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Criopass Laser Efficiency as an Enhancer of Caffeine and Caffeisilane C permeation: 3D Human Skin Equivalent as a Model Barrier

Patricia S. Lopes[1], Dayane P. Luco[2], Pedro G. Oliveira[3], Iasmin M.Rufino[1], Estela B. Herbst[1], Giulia Grecco[1], Monica B. Mathor[2], Heron D. T. Silva[1], Marcio A. Andreo[1], and Vânia R. Leite-Silva[1]

[1]Instituto de Ciências Ambientais Químicas e Farmacêuticas, Universidade Federal de São Paulo, Diadema, SP, *09913-030*, Brazil;[2]IPEN/CNEN - Instituto de Pesquisas Energéticas e Nucleares, São Paulo, SP, 05508-000, Brazil;[3]Universidade Anhembi Morumbi, São Paulo, SP, 03164-000, Brazil.
patricia.lopes@unifesp.br

ABSTRACT SUMMARY

A novel and efficient **full thickness** *in vitro* three-dimensional (3D) human skin model was developed to study the permeability of active ingredients that must reach deeper skin layers to achieve effective activity. This 3D model could allow the study of new forms and ingredients for different topical or transdermal treatments without the need of animal tests. The aim of this study was to evaluate the penetration of caffeine and Caffeisilane C® applied topically to a 3D human skin model, using Crio Laser Forese (CLF) or Criopass Laser (CL) as a permeation enhancer.

INTRODUCTION

The main barrier for topic treatments is related to the capacity of the drug or compound to penetrate the *stratum corneum*. Studies on permeation enhancers have intensified over the years, whether for the evaluation of the permeation properties of active substance in presence of an enhancer, or to achieve techniques able to reversibly modify the barrier function of the stratum corneum[1]. The present work evaluates the effectiveness of an *in vitro* biophysical method (CRL) to enhance the cutaneous permeation of caffeine. This biophysical method uses a laser radiation beam that focuses on a frozen gel containing the substance of interest[2]. The system releases photons that collide with electrons of the molecules resulting in an increase of its kinetic energy, allowing the gel molecules to penetrate the skin and reach the desired location[2]. Caffeine – a hydrophilic substance - was used as a model substance due to its difficulties to overcome the stratum corneum barrier[3].

EXPERIMENTAL METHODS

Human keratinocytes and fibroblasts were obtained based on the methodology proposed by Rheinwald and Green[4]. The reconstruction of the full thickness three-dimensional human skin models also followed a methodology already standardized in our laboratories proposed by Herson, et al.[5] with some modifications.

Fig.1. A – application of The CLS on 3D human skin model. B – CLS equipament.

The formulations 3% (w/w) Caffeine gel or 5% (v/w) Caffeisilane C® (Siloxanetriol Alginate and Caffeine and Butylene Glycol CAS 187175424) were applied topically in each of the test groups and its permeation rate evaluated after CLS 10 min of contact (fig.1 A, B). HLPC analyses were performed after 3 and 20 hours of contact using a THERMO Acella 600 HPLC-UV/DAD. The chromatographic conditions were optimized based on the literature[6]. It was used ACE C18 column (250 x 4.6 mm) with 5µm particles and HPLC grade solvents. Samples and standards (20 µL) were injected in isocratic system composed of 1% acetic acid and acetonitrile (90:10) for 12 minutes each run.

For the calibration curve, solutions were prepared with dilutions of the standard (caffeine) at 0.5, 1.0, 2.0, 4.0 and 8.0 $\mu g.mL^{-1}$. These samples were performed in triplicate. Sample's aliquots of 0.5 mL were taken and

diluted in a volumetric flask (10 mL) containing 70% methanol solution (v/v).

RESULTS AND DISCUSSION

This is a human cell based *in vitro* assay that represents a very plausible alternative way to replace animal models for permeability tests. An advantage presented by three-dimensional human skin models is the reproducible and trustworthy data acquisition if compared to those originated from ex-vivo models.

The *in vitro* model was composed by fibroblasts, keratinocytes and melanocytes allowing this 3D model to be use for cutaneous permeation tests with good efficiency to assay cosmetics, medicines and other drugs or ingredients of interest.

The chromatogram of standard caffeine (fig.2) allowed the calculation of the total amount of caffeine released in each condition.

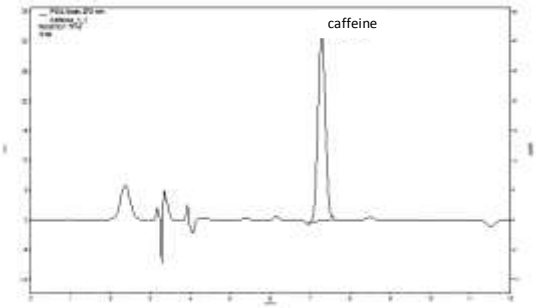

Fig.2. HPLC-UV/DAD chromatogram standard (caffeine).

The data obtained after 3h of exposure showed that the amount of caffeine that permeated the 3D equivalent was higher in absence of exposition to the criolaser, although this amount increased after 20h. This showed that the CLF could extend the activity for Caffeine and Caffeisilane C comparing to the usual formulation use.

There are a number of validated and recognized skin models that are commercially available. These models are generally based on reconstructed human epidermis (RhE), a three-dimensional epidermal model cultured from human keratinocytes.

The 3D equivalent model here presented is a full thickness model that is suitable to study active ingredients that must permeate the stratum corneum, epidermis and dermis to achieve their goal, as occurs to caffeine, a cellulite treatment ingredient.

CONCLUSION

In the present study, we developed procedures for building a novel *in vitro* skin model that could be used to reproduce satisfactorily the conditions of penetration and activity of cosmetic products intended to overcome the stratum corneum barrier, full epidermis and dermis.

It is expected that the standardization of this technique would allow, as an example, the assessment of the effect of caffeine and Cafeisilane C®, ingredients used to treat cellulite, to be assessed a lot easier, regarding their cytotoxicity and effects at the cellular level having as future prospect, the ability to achieve the real results in innovative dermocosmetic formulations as well as in those formulations already marketed.

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