



EVALUATION OF THE EFFECTIVENESS OF ANTI-WRINKLE COSMETIC PRODUCTS

Report number: S/10/1850

Test product: **Elicina**

Customer: Unipro Servizi s.r.l.
Via Accademia, 33
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Sponsor: Bioelisir S.r.l.
Via F.lli Kennedy, 40/b
07041 Alghero (SS)

Aim of the study

The study aims to investigate the effectiveness of the cosmetic formulation *Elicina* in the treatment of the imperfections of facial wrinkles by a 2 months “in use” test.

Experimental protocol

The study has been carried out according to Helsinki declaration (Ethical Principles for Medical Research Involving Human Subjects).

Twenty healthy volunteers, of both sex, between 40 and 67 years old, were selected according to the following inclusion criteria:

- good general health;
- absence of cutaneous diseases;
- people who has wrinkles and skin laxity in the face areas;
- people who does not show pigmentary lesions or other lesions on the interest area that could interfere with the study evaluation;



- people who does not present history of hypersensitivity to the common components in cosmetic formulations;
- women not being pregnant or breastfeeding;
- people who agreed not to use other skin products for the duration of the test in the tested areas;
- subjects who signed the consent form (see Annex I)

and following exclusion criteria:

- subjects that didn't meet the inclusion criteria above;
- some drug treatment that could interfere with the assessment of the study;
- participation in similar study by at least 60 days;
- subjects with allergies to some components of the product.

Drop out

During the study some case of drop out may occur. Possible causes of interruption of the test are:

- inability to contact the volunteer;
- free choice of volunteer;
- health reasons related to the treatment in study (i.e., onset of reaction of intolerance towards the product)

Length of Study

The study involves two months of treatment

Design of the study

The product is applied daily by massage until completely absorption over half face.

The contralateral half face is treated with a "placebo" formulation without active, as a control.

Instrumental evaluation

At the beginning of the trial, after 1 month and after 2 months of the product application the following instrumental image evaluation is carried out.

All measurements were made in an air conditioned room with controlled temperature and humidity (T 22 °C, r.h. 50 ± 5%); subjects were preconditioned for at least 15 minutes before the measurements.

These are techniques involving the contact between the skin and a series of probes free of discomfort, pain or individual damage.



Image evaluation

The image evaluation was performed by the apparatus PRIMOS^{pico} (GFM, Teltow, Germany), an optical analysis system, which can capture the 3D image of the skin surface.

The 3D image acquired with the instrument can be transformed by the software Primos 5.7 to obtain a colored image in which each shade of color (from blue to yellow) is related to a height, as shown below:



Thus, the presence of a groove will be indicated by a color gradient that can go from blue to green according to its depth.

Parameters characterizing skin microreliefs can be measured at the glance thanks to this three-dimensional topographic representation of the skin.

In particular, the parameter considered in this study is **Ra**, the average skin roughness, which is the integral of the function describing the skin profile curve and it corresponds to the curve area included above and below the average line (Figure 1).

This parameter is based on the sampling of all the points characterizing the profile, so it represents a true average because it is very significant in regard to the roughness of the skin.

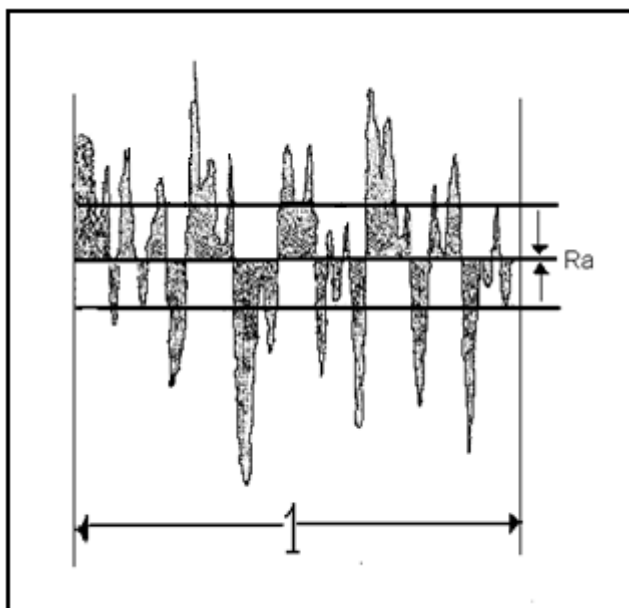


Fig.1 Roughness mean value (Ra)



The R_a is two-dimensional parameter. For skin with very anisotropic characteristics, the value of this parameter can be very different depending on the direction along which the profile being analyzed (Figure 2). R_a has a higher value in perpendicular orientation to respect to the grooves (X direction in the figure 2); in fact, $R_{ax} > R_{ay}$.

It must, therefore, to process simultaneously a large number of profiles that follow all possible directions and calculate the average of several measurements obtained.

The number of lines to be measured is an important factor; it is therefore very important to try as many lines as possible, to encompass the entire area of the captured image in the analysis; the higher the number of available profiles, the greater will be the precision of measurement.

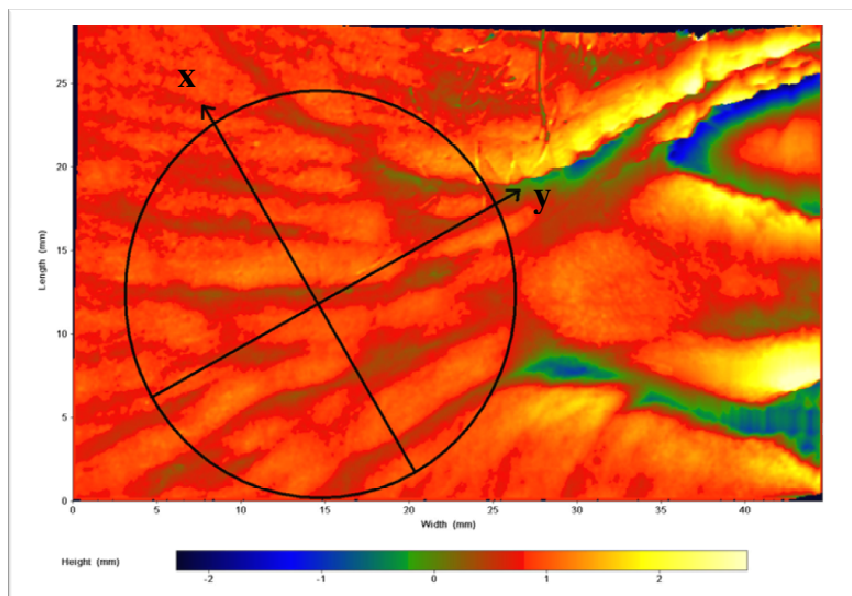


Fig.2 Microrelief image. In particular directions along which the profile being analyzed

With the PRIMOS^{pico} system up to 32 radial lines are processed in a few seconds (Figure 3).

The profiles must always be of the same size; in fact, a change in the length of the profile automatically lead to a difference in the values of the roughness parameter, R_a .

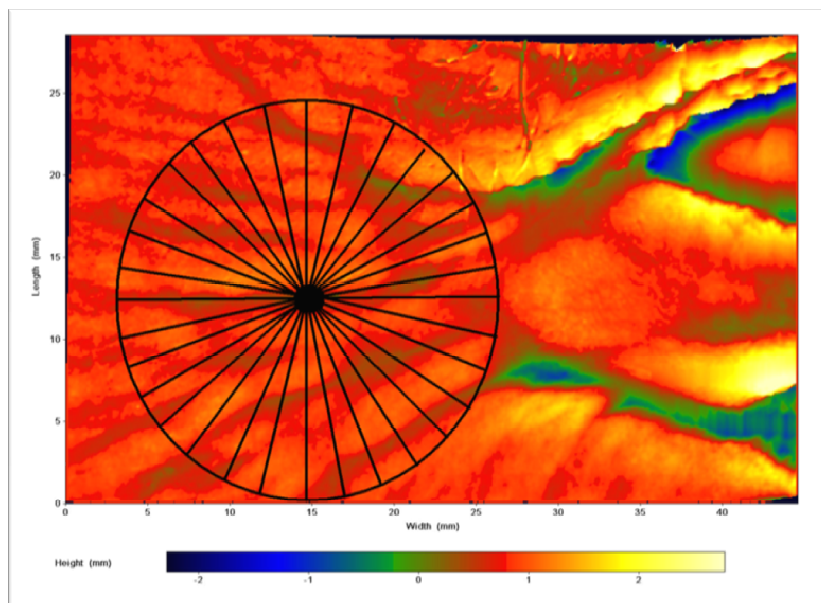


Fig.3 Microrelief image. Area analyzed by splitting up into 32 profiles

Subjective evaluation

The volunteers, at the end of the study, express an opinion regarding the subjective pleasantness, the cosmetic product effectiveness and tolerability using a questionnaire (Annex II).

Formulation INCI

Helix aspersa muller extract, paraffinum liquidum, propylene glycol, stearic acid, cetyl alcohol, sodium lauril sulfate, diazolidinyl urea, methylparaben, propylparaben, triethanolamine.

Statistical analysis

The study results are expressed as mean \pm S.D. On the data collected statistical analyses with the tests for non parametric data and on repeated measures were carried out. A significance level of 5% was chosen, so the changes were considered statistically significant for $p < 0.05$.



Confidentiality

The information contained in this document and any additional information supplied contains commercially sensitive information that is privileged and confidential and may not be disclosed unless such disclosure is required by law or regulations. Persons to whom information is disclosed must also be informed that the information is privileged or confidential and may not be further disclosed by them.

All documentation generated during the course of the study will be treated as strictly confidential.

Publication

The results of this study may be published externally only with written permission of the sponsor.

All information concerning the study products supplied by the sponsor and not previously published is considered confidential and shall remain the sole property of the sponsor. The investigator agrees to use this information only in relation to this study and will not use it for other purposes without written consent from the sponsor.

It is understood by the investigator that the information from the study will be used by the sponsor in connection with the development of the study products and, therefore, may be disclosed as required to other investigators or to government agencies. It is understood that there is an obligation to provide the sponsor with complete test results and all data developed in the study.

Investigators

The Study Director was Dr. Paola Perugini, PhD, Pharmacist, Professor of Cosmetic Products at the Faculty of Pharmacy at the University of Pavia; the investigator was Dr. Mariella Bleve, degree in Pharmaceutical Chemistry and Technology, Pharmacist.

The study was carried out at the Department of Pharmaceutical Chemistry of The Faculty of Pharmacy, University of Pavia, Via Taramelli 12, 27100 Pavia, Italy.



Results and discussion

The study aims to evaluate the effect of a cosmetic product in the treatment of facial wrinkles.

To this end, the product was applied on half face and a placebo on the other site daily for two months and the evaluation of skin roughness before, after 1 month and at the end of the trial was carried out.

One volunteer left the study for personal reasons not related to the product and no one showed skin problems.

In this experimentation the wrinkles studied have been both the texture lines always present in the chronological aging and the expression wrinkles on the eyes sides (chicken legs). These lines are particularly present in the accelerated aging and they are caused by the constant stress of the facial muscles.

They are wrinkles generally deeper than the skin texture lines and they appear also in relatively young subjects.

The Primos system has allowed us to evaluate, by matching, the same skin area in the three subsequent analysis times. Figures 4-7 show, such as example, color and black and white images obtained at 0 and 2 months time and their elaborations by the software both of skin texture lines (Figures 4, 5) and the expression wrinkles (Figure 6-7).

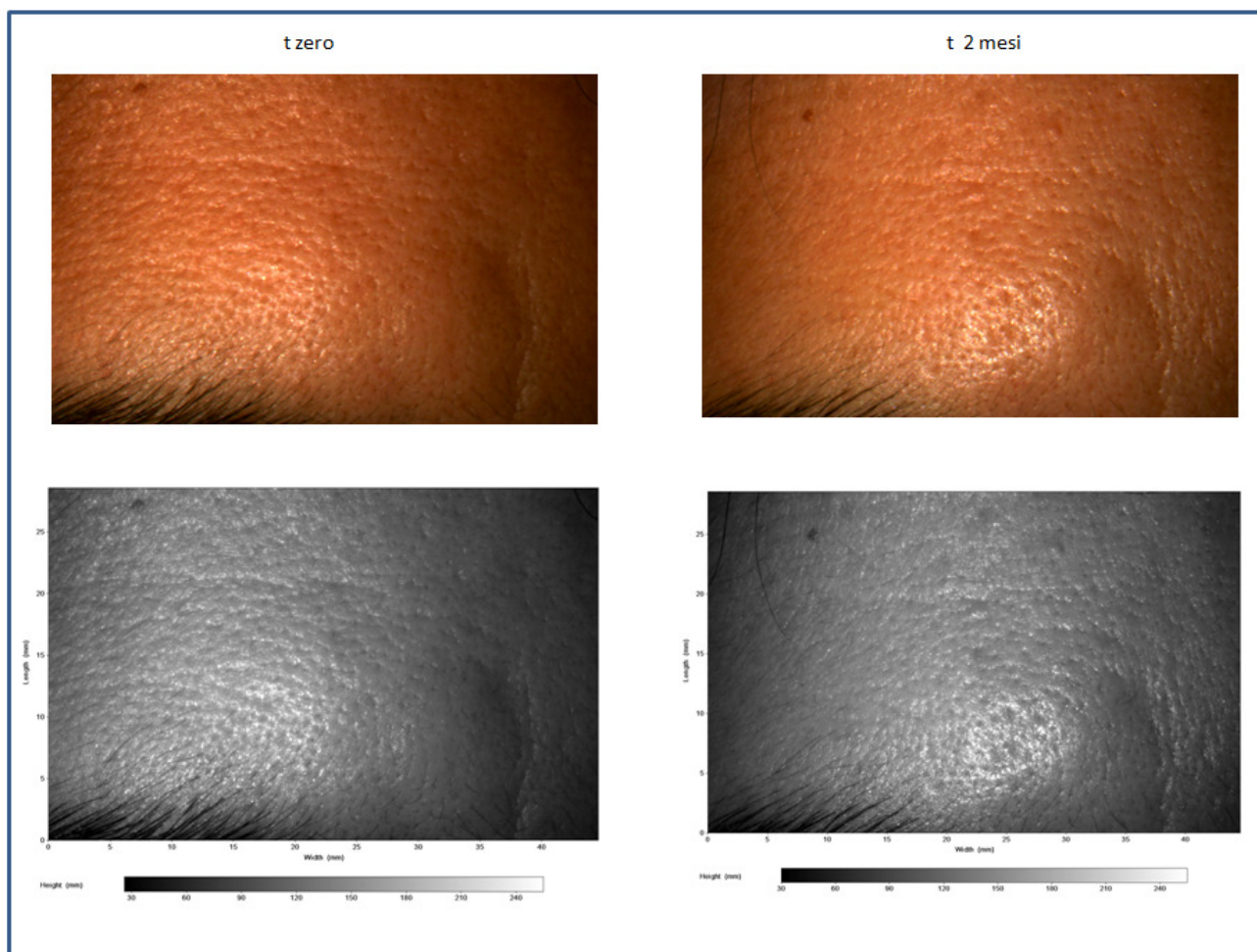


Fig.4: Image of skin texture wrinkles obtained at time zero and at 2 months of zone treated with the *Elicina* formulation

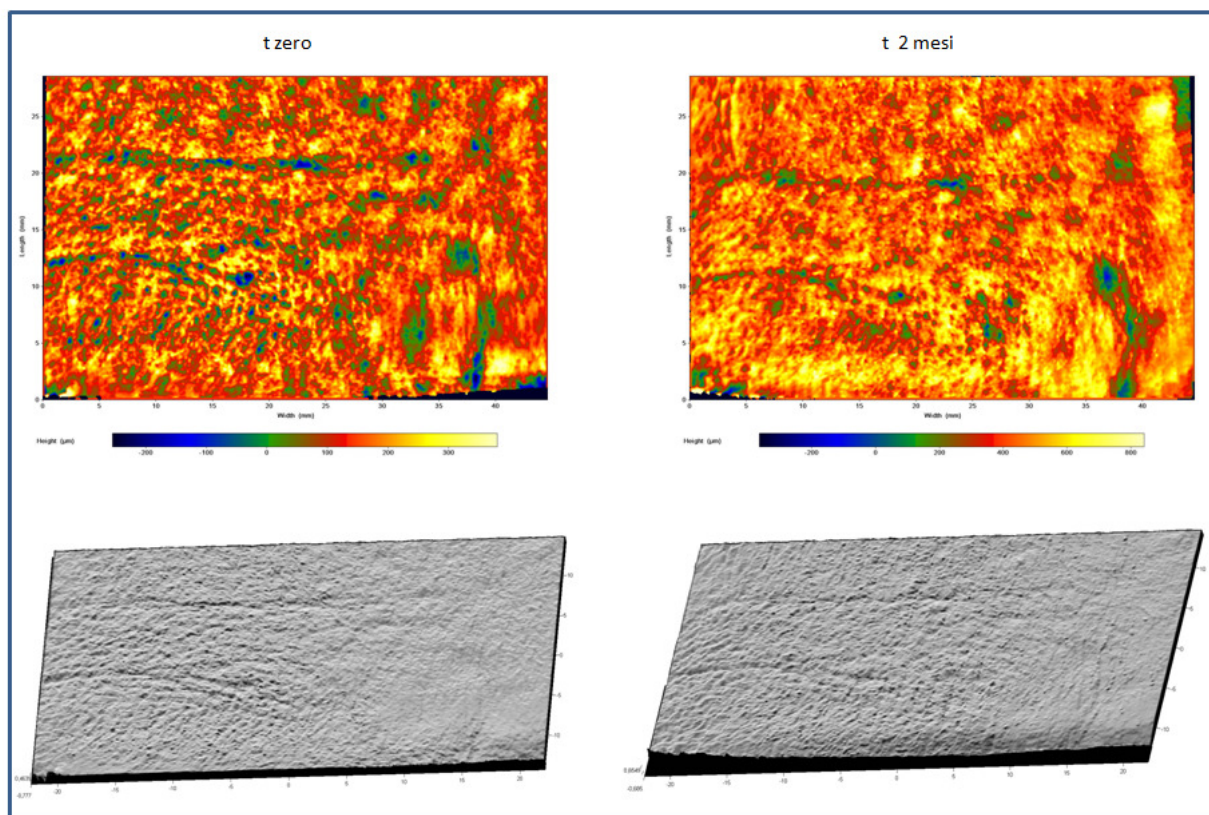


Fig.5: Elaboration of skin texture wrinkles obtained at time zero and at 2 months of zone treated with the *Elicina* formulation

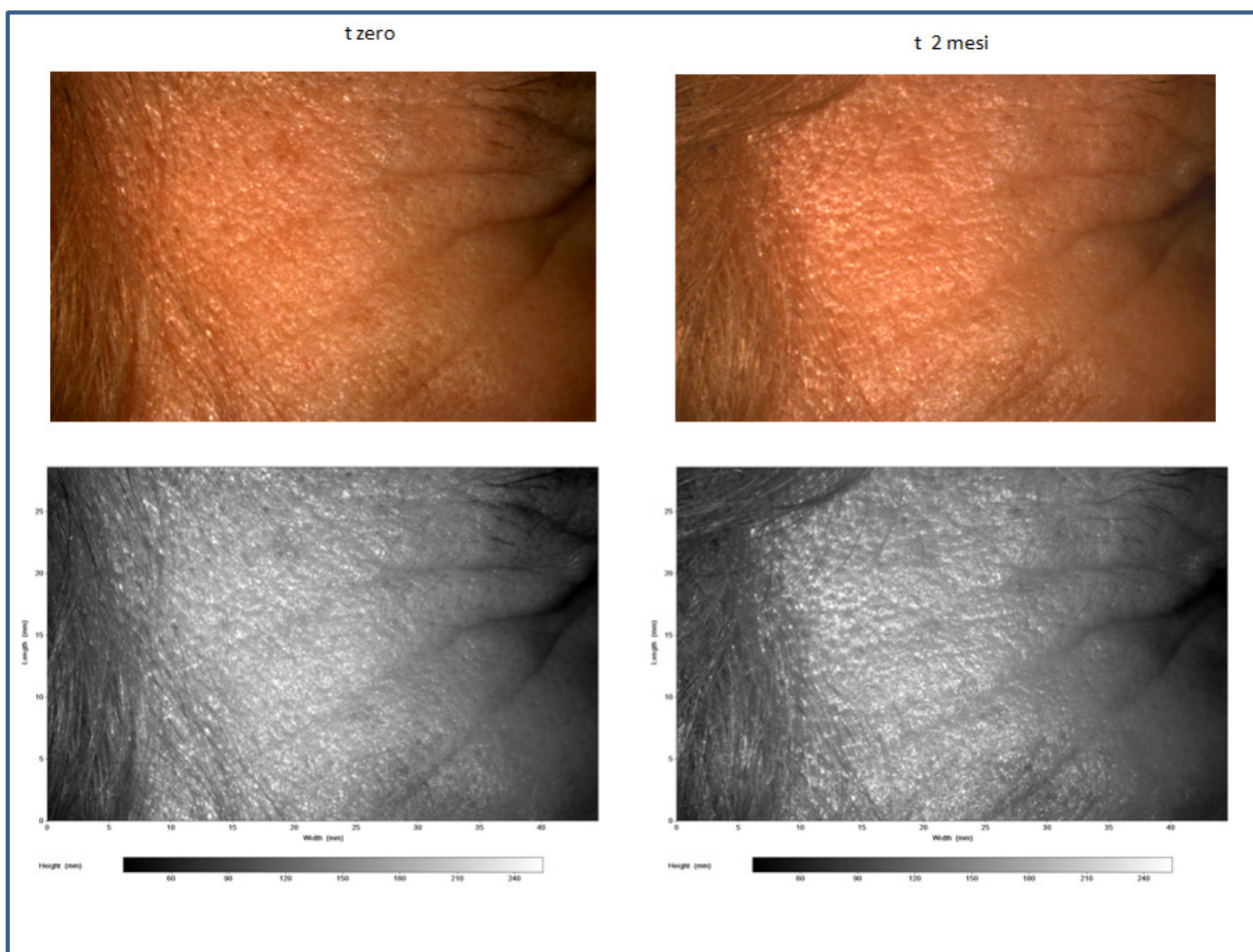


Fig.6: Image of expression wrinkles obtained at time zero and at 2 months of zone treated with the *Elicina* formulation

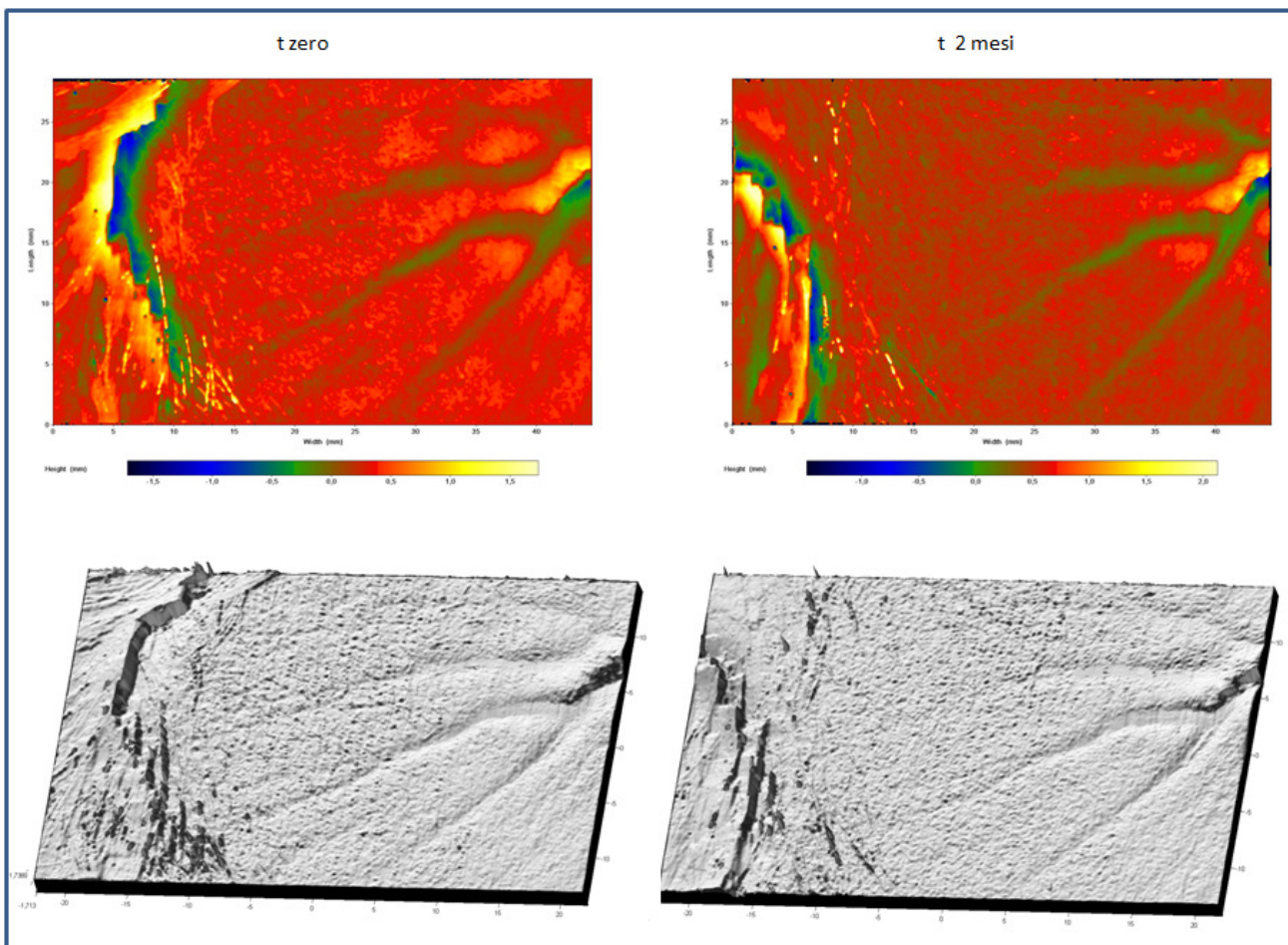


Fig.7: Elaboration of expression wrinkles obtained at time zero and at 2 months of zone treated with the *Elicina* formulation

The parameter Ra was obtained by processing 32 radial lines in order to obtain a measure of the average roughness correlated to sensory perception.

The results obtained are reported in the Tables 1-4.



Tab.1 Ra values (μm) of areas with expression wrinkles treated with placebo			
Volunteer	Ra t0	Ra t1	Ra t2
Vol1	40	47	48
Vol2	44	51	43
Vol3	32	21	21
Vol4	22	22	23
Vol5	31	31	27
Vol6	24	23	24
Vol7	20	25	24
Vol8	42	60	40
Vol9	38	31	30
Vol10	35	48	35
Vol11	40	40	40
Vol12	36	31	38
Vol14	32	36	40
Vol15	34	42	35
Vol16	70	68	63
Vol17	42	36	39
Vol18	33	32	32
Vol19	28	26	27
Vol20	51	64	61
Mean	36.53	38.63	36.32
S.D.	11.25	14.33	11.79
p	ns		



Tab.2 Ra values (μm) of areas with expression wrinkles treated with <i>Elicina</i>			
Voluntee	Ra t0	Ra t1	Ra t2
Vol1	33	30	32
Vol2	25	19	23
Vol3	25	25	20
Vol4	28	56	23
Vol5	27	25	26
Vol6	20	30	25
Vol7	26	28	27
Vol8	56	35	28
Vol9	33	29	31
Vol10	33	38	29
Vol11	44	33	42
Vol12	27	26	20
Vol14	35	28	35
Vol15	39	26	29
Vol16	42	45	45
Vol17	42	38	38
Vol18	42	37	54
Vol19	31	26	33
Vol20	35	31	36
Mean	33.84	31.84	31.37
S.D.	8.73	8.42	8.83
p	ns		



Tab.3 Ra values (μm) of areas with texture lines treated with placebo			
Voluntee	Ra t0	Ra t1	Ra t2
Vol1	33	35	33
Vol2	33	33	31
Vol3	53	27	27
Vol4	36	45	45
Vol5	24	24	21
Vol6	25	21	28
Vol7	28	27	33
Vol8	67	63	63
Vol9	29	30	28
Vol10	36	32	30
Vol11	35	33	40
Vol12	34	36	35
Vol14	48	45	41
Vol15	34	36	33
Vol16	48	60	64
Vol17	32	41	33
Vol18	39	35	30
Vol19	29	28	31
Vol20	46	46	47
Mean	37.32	36.68	36.47
S.D.	10.76	11.18	11.41
p	ns		



Tab.4 Ra values (μm) of areas with texture lines treated with <i>Elicina</i>			
Voluntee	Ra t0	Ra t1	Ra t2
Vol1	35	47	44
Vol2	29	37	33
Vol3	54	39	36
Vol4	43	36	36
Vol5	21	23	19
Vol6	27	23	25
Vol7	33	34	30
Vol8	72	70	72
Vol9	42	37	41
Vol10	39	53	36
Vol11	54	56	55
Vol12	53	47	49
Vol14	36	39	35
Vol15	38	37	37
Vol16	63	62	49
Vol17	40	39	36
Vol18	47	45	34
Vol19	28	27	28
Vol20	52	53	44
Mean	42.42	42.32	38.89
S.D.	13.12	12.53	11.82
p		0.0272*	

Statistical analysis by ANOVA test for repeated measures showed a statistically significant change in the results of the Ra values obtained on the area with skin texture wrinkles treated with *Elicina* formulation (Table 4).

These results show a reduction of the Ra parameter, indicating a smoothing of the skin surface, thus reducing the depth of fine lines, equivalent to 10% after two months of treatment.

As for the expression wrinkles, the trend of the Ra results is to diminish over time. However, this reduction was not statistically significant.

Probably, since the expression wrinkles are the deepest and the skin are always solicited by the underlying muscles, a longer time of treatment need to get a significant result.



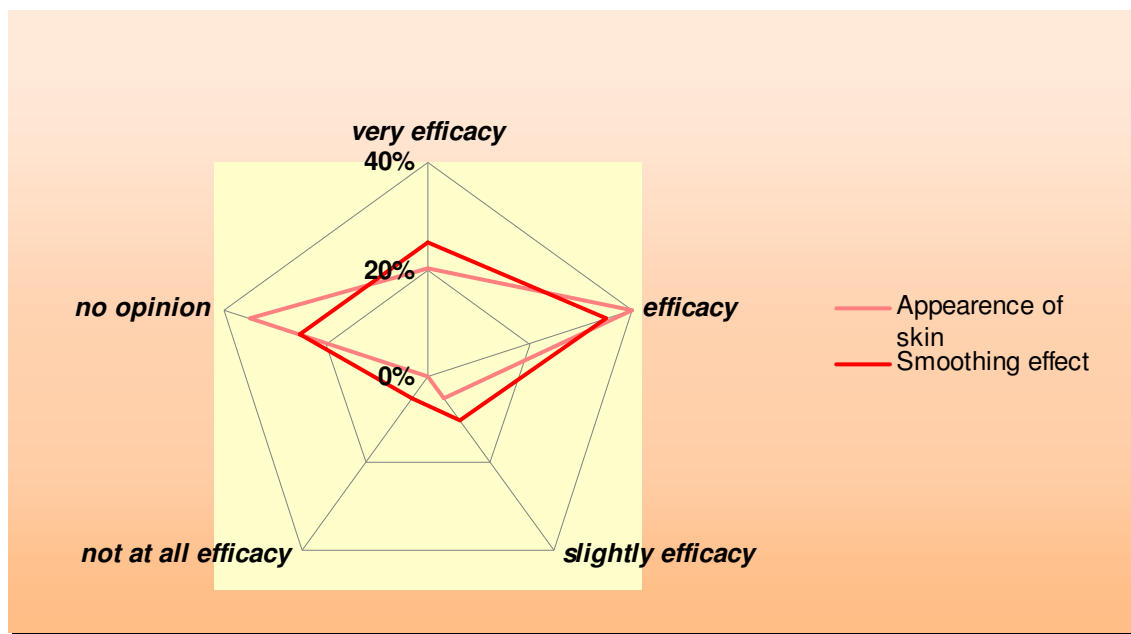
Subjective assessment

The subjective evaluation of effectiveness, pleasantness and tolerability of *Elicina*, was carried out through questionnaires completed by volunteers at the end of the study.

In the following graphs are reported the result of the subjective evaluation.

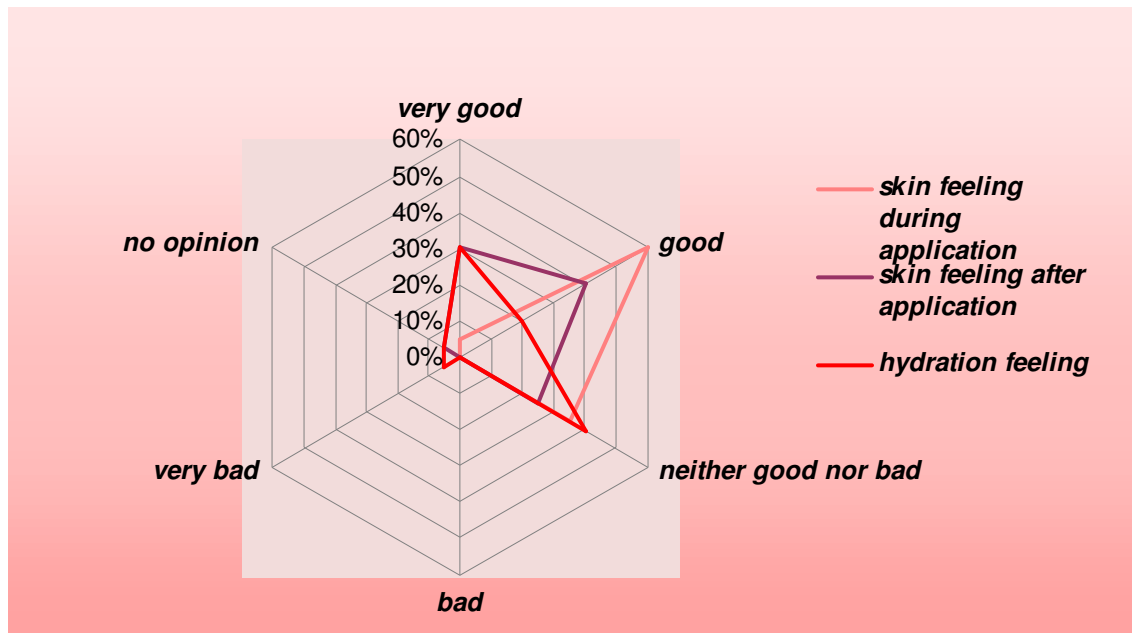
100% of subjects had tolerated well or very well the product and 80% were satisfied or very satisfied with the product *Elicina*. More than 50% of volunteers would buy the product *Elicina*.

Improvement of the skin appearance and smoothing effect

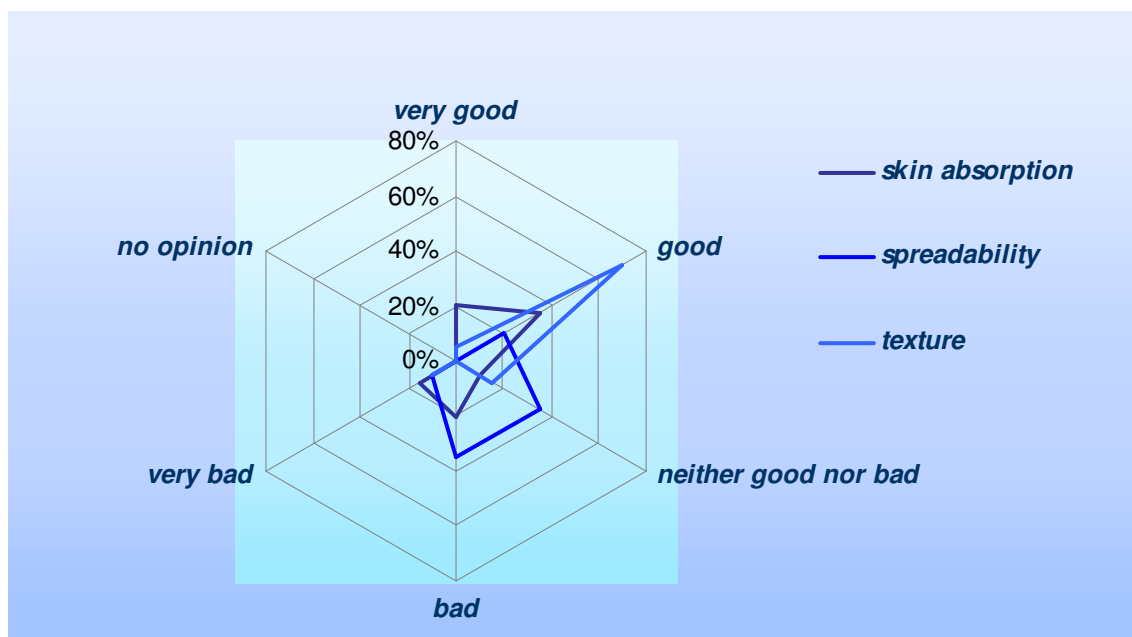




Sensation on the skin during and after application



Product characteristics





Conclusions

This study was intended to evaluate the tolerability and efficacy of anti-wrinkle cosmetic product *Elicina*.

At the end of this evaluation we can say that:

- The continued daily use for two months of *Elicina* product showed no problems of skin tolerance; therefore the product *Elicina* is very well tolerated;
- The continued daily use for two months of *Elicina* product has resulted in a statistically significant reduction of 10% of the depth of skin texture wrinkles, the fine wrinkles of chronological aging.

In conclusion, we can affirm that the cosmetic product *Elicina* has an anti-wrinkle efficacy on skin texture wrinkles, visible signs of chronological aging.

Date and signature:

Dr. Paola Perugini, Study Director



Annex I

DECLARATION OF CONSENT

The undersigned,, Born in on
residing at
States

To submit voluntarily to the study
Evaluation of efficacy of a cosmetic antiwrinkles product

Further states:

- To have been made aware of the usefulness of the test and the possible risks related to it;
- For not having taken part in similar tests over the past two months.

Pavia, By Faith

WRITTEN INFORMATION (PRIVACY)

We inform, within the meaning of 'Article 10 of Law 31 Dec. 1996, n.675 (Privacy Policy informatics) that the personal information you provide will be processed in compliance with the legislation mentioned above and the obligations of confidentiality imposed by the Act. The data collected will be used for purposes related to or useful for us to be carried out, using instruments that guarantee security and confidentiality and the treatment will be carried out with instruments that are not computerized. We inform you that the personal information you provide will not be covered nor communication, nor to spread and will be stored at our office for the time prescribed by law. Providing your personal information is an act of a voluntary nature and your refusal will not have any consequences. In relation to the data subject, you can still exercise their rights under Art. 13 of Law No. 675/1996.

CONSENT TO THE PROCESSING OF PERSONAL DATA GENERAL AND SENSITIVE.

Having understood the above, I agree, in accordance with Articles 11 and 12 of Law No 31 Dec. 1996 675, to process my personal data by the persons specified in the said information and in compliance with the same.

SIGNATURE



Allegato II ANNEX II

Name:

Vol-No.:

Study-No.:

Date:

**Questionnaire: Subjective Evaluation study of a cosmetic product for the treatment of wrinkles
(Questionario: Studio di valutazione soggettiva di un prodotto cosmetico nel trattamento delle rughe)**

Età(Age): _____

Sesso(Sex): M ☐
F ☐

Usa normalmente prodotti antirughe (Do you normally use an antiwrinkle product)?

Si(yes) ☐
no ☐

se sì, quale(if yes, which one(s) ? _____

Efficacia cosmetica (cosmetic efficacy):

La prego di valutare le proprietà cosmetiche del prodotto in accordo con i seguenti punteggi
(Please evaluate the cosmetic properties of the product according to the following criteria):

1=molto efficace (very efficacy) 2=efficace (efficacy) 3=poco efficace(slightly effective)
4= per niente efficace(not at all effective) 5=senza opinione(no opinion)

Punteggio(Criteria)	1	2	3	4	5
Miglioramento dell'aspetto visivo della pelle (appearance of the skin)					
Effetto levigante (firming effect)					

Come ha tollerato il prodotto(How did you tolerate the product)?

Molto bene(very well) ☐
Bene(well) ☐
Abbastanza bene(fairly well) ☐
Per niente(not at all) ☐

In caso di problemi quali reazioni ha avuto(in case of problems, which reactions)?

Gradevolezza cosmetica (cosmetic pleasantness):

La prego di valutare le proprietà cosmetiche del prodotto in accordo con i seguenti punteggi
(Please evaluate the cosmetic properties of the product according to the following criteria):

1=molto buono(very good) 2=buono(good) 3=nè buono nè cattivo(neither good nor bad)
4=cattivo(bad) 5=molto cattivo(very bad) 6=senza opinione(no opinion)



DIPARTIMENTO DI CHIMICA FARMACEUTICA

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Punteggio(Criteria)	1	2	3	4	5	6
Sensazione sulla pelle durante l'applicazione (skin feeling during application)						
Sensazione sulla pelle dopo l'applicazione(skin feeling after application)						
Profumo (parfum)						
Sensazione di idratazione sulla pelle dopo l'applicazione (Hydration feeling after application)						
Assorbimento sulla pelle (skin absorption)						
Spalmabilità (spreadability)						
Consistenza del prodotto (texture)						
Il prodotto in generale-commenti sul prodotto in generale(Product Overall (your mark for the product in general)						

E' soddisfatto delle proprietà cosmetiche(Are you satisfied with the cosmetic properties)?

- Molto soddisfatto(very satisfied) ☐
- Soddisfatto(satisfied) ☐
- Non proprio soddisfatto(not really satisfied) ☐
- Per niente soddisfatto(not satisfied at all) ☐

Comprerebbe il prodotto(Would you buy the product)?

- Si(yes) ☐
- no ☐
- Senza opinione(no opinion) ☐

Altri commenti(Any additional comment)?