In use double blind, randomized, placebo-controlled efficacy test of a cream to reduce the cellulite effects and increase the skin antioxidants.

Study Sponsor:

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Investigator:

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2. SUMMARY OF THE STUDY

2.1 Objective

The objective of this study is to evaluate the in-vivo efficacy of two anti-cellulite products coded:

- **ENDOR FORMULA A**
- **ENDOR FORMULA B**

The evaluation is performed using:

- **Weight Measurement**
- **Thigh Circumference Measurement**
- **Clinical Photography**
- **3D Analysis of Dimples with FOITS**
- **3D Analysis of Texture with FOITS**
- **Autofluorescence Spectroscopic Antioxidants Quantification**
- **50 MHz High Frequency Evaluation of the Epidermis and Dermis**

The study lasts 28 days following the first application of the products.

2.2 Population

24 subjects are selected for the study.
The subjects selected for this study are healthy females, aged between 32 and 52 years old.

These subjects are selected according to the inclusion / non inclusion criteria listed in paragraph 3.1.

2.3 Study Schedule and Duration

Pré-inclusion : 8-29/02/16
Beginning of the study: 7-11/03/16
End of the study : 25/04/16

Scheduled Procedures:

	D0	D28 (days)
Weight Measurement	**X**	**X**
Thigh Circumference Measurement	**X**	**X**
Clinical Photography	**X**	**X**
3D Analysis of Dimples with FOITS	**X**	**X**
3D Analysis of Texture with FOITS	**X**	**X**
Autofluorescence Spectroscopic Antioxidants Quantification	**X**	**X**
50 MHz High Frequency Ultrasound Evaluation of the Epidermis and Dermis	**X**	**X**

2.4 Study design

- Double blind study.
- Comparative study.
- Subjects serve as their own reference.

3. STUDY PROTOCOL

3.1 Subject selection

The study panel is composed of subjects selected on the basis of their medical record, which they agreed to be consulted for this purpose prior to the study, that provides details of their medical history, possible allergies, skin-care and make-up habits, as well as a certain amount of administrative information.

The selection procedures are elaborated in order to guarantee that the subjects receive all possible information about the aims of the study and the consequences of their participation.

This selection procedure includes:

-A preliminary interview, during which the following points are explained to the subjects: the study's modalities, its practical considerations, possible reward, as well as any possible cosmetic benefits, inconveniences or

potential risks.

- The information form, which is specific to the study, including all essential information is then read.

-The consent form is read, approved, and signed by the subject to substantiate the fact that they freely accept the conditions of the study, which has been described to them

- The consent form which was filled in freely and intentionally by the subject after it had been fully explained to them, in the event of any claims for damages, enables them to benefit from the terms of the insurance policies taken out by both the investigator and/or by the study sponsor as soon as the subject is accepted onto the study by the study manager.

The subject must respect the following conditions: (as well as those already mentioned):

- Available for the entire duration of the study
- Motivated to freely participate in the study
- Willing to follow the full product application procedure
- Able to justify a permanent address
- Capable of reading the consent documents and able to accept the participation conditions
- Benefiting from Social Security medical cover

The subjects selected for the study are chosen under the supervision of the investigator and study manager, on the basis of the inclusion/non inclusion and proscription/restriction criteria listed below.

A selection of 24 subjects is made for this study.

The results given include all of the present and assessable subjects at each examination.

3.1.1 Inclusion criteria

General criteria

- Female
- Healthy
- Between 30 and 70 years of age
- Skin at assessed area is healthy (free of psoriasis, eczema, erythema, edema, scars, wounds or lesions)

Specific criteria

- Having a Caucasian skin type
- Displaying a cellulite grade 1-2 (Müller Scale)
- Having had a stable lifestyle for at least three months

3.1.2 Non-inclusion criteria

General criteria

- Failing to meet the aforementioned inclusion criteria
- Being in remanence, at the beginning of the study, on the studied area(s), following another cosmetic, dermatological, or medical test
- Having undergone any major surgery in the previous year
- Having undergone plastic surgery on the studied areas
- Taking part or intending to take part in another study liable to interfere with this study
- Being diabetic
- Being asthmatic
- The refusal to give their assent by signing the consent form
- Being pregnant or breastfeeding in the past three months
- Intending to become pregnant during the study
- Having sun-tanned skin
- Intending to expose themselves to artificial UV light and/or to the sun during the study
- Having changed their cosmetic habits in the 14 days preceding the start of the study or intending to change them during the study on the concerned areas
- Having cutaneous hypersensitivity or a skin allergy to cosmetic products
- Following or intending to follow a chronic medicinal treatment comprising any of the following products taken orally: aspirin-based products, anti-inflammatories, anti-histamines, corticotherapy, anti-depressants, anti-migraine medication and neuroleptics, DHEA (the only medication permitted is paracetamol)

Specific criteria

- Having been on a weight-loss diet or received a treatment at a spa in the three months preceding the start of the study
- Having started, changed or stopped a hormonal treatment (Hormone Replacement Therapy, thyroid) in the past three months
- Intending to start, change or stop a hormonal treatment (Hormone

Replacement Therapy, thyroid) during the study
- Having started, changed or stopped a means of oral contraception in the past three months
- Intending to start, change or stop a means of oral contraception during the study
- Intending to modify their sporting habits during the study
- Having applied firming products or products with an anti-wrinkle or anti-ageing action, or A.H.A-based cosmetics (fruit acids, derivates) or retinol-based cosmetics in the two weeks preceding the start of the study

3.1.3 Proscriptions and restrictions

General proscriptions and restrictions

- Having changed their cosmetic habits during the study
- The use of any of the following products taken orally is proscribed for the entire duration of the study: aspirin-based products, anti-inflammatories, anti-histamines, corticotherapy, anti- depressants, anti-migraine medication and neuroleptics**,** DHEA (the only medication permitted is paracetamol)
- The days on which the measurements are to be taken, the application of any other cosmetic product and/or make up, including the usual cleanser and the tested products to the studied areas is proscribed (only face cleaned with water is accepted)
- During the study, the application of any other cosmetic product to the studied areas is proscribed (only the usual cleanser and make-up for the lips, eyes and blusher are accepted).
- Having exposed themselves to artificial UV light and/or to the sun during the study

Specific proscriptions and restrictions

- Having started, changed or stopped a hormonal treatment (Hormone Replacement Therapy, thyroid) during the study
- Having started, changed or stopped a means of oral contraception during the study
- Having begun a diet or a treatment at a spa during the study
- Having modified their sporting habits during the study

3.2 The products

3.2.1 Presentation of the products

The test products are supplied free of charge by the study sponsor.

References of the products	Constituent form	Packaging
ENDOR FORMULA A	CREAM	POT WITH ORANGE LABEL
ENDOR FORMULA B	CREAM	POT WITH BLUE LABEL

The study sponsor is in charge of product manufacturing and packaging. He/She is responsible for product identification, purity determination, composition, innocuousness, and any other characteristics of each product to be tested prior to the beginning of the study.

The study sponsor is responsible for supplying the exact amount of product needed to carry out the test(s).

For this study, the study sponsor agrees to supply:

- The appropriate quantity of the product required to treat all of the subjects;
- One product containing the real active per subject to be delivered as a reward at the end of the study

Products are stored in an ambient temperature away from light.

At the end of the study, the products used by the volunteers or the left over products can be sent back to the promoter if he has asked for it.

On the other hand, the investigator proceeds to eliminate the remaining products according to the method of their choice described in their procedures.

3.2.2 Product application

The application is carried out by the subjects themselves.

Product(s)	Application areas	Frequency of application	Application duration	Conservation
ENDOR FORMULA A	**One Thigh**	**Twice daily (morning and evening)**	**28 days**	**At an ambient temperature**
ENDOR FORMULA B	**One Thigh**	**Twice daily (morning and evening)**	**28 days**	**At an ambient temperature**

Application modalities:

The subjects themselves apply the products, twice daily during 28 days (morning and evening) on the whole thigh according to a pre-established randomized pattern.

Quantities of application should correspond to normal conditions of use. Volunteers have to wash their hands between applications of each product.

3.3 Study design

- This study is carried out as a "double blind test". Neither the participating subjects nor the investigator are aware of the type of product being applied throughout the study; only the sponsor is aware of the nature of the products.
- The tested is a gold nanoparticle conjugated to a commercially available hyaluronan polimer called Signal-10 (Principium Europe S.r.L., Italy).
- The placebo acts as a negative control and contains the same amount of hyaluronan polimer without the gold nanoparticle.
- This is a comparative study in which the results obtained at one treated area by one of the products are compared with those obtained at another treated area with a placebo.
- The subjects serve as their own reference and results obtained at various assessment times are compared with those obtained at T0.

3.4 Randomization

The selection of the areas to be treated by each of the products is determined at random for each subject. An algorithm designed for this purpose carries out this randomization. Due to a mislabeling, there was no Subject 11. The randomization patter for this study is as follows:

	PRODUCT A	**PRODUCT B**
VOL01	Left	Right
VOL02	Right	Left
VOL03	Left	Right
VOL04	Right	Left
VOL05	Left	Right
VOL06	Right	Left
VOL07	Left	Right
VOL08	Right	Left
VOL09	Right	Left
VOL10	Left	Right
VOL12	Right	Left

VOL13	Left	Right
VOL14	Right	Left
VOL15	Right	Left
VOL16	Right	Left
VOL17	Left	Right
VOL18	Right	Left
VOL19	Right	Left
VOL20	Right	Left
VOL21	Left	Right
VOL22	Right	Left
VOL23	Left	Right
VOL24	Right	Left

3.5 Study procedures

3.5.1 Weight Measurement

3.5.1.1 Acquisition of source data

Principle

The weight measurement is carried out with a digital scale BC-545 Inner Scanner (Tanita, Japan). The principle behind digital scales lies in high precision pressure sensors converting the mechanical pressure of the body weight into an electrical signal. The intensity of the electrical signal is proportional to that mechanical pressure.

Body weight is not constant. Fluctuations are to be expected based on the time of the day, the physical activity, menstruation, etc. Changes around ±1000g are not considered weight gain.

Acquisition methodology

- Subject

The subject stands on the device when the signal to proceed to the weighting appears onscreen. The subject wears her socks, disposable underwear and a t-shirt provided by the investigator.

- Studied areas and marking

In this case the whole body is evaluated and no marking takes place.

- Measures

The subject is placed in the scale as shown in the figure. A weight value is obtained and written down on the CRF.

3.5.1.2 Treatment of source data

Methodology and Treatment Software

On every visit the subject weight is obtained.

There is no analysis taking place in this case

.

Mathematical treatment

At each visit 1 measurement is taken.

Parameters

A weight in kg is obtained.

Exploitation

A change in weight greater than 1kg indicates that the subject may not have followed the study instructions related to physical activity or eating habits.

3.5.2 Thigh Circumference Measurement

3.5.2.1 Acquisition of source data

Principle

The subject is placed on the measuring bench Visio 4D (Eotech, France) that ensures that the subject is placed in the same position, angle and height every time. A green horizontal laser line signals the Region of Interest. Thigh circumference is measured with a measuring tape placed over this green laser line.

Weight and liquids fluctuations as well as small variations in the measuring tape may influence the result. Varioations of ±0,5cm are not considered as a real circumference change.

Acquisition methodology

- Subject

The subject stands still on the measuring bench while the investigator repositions everything at the same angles, heights and distances. Afterwards the measuring tape is placed on a horizontal green laser line projected on the thighs Region of Interest.

- Studied areas and marking

The Areas and laser marking is as shown in the following figure.

- Measures

Measuring tape surrounds the thigh and the length is written down on the CRF.

3.5.2.2 Treatment of source data

Methodology and Treatment Software

On every visit the subject the thigh circumference of both thighs is obtained.

There is no analysis taking place in this case.

Mathematical treatment

At each visit 1 measurement per leg is taken.

Parameters

A distance in cm is obtained for every thigh.

Exploitation

A change in length greater than 0,5cm indicates that the subject may not have followed the study instructions related to physical activity or eating habits.

3.5.3 Clinical Photography

3.5.3.1 Acquisition of source data

Principle

This technique consists in obtaining high-resolution photographs of the face at 0º, 180º, 90º and ±45º, in complete reproducible lighting conditions, in diffused light in order to produce shadows that allow to assess the skin appearance.

Photographs are taken with a Nikon D300 (Nikon Corp., Japan) using a lens Pro micro Nikkor 60mm (Nikon Corp., Japan). Two flashlights Elinchrom style 600 RX flashes (Elinca SA, Switzerland) provide the Lighting. The camera tripod and flashes are in a fixed position throughout the study.

In order for the photographs to be highly reproducible and consistent thorugh the study a setup with a positioning mat for the subject and fixed positions for the camera tripod, the flashes and he mat were established.

Acquisition methodology

- Environmental conditions

The evaluation is carried out in a room with grey walls, under controlled temperature and relative humidity (temperature 23,8ºC ± 2ºC; hygrometry: 43% ±10%).

- Subject

The subject is standing with her arms crossed over her chest on a mat where the different angles are indicated as shown in the figure bellow.

The camera tripod, the tripod height and the flashes remain in the same positions throughout the study

A 15-minutes period of acclimatization in the room is respected prior o the photographs.

- Studied areas and marking

No measurements are performed.

- Measures

The camera is positioned vertically. The visualization of the initial digital photograph (D0) at D28 ensures a good repositioning of the subject at each time.

3.5.3.2 Treatment of source data

Methodology and treatment software

The subject must wear as only garments clinical disposable underwear and a white t-shirt, keeping the arms resting on the column and looking straight ahead.

After the photographs are taken they are named according to image processing criteria: study name (5 alphanumeric characters)_volunteer code (5 alphanumeric characters)_camera position (1 letter)_type of lighting (2 letters)_time (2 numbers. Example: END02_VOL01_F_CP_00).

Mathematical treatment

There is no mathematical treatment.

Parameters

There are no parameters.

Exploitation

There is no exploration. The photographs are taken to have a visual reference of the subject state at each visit.

3.5.4 Autofluorescence Spectroscopic Antioxidants Quantification

3.5.4.1 Acquisition of source data

Principle

The Antioxidants quantification was assessed with the help of a specific device called Biozoom. Biozoom measures carotenoids present in the skin by means of fluorescence techniques (skin autofluorescence (skin AF)). Biozoom has multiple light sources, which illuminate the skin. Those lights will excite ß-carotenes in the tissue, which will emit light of a different wavelength. The wavelength band used for emission is 440-480nm and the wavelength band sampled for fluorescence was 390-970nm.

As the antioxidants form protective chains, which allow them to regenerate after neutralizing free radicals, the analysis of one main antioxidant substance is sufficient to yield information regarding the antioxidant status as a whole. Using electro paramagnetic resonance spectroscopy, it has been demonstrated that, for example, carotenoids represent indicator marker substances for the overall antioxidant status of the epidermis. In the case of Biozoom, several studies carried out at la Charité Hospital in Berlin have proven that Biozoom is as reliable as conventional laboratory spectroscopy techniques for the measurement of skin carotenoids.

Acquisition methodology

- Subject

The subject is standing on the Visio 4D acquisition bench.

- Studied areas and marking

The measurements are performed on both thighs. The site of the instrumental measurements and their location at the different points of the kinetics should be as the reproducible as possible.

- Measures

The handheld device is placed over the subject's face without compressing the skin while the measurement takes place.

One measurement is recorded on the CRF on a given measurement site, at each time point of the study.

3.5.4.2 Treatment of source data

Methodology and treatment software

On every site one measurement with the carotenoids autofluorescence is obtained.

The device is connected to a PC via a USB cable. The data from the device is transferred to the Biozoom servers where it is processed and then returns as a carotenoids autofluorescence index displayed onscreen.

Mathematical treatment

At each point of the kinetics 1 measurement is taken. There is no average and standard deviation of these measurements to be calculated for each site and time.

Parameters

Carotenoids autofluorescence is given on an arbitrary scale from 0 to 10.

Exploitation

An increase in the skin carotenoids autofluorescence index means an antioxidant effect is taking place and antioxidants are increasing in the skin.

3.5.5 50 MHz High Frequency Ultrasound Evaluation of the Epidermis and Dermis

3.5.5.1 Acquisition of source data

Principle

High Frequency Ultrasounds (HFUS) were performed with a DUB 75 HFUS system (TPM, Germany). Even if High frequency ultrasounds follow the same physical rules as traditional echography, the former uses probes ranging from 3 to 7 MHz in order to ensure good tissue penetration while the first uses frequencies ranging from 18 to 100 MHz as they prioritize resolution. Figure 5 illustrates the inverse relationship between penetration and resolution in the ultrasound domain. In the case of this study, A 50 MHz open probe with a 4mm penetration and a 31μm resolution was used in order to allow a reliable measure of the epidermal and dermal thickness as well as the epidermal and dermal echogenic density.

Acquisition methodology

- Subject

The subject lies on a bed on their belly.

- Studied areas and marking

The measurements are performed on both thighs. The site of the instrumental measurements and their location at the different points of the kinetics should be around the evaluated area in other tests. The location is determined by the marking performed during the circumference measurements.

- Measures

Water is put on the transducer after the probe is placed on the skin without compressing it.

Dozens of captures are recorded on a given measurement site, at each time point of the study. Each one corresponds to 12mm of scanned skin.

3.5.5.2 Treatment of source data (skin thickness)

Treatment software and Methodology

3 captures with optimal signal ratio are selected. The software analyses the length of the signal peaks though the A-Scan and establishes a distance based on it (on μm). The software provides the average thickness of both epidermis and dermis of the 12mm of scanned skin in each capture.

Mathematical treatment

The average of these measurements is calculated for each image.

Parameters

The skin thickness is expressed in μm and is subdivided in two parameters:

Epidermal Thickness

Dermal Thickness

Exploitation

An increase in the skin thickness characterizes an anti-ageing effect of the product.

3.5.5.3 Treatment of source data (skin density)

Treatment software and methodology

3 captures with optimal signal ratio are selected. The software analyses the amplitude of the signal though all points of the scan and establishes an amount of echo based on. This amount of echo is called echogenic density and is proportional to the amount of extracellular matrix present in the tissue. The software provides the average echogenic density of the epidermis and dermis (in Arbitrary Units) of the 12mm of scanned skin in each capture.

Parameters

The skin thickness is expressed in Arbitrary Units and is subdivided in two parameters:

Epidermal Density

Dermal Density

Exploitation

An increase in the echogenic density shows an improvement of the extracellular matrix al least partially related to a collagen and hyaluronic acid increase.

3.5.6 3D Analysis of Dimples and Texture with FOITS

3.5.6.1 Acquisition of source data

Principle

FOITS (Fast Optical *In vivo* Topometry of human Skin) technique allows us to objectify the modifications of the cutaneous topography.

The measurements are taken using an optical system dedicated to the metrology of the relief of surfaces. This system includes a measurement sensor associating a projector and a high resolution CCD camera (Dermatop system (Breuckmann, Germany - EoTech, France); field of view : 50 x 60 mm) - linked to the acquisition software Optocat (EoTech, France). The average axial and lateral resolutions are 10 μm.

Acquisition methodology

Environmental conditions

The evaluation is carried out in a dark room.

Subject

The subject must wear as only garments clinical disposable underwear and a white t-shirt, keeping the arms resting on the column and looking straight ahead.

- The positioning of the sensor and of the subject is made easier with the use of a measurement bench (Visio4D) enabling the body to be kept in the same position and a reproducible positioning of the sensor. In order to do so, the subject places his/her feet over two foot-shaped markings on a circular platform. This platform is then elevated until a fixed-position horizontal laser line reaches the Region of Interest. The elevation height is noted in the CRF in order order to place the subject in the same position on the following visit. A carpenter's square-shaped structure pivoting around the platform holds the optical system. A ruler allows to place it exactly at the same height every time while a protractor ruler placed in the border of the platform allows to place it at the same angle every time. In this case the height was 36cm and the angles were ±30º.

Studied areas

Thighs cellulite under the buttocks is selected as the area of choice.

Measurements

One acquisition is taken on a given area and time. The visualization on-screen of the initial measurement (T0), at Tn, ensures a good repositioning of the studied area.

3.5.6.2 Treatment of source data (texture analysis)

Treatment software and methodology

The analysis of the cutaneous topography of the surface consists in calculating the shape and size of "objects" (in this case the big depressions of the skin known as dimples) in order to obtain their volume, area, circumference, maximum depth and minimal depth. It also involves calculating typical roughness parameters used in metrology. In cellulite skin, roughness parameters not only correlate with the intrinsic quality of the skin but also with the nodules associated with the orange peel texture that characterizes cellulite. Concretely, SA, SQ and Stm. All these parameters are extracted from a surface of 50 x 60mm (30 cm^2).

The analysis of the data obtained by fringe projection on the studied areas is carried out using the Optocat analysis software. The analysis is performed on the "SDF" files obtained from the "ABS" files.

The principle involves quantifying the objects and micro-relief of the studied area by analyzing the deformation of high contrast networks of lines on this surface.

Mathematical treatment of the raw data

A numerical value of each of those variables is obtained for every volunteer, thigh and time. The results are gathered in distinct tables for Formula A and Formula B for every variable. Averages and Standard deviations are calculated

Parameters

Profile parameters

The parameters are quantified on a serial of profiles perpendicular to the studied structures on the area of interest.

Stm : maximum amplitude of the relief (mm): The 50x60mm area is subdivided in 25 areas. In each one of these, the amplitude between the highest peak and the lowest valley is measured. These 25 amplitudes are summed and averaged in order to obtain the Stm

SA: Average roughness (mm): Average variations in amplitude of the relief integrated into the studied surface.

SQ: Roughness with regard to the average quadratic variation (mm): Square root average of the variations in amplitude of the relief integrated into the studied surface. The interpretation of SQ is the same as SA. This parameter is less sensitive to artifacts.

Morphology parameters

Dimple areas are detected after the use of several filters and a polynomial correction in order to remove the local shape and flatten the Region of Interest (ROI).

Mean volume (VOL) of the dimples (mm^3): This parameter corresponds to the mean volume of objects (wrinkles and fine lines) detected in ROI.

Mean area (AR) of the dimples (mm^2): This parameter corresponds to the mean area of the objects detected in the ROI.

Mean circumference (CIRC) of the dimples (mm): This parameter corresponds to the mean perimeter of the objects detected in the ROI.

Mean maximum depth (Max. D.) of the dimples (mm^2): This parameter corresponds to the mean of the maximum depths of the objects detected in the ROI.

Mean average depth (Mean. D.) of the dimples (mm^2): This parameter corresponds to the average of the mean depths of the objects detected in the ROI.

Exploitation

A decrease in any of the roughness variables characterizes an anti-ageing effect and a nodule reduction effect of the product.

A decrease in any of the volume, max depth and mean depth characterizes a firmness effect of the product.

A decrease in the area and circumference characterizes a tightening effect of the product.

3.6 Examination schedule

The effect of the products is evaluated over a 28-day period. The scheduled measurement procedures are as follows:

Preinclusion

- Checking of the inclusion/non inclusion criteria
- Clinical observation and description of the quality of the skin at the measuring areas

At D0 before the application of the products:

- Acknowledgement, reading and signature of the consent form
- Checking of the inclusion/non inclusion criteria
- Clinical observation and description of the quality of the skin at the measuring areas
- Location of the measuring areas
- Weight Measurement
- Thigh Circumference Measurement
- Clinical Photography
- 3D Analysis of Dimples with FOITS
- 3D Analysis of Texture with FOITS
- Autofluorescence Spectroscopic Antioxidants Quantification
- 50 MHz High Frequency Ultrasound Evaluation of the Epidermis and Dermis

At D28 (28 days):

- Checking of the proscriptions and restrictions
- Discussion about the subject's tolerance towards the products
- Clinical observation of the quality of the skin at the measuring areas
- Weight Measurement
- Thigh Circumference Measurement
- Clinical Photography
- 3D Analysis of Dimples with FOITS
- 3D Analysis of Texture with FOITS
- Autofluorescence Spectroscopic Antioxidants Quantification
- 50 MHz High Frequency Ultrasound Evaluation of the Epidermis and Dermis

3.7 Data analysis and statistics

3.7.1 Data analysis of technical data

The results include:

- Raw values for each subject at each examination.
- Differences, in relation to D0 for each subject during the study (D28 – D0).

- Means, medians, maximum, minimum and standard deviations of the raw values and of the differences in relation to D0 obtained by all of the panel.
- Variations, in relation to T0 expressed as a percentage calculated from the mean values.
- Numbers and percentages of subjects presenting an improvement.

Comparison in time, for each product

Verification of the normality of the distributionsis carried out using the Shapiro-Wilk and Kolomogoev-Smirnov tests.

The statistical analysis of the evolution of the measured parameters during the study for each product is performed using the Student test (normality of distributions checked) or with the Wilcoxon test (normality of the distributions rejected). The significance threshold is fixed at 5%.

Comparison of the two products

Verification of the normality of the distributionsis carried out using the Shapiro-Wilk and Kolomogoev-Smirnov tests for the comparison of the two products at D0 and at D28-D0.

The statistical comparison of the two products, at D0 and on the differences (D28-D0), for each of the measured parameters, is performed with the Student test (normality of distributions checked) or the Wilcoxon test (normality of the distributions rejected). The significance threshold is fixed at 5%.

4. ETHICAL AND LEGAL CONSIDERATIONS

4.1 Study personnel

The investigator assures that the study manager and everyone who participates in this study have the required qualifications and abilities to carry it out.

4.2 Data archiving

The documents are archived for 10 years for non- interventional studies. Using both paper and IT storage media ensures dual archiving.
Paper files are archived by a service provider until the end of the archiving period.
Electronic files are archived on the cloud and a large capacity USB hard disk. The disk is stored for 10 years in one o the Instituto de Fotomedicina Facilities.

The investigator keeps the original case report form, questionnaires and all

associated documents, the consent forms, and all project-related documents of any type for a 10-year period following delivery of the final report.

All these documents are accessible upon request for inspection by the study sponsor, their representative or by administrative authorities.

The records of the undesirable events are stored for 10 years.

The documents for biomedical studies are archived for 15 years (food supplements and medical devices) or for 10 years (cosmetic products).

4.3 Insurance

The investigator is insured for civil liability under the terms of the following policy:

WR Berkley Insurance - Contract N°65g086103194

4.4 Declaration to the AEDP

In compliance with the organic law 15/1999 and 1720/2007 (LOPD) dated 13th December 1999 and21st December 2007, relating to the protection of natural person regarding the treatment of data of a personal nature and which amends the article 18 from Spanish Constitution from, files and freedom the automatic treatment of personal data are subject to a declaration to the AEPD (Agencia Española de Protección de Datos).

The study sponsor cannot have access to the confidential data relative to the subjects registered in the database of Instituto de Fotomedcina.

4.5 Anonymity of the subjects

The subjects are identified for the study sponsor using a thee-character alphanumeric code and a number. The investigator makes a commitment not to raise the anonymity of the subjects.

4.6 Consent to participate in the study

An information form is given to each subject providing full details about the study as well as:

- Its objectives, methods, and duration;
- Possible expected aesthetic benefits, constraints, and potential risks;
- The non-inclusion period, the right of access to data files and their later destruction.

This information enables the subjects to sign their participation consent form freely and unequivocally, in the knowledge that they are fully aware of the testing details.

4.7 Use of image

If the study involves the use of photographs, the volunteers are informed, in the consent form that Instituto de Fotomedicina may use their image without direct identification all over the world, with no time limit on this usage. The volunteers are also informed that Instituto de Fotomedicina may also provide images to the promoter for publishing or duplication.

The volunteers who refuse to allow their image to be used sign a refusal of image use form.

4.8 Confidentiality

All the information, data, and results of the study are confidential. Everyone having access to such data are informed of their confidentiality.

Any medical information concerning a subject's state of health and the results of the clinical examinations carried out during the recruitment, selection and admission phases before a study is subject to the medical secrecy regulations, in no case should such information be communicated to the study sponsor using a subject's identity.

4.9 Quality Assurance

The entire dossier of a study (quotation, protocol, results, report, and any other study-related documents) is subject to a Quality Management audit which conforms to the regulatory texts and procedures in force.

The investigator cooperates in ensuring any additional auditing required by the study sponsor to ensure that the study progresses in accordance with regards the protocol and the current procedures.

4.10 Regulations

This study is carried out in conformity with the most recent recommendations of the World Medical Association (Declaration of Helsinki 1964, amended in Seoul, Korea, 2008).

This study is qualified as "non interventional" because it does not comply with the public health code which defines "biomedical research" as any research organized and carried out on human beings in order to develop biological and medical knowledge.

This study does not fall under the field of application of the new Public Health law relative to the protection of subjects participating in biomedical research, the

counsel of the Advisory Board is not sought, and no information is communicated to the National file of subjects participating in biomedical research.

However, the spirit of law and Good Clinical Practices are respected.

5. RESULTS

5.1 Absence, Exclusions and Non Exploited Data

The subject n°02 (code CFI) was absent at D28 for a personal reason.

The subject n°06 (code CAN) didn't follow the study instructions the 10 days prior to the end of the study and thus, her data was discarded.

Due to technical issues, it was impossible to perform the antioxidants evaluation on subject n°1 (code CLA), thus she was not considered for that test-

The FOITS data at D0 of Subject n°09 (Code SAN) turned out to be partially unreadable, and thus she wasn't taken into account for that test

Because of some issues with the data capture at D28, the left FOITS analysis of subject n°17 (code VNA), had to be performed on 42x65mm area instead of 50x60mm.

5.2 Population considered in the expression of the results At D0, 20 subjects were recruited.

Considering the information previously mentioned in the paragraph 5.1, which led to "non exploited data" for several subjects, the number of subjects considered in the expression of the results, at each examination time, and for each technique, is presented in the following table:

Techniques		D0	D28
Weight Measurement	Formula A	24	23
	Formula B	24	23
Thigh Circumference Measurement	Formula A	24	23
	Formula B	24	23
Clinical Photography	Formula A	24	23
	Formula B	24	23
3D Analysis of Dimples with FOITS	Formula A	23	22
	Formula B	23	22
3D Analysis of Texture with FOITS	Formula A	23	22

	Formula B	23	22
Autofluorescence Spectroscopic Antioxidants Quantification	Formula A	23	22
	Formula B	23	22
50 MHz High Frequency Ultrasound Evaluation of the Epidermis and	Formula A	24	23
	Formula B	24	23

Table 1. Techniques and population for the tests carried out at D0 and D28.

Among this final group of volunteers, the worse results were discarded in order to obtain in all cases results based on the data of 20 volunteers.

5.3 Description of the exploited panel

The exploited panel consisted of **24 women**.
The following table summarizes the average age of the panel at D0.
The details of the characteristics of the panel are presented in appendix XX.

n = 20	Mean	Minimum	Maximum
Age (years)	41	32	52

Table 2. Study's subjects age demographics.

All the subjects displayed a degree 1 or 2 od Cellulite in both thighs (Müller Scale).

5.4 Weight Measurement

Studied parameter:
Body weight (kg)

5.4.1 Observed results on each side

The following tables present the means and the standard deviations on the raw values and and the percentages of body weight measured at D0 for the sides treated by the product A and by the product B, as well as the corresponding statistical results for the comparison of both sides (Student test, two-tailed for paired groups at 5%, after checking the normality of the distributions by a Shapiro-Wilk test and Kolmogorov-Smirnov test).

	D0	D28
Average	61,50	61,65
Std. Dev.	9,62	9,68

Table 3. Body weight Average and Standard Deviation.

	a	p
D0 vs D28	0,6499	p > 0,05

Table 4. Statistical results of all the comparison at D0 and D28.

No significant difference of the values of the body weight are observed between treatments and between D0 and D28. We can assume the volunteers maintained their eating and sporting habits throughout the study.

Both sides are then comparable.

5.5 Thigh Circumference Measurement

Studied parameter:
Thigh Circumference (cm)

5.5.1 Observed results on each side

The following tables present the means and the standard deviations on the raw values and and the percentages of the thighs circumferences measured at D0 for the sides treated by the product A and by the product B, as well as the corresponding statistical results for the comparison of both sides (Student test, two-tailed for paired groups at 5%, after checking the normality of the distributions by a Shapiro-Wilk test and Kolmogorov-Smirnov test).

		D0	D28	%ΔD28-D0
Formula A	**Average**	52,605	52,723	0,29
	Std. Dev.	4,454	4,112	1,66
Formula B	**Average**	53,277	52,473	-1,43
	Std. Dev.	4,236	4,062	3,35

Table 5. Body weight Average and Standard Deviation for the Formula-A-treated And Formula-B-treated forearms and cheeks.

	a	p
Product A D0 vs Product B D0	0,0984	p > 0,05
Product A D0 vs Product A D28	0,1995	p > 0,05
Product B D0 vs Product B D28	0,5200	p > 0,05
Product B D28 vs Product A D28	0,0595	p > 0,05
Product B %ΔD28–D0 vs Product A %ΔD28–D0	0,0592	p > 0,05

Table 6. Statistical results of all the comparison at D0 for forearms and cheeks for both treatments.

No significant difference of the values of thigh circumferences are observed between treatments and between D0 and D28. We can assume the volunteers maintained their eating and sporting habits throughout the study.

Both sides are then comparable.

5.6 3D Analysis of Dimples with FOITS

Studied parameters:
Dimples Volume (mm^3)
Dimples Circumference (mm)
Dimples Area (mm^2)
Dimples Max Depth (mm)
Dimles Mean Depth (mm)

5.6.1 Observed results on each side

The following tables present the means and the standard deviations on the raw values and the percentages of variation for the dimples volume, circumference, area, maximum depth and mean depth at D0 D28, for the sides treated by the product A and by the product B, as well as the corresponding statistical results for the evolution in time (Student, two-tailed for paired groups at 5%, after checking the normality of the distributions by a Shapiro-Wilk test and Kolmogorov-Smirnov; if the result is statistically significant the p-value will be colored in red).

Dimples Volume

		D0	D28	%ΔD28-D0
Formula A	**Average**	8,111	5,747	-31,16
	Std. Dev.	5,500	4,636	21,85
Formula B	**Average**	10,229	10,897	-1,67
	Std. Dev.	8,788	9,783	25,15

Table 7. Dimples Volume Average and Standard Deviation for the Formula-A-treated And Formula-B-treated cheeks at D0 and D28.

Formula A:

Responding subjects:
- 20 subjects out of 20 (i.e. 100% of the panel) show an improvement in the Dimples Volume.

Formula B:

Responding subjects:

- 9 subjects out of 20 (i.e. 45% of the panel) show an improvement in Dimples Volume. Subjects nº1 and nº16 presented and outlier result for **%ΔD28-D0** (282,51 and 196,72% respectively). As they clearly distort the trends we want to illustrate the **%ΔD28-D0** shown on table 7 doesn't take them into account.

Dimples Circumference

		D0	D28	%ΔD28-D0
Formula A	**Average**	190,001	160,503	-17,39
	Std. Dev.	64,596	72,184	26,49
Formula B	**Average**	196,262	197,739	-2,68
	Std. Dev.	44,059	58,728	19,04

Table 8. Dimples Circumference Average and Standard Deviation for the Formula-A-treated And Formula-B-treated cheeks at D0 and D28.

Formula A:

Responding subjects:

- 15 subjects out of 20 (i.e. 75% of the panel) show an improvement in the Dimples Circumference.

Formula B:

Responding subjects:

- 9 subjects out of 20 (i.e. 45% of the panel) show an improvement in Dimples Circumference. Subject nº1 presented and outlier result for **%ΔD28-D0** (108,01%). As it clearly distorts the trends we want to illustrate the **%ΔD28-D0** shown on table 8 doesn't take it into account.

Dimples Area

		D0	D28	%ΔD28-D0
Formula A	**Average**	137,732	109,348	-22,01
	Std. Dev.	63,663	55,010	23,80
Formula B	**Average**	151,928	164,194	2,30
	Std. Dev.	67,638	78,275	24,29

Table 9. Dimples Area Average and Standard Deviation for the Formula-A-treated And Formula-B-treated cheeks at D0 and D28.

Formula A:

Responding subjects:
- 16 subjects out of 20 (i.e. 80% of the panel) show an improvement in the Dimples Area.

Formula B:

Responding subjects:
- 9 subjects out of 20 (i.e. 45% of the panel) show an improvement in Dimples Area. Subjects nº1 and nº16 presented and outlier result for **%ΔD28-D0** (148,89 and 129,70% respectively). As they clearly distort the trends we want to illustrate the **%ΔD28-D0** shown on table 7 doesn't take them into account.

Dimples Max. Depth

		D0	D28	%ΔD28-D0
Formula A	**Average**	-0,112	-0,109	-3,54
	Std. Dev.	0,047	0,056	19,96
Formula B	**Average**	-0,141	-0,141	-3,48
	Std. Dev.	0,098	0,113	27,19

Table 10. Dimples Max. Depth Average and Standard Deviation for the Formula-A-treated And Formula-B-treated cheeks at D0 and D28.

Formula A:

Responding subjects:
- 9 subjects out of 20 (i.e. 45% of the panel) show an improvement in the Dimples Max. Depth.

Formula B:

Responding subjects:
- 10 subjects out of 20 (i.e. 50% of the panel) show an improvement in Dimples Max.Depth. Subject nº1 presented and outlier result for **%ΔD28-D0** (127,01%). As it clearly distorts the trends we want to illustrate the **%ΔD28-D0** shown on table 8 doesn't take it into account.

Dimples Mean Depth

		D0	D28	%ΔD28-D0
Formula A	**Average**	-0,038	-0,036	-4,53
	Std. Dev.	0,017	0,018	21,89
Formula B	**Average**	-0,047	-0,047	-1,27
	Std. Dev.	0,032	0,037	27,74

Table 11. Dimples Mean Depth Average and Standard Deviation for the Formula-A-treated And Formula-B-treated cheeks at D0 and D28.

Formula A:

Responding subjects:

- 20 subjects out of 20 (i.e. 100% of the panel) show an improvement in the Dimples Mean Depth.

Formula B:

Responding subjects:

- 9 subjects out of 20 (i.e. 45% of the panel) show an improvement in Dimples Mean Depth. Subject nº1 presented and outlier result for **%ΔD28-D0** (108,01%). As it clearly distorts the trends we want to illustrate the **%ΔD28-D0** shown on table 8 doesn't take it into account.

Dimples Volume

	a	p
Product A D0 vs Product B D0	0,4177	p > 0,05
Product A D0 vs Product A D28	0,0003	p < 0,0005
Product B D0 vs Product B D28	0,2612	p > 0,05
Product B D28 vs Product A D28	0,0590	p > 0,05
Product B %ΔD28–D0 vs Product A %ΔD28–D0	0,0115	p < 0,05

Table 12. Statistical results of all the comparison at D0 and D28 for both treatments.

A significant variation in the studied parameter is observed after 28 days of application of the Formula A.

No significant variation in the studied parameter is observed after 28 days of application of the Formula B.

A significant difference of evolution is observed between studied sides, 28 days after application of the products A and B on the studied parameters.

Dimples Circumference

	a	p
Product A D0 vs Product B D0	0,6880	p > 0,05
Product A D0 vs Product A D28	0,0133	p < 0,05
Product B D0 vs Product B D28	0,8861	p > 0,05
Product B D28 vs Product A D28	0,0339	p < 0,05
Product B %ΔD28–D0 vs Product A %ΔD28–D0	0,0161	p < 0,05

Table 13. Statistical results of all the comparison at D0 and D28 for both treatments.

A significant variation in the studied parameter is observed after 28 days of application of the Formula A.

No significant variation in the studied parameter is observed after 28 days of application of the Formula B.

A significant difference of evolution is observed between studied sides, 28 days after application of the products A and B on the studied parameters.

Dimples Area

	a	p
Product A D0 vs Product B D0	0,5455	p > 0,05
Product A D0 vs Product A D28	0,0011	p < 0,005
Product B D0 vs Product B D28	0,2283	p > 0,05
Product B D28 vs Product A D28	0,0212	p < 0,05
Product B %ΔD28–D0 vs Product A %ΔD28–D0	0,0053	p < 0,01

Table 14. Statistical results of all the comparison at D0 and D28 for both treatments.

A significant variation in the studied parameter is observed after 28 days of application of the Formula A.

No significant variation in the studied parameter is observed after 28 days of application of the Formula B.

A significant difference of evolution is observed between studied sides, 28 days after application of the products A and B on the studied parameters.

Dimples Max. Depth

	a	p
Product A D0 vs Product B D0	0,2694	p > 0,05
Product A D0 vs Product A D28	0,5517	p > 0,05
Product B D0 vs Product B D28	0,2814	p > 0,05
Product B D28 vs Product A D28	0,9959	p > 0,05
Product B %ΔD28–D0 vs Product A %ΔD28–D0	0,5725	p > 0,05

Table 15. Statistical results of all the comparison at D0 and D28 for both treatments.

There is no significant variation in the studied parameter is observed after 28 days of application of the Formula A.

No significant variation in the studied parameter is observed after 28 days of application of the Formula B.

A significant difference of evolution is observed between studied sides, 28 days after application of the products A and B on the studied parameters.

Dimples Mean Depth

	a	p
Product A D0 vs Product B D0	0,1141	p > 0,05
Product A D0 vs Product A D28	0,3187	p > 0,05
Product B D0 vs Product B D28	0,8191	p > 0,05
Product B D28 vs Product A D28	0,2602	p > 0,05
Product B %ΔD28–D0 vs Product A %ΔD28–D0	0,4313	p > 0,05

Table 16. Statistical results of all the comparison at D0 and D28 for both treatments.

No significant variation in the studied parameter is observed after 28 days of application of the Formula A.

No significant variation in the studied parameter is observed after 28 days of application of the Formula B.

There is no significant difference of evolution is observed between studied sides, 28 days after application of the products A and B on the studied parameters.

5.7 3D Analysis of Texture with FOITS

Studied parameters:
SA (mm)
SQ (mm)
Stm (mm)

5.7.1 Observed results on each side

The following tables present the means and the standard deviations on the raw values and the percentages of variation for the roughness parameters SA, SQ and Stm at D0 D28, for the sides treated by the product A and by the product B, as well as the corresponding statistical results for the evolution in time (Student, two-tailed for paired groups at 5%, after checking the normality of the distributions by a Shapiro-Wilk test and Kolmogorov-Smirnov; if the result is statistically significant the p-value will be colored in red).

SA

		D0	D28	%ΔD28-D0
Formula A	**Average**	0,095	0,088	-6,39
	Std. Dev.	0,034	0,032	5,20
Formula B	**Average**	0,102	0,103	-0,04
	Std. Dev.	0,042	0,044	5,35

Table 17. SA Average and Standard Deviation for the Formula-A-treated And Formula-B-treated cheeks at D0 and D28.

Formula A:

Responding subjects:
- 18 subjects out of 20 (i.e. 90% of the panel) show an improvement in the SA roughness parameter.

Formula B:

Responding subjects:
- 8 subjects out of 20 (i.e. 40% of the panel) show an improvement in the SA roughness parameter.

SQ

		D0	D28	%ΔD28-D0
Formula A	**Average**	0,120	0,112	-6,47
	Std. Dev.	0,043	0,041	4,76
Formula B	**Average**	0,130	0,131	0,13
	Std. Dev.	0,052	0,055	5,52

Table 18. SQ Average and Standard Deviation for the Formula-A-treated And Formula-B-treated cheeks at D0 and D28.

Formula A:

Responding subjects:
- 18 subjects out of 20 (i.e. 90% of the panel) show an improvement in the SQ roughness parameter.

Formula B:

Responding subjects:
- 9 subjects out of 20 (i.e. 45% of the panel) show an improvement in the SQ roughness parameter.

Stm

		D0	D28	%ΔD28-D0
Formula A	**Average**	0,459	0,425	-7,17
	Std. Dev.	0,142	0,135	8,64
Formula B	**Average**	0,484	0,479	-1,26
	Std. Dev.	0,151	0,163	9,38

Table 19. Dimples Area Average and Standard Deviation for the Formula-A-treated And Formula-B-treated cheeks at D0 and D28.

Formula A:

Responding subjects:
- 13 subjects out of 20 (i.e. 65% of the panel) show an improvement in the in

the Stm roughness parameter.

Formula B:

Responding subjects:
- 10 subjects out of 20 (i.e. 50% of the panel) show an improvement in the Stm roughness parameter.

SA

	a	p
Product A D0 vs Product B D0	0,4225	p > 0,05
Product A D0 vs Product A D28	0,0001	p < 0,0001
Product B D0 vs Product B D28	0,5369	p > 0,05
Product B D28 vs Product A D28	0,1107	p > 0,05
Product B %ΔD28–D0 vs Product A %ΔD28–D0	0,0115	p < 0,05

Table 20. Statistical results of all the comparison at D0 and D28 for both treatments.

A significant variation in the studied parameter is observed after 28 days of application of the Formula A.

No significant variation in the studied parameter is observed after 28 days of application of the Formula B.

A significant difference of evolution is observed between studied sides, 28 days after application of the products A and B on the studied parameters.

SQ

	a	p
Product A D0 vs Product B D0	0,3830	p > 0,05
Product A D0 vs Product A D28	0,0001	p < 0,0001
Product B D0 vs Product B D28	0,5050	p > 0,05
Product B D28 vs Product A D28	0,0968	p > 0,05
Product B %ΔD28–D0 vs Product A %ΔD28–D0	0,0004	p < 0,0005

Table 21. Statistical results of all the comparison at D0 and D28 for both treatments.

A significant variation in the studied parameter is observed after 28 days of application of the Formula A.

No significant variation in the studied parameter is observed after 28 days of application of the Formula B.

A significant difference of evolution is observed between studied sides, 28 days after application of the products A and B on the studied parameters.

Stm

	a	p
Product A D0 vs Product B D0	0,3757	p > 0,05
Product A D0 vs Product A D28	0,0193	p < 0,05
Product B D0 vs Product B D28	0,9066	p > 0,05
Product B D28 vs Product A D28	0,0631	p > 0,05
Product B %ΔD28–D0 vs Product A %ΔD28–D0	0,0053	p < 0,01

Table 22. Statistical results of all the comparison at D0 and D28 for both treatments.

A significant variation in the studied parameter is observed after 28 days of application of the Formula A.

No significant variation in the studied parameter is observed after 28 days of application of the Formula B.

A significant difference of evolution is observed between studied sides, 28 days after application of the products A and B on the studied parameters.

5.8 Autofluorescence Spectroscopic Antioxidants Quantification

The studied parameter is the Carotenoids Autofluorescence Score

5.8.1 Observed results on each side

The following tables present the means and the standard deviations on the raw values and the percentages of variation for the Carotenoids Autofluorescence Score measured on the cheeks at D0 D28, for the sides treated by the product A and by the

product B, as well as the corresponding statistical results for the evolution in time (Student, two-tailed for paired groups at 5%, after checking the normality of the distributions by a Shapiro-Wilk test and Kolmogorov-Smirnov; if the result is statistically significant the p-value will be colored in red).

		D0	D28	%ΔD28-D0
Formula A	**Average**	1,971	2,157	17,89
	Std. Dev.	0,893	0,859	36,39
Formula B	**Average**	2,643	2,052	-6,05
	Std. Dev.	1,433	0,905	45,62

Table 23. Carotenoids Autofluorescence Score Average and Standard Deviation for the Formula-A-treated And Formula-B-treated cheeks at D0 and D28.

Formula A:

Responding subjects:

- 16 subjects out of 19 (i.e. 80% of the panel) show an improvement in the Carotenoids Autofluorescence Score.

Formula B:

Responding subjects:

- 8 subjects out of 19 (i.e. 40% of the panel) show an improvement in the Carotenoids Autofluorescence Score.

	a	p
Product A D0 vs Product B D0	0,0128	$p < 0,05$
Product A D0 vs Product A D28	0,4143	$p > 0,05$
Product B D0 vs Product B D28	0,1183	$p > 0,05$
Product B D28 vs Product A D28	0,7301	$p > 0,05$
Product B %ΔD28–D0 vs Product A %ΔD28–D0	0,0472	$p < 0,05$

Table 24. Statistical results of all the comparison at D0 and D28 for both treatments on the cheeks.

A significant variation in the studied parameter is observed after 28 days of application of the Formula A.

No significant variation in the studied parameter is observed after 28 days of application of the Formula B.

A significant difference of evolution is observed between studied sides, 28 days after application of the products A and B on the studied parameters.

5.9 50 MHz High Frequency Ultrasound Evaluation of the Epidermis and Dermis

Studied parameter:
Epidermal thickness
Dermal Thickness
Epidermal Echogenic Density
Dermal Echogenic Density

5.9.1 Observed results on each side

The following tables present the means and the standard deviations on the raw values and the percentages of variation for the epidermal and dermal thickness and the epidermal and dermal echogenic density at D0 D28, for the sides treated by the product A and by the product B, as well as the corresponding statistical results for the evolution in time (Student, two-tailed for paired groups at 5%, after checking the normality of the distributions by a Shapiro-Wilk test and Kolmogorov-Smirnov; if the result is statistically significant the p-value will be colored in red).

Epidermal Thickness

		D0	D28	%ΔD28-D0
Formula A	**Average**	95,201	98,318	3,58
	Std. Dev.	6,819	5,131	6,51
Formula B	**Average**	94,136	98,818	6,87
	Std. Dev.	12,404	5,754	17,21

Table 25. Epidermal Thickness Average and Standard Deviation for the Formula-A-treated And Formula-B-treated cheeks at D0 and D28.

Formula A:

Responding subjects:
- 11 subjects out of 20 (i.e. 55% of the panel) show an improvement in the Epidermal Thickness.

Formula B:

Responding subjects:

- 14 subjects out of 20 (i.e. 70% of the panel) show an improvement in the epidermal thickness.

Dermal Thickness

		D0	D28	%ΔD28-D0
Formula A	**Average**	682,916	643,591	-5,07
	Std. Dev.	61,538	44,714	10,56
Formula B	**Average**	693,212	656,955	-4,88
	Std. Dev.	48,945	40,635	7,59

Table 26. Dermal Thickness Average and Standard Deviation for the Formula-A-treated And Formula-B-treated cheeks at D0 and D28.

Formula A:

Responding subjects:
- 6 subjects out of 20 (i.e. 30% of the panel) show an improvement in the Dermal Thickness.

Formula B:

Responding subjects:
- 8 subjects out of 20 (i.e. 40% of the panel) show an improvement in the Dermal thickness.

Epidermal echogenic density

		D0	D28	%ΔD28-D0
Formula A	**Average**	107,409	119,268	15,15
	Std. Dev.	18,727	13,081	26,57
Formula B	**Average**	109,653	119,030	11,79
	Std. Dev.	19,981	11,033	22,01

Table 27. Epidermal echogenic density Average and Standard Deviation for the Formula-A-treated And Formula-B-treated cheeks at D0 and D28.

Formula A:

Responding subjects:
- 14 subjects out of 20 (i.e. 70% of the panel) show an improvement in the Epidermal Echogenic Density.

Formula B:

Responding subjects:
- 13 subjects out of 20 (i.e. 65% of the panel) show an improvement in the Echogenic Density.

Dermal echogenic density

		D0	D28	%ΔD28-D0
Formula A	**Average**	24,527	36,487	51,74
	Std. Dev.	4,289	7,864	35,41
Formula B	**Average**	28,460	31,141	21,12
	Std. Dev.	8,362	9,185	53,13

Table 28. Dermal echogenic density Average and Standard Deviation for the Formula-A-treated And Formula-B-treated cheeks at D0 and D28.

Formula A:

Responding subjects:
- 20 subjects out of 20 (i.e. 100% of the panel) show an improvement in the Dermal Echogenic Density.

Formula B:

Responding subjects:
- 9 subjects out of 20 (i.e. 45% of the panel) show an improvement in the Dermal Echogenic Density.

Epidermal Thickness

	a	p
Product A D0 vs Product B D0	0,6718	p > 0,05
Product A D0 vs Product A D28	0,0225	p < 0,05
Product B D0 vs Product B D28	0,0971	p > 0,05
Product B D28 vs Product A D28	0,7830	p > 0,05

Product B %ΔD28–D0 vs Product A %ΔD28–D0	0,3676	p > 0,05

Table 29. Statistical results of all the comparison at D0 and D28 for both treatments.

A significant variation in the studied parameter is observed after 28 days of application of the Formula A.

No significant variation in the studied parameter is observed after 28 days of application of the Formula B.

No significant difference of evolution is observed between studied sides, 28 days after application of the products A and B on the studied parameters.

Dermal Thickness

	a	p
Product A D0 vs Product B D0	0,5148	p > 0,05
Product A D0 vs Product A D28	0,0242	p < 0,05
Product B D0 vs Product B D28	0,007	p < 0,05
Product B D28 vs Product A D28	0,2933	p > 0,05
Product B %ΔD28–D0 vs Product A %ΔD28–D0	0,9463	p > 0,05

Table 30. Statistical results of all the comparison at D0 and D28 for both treatments.

A significant variation in the studied parameter is observed after 28 days of application of the Formula A.

A significant variation in the studied parameter is observed after 28 days of application of the Formula B.

No significant difference of evolution is observed between studied sides, 28 days after application of the products A and B on the studied parameters.

Epidermal Echogenic Density

	a	p
Product A D0 vs Product B D0	0,4401	p > 0,05
Product A D0 vs Product A D28	0,0613	p > 0,05
Product B D0 vs Product B D28	0,0659	p > 0,05
Product B D28 vs Product A D28	0,9366	p > 0,05
Product B %ΔD28–D0 vs Product A %ΔD28–D0	0,4218	p > 0,05

Table 31. Statistical results of all the comparison at D0 and D28 for both treatments.

A significant variation in the studied parameter is observed after 28 days of application of the Formula A.

No significant variation in the studied parameter is observed after 28 days of application of the Formula B.

A significant difference of evolution is observed between studied sides, 28 days after application of the products A and B on the studied parameters.

Dermal Echogenic Density

	a	p
Product A D0 vs Product B D0	0,0191	p < 0,05
Product A D0 vs Product A D28	0,0001	p < 0,0001
Product B D0 vs Product B D28	0,3924	p > 0,05
Product B D28 vs Product A D28	0,0253	p < 0,05
Product B %ΔD28–D0 vs Product A %ΔD28–D0	0,068	p < 0,005

Table 32. Statistical results of all the comparison at D0 and D28 for both treatments.

A significant variation in the studied parameter is observed after 28 days of application of the Formula A.

No significant variation in the studied parameter is observed after 28 days of application of the Formula B.

A significant difference of evolution is observed between studied sides, 28 days after application of the products A and B on the studied parameters.

6. DISCUSSION

After the experimental part of the study was completed the study sponsor confirmed that the Formula A was the real product and the Formula b was the negative control-

6.1 Body Weight

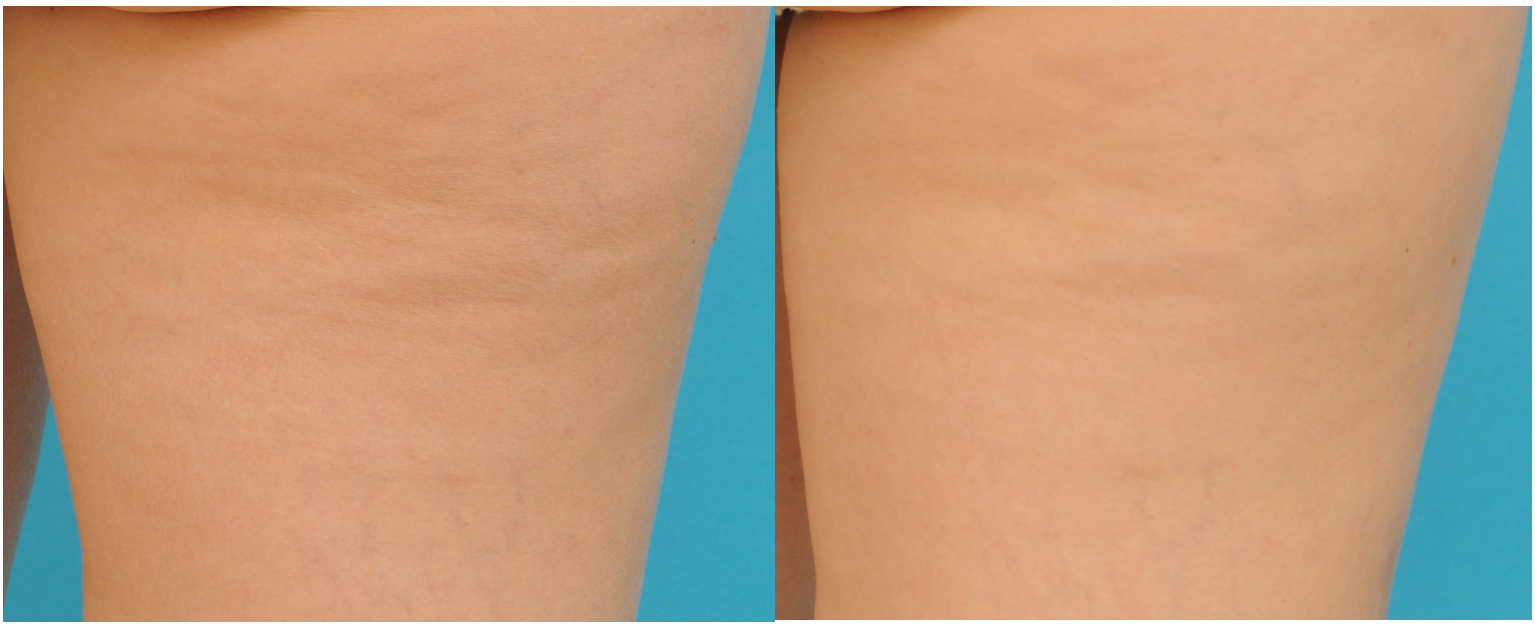
Body weight was one of the measures put I place in order to assess whether the subjects were following the study instructions and were not changing their habits by initiating some sport activities or a diet that could potentially alter the study results.

6.2 Thigh Circumference Measurement

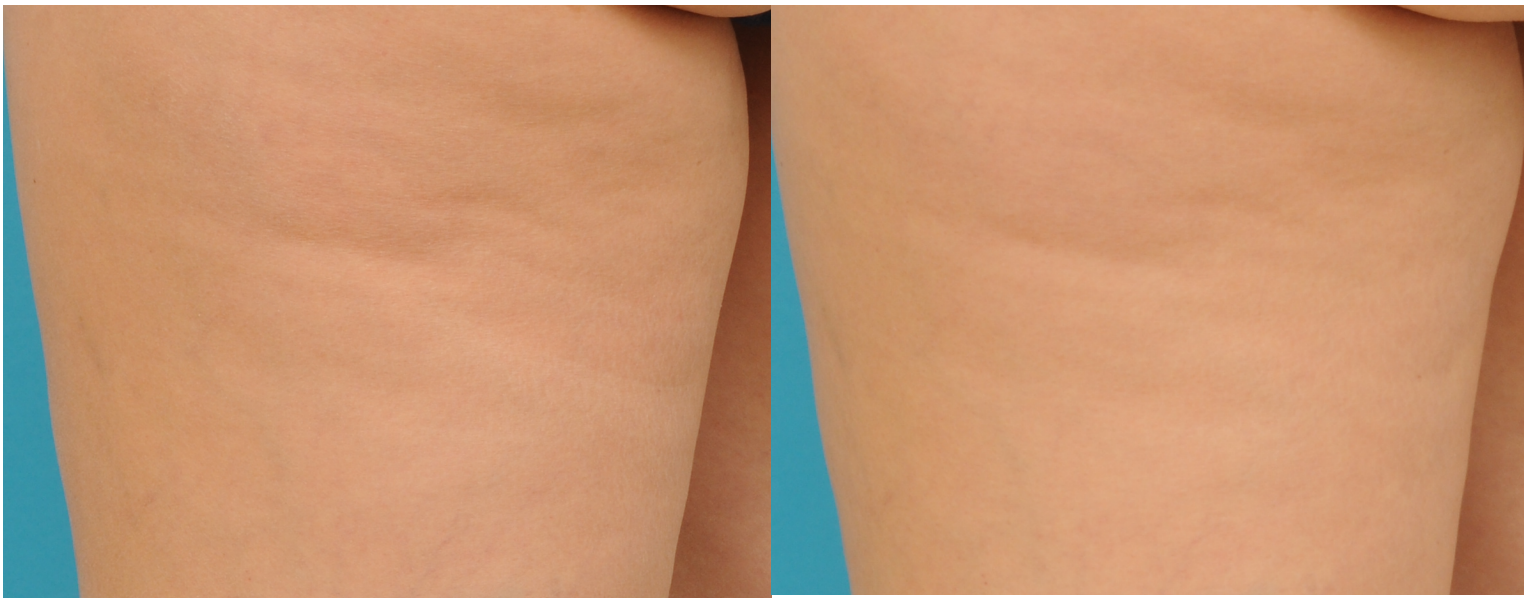
Thigh circumference measurement was the other procedure put in place to assess if the subjects of the study were following the rules regarding any slimming treatment. The results at D28 were almost identical to D0, proving that the study subjects respected the study protocol.

6.3 Clinical Photography

Clinical photographs of the patients were taken in order to have a visual reference of their aspect before and after the study. One of the aims was to obtain a very graphical way of showing which side was treated with the product and which side was treated with the negative control. Bellow some of those successful examples are visible:



Subject 16 Before and After on the leg treated with gold nanoparticles

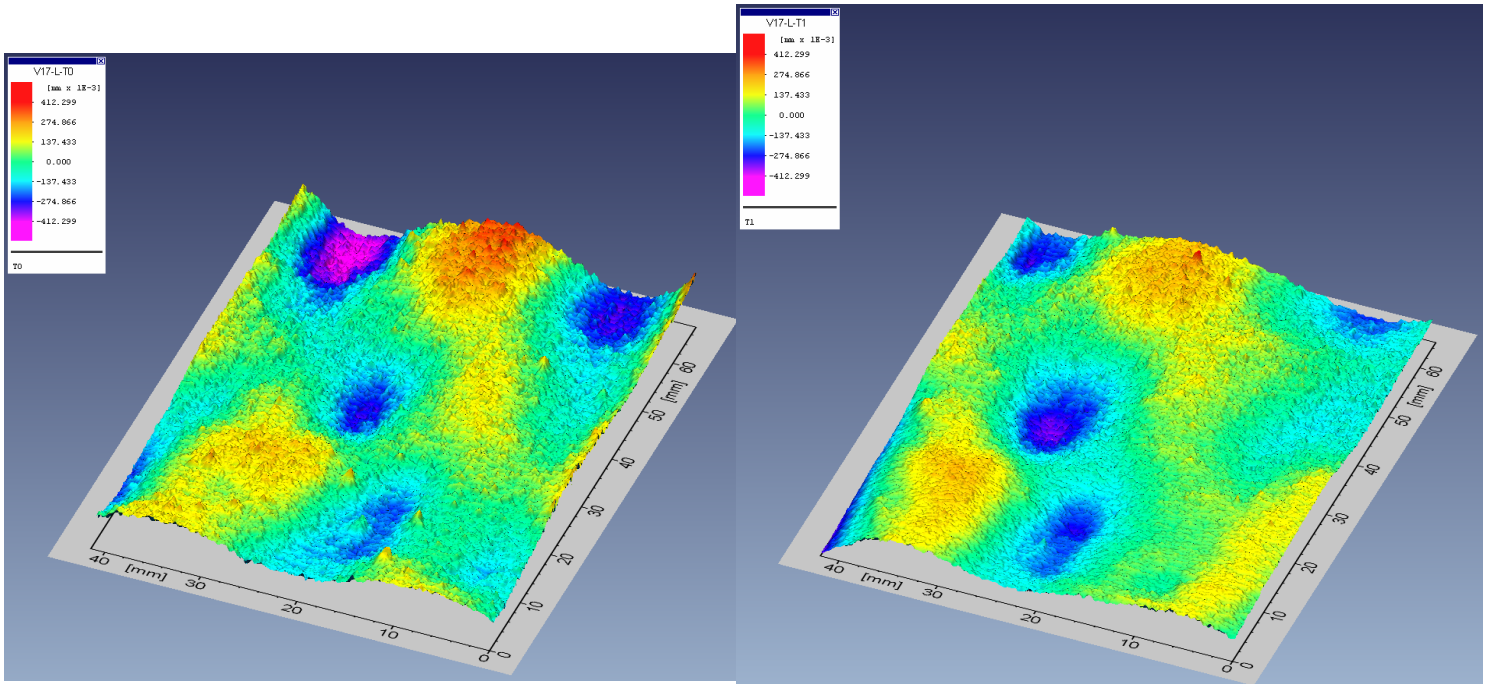


Subject 16 Before and After on the leg treated with the negative control

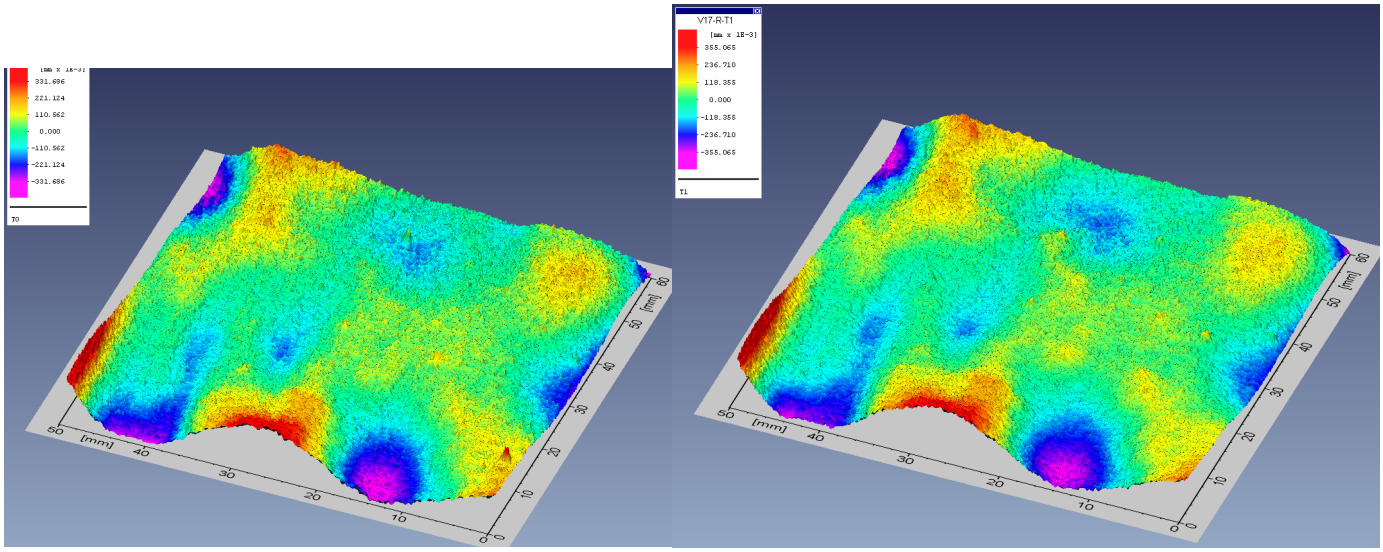
6.4 3D Analysis of Dimples with FOITS

Some outstanding results were obtained on this test, not only because the three main variables (volume, circumference and area) gave a statistical significance for the side treated with the gold nanoparticle while the negative control remained unchanged, but also because of the magnitude of the changes. A 31,1% reduction on the dimples volume is a huge reduction, more so when we know from the weight and thigh circumference follow-up that there was no slimming effect related to it that could justify such decrease. Additionally, the dimples volume is a variable that is easy to grasp and allows a very fast visualization of the kind of skin improvement experienced by the volunteers. Gain, 31,1% is an average. Some volunteers experienced results more modest than that while others achived much higher improvements, with two volunteers achieving dimples volume reductions above 70%.

When put together with the area and circumference results, it suggests a local tightening of the skin is taking place. The word local is of importance as the thigh circumference results prove there was no generalized skin tightening effect caused by the product. Moreover, both the mean and maximum depths of the dimples remain as a reminder that the cause of the dimples has not disappeared but has been rather mitigated by some mechanical support increase that is raising the skin firmness. Given the in vitro results obtained by the gold nanoparticle, that mechanical reinforcing may be related to an increase of collagen, hyaluronic acid and other components of the extracellular matrix.



Subject nº17 3d captures before and after on the leg treated with gold nanoparticles



Subject nº17 3d captures before and after on the leg treated with the negative control.

3D Analysis of Texture with FOITS

The texture analysis was also very successful as it yielded the statistical significance of the three tested parameters. In this case the obtained results were not as extreme as in the dimples volume case but a roughness reduction around 7% is still a good result, with some volunteers even doubling that number.

Let's not forget that in this case a roughness improvement does not only indicate an enhancement of the skin intrinsic qualities but also a reduction in the orange peel skin texture that characterizes cellulite. The Stm parameter was perhaps the most

telling in that regard as it reflects an improvement among the biggest structures having an influence over texture. Again, the pictures above allow to visualize that texture improvement

6.5 Autofluorescence Spectroscopic Antioxidants Quantification

The low ß-carotenes levels measured on the thigh skin since the start were surprisingly low in general, considering that the amount referenced as acceptable is around 5. This makes the increase experienced on the side treated with gold nanoparticles as even more important, no matter how modest the final number remains. Even more so when the antioxidant levels of the negative control side experienced a decrease during the same time. Most importantly of all, a statistically significant antioxidant activity increase could be demonstrated in vivo for the product.

6.6 50 MHz High Frequency Ultrasound Evaluation of the Epidermis and Dermis

The obtained ultrasound results are key to explain and justify the general findings of this study. On the thickness side, the humble but statistically significant increase of the epidermal thickness suggests a mechanical reinforcement responsible of the texture improvement is taking place. The probable underlying mechanism is a slight increase in extracellular matrix synthesis caused by the epidermal keratinocytes, which are the natural target of the gold nanoparticles. This effect is nevertheless moderate as no statistically significant difference was found between the epidermal echogenic density of the product side and the control side.

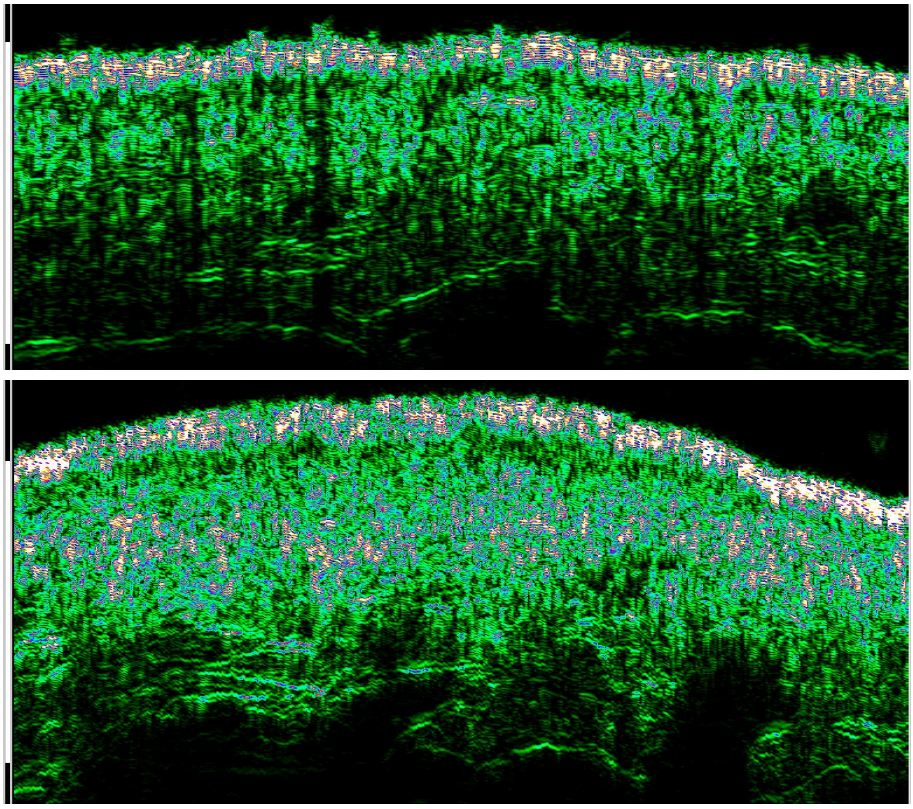
The dermal thickness results were surprising as both formulas caused a 5% statistically significant thickness reduction. Given the similarities and parallelisms obtained, these results cannot be attributed to the nanoparticles or the hyaluronan, but rather to the general ingredients shared by the two formulas. The cause of that reduction is intriguing, as it didn't affect the skin hydration, as the skin of both legs was found in good condition for all volunteers on all the visits. The most important part of these results, though, is that they clearly establish that no dermal thickening took place.

This is key given the dermal echogenic density results as the treated side obtained a 51,74% increase and the negative control obtained a 21,12% increase. The negative control increase, however, was found to be statistically not significant while remaining empirically important as it suggests that he hyaluronan per se is achieving an effect on the skin.

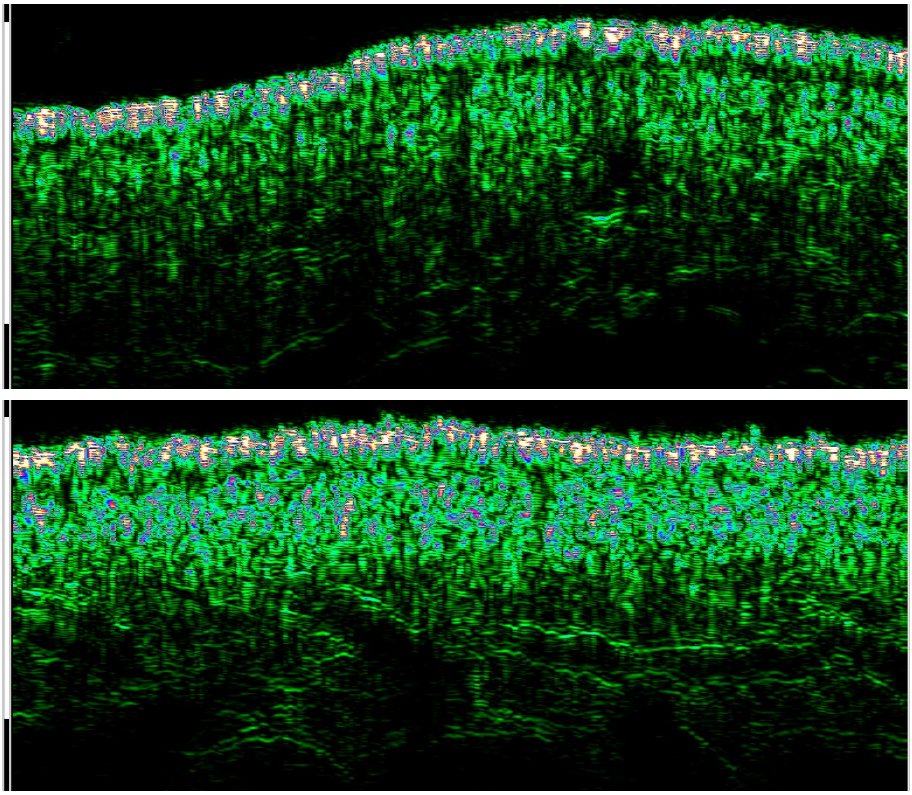
This effect, nonetheless, is much more potent when combined with the gold nanoparticles as they achieve a striking activation of dermal extracellular matrix activation while remaining in the epidermis. The mechanism for this activation remains partially unclear despite some in vitro tests suggesting the role o second messenger signaling.

This matrix synthesis strongly redensifies the dermis, providing it with improved

mechanical properties without altering its thickness. Those acquired mechanical properties allow the skin to buffer part of the pushing reaction caused by the fat and connective tissue protrusions associated wit cellulite, achieving in this way the improvements observed with the FOITS measurements.



Before and After 50 MHz High Frequency Ultrasound Images of subject nº17 on the side treated with gold nanoparticles.



Before and After 50 MHz High Frequency Ultrasound Images of subject nº17 on the negative control side.

7. CONCLUSION

To conclude, in the experimental conditions of the study, a significant anti-cellulite and antioxidant effects of the Formula A can be demonstrated through instrumental methods, after 28 days of application.

Gabriel Buendía Bordera
Investigator
Barcelona, 27th May 2016
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