the science of beauty

Vol 9 No 2 October 2019

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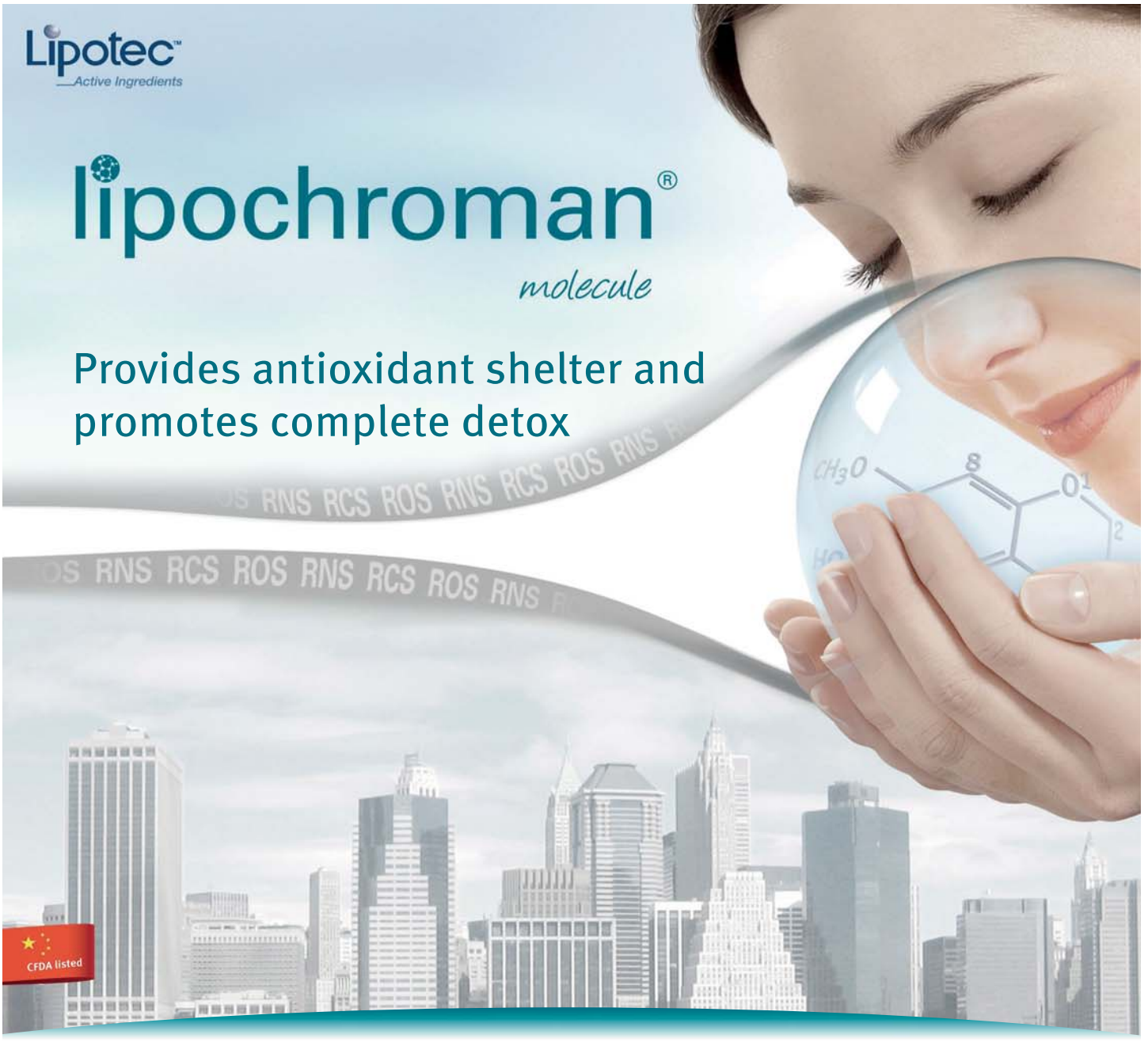

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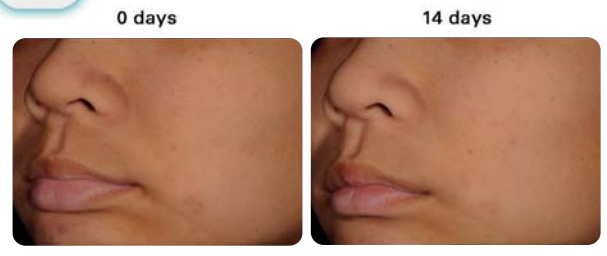

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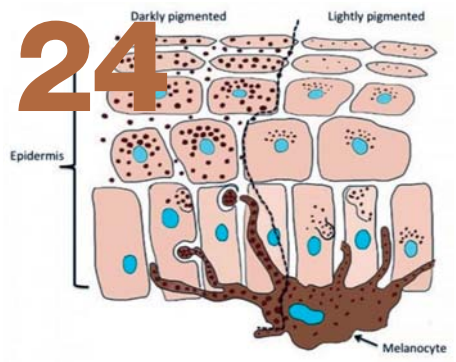

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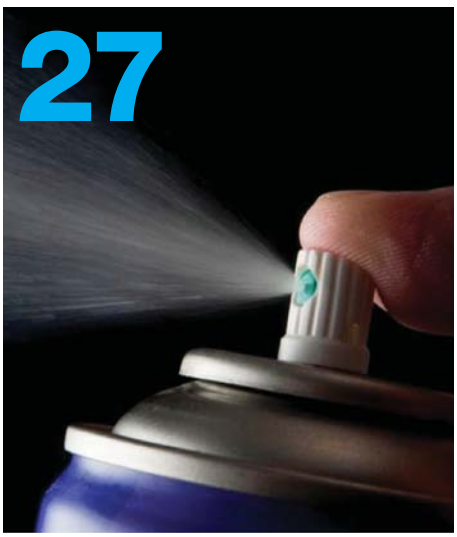

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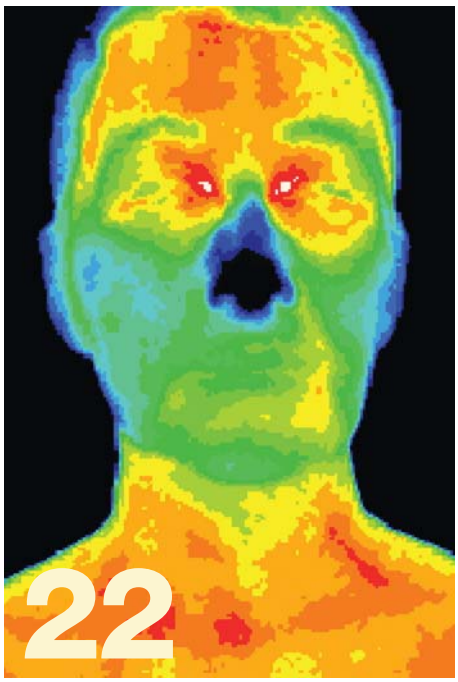

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Want to find out the latest innovations and advancements in the Cosmetic Industry?

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ASCC 2020 VISION

FOR A CLEAN AND SUSTAINABLE FUTURE

CROWN CONVENTION CENTRE, MELBOURNE,
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19th-21st May, 2020

In just over 6 months time the ASCC will be hosting the 52nd Annual ASCC Conference. Located in the heart of Melbourne CBD amongst the Crown Entertainment Precinct the ASCC 2020 Vision for a Clean and Sustainable Future Conference will be an event not to be missed.

I would like to introduce our Premium Sponsors for next years conference whose generous support will help make next years event a massive success:

- Gold Sponsor – Avenir Ingredients
- Silver Sponsors – Brentag and Concept Ingredients
- Bronze Sponsors – Trapeze, Ozderm and Coptis

Exhibition booths will be on sale by the time you read this but I would like to thank everyone who has sponsored and will be exhibiting at the conference. Next year we will have the largest exhibition space for a local conference with 40 booth spaces ready and waiting along with an Innovation zone and a newly created International zone.

The Call for Papers is out and we ask all interested speakers to send through your abstracts before the 30th November 2019. The technical team will be settling on the program in the coming weeks and we will be able to release further details of speakers including the excitement to be able to advise our Keynote and Plenary speakers.

I know the conference may seem a long way away but we will be looking at releasing the Registration packages before the end of the year. Keep an eye out for this as well as all other information as soon as it is released through the ASCC emailers as well as through the ASCC weebsite (www.ascc.com.au) and our Social Media posts on LinkedIn, Facebook and Instagram. We encourage everyone to get involved and share their thoughts for what they are looking forward to most at the Conference!

For now I think that is enough from me! A big thank you to all the 2020 Conference Organising Team for their hard work and continued dedication to making sure the 2020 ASCC Conference will be the best Conference yet. To everyone… we look forward to seeing you in Melbourne in May as the Australian Cosmetics Industry puts forward their Vision for a Clean and Sustainable Future.
Matthew Martens- Chairperson 2020 Conference

A.S.C.C. PULCHRITUDO PER SCIENTIAM

52nd Australian Society of Cosmetic Chemists Conference

To keep updated with all the latest conference information make sure you visit www.ascc.com.au

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meet the team...

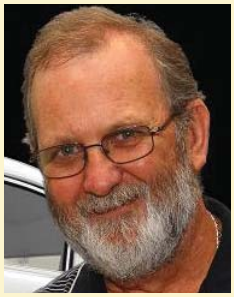
WENDY FREE has degrees in Science (B.Sc) and Technology Management (M.Tech Mngt) and is a member of a number of industry associations including Australian Society of Microbiologists, Royal Australian Chemical Institute, Association of Therapeutic Goods Consultants and is a Fellow of the Australian Organisation for Quality. With more than 25 years industry experience, Wendy's current roles include APVMA GMP auditioning, contributing to the Cochrane Collaboration and on a day to day basis, Scientific Director Quality Matters Safety Matters Pty Ltd (QMSM) that has over the last decade Wendy has provided expertise to over 400 Australian and International businesses. She specialises in regulatory compliance, commercialisation, troubleshooting and GMP systems, and considers cosmetics amongst the most challenging and enjoyable part of her work.

JULIAN JONES, the founder and Managing Director of ikonsulting Pty/Ltd, is Passionate about the Personal Care Industry in Australia and Globally.
Julian has been an active member of the ASCC for over thirty years. During this time he has served as President and Chairman of the Victorian Chapter of the ASCC. He is widely known and well respected both nationally and internationally for his knowledge and skills in developing and marketing the best Personal Care Products.

JOHN STATON has a background of over 40 years experience in the pharmaceutical and healthcare industries. John is a life member of the ASCC and serves in a number of industry representative roles with ASMI, ACCORD, TGA and Standards. He is the Australian representative to the ISO Committee on Sunscreen Testing-TC 217. (The committee for development of sunscreen standards). John is also in demand as a speaker on the International Conference Circuit.

TONI OVENELL is a formulation chemist and consultant for Queensland Cosmetic Formulators. She has worked in the cosmetic industry for many years in a range of roles covering areas of technical sales, quality, supply chain, manufacturing and product development. Most recently Toni has worked for a small contract manufacturer as technical manager, prior to setting up her own business. Toni is passionate about sharing her knowledge, maintaining a viable cosmetic industry in Australia and helping people bring their product ideas to market. She also likes champagne and hockey.

PAM JONES has worked in the Personal, Homecare and Pharmaceutical markets for more than 30 years. She has been working out of Asia since 1996 and is well versed and connected with the Asia Market.
Her experience covers technical, sales, marketing, management and training roles. She has qualifications in Chemistry, Marketing and Management. Her company PCA Consulting is well known for its training programmes. Pam has worked with and consulted to companies such as ICI, Croda, Ashland, Huntsman, Reed Exhibitions (in Cosmetics) and Connell to name a few. She is currently serving on the ASCC Technical Committee and volunteers as Technical Editor for this magazine.



RIC WILLIAMS was educated in Sydney obtaining his Bachelor of Science in Pure and Applied Chemistry from the University of New South Wales (1980) and a Diploma of Environmental Studies from Macquarie University in 1983.
Ric has had 40 years experience in the industry working for many companies and operating his own consultancy business for many years.
He has presented many lectures and workshops at national conferences for the Australian Society of Cosmetic Chemists (ASCC), the Association of Professional Aestheticians of Australia (APAA), Cosmetic and Pharmaceutical Special Interest Group (CAPSIG) and also beauty colleges nation wide.

MARG SMITH is the owner of Syndet Works – an Australian company established in 1984 to formulate and produce soap free skincare bars. Syndet has developed an enviable reputation for custom formulated and manufactured skincare that now extend well beyond the origins of the business.

JEN SEMPLE is Innovation & Education Manager at Accord Australasia, the peak national body for formulated chemical products. She is passionate about communicating the benefits of our industry's products to wider society and has authored a number of public education websites such as furphies.org.au, sunsible.org.au and hygieneforhealth.org.au. Jen also manages Accord's sustainability initiatives and seeks opportunities to build relationships between industry and academia. She has a PhD in Chemistry and Graduate Diploma in Education, and is a member of the Royal Australian Chemical Institute.

EMANUELA ELIA is the Director of Ozderm, which specialises in *in vivo* testing and clinical trials for cosmetic and personal care products. Emanuela Elia has a law degree from Rome and a Master of International Business from the University of Sydney. She had collaborated with Australia's longest serving Contract Research Organisation Datapharm for a few years before setting up a cosmetic and personal care products testing facility in 2009. Emanuela is enthusiastic about improving the quality of cosmetic and personal care products' research in Australia through science.

JAMES GILLARD is the Principal of Insurance Made Easy whose services include – business insurance, travel insurance and financial services. Insurance Made Easy has a client list of over 2000 businesses from all industries. The relevant major insurance schemes are – Hair and Beauty, Pharmaceutical Companies and Natural Therapists.

STEVE WELSH is a cosmetic packaging specialist with over 20 years experience across all mediums of packaging. As the director of Weltrade Packaging, Steve leads a team of designers, technicians, printers and supply chain professionals. To ensure the best exposure of your beauty, skincare or cosmetics brand. Steve's philosophy is to design your packaging correctly, right from the start, so you can elevate your brand and move more product. Steve works closely with leaders in the cosmetic industry to ensure that your packaging consistently stands out on the shelves within this highly competitive market.

TINA ASPRES has worked as a Pharmacist for almost 20 years in retail, industry and academia as well as being a Cosmetic Chemist. Currently she works in industry and has vast experience in both the pharmaceutical and healthcare arenas. In addition to this she is a casual academic at UTS, School of Health, (Faculty of Pharmacy in Pharmaceutics). Tina has a great interest in clinical research in dermatology and the treatment of skin disease and conditions and is Clinical Trial Coordinator at South West Sydney Dermatology. She is a keen researcher in transdermal drug delivery systems. Tina is a Member of the Pharmaceutical Society of Australia and a Member of the Australian Society of Cosmetic Chemists. She regularly consults pharmaceutical companies in the area of acne, eczema and skincare especially in the area of cosmeceuticals and has devised and written numerous support, training and education material for companies aimed at both professionals and consumers. Tina consults for the Eczema Association Australasia and is on their Integrity Assessment Panel and has worked with Choice Magazine on numerous reports. Tina has presented at the Annual Scientific Meeting of the Australasian College of Dermatologists and has published within the pharmacy and medical literature in the area of sun protection, Vitamin D, skin cancer prevention and eczema as well as co-authoring the book 'All About Kids' Skin – The Essential Guide' published by ABC Books

GINT SILINS is a registered patent and trade marks attorney, and a principal of Spruson & Ferguson Patent & Trade Mark Attorneys (incorporating Cullens). He holds a Bachelor of Science degree in chemistry with honours in biochemistry, and a Doctor of Philosophy degree in biochemistry. Gint specialises in protecting branding and innovations largely in the health care, personal care, animal health, food and beverage, biotechnology, industrial chemical, clean energy and agricultural sectors. His practice includes: conducting brand and innovation availability and registrability searches; IP audits; registering patents, trade marks and designs worldwide; enforcing intellectual property rights; resolving IP disputes; and, providing infringement and validity advice.

anti-social media

by Julian Jones

Okay, I've used a click bait headline to get your attention!

So, now you're going to call me a dinosaur… Correct!

And then, a Luddite… Definitely, incorrect!

Whether you are a brand owner, work for a brand, or are a consumer, we all have to deal with Social Media.

In a perfect world social media would be for the benefit of society. It would provide factual information and genuine opinions to everyone so that we can all make well-informed decisions, particularly about what we decide to purchase.

There's nothing new about seeking advice before we make a purchase!

In the past (before social media) we looked to friends, acquaintances that we trusted and published reviews from informed and qualified sources. Importantly though, these sources were credible and known to us. We knew who these people or companies were and could decide if they were trustworthy.

Word of mouth recommendations came from our social networks with whom we had established trust. Importantly, these contacts knew that if you made a decision based on their recommendation, and you ended up not happy with that decision, there would be consequences!

Fast-forward to today's "Social Media" and the first thing you notice is that overwhelmingly, the information comes from anonymous sources.

Who knows who "Happy Feet 73" is or "Green Frog 48"? (I'm making these names up - I think!)

As one of my previous employers once said, "Opinions are like rear-ends … everyone's got one!"

Genuine reviews about products and services are very useful when deciding about who to engage or which product to buy. But, I would argue that the first thing you need to know is, who is posting this review, what are their qualifications to offer an opinion and, ultimately - do you trust them? If you don't know anything about them, why would you trust their opinion?

Many people are well-meaning and want to share their experiences with a product or service to reward that company or person for doing a great job in the same way that we share word of mouth stories about our great (and bad) experiences. This is genuine social behaviour and we should all do it! Just have the confidence and commitment to identify who you are!

The worst kinds of online Social Media posts are, of course, from trolls. These are people who deliberately post incorrect or misleading information about almost anything - either because they think it is funny, or more menacingly, to deliberately denigrate a person or product for whatever reason. Before we had "Social Media", we would have called these people bullies. They were cowards then, and they are cowards now. The difference is they are now hiding behind a veil of anonymity.

I am sure this idea is contentious: but why can't we change the rules of Social Media to require confirmed identification before anyone can start posting opinions and comments? I know there are limited circumstances where this could be problematic, particularly in certain political jurisdictions where open criticism is likely to result in harsh consequences. In these situations, anonymity is important to preserve freedom of speech, but overwhelmingly, being prepared to back your posts with your identity would, I believe, reduce false information greatly.

So please don't give up your use of Social Media, just think about where your information is coming from and whether you believe it is credible! We all have responsibility for making the medium social – not anti-social!

Till next time! Cheers,

Julian

make **your products stand out** from the crowd!

by Steve Welsh

The cosmetic and wellness industry is starting to become flooded with new and smaller brands making an impact in both retail and online markets. Consumers have never had as many choices in products as what they have today, and brands are finding it harder to market to customers.

It has never been as important for brands to make a visual impact of their products. Whether that is on the shelf, online on e-commerce websites or even through Influencers on Social Media. The product must be able to visually appeal to the targeted demographic otherwise the brand will find it hard to sell.

Fortunately, as time goes on, technology improves and there has never been so many different ways to package and decorate a product. In this article we are going to discuss some of the different choices a company will have when decorating their packaging.

Direct Printing

Probably the most popular choice when decorating cosmetic products is a direct print. This is printed with UV ink straight onto the packaging, whether that is a bottle, jar, tube, pouch, or cap. This printing dries instantly and gives the product a premium look.

There a few different printing processes with cosmetic packaging and some of them are:

- Silk Screen Printing
- Offset Printing
- Hot Stamping (foiling)
- Flexographic

When printing, the artwork will generally need to be setup in Pantone colours and the brand will be charged the number of different colours they

use rather than the size or complexity of the artwork. Weltrade Packaging specialises in packaging printing, so if you have any questions please don't hesitate to ask!

Labelling

Another popular form of decoration is labelling. This is when artwork is printed in CMYK onto a sticker and then the sticker is later applied to the packaging. This is more traditional way of decorating and is great if you're a company that has multiple skus in the same package or if you are ordering smaller amounts of qty's. Labelling is also great if you have complicated and busy artwork as direct printing is limited in what you can design.

- Even though labelling may seem the better option for some, there are some downsides.
- Labels can cost more due to extra packaging and the application fee
- Labels may not seem as premium
- Standard labels will fail when wet
- The extra packaging of the label can be seen as a negative impact to the environment

Shrink Sleeving

Shrink Sleeving is proving to be a popular way in decorating for a lot of wellness and cosmetic brands. This process is where the artwork is printed on to a piece of plastic film, this film is then heated and shrunk onto the packaging.

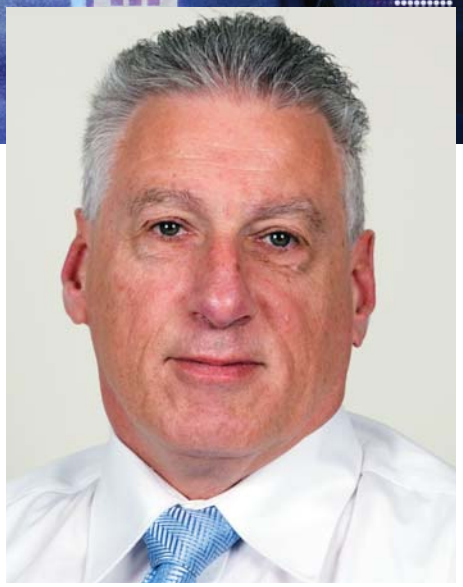
Shrink Sleeving can cover all of the packaging and is great if the brand wants the focus to be on their artwork. Shrink Sleeving can also act as tamper evidency and can be applied to almost any shape of packaging large or small.

If you are interested in our decoration options/packaging and would like to know more, please don't hesitate to reach out. You can contact us via our website weltradepackaging.com.au

Looking forward to talk more regarding your packaging requirements.

Steve Welsh

Social Engineering isn't just a buzz word



by James Gillard

So, what is Social Engineering anyway?

It's the use of deception to manipulate individuals into divulging confidential or personal information that may be used for fraudulent purposes.

Hopefully by reading this article it will alert you to the fact, whatever the size of your business , you are a target and need to be constantly aware of IT Risks.

Deception by hackers wanting to steal your identity, or destroy your hard earned business reputation , disrupt your day to day business activities , steal/ lock your data, demand ransoms to get it back, and possibly spread or sell your client's information to all and sundry.

So, what types of Social Engineering are there?

Phishing, where attackers use emails, social media, instant messaging, and SMS to draw in targeted victims into providing sensitive and private information with an aim to compromise business systems. An example of this is "Business Email Compromise" (BEC), where hackers create a fictitious appearance as known Executives of a firm to force those in Account Payable areas into transferring significant sums of the company's money into the hackers own bank account.

Watering hole means implanting malicious code into the public website pages of sites that the targets visit such as online shopping sites.

Whaling attack. Same as phishing but this time the hackers are out their trawling with an aim to catch a whale which are big PHISH, in other words, Board Members & Senior Executives of private business and government agencies.

Pretexting. Hackers create a fictitious identify of a person to gain private information then once in hand manipulate that information for criminal intent.

Baiting where hackers use an infected computer file which looks just like an authentic software update. Case in point, a hacker infects USB sticks , leaves them around public areas and the unknowing public insert them into their own computers potentially destroying data on their hard drive due to an infected program on the USB stick.

Tailgating where a hacker can infiltrate entry to a restricted area by walking in behind a person who is authorized to access the area. Someone for example in a building who enters the lift but gets off behind the person that works on that floor. Possibly disguised as a tradesman where no questions are asked of the intruder.

Businesses are not bullet proof

Businesses MUST do everything in their power to protect themselves from being exposed and vulnerable when it comes to forms of social engineering by 3rd parties. Undertake checks on vendors, install fraud detection systems, separate financial duties of employees, and roll out education programs for staff on how to detect fraud and what steps to take.

Despite these measures' businesses can still and do become victims of social engineering.

Three example cases

A. *The case of the fake invoice*

An employee for a distributor of component parts was accountable for making regular vendor payments. After several months of this arrangement in place the employee one day received an email appearing to come from the vendor. The proposed vendor said they were having banking issues and asked for the payments to be made to a new bank and attached a new invoice. The employee was finding it difficult to verify the request and the proposed vendor applied pressure for the payment to be made. The employee paid the false invoice. The real vendor chased a payment they did not receive and realised through social engineering techniques they had been hacked and the cost to the distributor was $250,000.

B. *The case of the imaginary CEO*

A regional CFO of a subsidiary of a large public listed company received an email purporting to be the Assistant of the CEO in the United States. The email requested the CFO transfer a significant sum of money immediately to cover a tax payment in China. The CFO questioned the request and then the fake Assistant of the CEO made a phone call to the CFO insisting and proving over the phone the request was legitimate. The fake Assistant to the CEO knew all about the companies polices, operations, people, processes, etc. The CFO transferred the money however the scam was detected after another attempt at transferring the funds was stopped by the subsidiaries bank. The company only recovered a small portion of the transfer and the resultant loss to the company was $1,000,000.

C. *The case of the Hacked emails*

A Business Manager (external consultant) who is responsible for Account Payments received an email, purportedly from their client enquiring about her bank balances and availability of funds. The email seemed very authentic as it included details regarding full descriptions of account numbers, names and some recent transactions. The Business Manager communicated back to their client with the requested details. A further email came back from the client requesting $100,000 be sent to a specific account offshore to purchase real estate. The Business Manager trusted the instructions and the payment was made into the hacker's bank account. All too late, as the $100,000 had been sent.

Can Cyber Insurance help?

Cyber Insurance can at least be a safety net consideration which business should seriously look at. In the event a business falls victim to being hacked, Cyber Insurance cover provides some relief to the devasting repercussions to the Business and provides financial reimbursement to rectify the damage done under the terms and conditions of the insurance policy.

There is standalone Cyber Insurance and Cyber cover is also part of an overall Management Liability insurance policy.

If you would like to know more about Cyber Insurance and Management Liability Insurance please contact the friendly team at IME Insurance Brokers – Insurance Made Easy for personal assistance to discuss your own individual circumstance 1800 641 260 or visit us www.imeinsurance.com.au

Collaboration for innovation in beauty and personal care –

Accord partnership with Monash University

by Jennifer Semple

It's a well-known saying that "two heads are better than one".

But, did you know that businesses that collaborate on innovation are 70% more likely to create new-to-world innovations? And, have 4% annual increase in productivity growth? That's according to the Australian Systems Innovation Reports 2014 and 2017, respectively.

Collaboration enhances innovation in several ways.

First, collaboration means more brains. More brains means more ideas, more diverse but complementary perspectives and more skills working together towards a common goal. Collaboration can lead to a much larger network to draw upon for current and future projects.

Secondly, collaboration means more stuff. This could be a wider range of equipment or facilities through pooled resources.

There can be many positive outcomes of collaborative research. The ability to move beyond day-to-day operations and undertake blue sky, disruptive research. The creation of new chemical technologies to solve social and environmental challenges, provide enhanced sensory experiences, health benefits and cater for diverse needs. Mutual growth in personal knowledge and skills.

All of these are essential in Australia's transition to a knowledge-based economy.

One of Accord's primary goals is to strategically assist our Member companies enhance their innovation capacity. One key way in which we do this is by identifying and building relationships with research institutions and hubs that are keen to work with our industry. We then facilitate regular dialogue and information sharing with these organisations.

Therefore, we are thrilled to announce that Monash University has recently become our first official Contributing Innovation Partner – formalising the relationship built between our organisations over the last few years.

Accord has been a supporter of industry-aligned Monash initiatives for many years. We contribute in an advisory capacity to the Pharmaceutical Sciences program; formerly known as Formulating Science, this is a key pathway for graduates in the beauty industry with formulation expertise.

We are also a supporter of Monash's Chemicals and Plastics Manufacturing Innovation Network (C&PMIN), a key hub for industry-led collaborative innovation including through industry-led PhD projects, knowledge sharing and technical/business training. We are also supporting Monash in other future opportunities to encourage industry-university collaboration.

Other highlights of our interactions with Monash have included Accord participation at careers days, Monash presentations at Accord events, e.g. on new green chemistry innovations, and regular interface between Monash and Accord members to build mutual understanding, share knowledge and explore opportunities.

Having a formal partnership now in place will ensure more opportunities for the beauty industry and academic expertise to come together, learn from each other and capitalise on each other's strengths.

Accord Australasia is the peak body representing companies operating in the cosmetic, fragrance, personal care and toiletries sector – from multinationals to small Australian-owned businesses, importers to local manufacturers. www.accord.asn.au

- Sunscreen Testing
- UVA Testing
- Chemistry
- Stability Testing
- In-Vitro Tests
- Clinical Studies
- Hair Testing
- Consumer Research

Dermatest has recently joined the Eurofins testing group. With over 45 years experience in cosmetics and 27 laboratories worldwide, serving the cosmetic industry and in continuous expansion. This dynamic resource allows us to offer an even broader scope of testing and development services.

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https://www.eurofins.com/cosmetics/

Singapore people and events

by Pam Jones

For this edition of the magazine, I would like to take you to Singapore for the Society of Cosmetic Scientists Singapore (SCSS) Suppliers Day. This is the 10th Anniversary for the SCSS and each year the event grows. For the first time this year, it was a two-day event held on the 29th and 30th August. The personal care manufacturers in Singapore are a small minority, according to Philip Jacobs who has been organising this event for the past 10 years, "we are seeing an increase in investment by major players in the Flavour and Fragrance sector as well as an expansion of cosmetic & personal care raw material suppliers in Singapore These suppliers are increasing their manufacturing facilities. There is also an increasing number of personal care support industries including efficacy testing facilities and laboratory support." The expansion is partly due to the Singapore Government who actively seek out and reward companies with benefits such as decreased taxes plus many other incentives for research. The Singapore Government is also building bridges between academia and industry through their A*Star organisation and Polytechnic education institutes.

Singapore is also the home to major multinationals who locate their research centres in Singapore such as P&G, J&J and Shiseido.

More than 300 visitors registered for the Suppliers Day, with delegates from Indonesia, Japan, Malaysia Philippines, Singapore, Thailand and Viet Nam.

Day 1 of the event had a ½ day workshop. This included an introduction and tour of the Singapore Skin and Scalp Research Centre which is part of the Skin Research Institute of Singapore SRIS. https://www.a-star.edu.sg/sris

Day 2 also featured a workshop (conducted by Belinda Carli) as well as Podium sessions (9 speakers including a Guest Speaker) and of course the interesting tabletop presentations offered by the 30 exhibitors (*regional & International suppliers*) who supported the event.

Guest speaker for the event was Dr Tomonobu Ezure, Principal Scientist, Shiseido Global Innovation Centre with a paper entitled "Cutting-edge Skin Research: the key to innovative cosmetic product development" (anti-ageing skincare)

Congratulations to the SCSS who despite a small consumer manufacturing base and a small membership were able to present such an outstanding Suppliers Day.

Save the date for the next Singapore

Suppliers Day Thursday 27th & Friday 28th August 2020.

The team of the SCSS is composed of the following

Dr Nobuaki Matsuoka – SCSS President

Philip Jacobs – Event Leader

Dr Gen Nonaka – Scientific Chairperson

Alex Neo – Event Treasurer

TAN Ming Jie – Day 1 Coordinator

Angelito Delos Reyes, Frederica Lam, Marty Lumain, Tanu Kustandi, TIO Phooi Siang – Committee Members,

SCSS Secretariat – Michelle Soh

My thanks to Philip Jacobs for his input into this article. I would like to refer Philip to anyone who is looking for a consultant re cosmetic raw materials and any question re Singapore and or Viet Nam cosmetic industry.

Philip is also an Adjunct Lecturer at Singapore Polytechnic for UV Protection.

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Every plant has a season …

Every plant has a season, a purpose and a story that helps us to celebrate and capture the diverse and resilient beauty of our country. Australian native plants have responded to our varied and, at times harsh climate by producing highly concentrated and complex phytochemicals signatures which the cosmetic world recognises as antioxidants, anti-inflammatories, anti-microbials, micronutrients, humectants and emollients.

In terms of bush medicine, it is important to recognise and celebrate the diversity that exists within Aboriginal and Torres Strait Islander plant/ human relationships. Each nation has its own unique medicine practices and different people within each nation have different roles to fulfil. This complex and interwoven cultural relationship helps to maintain the balance between supply and demand, wants and needs.

In the wider context, Australian extracts are earning a name for themselves in the organic and natural skincare market for their beauty, potency and intrigue. The thirst for new native botanicals in the cosmetic space is fuelling new research both in extraction techniques and in their applied benefits.

New Directions is excited to present our recently expanded selection of Australian Native Ingredients and is looking forward to working with you, whatever your interest.

Native Extracts category:

Mountain Pepper Berry – high antioxidant capacity, anti-Inflammatory properties, Vitamin E, Lutein, Trace Minerals Zinc, Magnesium, and Calcium.


Desert Lime Powder – High levels of phenolic antioxidants, Vitamin C, Vitamin E and trace minerals making it perfect for anti-ageing skin care.

Wattle Seed Powder – Protein, fibre and carbohydrate source, wide range of Micronutrients such as Magnesium, Iron, Zinc and Potassium. Used to soothe and calm irritated skin.


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LIPOCHROMAN® *molecule*

has shown to help reduce pigment, homogenizing skin tone

Pollution is one of the most serious problems we have in the modern society, negatively affecting not only the health of our body but also that of our skin. The skin is the first organ that faces the elements and thousands of chemicals and reactive species coming from synthetic chemicals and pollutants as well as those of natural origin like pollen and radiation.

Our lifestyle not only involves exposure to these foreign substances but also the production of reactive species within the body itself. These substances, very stable and difficult to eliminate, can accumulate in the body over time causing allergies, inflammation and accelerating external signs of aging like wrinkles, sagging and pigmentation.

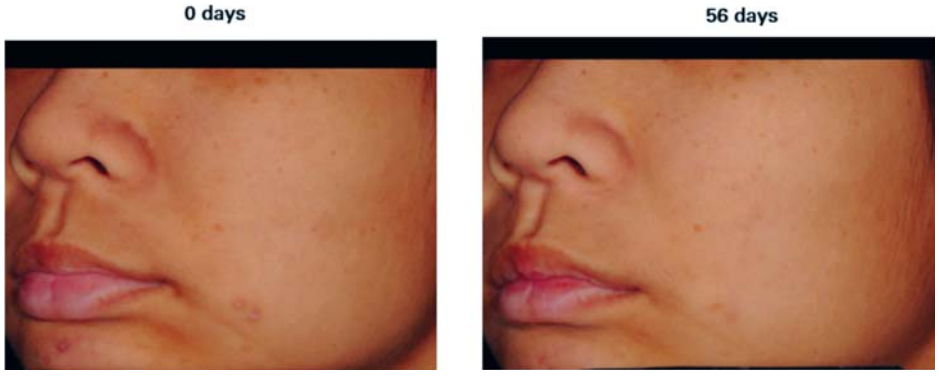
Inspired by natural vitamins, LIPOCHROMAN® *molecule* confers triple protection from reactive species. This powerful antioxidant with detoxification properties showed to improve wrinkles and to reduce melanin index.

A recent clinical test was performed with Asian volunteers between 18-44 years old that applied to the face a cream containing 0.05% LIPOCHROMAN® *molecule* on a randomized half side of the face and a placebo cream on the other side. After 14, 28 and 56 days, the melanin index in the skin of the volunteers was measured by mexametry, which is based on measuring the light reflected by the skin. In only 14 days the melanin index was reduced up to 6.9%. This percentage increased in 28 days to 9.4% and in 56 days to 13.3%, showing the demelanizing effect of the ingredient and suggesting a fairer complexion.

Bio-inspired antioxidant, LIPOCHROMAN® *molecule* can be included in cosmetic formulations aiming to prevent premature skin aging due to environmental aggressors as well as in products to help reduce the accumulation of melanin and to protect from oxidation.

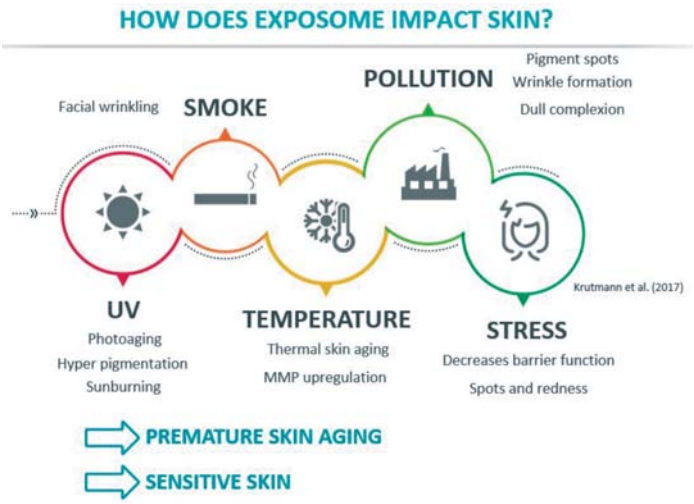
A comprehensive solution to fight pollutants and prevent premature skin aging.

For more information, please contact Robert McPherson, Account Manager for Australia and New Zealand, at Robert.McPherson@Lubrizol.com or Tel: +61 (02) 9741 5237.



what is Exposome and what you can do to conquer it?

How many people live without exposure to pollution today? Apparently only around 8% of the world's population, but we can safely assume that it would be zero percent of anyone reading this article. We are well aware of the damaging effects caused by UV rays on the skin, but the effects of other urban pollution can potentially be more dangerous – air pollution, light, temperature, smoke and stress.



All these stressors named exposome are particularly aging to the skin and can result in sensitive skin, and irritation such as burning or pain. Recent reports also show the link between dysbiosis (microbial imbalance) and neuroinflammation induced by exposome.

What can you do to minimise the effects of Exposome?

Exposome are present everywhere – walking down the street, sleeping, shopping, working and, short of living in a sterile bubble, there is very little you can do to avoid their impact. What you can do is use an active ingredient that is able to strengthen the skin barrier and improve skin comfort.

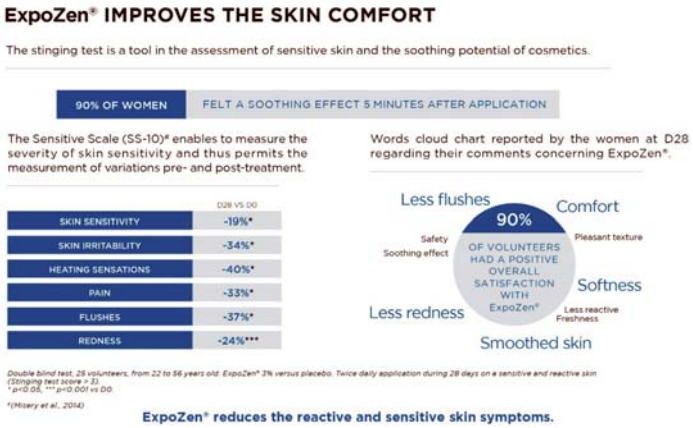
ExpoZen® is this active. An alga-based ingredient, designed to fight against reactive and sensitive skin and focuses on daily environment stress attacks, ExpoZen® soothes the skin, makes it more Zen by reducing stresses on the skin.

This active compound is derived from *Halymenia durvillei,* found in the depths of the Indian Ocean, rich in biologically active metabolites, such as polysaccharides, well known for their anti-inflammatory effects. These polysaccharides help skin resist and endure sensitisation and to rebalance skin microbiota.

What can Expozen® achieve?

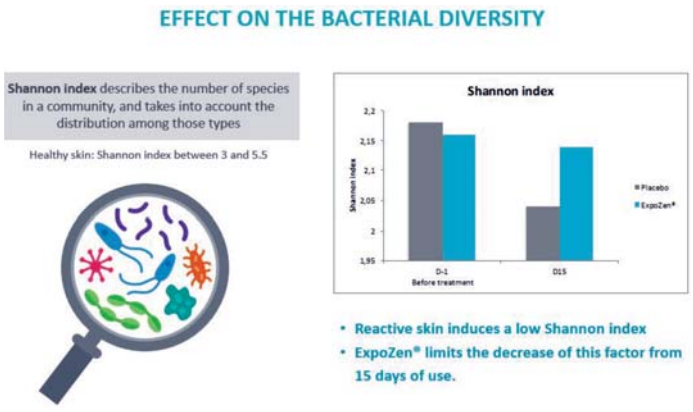
Briefly, ExpoZen® makes skin more resilient to stress exposome attacks day-to-day because:

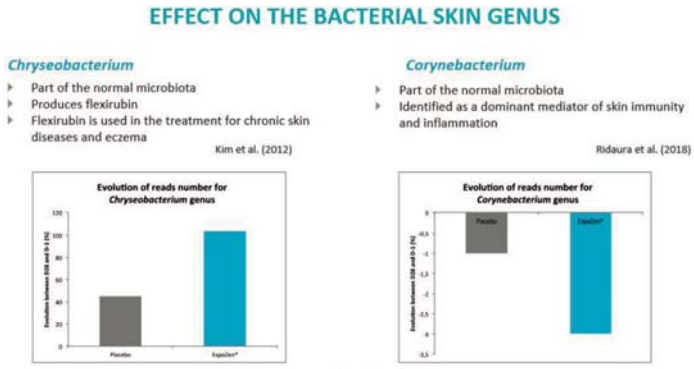
- It delivers its soothing properties by inhibiting the substance P receptors. It also acts on TRPV1, which provides a

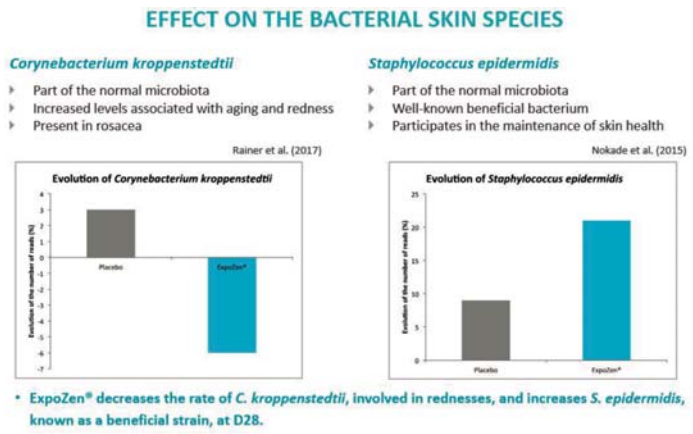


sensation of heat and pain (nociception). The skin is then more radiant.

- It stimulates healing processes by stimulating the activity of VEGF growth factor, a protein that plays an important role in the growth of new tissues and organs.
- It reduces and soothes the feeling of discomfort.
- It maintains bacterial diversity, reduces species involved in inflammation and redness and promotes strains known to be beneficial for skin as *Staphylococcus epidermidis*. At the same time, ExpoZen® decreases levels of *Corynebacterium Kroppenstedtii*, a novel target for the control of skin redness.







This results in the skin being less flushed, more comfortable and more radiant.

Where to use ExpoZen®? Or more importantly, where NOT to use ExpoZen®?

ExpoZen® is a universal active suitable for reducing the harmful effects of exposome across all ages – from babies to mature age. It helps to reduce the effects – anti aging, anti-wrinkle, reduces redness and lowers the effects of exposure to pollution.

Expozen® – is this the new HERO active for your next skin care range?

A S Harrison & Co has had the pleasure of partnering with GREENTECH for nearly 10 years supplying the industry with innovative, quality products based on plant biotechnology.

GREENTECH has been developing and producing high-tech active ingredients originating from the plant, marine and microbial worlds for the cosmetic, pharmaceutical and nutraceutical industries since 1992. They were the first cosmetics supplier to be certified ecological and organic by ECOCERT Greenlife and are the number one supplier of organic cosmetics ingredients in terms of volume.

A S Harrison & Co offers a comprehensive range of GREENTECH products – Actives for skin, body and hair care, Scrubs, Aquasoils®, Extracts, Butters & Oils, Soft Butters® and Greensoft®. By purchasing from A S Harrison & Co, you are not just buying quality ingredients but also supporting a company that operates to the highest ethical standards whilst giving back to the community.

For more information and samples please contact your A S Harrison & Co account manager or email performanceingredients.ash@harrison.com.au or call us on +61 (0)2 8978 1016

new ways to bring the skin into focus

by Emanuela Elia

Both cameras and imaging technologies have advanced rapidly in the last few decades, leading to the production of some fascinating images. As a result, the visual impact of the images associated with research on humans attracts a lot of interest. Here we go through some of the technologies used that have been found to be extremely useful in skin care research and still hold huge potential for further developments.

3D Imaging

Nowadays, a number of systems are available for the clinical practice or for researcher using 3D imaging technology. Coming in either handheld or fixed varieties, there are a number of ways to generate 3D imaging - multiple cameras linked to fire simultaneously, a singular camera as part of a turntable system, or a laser scanner that builds a true 3D topographical map of the skin. All these methods allow for quick and accurate capture of a 3D body or face image. They are also reproducible as subjects' distance and lighting conditions are standardised within the apparatus and do not vary across multiple visits.

These systems have many applications in cosmetic practice. Analysis of the skin or body before and after cosmetic procedures can occur, with some treatments being informed by the results of the initial analysis. 3D cameras have also been adopted in studies involving topical preparations for the objective assessment of the effects on skin topography (lines and wrinkles, skin puffiness, acne, scars etc.) which provides an extra level of accuracy that is not possible using traditional 2D methods.

UV Cameras

Ultraviolet (UV) light is a form of light emitted by the sun that is invisible to the human eye. It occupies the portion of the electromagnetic spectrum between X-rays and visible light. Most part of the UV light is absorbed by the earth's ozone layer. In fact, UV light constitutes about 5% of terrestrial sunlight. There are three types of UV rays - A, B, and C, with increasing penetrative ability and risk for harm.

Although UV has some important benefits for humans such helping improve mood, triggering vitamin D (which in turn helps strengthen bones, muscles and the body's immune system), and helping some skin conditions such as psoriasis, in more recent years, great emphasis has been given to the harmful effects of UV such as skin ageing, sunburn and skin cancer. UV related skin damage is a widespread issue. Australia has one of the highest rates of skin cancer in the world, in which UV radiation is attributed as the main preventable cause. As such, formulating efficacious and protective sunscreen products is vital for both the consumer and pharmaceutical markets.

While the levels of sunscreen ingredients present will obviously have an impact on the overall protection capability of the product when applied to skin, an area which is often overlooked is formulation from which these ingredients are being delivered, and the impact it has on how the sunscreens spreads and moves on the skin once applied. Studies use UV reflectance photography to assess how the sunscreen spreads across the body, both directly

after self-application and after a period of use. The spreading of the sunscreen ingredients will have a dramatic impact on the overall protection offered by the film, as areas of the applied film where the layer is thinner will more readily allow an increased level of UV light to penetrate and damage the skin.

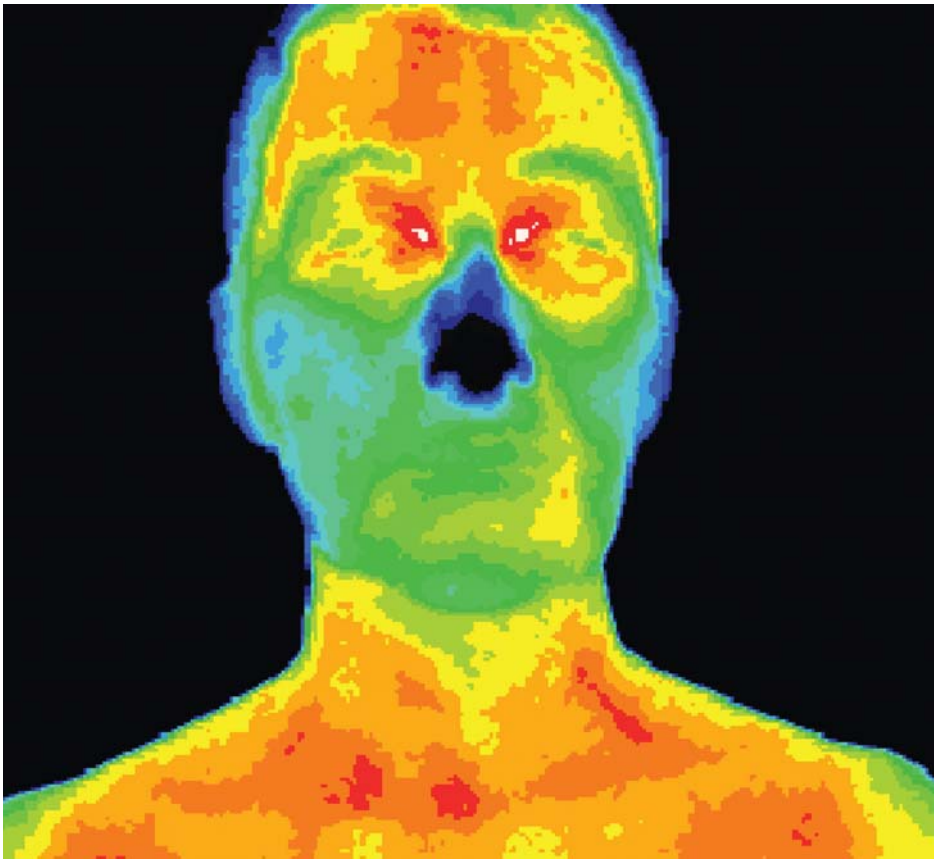
Another popular application for UV photography is analysis of UV skin damage. In fact, this type of application has been shown to a have a positive impact on individual skin health and protection behaviours. A randomized clinical trial in university students showed that a UV photography intervention resulted in significantly stronger sun protection intentions ($P < .01$) and greater sun protection behaviours ($P < .05$). These findings demonstrate the importance of broadening the use of UV photography to improve skin health.

Infrared Cameras

Infrared radiation is radiation with longer and less energetic than visible light, and transmits heat over long distances. Thermography, or thermal imaging, uses a special camera that is able to measure these waves, and thus the temperature of objects, surfaces, and living organisms. The difference between the values of temperature traditionally measured with contact probes (the standard technique) and the ones measured by thermal imaging lies in the fact that the former produces a singular value based on the contact point, while the latter gives a distribution over a continuous area, producing 2D heatmaps.

Thermography imaging does not involve ionizing radiation - it is fast and non-invasive. For these reasons, it is used in diagnostic medicine for detecting, recording and producing infrared images that reflect the microcirculatory dynamics of the skin surface of individuals in real time, comprising the vascular, nervous and musculoskeletal systems, in addition to inflammatory processes, as well as endocrine and oncological conditions.

In healthy subjects, the hypothalamus homogenises skin blood flow using



the central nervous system in order to maintain normal thermoregulation. This results in a symmetrical thermal pattern between the right and the left side of the body. On the other hand, qualitative and quantitative changes in thermal distribution are indicative of abnormality. This principle can be applied even on smaller scales - anomalous skin function can be detected by the presence of unusually hot or cold patches on the skin. There are many other uses for thermography, such as: in dental imaging; diagnosing specific pathologies; as an indicator of the muscle activity during physical exercise; post-surgery monitoring skin grafts to identify positive blood flow in the new flap of skin, and so on.

In scientific studies assessing the effects of topical preparations, thermography has most recently been used to investigate the stimulation of cooling effects and blood flow increases in the areas of the skin treated with the study product.

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EMANUELA ELIA is the Director of Ozderm, which specialises in *in vivo* testing and clinical trials for cosmetic and personal care products. Emanuela Elia has a law degree from Rome and a Master of International Business from the University of Sydney. She had collaborated with Australia's longest serving Contract Research Organisation Datapharm for a few years before setting up a cosmetic and personal care products testing facility in 2009. Emanuela is enthusiastic about improving the quality of cosmetic and personal care products' research in Australia through science.

the epidermis – engine room of your skin

by Tina Aspres

Look at your skin's surface. Chances are you can't notice anything happening but your epidermis is hard at work for you every second of the day!

The epidermis (figure 1) has approximately 40-50 million cells of which 95% are keratinocytes. The next most populous cell is the melanocyte followed by Langerhan cells and then Merkell cells.

The epidermis has many functions – most importantly providing protection against injury, microorganisms and ultraviolet radiation. In addition, the epidermis is responsible for making new skin cells, the colour of our skin, our light touch sensation and the production of vitamin D. How is this so?

Keratinocytes

The epidermis is a continuously renewing squamous epithelium that consists of functionally distinct layers (figure 1). The cells of the epidermis (keratinocytes) undergo a process of cell proliferation (stratum basale) then differentiation (stratum spinosum, stratum granulosum and stratum lucidum) to ultimately produce a hydrophobic protective barrier (stratum corneum), which is continuously shed into the environment.

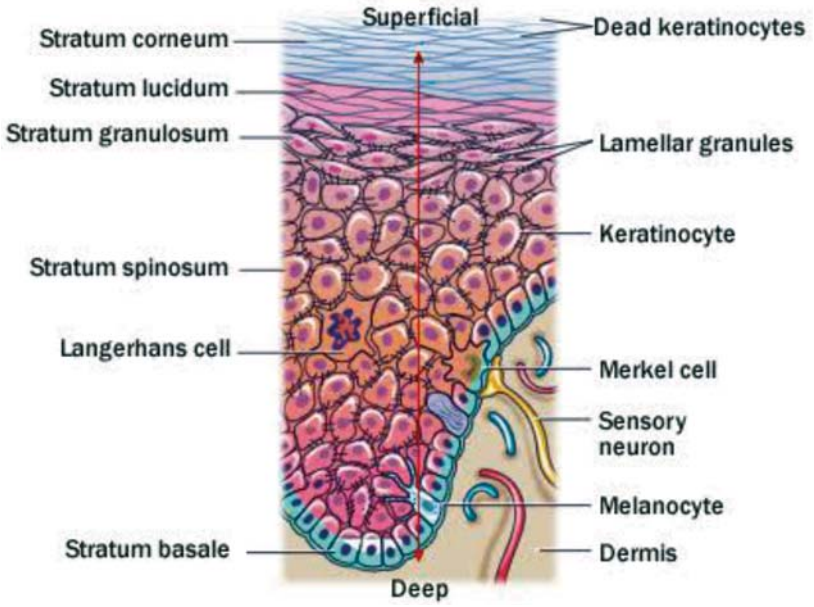


Figure 1: Cells of the epidermis

The stratum basale contains a single layer of cuboidal shaped keratinocytes that contain a nucleus and the usual assortment of cytoplasmic organelles. The stratum basale, also referred to as the stratum germinativum, is the production house for new skin cells where continuous mitosis occurs producing all of the keratinocytes of the epidermis. As new cells are being formed, existing cells are pushed upwards towards the surface during which they are undergoing cellular changes – termed keratinocyte differentiation.

The next layer is the stratum spinosum which consists of 8 to 10 layers of keratinocytes, formed as a result of cell division in the stratum basale. Keratinocytes in the stratum spinosum produce protein filaments termed desmosomes that function as intercellular bridges to tightly bond adjacent keratinocytes to each like a tightly woven fabric.

As more keratinocytes are formed in the stratum basale, the stratum spinosum cells are pushed higher to form the stratum granulosum layer which is 3-5 cell layers thick. The keratinocytes of the stratum spinosum begin to shrink in size and flatten in shape. In this layer keratohyalin granules appear. Keratohyalin granules contain two important structural proteins: a fibrous protein called keratin which is secreted outside the cell into the intercellular space and the protein filaggrin whose function is to align and glue the keratin protein filaments together.

In both the cells of the upper stratum spinosum and stratum granulosum layers, secretory organelles called lamellar bodies are found. Lamellar bodies are responsible for the secretion into the extracellular space of epidermal lipids such as ceramides, cholesterol and fatty acids that together with the structural protein keratin will result in the formation of an impermeable, lipid-containing membrane at the surface of the epidermis.

The next layer is the stratum lucidum, a layer only found in the thicker skin of the palms, soles and digits. The stratum lucidum keratinocytes are flatter in shape compared to the stratum granulosum and are no longer viable (dead) having lost their nucleus. The stratum lucidum contains additional lipids that make the skin of the extremities waterproof and supple to resist the effects of friction.

The stratum corneum is the most superficial layer of the epidermis and is the layer exposed to the outside world. There are usually 15 to 30 layers of cells in the stratum corneum. The cells of this layer (termed corneocytes) are dead, flat, dehydrated keratin filled

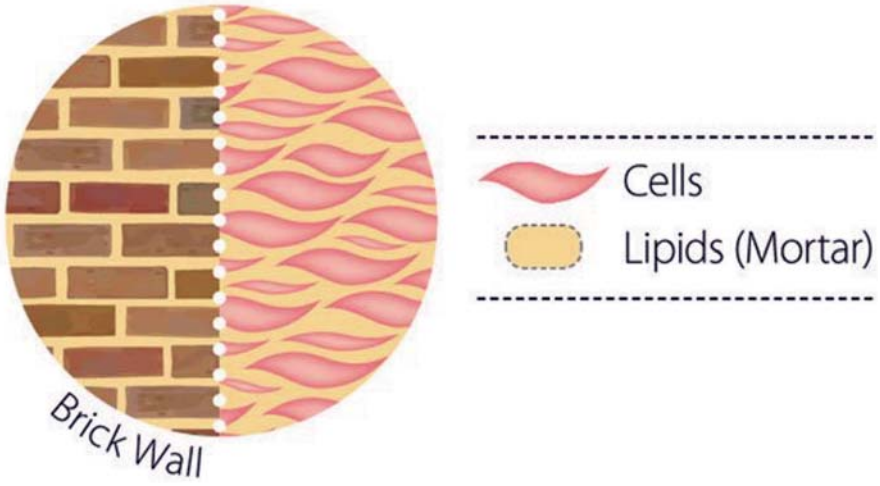


Figure 2: Bricks and mortar appearance of stratum corneum

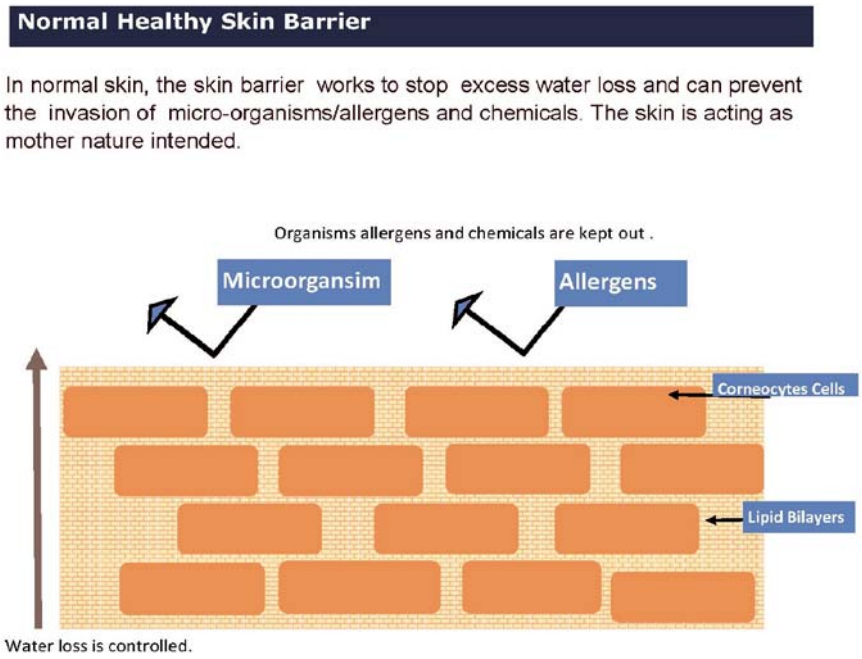


Figure 3: Function of normal healthy skin barrier

cells that lie embedded in lipids such as cholesterol, ceramides and free fatty acids produced from the layers below during keratinocyte differentiation. The corneocytes and the surrounding lipids create a bricks and mortar structure (figure 2) responsible for preventing mechanical abrasion, microbial and allergen penetration and excessive transepidermal water loss (figure 3)

The entire process from birth (stratum basale) to desquamation (stratum corneum) from the surface takes approximately 28 days.

Some interesting facts about epidermal (keratinocyte) differentiation include:

- Every minute of the day our skin sheds 30,000-40,000 skin cells
- Our skin sheds 500 million skin cells in a day
- 3-4 kilograms of skin cells are shed each year from our skin
- We renew 1000 skins in our lifetime
- Dead skin comprises a billion tons of dust in the atmosphere

Melanocytes

Melanocytes are found at the base of the epidermis where they occupy one in every ten cells in the basal layer (figure 4). Melanocytes are responsible for the production of melanin – the pigment responsible for each person's natural skin color. Once produced, melanin is then packaged into specialised compartments called melanosomes through which melanin is transported to nearby keratinocytes after which it is dispersed evenly throughout the epidermis. Each melanocyte in the basal

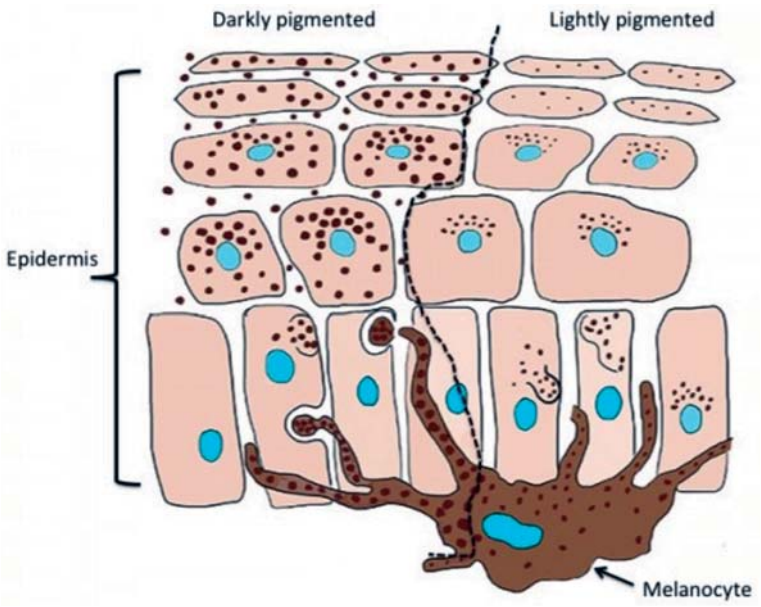


Figure 4: Melanocyte and surrounding keratinocytes

layer communicates with 30-40 nearby keratinocytes. According to one's level of skin pigment and the response of the skin to ultraviolet radiation, skin types are classified as Fitzpatrick skin types one to six (table 1). The number of melanocytes is the same in all individuals irrespective of their natural skin colour. Darker skin however contains larger numbers of larger melanosomes containing greater amounts of melanin (Figure 4)

Langerhan cells

Langerhans cells (figure 1) are present in all layers of the epidermis but are most prominent in the stratum spinosum. They can also be found in the epithelia of the digestive, respiratory and urogenital tract, and lymph nodes. Langerhan cells function in the skin immune system by processing and presenting antigens (such as pathogenic organisms) for recognition and destruction by white blood cells such as T-lymphocytes.

Merkel cells

Merkel cells (figure 1) are found in the basal layer of the epidermis. They are a specialised neural cell in the skin important in detection of light touch stimuli.

Vitamin D production

The skin is responsible for producing vitamin D needed for the development of strong bones as well as modulation of cell growth, neuromuscular and immune functions in the body.

During exposure to sunlight, ultraviolet B radiation (290-320 nanometres) penetrates into the epidermis and within keratinocytes of the stratum basale and stratum spinosum photolyzes the conversion of 7-dehydrocholesterol to cholecalciferol. Once formed, cholecalciferol enters the circulation and is sequentially metabolized to 25-hydroxyvitamin D3 in the liver and 1,25-dihydroxyvitamin D3 (active vitamin D) in the kidneys.

In summary, the epidermis – as the principal source of cellular activity within the skin, maintains homeostasis and functions as the skin's primary physical barrier to the outside world. Due to the housing of melanocytes and Langerhans cells, the epidermis is also responsible for protections against ultraviolet radiation and the entry of pathogenic microorganisms, at the same time allowing us to appreciate fine touch through the addition of specialised Merkell cells.

SKIN TYPE	SKIN COLOUR	CHARACTERISTICS
1	White; very fair, red or blonde hair; blue eyes; freckles	Always burns, never tans
2	White, fair, red or blond hair; blue, hazel or green eyes	Usually burns, tans with difficulty
3	Cream white; fair with any eye or hair color (common)	Sometimes mild burn, gradually tans
4	Brown; typical Mediterranean Caucasian skin	Rarely burns, tans with ease
5	Dark Brown; mid-eastern skin types	Very rarely burns, tans easily
6	Black	Never burns, tans very easily

Table 1: Fitzpatrick skin types

sunscreen highlights

by John Staton

how do we SPF test aerosols?

Laboratories providing testing of sunscreens have to deal with a broad range of formulation types and this is actually more complex that it would appear.

The review of the ISO sunscreen standard 24444 reaches finality with the issue of the Final Draft International Standard in September this year. Amongst the improvements, designed to reduce inter-lab variability, is a far more detailed description of how each type of sunscreen formulation is managed for sampling and application to the skin. I have set out below, a summary of these and how it is required that they be handled according to one of three methods.

Out of all of these various product type, aerosols stand out as the most difficult and controversial. To understand this, the basic steps of the test need to be explained.

In order for testing to be consistent, a very accurate amount of test product has to be applied – 2 mg/sq cm +/- 0.05 mg/sq cm over an area of the back which is 30 to 60 sq cm. For most products, this is simply a matter of weighing and applying from syringe, finger or spatula. However, the first issue for aerosols is how to manage the instantly volatile gas component.

It might appear that the product could simply be sprayed onto the designated area of the back. However, several issues arise when doing this. Firstly, the spray rate of aerosols typically ranges between 0.5 and 1 gm per second – resulting in a significant overdose if even sprayed for one second. Secondly, the applied film needs to be even over the application area in order to allow for the at least five exposure spots needed for the UV light irradiation challenge step of the test. It is not possible to achieve this directly

Method	Categories
A	**Fluid Products**
	Lotions, creams, oils, Liquids, Gels, pump sprays - aerosols after degas
	Droplets deposited by syringe, then spread in circular motion
B	**Non-flowing viscous liquids and semi-solids**
	Sticks, Balms, Roll on (A or B)
	Weighing boat and applied by finger in circular motion
C	**Powders**
	Transfer by spatula or sponge. Tapped and spread with finger. Skin may be first wet

from the can. A further issue is the rapid weight loss when handling the sample on an analytical balance, as the gas component of the aerosol typically forms at least 25% of the content.

As well as incorporating a gas content in the free space, some propellant is also dissolved in the carrier or co-solvent in which the actives are dissolved. This dissolved gas, plus the solvent, can add another 50% to volatile components. The net effect is that the dried down film on the skin , which is what is tested, can be a little as 5 microns skin cover, inherently much thinner that that from an anhydrous stick 20 micron from 20 mg/sq cm or a typical emulsions 10 microns.

The only viable approach to managing these types of formulations is as described in the new ISO document. Whilst this may not reflect in-use application of the product, it does at least indicate the performance of the final dried down film on the skin and this is to some degree in line with products such as creams and lotions which are also recognized to be subject to under application in actual use.

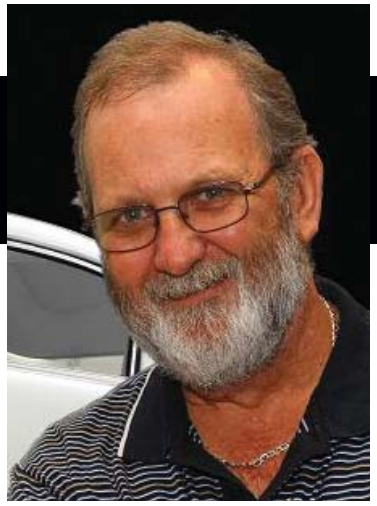
The same issues apply for conduct of the associated "Broad Spectrum" ISO 24443 test which is also part of AS/NZS 2604, as sample weighing and application to the PMMA slide are much the same as skin application on the SPF test.

The reality is that there is a margin for underuse inherent in the SPF test number, as the maximum UV dose recorded over 14 years of UV Index data is just over 70 SEDs. This is the equivalent of 35 Minimal Erythemal Doses for a Type 1 skin. In effect, an SPF 50+ product at half the test application rate and reapplied every 2 to hrs would still provide very high protection over the most challenging of summer days.

It is recognised that aerosols and pump sprays are difficult to apply evenly all over the body and that users tend to apply more like an insect repellant than a spray paint. Calculating the required full body application requirement recommendation of 30 gms of SPF products and discounting the gas component, this would require pressing the actuator on the can for at least 30 seconds and even up to 1 minute for low dispense rate products.

Inherent efficacy always needs to be supplemented by clear and appropriate instructions for use. In the same manner as for other therapeutic product categories, this is no different for sunscreens and is a requirement of TGA, FDA and regulators in most countries. Dose, frequency and other relevant directions for use are necessary. Compliance, of course, is another issue!

ISO 24444 is a laboratory method that reflects the latest state of the art in determining an SPF number and estimates comparative performance of formulations. The new version focuses on providing methodology improvements for accuracy and reproducibility.



Part 48 –

by Ric Williams

Biogradation

Biodegradation is the process by which organic substances are broken down by the enzymes produced by living organisms. Biodegradation is the most important mechanism for the total removal of chemicals from the environment. It is nature's way of getting rid of wastes by breaking down organic matter into nutrients that can be used by other organisms. The term is often used in relation to ecology, waste management and environmental remediation (bioremediation). Organic material can be degraded aerobically, with oxygen, or anaerobically, without oxygen. A term related to biodegradation is biomineralisation, in which organic matter is converted into minerals.

Biodegradable matter is generally organic material such as plant and animal matter and other substances originating from living organisms, or artificial materials that are similar enough to plant and animal matter to be put to use by microorganisms. Some microorganisms have the astonishing, naturally occurring, microbial catabolic diversity to degrade, transform or accumulate a huge range of compounds including hydrocarbons (e.g. oil), polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), pharmaceutical substances, radionuclides and metals. Major methodological breakthroughs in microbial biodegradation have enabled detailed genomic, metagenomic, proteomic, bioinformatic and other high-throughput analyses of environmentally relevant microorganisms providing unprecedented insights into key biodegradative pathways and the ability of microorganisms to adapt to changing environmental conditions.

Bioremediation is the engineered process of application of biological means (including bacteria, algae, fungi, etc.) to degrade a material. Biodegradation is a slow process process, while bioremediation is a faster process. ... Biodegradation, on the other hand, is controlled by nature.

Aerobic Biodegradation

It is the breakdown of organic contaminants by micro-organisms when oxygen is present. Organic contaminants are rapidly degraded under aerobic conditions by aerobic bacteria called aerobes.

Anaerobic biodegradation in landfill

Biodegradable waste in landfill degrades in the absence of oxygen through the process of anaerobic digestion. The byproducts of this anaerobic biodegradation are biogas and lignin and cellulose fibres which cannot be broken down by anaerobes (anaerobic microbes)

Engineered landfills are designed with liners to prevent toxic leachate seeping into the surrounding soil and groundwater. Paper and other materials that normally degrade in a few years degrade more slowly over longer periods of time. Biogas

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This column is intended not only as an education tool for non-technical people or beginners in our industry, but as a forum for those wishing to enlighten all about recent technology advances and new ideas. I hope experienced scientists will also contribute to this ideal and if you wish to do so please email me at: ric@cosmepeutics.net.au and I will publish your comments.

contains methane which has approximately 21 times the global warming potential of carbon dioxide. In modern landfills this biogas can be collected and used for power generation.

Methods of measuring biodegradation

Biodegradation can be measured in a number of ways. The activity of aerobic microbes can be measured by the amount of oxygen they consume or the amount of carbon dioxide they produce. Biodegradation can be measured by anaerobic microbes and the amount of methane or alloy that they may be able to produce.

Measurement of aerobic decomposition

The DR4 test or 4-day dynamic respiration index test is a test to measure the biodegradability of a substance over 4 days. The substance is aerated by passing air through it. This definition is used to determine the method from those where aeration is by diffusion of air into and out of the test material which is referred to as the SRI or static respiration index test. Microbes are introduced to the test material while incubating it under aerobic conditions by aerating the mixture in a vessel through which air is blown. The microbes degrade the material producing CO_2 as the product of biodegradation. This CO_2 production can be monitored as a measure of the biodegradability of the test material and converted into oxygen consumption units.

Measurement of anaerobic decomposition

BMP100 test, 100 day biogenic methane potential test, is a test method that determines the potential biodegradability of biodegradable wastes under anaerobic conditions by measuring the production of biogas. The test has not been peer-reviewed by the international community and no known official publication exists for it. Other published tests that accomplish this in shorter time are the GB21 protocol (DIN 38414).

Under anaerobic methanogenic conditions the decomposition of organic carbon proceeds by producing biogas (containing methane and carbon dioxide)from the organic carbon. The amount of biogas production therefore measures directly the carbon which is mineralised. The test is set up in a small vessel containing the test substrate, a mineral aqueous medium and an inoculum of methanogenic bacteria taken from an active anaerobic digester. The test is monitored by collecting and measuring the biogas produced. The test is incubated for an extended period until gas production ceases which may be up to 100 days or more; for all practical purposes most organic materials reach the majority of decomposition in less than 45 days. By being run so long, however, the BMP100 test therefore measures the complete degradation of the waste.

Plastics

Biodegradable plastics made with plastarch material (PSM), and polylactide (PLA) will compost in an industrial compost facility. There are other plastic materials that claim biodegradability, but are more often (and possibly more accurately) described as 'degradable' or oxi-degradable; It is claimed that this process causes more rapid breakdown of the plastic materials into CO_2 and H_2O.

Indicative lengths of degradation

The following table should be read with the above comments in mind, and care should be taken before accepting claims of biodegradability in view of the (dubious) claims being made. This is how long it takes for some commonly used products to biodegrade: (from http://www.worldwise.com/biodegradable.html)

Banana peel,	2 – 10 days
Cotton, rags	1 – 5 months
Sugarcane Pulp Products,	30 – 60 days
Paper,	2 – 5 months
Rope,	3 – 14 months
Orange peels,	6 months
Wool socks,	1 – 5 years
Cigarette filters,	1 – 12 years
Tetrapaks (plastic composite milk cartons),	5 years
Plastic bags,	10 – 20 years
Leather shoes,	25 – 40 years
Nylon fabric,	30 – 40 years
Plastic six-pack holder rings,	450 years
Diapers and sanitary napkins	500 – 800 years
Tin cans	50 – 100 years
Aluminium cans	80 – 100 years
Plastic Bottles	non-biodegradeable
Styrofoam cup,	non-biodegradeable

Botanical skin care bases (esp. body lotion, moisturiser, mild face wash gel, cleanser cream, night cream) generally contain all biodegradable materials however in some cases there will be minor components (eg EDTA, Carbomer and Dimethicone) that are not biodegradable. These are being changed as alternate materials are evaluated.

Oils

Biodegradability of common cosmetic ingredients (CEC) %

Mineral Oil	10 to 35
Glycols	10 to 100
Vegetable Oils	70 to 100
Synthetic Esters	10 to 100

100% pure essential oils, fragrant & carrier oils are biodegradable

Surfactants

Soap is still a commonly used surfactant. However, the poor solubility of soaps precipitated with metal ions influences biodegradation rates. Soaps are susceptible to precipitation within hard water environments. Schöberl et al. [55] reported that Sturm-tested sodium soap salts exhibited mineralisation

rates of 80–90% whereas calcium soap salts were significantly less biodegradable with only 67% mineralised. Association of soaps with metal ions leads to precipitation of a significant quantity of soap. Precipitation leads to sedimenting of soap in the primary settling tanks at WWTP. Therefore, depending upon the hardness of water at an individual WWTP sewage sludge may contain a significant amount of soap. Anaerobic degradation of soap is an important factor in determining the effectiveness of WWTP and the degradation of these compounds. Results suggested that the soap was 92–97% degraded during a 28 day time period. Birch et al. observed a 79–94% degradation of sodium palmitate during a 3–4 week period of anaerobic digestion of sludge. The data suggest that soap is readily biodegradable in both aerobic and anaerobic environments and therefore is ultimately treatable within the conditions and residence time of wastewater.

Surfactant	aerobic degradation	anaerobic degradation
Plant-derived oil soaps	Yes	Yes
Fatty alcohol sulfates (FAS)	Yes	Yes
Sugar surfactant (APG)	Yes	Yes
Linear alkyl benzene sulfonate	Yes	No

- Triclosan (sometimes abbreviated as TCS) is an antibacterial and antifungal agent present in some consumer products, including toothpaste, soaps, detergents, toys, and surgical cleaning treatments. Its efficacy as an antimicrobial agent, the risk of antimicrobial resistance, and its possible role in disrupted hormonal development remains controversial. Triclosan was developed in the 1960s. Despite its high chemical stability, being extremely resistant to high and low pH, triclosan is readily degraded in the environment via photodegradation.
- minerals (including dead sea mineral mud) is not biodegradable as the mud itself is inorganic and not susceptible to microbial attack.

Thanks for your attention.

Next Issue Emollients

Technical Committee – outline of activities/responsibilities

The ASCC Technical Committee is a voluntary body of independent technical (Full) members of the Australian Society of Cosmetic Chemists, which acts as the scientific committee for the benefit of all Australian Society of Cosmetic Chemists' members.

They meet five to six times per year and communicate regularly by email.

The Committee may take up any technical/scientific aspect which may impinge upon the cosmetics/personal care industry. As noted earlier they act as the scientific advisory committee for the benefit of members. As such they may respond to a wide range of matters, from members, and respond to inquiries for information or assistance, from outside sources (such as Government or Statutory Bodies, Special Interest Groups or the public), on general scientific matters.

The ASCC Technical Committee liaises with other relevant bodies such as ACCORD in areas of mutual concern (e.g. certain regulatory areas).

The Technical Committee reports to the ASCC Council, as do the various State Chapters.

Nature and Scope

Regulatory

Input/comment/ participate in the review/drafting of ISO Standards and AUS/NZ Standards as well as recommendations on matters which impact on the industry such as NICNAS, TGA, and other proposed regulations (e.g. Microbeads, Animal Testing)

Sunscreens

Australia was the frontrunner in sunscreen testing and ASCC plays a major role in the updating of the ISO and Australian standard on Sunscreens.

Position Papers and Position Statements

These are prepared, under the guidance of the ASCC Technical Committee for specific topics to present the position of the ASCC clearly and scientifically, to inform & educate both members of the ASCC and the public at large.

The Technical Committee addresses various 'Hot Issues' such as Sustainability/RSPO, Claim Substantiation, Sunscreens and Vitamin D, Natural/Organic and Cannabis in Cosmetics, often these becoming position papers or position statements.

Conference Papers

The ASCC Technical Committee is responsible for classifying and approving conference papers, from the ASCC Conference, as well as some external sources, conferences and magazines. These are published in The Science of Beauty and bearing the ASCC logo.

Classifications are;

– *Technical*

A paper containing all the elements of what is considered a scientific paper (with no advertorial content).

If not a pure technical paper then the ASCC Technical Committee may decline the publication, or classify the submitted paper as

– *Educational*

Not a true scientific paper but deemed of interest to educate the readership.

or

– *Advertorial*

Has some small amount of commercial content but worthy of publication.

Current members (as at Sept 1, 2019)

Chairperson **Ric Williams**, (NSW)

Vice Chairperson **Ranelle Anderson** (Vic)

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Active Beauty

Skin microbiota and stem cells: delicate balance for healthier skin

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1. Abstract

Healthy skin is a delicate balance between skin renewal and microbiota homeostasis, and its imbalance promotes premature ageing and dermatological disorders. Skin stem cells are key actors in this process and unfortunately are very sensitive to ageing and external stressors such as UV, pollution, chemical agents which promoted their cell death while reducing significantly the skin renewal power. The Stratum microbium™ has been recently described as active in the skin healthier. Its imbalance could trigger some skin disorders and promoting skin ageing. In this study, we hypothesized that reactivation of skin stem cells and maintenance of skin microbiota could be an disruptive strategy for younger and healthier skin. We developed a new plant extract that restores the entire skin renewal process by sequential activation from stem cells stimulation to Stratum microbium™ protection. First, we demonstrated an active protection of skin stem cells through the maintenance of their clone-forming capacity and resistance to UV trough the over-expression of surviving coupled to caspases inhibition. The activation of the stem cells generated an increase of high proliferative multilineage cells. Furthermore, we showed the high rejuvenation potential of the product through the restoration of epidermal differentiation markers and ceramide biosynthesis favorable to orthorhombic organization. Clinical studies, including Metagenomic analysis showed an active skin surface renewal coupled with Stratum microbium™ protection leading to younger and healthier skin aspect. For the first time, we evidenced that an active ingredient is able to stimulate skin rejuvenation while protecting the cutaneous microflora, creating healthier skin and thereby beauty.

2. Introduction

The epidermis is a stratified squamous epithelium whose outermost layer is the skin surface. The basal layer is the most mitotically active and produces, secretes and assembles an extracellular matrix (ECM), which constitutes the basement membrane. Cells from basal layer express β1-integrin which participates to establishment of epidermal polarity and tissue architecture. During the skin renewal, cells leave the basal layer and move toward the skin surface during this process cells is subjected to molecular modifications as switch off integrin and execute the terminal differentiation program to finish by corneocytes. In the basal layer, there is the presence of progenitor cells able to ensure the tissue self-renewal in the absence of injury and ensure the tissue repair during aggressions. Moreover, the stem cells contained in the hair follicle epidermis are mulitpotent and participate to a new hair cycle, hair follicle regeneration and repair the epidermis. The balance of skin renewal mechanism is important to conserve a perfect equilibrium between the elimination of dead cells and the epidermal differentiation permitting to have a healthy skin aspect.

The differentiation of the epidermis is a process in which keratinocytes of the basal layer undergo metabolic and structural changes during their transfer to the surface of the skin. The cornification is the last step of this process and results in the stratum corneum formation. The stratum corneum contains large quantities of lipids (ceramides, cholesterol and free fatty acids) and proteins responsible for epidermal barrier homeostasis. Ceramides (CERs) consist of a long amino alcoholic chain, sphingoid moiety (sphingosine [S], DihydroSphingosine [DS] and PhytoSphingosine [P]) covalently bound via an amide linkage to a fatty acid

Fatty acid / Sphingoid	Non-hydroxy fatty acid [N]	α-hydroxy fatty acid [A]	Esterified ω-hydroxy fatty acid [EO]
Dihydrosphingosine [DS]	CER[NDS]	CER[ADS]	CER[EODS]
Sphingosine [S]	CER[NS]	CER[AS]	CER[EOS]
Phytosphingosine [P]	CER[NP]	CER[AP]	CER[EOP]
6-hydroxy sphingosine [H]	CER[NH]	CER[AH]	CER[EOH]

Figure 1: organization of ceramides according their fatty acid and sphingoid base components.

moiety ([N] and [A]). Ceramides have important physicochemical roles in the barrier function and water-holding property of skin. The main ceramides identified were linked to a chain length of 16 carbon to 24 carbon atoms for the fatty acids and a chain lengths of 18 carbons for basis.

The stratum corneum formation is also dependent to transglutaminase-1, 3 and 5 expressions. They ensure the covalent bonds between the different proteins forming the stratum corneum such as involucrin, and loricrin. Loricrin is synthesized in the granular layer. During the formation of the stratum corneum, loricrin migrates to the cell periphery and is covalently linked by transglutaminase-1 to involucrin to form the cornified envelope.

- Involucrin is a structural protein of the horny layer, involved in the initiation stages of corneocytes maturation.
- Caspase-14 is expressed and activated mainly in the epidermis. It plays a role in the programmed cell death of keratinocytes leading to formation of the stratum corneum. It is also responsible for "processing" and degradation of pro-filaggrin
- Hornerin is a protein of the stratum corneum and the stratum granulosum. Hornerin contributes to maintaining the barrier function of the epidermis.
- Filaggrin is a key protein required for the formation of the stratum corneum (SC) and also is essential for SC hydration as a source of hygroscopic amino acids and their derivatives, known as natural moisturizing factors (NMFs).

Tight junctions and desmosomes are cell-cell junctions essential for maintaining the barrier function and the cohesion of the epidermis. Claudin is a protein found in tight junctions. Desmocollin-1 (DSC1) and desmoglein-1 (DSG1) are two components of the desmosomes. During differentiation, desmosomes mature in corneodesmosomes in the stratum corneum. Corneodesmosin, a component of these structures, is cleaved and degraded by kallikreins 5 and 7 to allow the release of corneocytes and hence desquamation of the skin. Increased expression of genes KLK5, KLK7 and SPINK5 would therefore promote epidermal renewal.

The skin desquamation process is located in the stratum corneum. The turnover time of the stratum corneum is normally two to four weeks. As epidermis is constantly in self-renewal, the superficial desquamation is adequately compensated by keratinocyte proliferation in epidermal basal layer. In order to maintain a constant stratum corneum thickness at a given body site superficial parts of the stratum corneum must be continuously shed in the process of desquamation at a rate,

Exhaustive *in vitro*, *ex vivo* and 4 clinical studies have demonstrated that Revivyl™ has an overall reactivation effect of the natural skin renewal cycle from bottom to top by means of 6 synergistic actions:

1. Protection and activation of skin stem cells
2. Reactivation of cellular metabolism
3. Stimulation of cellular differentiation
4. Reinforcement of skin barrier components
5. Reactivation of natural exfoliation
6. Active protection of skin microflora

This multifunctional active ingredient delivers consumers benefits in only 2 weeks, showing a better skin moisturisation and an improvement of skin surface differentiation with up to 30% wrinkle reduction, *versus* the placebo.

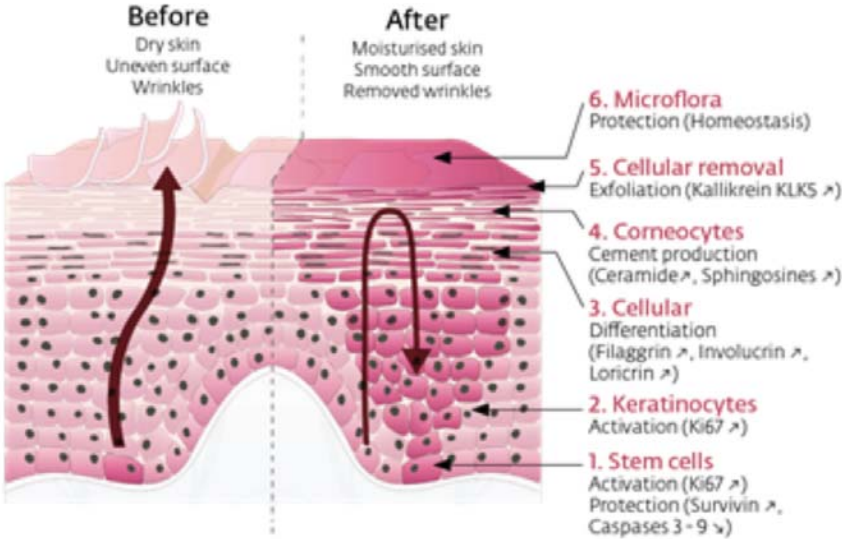


Figure 2: Mechanism of action

which balances de novo production of corneocytes. A central event in desquamation is elimination of corneocyte cohesion by degradation of corneodesmosomes by enzymes. The enzymes (KLK5 and KLK7) involved in desquamation were delivered into the intercellular spaces through the lamellar bodies. In physiological conditions, corneodesmosome degradation that leads to a controlled desquamation is a prerequisite to normal recycling of the stratum corneum and epidermal barrier function. Desquamation normally occurs invisibly with shedding of individual cells (corneocytes) or small aggregates of cells. Disturbances in this process results in the accumulation on the skin surface of only partially detached cells with or without a concomitant thickening of the stratum corneum. The accumulation on the skin surface of the cells and debris is combined with a feeling of roughness and dryness of the skin surface, to the accumulation of thick scales.

Orobanche rapum extract was discovered during a bio-guided fractionation of a unique chlorophyllfree plant called Orobanche rapum, living in synergy with the "golden butterfly" plant, Cytisus scoparius. This novel active ingredient has been specifically enriched in two specific polyphenol sugar esters: crenatoside and acteoside, known for their very high antioxidant activities.

3. Material and methods in tubo and in vitro assessments

In vitro assessments of Orobanche rapum extract were done on human stem cells and normal human keratinocytes throughout two studies.

- The first was related to the impact of the active on surviving expression in presence of UVs exposure on stem cells from hORS (human Outer Root sheath).
- The third study was based on the effect of the active on gene expression in normal human foreskin keratinocytes.

In tubo assessment of Orobanche rapum extract was done without cells, to determine the capacity of the active to inhibit enzymatic activities of caspase 3 and caspase 9.

3.1 Enzymatic inhibition assays for caspase 3 and caspase 9

The assay systems used for the evaluation of caspase 3 and caspase 9 enzymatic activities were composed by purified caspase 3 or caspase 9 enzyme on one hand, and their specific luminescent substrates on the other hand. Caspase 3 or caspase 9 plus their specific substrate were incubated in absence (control) or in presence of reference product or of increasing concentrations of the active (0.1; 0.5 and 1%). The active was diluted directly in the reaction buffer. Ac-DEVD-CHO at 5nM was used as reference inhibitor of Caspase 3 enzyme. Ac-LEHD-CHO at 0.5µM was used as reference inhibitor of Caspase 9 enzyme.

At the end of the incubation period (20 minutes for caspase 3 and 30 minutes for caspase 9), the product formed from the cleavage of the specific substrate by caspase, was measured by using a luminometer.

Results are expressed as RLU (mean ± S.D.). Level of significance between "Control" and "reference product" has been assessed using a Student t test (*: $p<0.05$). Level of significance between "Control" and "test product" has been assessed by a one factor variance analysis (One way ANOVA) followed by a Holm-Sidak test (*: $p<0.05$).

3.2 Cellular culture conditions

For Stem cells from hORS:

Cells used in this study are primary normal human hair follicle Outer Root sheath cells (hORS) provide by Celprogen (USA). The active was applied in the culture medium for 24h or 48h at 0.5%.

For gene expression study:

The study was carried out on normal human foreskin keratinocytes. The active was applied in the culture medium of keratinocytes for 24 hours at 1 mg/mL.

3.3 In vitro clone forming observation

hORS were seeded at 5000 cells/well in 96-wells microplates and were left to adhere for minimum 24h at 37°C/5% CO2 in complete medium. Cells were treated with the product at 0.5% or not in serum free medium for an incubation of 48h at 37°/5% CO2. The morphological aspect was analysis by optical microscopy in phase contrast (Olympus CK40). The capacity to forming in vitro clones was only appreciated visually.

3.4 Survivin, β1-Integrin and p63 immunostaining on human stem cells (hORS)

hORS are seeded on 8-wells slides or Petri dishers (between 10 000 to 60 000 hORS/cm²) and were left to adhere for 1 to 3 days at 37°C/5% CO2 in complete medium. Cells were pre-treated for 24h with or without the product at 0.5% and were then irradiated or not for 25 min at UVA (8J/cm²) and UVB (150J/cm²) in a saline buffer. After the UV exposure, cells were treated again with or without the active at 0.5% for 24h. Cells were incubated at 37°/5% CO2 for all the treatment times and applications were done in serum-free medium. Cells were finally fixed with formaldehyde (4%) and only for surivin they were permeabilized with 0.1% Triton X-100. Unspecific fixation sites were blocked with serum from the same species as the secondary antibody (santa cruz). After primary antibodies incubation (Survivin Novus-biologicals NB500-237, β1-integrin Santa Cruz sc-9970 and p63 Abcam) for room temperature or 4°C, cells were incubated with secondary antibody coupled to a fluorochrome (Alexa type, Invitrogen 546 nm) and DAPI (Roche) coloring nucleus. Finally, slides were mounted with a mounting medium (DAKO) that provided fluorescence protection and coverslips adhesion on the materials.

The fluorescence was analyzed with a fluorescence optic microscope (Olympus CK40) with appropriate filters to the fluorochrome. Images acquisition was done with Archimed logiciel (Microvision). The quantification was performed by Colombus software

(PerkinElmer) by HCS Pharma (Rennes, France), as mean intensity (12 images for each condition)/ It was the mean value of intensity for each pixel of staining, contained in the red-stained cell area.

3.5 qRT-PCR TaqMan array: Gene expression analysis

At the end of treatments, total RNA were extracted using RNeasy mini Kit (Qiagen) and sample integrity was analysed by spectrophotometry and capillary electrophoresis. cDNA were then synthesized from mRNA by reverse transcription the high capacity RNA-to-cDNA kit (Applied Biosystems, 438706) according to the manufacturer's instructions, from 2 µg of total RNA. This reaction was carried out at 37 ° C for 1 hour, then 5 minutes at 95 ° C. The cDNA were then stored at -20°C.

The effects of test compound on gene expression was analysed by using 96-genes microfluidic TaqMan qPCR arrays designed by StratiCELL and manufactured on demand by Applied Biosystems. Among them, 2 internal controls or reference genes, and 94 genes of interest are represented. The TaqMan arrays were processed as described by the manufacturer's instructions (Micro Fluidic Card Getting Started Guide, Applied Biosystems). PCRs were executed with the 7900HT Fast Real-Time System (Applied Biosystems). The thermal cycles were programmed with one incubation step at 50°C during 2 min followed by a first denaturation step at 95°C during 10 min. The amplification protocol was followed with 40 cycles (15s at 95°C and 1min at 60°C). The relative expression level was calculated by the comparative Ct (DDCt) method (Pfaffl, 2001 & Livak and Schmittgen, 2001). The calculation consisted in comparing the Ct values obtained for conditions treated by the active with the reference condition (untreated control). These Ct values wer first normalized relatively to the housekeeping gene B2M (β2-microglobuline). The maximum accepted Ct value used for calculation was set to 36 cycles.

4. Results of in tubo and in vitro investigations

4.1 Survival and apoptosis resistance of skin stem cells

4.1.1 Maintenance of the clone forming capacity of human stem cells in vitro (hORS:human Out Root Sheath)

Human Stem cells from Out Root sheath are cultivated for 48h in absence or with Orobanche rapum extract at 0.5%. Optical microscopy investigation demonstrates that the product promotes the in vitro clone forming in comparison with untreated cells. This result shows that Orobanche rapum extract induced the maintenance of stem cells status in vitro (figure 3).

4.1.2 Human Stem cells (hORS) protection against UV stress by overexpression of surviving and b1-integrin

Survivin expression:

Orobanche rapum extract shows no impact on the survivin expression on stem cells in comparison with untreated cells at basal level.

The expression of survivin is drastically and significantly decreased after UV exposure (-66%). Orobanche rapum extract protects the stem cells from UVs exposure as observed by the

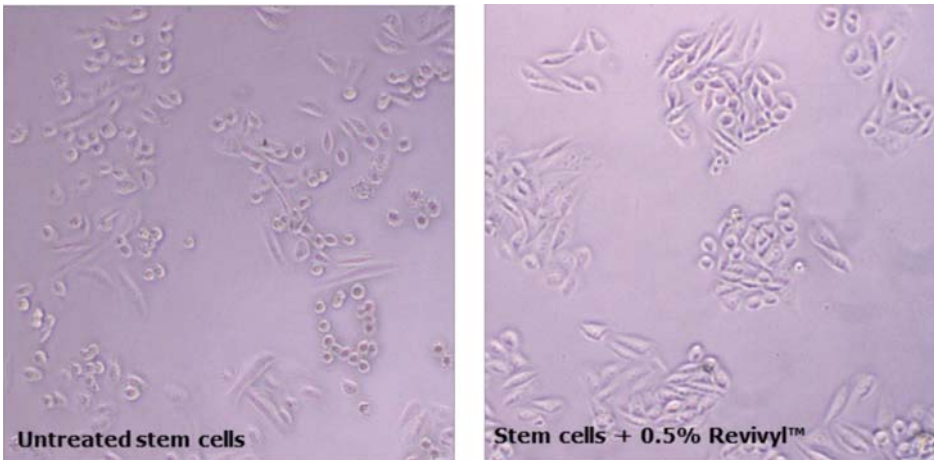


Figure 3: Human stem cells cultivated without or with Orobanche rapum extract at 0.5%

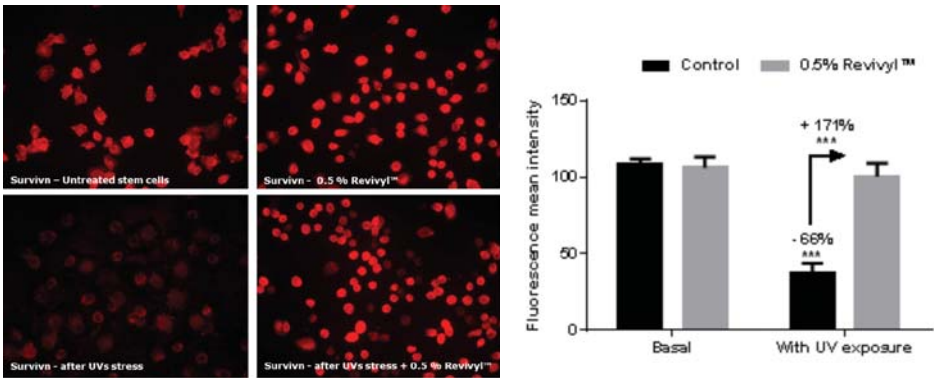


Figure 4: Survivin immunostaining on human Stem cells in absence or in presence of Orobanche rapum extract at 0.5% in basal and after UV exposure.

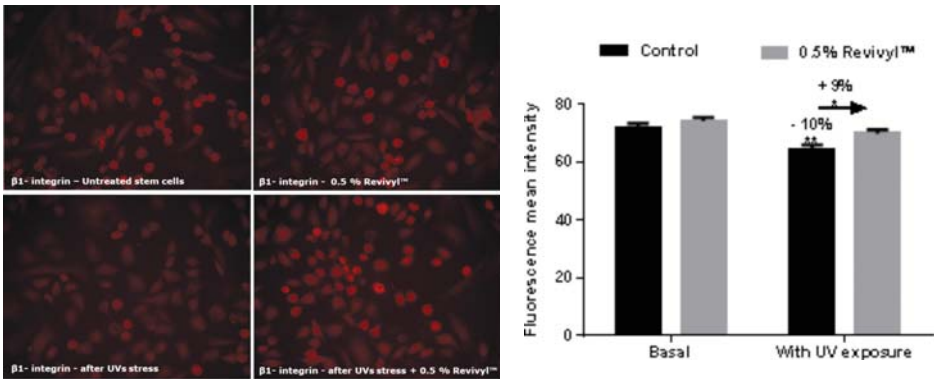


Figure 5: β1-Integrin immunostaining on human Stem cells in absence or in presence of Orobanche rapum extract at 0.5% in basal and after UV exposure.

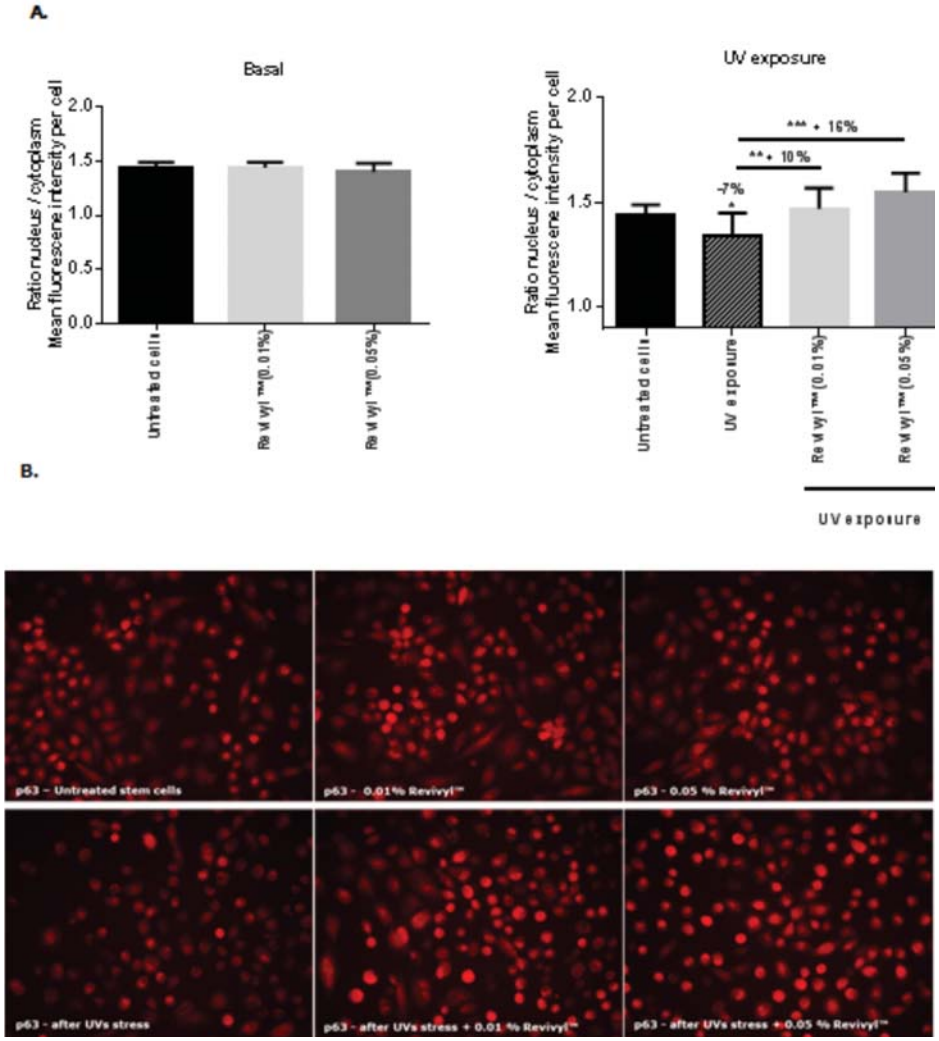


Figure 6: p63 immunostaining on human Stem cells in absence or in presence of Orobanche rapum extract at 0.01 and 0.05% in basal and after UV exposure.

survivin restoration (+171%) (figure 4).

Surivvin is a stem cells biomarker and present two different cell localizations associated with its both biological functions. Its nuclear localization is involved in the control of cell division and it is correlated to stem cells status. But survivin can be expressed in cytoplasm where it plays an active antiapoptotic role by direct effect on caspase 9 inhibition and indirectly on caspase 3 inhibition. Consequently, an overexpression of survivin is favorable to a strong defense against proapoptotic stress like UV exposure.

Orobanche rapum protects stem cells against UV stress through an overexpression of surviving.

β1-Integrin expression:

Orobanche rapum extract slightly increases the β1-Integrin expression in comparison of untreated cells.

The expression of β1-Integrin is significantly decreased after UV exposure (-10%). Orobanche rapum allows to maintaining the stem cell status after a UV stress as observed by the significant overexpression of β1-Integrin (+9%) (figure 5).

p63 expression:

p63 is a molecule involved in maintenance of the proliferative potential of embryonic and adult epithelial stem.

At basal level, our product didn't induced modification of p63 expression on Stem cells.

The UV exposure significantly decreased p63 expression (-7%). The preincubation with the product at 0.01% and 0.05% promoted a significant up of p63 expression after UV-exposure. These results proved that the product protected the Stem cells against UV exposure and permitted to maintain their status (figure 6).

4.1.3 Caspase 3 and caspase 9 enzymatic activities

The active tested at 0.1%; 0.5% and 1% significantly inhibits (table 1 and 2):

- caspase 3 enzymatic activity respectively by 12.3%,17.8% and 31.3%
- caspase 9 enzymatic activity respectively by 7.8%, 14.7% and 25.2%

Orobanche rapum extract is able to inhibit directly both caspases 3 and 9 reinforcing the cells protection and cell resistance to apoptosis.

Conclusion

- Orobanche rapum extract allows the enrichment of the stem cells pool by the maintenance of clone forming capacity in vitro

Caspase 3 inhibition (luminescent signal in arbitrary unit)	Control	Inhibitor "Ac-DEVD-CHO" (5nM)	Revivyl™		
			0.10%	0.50%	1%
Mean	402397	210693***	352854***	330805***	276525***
S.D	12000	18532	12387	5404	10012
% of inhibition versus "Control"	0	47.6	12.3	17.8	31.3

Table 1: Evaluation of inhibitory effect of the active ingredient on caspase 3 enzymatic activity. *(***: mean significantly different from "control" group (p<0.001))*

Caspase 7 inhibition (luminescent signal in arbitrary unit)	Control	Inhibitor "Ac-LEHD-CHO" (0.5µM)	Revivyl™		
			0.10%	0.50%	1%
Mean	18031	4678***	16631**	15380***	13493***
S.D	74	92	636	425	107
% of inhibition versus "Control"	0	74.1	7.8	14.7	25.2

Table 2: Evaluation of inhibitory effect of the active ingredient on caspase 9 enzymatic activity. *(**: mean significantly different from "control" group (p<0.01); ***: mean significantly different from "control" group (p<0.001))*

- The product induces stem cells survival and their resistance against UV stress through a relevant overexpression of survivin and β1-integrin.
- In addition, Orobanche rapum extract is able to limit the apoptosis by direct inhibition of two key caspases including Caspases 3 and 9.

4.2. Epidermal differentiation and skin barrier function

4.2.1. Gene expression analyses studies

The active at 1mg/mL regulates positively several genes involved in the differentiation of keratinocytes. This is the case of TGM-1 genes (Transglutaminase-1: x3.1), TGM-3 (Transglutaminase-3: x5), FLG (Filaggrin: x4.5), LCE2B (Late cornified envelope protein 2B: x3.1), HRNR (Hornerin:x 3.5), CASP14 (Caspase 14: x 4.3), FLG2 (Filaggrin-2: x 5), IVL (Involucrin: x3.3), SPRR1A (Small Proline-Rich protein 1A, or Cornifin-A: x3.6), ZNF750 (protein Zinc Finger 750: x 3.1), and LOR (Loricrin: x15.1). Among other genes positively regulated by the active, we have observed the overexpression of DSC1 (Desmocollin-1: x6.9), DSG (Desmoglein-1: x7.2), KLK5 (Kallikrein-5: x2.7), KLK7 (Kallikrein-7:x 2.5), and SPINK5 (Serine protease inhibitor Kazal type 5: x3.7).

We also observe a positive regulation of mRNA expression of gene involved in lipid synthesis ABCA12 (ATP-binding cassette subfamily A member 12: x 1.8) (table 3).

The active induces a strong expression of LOR at more than 15X the level observed in the control and enables simultaneous inductions (more than 3x) of TGM1 and TGM3.

Functions	Name of genes	Fold change	*p* value
Desquamation of stratum corneum	**KLK5** (kalikrein 5)	2.7	0.0068
	KLK7 (Kalikrein 7)	2.5	0.0149
	SPINK5 (Serine Protease inhibitor Kazal type 5)	3.7	0.0066
Formation of cornified layers	**FLG** (Filaggrin)	4.5	0.0029
	FLG2 (Filaggrin-2	5	0.0016
	LOR (loricrin)	15.1	0.0007
	IVL (Involucrin)	3.3	0.0074
	CASP14 (caspase 14)	4.3	0.0134
	HRNR (Hornerin)	3.5	0.0398
	SPRR1A (Small Proline-Rich protein 1A or cornifin A)	3.6	0.0141
	ZNF750 (Zinc Finger Protein 750)	3.1	0.0152
Lipid synthesis	**ABCA12** (ATP-binding casseette sub-family A member 12)	1.8	0.029
Granulosum layers	**LCE2B** (Late cornified envelope protein 2B)	3.1	0.0397
	TGM3 (Trasnglutaminase 3)	5	0.0043
	DSG1 (Desmoglein-1)	7.2	0.0077
	DSC1 (Desmocollin-1)	6.9	0.0001
Spinosum layers	**TGM1** (Transgluatminase 1)	3.1	0.0033

Table 3: Genes modulated by Orobanche rapum extract at 1 mg/mL (model: normal human foreskin keratinocytes). RQ means relative gene expression in comparison to untreated cells (RQ > 1: increase gene expression and p values were mentioned to confirmed the statistically significance of the results (p-value < 0.05)).

The biological function of each gene is described in the section 3 and briefly summarize in the schema below:

The table 3 reports all the significant results obtained on gene expression organized according to their biological function in epidermal differentiation and skin barrier property:

4.3 Conclusion of in tubo and in vitro studies

- The active protects the stem cells (ORS) through UV exposure by Survivin and p63 overexpression.
- The overexpression of β1-integrin expression after UV exposure confirms the protection and the maintenance of Stem cells
- The active demonstrates an efficacy regarding the inhibition of enzymatic activities of two caspases involved in cell apoptosis: the "caspase 3" and "caspase 9". Moreover, this inhibition was dose dependent.
- Stimulation of mRNA expression of several genes involved in the differentiation of the epidermis and maintenance of barrier function

These results indicate that the active could be a potential candidate for the maintenance of skin renewal and barrier function. Increased expression of genes such as survivin, KLK5, KLK7 and SPINK5 would therefore promote epidermal renewal.

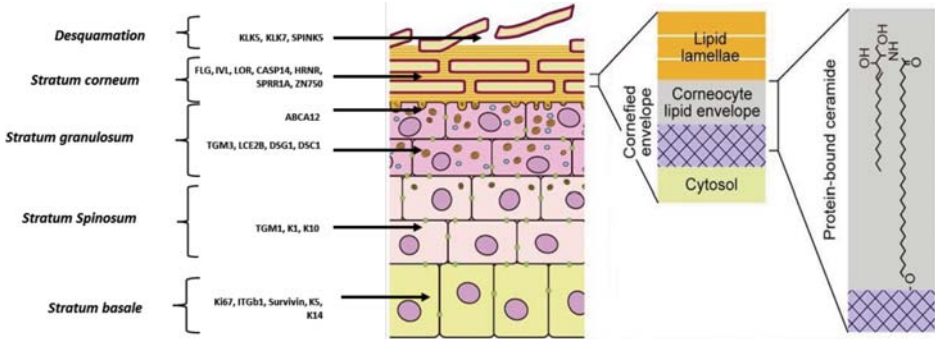


Figure 7: Schematic representation of expression of the epidermal differentiation markers

5. Material and methods ex vivo assessments of orobanche rapum extract

Ex vivo assessments of Orobanche rapum extract were done on skin explants maintained in survival throughout one study and on human epidermis reconstructed in two studies.

o Study 1: The aim of the first study done on skin explants was related to the study of the effect of the active to modulate the stratum corneum desquamation, the expression of enzymes involved in desquamation process (KLK5, KLK7) and the

expression of Ki67 a wellknown proliferation marker.

- o Study 2: The aim of the second study done on human epidermis reconstructed was related to the effect of the active on markers involved in epidermis differentiation.
- o Study 3: This study was done on inflammatory human epidermis reconstructed to mimick an epidermis having a barrier default. In this model, we studied the effect of the active to repair this barrier default at the lipids level.

5.1 Study 1 related to desquamation process and epidermis renewal (stratum corneum cohesion, kallikrein enzymes and cell proliferation)

5.1.1. Skin explants treatment:

Human skin explants were obtained from abdominoplasty or mammal reduction from 8 healthy donors (aged from 31 to 67 years old). Skin explants were placed on inserts composed by a porous membrane (8 µm) allowing the diffusion of survival culture milieu. Explants were either left untreated (control) or treated with the active at 0.5% or with the placebo at D0 and D1. The products after application (2 mg/cm²) were left on skin surface without any cleaning (to avoid stratum corneum shedding due to mechanical cleaning). The products were applied on days 0, and D1 in the mentioned concentrations topically on the basis of 2 mg per cm2, using a small spatula. The culture medium was renewed at day 1.

These skins explants were used for the assessment of stratum corneum cohesion, KLK5 and KLK7 expressions and Ki67 expression.

5.1.2. Assessment of the effect of the active on stratum corneum cohesion to determine its desquamation properties

Histological analysis:

Preparation of explants for histological analysis:

After treatment at D2, 3 explants per donor were fixed in buffered formalin. After the fixation in formalin, the samples were dehydrated and impregnated in paraffin. The samples were then embedded and sections were made using a microtome. The observation of the general morphology was performed after staining of formalin fixed and paraffinized sections by hemaluneosin.

Grading of stratum corneum cohesion:

The assessment of stratum corneum cohesion is directly related to exfoliation activity of the products. Based on skin histological sections, stained with hemalun eosin the anatomopathologist scored the stratum corneum cohesion following this grading:

- Score 0: no modification of stratum corneum cohesion
- Score 1: slight decrease of stratum corneum cohesion
- Score 2: moderate decrease of stratum corneum cohesion
- Score 3: important decrease of stratum corneum cohesion
- Score 4: very important decrease of stratum corneum cohesion

The cohesion assessment was performed on the whole section (10 to 15 fields at the magnification x40 were analysed).

5.1.3. Assessment of the effect of the active on the expression of enzymes involved in desquamation process (KLK5 and KLK7)

Kallikrein 5 (KLK5) and Kallikrein 7 (KLK7) expressions were analyzed by immunohistochemistry using rabbit polyclonal antibodies antiKLK5 (SIGMA, ref HPA014343) and antiKLK7 (SIGMA, réf HPA06226). The technic used was based on indirect immunoperoxydase (kit Impress, vector) and revealed with AEC (3-amino-9-éthylcarbazole). Then semi-quantitative scores were determined according the staining intensity at the level of stratum granulosum and stratum corneum. The scores were from 0 to 4 corresponding to a low to high intensity.

5.1.4. Assessment of the effect of the active on the expression of ki67 (a proliferation marker)

Cell proliferation was analyzed by immunohistochemistry using antiKi67 antibody. The technic used was based on indirect immunoperoxydase (kit Impress, vector) and revealed with AEC (3-amino-9-éthylcarbazole). Cells stained were counted and reported to the total cell number in the basal layer to determine the percentage of stained cells.

5.2. Study 2 related to epidermis differentiation process

This study followed the gene expression study and was done to confirm the results at the protein level for some markers after topical application of the formulated Orobanche rapum extract active ingredient. The study consisted in immunostaining of reconstituted human epidermis sections, and fluorescence measuring of the abundance of 3 differentiation markers: Involucrin (IVL), Loricrin (LOR), and Filaggrin (FLG). The abundance of markers was compared between epidermis treated with the active agent and those treated with the placebo, in presence or absence of a disrupted barrier function induced by stripping.

5.2.1. RHE treatment:

The study was performed using Reconstructed Human Epidermis from primary keratinocytes in a serum free, differentiation culture medium (RHE batch RH0615/1, StratiCELL® EPI/001). The reconstructed tissues were cultivated at the air-liquid interface for 14 days in a humidified incubator at 37°C in a 5% CO2 atmosphere. The epidermises were stripped on the eleventh day, before addition of topically applied creams. Hyaluronic acid solubilized in the culture medium (50µg/ml) was used as a reference molecule promoting the development of the epidermal barrier (Gu et al., 2010 and internal data).

5.2.2. Histological analysis (morphological analysis) and immunohistological (abundance measurement of marker of epidermal differentiation by immunostaining):

Morphological analysis

At the end of the treatment, tissues were fixed in formalin and then

dehydrated in various alcohol baths before paraffin inclusion. Subsequently, 6 µm sections were cut and stained with hematoxylin and eosin. The slides were observed using a Leica DM2000 microscope equipped with a camera (Zeiss). This histological analysis confirmed the stress induced by stripping generates a limited perturbation of the cellular organization and the morphology of the epidermis is preserved.

Abundance measurement of marker of epidermal differentiation by immunostaining):

The effects of the Orobanche rapum extract on the 3 proteins of interest were then studied under the various culture conditions, by fluorescence immunolabeling. During the immunostaining, the first step consisted in removing the paraffin from tissue sections and placing the slices in a 0.01 M citrate buffer, pH 6. The slices were then washed with PBS and incubated in a saturation solution containing 5% serum for 1 hour at room temperature, then in presence of anti-filaggrin (Acris, AM00245PU-N), anti-involucrin (abcam ab53112) and anti-loricrin primary antibodies (abcam ab24722), for 1 h in a humid chamber and at room temperature. The samples were then washed twice in 0.1% PBS-Tween and once in PBS before being incubated for 30 min in PBS in presence of fluorescein-conjugated anti-mouse or anti-rabbit secondary antibodies. 4', 6'-diamidino-2-phenylindole, or DAPI, a fluorescent molecule capable of binding to the adenine and thymine bases of DNA, was used to detect nuclei of NHEKs keratinocytes. The slices were then mounted in Mowiol medium. For each immunostaining, 3 photos were captured using a Leica DM100 microscope and a DCF290 camera at 40X magnification. Image quantification was performed using the QWin 3 analysis software (Leica). The detection thresholds have been previously determined, using the most marked slices, in order to avoid saturation of detection. For each photo, an area of interest has been delimited manually and its surface was measured. The surface corresponds to the viable layers of the epidermis with the exception of the basal layer. The software then measured the marked area within the delimited area, in number of pixels. The mean intensity of the marked surface multiplied by the ratio "marked / total surface" determines the intensity of marking for a given slice.

Type of condition assessed	Stratum corneum cohesion score (mean ± standard deviation)
Control skin (untreated skin)	1.35 ± 0.28
Skin + active at 0.5%	2.10 ± 0.61 (vs untreated skin * p = 0.003) (vs placebo *p =0.001)
Skin + placebo	1.56 ± 0.37 (vs untreated skin * p =0.03)

Table 4: Histological assessment of stratum corneum cohesion (*: statistically significant difference in comparison to control skin (Student test, p < 0,05)).

5.2.3. Statistical analysis

Paired Student t-tests were performed to compare each condition with the unstripped control condition and to compare the stripped or non-stripped conditions with their respective controls as well as the condition treated with the active agent relative to the condition treated with placebo. All significant comparisons are shown on the graph (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$).

5.3. Study 3: related to barrier function maintenance in inflammatory condition

5.3.1. Reconstruct human epidermises treatment:

The model used was the inflammatory reconstructed human epidermis (RHE) model. This model is suitable for barrier default study. Indeed the inflammation leading to barrier default is triggered by cytokines mixture. The study was done in triplicate on normal RHE (unstressed) and on RHE stressed under cytokines mixture. The RHE were prepared with keratinocytes obtained from a 24 years old women donor.

For the inflammatory RHE, the culture protocol was the following:

On day 6 of air exposure, a mix of cytokines (IL-4, IL-13, IL-17E, IL-31 and TNF-α at 15 ng/ml) was supplemented to the medium until day 10 with and without the product at 0.5%. The reference molecule used in this study was the sodium butyrate at 0.1%. Normal RHE were cultured 10 days.

5.3.2. Biochemical analysis:

Characterization and evaluation of the product efficacy were performed using biochemical approach on lipids involved in the barrier function:

- Polar lipids with ceramides (Sphingosine [S], DihydroSphingosine [DS], PhytoSphingosine [P] base with a chain lengths of 16 and 18 carbon atoms) were analyzed by LC/MS.
- Neutral lipids with free fatty acids, cholesterol and glycerides were analyzed by GC/MS.

6. Results of ex vivo studies

6.1. Study 1: Desquamation process

6.1.1 Stratum corneum cohesion study

When the skin is treated with the active at 0.5%, a significant decrease in stratum corneum cohesion was seen in comparison to control and placebo

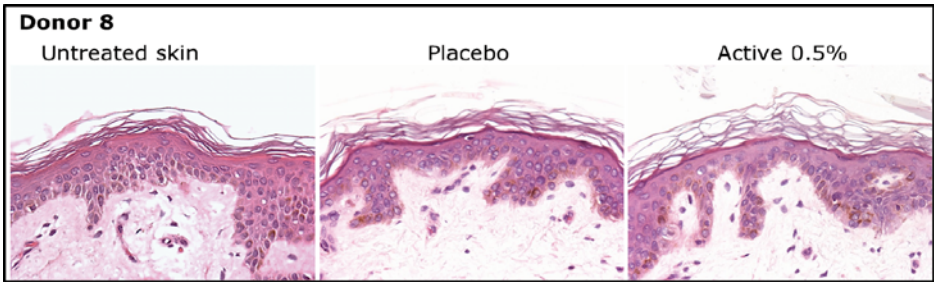


Figure 8: Histological observation of skin untreated (control skin) and treated skin with the active at 0.5% or the placebo (Staining Hemalun Eosine, magnification X 40).

treated skins as shown by a higher score obtained with the active (+56% versus control). The placebo induces also a significant but slight decrease of the stratum corneum in comparison to control skin (+16% versus control) (table 4 and figure 8). A decrease in stratum corneum cohesion means biologically an increase in the desquamation process.

6.1.2. Study of the expression of enzymes involved in desquamation process (KLK5 and KLK 7) and epidermis renewal (ki67)

A significant increase in KLK5 expression (score of 3.65) was observed with the active at 0.5% in comparison to untreated skin (score 3.29) and placebo treated skin (score of 2.96). This result is statistically significant in comparison to untreated skins (p=0.04). For KLK7 no difference was seen between the conditions studies.

An increase in epidermis renewal is observed with the active treatment (5% of ki67 positive cells) in comparison to untreated skin (2.71%). This result is statistically close to significance in comparison to untreated skins (p=0.06). (table 5, figure 9 and figure 10)

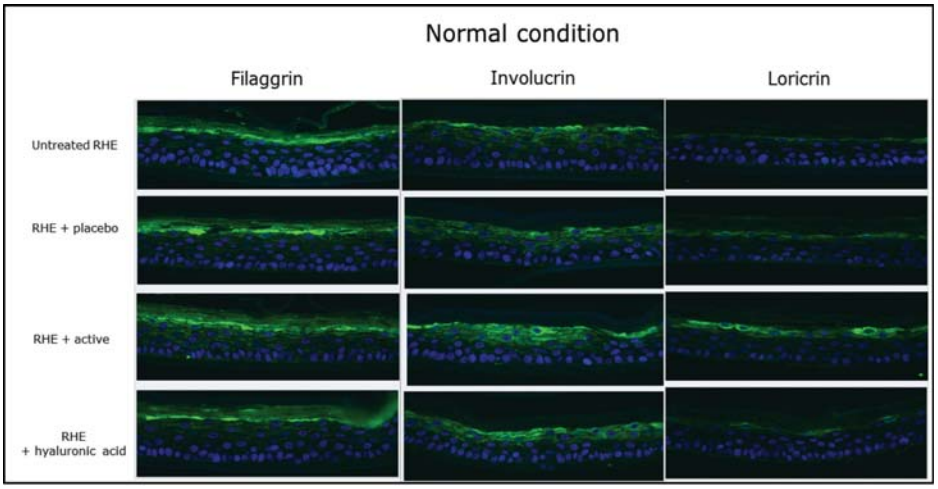


Figure 11: Immunostaining of the Filaggrin, Involucrin and Loricrin proteins on normal RHE after treatment of 72 hours with the active ingredient, placebo, or reference (Hyal: hyaluronic acid).

6.2. Study 2: Epidermis differentiation process

The results obtained after immunofluorescence labeling of filaggrin (FLG), involucrin (INV) and loricrin (LOR) are presented in figure 5-8, with a representative image by condition and by biological replicate (n = 3).

The active induces a statistically significant overexpression of FLG, IVL and LOR on the unstripped RHE compared to the placebo-treated RHE (not significant). The results are much more pronounced on RHE which have undergone stress by stripping. Of note, hyaluronic acid confirms its role of positive reference only in stripped condition.

The difference for all markers FLG, IVL and LOR in comparison with placebo are also statistically significant for all the conditions (unstripped and stripped). (Figures 11, 12, 13 and 14).

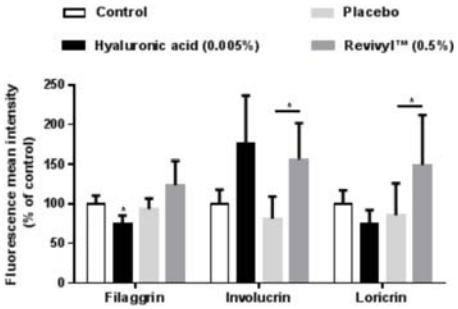


Figure 12: Relative quantification of the Filaggrin, Involucrin and Loricrin proteins abundance on normal RHE after treatment of 72 hours with the active ingredient, placebo, or reference (hyaluronic acid). All significant comparisons are shown on the graph (*: p <0.05, **: p <0.01, ***: p <0.001).

Conditions	Klk5 (mean score on 8 donors)	Klk7 (mean score on 8 donors)	% of ki67 positive cells (mean on 8 donors)
Untreated skin	3.29 ± 0.43	2.89 ± 0.23	2.71 ±1.72
Skin + placebo	2.96 ± 0.5	2.46 ± 0.43	3.25 ± 1.32
Skin + active at 0.5%	3.65 ± 0.26	2.88 ± 0.49	5 ± 3.46

Table 5: Quantification of KLK5, KLK7 and ki67 expression in skin explants maintained in survival and after treatment with the active ingredient or placebo (immunostaining and image analysis).

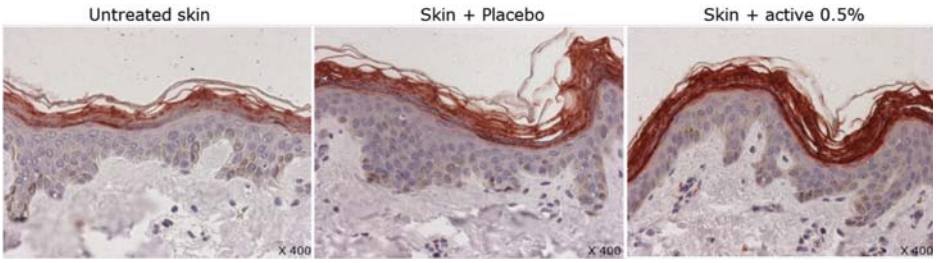


Figure 9: Immunostaining of KLK5 on skin explants maintained in survival and after treatment with the active ingredient or placebo.

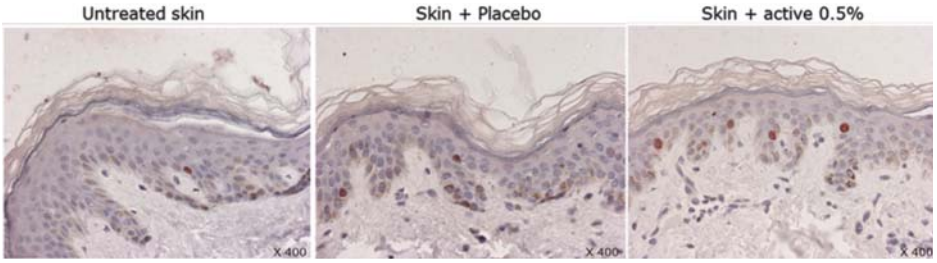


Figure 10: Immunostaining of ki67 on skin explants maintained in survival and after treatment with the active ingredient or placebo.

6.3. Study 3: Barrier function maintenance

6.3.1. Ceramides analysis

Ceramides content

The product doesn't modify the total ceramide content in untreated RHE. Ceramides content decreases with cytokines exposure (figure 15A). Cytokines effect is reduced with application of the product and reference

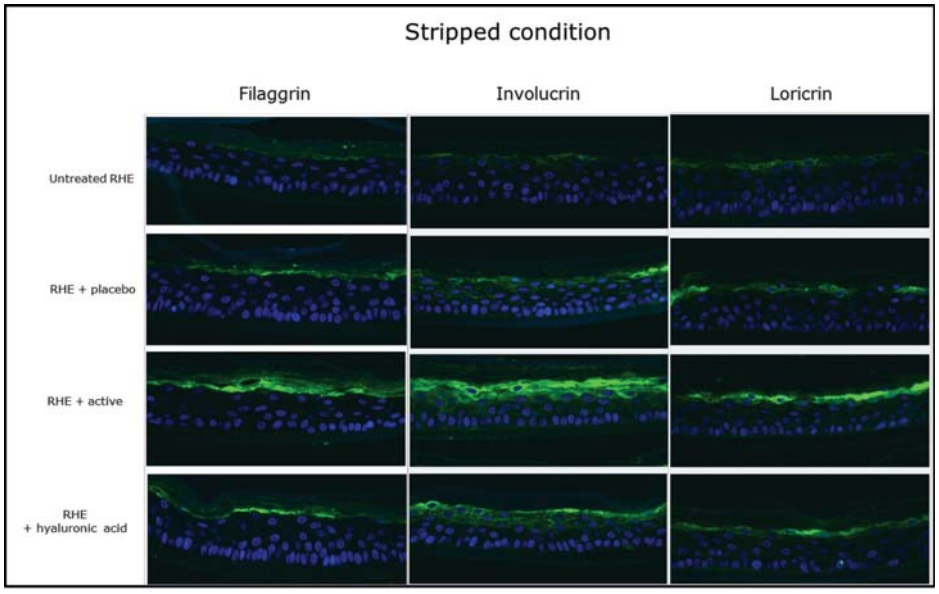


Figure 13: Immunostaining of the Filaggrin, Involucrin and Loricrin proteins on stripped RHE after treatment of 72 hours with the active ingredient, placebo, or reference (Hyal: hyaluronic acid).

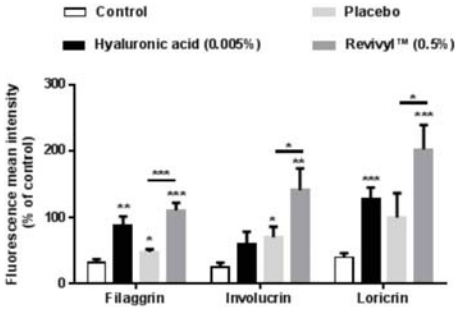


Figure 14: Relative quantification of the Filaggrin, Involucrin and Loricrin protein abundance on normal RHE having undergone stress by stripping and after treatment of 72 hours with the active ingredient, placebo, or reference (hyaluronic acid). All significant comparisons are shown on the graph (*: p <0.05, **: p <0.01, ***: p <0.001).

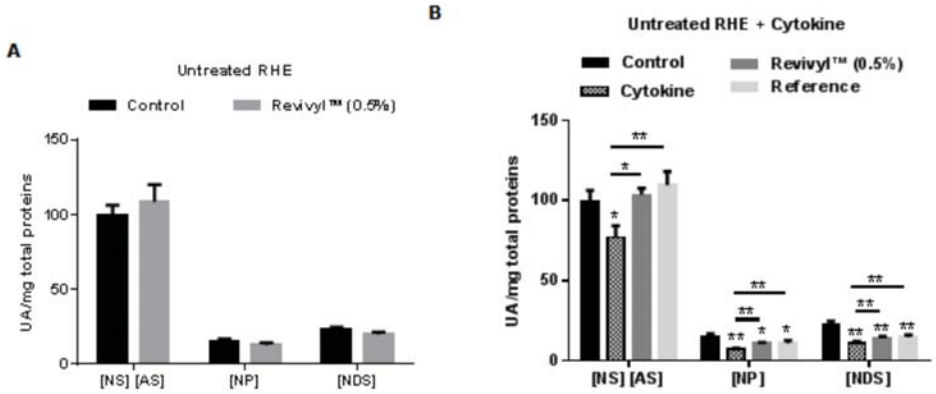


Figure 16: Content of Ceramides species dosed in untreated RHE and cytokines treated RHE with and without Orobanche rapum extract (Biochemical analysis). NS and AS: non hydroxy and α-hydroxy sphingosines, NP: nonhydroxy phytosphingosine and NDS: non hydroxy dihydrospingosine

respectively by -6% and -1% (figure 14 B and table 6). Product application reduces the impact of cytokines and particularly for sphingosine [NS AS] in which the evolution is positive (+5%) (Figure 16 and table 6). Sphingosine [NS AS] represents the predominant form of

Condition	[NS] [AS] Evolution%	[NP] [AP] Evolution%	[NDS] [ADS] Evolution%	Global ceramides evolution (%)
Cytokines vs untreated RHE	-22	-55	-51	-31
Product vs untreated RHE	10	-17	-13	3
Product + Cytokines vs untreated RHE	5	-29	-37	-6
Reference + Cytokines vs untreated RHE	11	-28	-36	-1

Table 6: Ceramides content evolution calculated in percentage for each condition in comparison to untreated RHE.

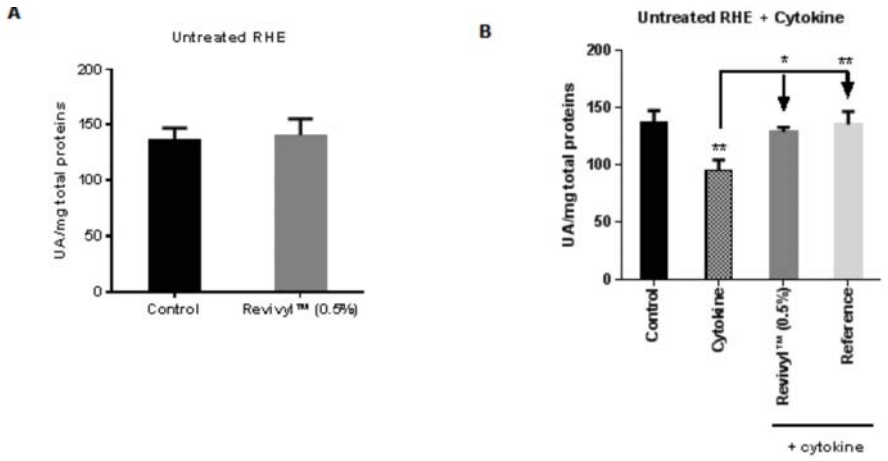


Figure 15: Total Ceramides content dosed in untreated RHE (A) and cytokines treated RHE (B) with and without Orobanche rapum extract (Biochemical analysis).

ceramides from this tissue (RHE). The barrier function of the skin is enhanced with the product treatment.

Analysis of the evolution of chain length of fatty acids linked to the main ceramides basis:

According to the results obtained in ceramides analysis, a complementary analysis was performed on chain lengths of the fatty acid linked to the main basis (Sphingosine [AS NS], Phytosphingosin [NP], and Dihydrospingosine [NDS]) with a chain length of 18 carbons. This approach focused on the determination of the ratio between a chain length of 18 carbons and 24 carbons for the fatty acids. This ratio illustrates the barrier function. Indeed more the length of the fatty acid chains is important, more the barrier function is better through a lipids' orthorhombic organization. Evolution percentages of this ratio in each tested condition were determined compare to the normal condition (untreated RHE) (table 7).

Ceramides [AS] was more reduced (-18.5%) for this ratio under cytokines exposure than ceramides [NS] (-10%) (Figure 17). Product application limited the impact of cytokines. Furthermore, product and reference applications reduced the impact of cytokines and

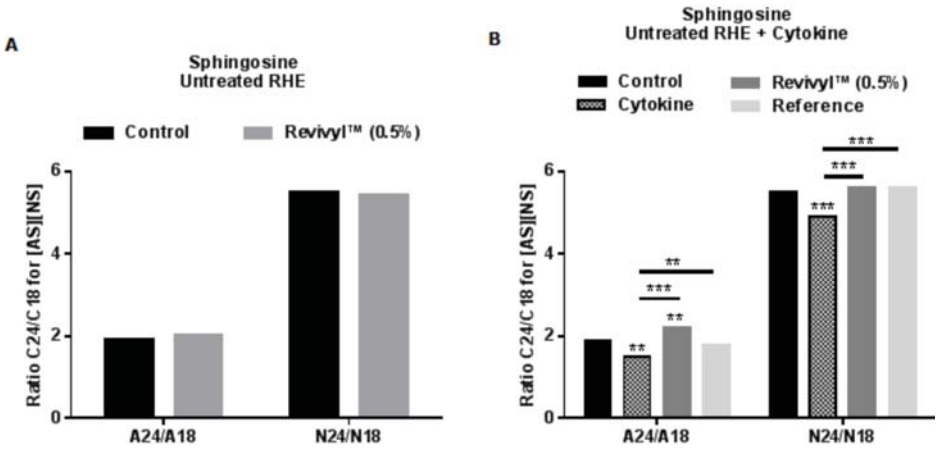


Figure 17: Evolution of chain length of fatty acid linked to Sphingosine in untreated RHE (A) and cytokines treated RHE (B) with and without Orobanche rapum extract. A24/A18: ratio α-hydroxy sphingosine and non-hydroxy sphingosine.

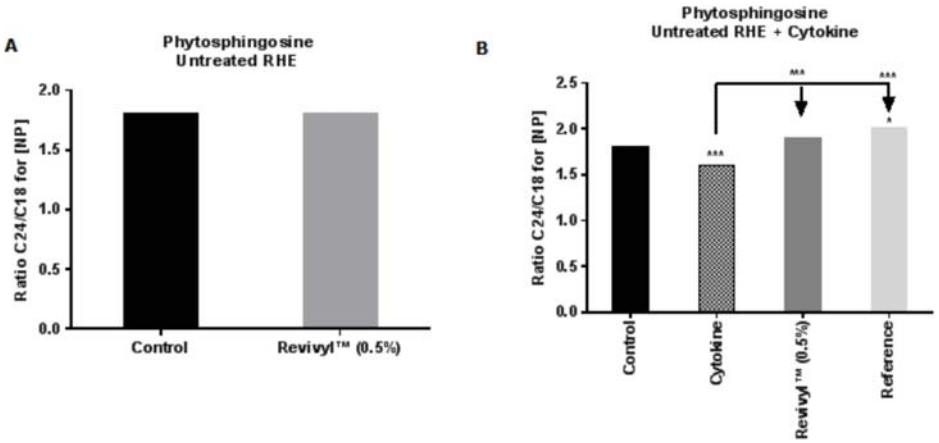


Figure 18: Evolution of chain length of fatty acid linked to Phytosphingosine in untreated RHE and cytokines treated RHE with and without Orobanche rapum extract. NP: non-hydroxy phytosphingosine.

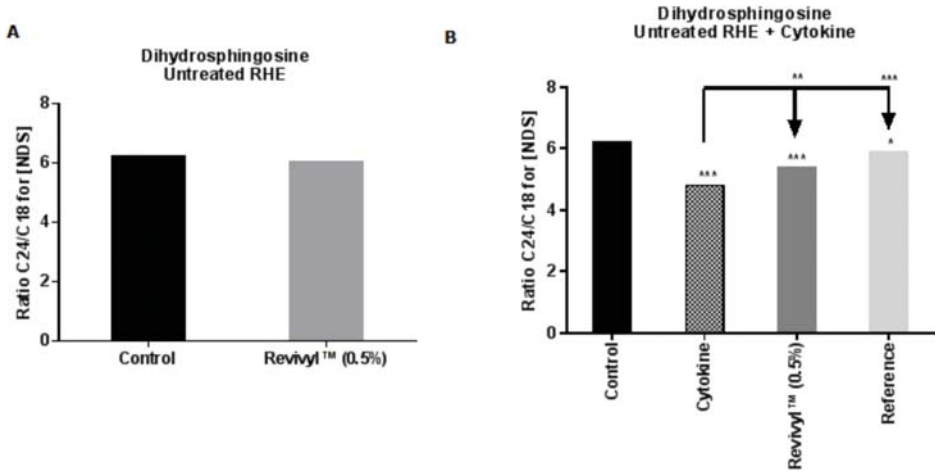


Figure 19: Evolution of chain length of fatty acid linked to Dihydrosphingosine in untreated RHE and cytokines treated RHE with and without Orobanche rapum extract. NDS: non-hydroxy Dihydrosphingosine.

Condition	Ratio A24S18/A18S18 (%)	Ratio N24S18/N18S18 (%)	Ratio N24P18/N18P18 (%)	Ratio N24DS18/N18DS18 (%)
Cytokines vs untreated RHE	-18.5	-10.0	-10.3	-23.0
Product vs untreated RHE	6.3	-2.0	-1.1	-3.9
Product + Cytokines vs untreated RHE	15.9	2.7	0.5	-14.0
Reference + Cytokines vs untreated RHE	-3.2	1.5	10.9	-4.8

Table 7: Evolution of chain length of fatty acids linked to the main ceramides basis in comparison to untreated RHE calculated in percentage.

particularly for Sphingosine [AS] in which the evolution is positive (+15.9%) for product (figure 17 and table 7).

Regarding the other ceramides contents, we observe that Orobanche rapum extract doesn't modify the ratio C24:C18 of Phytosphingosine on untreated RHE. Cytokine cocktail significantly decreases it (-10.3%) demonstrating a barrier default involving a decreasing of C24:C18 ratio. The product and Reference restore it at the untreated level with +0.5% and +10.9% respectively (Figure 18 and Table 7).

Same results are obtained on the study of chain length of Dihydrosphingosine witht doesn't modification by the product on untreated RHE. Cytokines cocktail significantly decreases the C24:C18 ratio proving a barrier default through a modification of chain length of fatty acid that is shorter. (-23%). Orobanche rapum and reference significantly improve it but don't induce a fully restoration of the C24:C18 ratio with -14% and -4.8% respectively (figure 19 and table 7).

These results confirm that Orobanche rapum extract restores the skin barrier function with a ceramide contents quality's increasing in term of chain length of fatty acid.

Neutral lipids

Illustration of neutral lipids content is reported in the figure below. Cholesterol, Glycerides and so neutral lipids content decreased under cytokines whereas free fatty acids content increased. Product and reference application reduced the effect of (figure 20 and table 8).

Evolution percentages of neutral lipids in each condition tested were determined compare to the normal condition (untreated RHE). Cholesterol, Glycerides and so neutral lipids content presented a similar evolution with a reduction effect of cytokine under product and reference applications (table 9).

6.4. Conclusion on ex vivo assessments

We demonstrate that Orobanche rapum extract positively actives the biological mechanisms involved in

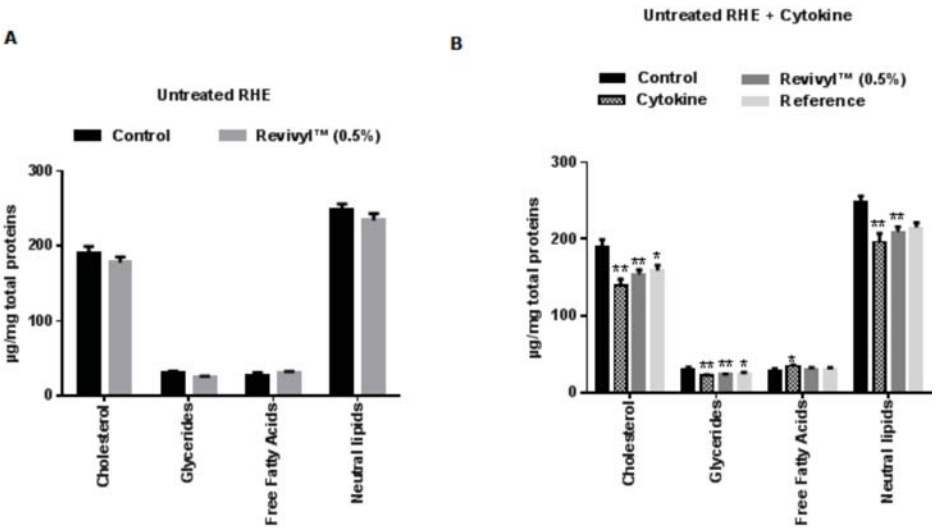


Figure 20: Neutral lipids content dosed in untreated RHE (A) and cytokines treated RHE (B) with and without Orobanche rapum extract (cholesterol, glycerides and free fatty acids). (Biochemical analysis).

Conditions	Neutral lipids			Neutral lipids
	Cholestrol	Glycerides	Free fatty acids	
Untreated RHE	189.1 ± 9.9	30.8 ± 2.2	27.9 ± 3.1	247.9 ± 7.9
RHE + cytokines	139.3 ± 8.6	22.1 ± 0.9	34.6 ± 1.6	196.1 ± 11.1
RHE + active	177.7 ± 7.5	25.6 ± 1.1	30.4 ± 2.4	233.7 ± 9.2
RHE + cytokines + active	153.9 ± 5.7	24 ± 0.3	31.2 ± 1.6	209.1 ± 6.6
RHE + reference + cytokines	159.4 ± 8.4	25.5 ± 0.9	30.9 ± 1.7	214.8 ± 6.3

Table 8: Neutral lipids content dosed in untreated RHE and cytokines treated RHE with and without active (cholesterol, glycerides and free fatty acids). (The neutral lipids content is expressed un µg/mg total proteins (Biochemical analysis).

Condition	Cholesterol Evolution (%)	FFA Evolution (%)	Glycerides Evolution (%)	Neutral lipids Evolution (%)
Cytokines vs untreated RHE	-26.3	23.9	-28.2	-20.9
Revivyl™ vs untreated RHE	-6.0	8.9	-17.0	-5.7
Revivyl™ + Cytokines vs untreated RHE	-18.6	11.6	-22.0	-15.6
Reference + Cytokines vs untreated RHE	-16.3	10.6	-17.2	-13.4

Table 9: Neutral lipids content evolution calculated in percentage for each condition in comparison to untreated RHE.

improvement of epidermal maturation and skin barrier property through:

- Decreasing of stratum corneum cohesion promoting the desquamation process
- Increasing of KLK5 expression in stratum corneum and KI67 in basal layer
- Rising of Filaggrin, Involucrin and Loricrin expression un normal condition and that are more pronounced in stripped condition
- Total ceramide content is significantly increases with the product what showed on different type of ceramides including Sphingosine [NS AS], Phytosphygosine [NP] and Dihydrosphingosine [NDS].
- The C24:18 ratio corresponding to the chain length of fatty acid of ceramides is significantly increased for all the ceramides identified.

The active is a potent full activator of epidermis maturation from basal layer to stratum corneum until desquamation respecting perfectly the equilibrium between skin renewal and desquamation.

Futhermore, we also demonstrate the full reconstruction of efficient and permeable skin barrier by content ceramides and thier quality suitable for ordered, densely packed lipid organization.

7. Material and methods clinical investigations

Clinical assessments of Orobanche rapum extract were done on human volunteers throughout four studies.

o Study 1 (Active beauty study) and study 2 (Dermscan study): The studies were performed on aged volunteers (face area). The first study analyzed the evolution of cutaneous relief using silicone replicas (after 14 and 28 days of use), whereas the second one analyzed the cutaneous relief using Primos 3D (after 28 days of use).

o Study 3 (Active beauty study): This study was done on volunteers having dry skins (forearm area). The study 3 was followed after 14 and 28 days of use.

o Study 4 (Bio-EC study): This study was done on volunteers having dry skins (leg area) and studies after 1, 7, 28 and 56 days of use.

7.1. Description of the creams used for clinical investigations

Study 1, study 2, and study 3:

Cream compositions (INCI composition):

Aqua/Water, Capric/Caprylic Triglyceride, Cetearyl Wheat Straw Glycosides, Cetearyl Alcohol, ± Propanediol And Orobanche Rapum, Phenoxyethanol, Dimethicone, Methyl Paraben, Propyl Paraben, Ethyl Paraben, Fragrance, Hexyl Cinnamal, Butylphenyl Methylpropional, Citronellol, Alpha Isomethyl Ionone, Hydroxyisohexyl 3-Cyclohexene Carboxaldehyde, Sodium Hydroxide.

Study 4:

Cream Compositions (Inci Composition):

Aqua/Water, Capric/Caprylic Triglyceride, Cetyl Alcohol, Glyceryl Stearate, Peg-75 Stearate, Ceteth-20, Steareth-20, ± Propanediol And Orobanche Rapum, Phenoxyethanol, Methyl Paraben, Propyl Paraben, Ethyl Paraben, Fragrance, Hexyl Cinnamal, Butylphenyl Methylpropional, Citronellol, Alpha Isomethyl Ionone, Hydroxyisohexyl 3-Cyclohexene Carboxaldehyde, Sodium Hydroxide.

7.2. Study 1 and study 2 related to aged skins

7.2.1. Description of the panel and study condition

Study 1: A simple blind and placebo-controlled clinical evaluation was carried out with 15 women (Age: between 35

and 55 years old, mean age: 43 years ± 1) showing clinical signs of ageing (wrinkles on crow's feet). All of the subjects participating in the study gave their informed consent signed at the beginning of the study. The measurements were done after 14 and 28 days of use. In this study the parameters studied were the evolution of cutaneous relief using silicone skin's replica technic after formula application containing or not 0.5% Orobanche rapum extract.

Study 2: The study was a single blinded and on two parallel groups (at least 20 volunteers were enrolled) showing clinical signs of ageing (wrinkles on crow's feet). The mean age of the group with active product was 55 ± 1 year (from 45 to 63 years old). The mean age of the group with placebo product was 56 ± 1 year (from 47 to 65 years old). The study of the cutaneous relief parameters (average roughness Ra, maximum amplitude Rt and average relief Rz was determined using 3D Primos compact system. The measurements were done after 28 days of twice daily application of formula containing or not 0.5% Orobanche rapum extract.

7.2.2. Cutaneous relief analysis

Silicone skin's replica technic (study 1):

Cutaneous relief analysis was done using polymer silicone skin's prints (Silflo®) also called replica. The polymer silicone skin's prints were taken on crow's feet zones, before product use and at each time of measurement, then analyzed using the Skin Image Analyser® (S.I.A®). An oblique lighting of 35° brings shadows on the replica surface. These shadows are observed with a digital camera linked to a computer. A 1 cm^2 area was studied. The digitized picture obtained was analysed in grey levels and allows to obtain different parameters of the skin surface relief. The parameters studied with the Quanti-Rides® software (Monaderm, Monaco) were the total wrinkled surface (in mm^2) and the total wrinkles number. A decrease in these parameters characterizes an anti-wrinkle effect.

Primos 3D technic (study 2):

Measurements are done directly in vivo, using PRIMOS ® 3D (Phaseshift Rapid In vivo Measurement Of Skin). This technique consists in calculating a phase image from images with interference fringe projection. The acquisition software allows to obtain 2D and 3D measurements and to determine parameters of the cutaneous relief on 32 radiuses distributed like a star on the zone of interest. An automatic system of repositioning allows the precise re identification of the zone of measurement.

Parameter studied on each profile.

- Ra: the average roughness (in µm) is defined like the ratio between the surface integrated around the mean value on the profile length. A decrease in Ra characterizes a smoothing effect.
- Rz: the average relief (in µm) is defined as the average value of all the maxima (between peaks and hollow) on the profile length. A decrease in Rz characterizes a tensor, anti-wrinkle effect.
- Rt: the amplitude (in µm) is defined as the average value of the five highest maxima (between peaks and hollow) on the profile length. A decrease in Rt characterizes a tensor, anti-wrinkle effect.

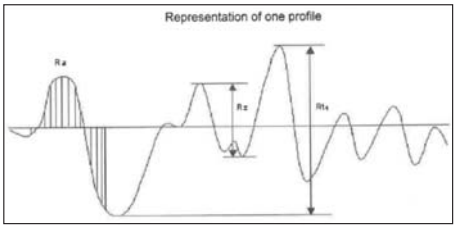


Figure 21: schematic representation of the parameters measured with Primos 3D.

7.3. Study 3 and study 4 related to dry skins

7.3.1. Description of the panel and study condition

Study 3 (Active beauty study): A double blind and placebo-controlled clinical evaluation was carried out with 19 women (Age: between 18 and 50 years old, mean age: 39.5 years) having dry skins (corneometer value below 40 AU). All of the subjects participating in the study gave their informed consent signed at the beginning of the study. The measurements were done after 14 and 28 days of use with formula containing or not 0.5% Orobanche rapum extract. In this study the parameters studied were the evolution of skin hydration by corneometry and by raman spectroscopy but also the biochemical content evolution of the skin (lipids and proteins). The evolution of dry skin surface was observed by OCT tool (Optical coherence tomography) and by Visioscan. In this study, the effect of the products on skin metagenome and skin pH was also studied. The body area studied was the forearm.

Study 4 (Bio-EC study): A double blind and placebo-controlled clinical evaluation was carried out with 20 women (Age: between 30 and 50 years old, mean age: 44 years) having dry skins and squams on legs (corneometer value below 35 AU). All of the subjects participating in the study gave their informed consent signed at the beginning of the study. The measurements were done after 1, 7, 28 and 56 days of use with formula containing or not 0.5% Orobanche rapum extract. In this study the parameters studied were the evolution of skin hydration by corneometry and . The body area studied was the leg.

7.3.2. Skin hydration measurements

Corneometry technic (Study 3 and 4): This method is based on the skin electrical conductivity measurement. The dielectrical constant of the skin is correlated to the water content of the epidermis. The corneometer measures the hydration degree of the upper layers of the epidermis: the stratum corneum. The corneometer CM825® (Courage & Khazaka) was used.

For study 3 (forearm area): Measurements were taken at all study time (D0, D14 and D28) and after twice daily application.

For study 4 (leg area): Measurements were taken at all study time (D0, D7, D28 and D56) and after twice daily application.

Raman spectroscopy technic (study 3): The

set-up included a confocal Raman Probe (Horiba Jovin Yvon, France) coupled to a dispersive Raman spectrograph (Micro HR, Horiba Jobin Yvon, France). The probe was equipped with a 100X long working distance objective (MPlan FLN Olympus, Japan) operating in air with a numerical aperture of 0.9.A piezo-electric device (Physics instrument, Germany) allowed to collect Z Raman profiles by assuring axial measurements from the surface down to a defined depth in the skin. The axial resolution of the system was given to about 3 µm by the device manufacturer. A color video camera integrated in the probe permitted to visualize the skin surface. The spectrograph was equipped with a CCD (Coupled Charge Detector) camera (Synapse, Horiba Jobin Yvon, Edison, NJ, USA) of 1024 X 256 elements cooled by Peltier effect, and a 830 grooves/mm grating which allows to cover a large spectral range from 550 to 3700 cm-1 in a single shot acquisition with a spectral resolution of about 7 cm-1. The excitation source was a 660 nm laser diode (Ignis Laser Quantum GmbH Germany). The power at sample was fixed at 30 mW in accordance with protection standards for radiation. The 660 nm excitation was chosen as the optimal compromise between generation of parasitic fluorescence and sensitivity of the CCD camera over the whole spectral range. For skin characterization, the measurement of high wavenumber vibrations is important to access information on water content. Water was identified using band intensity from 3100-3600 cm-1, lipids were identified using band intensity at 1450 cm-1 and proteins were identified using Amide I band intensity at 1650 cm-1. Before measurement, the skin surface was gently pressed to assure a stable contact between the skin and the probe. Raman profiles were recorded by collecting spectra from the skin surface to 40 µm in depth. The pre-processing of spectral data was performed using Matlab 7.2 (The MathWorks Inc., USA). Aberrant profiles were excluded by visual inspection of the data. Each non-aberrant profile was submitted to background corrections, which allowed to clean up the Raman signal of the skin. These background corrections included a notch correction for laser wavelengths removal, followed by a baseline correction, using a polynomial function of degree 4, meant to remove the skin fluorescence. Raman spectra were thereafter spatially registered onto a reference spectra (lysopaïne), correcting for instrumental Raman shift, as well as a spatial realignment of the Raman profile. This spatial correction was often necessary since a slight laser defocalization can occur between the probe adjustment on skin surface (visually controlled by the laser spot) and the profile acquisition. For this, the Stratum corneum (SC) surface was located from the Raman profile by determining the Z position corresponding to the half of maximum of CH intensity (integrated intensity between 2910 and 2965 cm-1). Finally, Raman profiles were spatially smoothed with a 9-mm Savitzky-Golay filter (polynomial function of degree 2), and normalized on the intensity of the entire wavenumber range, with a vectorial function. After these pre-processing steps, corrected data were processed using statistical multivariate analyzes.

7.3.3. Skin desquamation analysis

Diagnoskin analysis (study 4):
Assessment of skin moisture through DIAGNOSKIN® was done by study of desquamation. Stratum corneum moisturizing and quality of corneocytes physiological desquamation (indispensable to the preservation of the skin barrier function) are two parameters closely linked:

- An abnormal desquamation induce a trouble of barrier function and so a tendency to dehydration of the stratum corneum,
- A cutaneous dryness will disturb the desquamation.

The closely link of the stratum corneum moisture level with desquamation phenomenon was confirmed through a comparative assessment of corneometrics and squamometrics data by DIAGNOSKIN® la semiology (BIO-EC owner). The DIAGNOSKIN® skin diagnosis system is automated and allows assessment of skin moisture level and micro-relief structure. Surface corneocytes were removed using of a flexible adhesive – D Squam®, and analyzed through the automated DIAGNOSKIN® skin diagnosis system. This system allows the microscopic assessment of the stratum corneum moisture statement, according to a specific scoring of 1 to 100 % (BIO- EC owner) that takes into account desquamation quality (isolated cells, cellular groups and skin flakes).

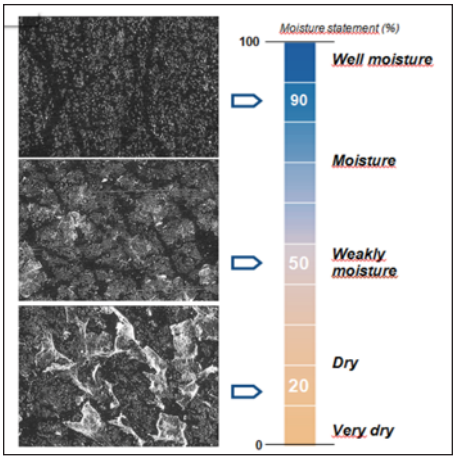


Figure 22: Explanation of Diagnoskin analysis

7.3.4. Skin pH analysis

The pH meter measures the pH at the skin surface. The pH meter 905 was used in this study. Five measures were taken on each volunteer for each product tested, at all study time (D0, D14 and D28). The average and standard deviation were calculated.

7.3.5. Skin structure analysis

Optical coherence tomography technic (OCT, Study 3): OCT uses light and captures the light reflectance to generate an image. OCT images are 2-dimensional images that provide a cross-sectional image of the superficial skin resembling histological images. Therefore, OCT is sometimes referred to as an "optical biopsy," as it aims to provide histologic information non-invasively. OCT imaging systems contain an interferometer illuminated by light. OCT-emitted light is split into two

fractions, one fraction is directed to a reference mirror and the other is directed to the tissue. Light reflected back from the mirror and the tissue are recombined and guided to a detector that collects the interference signal and transmits to a computer to generate the image. It offers maximum penetration depth of 1 mm and a field of view of 1.8*1.5mm. The images are of high resolution with rendering structures up to 3µm. One measure was taken on each volunteer at each study time (D0, D14 and D28). In this study, this tool was used to study the evolution of flaky skins treated with and without the active.

7.4. Metagenomic analysis (study 3)

7.4.1. Microbiotia sampling and storage

Skin samples of cutaneous microflora were collected from the forearms of healthy volunteers (50 cm²), by a non-invasive swabbing method, using sterile swabs moistened with a sterile solution of 0.15 M NaCl. Swabs were transferred at -20°C and kept frozen until DNA extraction.

7.4.2. DNA extraction

DNA extraction was performed using the PowerLyzer® PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, USA), with the following modifications. The tip of each swab was detached with a sterile surgical blade and transferred in a 1.5 mL tube to which 750 µL of Bead Solution has been added. The sample biomass was resuspended by stirring and pipetting and the biological suspension was transferred to a bead beating tube. The remaining steps were performed according to the manufacturer instructions.

7.4.3. Sequencing and data analysis

16S rRNA gene sequencing:

Sequencing was performed with the MiSeq device (Illumina, Inc., San Diego, CA, USA) through a 500 cycles paired-end run, targeting the V3V4 16S variable regions using the following primers: 16S-Mi341F forward primer 5'- CCTACGGGNGGCWGCAG-3' and 16S-Mi805R reverse primer 5'-GACTACHVGGGTATCTAATCC -3', producing about 460 bp amplicons.

PCR1s were performed as follows: 8 µL of template DNA (0.2 ng) were mixed with 5 µL of each reverse and forward primers (1 µM), 5 µL of KAPA HiFi Fidelity Buffer (5X), 0.8 µL of KAPA dNTP Mix (10 mM each), 0.7 µL of RT-PCR grade water (Ambion), and 0.6 µL of KAPA HiFi hotstart Taq (1 U/µL), for a total volume of 25 µL. Each amplification was duplicated, and duplicates were pooled after amplification. PCR1 cycles consisted of 95°C for 3 min and then 32 cycles of 95°C for 30 s, 59°C for 30 s, and 72°C for 30 s, followed by a final extension at 72°C for 3 min, with a BioRad CFX1000 thermocycler. Negative and positive controls were included in all steps to check for contamination. All duplicate pools were controlled by gel electrophoresis, and amplicons were quantified using fluorometry.

Libraries ready for analysis were then produced following the Illumina guidelines for 16S metagenomics libraries preparation. Briefly, the PCR1 amplicons were purified and controlled using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, USA). To enable the simultaneous analysis of multiple samples (multiplexing), Nextera® XT indexes (Illumina) were added during PCR2 using between 15 to 30 ng of PCR1 amplicons. PCR2 cycles consisted of 94°C for 1 min and then 12 cycles of 94°C for 60 s, 65°C for 60 s, and 72°C for 60 s, followed by a final extension at 72°C for 10 min. Indexed libraries were purified, quantified and controlled using an Agilent 2100 Bioanalyzer. Validated indexed libraries were pooled in order to obtain an equimolar mixture.

The run (500 cycles) was achieved on MiSeq sequencer (Illumina) using the MiSeq Reagent Kit v3 600 cycles (Illumina). The sequencing run produced an output of 25 million of paired-end reads of 250 bases, i.e. up to 6 Gigabases. The libraries and the MiSeq run were performed by libragen, at the GeT-PlaGe platform (INRA, Auzeville, France).

After MiSeq run, raw data sequences were demultiplexed and quality-checked to remove all reads with ambiguous bases. Indexes and primers sequences were then trimmed, and the forward and reverse sequences were paired. The paired-sequences were then treated using in-house pipeline to remove chimeras and reads with PCR errors and to split sequences into Operational Taxonomic Unit (OTU) at a 1% dissimilarity level. Good quality binned paired-sequences were mapped to the SILVA SSU Ref database (Release 123; https://www.arbsilva.de/) for taxonomic assignation. Data were then normalized, and compared using White's non-parametric test (White et al., 2009).

8. Results clinical investigations

8.1. Study 1 and study 2: anti-ageing studies

8.1.1. Wrinkles evolution assessment

Silicone replica technic: In this study, we have shown that the active reduces the wrinkles as confirmed by the decrease in the total wrinkled area (-12.1 % after 28 days of use) and the total wrinkles number (-28.5% and -30.6% after 14 and 28 days). The difference is statistically significant in comparison to placebo after 14 days (for both parameters) and 28 days of use (for the parameter total wrinkles number). (table 10 and figure 23).

Parameters		Active			Placebo			Student
		Δ Mean +/- SEM	Δ % (mean)	Student t-test versus D0 (p)	Δ Mean +/- SEM	Δ % (mean)	Student t-test versus D0 (p)	t-test versus placebo (p)
D14-D0	Total wrinkles (Number)	-3.7 ± 4.3	-3.6%	0.402	7.9 ± 5.0	10.8%	0,136	**0.044**
	Total wrinkled area [mm²]	-5.2 ± 1.6	-28.5%	**0.006**	0.2 ± 1.7	1.4%	0,911	**0.014**
D28-D0	Total wrinkles (Number)	-12.1 ± 5.4	-11.9%	**0.041**	8.9 ± 4.7	12.2%	0.082	**0.003**
	Total wrinkled area [mm²]	-5.6 ± 2.1	-30.6%	**0.017**	-2.5 ± 1.5	-18.4%	0.126	0.119

Table 10: Wrinkles analysis using silicone replica pictures.

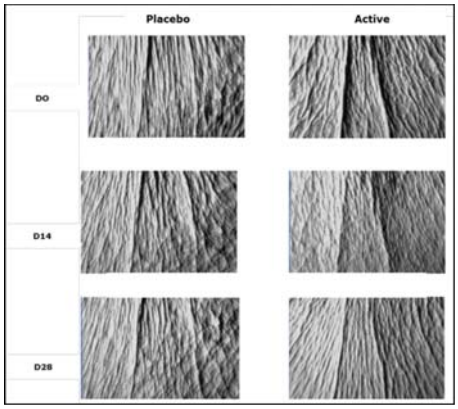


Figure 23: Silicone replica pictures

Primos 3D technic: In this study, we have shown in comparison to placebo that the active reduces the wrinkles. Indeed, a limit significant difference in the average roughness (Ra), and in average relief (Rz) between the products after 28 days of use (respectively with p = 0.0748 and p = 0.0689) were calculated (table 11). The effect is statistically significant for Rt (maximal amplitude) in comparison to placebo (p= 0.0251). The effect is also clearly visible in the 2D and 3D pictures below (figure 24).

8.2. Study 3 and 4: Dry skins

8.2.1. Skin hydration

Raman spectroscopy (study 3):

In the active group the level of total water is increased by +71% and 109% respectively after 14 and 28 days. In the placebo group the level of total water is increased by +53% and +73% respectively after 14 and 28 days. The comparison between active and placebo were statistically significantly different after 28 days (figure 25).

Figure 25: Water content determination using Raman spectroscopy

Corneometry analysis (study 4):

- Study 4 (legs, table 12):

For placebo: A statistically significant increase of the skin hydration rate (upper layers of the epidermis) was highlighted 1, 7, 28 and 56 days after twice a day application of the active product when compared to the baseline value (D0).

For active: A statistically significant increase of the skin hydration rate (upper layers of the epidermis) was highlighted 1, 7, 28 and 56 days after twice a day application of the active product when compared to the baseline value (D0). The effect is more pronounced with the active in comparison to placebo after 28 and 56 days.

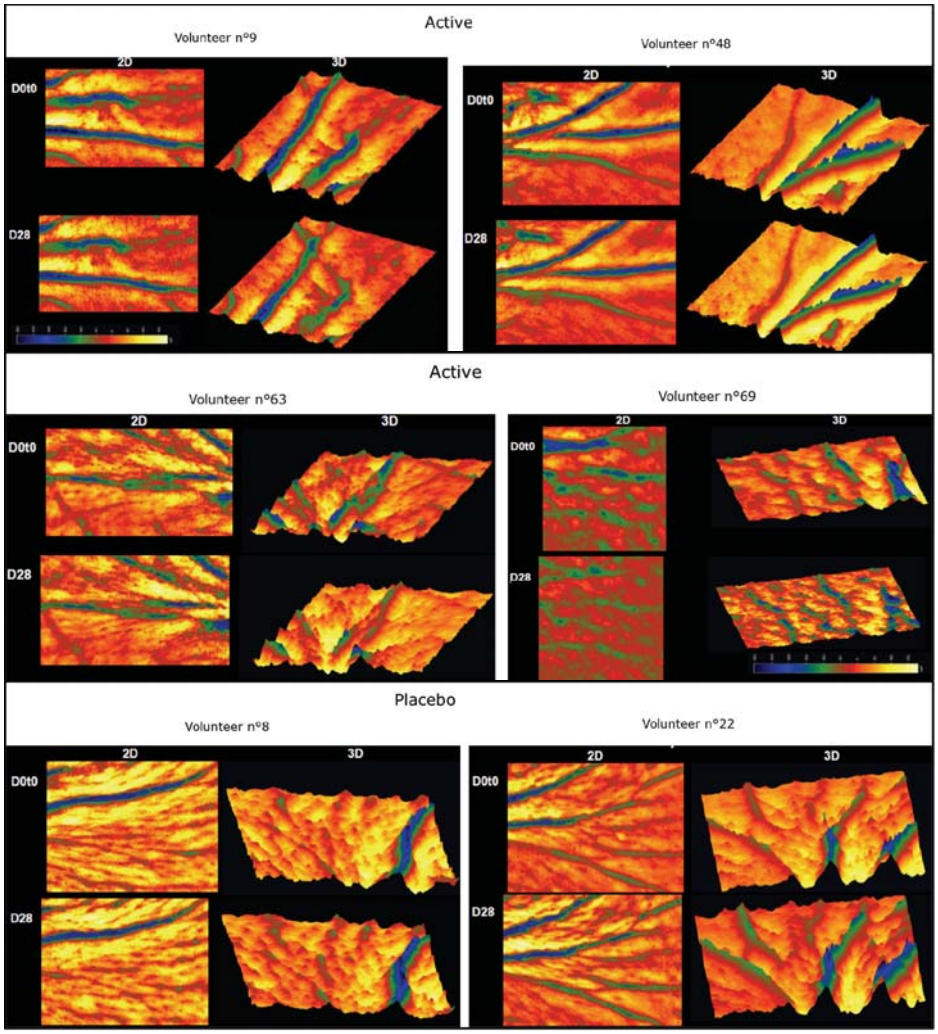


Figure 24: Primos 3 D representation of wrinkles area.

Parameters		Active			Placebo			Student
		Δ (Mean +/- SEM)	Δ % (mean)	Student t-test versus D0 (p)	Δ Mean +/- SEM	Δ % (mean)	Student t-test versus D0 (p)	t-test versus placebo (p)
D28-D0	Ra (µm)	0.2 ± 0.2	1%	0.5589	1.1 ± 0.5	8%	0.0022	0.0748
	Rz (µm)	0.5 ± 1.1	1%	0.6721	4.9 ± 2.1	8%	0.0030	0.0689
	Rt (µm)	-2.1 ± 4	-4%	0.6235	11.3 ± 4.2	11%	0.0066	**0.0251**

Table 11: Determination of parameters Ra, Rz and Rt using primos 3D

Diagnoskin tool (Study 4 on legs, table 13): The skin desquamtion results obtained with Diagnoskin tool were reported in the table below.

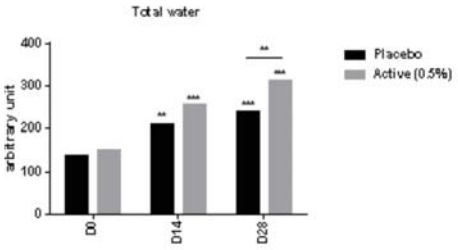
- For active: A statistically significant increase of the skin hydration rate (upper layers of the epidermis) was highlighted 1, 7 and 28 days after twice a day application of the active product when compared to the baseline value (D0) respectively by +11.15%, 14.80% and 13.10%.
- For placebo: No statistically significant increase of the skin hydration rate (upper layers of the epidermis) was recorded.

A statistically significant difference between both products was highlighted 1, 7 and 28 days after product application, in favour of the active. We can conclude that the active has a significant moisturizing effect until 28 days of application (table 13 and figure 26).

Diagnoskin tool:

In the figure below, we observed that

8.2.2. Skin desquamation



Total water	D0	D14	D28
Active (mean arbitrary unit)	150.03	256.44	313.16
Placebo (mean arbitrary unit)	138.19	210.75	239.01
% variation active		71	109
% variation placebo		53	73
t test vs D0 active		4.0E-05	8.9E-07
t test vs D0 placebo		0.0020	0.0004
t test active vs placebo	0.36	0.109	0.0074

Table 12: Corneometer measures (legs)

Parameters	Active		Placebo		Student
	Δ % (mean)	Student t-test versus D0 (p)	Δ % (mean)	Student t-test versus D0 (p)	t-test versus placebo (p)
T1 day	36%	0.004	33%	0.0001	ns
T7 days	46%	<0.0001	49%	<0.0001	ns
T28 days	34%	<0.0001	26%	0.0002	ns
T56 days	29%	0.0006	19%	0.0091	ns

Table 13: Diagnoskin analysis (legs)

the active improve the skin quality with less squams at the skin surface.

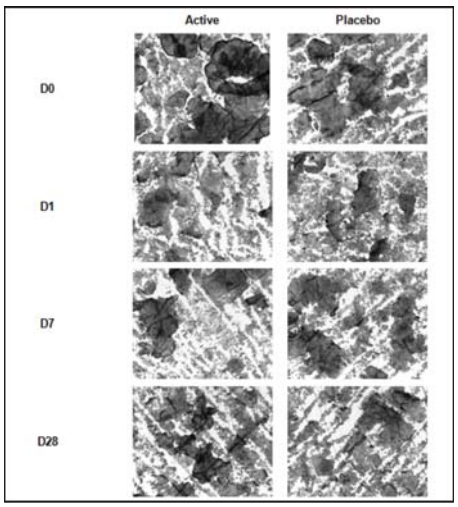


Figure 26: Skin surface observed using Diagnoskin

8.2.3. Skin lipids and proteins content (Raman spectroscopy)

Lipids:

In the active group the level of lipids is increased by +137% and 118% respectively after 14 and 28 days. In the placebo group the level of lipids is increased by +48% and +51% respectively after 14 and 28 days. The comparison between active and placebo were statistically significantly different a both study times (figure 27).

Proteins content:

In the active group the level of lipids is increased by +101% and 105% respectively after 14 and 28 days. In the placebo group the level of lipids is increased by +44% and +48% respectively after 14 and 28 days. The comparison between active and placebo were statistically significantly different at both study times (figure 28).

8.2.4. pH analysis

The following table presents the results corresponding to mean pH calculated after 14 and 28 days of twice-daily use of active and placebo. In comparison to D0, for the active group, statistically significant increase of +13.93% and + 11.57% were calculated respectively after 14 and 28 days of use. In comparison to D0, for placebo group, no statistical significant modification was seen after 14 and 28 days of use.

There were significant differences between the two products after 14 and 28 days of use (table 14).

8.2.5. Skin morphology by Optical coherence tomography (OCT) analysis

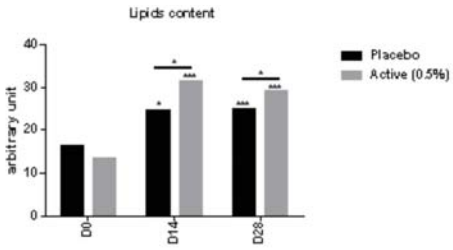
Orobanche rapum extract enhances the structure morphological of skin to a more healthy skin aspect in comparison with placebo after D14 and D28:

- Increasing of density of Stratum corneum
- Epidermis is more defined with a visible DEJ
- Dermis is more organized and brightness

8.3. Study 3: skin microbiota analysis by Metagenomic

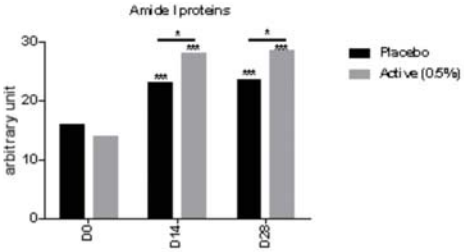
Phyla level:

After 14 Days of treatment, the microbiota evolves differently regarding the treatment. At the phylum level, when treated with vehicle, a significant



Lipids	D0	D14	D28
Active (mean arbitrary unit)	13.30	31.58	29.02
Placebo (mean arbitrary unit)	16.46	24.42	24.92
% variation active vs D0		137	118
% variation placebo vs D0		48	51
t test active vs D0		3.8E-06	3.44E-07
t test placebo vs D0		0.01076	2.40E-05
t test actif vs placebo	0.076	0.022	0.038

Figure 27: Lipids content determination using Raman spectroscopy



Proteines Amide I	D0	D14	D28
Active (mean arbitrary unit)	13.89	27.92	28.42
Placebo (mean arbitrary unit)	15.95	23.05	23.59
% variation active vs D0		101	105
% variation placebo vs D0		44	48
t test active vs D0		6.9E-07	5.1409E-09
t test placebo vs D0		0.00034	0.0004
t test actif vs placebo	0.086	0.012	0.019

Figure 28: Proteins content determination using Raman spectroscopy

Time	Active			Placebo			p value active vs placebo
	Mean +/- standard deviation (arbitrary unit)	Average variation (%) vs D0	p value (vs D0)	Mean +/- standard deviation	Average variation (%) vs D0	p value (vs D0)	
D0	4.23			4.27			0.898
D14	4.82	13.93%*	0.002	4.31	0.99%	0.828	0.000
D28	4.72	11.57%*	0.003	4.17	-2.22%	0.735	0.000

Table 14: Determination of skin pH

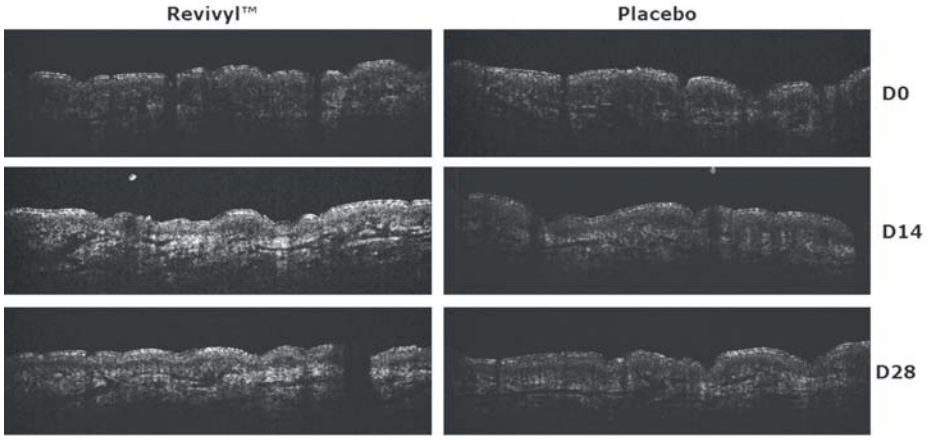


Figure 29: skin structure observed using Optical coherence tomography tool.

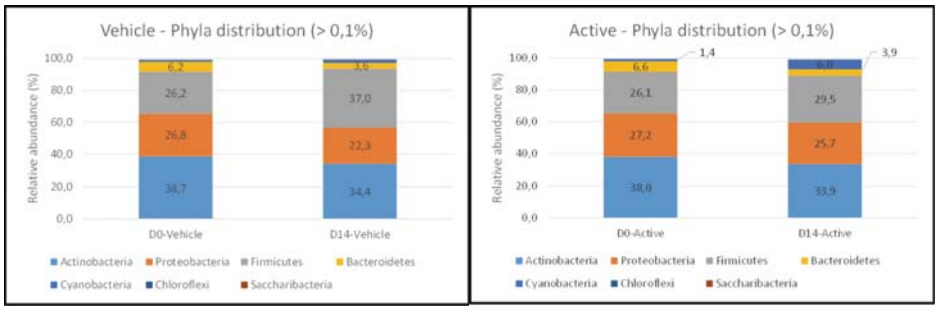


Figure 30: Microbial evolution at phyla level, after 14 days of treatment with Vehicle treatment (A) Vehicle + Active treatment (B).

increase of Firmicutes is observed from 26.2 % (D0) to 37.0 % (D14), in the same condition the other phyla stay stable (Figure 30A and Table 15).

After 14 days of treatment with vehicule+active, all phyla stay stable, even the Firmicutes (Figure 30B and Table 15).

At the phylum level, the active mitigates the vehicle impact on the observed microbial composition. Thus the active stabilises the microbiota, and maintains the essential balance of the skin microflora.

Genus level:

Considering the genus level, among the most abundant genera (> 1%) Finegoldia is the only genus significantly impacted by the active (see Table 16). Finegoldia magna is the only characterized species among the Finegoldia genus.

	Vehicle			Vehicle + Active		
Phylum	**D0-Vehicule: mean rel. freq. (%)**	**D14-Vehicule: mean rel. freq. (%)**	**p-values**	**D0-Active: mean rel. freq. (%)**	**D14-Active: mean rel. freq. (%)**	**p-values**
Actinobacteria	38.71	34.42	0.387	38.05	33.88	0.443
Proteobacteria	26.78	22.27	0.372	27.25	25.73	0.775
Firmicutes	**26.19**	**37.00**	**0.025**	**26.10**	**29.47**	**0.486**
Bacteroidetes	6.18	3.61	0.144	6.62	3.94	0.176
Cyanobacteria	1.15	1.91	0.496	1.36	6.04	0.272
Saccharibacteria	0.14	0.09	0.092	0.09	0.08	0.852
Chloroflexi	0.11	0.09	0.794	0.07	0.14	0.061

Table 15: Forearm microbiota phyla composition, after 14 days of treatment with Vehicle and vehicle + Active

	Active 0.5%			Placebo		
Genus	**D0-Active: mean rel. freq. (%)**	**D14-Active: mean rel. freq. (%)**	**p-values**	**D0-Vehicle: mean rel. Freq. (%)**	**D14-Vehicle: mean rel. Freq. (%)**	**p-values**
Finegoldia	**3.1**	**1.3**	**0.043**	**1.9**	**2.1**	**0.741**

Table 16: comparison of the relative abundances of Finelgoldia magna at D0 and D14 regarding the treatment (Vehicle and vehicle + Active).

This species is a normal inhabitant of human skin and is the most frequently Gram positive cocci isolated from infected lesion (Murphy E. et al. 2014). F. magna can be involved in mono- and poly-microbial infection of skin, bones, heart and meninges (Murphy E. and Frick I, 2013). A case of toxic shock syndrome caused by F. magna has also been reported by Rosenthal M. et al. (2012).

During the treatment with the active, the average relative abundance of Finegoldia significantly decreases from 3.1% (D0) to 1.3% (D14).

Without Active (vehicule only, see Figure 29 and Table 16), Finegoldia stays stable, thus the active inhibits the growth of the opportunistic pathogen Finegoldia (these inhibitory effect has also been observed on Finegoldia magna, regarding the species level, see figure 31).

9. Conclusion

9.1. Conclusion on in tubo and in vitro studies

In tubo: The active demonstrates an efficacy regarding the inhibition of enzymatic activities of two caspases involved in cell apoptosis: the "caspase 3" and "caspase 9". Moreover, this inhibition was dose dependent.

In vitro: The active demonstrates efficient results on stem cell keratinocytes and normal human keratinocytes:

- o Stimulation of nuclear expression of survivin
- o Stimulation of mRNA expression of several genes involved in the differentiation of the epidermis and maintenance of barrier function (filaggrin, involucrin, loricrin, transglutaminase, KLK5, KLK7…)

9.2. Conclusion on ex vivo studies

The active demonstrates an efficacy regarding:

1. The desquamation process:

- o Increase expression of KLk5
- o The stratum corneum is less cohesive

This desquamation process is improved probably by the regulation of the skin pH. The active increase the pH from the

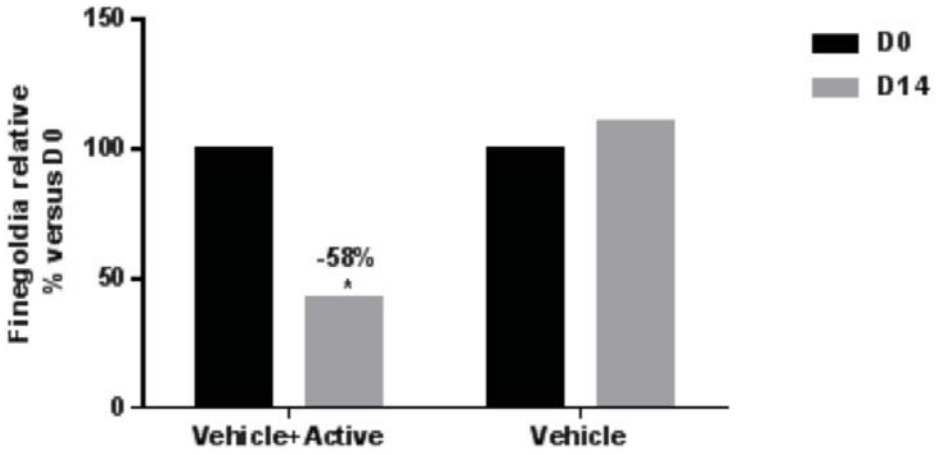


Figure 31: comparison of the relative abundances of Finelgoldia magna at D0 and D14 regarding the treatment (Vehicle and vehicle + Active).

value 4.2 to 4.7.

2. The differentiation process (in normal reconstructed epidermis):

o Increase expression of filaggrin, involucrine and Loricrin

3. The barrier repair recovery (in normal stripped and/or inflammatory reconstructed epidermises):

o Increase expression of filaggrin, involucrine and Loricrin

o Increase of ceramides content

o Increase of neutral lipids content

o Increase of long chain fatty acids

9.3. Conclusion on clinical studies

The active demonstrates an efficacy regarding:

1. Anti-aging properties:

o Decrease the wrinkles number and the wrinkled area.

2. Hydration properties:

o Increase total water content measured by Raman spectroscopy and by corneometer

3. Skin surface aspect:

o The skin is less flaky. This effect is confirmed by several complementary tools such as, D-squams analysis (Diagnoskin tool), optical coherence tomography, and Visioscan.

4. Skin microbiota analysis:

o The active maintains the balance of skin microbiota for 14 days

o It improves the skin microbiota composition by inhibition of opportunists bacteria: Finel-goldia magna

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