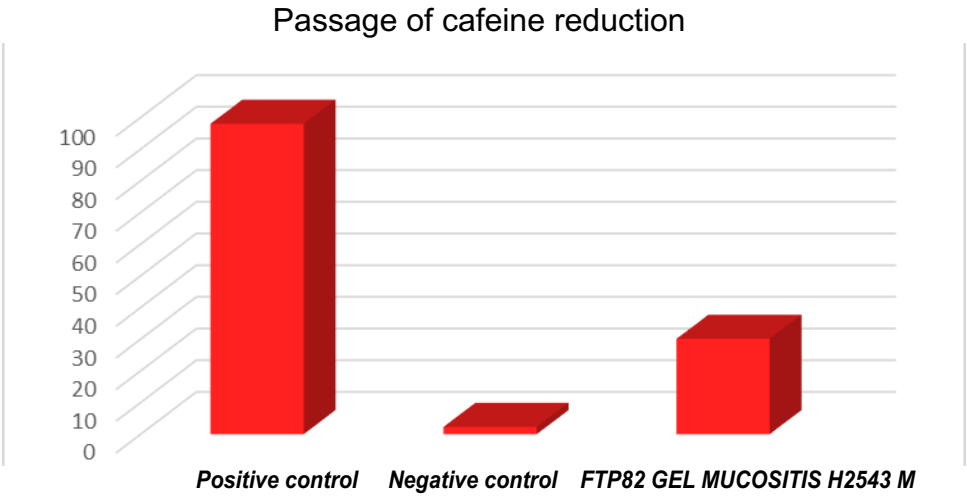

Figure 2
Positive Control: Vaseline;
Negative Control: saline solution (NaCl 0,9%)

*Figura 3. TEER expressed in ohm*cm^2 at time 0 and after 6 h treatment.*

The TEER recorded after 6 h of treatment with **FTP 82 GEL MUCOSITI H2543 M** does not differ much from the value recorded at time 0. A similar result was recorded for the positive control and for the negative control.

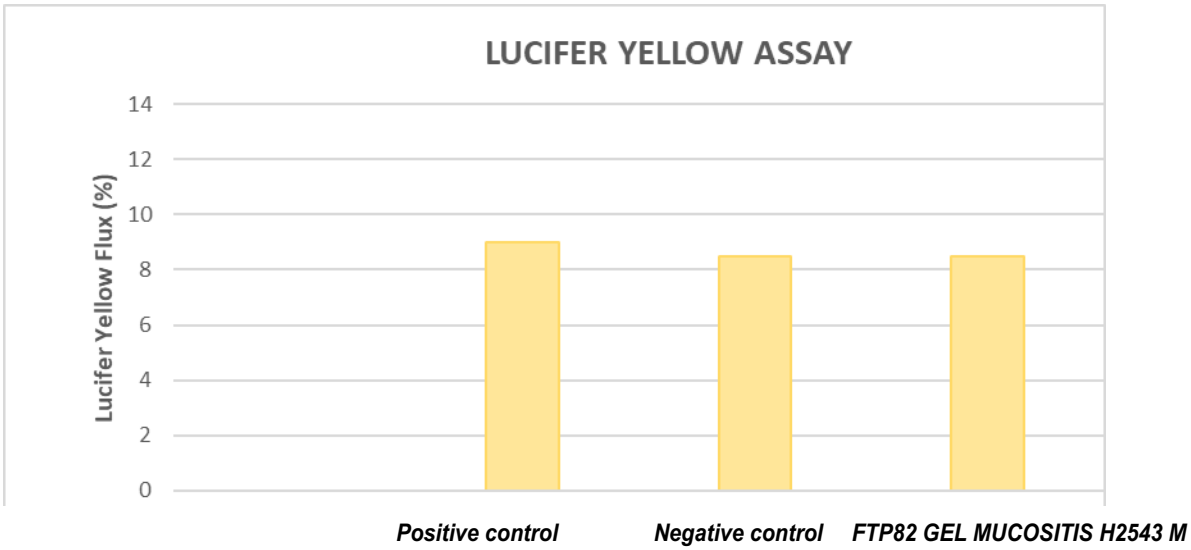

Figura 4. Lucifer yellow flux after 6 h.

The values obtained for the negative control, the positive control and the sample **FTP 82 GEL**

MUCOSITI H2543 M are comparable. This indicates that the integrity of the membrane has been

preserved.

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