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"Versatile biomimetic approach to conceive new sustainable formula to fight skin imperfections"

Thomas POCARD [1*], Anne MAGNAN [1*] , Annie BOSSIERE [2] and Catherine MARION [1]
[1] GSF, DI SPF, l'Oréal, Chevilly-Larue
[2] Physico-chimie, DISPF, l'Oréal, Chevilly-Larue
* The authors equally contributed to the work

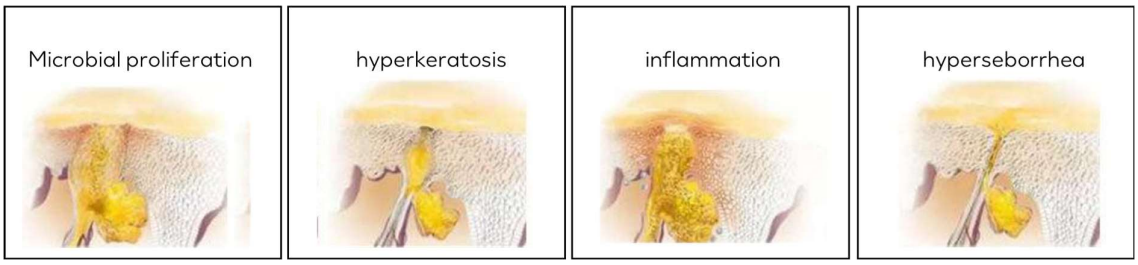
1. Introduction

Acne vulgaris, or common acne, is an extremely prevalent disorder that is frequently encountered in the practice of most medical disciplines. Acne is the most common dermatologic disorder and affects 80% of people between the ages of 11 and 30 years [1]. Although acne is usually thought of as a condition of adolescence, 8% of the 25- to 34-year and 3% of the 35- to 44-year age groups are affected [2]. Acne remains a major component of a dermatologist's practice, involving approximately 20% of all visits [1] but is often encountered in the practice of non-dermatologists as well. The diagnosis is often readily apparent, and of the 20 most common dermatoses, acne is the one that primary care physicians have the least difficulty correctly diagnosing [3].

The pathogenesis of acne is thought to be due to a complex interaction between abnormal follicular differentiation, androgens, sebum, and the anaerobic microorganism Cutinibacterium acnes (C. acnes) in the sebaceous follicles of the face, chest, and back (Figure 1).

(Figure 1. C. acnes)

Abnormalities in the follicular epithelial differentiation also are thought to play a pivotal role. Abnormal epithelial desquamation in the upper canal of the follicle leads to a thickened stratum corneum and follicular plugging. This occluded follicle is the precursor of inflammation (Figure 2).

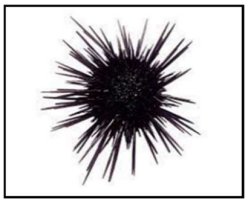


(Figure 2. Acne desorder)

In cosmetics, topical therapy is the only appropriate therapy for acne. The most frequently used topical agents include keratolytics, alpha-hydroxy acids, benzoyl peroxide, retinoid analogues, azelaic acid, and topical antibiotics. Those treatments are neither green nor without danger for the skin homeostasis.

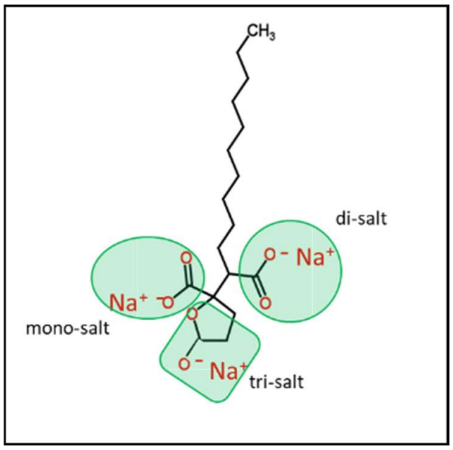
In the GSF group, we work with green ingredients, from natural origins, and we are also interested in biomimicry. As acne is partially caused by a microorganism, we looked if we could find ingredients used by microorganisms to fight against other microorganisms.

Interestingly, microorganisms of marine origin have proven to be a rich source of novel and/or biologically active natural products with promising pharmacological properties [4,5,6,7]. In related studies, the Tsukamoto laboratory reported a new antimicrobial anthraquinone (monodictyquinone A) from a sea urchin-derived fungus Monodictys sp. [8]. Another microorganism, the fungus Aspergillus sp. HDf2 was isolated from the sea urchin Anthocidaris crassispina (Figure 3), collected from the seashore of Qionghai, Hainan, China. The genus Aspergillus (Trichocomaceae) is one of the most prolific fungi that produce a variety of secondary metabolites with novel structures and interesting bioactivities [9]. Subsequent chemical study on the fermentation broth of the fungus Aspergillus sp. HDf2 led to the isolation of three new γ-butenolide derivatives, the structures of which were similar to that of spiculisporic acid [10,11], and these compounds were thus named as spiculisporic acids B–D).



(Figure 3. Anthocidaris crassispina)

Spiculisporic acid; ei S-Acid (4,5-dicarboxy-4-pentadecanolide) (Figure 4); is also produced by Penicillium spiculisporum [12]. It is soluble by neutralization in a pH range from 5 to 7: better to mild basis than strong ones and be used under conditions compatible with the constraints of cosmetic product formulation. S-acid is also a very good emulsifier and enables the stabilization of highly diluted emulsions (5% internal phase). Finally, S-Acid being efficient in killing C. acnes, makes it a perfect candidate to work on a green cosmetic formula against Acne.



(Figure 4. S-acid : 4,5-dicarboxy-4-pentadecanolide)

2. Materials and Methods

In vitro evaluation protocols

In vitro microbial proliferation efficacy - CMI determination by Keybio®:

- 100 µL of the product at twice the concentration to be tested was put into contact with 100 µL of nutrient broth at twice the concentration titrating approximately between 2 and 6,105 cfu/ml.
- After incubating the microplate for a defined period, the optical density was read at 620 nm and the results were expressed as a percentage of growth calculated relative to a growth control according to the following equation:
  Percentage of growth = (measured OD of the product at concentration C – measured OD of the control absorbance of the product at concentration C) / measured OD of the growth control
- The first concentration of product tested that achieves a percentage of growth less than or equal to 20% is considered inhibitory.

In vitro keratolytic efficacy by Qima®:

- Biological model: tissues human skin explants (2cmx2cm) from abdominal plastic surgery of a 53-yo female, donor ref. E2207 with culture conditions at 37°C, 5% CO2 and bioalternatives maintenance medium
- Upon receipt of the biopsy, the adipose tissue was removed; skin explants were then cut into pieces (~4 cm²) and placed in assay medium (Day 0).
- Test compounds: Skin explants were topically treated or not (control) with the test compounds. The explants were then incubated for 3 days with a culture medium and treatment renewal every day (D1, D2 and D3 with incubation post treatment for 7 hours).
- Potential reference: Skin explants were topically treated with the potential reference (lactic acid). The explants were then incubated for 3 days with a culture medium renewal every day (D1, D2 and D3 with incubation post renewal for 7 hours).

- After incubation (D3), skin explants were washed in a phosphate buffered saline solution (PBS) and punches (8 mm diameter) were made for histological staining.
- All experimental conditions were performed in n=3.

In vitro anti-inflammatory and anti-pro pigmentation efficacy by Qima®:

- Biological model: normal human epidermal keratinocytes NHEK bioalternatives reference K341 and K882 used at 3rd passage and NHEM normal epidermal melanocytes lightly pigmented NHEM-LP or moderately pigmented NHEM-MP bioalternatives reference NHEM-LP used at the 12th passage and NHEM with culture conditions at 37°C, 5% CO2. The assay medium is supplemented M254 without PMA optimized for both and additional with Epilife for NHEK.
- NHEK + NHEM-MP (ratio 10:1) were seeded in 24-well plates and incubated in co-culture medium 1 for 24 hours and then in co-culture medium for a further 24 hours. The medium was then replaced by co-culture medium containing or not (stimulated control) the test compounds or the reference (dexamethasone) and containing the specific stimulating mix (containing notably pro-pigmenting factors). The cells were incubated for 48 hours. Then, half of the medium was discarded, the treatments and the stimulation were renewed and the cells were incubated for 72 hours. A non-stimulated control condition was carried out in parallel.
- All experimental conditions were performed in n=3.

In vitro adsorption efficacy – internal Sweat & sebum resistance (SSR):

- The reference is composed with 1% Oleth-10 + 20% oleic acid + 79% Vichy water.
- A 100µm thick-film in spread on map of contrast ERICHSEN with a film applicator and let dry by incubator at 37°C during 24h.
- The gloss is measured on the white part at 60° for immediate matt effect. Then the artificial sebum is spread and the gloss is measured on the white part at 60° at T6min.
- Results are compared to internal positive and negative references.

In vivo evaluation protocols:

In vivo sensorial efficacy (Quali test):

- Home use test with 56 men & women between 18 to 35 yo for 7 days across 3 countries (Indonesia, Germany, USA)
- Good distribution of different skin types (normal, combination, greasy) and all suffering from light to moderate acne, a large part of the year. They’re having a light to moderate acne, on the face / skin with imperfections on the face / neck (nodules or inflammatory pimples/cysts localized on the lower part of the face)

In vivo clinical efficacy (Clinic test):

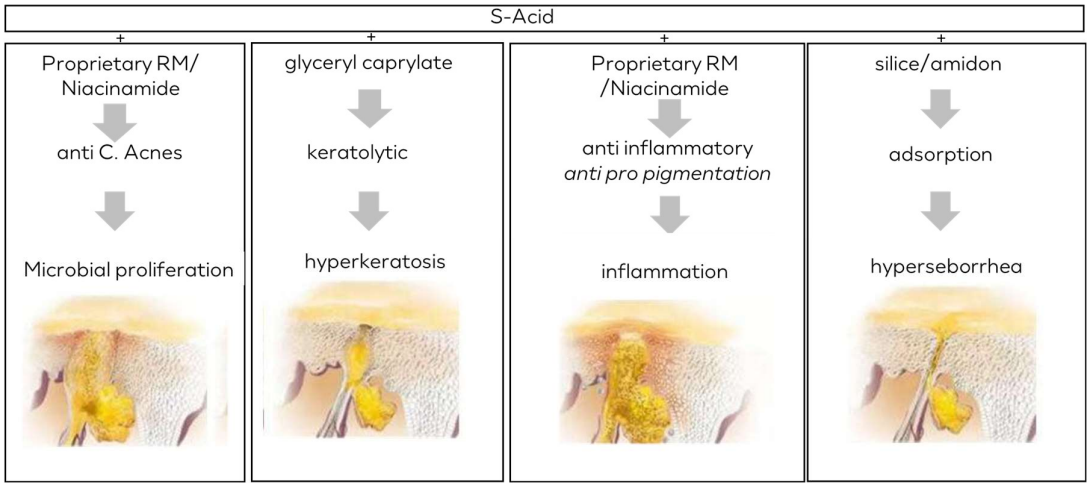
- 2 groups of 40 healthy European women from 16 to 40 yo, with phototype I to IV, who present inflammatory lesions (≥ 10 to ≤ 25 on the whole face (except the nose)) and non-inflammatory lesions (≥ 25 to ≤ I00 on the whole face (except the nose)) and IGA score for acne severity from 2 to 3 (mild to moderate)
- 56 days of use vs benchmark Effaclar Duo+

Confocal microscopy:

- Fluorochrome solutions are Fluorescein Salt (Sigma Aldrich) dissolved in water, at a concentration of [0.01 M], solubilization is carried out at room temperature, with gentle stirring and Pyrromethene 650 (Exciton) dissolved in caprylic/capric triglyceride at a concentration of [0.1 mg/L], solubilization is carried out at room temperature, with gentle stirring.
- Microscope Settings: a confocal laser scanning microscope (TC SP8, Leica Microsystems®) is used for observations. Two lasers (Argon - λAr = 488 nm and He/Ne - λHe/Ne = 561 nm) are used to excite respectively the fluorochromes Fluorescein (λF - abs max = 515 nm) and Pyrromethene 650 (λP - abs max = 612 nm). Two PMT (PhotoMultiplier Tube) detectors are configured to recover the fluorescence emission signal respectively on the bandwidths ΔλF - em = 497 - 530 nm and ΔλB - em = 620 - 720 nm.
- STRAT-M: 150µm is spread on a STRAT-M substrate. This is left to dry for 1 hour at room temperature. STRA-M is a substrate consisting of a lipid gel layer (70% PDMS 100cst / 30% isopropyl myristate) and a polymeric fibrous layer (polyolefin / polyethersulfone). The surface energy of strat-M is close to that of the stratum corneum.

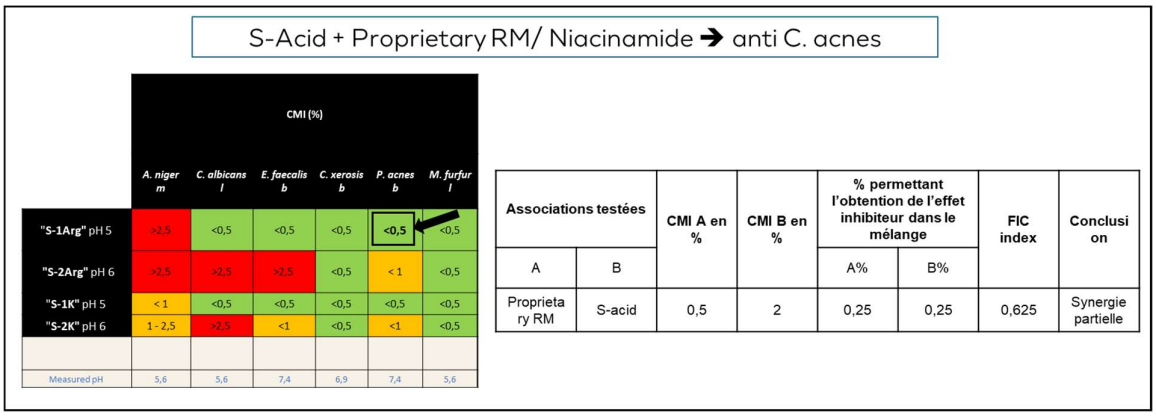
3. Results

Our efficacy engine is a mix of 4 ingredients that will help fighting against the 4 disorders of acne : a proprietary raw material (RM) mixed with Vitamin B3 (Niacinamide) against C. acnes and inflammation, Glyceryl caprylate for keratolysis, and silica and corn starch for sebum absorption (Figure 5).



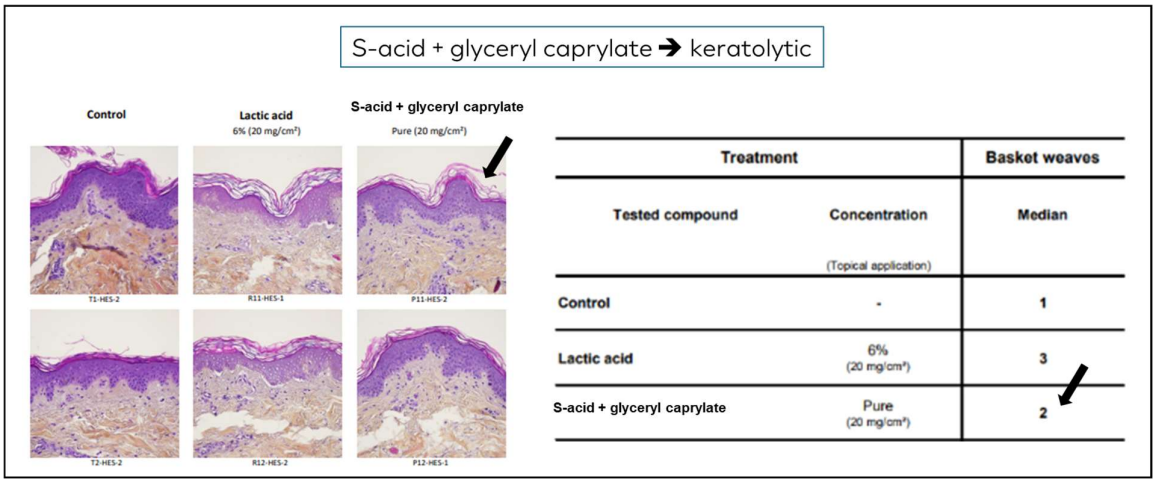
(Figure 5. 4 acne desorders targets)

In vitro efficacy testing
(anti-microbial, keratolytic, anti-pigmentation/inflammation and anti-sebum)
4 different in vitro tests were conducted to access the 4 disorders of acne.



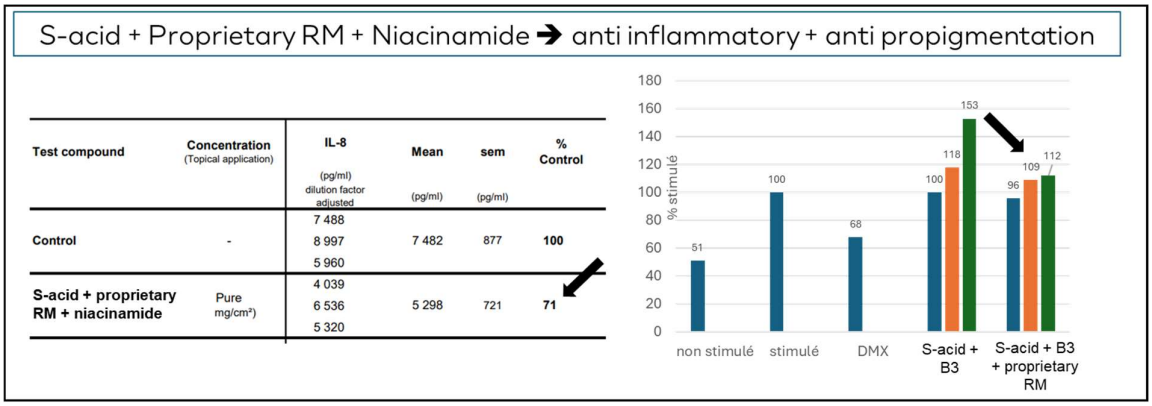
Associations testées		CMI A en %	CMI B en %	% permettant l'obtention de l'effet inhibiteur dans le mélange		FIC index	Conclusion
A	B			A%	B%		
Proprietary RM	S-acid	0,5	2	0,25	0,25	0,625	Synergie partielle

(Figure 6. Anti microbial target)



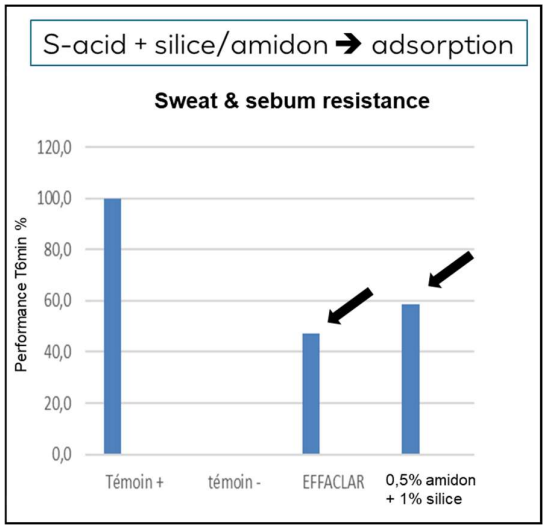
Treatment		Basket weaves
Tested compound	Concentration (Topical application)	Median
Control	-	1
Lactic acid	6% (20 mg/cm²)	3
S-acid + glyceryl caprylate	Pure (20 mg/cm²)	2

(Figure 7. Keratolytic effect target)



Test compound	Concentration (Topical application)	IL-8 (pg/ml) dilution factor adjusted	Mean (pg/ml)	sem (pg/ml)	% Control
Control	-	7 488 8 997 5 960	7 482	877	100
S-acid + proprietary RM + niacinamide	Pure mg/cm²)	4 039 6 536 5 320	5 298	721	71

(Figure 8. Anti inflammatory and anti propigmentation target)



(Figure 9. Adsorption target)

Anti-microbial activity was accessed using 2 different tests (Figure 6) to demonstrate the anti-microbial valence of our formula. We first found that S-acid by itself has an anti-microbial effect against C. acnes in vitro. Moreover, we found a synergistic effect of S-Acid with our proprietary raw material.

Keratolytic efficacy was accessed using a desquamation test on ex-vivo in survival skin ex-plants (Figure 7), we demonstrated that compared to the positive control (ei, lactic acid, score 3), our formula was able to induce a desquamation of score 2, with significant detachment of keratinocytes.

Finally, anti-pigmentation/inflammation was investigated using ex vivo in survival skin explants (Figure 8) that our formula had an anti-inflammatory effect (29% decrease of IL8 compared to control). Interestingly, this anti-inflammatory effect was able to induce a 40% decrease of mel-anin transfer in a melanocyte/keratinocyte co-culture, indicating that our formula could reduce post-inflammatory hyperpigmentation

Anti-sebum was accessed using silica and corn starch for sebum absorption, we tested the resistance towards sebum in a sweat and sebum resistance (SSR) test (Figure 9). Compared to the positive control (100%) where there is no absorbance of sebum, our formula was able to absorb 40% of sebum, being better than the formula on the market, Effaclar duo+.

In vivo sensorial efficacy (Quali test in house)

A quali home use test test has been conducted within 3 different countries (Germany, Indone-sia & USA) during 7 days in order to collect the sensorial opinion about our formula.

Conclusion is very positive because the prototype stands out as a very attractive anti-acne product, and in the 3 countries is for most preferred comparing to treatments currently used by consumers. While conventional products primarily focus on curing acne, they often lead to numerous side effects that aggravate the main concern for users: acne breakouts. These

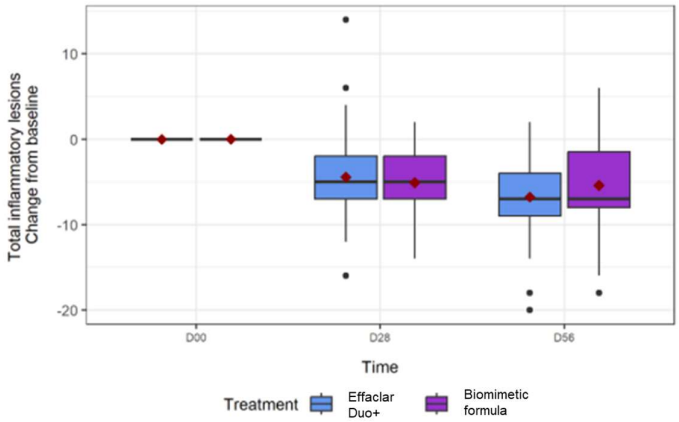
products can be harsh, causing additional issues such as skin irritation, dryness, redness, and even pain.
Over a period of 7 days, the prototype seems to be performing well in treating acne in a gentle way, without causing side effects and even more, enhancing natural beauty. Its protective layer offers efficient preventive action, reducing the frequency of acne flare-ups compared to usual. It also offers good hydrating properties, enhancing the overall skin condition by providing a radiant appearance, unlike the typically dull and dry skin resulting from conventional products. The skin feels softer and smoother, looks more even and uniform, and shows fewer scars. Last but not least, it provides a very pleasant sensory experience delivering well-being, care feelings/sensations far from feelings of medical treatment with more neutral sensoriality.

Consumers view this product primarily as an anti-acne treatment with the added benefit of hydration. It also can regenerate and improve the skin overall, both in texture and appearance.

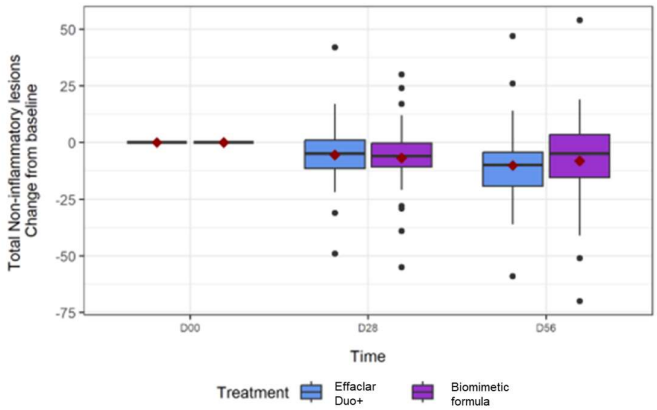
As a result, if the majority would replace their anti-acne solution with it , this product is not yet intended to replace their regular moisturizer or even their anti-acne moisturizer, given that habits are yet established and moisturization is not its primer function.

However, replacement may be later considered by some individuals after few days/weeks of experience, depending on their personal feelings and results, esp. for men more driven by convenience and simple routines.

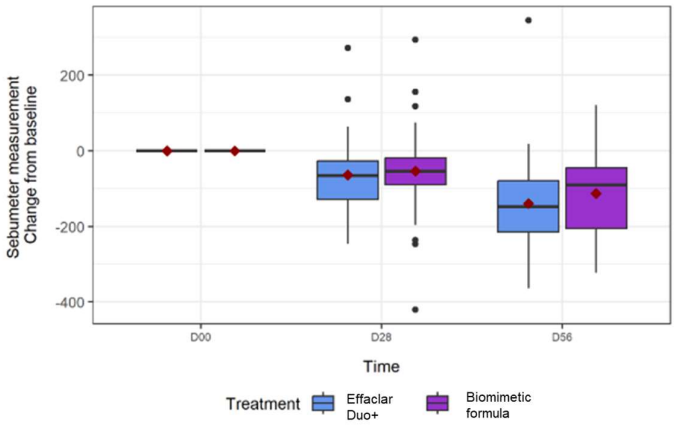
In vivo clinical efficacy (Clinic test in house)
We conducted a 2-month clinical test within 80 consumers. We demonstrated that our new formula is as performant as our internal and on the market bench, Effaclar Duo+ on the inflammatory lesions (Figure 10) and non-inflammatory lesions (Figure 11). Our formula is also on parity with the benchmark on sebumeter measurement (Firgure 12).



(Figure 10. In vivo clinical results for inflammatoy lesions)



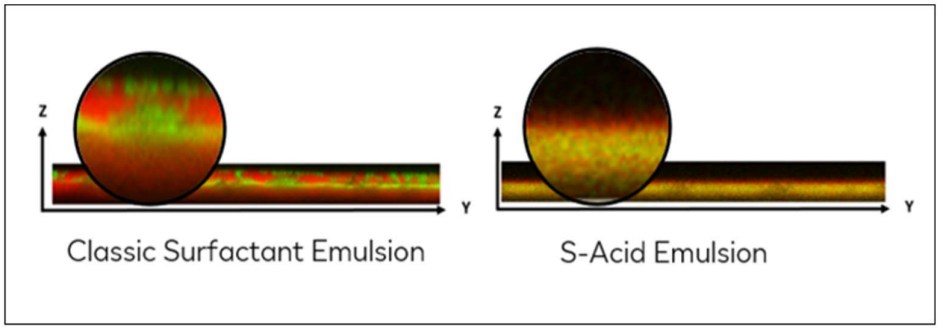
(Figure 11. In vivo clinical results for non-inflammatory lesions)



(Figure 12. In vivo clinical results for sebumeter measurement)

Confocal microscopy

We analyzed our formula with the confocal microscope in order to understand more this particular sensoriality described during the in vivo tests. Looking at z section, we observed a very homogeneous repartition of the oily phase (Figure 13, right panel) characterized by the merge of our dyes. Interestingly, with a classical surfactant, we didn't find this homogeneous repartition of oily phase (Figure 13, left panel), leading to a clear separation of the 2 dyes.



(Figure 13. Confocal microscopy analysis)

4. Discussion

In order to go more deep in the comprehension of our formula and to understand the feedbacks we had from our consumers, we decided to undergo confocal analysis. Interestingly, we discovered a very homogeneous repartition of the lipidic phase consistent with the very pleasant sensoriality described in the verbatims of our consumers. We decided to give to this new sensoriality the name of evanescence, in order to describe this almost invisible film, generated by S-Acid.

S-Acid was known to be a surfactant and a preservative, as it was shown killing bacteria in killing tests. We had, by the past, demonstrated that S-acid could also potentize the keratolytic effect of GMCY (patent). We confirmed ex-vivo this keratolytic effect with our full formula, and then demonstrated that it was also able to augment anti-inflammatory effects, as well in vitro than in vivo, resulting in the total disappearance of non-inflammatory lesions and inflammatory lesions in our clinical test. Finally, our fillers were performant in clinic as we observed a strong sebum reduction.

We hypothesize thet the multiple functionalities of our surfactant, the S-Acid. could resulting in this evanescence

Formulating new categories of actives that possesses different functionalities will help to build a new way of constructing efficient architectures.

Finally, better and stronger biological effects will open a new and greener way of creating formulas for tomorrow and investigating other cosmetic fields than acne.

5. Conclusion

We showed in vitro on the four biological disorders ; sebum, hyperkeratosis, bacterial proliferation and inlflammation; and in vivo with consumers medically monitored on every clinic endpoints, that our new and green anti acne formula was extremely efficient as well as sensorially never highlighted by our consumers. Because the consumers that used our formula were so found of it, in different tests, we decided to change our investigation latter and started to study our formula at the microscopic level in order to better characterize this new sensoriality.

By analyzing our formula on StratM subtrate with confocal microscopy, we discovered a very homogeneous repartition of our lipidic phase, highlighted by the merge of our 2 dyes resulting in a thin yellow film on the Z section. When using a classical surfactant, we’re not able to find this homogeneous repartition, but an heterogeneous dispersion of our 2 dyes resulting in red and green colors on the Z section.

This allowed us to hypothesis a new sensoriality. We decided to name this very thin film leaving almost no sensation on the skin, coupled with a very rapid water evaporation due to the high rate of hydrophilic phase. This was corroborated by our sensorial panels, where consumers were describing an ultra-light, smooth uniform and soft almost imperceptible film. We decided to call this imperceptible film evanescence.