

# Evaluation of the anti-aging and skin rejuvenation effect of a novel facial cream containing small synthetic peptide which to unlock TGF-β potential

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## Introduction:

Nowadays, the transforming growth factor beta (TGF-β) pathway, which is known to play a pivotal role in the pathogenesis of skin anti-aging. TGF-β is one of these cellular master switches, and it is the master regulator of the ECM, and itself acts as a multifunctional cytokine to regulate growth, differentiation, and other functions in various cell types. And TGF-β is known to stimulate the fibroblasts in the dermis to synthesize several proteins such as collagen, the main constituent of dermal ECM. TGF-β triggers synthesis of some components of the dermal-epidermal junction such as laminin V, collagen IV and collagen VII. TGF-β inhibits the expression of protein-degrading matrix metalloproteinases (MMP) such as MMP-1, -2, -3 and -13.

The best performers peptide in the screening Palm-Lys-Val-Lys-OH (Pal-KVK), as an anti-aging and skin rejuvenation effect active ingredient has been studied. We design in-vitro LAP-TGF-β activation testing and the quantification of cytokines and MMPs testing to clear activation of TGF-β and inhibition of TNF-α, IL-8, MMP-1, and MMP-3 expression of the peptide Pal-KVK. And the in-vivo 28 Days clinical testing, we study the smooth and wrinkles of crow's feet after 28 days treatment of sample with Pal-KVK using DermaTOP. Those Several in-vitro activity tests and in-vivo application studies were performed to demonstrate the effectiveness of Pal-KVK peptide as an anti-aging and skin rejuvenation active.

## Materials & Methods:

In-vitro LAP-TGF-β activation, the samples were tested with TGF-β Emax immunosay system (promega). TGF-β and the peptide suppress pro-inflammatory factors (TNF-α, MMP-1, and MMP-3 and IL-8) in human normal keratinocytes. Keratinocyte culture and treatment phorbol 12-myristate 13-acetate (PMA) experiments were test. Quantitation of cytokines and matrix metalloproteinases (MMPs) were quantified from supernatant of cell cultures with a multiplex bead array system (Luminex 100 xMAP) using the Procarta Cytokine Assay Kit (Panomics, Fremont USA). Clinical evaluation with 36 female subjects is to evaluate the anti-wrinkle effect of Sample B with Pal-KVK. Study of the variations, directly in vivo, of the cutaneous relief parameters on crow's feet (average roughness Ra, maximum amplitude Rt and average relief Rz) using DermaTOP (EOTECH - France). Illustration of the visual effect by realization of macrophotographs.

Table 1 The experimental design schedule of 28-Days Clinical testing

	D0	D7	D14	D28
Information of the subject about study conditions and collection of their informed consent.	•			
Verification of inclusion and non-inclusion criteria.	•			
Acquisition of a 3D-profile of each crow's feet and each under eyes area using DermaTOP	•	•	•	•
Acquisition of a photograph of each hemi-face and of the entire face for:				
- Illustrations and further analysis by NEWTONE Technologies®	•	•	•	•
- scoring of lids sagging	•	•	•	•
Distribution of a daily log.	•	•	•	•
Distribution of the study products.	•	•	•	•
Subjective evaluation questionnaire.	•	•	•	•

Table 2 The Primary criterion of DermaTOP evaluation

DERMISCAN	Acquisition with DermaTOP
NEWTONES Technologies	Crow's feet wrinkles Under eyes wrinkles Wrinkles around eyes Bags under eyes

Table 3 The Secondary criteria of DermaTOP evaluation

DERMISCAN	Photographs
NEWTONES Technologies	Scoring of lids sagging Dark circles analysis

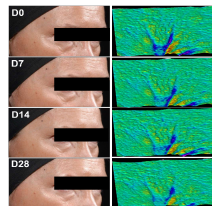
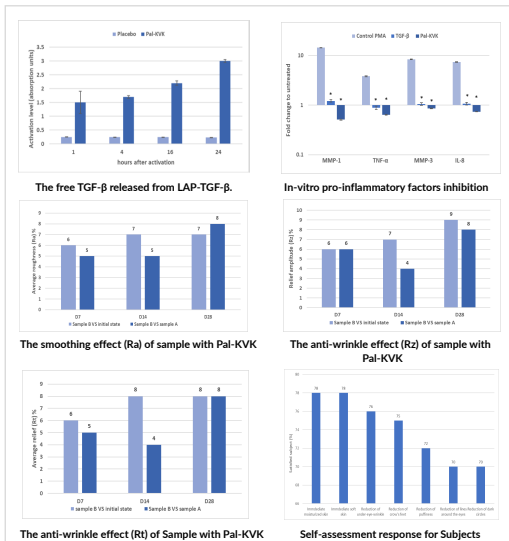
## Results & Discussion:

The result of the in-vitro shows a significant higher time-dependent increase release of TGF-β from LAP-TGF-β complex after incubation with the peptide than control group. TGF-β and the peptide Pal-KVK strongly in habited up-regulation of TNF-α, MMP-1, and MMP-3 and IL-8 expression. As clinical in-vitro testing results, there were no noticeable variation in the cutaneous relief parameters on underneath eye wrinkles and on the lids sagging.

After 28 days formulation with Pal-KVK and control sample treatment, subjective evaluation with self-assessment questionnaire and skin conductance response were analyzed. The products satisfied many of the subjects for their organoleptic characteristics and their efficacy especially on wrinkles. The self-assessment showed that after 28 days of application, up to 78% subjects satisfied with the Pal-KVK formulation for immediate moisturized and soft skin, up to 76% subjects report reduction of under-eye-wrinkle, 75% subjects report crow's feet reduction, with 72% subjects reporting puffiness reduction, lines around the eyes and dark circles were also reduced for 70% subjects.

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Example of DermaTOP artificially colour-coded microrelief record from the crow's feet area from one volunteer before treatment (day 0) and after 7, 14, 28 Days treatment with formulation with 100 ppm Pal-KVK

As Pal-KVK induced a time-dependent release of active TGF-β during in-vitro LAP-TGF-β activation testing. The palm-KVK (10 μM) peptide shows stronger inhibited up-regulation of MMP-1, MMP-3, and pro-inflammatory cytokine expression (TNF-α and IL-8) than TGF-β at 12.5 ng/ml. The self-assessment showed that after 28 days of application, more than 70% subjects reporting the reduction of puffiness, lines around the eyes and dark circles. The Pal-KVK peptide showed a high TGF-β potential function relate with time, and inhibit pro-inflammatory factors (TNF-α, MMP-1, MMP-3, and IL-8) in human normal keratinocytes. In clinical testing, the cream containing the peptide showed a significant smoothing and anti-wrinkle efficacy on crow's feet and underneath eye after 28 days twice-daily application. And in Subjective-self-evaluation, the Sample B with Pal-KVK satisfied most of the subjects for their organoleptic characteristics and their efficacy, especially on wrinkles.

## Conclusions:

The small synthetic peptide Pal-KVK showed an unlock TGF-β potential function, and suppress pro-inflammatory factors (TNF-α, MMP-1, MMP-3, and IL-8) in human normal keratinocytes. In clinical testing, the cream containing the peptide showed a significant smoothing and anti-wrinkle efficacy on crow's feet and underneath eye after 28 days twice-daily application.

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