EFFICACY REPORT

OXYGENATE THE SKIN TO REDUCE ACNE SCARS ON A CAUCASIAN PHOTOTYPE

Product: Fiflow® AA

Beginning of the study: 01/23/2020

End of the study: 03/12/2020

PURPOSE OF THE STUDY

Acne is a common skin disease that generally appears with puberty. The limited exposure of damaged tissues to oxygen (from air) leads to poor wound healing of acne scars. The severe sequel of acne scarring can lead to long-term psychological injury. There are a wide range of treatments for acne scars such as surgical techniques, chemical peels, ablative lasers, fractional lasers and collagen induction therapy. However, these solutions are often scarying and very intrusive for the patients. It is widely prefered to use topical applications.

Oxygen therapeutics are derived from hemoglobin or perfluorochemical-based compounds. Fully fluorinated perfluorocarbons (hydrogen atoms have been replaced by fluorine) are stable and inert due to the strong C-F bond. They have the key property for carrying gases, at 9 times superior to the function of respiratory gases in the human body. The solubility of the gases, notably oxygen and carbon dioxide, in Perfluorocarbons is a fully reversible phenomenon based on physical gas diffusion (Fick's law).

Dual Laser Confocal Microscopy is the new non-invasive examination technique for the skin *in vivo* in real time, which is useful to evaluate the effectiveness of this advanced therapeutic approach.

This study focuses on the treatment of acne scars using Perfluorocarbons and the follow-up observations with Dual Laser Confocal Microscopy.

Our subject was a 21-years+ (non-smoker) woman with a fair phototype. Our subject showed a lot of acne scars on her face since she takes contraception.

The testing had been performed according to a half-face method. The products (one serum and one cream) containing respectively 15 and 10% of Fiflow® AA *(Perfluorohexane (and) Perfluoroperhydrophenanthrene (and) Perfluorodecalin (and) Perfluorodimethylcyclohexane)* have been applied twice a day (0.2g/0.15ml; morning and evening) on the left side of the face. Macroscopic pictures have been taken with two different macro-cameras (one is linked to the confocal microscope) before starting the study, then again after 1 week, 2 weeks, 4 weeks and 6 weeks.

Hydration, skin elasticity, melanin index and ultrasounds (collagen density and skin thickness) measurements were taken using a multiparameter skin analyser. Three repeatability measurements have been done for each parameter.

Reflectance Confocal Microscopy images and microcirculation videos have been recorded with the Reflectance Confocal Microscope, focusing on the *Stratum Corneum*, Dermal-Epidermal Junction and upper dermis.

ENVIRONMENTAL CONDITIONS

Time of measurement	Date of testing	Weather	Atmospherical pressure	Air Quality Index	Outside temperature	Outside relative humidity	Room temperature	Room relative humidity
W0 (Before starting products application)	01/30/2020	Rainy	1010 hPa	40	7°C	95%	21.6°C	33%
W1	02/06/2020	Cold	1031 hPa	60	3°C	83%	21.6°C	29%
W2	02/13/2020	Rainy	1006 hPa	37	6°C	87%	21.5°C	34%
W4	02/27/2020	Rainy	1000 hPa	39	5°C	93%	21.2°C	28%
W6	03/12/2020	Cloudy	1017 hPa	22	8°C	85%	21.8°C	41%

TESTING EQUIPMENT

Skin property to assess	Test location	Tool used	Device used
Macroscopic aspect	Defined scar on each side of the face	Macro-camera	Canon EOS
			DermaLab® SkinLab Combo
	Entire face then each side of the face	Dermatoscopy	Dual Laser Confocal Microscope (DLCM)
Melanin content	Defined scar on each side of the face	Skin colour probe	DermaLab® SkinLab Combo
Erythema index			
Skin luminosity			
Skin hydration	Defined scar on each side of the face Cheek of each side of the face	Hydration pin probe	
Sebum content	Nostril of each side of the face	Sebum collecting device	
Collagen density	Defined scar on each side of the face Cheek of each side of the face	Ultrasounds probe	
		Microscope	Dual Laser Confocal Microscope (DLCM)
Skin thickness		Ultrasounds probe	DermaLab® SkinLab Combo
Skin elasticity		Skin elasticity probe (suction cup)	
***Stratum Corneum* structure**		Microscope	Dual Laser Confocal Microscope (DLCM)
***Stratum Granulosum* structure**			
Dermal Epidermal Junction structure			
Dermis structure			
Microcirculation		Microscope (20-s video)	

SUBJECT

First Name	**Mélanie**
Gender	F
Birth date	2/23/1999
Phototype	II
Origin	France
Smoker	No
Skin Type	Mixte with acne
Work	Inside
Cosmetic use	Everyday
Sun exposure	Summer
Sunscreens use	Yes
Acne	For more than 2 years now / Began with contraception

PRODUCTS AND APPLICATION

Product number	1	2
Type of product	Serum	Cream
Batch number	PFI3696CS	PFI3695CS
Fiflow® AA content	15%	10%
Location of application	Left side of the face and neck	Left side of the face and neck
Amount of product applied	0.2g / 0.15 ml	0.2g / 0.15 ml
Time of application	Every day / Twice a day (morning and evening)	Every day / Twice a day (morning and evening)

CONCLUSIONS

The long-term study shows a reduced inflammation and redness of acne scars as soon as 2 weeks after having started the study. On the right side of the face (control area), acne scars are still present forming bumps.

After 6 weeks of application, skin hydration, skin luminosity, skin thickness and collagen network density have significantly increased due to the oxygenating power of perfluorocarbons on the skin cells. It consequently improves cell breathing and moreover, it stimulates fibroblasts and collagen network density. These results improved week after week for the both sides of the face. It has also been noticed that the cycle period has a strong impact on the woman skin : more pimples and greasy skin at menstruations time. The Air Quality Index has a strong impact on skin properties mainly on skin surface properties such as hydration, skin colour and sebum. This is due to the pollutant gases containing in air that can

be removed with the use of perfluorocarbons products.

The following table summarizes the results of the entire study after 6 weeks of products application :

Skin property	Control Skin Area (Right side of the face)	Testing Area (Left side of the face)
Macroscopic aspect	No significant change. Pimples and comedo growing up Inflamed and swollen skin.	Less inflamed and swollen skin. Less comedo.
Melanin content	Reduced by 10%	Reduced by 7%
Skin luminosity	Increased by 12%	Increased by 14%
Erythema index	Decreased by 14%	Decreased by 24%
Skin hydration	Decreased by 17%	Increased by 8%
Sebum content	Decreased by 15%	Decreased by 53%
Collagen density	Increased by 10%	Increased by 24%
Skin thickness	Increased by 60%	Increased by 99%
Skin elasticity	Decreased by 5%	Increased by 7%
***Stratum Granulosum* structure**	Inflammatory cells have been reduced by 70%	Inflammatory cells have been reduced by 91%
Dermal Epidermal Junction structure	No changes	Visibly more regular and restructured
Dermis structure	Elastic fibers density has been reduced by 10%	Elastic fibers density has been increased by 144%
Microcirculation	Increased by 2%	Increased by 45%

I. PROTOCOLS

Dual Laser Confocal Microscope (DLCM)

The Dual Laser Confocal Microscope (DLCM) is equipped with a laser sweep with an imaging software. This non- invasive device makes it possible to have in vivo measurements by optical biopsies and to observe internal skin layers up to the deep dermis. It has a laser probe for observing deep skin layers and blood microcirculation, a macro digital camera to capture macroscopic skin images and has a digital high-resolution screen.
Reflectance Confocal Microscopy is the only technique that enables the en-face (horizontal plane) visualization of the skin with a resolution at the cellular level and therefore may provide an alternative to histopathology.
The contrast in RCM images relies on the differences in the reflectivity of the tissue, which is depending on chemical and molecular structures. Due to these variations of the refractive index, only a certain portion of the light is reflected. Structures with a higher refractive index appear bright in RCM. Melanin and melanosomes are the strongest natural sources of contrast resulting white in RCM.
The deeper the skin section is the more laser power is needed to penetrate the skin. The VivaScope® 1500 uses a laser diode with a near-infrared wavelength of 830 nm. Laser power of 830 nm causes no damage to tissue, but limits the imaging depth to 200-300 µm, which corresponds to the papillary dermis, depending on the skin site. Higher laser power would provide an increased signal and contrast but is hazardous for the skin or the eyes.
Confocal observations are performed at each time of measurement according to this method :

- After the subject is ensconsed in the chair and is relaxed, the experimenter placed on the test area, the ring with the adhesive patch oiled beforehand in the center of the side that will be in contact with the skin.
- The experimenter placed the macro camera onto the ring to take a picture of macroscopic skin aspect of the subject
- After removing the macro camera from the ring, the experimenter applied a gel on the patch center where the laser will be in contact with.
- The assessor then positioned the removable device of the microscope on the ring.
- When corneocytes are detected, the depth is set to 0 µm.
- The assessor looked for an area where the blood flow is easily viewable (it is possible to adjust magnification). This zone is sometimes identifiable in a tiny way. It must ensured that the flow is clear and definite.
- The Stack (optical biopsy in vivo) is then started. According to the programming software, the device will focus on the selected location and take multiple pictures (around thirty) while gradually increasing its observation depth. Images can be observed like a video. The experimenter can then zoom again on one or the other of each image to take photos or videos the most highlighting the blood circulation.
- The assessor removed the device from the ring and patch of the skin.
- All of the previously steps are repeated to study. The microcirculation on the patient after applying the product.

Macroscopic picture of a defined scar on each side of the face is taken. Confocal observations of skin structures (Stratum Corneum, Stratum Granulosum, Dermal Epidermal Junction and collagen network) have been performed at each time of measurement. Microcirculation has been quantified by means of a 20-s video to count the number of blood cells in a capillary.

DermaLab® SkinLab Combo

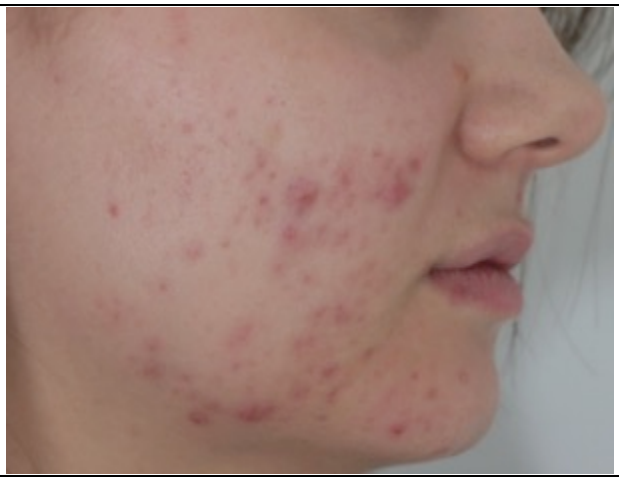
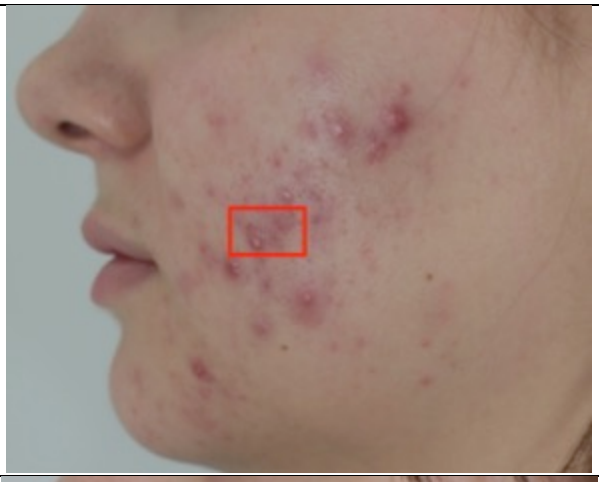
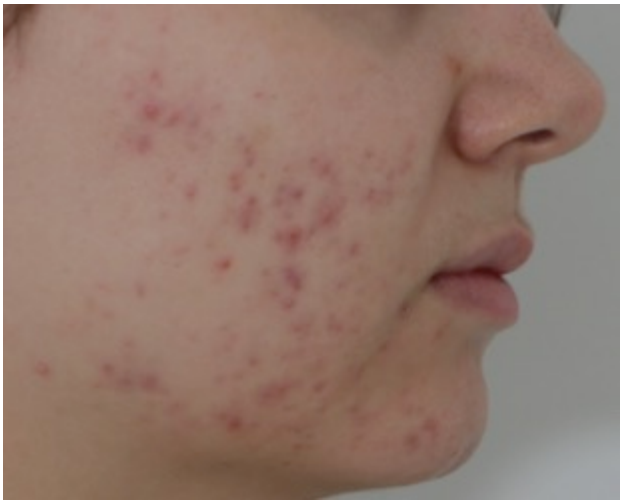
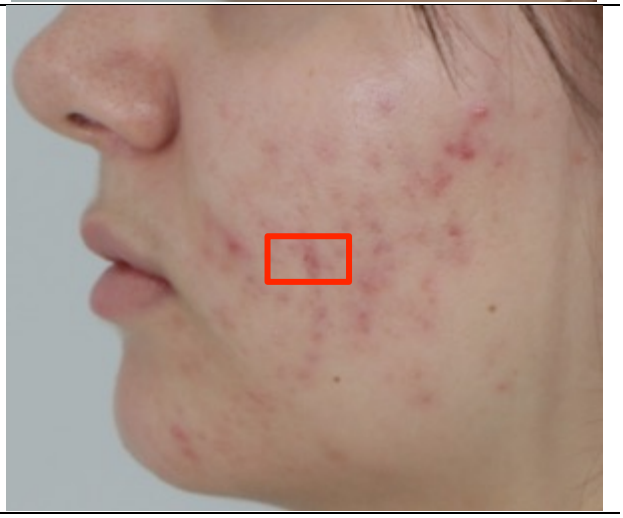
The DermaLab® SkinLab Combo (multiparameter skin analyzer) is a non-invasive method to perform *in vivo* measurements of skin properties by means of different probes :

- Macro camera magnifies (x50) and vizualizes the surface of the skin with a polarized light (in this case)
- Skin colour tool gives the spectrocolourimetric parameters L*a*b* and erythema-melanin indexes for skin application. Melanin index is defined as the pigmentation index of the skin
- Ultrasounds probe is based on measuring the acoustic response from the skin acoustic pulse is sent into the skin. It determines skin thickness and collagen density
- Hydration pin probe measures the conductance to assess the water binding capacity of the *Stratum Corneum*
- Skin elasticity is based on the suction cup method upon measuring the necessary force to lift the skin a certain distance using negative pressure as well as the time it takes for the skin to retract, when the negative pressure is released
- Sebum surface quantity is measured by means of a sebum collecting device. The transparency of the film increases with sebum

Hydration, skin elasticity, sebum, melanin index, skin luminosity and ultrasounds (collagen density and skin thickness) measurements were taken at each time of the study.
Three repeatability measurements have been done for each parameter.

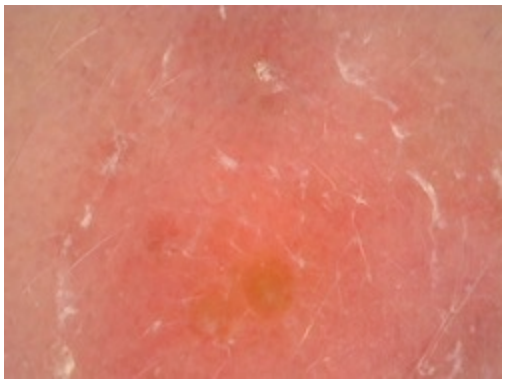
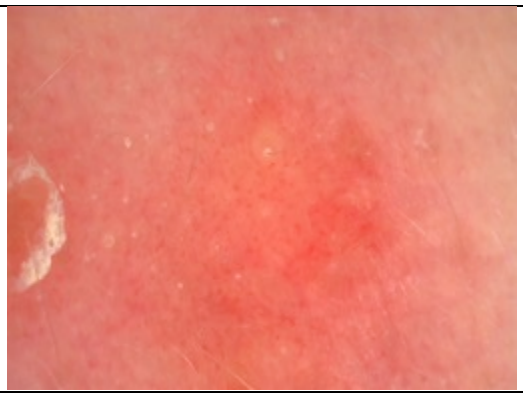
II. RESULTS - MACROSCOPIC ASPECT AND SKIN COLOUR

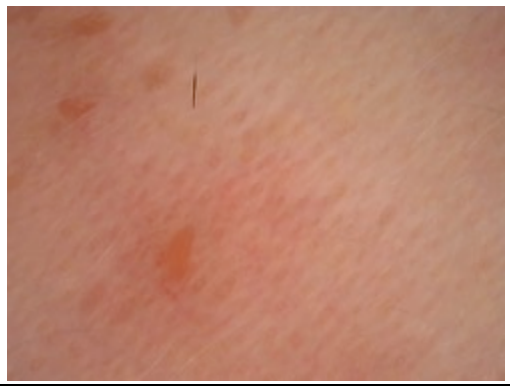
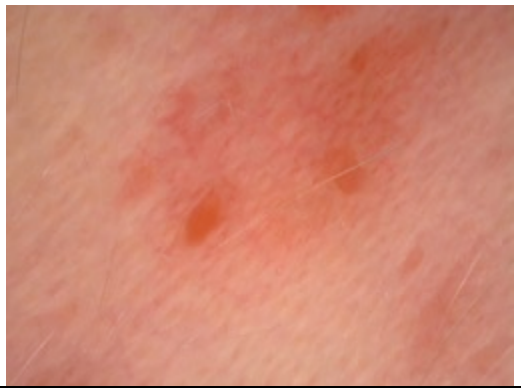
Camera – Canon EOS

Time of measurement	Control Area (Right side of the face)	Testing Area (Left side of the face)
Before having started the study (Wo)		
After 6 weeks (W6)		

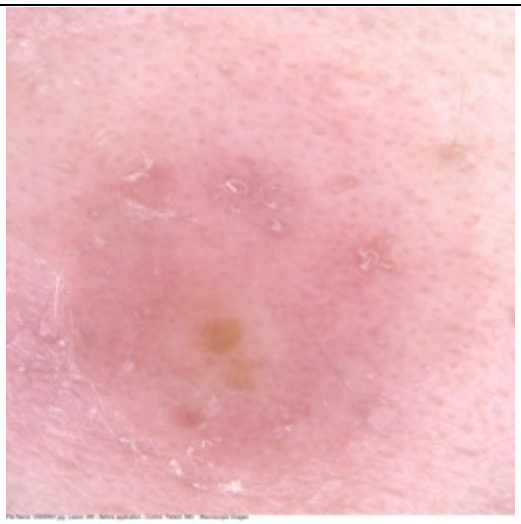
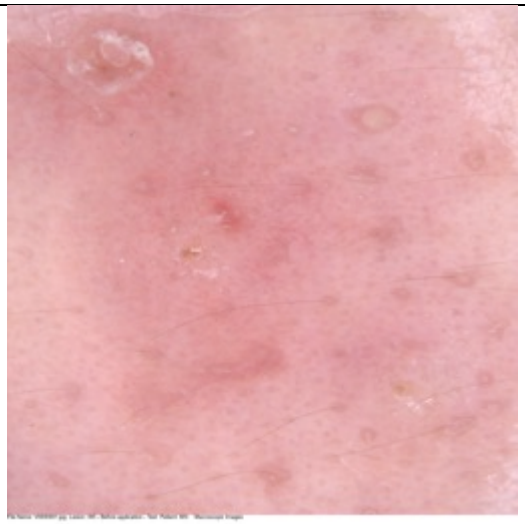
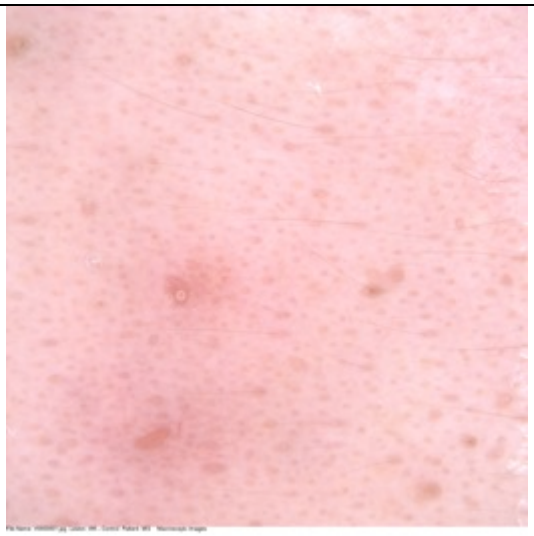
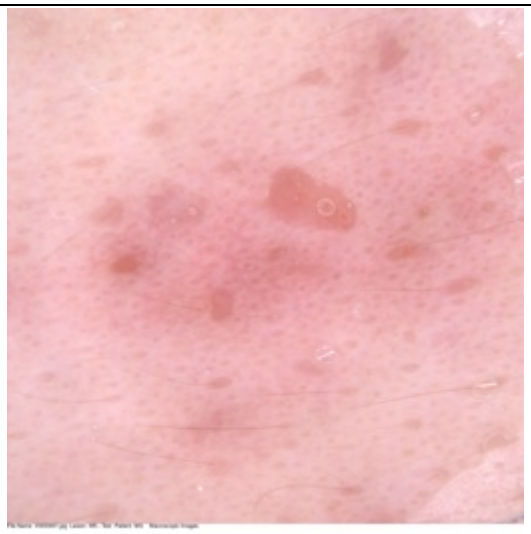
On the control skin area, skin is still visibly red and inflamed. New acne spots are visible.
On the testing area, the skin is visibly less inflamed and less red. Few acne spots and acne scars.

Macro-camera of the DermaLab® SkinLab Combo

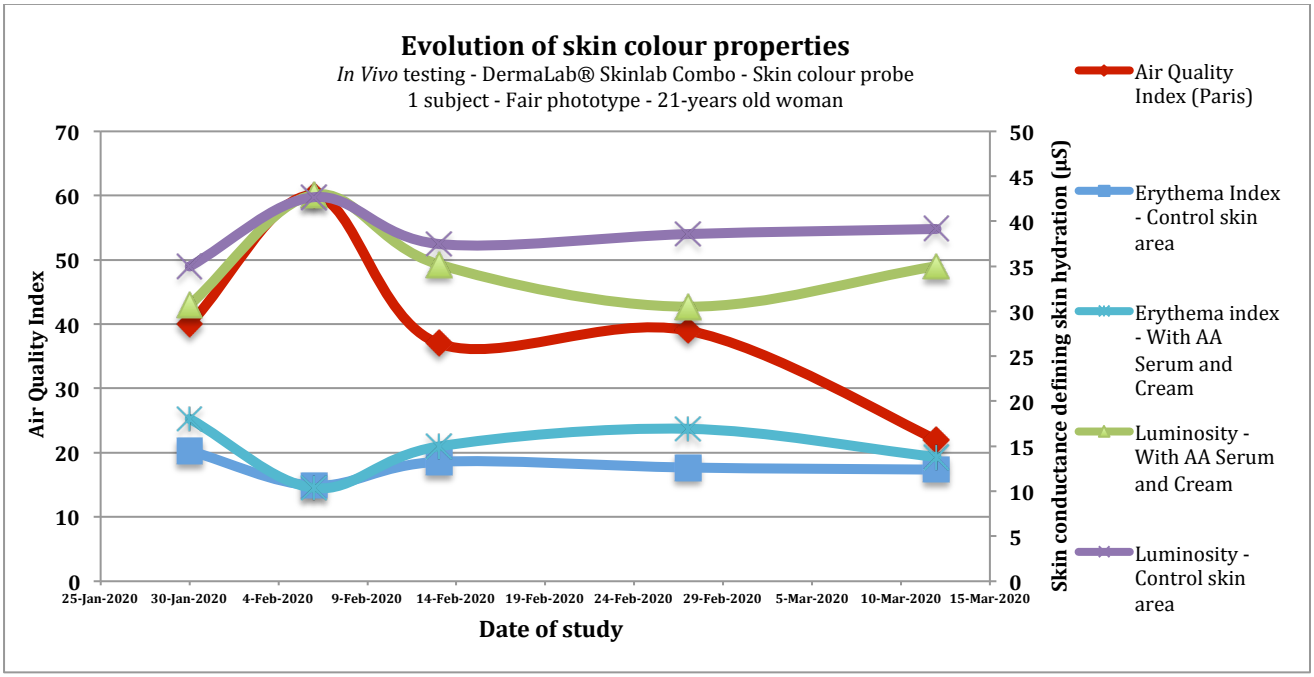
Time of measurement	Control Area (Right side of the face)	Testing Area (Left side of the face)
Before having started the study (Wo)		

After 6 weeks (W6)		

Macro-camera of the Dual Laser Confocal Microscope

Time of measurement	**Control Area (Right side of the face)**	**Testing Area (Left side of the face)**
Before having started the study (Wo)		
After 6 weeks (W6)		

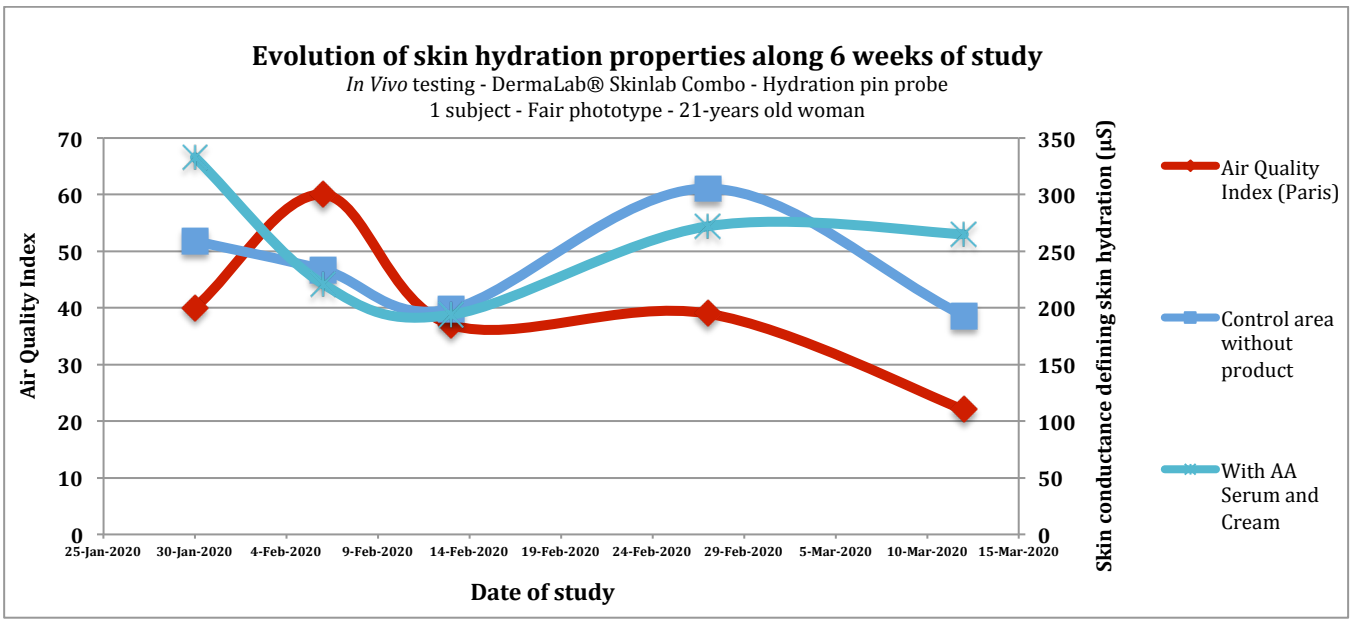
Skin Colour



The previous graph shows the impact of AA serum and cream application on skin colour. Due to the reduction of skin inflammation, we can easily observe that skin luminosity is higher on the tested area of the face. Erythema index has also decreased due to the oxygenation of the skin cells. The Air Quality Index (AQI) impacts the results.

Skin luminosity has increased by 14% on the testing area vs. 12% on the control side. Erythema index decreased on both areas : by 14% on the control vs. by 24% on the testing area.

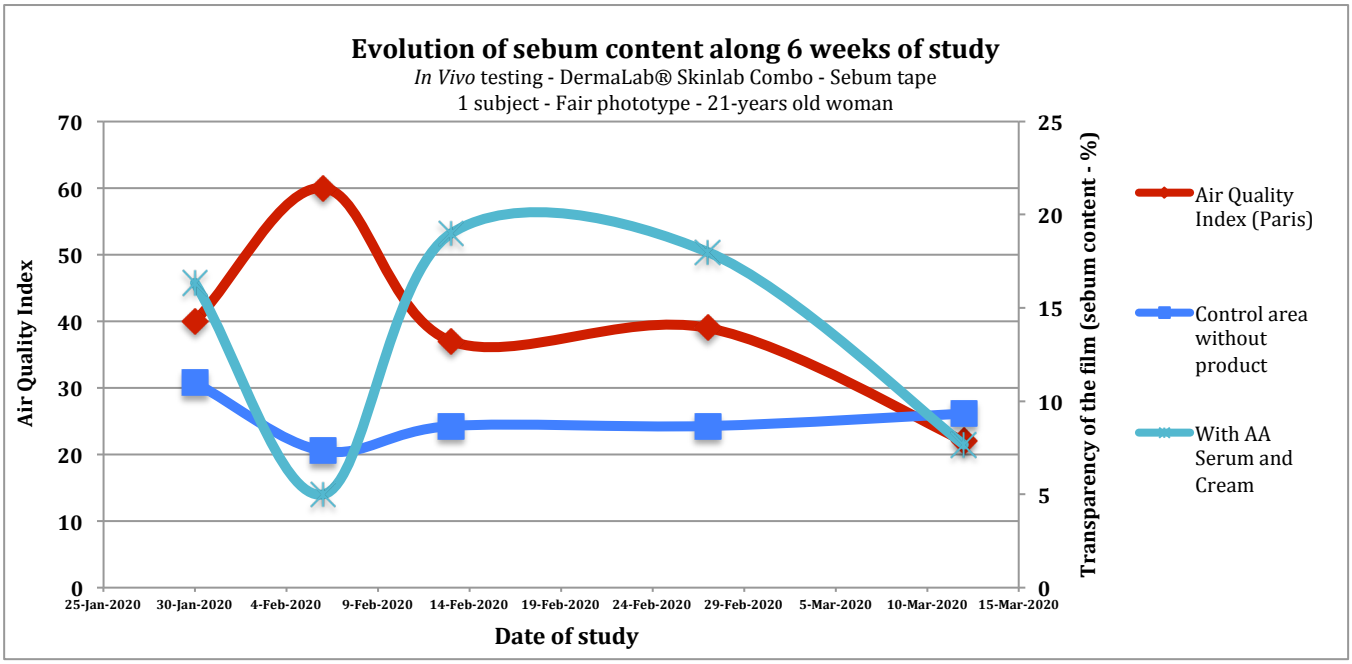
III. RESULTS - SKIN HYDRATION



The previous graph clearly shows that skin hydration is impacted by Air Quality Index (AQI). An increase of AQI seems to decrease skin hydration. This is due to the increase of pollutants affected the skin.

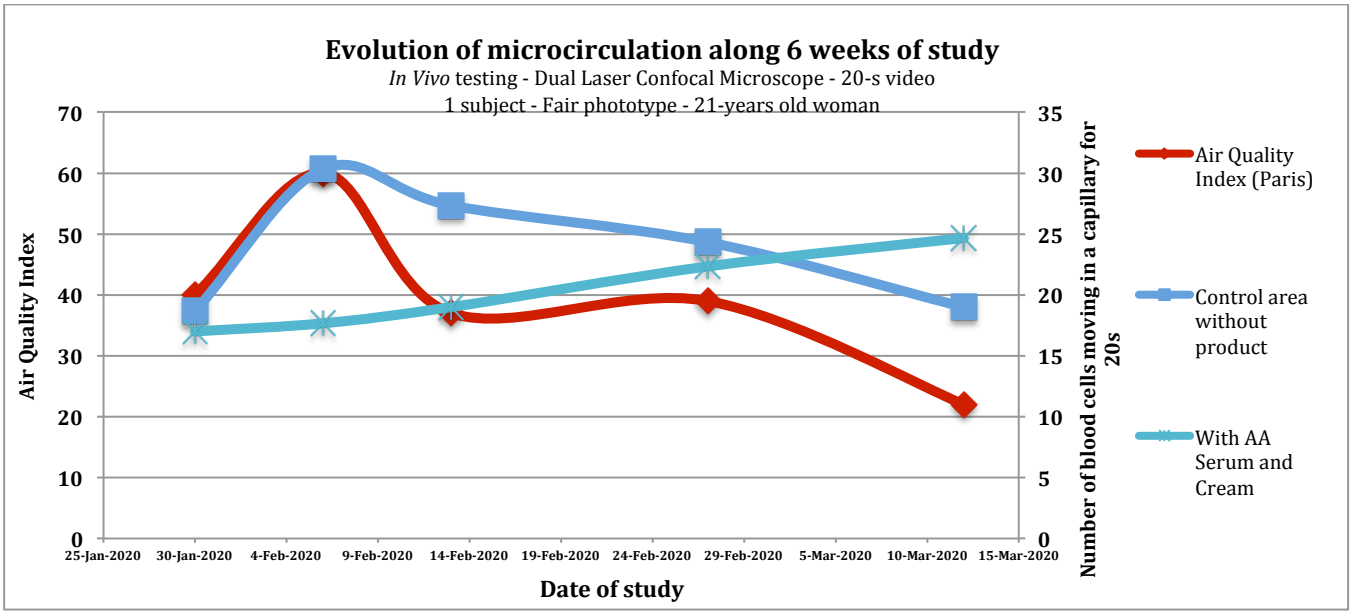
After 6 weeks of study, skin hydration has increased by 8% on the testing area compared to a reduction by 17% on the control area.

IV. RESULTS – SEBUM



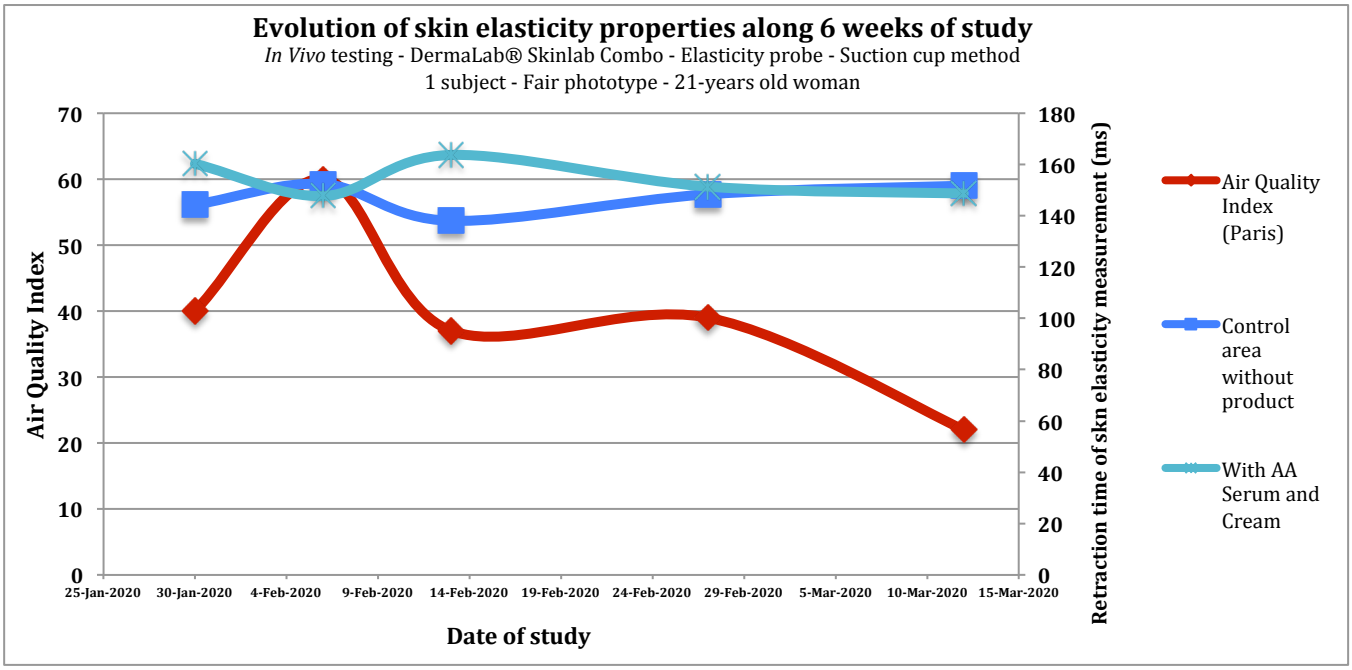
The previous graph shows the impact of AA serum and cream application on sebum reduction. This property is also drastically impacted by AQI. Higher the AQI, higher the sebum content. This is due to the increase of pollutants affected the skin. Sebum content varies a lot on the testing area.

V. RESULTS – MICROCIRCULATION



The previous graph highlights the huge impact of oxygenating products on the increase of microcirculation. This is interesting to notice that the control area without product is depending on the AQI whereas the assessed area seems to not be affected by the AQI. The microcirculation increases weeks after weeks with the use of AA serum and cream up to an increase by 45% after 6 weeks.

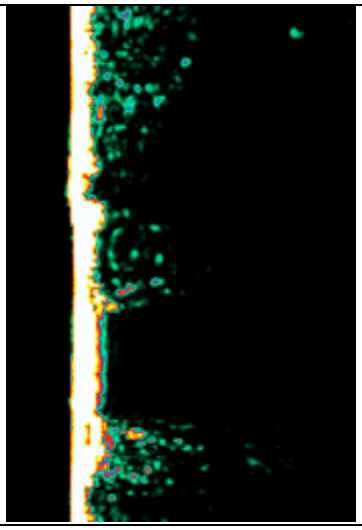
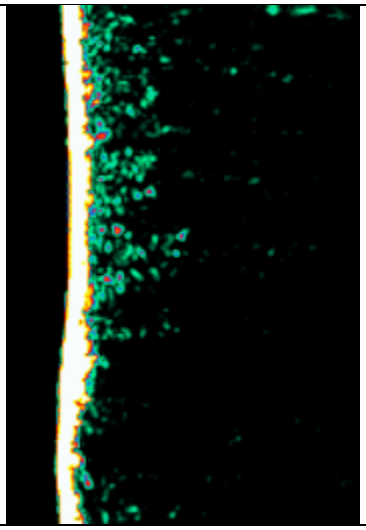
VI. RESULTS – SKIN THICKNESS – ELASTICITY - SKIN LAYERS RESTRUCTURING -

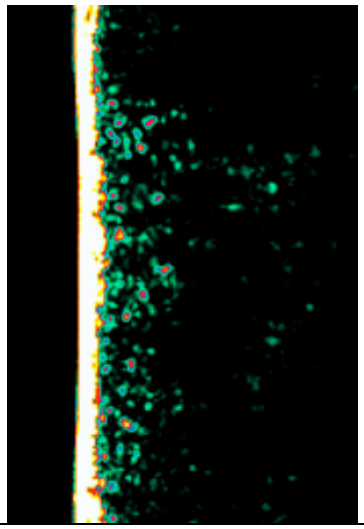
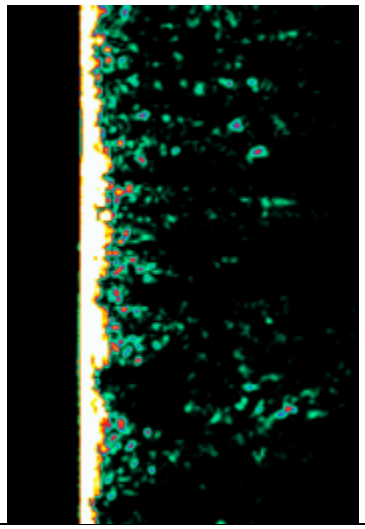


The previous graph highlights the huge impact of oxygenating products on skin elasticity. We can notice that retraction time is reduced when using AA serum and cream. However, this properties is depending on the dermis fibers. It is a long-term reaction to observe modifications.

The skin elasticity results are taken by precising the skin thickness value. The skin thickness is determined by means of the ultrasounds measurement.

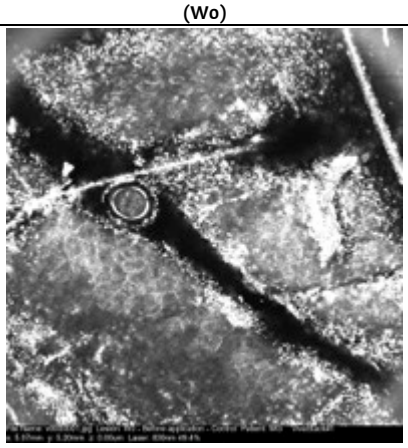
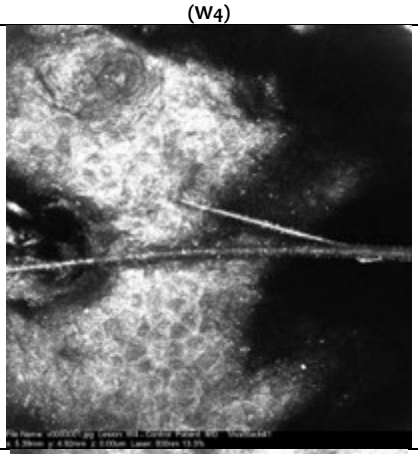
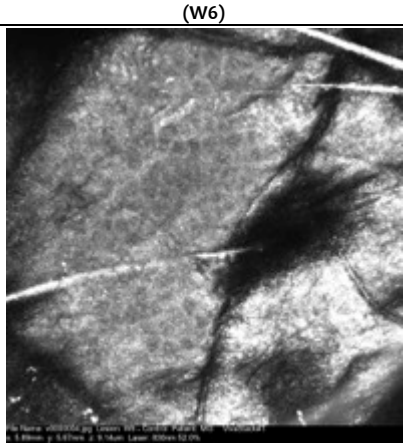
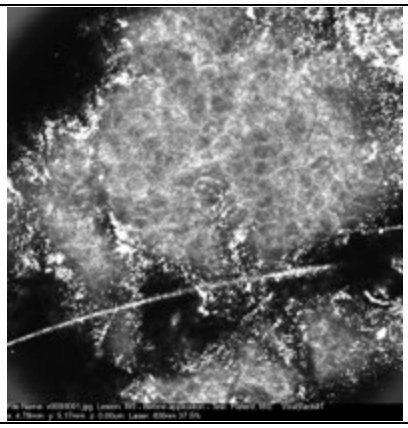
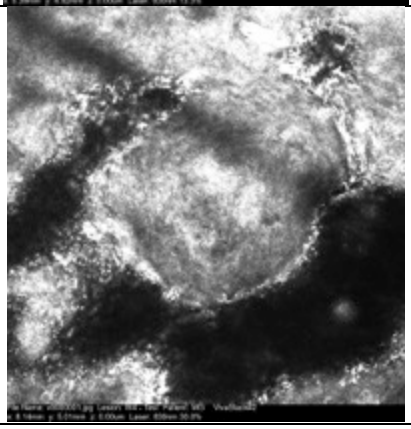
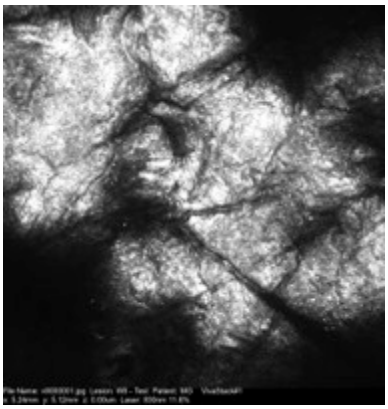
The following table shows the ultrasounds pictures of the both sides of the face on the acne scar/comedo previously defined. The lightest/yellow area represents the epidermis, the green to red spots stand for the dermis and the black area is the subcutis.

Time of measurement	Control Area (Right side of the face)	Testing Area (Left side of the face)
Before having started the study (Wo)		

After 6 weeks (W6)		

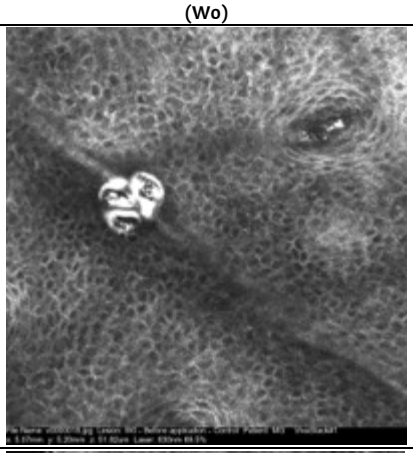
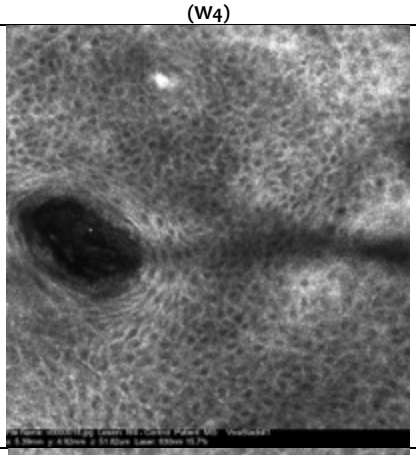
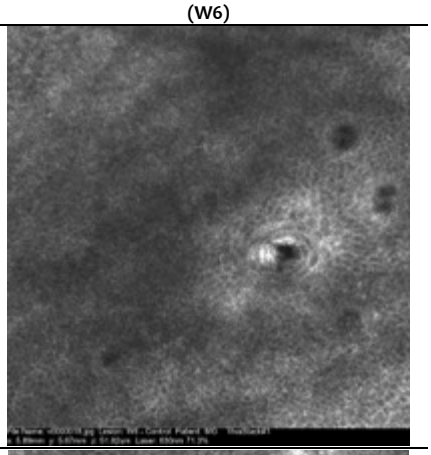
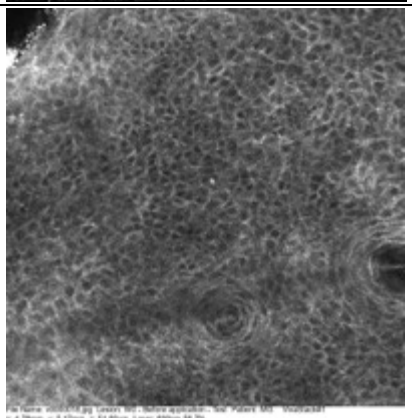
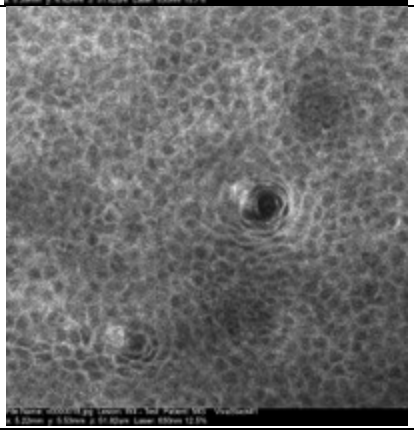
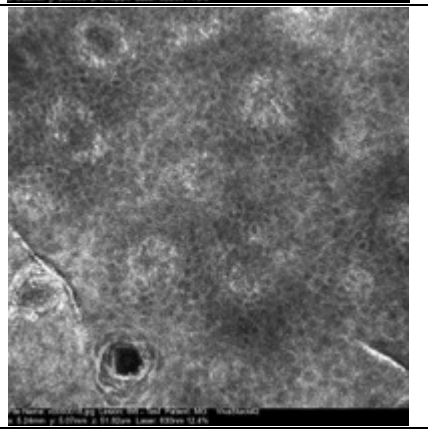
The results are comparable on the control skin area. However, the dermis fraction is denser and more intense on the testing area after 6 weeks of study. It means that dermis fibers such as collagen and elastin have been created ; skin thickness has been increased by 99%.

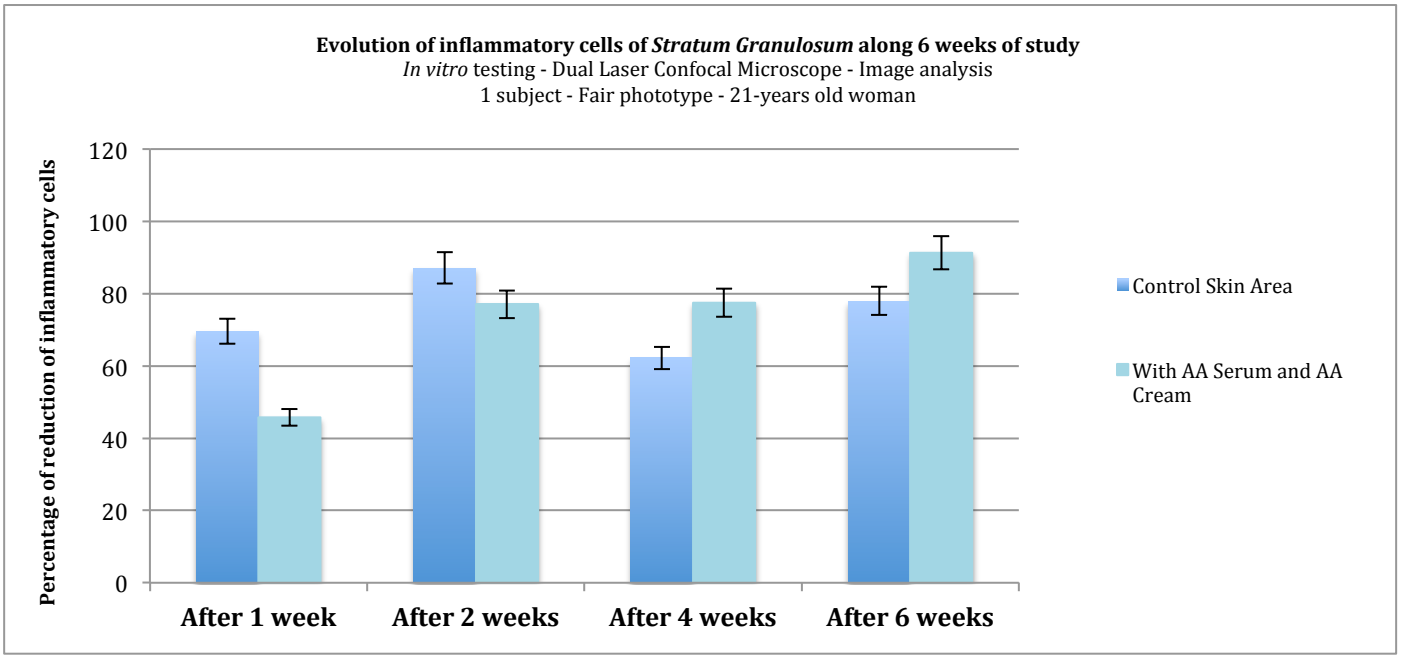
Stratum Corneum structure (0 micron depth)

Time of measurement	**Before having started the study (W0)**	**After 4 weeks (W4)**	**After 6 weeks (W6)**
Control Area (Right side of the face)			
Testing Area (Left side of the face)			

The face has a lack of hydration at the beginning of the study with desquamating corneocytes. Weeks after weeks, the skin layer is moisturized on the testing area: no more desquamating corneocytes, regular layer and thinner skin furrows.
Results are comparable on the control skin area.

Stratum Granulosum structure (50 microns depth)

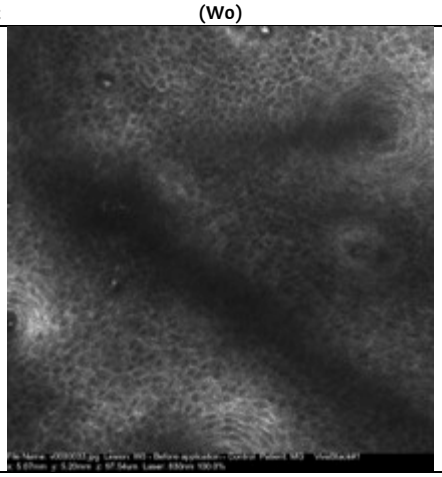
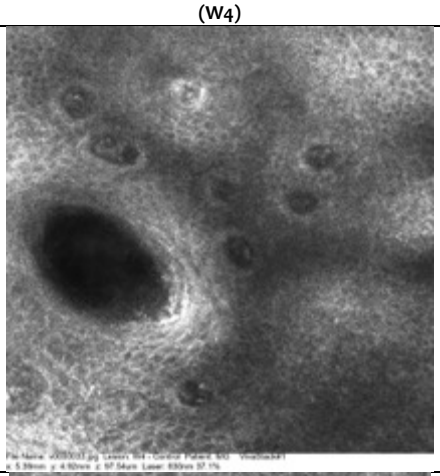
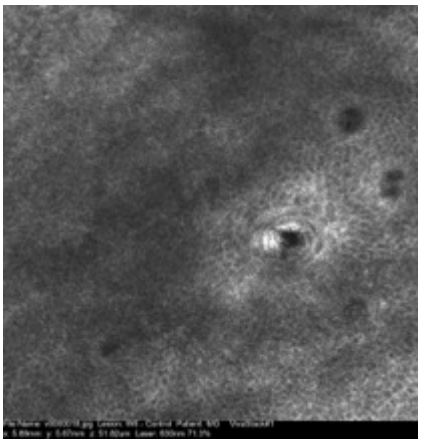
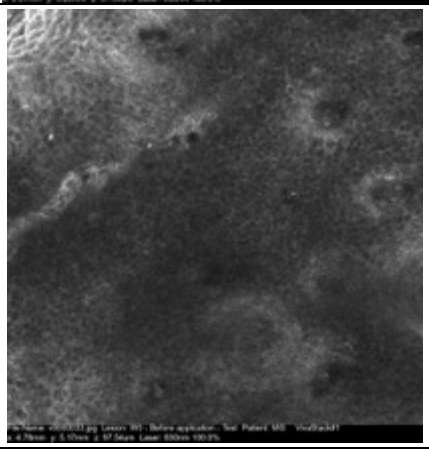
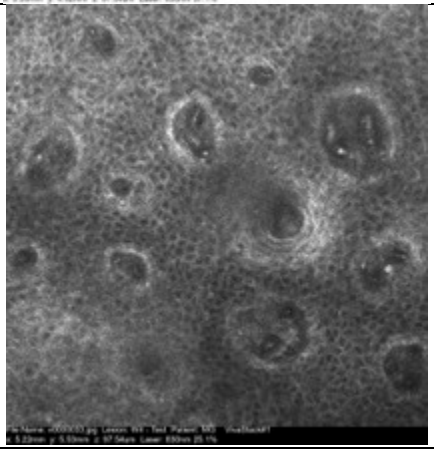
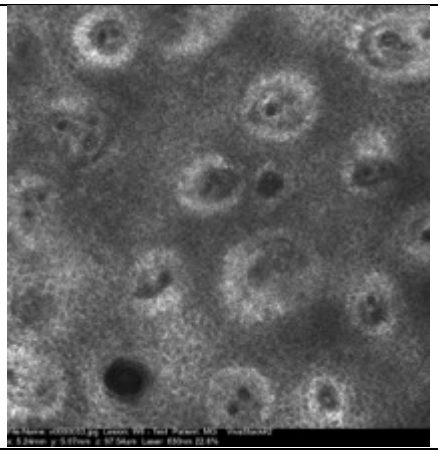
Time of measurement	Before having started the study (W0)	After 4 weeks (W4)	After 6 weeks (W6)
Control Area (Right side of the face)			
Testing Area (Left side of the face)			



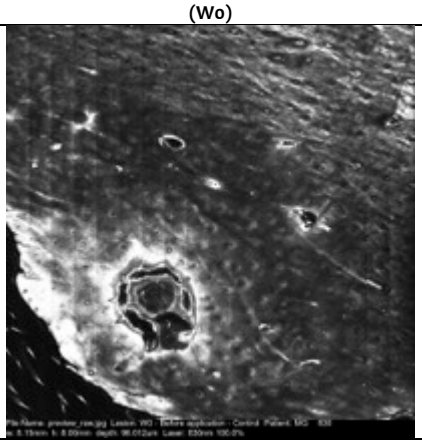
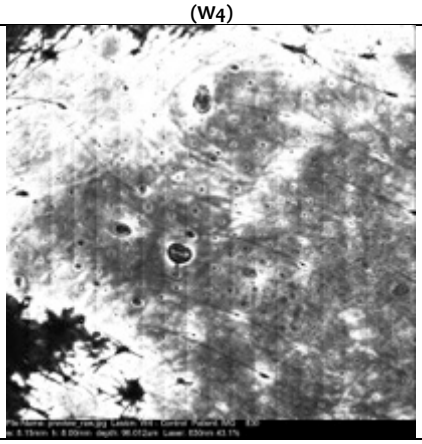
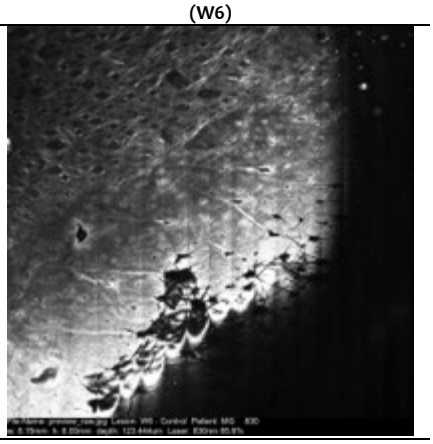
Before starting the study, *Stratum Granulosum* was not well structured with amorphous keratinocytes although they were already organised in honeycomb pattern. With the use of serum and cream containing perfluorocarbons, the skin layer became well-defined with a visible honeycomb pattern of regular keratinocytes. Moreover, at the begenning of the study, we can notice bright inflammatory cells between keratinocytes that are no more visible after 6 weeks of products application.

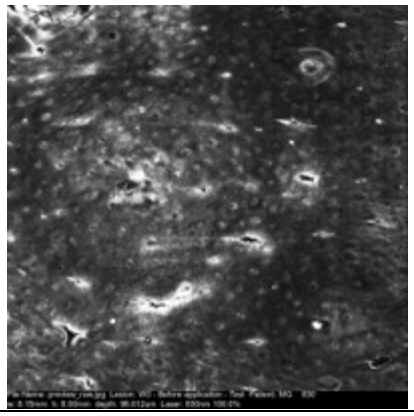
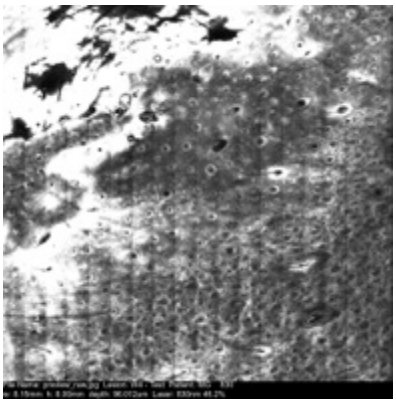
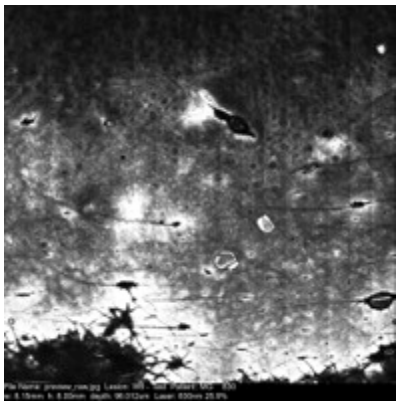
This reduction of inflammatory cells is visible on both sides of the face. This is probably due to the cycle period of the subject. On the control area, inflammatory cells have been reduced by 70% vs. 91% for the testing area.

Dermal Epidermal Junction structure (96 microns depth)

Time of measurement	Before having started the study (W0)	After 4 weeks (W4)	After 6 weeks (W6)
Control Area (Right side of the face)			
Testing Area (Left side of the face)			

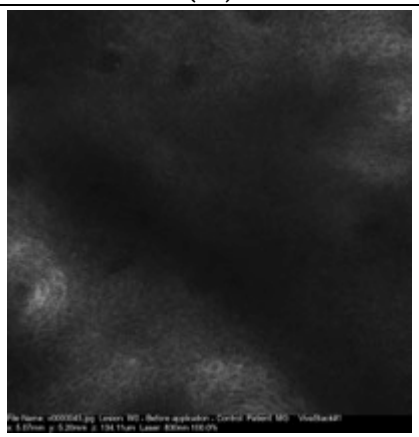
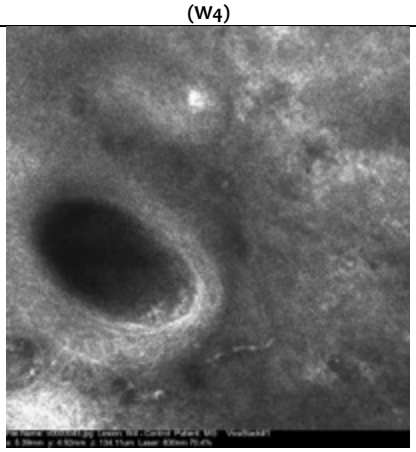
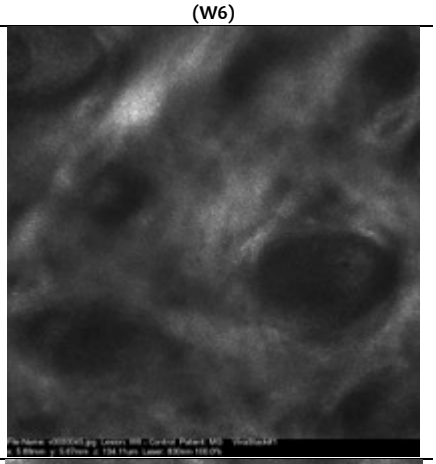
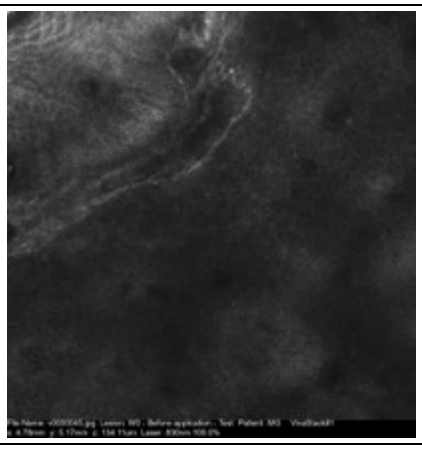
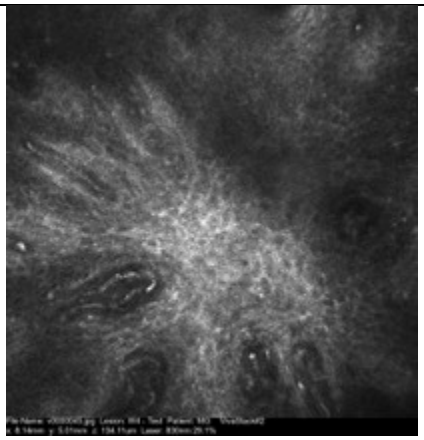
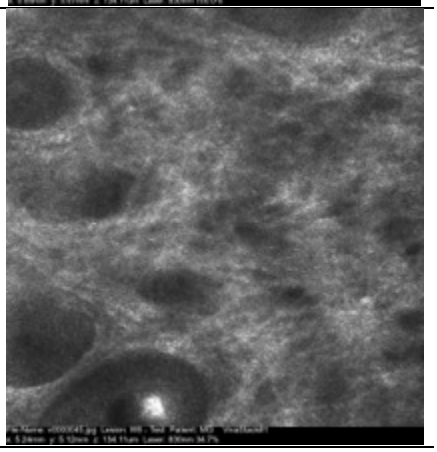
On the control area, few dermal papillae are visible. They are small and not well-defined. On the testing area, the skin layer is amorphous before applying the products. Few dermal papillae are visible. After 4 weeks, the layer was restructured and there are much more dermal papillae visible and clearly defined. This was enhanced after 6 weeks of study with round and clear papillae with more blood cells inside.

Time of measurement	Before having started the study (W0)	After 4 weeks (W4)	After 6 weeks (W6)
Control Area (Right side of the face)			

Testing Area (Left side of the face)			

The previous mozaics show the structure of Dermal-Epidermal junction on a wide area. Black area are visible for the control skin area. They are due to the irregular skin surface because of the inflammation of acne spots. On the testing area, the layer was not regular before starting the study. We can already see fibers structures on the right part of the image because the skin is inflamed. Weeks after weeks, the layer is more defined and regular because the inflammation is reduced.

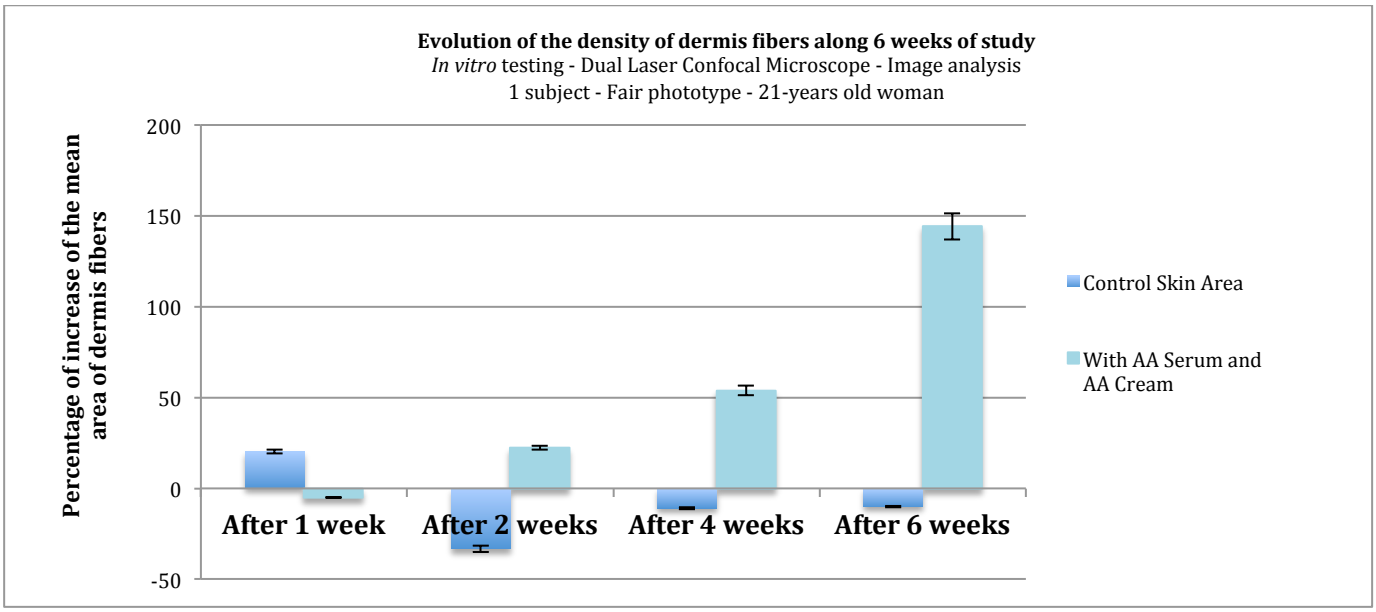
Density of dermis fibers (134 microns depth)

Time of measurement	**Before having started the study (W0)**	**After 4 weeks (W4)**	**After 6 weeks (W6)**
Control Area (Right side of the face)			
Testing Area (Left side of the face)			

Before starting the study, both sides do not show any clearly defined elastic fibers. The skin is bumped and inflamed so we mainly see the end of the dermal-epidermal junction. No bright structures.

The results are not significant on the control skin area even if some blurry elastic fibers are visible after 6 weeks of study.

On the testing area, we clearly see a restructuring step weeks after weeks until obtain a dense and strong network of elastic fibers, long and bright.



The previous graph shows the quantification of the density of dermis fibers on 6 weeks. We can easily notice that there is no difference on the control skin area, the results are not significant.

On the testing area, the results are comparable after 1 week. As soon as 2 weeks after products application, elastic fibers are created and their density is increased weeks after weeks up to 144% after 6 weeks.