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DEMONSTRATION OF THE ANTI-WRINKLE EFFICACY OF A COSMETIC PRODUCT. CORRELATION BETWEEN CLINICAL OBSERVATIONS AND INSTRUMENT METHODS.

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Synopsis

Demonstration of the anti-wrinkle efficacy of a cosmetic product was carried out in 41 women presenting with photoageing of facial skin. The subjects applied the product to the whole of the face, twice a day, for 8 weeks. Evaluation of the state of the skin and cutaneous relief was carried out before the start of treatment and after 2, 4 and 8 weeks of treatment using a clinical score method, together with instrument measurements: replicas of the area around the eye and Image Analysis. Evaluation by the subjects was recorded. Skin tolerability was also verified.
The clinical observations showed a significant and progressive improvement in cutaneous relief as well as a significant increase in the firmness and cosmetic qualities of the skin during treatment.
These results were confirmed by Image Analysis of the replicas: reduction in the number and depth of the wrinkles, as well as evaluation of product efficacy by the treated subjects.

Riassunto

La dimostrazione dell'attività anti-rughe di un prodotto cosmetico è stata svolta su 41 donne che presentano fotoinvecchiamento della pelle del viso. I soggetti hanno applicato per 8 settimane il prodotto sull'intera superficie del volto, due volte al giorno. La valutazione dello stato della pelle e del rilievo cutaneo è stata svolta sia prima dell'inizio del trattamento che dopo 2, 4 e 8 settimane di trattamento utilizzando un metodo di punteggio clinico, insieme a misure strumentali: riproduzioni dell'area intorno agli occhi e Immagine Analitica Computerizzata. Le valutazioni dei soggetti sono state registrate. É stata anche verifica la tolleranza della pelle. Le osservazioni cliniche hanno dimostrato un significativo e progressivo miglioramento del rilievo cutaneo, insieme ad un aumento significativo del tono e delle qualità cosmetiche della pelle durante il trattamento. Questi risultati sono stati confermati dalla Immagine Analitica Computerizzata delle riproduzioni: riduzione nel numero e nella profondità delle rughe, insieme alla valutazione dell'efficacia del prodotto data dai soggetti trattati.

INTRODUCTION

Cutaneous ageing is the result of two distinct processes: intrinsic or chronological ageing and photoageing which is induced by repeated exposure to UV radiation from the sun (1, 2, 3). In exposed areas, particularly on the face, numerous cutaneous alterations are found. In the epidermis, an effect on division (4) and differentiation of keratinocytes leads to cutaneous dryness and a loss of elasticity in the Stratum Corneum. In the dermis, a disorganisation of the fibre network can be observed with accumulation of abnormal elastic fibres (5) and degeneration of collagen (6) which is seen as the appearance of deep wrinkles and loss of firmness, suppleness and elasticity of the skin.

Vitreoscilla filiformis, a bacterium obtained from sulphur thermal springs, has been cultivated *in vitro* by biotechnology (7); recent studies have shown that an extract of *Vitreoscilla filiformis* was able to stimulate the proliferation of human keratinocytes *in vitro* (C.M. Lapière et al, unpublished observations). Its activity has also been demonstrated in human fibroblast cultures which it stimulates to proliferate and produce IL1β (J.A. Grimaud et al, unpublished observations), and in cultures of human macrophages where it induces cellular activation and production of IL1b (V. Bayer et al, unpublished observations). In the complex mechanisms of dermal repair, IL1 induces proliferation of fibroblasts, probably by the intermediary of the autrocrine production of PDGF (8) which in turn, indirectly stimulates the production of collagen (9). IL1β also acts on regulation of elastin expression (10) and in the epidermis, it induces proliferation of keratinocytes (11).

The properties of *Vitreoscilla filiformis* observed *in vitro* suggest a potential *in vivo* action on the restructuration of cutaneous tissue and thus improvement in the relief and state of the skin. In to order to verify this hypothesis, an extract of *Vitreoscilla filiformis was* introduced into a cosmetic product and its efficacy was evaluated over 8 weeks of treatment in a group of women presenting cutaneous photoageing of the face. The evolution of cutaneous relief and the state of the skin was determined by clinical observations (scoring method) together with methods of instrumental analysis of the skin surface (replicas and Image Analysis) and by a subjective evaluation (evaluation of the efficacy by the subjects in the study).

MATERIAL AND METHODS

1 - Protocol

An open, non-randomised study was carried out in subjects presenting facial cutaneous photoageing.

2 - Subjects in the study

43 female volunteers in good health, of Caucasian origin, aged between 35 and 58 years (mean age: 44 years) participated in this study. The subjects had a normal skin and presented moderate to severe facial photodamage corresponding to degrees 3 to 5 on the photonumeric scale defined by Larnier et al (12). All the subjects gave their written informed consent in conformity with the ethics of cosmetic experimentation.

3 - Product

The product studied was an oil in water emulsion containing 1% Vitreoscilla filiformis extract together with traditional cosmetic active ingredients.

4 - Treatment

- Method of product application

The subjects had to apply the product to the whole of the face, morning and evening, for 8 weeks. No other cosmetic product was allowed during the course of the study with the exception of cleansing products.

- Conduct of the study

This study including four control visits: before

the beginning of the treatment (TO), after 2 weeks (T2S), after 4 weeks (T4S) and after 8 weeks of treatment (T8S). Evaluation of the state of the skin and cutaneous relief was carried out at each visit. A questionnaire concerning the efficacy and cosmetic aspects of the product was given to the subjects after 4 and 8 weeks of treatment.

5 - Evaluation methods

- Clinical observations: scoring method (13)

Evaluation of the state of the facial skin was carried out using clinical scores defined on four areas of the face: forehead, crow's feet area, cheeks and medio-facial region. The criteria studied were the following: cutaneous relief, suppleness, firmness, hydration, complexion. Each criterion was evaluated individually by the investigator using a scale of 0 to 10 (0: negative value for the criterion, 10: positive value for the criterion). The mean of the scores obtained for the four zones was calculated for each criterion.

- Instrumental methods: replicas and Image Analysis

Silflo replicas of the cutaneous surface (14) were made from the chosen zones and delineated precisely in the area around the eye (crow's feet area). These replicas were photographed and analysed qualitatively by observing macrophotographs and quantitatively by Image Analysis according to the technique described by Corcuff et al (15, 16), using a video-camera (Cohu) together with a microcomputer using Image Analysis software (Quantrides, Monaderm). This technique enabled evolution in the two parameters characterising relief to be evaluated: the number of wrinkles per unit surface area (u.s.) and their depth (mm).

- Subject evaluation

The efficacy of the product was evaluated by the subject after 4 and 8 weeks of treatment. The subjects had to judge the efficacy of the product on cutaneous relief (satisfactory or unsatisfactory results obtained), as well as its activity on firmness, softness and hydration of the skin, according to a scale of 0 to 4 for each criterion (0: unsatisfactory; 4: satisfactory).

6 - Tolerability

Skin tolerability was evaluated by the investigator after 2, 4 and 8 weeks of treatment.

7 - Statistical Analysis

A two-tailed Student's t test on paired series was used to analyse the differences between the values obtained before treatment and after 2, 4 and 8 weeks of treatment (clinical scores and Image Analysis). The differences were considered to be significant when $p < 0.05$. The correlation coefficient r and its level of significance p were calculated using linear regression analysis in order to determine the correlation between the results recorded by the scoring method and those obtained by Image Analysis.

RESULTS

1 - Early Discontinuations

Two subjects did not present for the control visit at 2 weeks; these subjects were excluded from the study. No other observations concerning them were taken into account during analysis of the results. The results therefore include 41 subjects who finished the study.

2 - Evolution in cutaneous relief and state of the skin during treatment

- 2.1 Clinical observations

The treatment induced significant and progressive improvement in cutaneous relief.

The mean of the scores obtained for all subjects, at each control visit, for each parameter studied, is given in Table 1. The variations, expressed as

Table I

CLINICAL SCORES
(mean +/- standard deviation of the mean, % of evolution with respect to time T0 and p determined using Student's t test)
T0: Before starting the treatment; T2S: After 2 weeks of treatment; T4S: After 4 weeks of treatment; T8S: After 8 weeks of treatment

CRITERIA	T0	T2S	T4S	T8S
CUTANEOUS RELIEF	4.5+/-0.3	5.0+/-0.3	5.8+/-0.3	6.8+/-0.2
% evolution		+10.2%	+29.0%	+49.4%
p		< 0.05	< 0.05	< 0.05
FIRMNESS	5.6+/-0.3	6.4+/-0.3	7.0+/-0.3	7.6+/-0.3
% evolution		+13.0%	+23.5%	+34.6%
p		< 0.05	< 0.05	< 0.05
SUPPLENESS	5.2+/-0.3	5.8+/-0.3	6.5+/-0.3	7.1+/-0.3
% evolution		+11.4%	+25.2%	+36.5%
p		< 0.05	< 0.05	< 0.05
HYDRATION	5.6+/-0.3	6.4+/-0.3	7.0+/-0.3	7.6+/-0.3
% evolution		+13.0%	+23.5%	+34.6%
p		< 0.05	< 0.05	< 0.05
COMPLEXION	5.1+/-0.2	5.9+/-0.2	6.7+/-0.2	7.5+/-0.2
% evolution		+15.0%	+30.7%	+47.3%
p		< 0.05	< 0.05	< 0.05

a percentage with respect to T0 (before treatment) as well as the results of the statistical comparison with respect to T0, are also given in the same table. An improvement in cutaneous relief of 10, 29 and 49% respectively after 2, 4 and 8 weeks of treatment was observed.

In the same way, the firmness of the skin increased significantly during treatment. The use of the product also progressively and significantly improved the cosmetic qualities of the skin: suppleness, hydration, complexion.

- 2.2 Qualitative and quantitative analysis of the replicas

The observation of replicas by macrophotographs taken of all subjects enable a qualitative visual observation in the progressive improvement of cutaneous relief during treatment to be made. For example, the macrophotographs of replicas carried out on two subjects are given in Figure 1 (a and b).

The quantitative evolution in cutaneous relief was determined by Image Analysis of the replicas. Table 2 presents the evolution in the num-

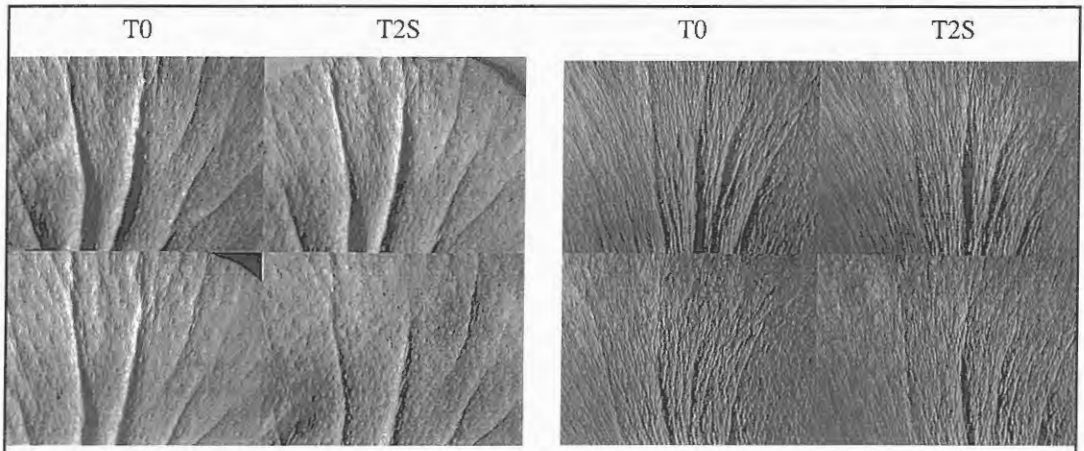

Figure 1a: Visualisation of replicas from crow's feet area carried out on a woman 50 years of age

Figure 1b: Visualisation of replicas from crow's feet area carried out on a woman 58 years of age

FIGURE 1:
Macrophotographs of replicas carried out on two subjects (Final enlargement: x 10)
Figure 1a: subject n° 1 - Figure 1b: subject n° 2
T0: Before the start of treatment; T2S: After 2 weeks of treatment; T4S: After 4 weeks of treatment; T8S: After 8 weeks of treatment;

Table II

QUANTITATIVE ANALYSIS OF REPLICAS: NUMBER OF WRINKLES PER UNIT SURFACE AREA AND DEPTH OF WRINKLES IN μM
(mean +/- standard deviation of the mean, % evolution with respect to time T0 and p determined by Student's t test).
T0: Before starting the treatment; T2S: After 2 weeks of treatment; T4S: After 4 weeks of treatment; T8S: After 8 weeks of treatment

	T0	T2S	T4S	T8S
NUMBER OF WRINKLES per u.s.	92.9+/-1.8	85.4+/-1.9	81.8+/-2.2	74.6+/-2.6
% evolution		-8.1%%	-12.0%	-20.0%
p		< 0.05	< 0.05	< 0.05
DEPTH OF WRINKLES (μm)	116.9+/-7.9	98.1+/-6.7	79.5+/-6.1	61.0+/-5.6
% evolution		-16.1%%	-32.0%	-47.8%
p		< 0.05	< 0.05	< 0.05

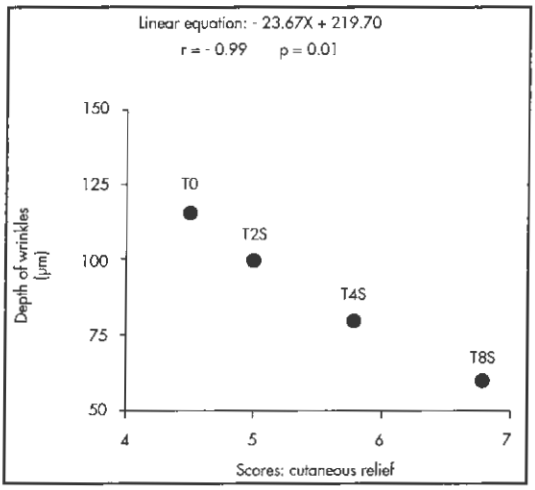

Figure 2a: Correlation between scores (cutaneous relief) and depth of wrinkles.

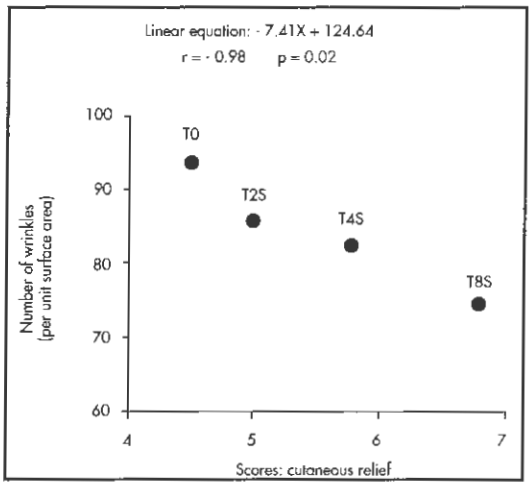

Figure 2b: Correlation between scores (cutaneous relief) and number of wrinkles.

Figure 2: Correlation between scores and the results obtained by Image Analysis.
T0: Before the start of treatment; T2S: After 2 weeks of treatment; T4S: After 4 weeks of treatment; T8S: After 8 weeks of treatment;

ber and depth of the wrinkles for all subjects. The number of wrinkles was reduced in a significant fashion during the treatment (-8, -12 and -20% after 2, 4 and 8 weeks of treatment respectively) as well as the depth of the wrinkles (-16, -32 and -47%).

- 2.3 Correlation between the clinical scores and results obtained with Image Analysis

Figure 2a represents correlation over time between the mean of the clinical scores (cutaneous relief) and mean of the values corresponding to the depth of the wrinkles.
The correlation coefficient r = -0.99 is significant at the 0.01 level.
There also exists a significant correlation between the clinical scores and the number of wrinkles per unit surface area (Figure 2b):
r = -0.98, p = 0.02.

- 2.4 Subjective evaluation

Analysis of the questionnaires given to the subjects confirms the efficacy of the product with respect to improvement in cutaneous relief: in terms of wrinkle improvement, 92% of subjects judged the result obtained to be satisfactory from the fourth week of treatment onwards. The subjects also noted a good activity of the product on the firmness, softness and hydration of the skin.

3 - Tolerability

No undesirable cutaneous reaction was observed during use of the product: tolerability was excellent.

DISCUSSION

This study showed the efficacy of the product studied based on the most characteristic signs of photoageing of the face: wrinkles and loss of firmness. The clinical observations showed an improvement in these criteria and the cosmetic qualities of the skin during treatment. They were confirmed by the results obtained after quantitative analysis of replicas: reduction in the number and depth of wrinkles. The evalua-

tion of the subjects also corroborates the observations made.

The correlation between the clinical observations and the results recorded using instrumental methods demonstrates the reliability and value of the clinical score method when attributed by a dermatologist who is expert in the evaluation of cutaneous relief.

Insofar as activity of the product is concerned, all the results confirm the basic hypothesis, i.e. that the activity of *Vitreoscilla filiforma* measured *in vitro* on cutaneous cells has been verified in vivo: cellular activation induced by the bacterial extract can induce an increase in renewal and cellular cohesion *in vivo* in the epidermis as well as an increase in density of connective tissue, leading to an improvement in cutaneous relief and the state of the skin.

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