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Senotherapeutic approach revealed on human skin cells as a skin anti-aging strategy

Frechet Mathilde; Clariant Active Ingredients

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Introduction of research

Cellular senescence acts physiologically as a tumor suppression mechanism by limiting aberrant proliferation of cancer cells. But cellular senescence can occur also independently of cancer and contribute to the physiological processes of normal organismal aging. In skin, cellular senescence contributes to a myriad of effects such as accelerating skin aging, disbalancing wound healing process, regenerative efficiency. Indeed, the number of senescent cells (SC) increases in chronologically aged and photoaged skin (1). In skin, senescent fibroblasts occupy between 20% to 60% of the cells. Some characteristics of senescent cells, such as their ability to release growth factors, cytokines, chemokines, proteases contribute to the alteration of their extracellular environment. For example, matrix metalloproteinases (MMP-1 and MMP-3 for example) or proinflammatory cytokines such as IL-6 and IL-8 secreted from senescent fibroblasts are well known senescence associated secretory phenotype (SASP) play a critical role in degradation of ECM (2) leading to worsening skin conditions. Moreover, recent searches have identified that selectively eliminating senescent cells increases lifespan that improves health span and benefits the outcomes of a wide range of diseases (3; 4; 5). These findings indicate that suppression of senescent fibroblasts is a good target to counteract skin aging. Another type of anti-senescence compound corresponds to senomorphics, which can modulate the phenotypes of SCs to those of non-senescent cells through interfering with their secretory profile (decrease SASP and modulating senescence markers such as p21) (6). The selective elimination of senescent cells (senolytic) and the modification of secretory profile (senomorphic) opened the way to an exciting new field of research aiming at the development of senotherapeutics-like compounds in the cosmetic industry.

Sylibum Marianum, commonly called milk thistle is a plant from the daisy family. Originally a native of Southern Europe through to Asia, it is now found throughout the world. Here, we investigate the senotherapeutic-like effect of *Sylibum Marianum extract (SME).*

Methodology

Senescent HDFs was induced by cumulative population doubling (CPD) corresponding to 40 passages. Non senescent HDFs from passages 8 to 10 were used for the experiments. SA-β-gal positive cells were assessed after incubation with treatment of test materials for 3 days using Senescence β-Galactosidase staining kit (CST, MA, USA). The average number of stained and total cells was determined. HDFs viability was determined using MTT assay. p-H_2AX, p21, IL-6 and TNFa were assessed by immunofluorescence staining (1:50 dilution; CST, USA). The level of MMP-1 in the cell supernatants was measured by Human MMP-1 Quantikine ELISA Kit (R&D system Inc., USA). Clinical assessment was performed using a panel of 30 Caucasian women (aged 50–60) with wrinkles on the neck and décolleté. On the face area, volunteers used a 3% formula cream and a placebo cream on each half-face, twice a day for 14 days. Concerning the entire neck and décolleté areas, 15 volunteers used a 3% formula cream and 15 volunteers used a placebo cream twice a day for 56 days.

Results

Senolytic activity

To examine whether SME has senolytic activity on human dermal fibroblasts (HDF), we observed the cell viability in replicative senescent cells and non-senescent cells. The senescent and non-senescent HDFs were treated with SME for 3 days. Cell viability was reduced by 35% in SME-treated senescent cells and no change was observed in non-senescent cells (Fig.-1A). Furthermore, staining SA-β-gal in senescent cells was particularly reduced upon treatment with SME (100, 200 µg/mL) (Fig.-1B). the effect observed was dose dependent. Taken together, SME showed a mild senolytic effect in senescent HDF cells.

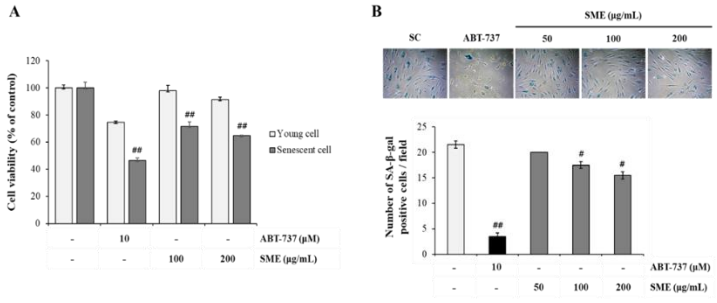

Fig.-1 (A) Effect of SME on the viability of non-senescent cells (p8) and senescent cells (p40) (B) SA-β-gal activity in senescent cells. Representative microphotographs are shown. Quantitation is presented to the below of the representative images. Data are represented as mean ± SEM of three independent assays. *p < 0.05; **p < 0.01 compared to non-senescent cell control, #p < 0.05; ##p < 0.01 compared to senescent cell control. **SC, senescent cell control; RS, replicative senescent**

Senomorphic activity

Next, we assessed the senomorphic effect of SME by the quantification of two established markers of senescence, using immunofluorescence: p21, and γH2A.X. As expected, in comparison to non-senescent cells, the senescent cells showed a significant increase of γH2A.X and p21 expression. SME reduced the level of γH2A.X and p21 in a dose dependent manner (Fig-2A, B), demonstrating the ability of SME to decrease senescence-related signal pathways.

In parallel we measured the expression levels of key well known SASP molecules, using immunofluorescence staining and ELISA assay. Senescent cells increased the level of IL-6, TNF-α and MMP-1 expression. However, SME reduced the expression of IL-6, TNF-α and MMP-1 in a dose-dependent manner (Fig.-2C, D, E), demonstrating the capacity of SME to modulate the secretome of senescent cells.

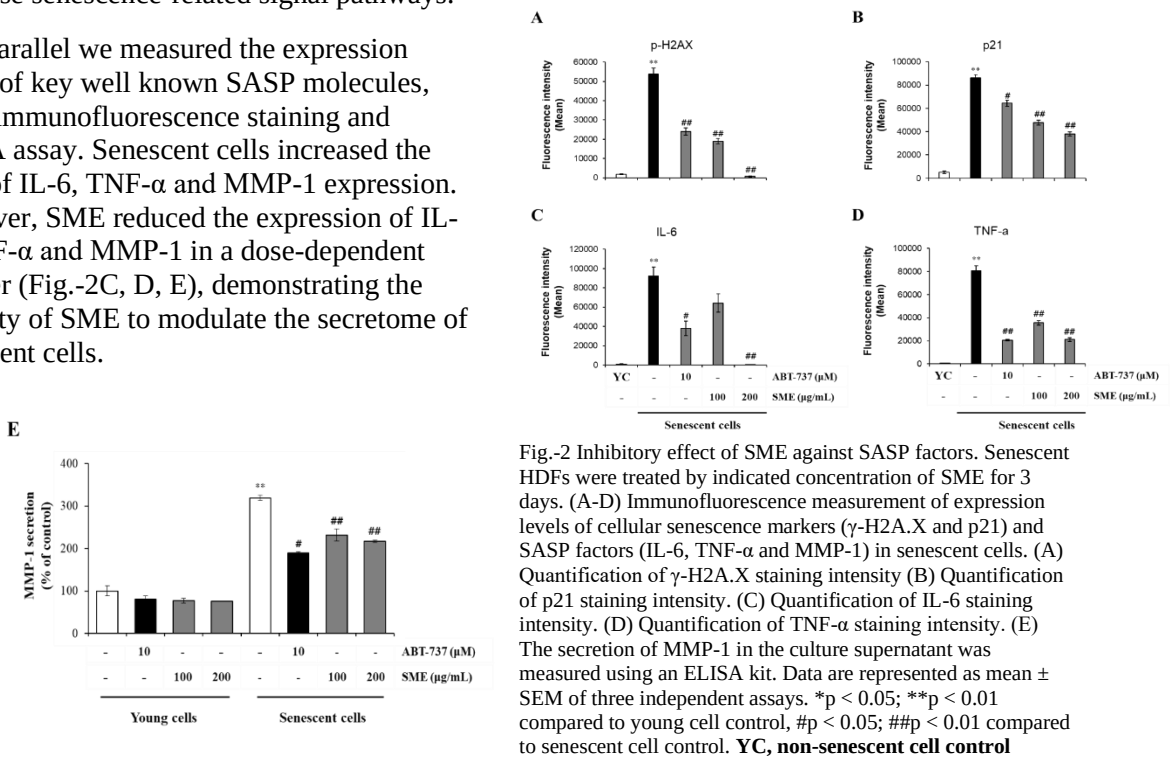

Fig.-2 Inhibitory effect of SME against SASP factors. Senescent HDFs were treated by indicated concentration of SME for 3 days. (A-D) Immunofluorescence measurement of expression levels of cellular senescence markers (γ-H2A.X and p21) and SASP factors (IL-6, TNF-α and MMP-1) in senescent cells. (A) Quantification of γ-H2A.X staining intensity (B) Quantification of p21 staining intensity. (C) Quantification of IL-6 staining intensity. (D) Quantification of TNF-α staining intensity. (E) The secretion of MMP-1 in the culture supernatant was measured using an ELISA kit. Data are represented as mean ± SEM of three independent assays. *p < 0.05; **p < 0.01 compared to young cell control, #p < 0.05; ##p < 0.01 compared to senescent cell control. **YC, non-senescent cell control**

Clinical assessment

Senescence causes in mature skin a decrease in skin brightness. We demonstrated that SME at 3% significantly improves the chest brightness (L* parameter) in comparison to placebo, leading to a more luminous and even skin (fig 3A). We were also able to demonstrate a benefit in skin hydration (corneometer) already after 14 days. IL-1α pro-inflammatory cytokine is an important SASP involved in the cellular contagiousness of senescence, and its high presence is correlated with a high number of senescent cells. We have previously shown *in vitro* that SME is able to reduce the expression of this cytokine. We show here using an innovative approach relying on patch sampling on the décolleté surface and ELISA assay to determine if IL-1α secretion that SME performance is confirmed *in vivo*. This result confirms at the clinal level the senomorphic ability of SME to counteract the hypersecretory phenotype of senescent cells.

A B

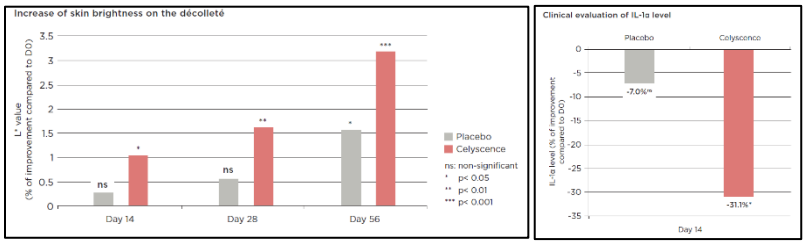

Fig 3: A. Skin brightness was measured using the L* parameter (Antera 3D device).* p<0.05, **p<0.005, ***p<0.001 compared to D0 for both groups. B. The IL-1α secretion from the décolleté of 15 subjects of each group for subsequently analysis before and after 14 days of a twice daily application of the body products using transdermal patches (FibroTx) (* p<0.05 compared to D0).

Conclusion

Cellular senescence is a hallmark of aging and the senescent cells accumulation contributes to tissues dysfunction in human, including the skin. Alleviating senescence induced skin damages is a huge challenge handled efficiency by SME. Thanks to its senotherapeutic activity (preventive, senolytic and senomorphic effects), SME demonstrates a positive impact on signs of aging such as hydration and skin complexion after only 14 days of application and an improvement of brightness after 56 days of use. Very interestingly, SME induces a rapid healthier look of the décolleté, an area important for human feminine feeling and strongly impacted by ageing process.

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About the speaker

Dr. Mathilde Fréchet received a Ph.D in Molecular and Cellular Pharmacology. She is an expert in cosmetic active ingredients with more than 17 years of experience in this peculiar and technical field. After 9 years in fundamental research studying skin cancer biology (France, Italy, England), she joined the industry of cosmetic active and broaden her expertise in cutaneous biology, dermatology and cosmetology to design active ingredients for cosmetics, as the R&D manager of Exsymol (Monaco) for 11 years and now the scientific director of the business segment Active Ingredients in Clariant (France).

Microfluidic arrays of dermal spheroids: a high-throughput screening platform of skincare products

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Introduction of research

Alternative *in vitro* skin models are highly recommended and favored to screen and select cosmetic active ingredients. Currently, the most advanced skin model is the full-thickness skin equivalent, a 3D cell-laden tissue scaffold that replicates at least the stratified structure of human skin including a reconstructed epidermis and dermis. It could be implemented with other cell types such as adipocytes to have a hypodermis layer, melanocytes to be pigmented, nerves and/or endothelial cells to be innervated and vascularized or immune cells to implement the skin model's immunity potential [1].

Organotypic micrometer-size 3D aggregates of skin cells, named multicellular spheroids [2-3], have emerged as promising *in vitro* models to test active ingredients (AIs) for dermocosmetic applications [4-5]. As they are mostly made by the hanging drop method [6] or using non-adherent U-bottom shaped plates [7], current spheroids-based *in vitro* models have the limitations of a broad distribution of spheroid dimensions, lack of control on the spheroid growth environments, and low throughput capacity of screening.

Objective

Here, we report a microfluidic (MF) skin-on-a-chip platform for the growth of dermal fibroblast spheroids (DFSs) in a biomimetic hydrogel under close-to-physiological flow conditions for throughput screening of AIs.

Methods

A75 mm × 50 mm MF chip was designed to contain 2,400 microwells in 12 replicates of 200 wells, with each replicate having a common inlet and outlet and offering the capability of simultaneously screening the effect of 12 distinct AIs with a high number of spheroid replicas (Figure 1A). The DFSs formed after 2 days of on-chip culture were grown for 3 additional days. The size of spheroids, fibroblasts viability and extracellular matrix (ECM) synthesis were evaluated. Procollagen type I and fibronectin were measured in the media by immunoassay; E-cadherin, F-actin, collagen type I and fibronectin were immunostained in spheroids at different timepoints. For the validation purpose, the DFSs were used in a time-efficient manner for testing the effects of vitamin C on the synthesis of the two most important ECM proteins, namely, collagen type I and fibronectin.

Results

By altering the size of the microwells, hydrogel composition, spheroid diameter and the loading densities of fibroblast-suspension, we achieved a 90% success rate in forming the cell-laden droplets and an 89% of survival rate of the DFSs (Figure 1B), without the formation of a necrotic core.

Despite 40% contraction observed on day 2, the spheroid viability remained excellent in the 100µm-large microwells up to 5 days with or without vitamin C (Figure 1C). DFS was also stained for E-cadherin demonstrating cell-cell adhesion junctions (data not shown).

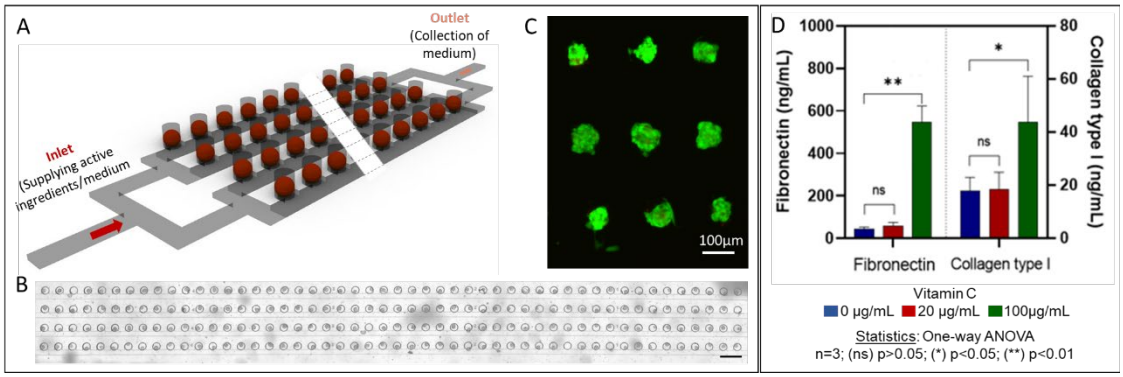

Figure 1: A-Illustration of a 200-well replicate among the 12 per chip; B-Spheroids at day 5; C-Live (green)/Dead (red) assay at day 5 (100µg/ml vitamin C) and D-Fibronectin and collagen type I synthesis at day 3 with vitamin C at different concentrations for 24h.

Vitamin C increased both the secretion and deposition in the DFSs of collagen type I and fibronectin in a dose and time-dependent manner (data not shown). The 100µg/mL concentration was chosen as a positive control as it was the minimal concentration inducing both fibronectin and collagen significantly in a reproducible manner (Figure 1D).

Conclusions

With this first case study, we have shown that our DFS-based MF skin-on-a-chip platform presents a high screening potential for AI discovery in skincare or for drugs in dermatology. The formation of DFSs was reproducible and the cell survival for more than 5 days without necrosis or exaggerated contraction allowed the fibroblasts to grow and produce ECM proteins. Moreover, DFSs are suitable to screen for ECM modulators as they were inducible by vitamin C.

For broader possible claims, we envision that this MF platform can be further adapted to more complex 3D skin models by first growing fibroblasts with keratinocytes to generate skin, then adding other cells such as sebocytes, adipocytes, dermal papilla cells and so on. The chip will also be upgraded to increase the number of AIs tested per chip into a scale-up industrial setting, thereby facilitating the high-throughput screening of diverse AIs in 3D conditions. When implemented, the MF skin-on-a-chip platform will generate large and accurate data sets that may be integrated into machine learning strategies.

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About the speaker

Oussama EL BARAKA is the manager of the organoids and hair model team for BASF Beauty Care Solutions in the R&D Department. He has a master's degree in Biology and Cell Engineering from Université de Lorraine (France) and he is finishing his PhD at the Technische Universität Berlin (Germany). He has a background in cell biology and cell engineering with a focus in three-dimensional, hair and microfluidic models.

Quantitative sensory interpretation of rheological and tribological parameters of emulsions: impact of the nature of the emollient on key sensorial attributes

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Introduction of research

The success of a cosmetic product is highly dependent on the consumer's perception and the sensorial profile of the product. Previous studies have shown that both emulsifiers and emollients affect the skin feel of emulsions, in the early phases (appearance, pick-up and rub-out) but also in later stages (after-feel) of the consumer's experience [1,2].

There is an increasing body of evidence in the literature that instrumental methods like rheology (science of flow and deformation of matter) and tribology (science of interacting surfaces in relative motion) can be used during the formulation developmental stage for the quantitative assessment of sensorial attributes of cosmetic emulsions [3,4]. Some of these methods are well correlated with visual or tactile perceptions such as *softness/hardness/firmness, thickness, spreadability, slipperiness, greasiness,* or *stickiness* of skin care products, as assessed by trained sensory panels [4-7].

The main objective of this study is to use a simple, reliable, and cost-effective tribo-rheological protocol [8] to assess the impact of a novel natural-derived multifunctional emollient, Dimethicone PG-Sunflowerseedate (DSF) [9], on the sensorial properties of oil-in-water emulsions, in comparison with other natural (sunflower seed oil), synthetic (mineral oil) and silicone (dimethicone) oils.

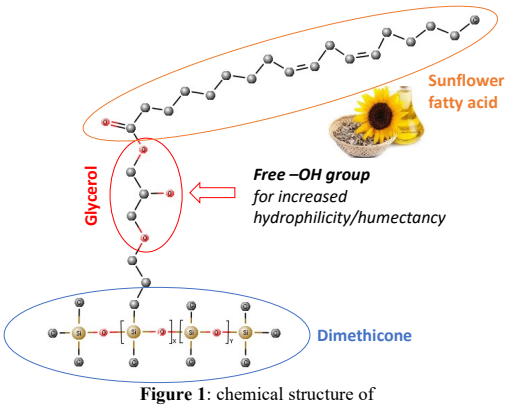


Figure 1: chemical structure of Dimethicone PG-Sunflowerseedate (DSF)

Materials & Methods

DSF is a multifunctional sunflower-derived emollient in which sunflower fatty acids, dimethicone and glycerol have been grafted together to provide enhanced sensorial benefits and ease of formulation in skin and hair products. Its chemical structure is shown on Figure 1.

Seven oil-in-water emulsions were prepared according to the same ingredient list and process. They differed only by the nature of the Emollient 1 in the oil phase, used at a concentration of 10% w/w (Table 1).

Phase	Function	INCI	Composition (% w/w)						
			I	II	III	IV	V	VI	VII
A	Emollient 1	Sunflower Seed Oil	10					5	8
		Dimethicone (10cS)		10					
		Dimethicone (350cS)			10				
		Mineral Oil				10			
		Dimethicone PG-Sunflowerseedate					10	5	2
	Emollient 2	Isopropyl Myristate	10	10	10	10	10	10	10
	Emulsifier	Cetearyl Alcohol (and) Polysorbate 60 (and) Oleth-10 (and) PEG-75 Lanolin (and) PEG-100 Stearate (and) Ceteth-20	5	5	5	5	5	5	5
B	Humectant	Glycerine	5	5	5	5	5	5	5
	Thickener	Carbomer	0.3	0.3	0.3	0.3	0.3	0.3	0.3
	Carrier	Water	68.6	68.6	68.6	68.6	68.6	68.6	68.6
C	pH adjuster	Triethanolamine	q.s. pH 6						
D	Preservative	Benzyl Alcohol (and) Dehydroacetic Acid	1	1	1	1	1	1	1
D	Chelating agent	EDTA	0.1	0.1	0.1	0.1	0.1	0.1	0.1

Table 1: Composition of oil-in-water emulsions I to VII. Process: mix Phase A items to 60°C until appearance is clear; mix Phase B items to 60°C for 30 min; slowly add Phase A into Phase B and mix for 30 min at 60°C; begin cooling while mixing; at temperature less than 50°C, add Phase C until pH 6; at temperature less than 40°C, add Phase D and keep mixing until 25°C. Homogenize for 15min at 25°C.

An instrumental protocol was designed and applied for the quantitative assessment of the sensorial attributes of the different oil-in-water emulsions, by correlating simple sensory lexicons to rheological and tribological parameters (Table 2).

For each sensorial attribute, a score of 1 to 4 was defined according to the range of the corresponding physical parameter, as described in Table 3.

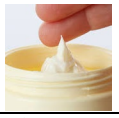
Moment of User Experience	Sensorial Attribute	Description of Physical-Sensorial Relationship	Instrument / Protocol / Physical Parameter	Level of Physical-Sensorial correlation
Appearance & Pick-up 	*Firmness*	The degree to which the product can keep its shape or structure in the presence of force.	Rheometer / Oscillatory stress sweep, 25°C / Yield Stress (Pa)	High [5]
Rub-out & After-feel 	*Spreadability*	The force required to make the product flow or to spread it on a surface.	Rheometer / Shear rate sweep, 32°C / Viscosity (Pa.s) under high shear rate (1000 s^{-1})	High [6]
	Slipperiness	The ability of the product to reduce friction between contacting sliding surfaces.	Tribometer / Sliding speed sweep, 32°C / Coefficient of friction at 10mm/s	Low to Medium [4,7]

Table 2: Proposed protocol of rheological and tribological parameters-sensory attribute pairs, their description, and level of correlation.

Score	Yield Stress (Pa) - *Firmness*	Viscosity (Pa.s) under high shear rate (1000 s^{-1}) - *Spreadability*	Coefficient of friction at 10mm/s - *Slipperiness*
1-low	<100	>0.425	0.10-0.11
2-medium	100-140	0.350-0.425	0.09-0.10
3-high	140-180	0.275-0.350	0.08-0.09
4-very high	>180	0.200-0.275	0.07-0.08

Table 3: Correlation of the range of yield stress, viscosity under high shear rate and coefficient of friction to *firmness, spreadability* and *slipperiness* scores (1-4), respectively

Results

Appearance & Pick Up: the nature of the emollient has a slight impact on the *firmness* of O/W emulsions. Among the five oils tested as Emollient 1, mineral oil gives the thicker and firmer structure to the emulsion.

Rub-Out & After-feel: results show that the nature of the emollient impacts both the *spreadability* of the product and the *slipperiness* of the cosmetic film during the later rub-out stage of the application.

As shown in Figure 2, the sensory profiles of emulsions IV and V, obtained with Dimethicone PG-Sunflowerseedate and mineral oil, respectively, are very similar. Both have medium-high *firmness,* very high *spreadability* and high *slipperiness*, an appealing sensorial profile which can also be found in existing commercial benchmark day creams.

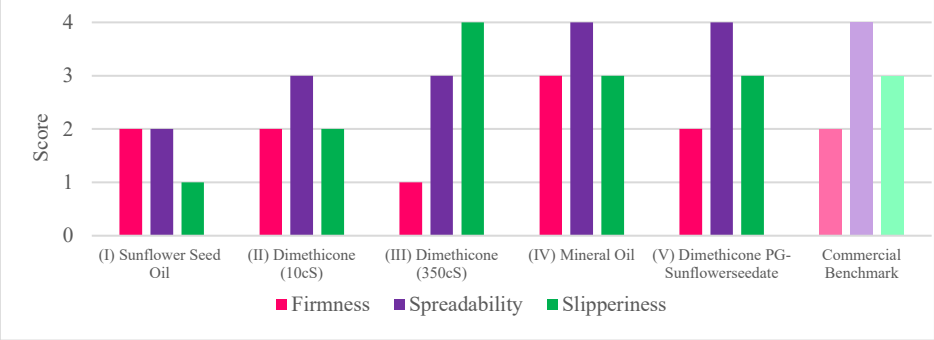


Figure 2: Influence of Emollient 1 on *firmness, spreadability* and *slipperiness* scores of O/W emulsions I-V, compared to a commercial benchmark day cream (Estée Lauder Revitalising Supreme Light).

Multi-functional properties of Dimethicone PG-Sunflowerseedate (DSF):

DSF is a 3-in-1 ingredient, which was designed to have triple benefits in cosmetic formulation: 1/ the sunflower oil-derived lipophilic pendant chains provide moisturizing, conditioning, emollience and compatibility with other polar oils ; 2/ the silicophilic dimethicone backbone provides glide, occlusive skin barrier, shine, smoothing characteristics, non-greasy feel and compatibility with other silicones; 3/ slightly hydrophilic di-substituted glycerol linkers are used to bridge the lipophilic and silicophilic moieties and provide increased water compatibility, moisturizing and humectancy (Figure 1).

As detailed in Figure 3, the *in vitro* sensory profile of O/W emulsions can be also strongly influenced by the concentration of DSF in the oil phase. The higher the DSF content, the easier to spread and the more slippery the emulsion is upon rub-out.

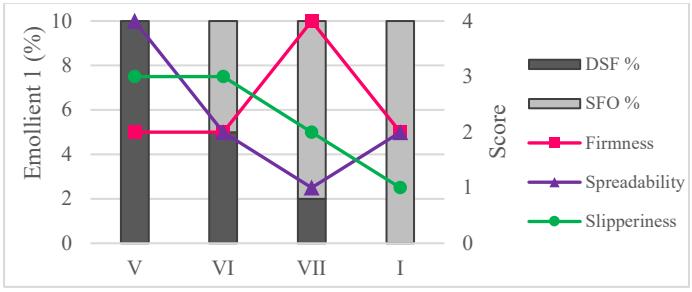


Figure 3: impact of different ratios of Dimethicone PG-Sunflowerseedate (DSF) vs Sunflower Seed Oil (SFO) on *firmness, spreadability* and *slipperiness* scores of O/W emulsions I, V, VI and VII.

Interestingly, when used at lower concentration (2%), DSF can bring dramatic viscosity/*firmness* enhancement benefits to emulsions (Formula VII, Figure 3). The reasons for the unexpected properties of the emulsion VII with 2% DFS need to be further investigated, but given its slight hydrophilicity, one can assume that DSF probably acts more as an emulsifier (positioned at the oil/water interface) than an emollient (in the oil phase) at this concentration.

Conclusion

Using a convenient and cost-effective instrumental protocol, which correlates the rheological and tribological parameters of emulsions with perceivable attributes such as *firmness*, *spreadability*, and *slipperiness*, the sensorial impact of a new multi-functional ingredient, Dimethicone PG-Sunflowerseedate was assessed. When used at 10% in the oil phase of O/W emulsions, it functions as an emollient and gives similar sensorial profile as mineral oil in terms of *firmness, spreadability* and *slipperiness*. Compared to sunflower seed oil it brings more *spreadability* and lubrication in the rub-out experience. Interestingly, it can also be used as a thickening agent at lower concentration (2%).

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About the speaker

Séverine Jeulin, is the Director of New Product Development for Personal Care at Elé Corporation. She holds a Ph.D. in Organic Chemistry from Paris 6 University, France and has 15 years of international experience as R&D team and project leader in cosmetic and pharmaceutical industries, across Asia, Europe, and the USA. Her main expertise is the design, development, and evaluation of innovative cosmetic ingredients, leading to 8 patents and over 15 product launches for international cosmetic brands.

Screening of personal care ingredients for potential in immediate wrinkle blurring

Daphne Benderly, PhD; Presperse

Introduction of research

There is great consumer interest in personal care products that provide immediate wrinkle blurring/ soft focus, in which the appearance of facial fine lines and wrinkles is demonstrably blurred. This should be achieved without the formulation giving high coverage or an unnatural appearance. One method to achieve this effect is by use of an appropriate soft focus powder in the formulation.

Guidelines for a good soft focus powder were summarized by Emmert[1] – minimal light absorption; high total transmission with most of the transmission being diffuse; minimal specular reflection and high scattered reflection.

Methodology

Several methods for evaluation of soft focus potential of powders are given in the literature and in supplier publications[2-4]. The goal in the method described here is to use hand-held/benchtop instrumentation and realistic powder use levels.

The basis for this method is the difference in reflectance between a mirror-like surface and a "blurring" soft focus surface. A perfectly mirror-like surface has high reflectance at an angle equivalent to the specular angle, and much lower reflectance at other observation angles. A nonglossy surface with good soft-focus characteristics would exhibit less reflection at the specular angle, and more reflection at other observation angles. These are two of Emmert's criteria for soft-focus potential. Experimentally, the intensity of the reflectance spectrum should be similar at the different measurement angles so that the curves obtained from different angles should overlap.

The instrumentation used here (multi-angle spectrophotometer) is used also in the evaluation of effect pigments. One learning from interference pigments is that reflection at a *trans* angle, which is "behind" the specular angle, gives important information about color, and gives different results than reflection at a *cis* angle[5,6]. This finding is incorporated into the ASTM standard E2539-14 for measuring color of interference pigments. During the development of this soft focus method, this differentiation provided useful guidance. These angles are show in Figure 1.

In this work, drawdowns were prepared on an artificial leather substrate (EnviroLeather, LDI Corporation) rather than glass plates or transparencies. This substrate is more similar to skin and since it is textured, the soft-focus effects of the applied film can more easily be visualized. Powders were used at a level of 10%. Several different colors of artificial substrate were used, to examine the effect, if any, on the ranking of cosmetic powders. Powders are incorporated into either a commercially available aqueous based clear wood coating (Minwax) or a commercially available lotion (Nivea Essentially Enriched lotion). For evaluation of rheology modifiers, simple model emulsions were prepared. The Minwax carrier was chosen rather than nail lacquer, which has been used in many of the studies cited in the literature, as it avoids the flammability and odor hazards associated with lacquer.

Once measurements are taken, the ratio is calculated of the spectral results at angles 45as-15 (*trans* angle) and the 45as110 angle. As this ratio *decreases*, the soft focus performance is expected to *increase*.

Results

The first step was to demonstrate that this method can differentiate between powders of known performance, based on formulators' experience. Four powders were evaluated: Talc, Mica (sericite), Calcium Aluminum Borosilicate and Acrylates Crosspolymer. Talc and mica are bulking agents, and are not expected to show soft focus potential. The two other powders have proven soft-focus performance.

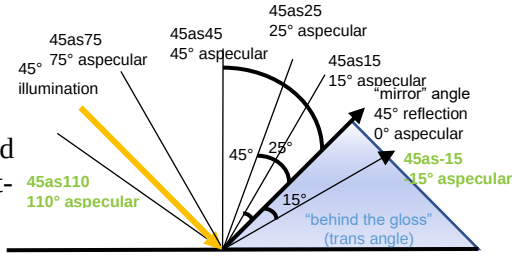

Figure 1 Angle definition

Typical results are shown in Figure 2. Similar trends were obtained from different color substrates. This test method clearly differentiates between powders expected to give soft focus effects, and powders that do not. This method has been applied to hydrophobically modified powders by using an appropriate non-aqueous carrier. A surprising use of this method was to extend beyond powder raw materials and evaluate rheological modifiers. Evaluation of rheological modifiers with similar chemistries was conducted in an emulsion formulation, and differences are seen (Figure 3).

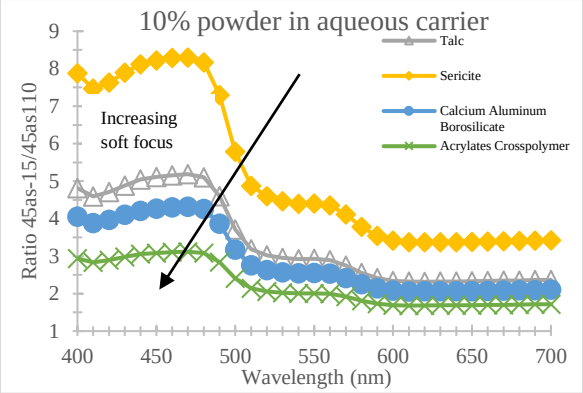

Figure 2: Typical results, powders in a Minwax carrier

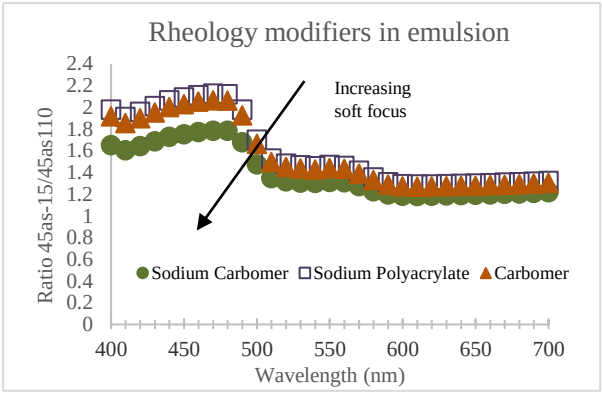

Figure 3: Emulsions with different rheology modifiers

Conclusion

This proposed method gives a good indication for soft focus potential. For powders, films with either Calcium Aluminum Borosilicate or Acrylates Crosspolymer showed a more uniform reflected light scattering, compared to Talc or Mica (sericite). For rheology modifiers, differentiation was seen between products of similar chemistries. Results not shown include evaluation of hydrophobically modified powders by using a water-in-oil based carrier, rather than an aqueous carrier. Results agreed with formulators' visual assessments.

In conclusion, this method for soft focus assessment is easy to implement and can give valuable data to chemists and formulators in the early stages of new product evaluation or formulation development.

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About the speaker

Graduated with a PhD in Materials Engineering from the Technion Institute of Technology, and BS in Mechanical Engineering from the Massachusetts Institute of Techonlogy. Daphne has 30 years experience in materials R&D and development, in a range of industries. She is currently Senior Manager Research and Discovery at Presperse Corporation.

Predict the local tolerance of ingredients for gentler personal care applications

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Introduction of research

What consumers need are efficient and well tolerated products. Beyond conducting classical safety evaluation on pure ingredients, tests are done at use levels, intended for healthy mature skin. However, personal care products target different populations, body area and skin conditions, with more or less sensitivity. How can we address the skin specificities to demonstrate the tolerance of ingredients? Tailored predictive in vitro protocols were explored in this perspective.

Objective: Former publications on 3D models mainly focused on formulations. The objective is therefore to investigate upstream the behavior of ingredients with a well known tolerance using different human reconstructed models intended to be closer to different real situations (baby skin, impaired epidermis...). Final goal is to provide the manufacturers of finished products with more dedicated tolerance data.

Materials & methods: Ingredients from different chemical structures and functions were applied at usual doses: surfactants with cleansing, solubilizing or emulsifying properties, thickening-stabilizing polymers, active ingredients with moisturizing, soothing and skin repair action. Controls and benchmarks were chosen for comparison and to challenge the model's predictivity. Four 3D biological systems based on cultivated human cells were tested. Two models, dedicated to skin tolerance, were developed from standard Reconstructed Human Epidermis (RHE): an “immature” epidermis intended to be closer to baby skin and an impaired epidermis expected to model damaged skin. Two existing biological systems were also evaluated. A first “gingival epithelium”, histologically similar to the outer cell layers of the human gum, was selected for ingredients used to clean the teeth or refresh the mouth. A second “vaginal epithelium”, histologically similar to vaginal mucosa, was selected for ingredients used for intimate areas. The biological parameters evaluated were selected for their relevance against the targets of the experimental models. Cellular viability recognized for its predictivity of irritation potential was measured on all the in vitro models, using the same protocol and controls for calibration. For mucosa models, the cell viability performed at different exposure times was able to predict, as a stand-alone, the irritation potential conclusion. For the epidermis models, tolerance conclusion came from a tailored combination of biological parameters.

Results & Discussion: The results on controls and benchmarks confirmed the greater sensitivity of each model compared to the standard RHE, in accordance with specificities in tissue structure and biological functions. Differences in dose-response relationship on cell viability were also observed on positive controls between the models, suggesting adapted reactions and interest of each of them. Ingredients provided specific responses, with a dose-effect, according to the model sensitivity. High sensitivity to surfactants was overall noticed, both with ingredients alone and with formulations that contain them. For example, different reactions have been observed between cream gels and emulsions with identical oil.

Furthermore, slight differences in the chemical structure could lead to distinct effects, such as the length of the fatty chain of an emulsifier. The multiparametric approach carried out on the “immature” and “impaired” epidermis models enriched tolerance conclusion with cellular, morphological and functional effects. Finally, some benefits of the tested ingredients could be observed on the “impaired” model. For example, an enhancement of the tissue recovery could be qualitatively highlighted with a sugar-based moisturizer (Figure 1). Some improvement of the barrier functionality is also clearly seen with the skin repair and the soothing active ingredients as illustrated by Trans-Epithelial-Electrical-Resistance signal evolution (TEER in Figure 2)

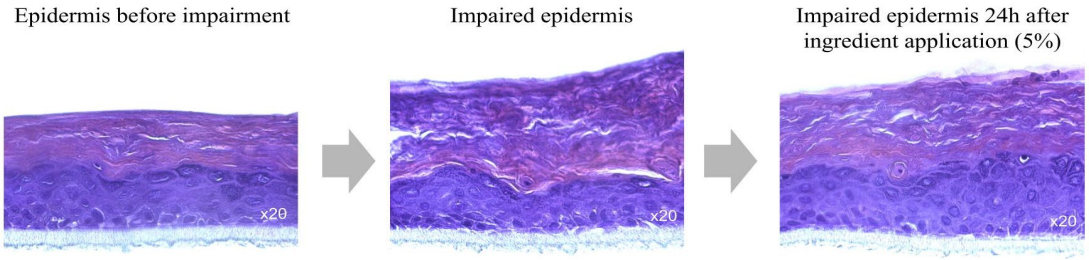

Figure 1. Histological section, H&E staining; Photo magnification x 20.

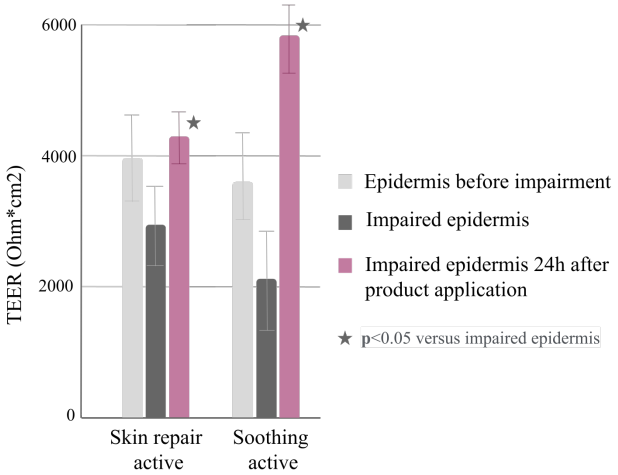

Figure 2. TEER kinetics.

Conclusion

These models have proven to be useful screening tools to support the selection of ingredients, at the right dosage, according to the final area of application. The use of multiparametric analysis on the “immature” and “impaired” reconstructed epidermis enable better understanding of the ingredient mechanisms before using it on new body targets. Mucosal models, tested first, should be optimized in the same way.
This approach favors customizations of formulation according to the final use from the early stages of development, saving time and reducing cost and reinforces the safety of the next new trend or inspiring concept.

About the speaker

Alicia Roso works at Seppic since 1986, currently as scientific communication manager in the Research & Innovation team. Chemical engineer, she has worked for twenty years in the cosmetic R&D team, firstly as lab technician then as lab manager. She joined the marketing team in 2006 as product manager and gained a marketing MBA from ESSEC business school in 2012. She was named as Air Liquide International Expert for health care formulation and emulsions in 2010. She is co-author of 23 patents on new ingredients or formulation technologies dedicated to cosmetology and dermopharmacy applications.