J. Cosmet. Sci., **62,** 421–452 (July/August 2011)

Papers Presented at the Annual Scientific Seminar of the Society of Cosmetic Chemists

June 2–3, 2011
The Bellagio
Las Vegas, Nevada

HENRY MASO KEYNOTE AWARD LECTURE

SPONSORED BY THE HENRY MASO FAMILY AND SILTECH, LLC

BIOMECHANICS OF THE BARRIER FUNCTION OF HUMAN SKIN: PREDICTING SKIN DAMAGE AND THE EFFECTS OF COSMETIC TREATMENTS

Professor Reinhold H. Dauskardt

Stanford University, 496 Lomita Mall, Durand Building, Room 121, Stanford, CA 94305
dauskardt@stanford.edu

The biomechanical properties of human skin are crucial in understanding the mechanical and biophysical function of skin, its cosmetic "feel" and appearance, and play a central role in skin damage processes like chapping and cracking. Daily exposures to variable temperature and moisture conditions, together with application of cleansing agents, lead to the perception of skin "dryness" and "tightness." However, the connection to the mechanical properties and stresses in the skin remains elusive due in part to a paucity of mechanical properties of the skin layers following such exposures.

Dry skin conditions are accompanied by significant changes in the stratum corneum (SC) biomechanical properties including the "so-called" drying stress, σ_{sc}, which leads to the perception of skin stiffness/tightness and provides a mechanical driving force for skin damage processes like cracking and chapping (**Fig. 1**) [1-8]. Surprisingly, the effects of moisturizers on these properties as well as their role in reducing the mechanical driving force for dry skin damage are not well characterized.

Our objective has been to develop a suite of novel thin-film methodologies in which moisture and moisturizer effects on skin stiffness [1-3, 5-8], stress [5-8] and fracture resistance [1-4, 7,8] can be directly quantified. We have particularly focused on the outermost stratum corneum (SC) layer. We show how water loss determines SC drying stresses and is linked to hydration and chemical state of the SC components [5]. Using a combination of micro-tension, substrate curvature, bulge and delamination techniques, we show how a range of moisturizing molecules reduce drying stresses and alleviate skin damage [6-8]. We show how drying stresses develop as a function of time and how they change following the application of classes of humectant, occlusive and emollient molecules. They included concentrations of the trihydroxylated humectants (glycerol) widely used as a hydrating agent, purified mixtures of occlusive hydrocarbons (petrolatum), a range of silicone homopolymers (dimethicone) at varying viscosities, and a range of ester based emollients.

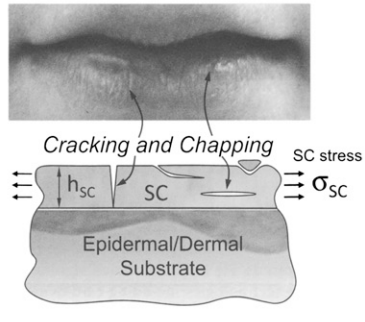


Figure 1: A schematic illustration showing typical dry skin cracking and chapping processes that result from the development of drying stresses in SC.

We finally demonstrate how damage processes in human skin can be quantitatively modeled and predicted based on thin-film biomechanics and cracking processes [5-8]. We believe that this represents a new approach to characterize and model the fundamental biomechanics of human skin.

References

1. K.S. Wu, W.W. van Osdol, and R.H. Dauskardt. "Mechanical Properties of Human Stratum Corneum: Effects of Temperature, Hydration, and Chemical Treatment," Biomaterials, 27 [5], 785-795, 2005.
2. K.S. Wu, M. M. Stefik, K. P. Ananthapadmanabhan and R.H. Dauskardt, "Graded Delamination Behavior of Human Stratum Corneum," Biomaterials, 27, 5861-5870, 2006.
3. K. S. Wu, J.Li, K. P. Ananthapadmanabhan, and R. H. Dauskardt, "Time-Dependant Intercellular Delamination of Human Stratum Corneum," Journal of Materials Science, 42, 8986 – 8994, 2007.
4. K. Levi, J. Baxter, H. Meldrum, M. Misra, E. Pashkovski, and R. H. Dauskardt, "Effect of Corneodesmosome Degradation on the Intercellular Delamination of Human Stratum Corneum," Journal of Investigative Dermatology, 107, 2008.
5. Kemal Levi, Robert J. Weber, James Q. Do, and R. H. Dauskardt, "Drying Stress and Skin Damage in Human Stratum Corneum," International Journal of Cosmetic Science, 1 - 18, 2009.
6. K. Levi and R. H. Dauskardt, "Application of Substrate Curvature Method to Differentiate Drying Stresses in Topical Coatings and Human Stratum Corneum," International Journal of Cosmetic Science, 1 - 5, 2010.
7. K. Levi, A. Kwan, A.S. Rhines, M. Gorcea, D.J. Moore, and R.H. Dauskardt, "Emollient Molecule Effects on the Drying Stresses in Human Stratum Corneum," British Journal of Dermatology, 2010.
8. Kemal Levi, Alice Kwan, Allison S. Rhines, Mihaela Gorcea, David J. Moore, and Reinhold H. Dauskardt, "Effect of Glycerin on Drying Stresses in Human Stratum Corneum," Journal of Dermatological Science, 61[2], 129 - 131, 2011.

ARABINOXYLO-OLIOGOSACCHARIDE: A LIGHTENING ACTIVE INGREDIENT WITH A NEW MECHANISM OF ACTION THAT INHIBITS MELANOGENESIS TARGETING TYRONSINASE AND TRP-1

Romain Reynaud, Anne Sophie Dutailly, Julie Corfa and Paolo Marchesi
Soliance, Pomacle, France

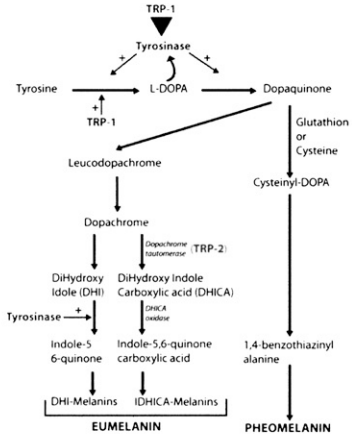
INTRODUCTION:
For a long time TRP-1, a major enzyme involved in melanogenesis, was thought to have an activity DHICA oxidase and to be involved in the way of eumelanins are generated . New scientific data are showing this activity remains true only in the murin model, but not in the human one[1].

In humans, TRP-1 acts on two levels in the melanogenesis:

- At the level of tyrosine hydroxylation: this reaction is the limiting stage of the melanogenesis because it is a slow reaction. At low levels of tyrosine, TRP-1 has a Tyrosinase Hydroxylase activity that transforms tyrosinase into L-DOPA. The increase of L-DOPA then activates tyrosinase[2].
- At the level of the stabilization of tyrosinase: after the activation of the tyrosinase, TRP-1 forms a complex with tyrosinase to stabilize it and increase its activity[3].

TRP-1 is involved in the first step of the melanogenesis: it is a key enzyme essential for the melanin synthesis.

Through this new data, the objective of our study was to understand the way of action of Arabinoxylo-oligosaccharide in the melanogenesis in the human model.



OBJECTIVE OF THE STUDY:
To study and understand the mechanisms of action of the arabinoxylo-oligosaccharide in the inhibition of melanogenesis (using the new scientific data) and to validate its activity with in vivo results.

METHODS:
Inhibition of melanogenesis by Arabinoxylo-oligosaccharide was initially evaluated *in vitro* by spectrophotometry on normal human melanocytes. The key enzymes of melanogenesis were then tested to understand which steps of melanin synthesis are inhibited by Arabinoxylo-oligosaccharides:
- Tyrosinase activity was evaluated by spectrocolorimetry on human melanocytes in culture.
- TRP-1 activity was measured by immunolabelling on treated and irradiated skin explants.

To confirm the results, skin luminosity of treated skin explants was evaluated *ex vivo* by chromametry. Explants from an abdomino plasty (about 10 mm in diameter) on a 33 year-old Caucasian woman were prepared and kept alive in a specific BEM medium (BIO-EC's explants medium). At D0, D2, D4, D6 and D8, skin explants were applied with 2 mg/cm^2 of cream containing 3% of Arabinoxylo-oligosaccharides. The treated control explants were irradiated every day for 10 days with UVAs (6-8% UVB). Before irradiations, the culture medium was temporarily replaced with a Hank's Buffered Saline Solution. After irradiations, the explants were put back in the culture medium. At D10, the following parameters were analyzed:
- Explants discoloration, measured using a Minolta CM 2600 D chromameter.
- The quantity of melanin in the basal layer, visualized on paraffin sections formalin-fixed by silver impregnation according to the Masson-Fontana method (cf. results fig 1.).
- The number of TRP-1 positive melanocytes, measured by immunolabelling on paraffin sections with an anti-TRP-1 monoclonal antibody (clone Ta99, CA1005, Calbiochem) at 1/25 for 2 hours at room temperature with a streptavidine biotin amplifier system (Vector, PK-7200) revealed in VIP (SK-4600, Vector). The nuclei were counterstained with Masson hemalun.

Also clinical study on Asian volunteers was done to observe lightening efficacy. A panel of 22 middle-aged Asian women (37 years old) applied the following creams on their forearms twice a day for 56 days: a cream containing 3% of Arabinoxylo-oligosaccharides and a cream containing 2% of arbutin as a positive reference (cf. results fig. 2).

RESULTS:
Melanogenesis inhibition was measured at 80%. As a comparison, kojic acid (a reference for skin lightening) inhibits melanogenesis by 35,6%. Despite this, the inhibition of the tyrosinase appears to be quite low (25%) compared to kojic acid. At the same time, the inhibition of TRP-1 is as high as 88%. This confirms the preliminary idea on the mechanism of action of Arabinoxylo-oligosaccharide.

Visualization of melanin and image analysis: Applying the cream containing 3% of Arabinoxylo-oligosaccharides for 10 days, coupled with chronic UV irradiation, induces a significant reduction of 18% ($p<0.05$) of the surface containing melanin in the basement layer, compared to the control at D10. Image analysis carried out on 18 microscopic fields for each batch enabled us to discover the surface percentage containing melanin in the basement layer.

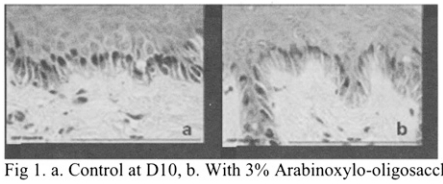


Fig 1. a. Control at D10, b. With 3% Arabinoxylo-oligosaccharides at D10

The clinical study shows significant and visible results, within only 28 days. At 28 days, the cream containing 3% of Arabinoxylo-oligosaccharides had significantly increased the CIE 1976 lightness. The variation observed with the cream containing 2 % of arbutin was not significant. At 56 days, the both creams had significantly increased the CIE 1976 lightness but there is no significant difference between the two molecules.

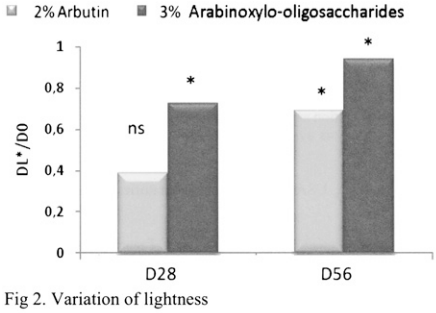


Fig 2. Variation of lightness

CONCLUSION:
Arabinoxylo-oligosaccharides efficiently inhibit melanogenesis through its action on TRP-1 and tyrosinase which are involved in the first steps of melanin synthesis. This conclusion was confirmed by the new scientific data. Soliance arabinoxylo-oligosaccharide is also environmentally-friendly. This new lightening active ingredient combines innovation and efficiency to reduces age spots and lighten up the skin for a radiant complexion.

1. Boissy RE, and all. *Exp Dermatol*, **7**: 198–204*(1998)*
2. Zhao and all. *Pigment cell res*, **7**: 131-140*(1994)*
3. Wu and all. *Biochemical and Biophysical Research Communications,* **311**: 948–953 *(2003)*

IMAGING QUANTIFICATION AND COSMETIC APPLICATIONS

David Boudier, Josselin Breugnot, Eugenie Vignau,
Maud Le Guillou and Brigitte Closs
R&D Department, SILAB

INTRODUCTION:

Imaging techniques originally limited to a purely illustrative role can now be used in dermo-cosmetology in a more objective approach, especially for purposes of quantification. Recent progress involving both the quality and precision of image sensors, and the immense computer power now available on the market, are consistent with envisaging studies no merely qualitative, but quantitative. Three steps are essential in imaging quantification: acquisition, processing of images and statistical analysis of results (**Figure 1**). This presentation will address the different approaches we developed to succeed in quantifying digital images captured with different devices according to the targeted cosmetic application. A particular lightening will be address to the difficult step of images processing for each application.

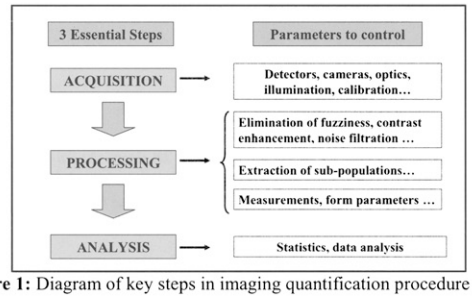


Figure 1: Diagram of key steps in imaging quantification procedure

MATERIAL AND METHODS:

Biological Approach and Acquisition of images by fluorescence microscopy:

Corneocytes were removed by tape stripping on the skin of volunteers pre-selected according to their age or to their skin hydration level (Corneometer®). Lipids covalently bound to corneocytes were stained with Nile Red. Visualization and capture of images were made with an IX 70 microscope (Olympus, Japan – Camera DXM-1200C, Nikon) coupled to an image analysis system (Nikon NIS-Elements AR software). Fluorescence intensity of images is representative of the rate of corneocytes lipids.

Multiparametric Approach and Acquisition of digital photographs:

Digital photographs of the face of the volunteers were taken with the VISIA CR® (Canfield, USA) device in our laboratories on volunteers selected according to different parameters (young, aged, smokers, non-smokers).

3D Approach and Acquisition of images by Fringes Projection:

Images were acquired using Fringes Projection apparatus dedicated specifically to 3D measurement of skin relief (Eotech, France). The system (dermaTOP-blue, Breuckmann Gmbh, Germany) comprised a measurement device combining a fringe projector, coupled with a high resolution digital camera mounted with 50mm field objectives. The acquisitions were made thanks to the dedicated EOTech software named DermaTOP (EOTech, France). A three-axis system for positioning the head of the volunteer ensured that the same measurement area was examined at the different time-points of the study. The study was conducted on a panel of male and female Caucasian volunteers aged between 22 and 67 years, average age 41 ± 12 years, pre-selected as having dilated pores on the cheeks (sensorial evaluation by experts) in our *in-vivo* laboratory.

Common step: enhancement and processing of images:

Quantitative analyses of images were conducted with software provided with each tool, with Matlab® software, release R2009a (Matworks), or with our own developments of programs (C Langage) by our specialist in digital image processing.

Statistic evaluation of results: the software used by our statisticians was StatgraphicsTM Centurion, release XV.

RESULTS and DISCUSSION:

BIOLOGICAL APPROACH: QUANTIFICATION OF IMAGES OBTAINED BY FLUORESCENCE MICROSCOPY

Cosmetic application: quantitative analysis of corneocytes lipids involved in the maintenance of the epidermal barrier function

By applying the method described above, it is possible to evaluate non-invasively the quality of the hydrophobic assembly in the *stratum corneum*, mainly due to lipids covalently bound to the cornified envelope. [1] The fluorescence intensity observed in samples is representative of the lipids rate. Qualitatively, we have observed some differences of the fluorescence intensity i.e. the lipid content in corneocytes, between young and aged volunteers, or between volunteers with a dry skin or a hydrated skin.

Innovation resides in our quantitative approach of the level of fluorescence in samples. We have developed our own images processing step to succeed in this quantification. This innovative method is based on the use of the LIP (Logarithmic Image Processing) grey level model, method discovered and published by the group of Pr M. Jourlin, France. [2]; [3]

This method allowed us to improve the dynamic of images. We thus obtained a better segmentation of corneocytes, whatever the degree of emitted fluorescence. Consequently, we have limited the loss of information that we had observed with a classic approach (use of the double threshold Otsu).

The resulting quantification has shown that there was a significant decrease of the quantity of lipids covalently bound to the corneocytes by 34% (**Figure 2**) between young and aged volunteers and by 24% between volunteers with a hydrated and a dry skin. This innovative *in-vivo* approach is fast and non invasive, and has been used in our laboratories to show efficacy of a purified fraction of *Pichia anomala* mannans on the synthesis of corneocytes lipids.

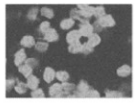
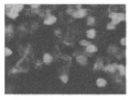
	Young Volunteers (mean age 24)	Aged volunteers (mean age 71)
Qualitative Approach: Visualisation of lipids (red) covalently bound to corneocytes		
Quantitative Approach: Level of emitted fluorescence representative of the rate of lipids (Arbitrary Unit – Grey levels).	61.16	40.09
Variation Young / Aged	/	-34% (P=0.0315)

Figure 2: Visualization & quantification of corneocytes lipids stained with Nile Red between young and aged volunteers.

MULTIPARAMETRIC APPROACH: QUANTIFICATION OF DIGITAL PHOTOGRAPHS OF THE FACE:

Cosmetic application: Overall evaluation of the skin complexion:

The VISIA CR® device allows capturing rapidly images of the face, with multiple and reproducible lighting modalities conditions. These images are widely used to illustrate qualitatively the effect of a cosmetic product or a facial plastic or aesthetic surgery treatment. Our objective was to develop our own images processing to quantify different parameters of interest from captured images. These quantified parameters can be used independently to investigate a particular field of research: skin colour, wrinkles, aged spots… or more globally to study and to give an overall evaluation of the skin complexion.

In this objective we applied different images processing to evaluate the following parameters: irregularities of the brightness, clarity of the complexion, intensity of the skin colour, imperfections of the skin (spots, scars…), circles and wrinkled aspect.

This quantitative approach enabled us to define references profiles characteristic of different stages of the skin complexion, in accordance with the results previously obtained by experts in sensorial evaluation.

By applying this quantitative analysis on images captured with the VISIA CR® device, we have shown that the skin complexion was modified with age. Moreover, the panel of smokers tends to have a dull complexion in comparison with non-smokers.

3D APPROACH: QUANTIFICATION OF IMAGES OBTAINED BY FRINGES PROJECTION:

Cosmetic application: quantitative analysis of the size of dilated pores on the cheeks

To date, measurement of pores size by Fringes Projection was few investigated in cosmetology. For pores acquisition, a reproducibility study demonstrates easily that even with a positioning bench, limited motion from the volunteer gives large distortion between two volume images acquired at two successive times. Consequently, when we applied the "Texture" Approach (no geometry alignment performed) during the images processing step, the variation of parameters quantified between the two time acquisitions is superior to 10%, which is non-satisfactory. In order to correct this shift between two acquisition times, we firstly used registration technique based on Fourier transforms and the associated cross power spectrum. Thanks to this modification in the processing step, we can approach the translational shift. Secondly, with the help of an additional log-polar transform, we can approach the rotational shift. This calculation enables to register images correctly.

By implementing this modification in the images processing step, we have shown that the variation of parameters quantified between two acquisition times was less than 5%, which provides a very satisfactory result. [4]

This modification in the images processing step enables to use the Fringes Projection to register accurately two acquisition times and to quantify correctly the size of pores. This innovative approach allowed us to prove efficacy of a natural active ingredient to minimize enlarged pores.

Finally, all the results we presented were supported by a suitable and controlled statistical analysis (study of the distribution of populations, comparison of variances of paired or independent samples, significance, etc.) by statistics experts.

CONCLUSION:

Imaging quantification is among the methods widely used in our *in-vivo* laboratory to highlight the cosmetic efficacy of a natural active ingredient. Images designed for quantification result from different approaches according to the target cosmetic application. A biological approach of skin surface markers (rate of lipids, rate of carbonylated proteins…) is feasible *via* the quantification of fluorescence images. To estimate in a quantitative manner several skin parameters, we developed a multiparametric approach from digital photographs of the face. Last, interest of images captured by fringes projection is to be able to quantify the skin topography (wrinkles, pores, cellulite). The accurate registration of two times acquisition to measure the size of pores with this tool is an innovative approach.

Finally, we underline importance and expertise necessary during the difficult step of images processing. This key step, from which we have seen an application with the LIP grey-level model, is still the subject of considerable basic research. This model is currently finding new applications with the development of the Color LIP model. A joint publication with the group of Prof. Michel Jourlin involving this model has been submitted to specialized journal (Advances in Imaging and Electron Physics) and we are convinced this innovative model will rapidly be applied to the cosmetic field.

References:

[1] Hirao T et al, *Exp Dermatol*, 10 35-44 (2001)
[2] Jourlin, M., Mayet, F. & Pinoli, J. *Traitement du signal* **13**, 251-262 (1996)
[3] Jourlin, M. & Pinoli, J. *Advances in imaging and electron physics* **115**, 129-196 (2001)
[4] Boudier D et al, Podium presentation, Book of IFSCC 2010, Buenos Aires.

MANIPULATING THE BULK PROPERTIES OF HAIR

Trefor Evans, Ph.D.
TRI-Princeton, 601 Prospect Avenue, Princeton, NJ 08540

Introduction

The hair care industry is dominated by large molecules such as surfactants, polymers and oils. However, due to their size, it is expected that activity must be restricted to the hair surface – as diffusion rates into the fibers would presumably be extremely slow. This leads to the notion that a whole next-generation of hair care products may still be waiting to be discovered that involve changing the internal properties. Moreover, it would seem that such formulations must be based on small molecules that are more-readily able to penetrate into the bulk. By means of illustration, it is difficult to think of a molecule that changes the properties of hair more than water. Water readily penetrates into the hair, leading to plasticization (that changes the mechanical properties) and swelling (that changes the feel properties). Therefore, if we can understand how water manipulates the properties of hair, there is the possibility of finding other molecules that provide similar effects. Alternatively, there may be ways of manipulating the moisture content of hair, such that the bulk properties can be controlled.

Methodology

Quantification of water content was attained by generating adsorption isotherms using commercially-available Dynamic Vapor Sorption (DVS) equipment from Surface Measurement Systems. Changes in the mechanical properties of hair were measured by generating stress-strain curves for individual fibers via constant extension rate experiments. This testing was performed using either a Dia-stron 600 or 675 Mini Tensile Tester (MTT). Fatigue testing results will also be shown that were generated using a Dia-stron CYC800.

Results:

Perhaps the most obvious molecules to change the moisture content of hair are so-called “humectants”. Figure 1 shows the adsorption isotherm of hair soaked in a 5% glycerol solution relative to the virgin state. As possibly anticipated, significantly higher water adsorption can result; although such changes only appear to occur at elevated humidity. Nonetheless, mechanical testing suggests considerable plasticization (softening) of the hair structure – presumably as glycerol molecules adsorb in a similar manner to water and produce a comparable effect. There is also precedence in the scientific literature for potentially being able to lower the water content of hair. Papers appeared in the 1960s pertaining to the ability for ninhydrin[1] and various phenolic compounds[2] to reduce the moisture content of keratinous materials. In each case, the mechanism was presumed to involve the foreign molecules occupying the same adsorption sites, and consequently occluding water by steric means. Figure 2 shows an adsorption isotherm demonstrating how soaking hair in a 5% resorcinol solution can produce an approximate 25% reduction in moisture content. Furthermore, our studies are suggesting that other classes of molecules (for example, carboxylic acids) are also able to produce comparable effects. By means of illustration, Figure 3 shows an adsorption isotherm for hair after soaking in a 5% glycolic acid solution.

It may be surmised that diminished moisture content would result in reduced plasticization and subsequently lead to increased mechanical properties. This premise appears to be partially true. Treatment of hair with aqueous solutions of certain molecules can indeed produce this effect; however, in other instances, an overall plasticization arises. The current hypothesis involves the adsorption of especially small molecules (e.g. glycerol, glycolic and lactic acid) giving rise to their own plasticization effect. That is, while these molecules do lower the moisture content, the overall effect is still lesser tensile properties. Conversely, adsorption of somewhat larger molecules (e.g. resorcinol) can indeed increase fiber stiffness – with some novel consequences.

Nonetheless, the magnitude of these effects still depends on the rate at which molecules can diffuse into the hair. Namely, these larger molecules do require somewhat longer soaking times to attain their full potential. This said, as may be expected, the use of elevated temperature, and/or the presence of chemically-damaged hair, has been found to increase penetration rates.

Conclusion:

Studies are continuing in an attempt to better understand how small molecules can diffuse into the hair and change the bulk properties.

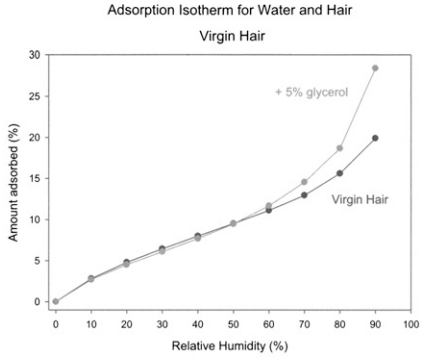


Figure 1

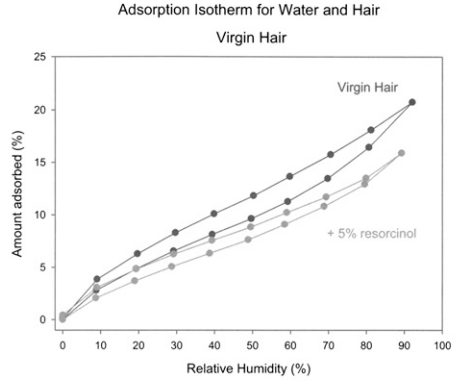


Figure 2

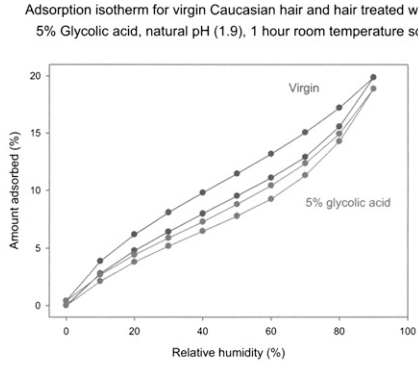


Figure 3

1) I.C.Watt and J.D.Leeder, Effect of Chemical Modifications on Keratin + Water Isotherm, Trans.Farad.Soc., 60, p1335, (1964)

2) M.M.Breuer, Binding of phenols by hair, Parts I, II and III, J.Phys.Chem, 68, p-2067-2084, (1964).

MALTODEXRIN BASED STYLING POLYMERS

Michael Philbin, Ph.D., Anthony Adamo, Crystal Priester
Norman Rackison, John Thomaides, Ph.D. and Samuel Vona
AkzoNobel Surface Chemistry, Bridgewater, NJ

INTRODUCTION

This work is directed towards developing a styling polymer with a higher content of natural polymer, compared to the typical synthetic fixative polymers used in hair gels. Polyvinylpyrrolidone (PVP) and polyvinylpyrrolidone/vinyl acetate copolymers (PVP/VA) are typically used as the fixative polymer in a hair gel. Polymerization of vinyl pyrrolidone (VP) in the presence of 2 different maltodextrins was investigated. Maltodextrins are polymers of D-glucose linked primarily by α–1,4 bonds with a Dextrose Equivalent (DE) typically ranging from 5 to 20.

A DE10 maltodextrin and a DE15 maltodextrin were used. The ratio of the two maltodextrins and VP was varied according to an Augmented Simplex Centroid Mixture Experimental Design. This included binary combinations of each maltodextrin with VP as well as ternary combinations of the two maltodextrins with VP. Compositions of the various polymers are shown in Table I. Hair gels containing each polymer composition were made up in a carbomer thickened system. Hair gels were evaluated for clarity, and stiffness on hair. Hair gel performance is shown in Table I.

MATERIALS AND METHODS

Polymers were made by polymerizing vinyl pyrrolidone in the presence maltodextrin using water as the solvent.

Hair gels were made up in water at 3.0% polymer solids with 0.6% carbomer neutralized with 2-amino-2-methyl-1-propanol and preserved with 1,3-dihydroxymethyl-5,5-dimethylhydantoin and 3-iodo-2-propynyl butyl carbamate.

Turbidity of the hair gels was determined using a Hach (Loveland, CO) Model 2100N turbidimeter by placing a 30 mL vial containing the hair gel in the sample compartment of the turbidimeter. The reading is given in nephlometric turbidity units (ntu). Hair gels were centrifuged to remove all air prior to taking turbidity readings.

Swatch stiffness was measure by applying 0.5g of hair gel to a wet 6" virgin brown hair swatch. The swatch was allowed to dry overnight in a room maintained at 72F/50% RH. Swatch stiffness was determined using a Diastron (Andover, United Kingdom) MTT160 miniature tensile tester equipped with a 3 point bend test stand. Swatch stiffness was determined as the work required to deflect the swatch 10mm at a rate of 50mm/min. Five swatches were made up and analyzed for each hair gel sample and the average was determined. A hair gel control based on PVP with a K value of 30 was run. All swatch stiffness results were normalized to this value.

The Augmented Simplex Centroid Mixture Experimental Design was setup and analyzed using Design-Expert Software available from Stat-Ease (Minneapolis, MN).

RESULTS AND DISCUSSION

A mathematical model was determined for each response by doing an Analysis of Variance (ANOVA) on the polymer compositions and hair gel performance shown in Table I.

$$\text{Swatch Stiffness}^{*} = 5.0\times10^{-3}\text{DE10Maltodextrin} + 4.9\times10^{-3}\text{DE15Maltodextrin} + 2.0\times10^{-2}\text{VP}$$
$$\text{Turbidity} = 2.9\text{DE10Maltodextrin} + 4.5\text{DE15Maltodextrin} + 27.1\text{VP}$$

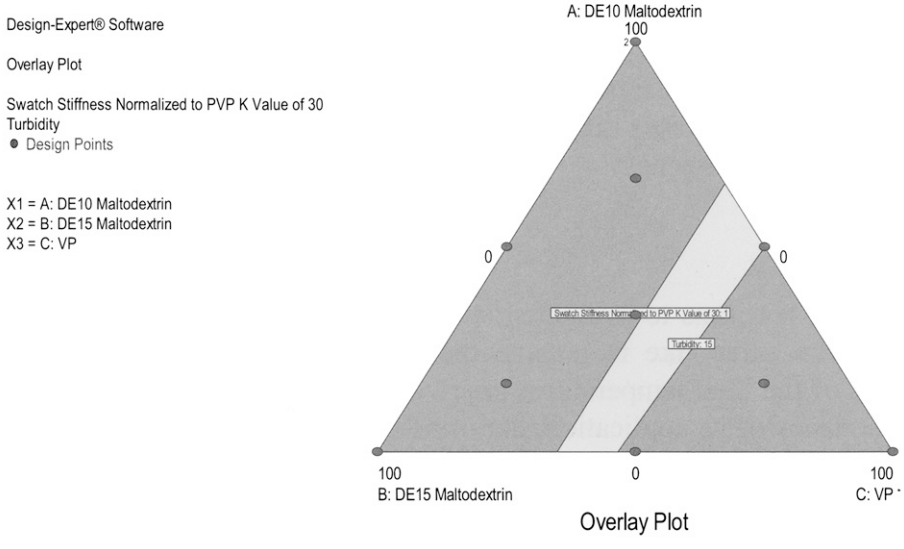
Based on the equation generated for each response from the ANOVA, a contour plot for each response relative to the polymer composition was generated. An overlay of the contour plots shows polymer compositions expected to give similar or better swatch stiffness normalized to PVP with a K value of 30, and similar or better clarity to PVP with a K value of 30. Polymer compositions meeting this performance criterion contained from approximately 50%-65% maltodextrin and approximately 35% to 50% PVP. See Figure I for the overlay of the turbidity and swatch stiffness contour plots.

Table I

Polymer Composition			Hair Gel Performance	
DE10 Maltodextrin	DE15 Maltodextrin	Vinyl Pyrrolidone	Hair Gel Turbidity (ntu)	Swatch Stiffness*
100.0	0.0	0.0	4.3	0.54
100.0	0.0	0.0	2.5	0.52
0.0	100.0	0.0	7.4	0.52
0.0	0.0	100.0	25.5	2.27
50.0	50.0	0.0	4.2	0.69
50.0	0.0	50.0	16.2	1.13
0.0	50.0	50.0	13.7	1.10
33.3	33.3	33.4	11.0	1.12
16.7	16.7	66.6	24.1	1.13
16.7	66.6	16.7	4.5	0.69
66.6	16.7	16.7	4.4	0.59

*Normalized to PVP with a K value of 30

Figure I



CONCLUSION

Polymer compositions made by polymerizing vinyl pyrrolidone in the presence of maltodextrin were developed that gave similar performance in a hair gel to PVP with a K value of 30. This indicates that a performance equivalent to commonly used synthetic polymers can be achieved from a polymer having a natural component as a significant percentage (>=50%) of its composition.

HAIR SETTING WITH HOT IRONS AND HEAT ACTIVATION

Manuel Gamez-Garcia, Ph.D
BASF Care Chemicals, 500 White Plains Road, Tarrytown, NY 10591

Abstract

An analysis is made of the various factors involved in the process of hair setting with hot irons. The effects of temperature, water, solvents, and other ingredients on hair setting are evaluated. The role of moisture and the hair denaturation temperature are also analyzed. The experiments show that hydrogen bond breakage by water absorption and hydrogen bond reformation by fiber dehydration is predominant in the setting of hair at low temperatures. The low temperature setting process is fully reversible and is seen to change at high temperatures suggesting a different hair setting mechanism. For instance, at high temperatures water is also needed for the setting of hair, however, the appearance of fiber super-contraction and the associated resistance to shape reversion indicates a different role for water. The experiments also show that the physical changes occurring in the hair cuticle sheath and cortex during the setting process do not necessarily reflect the hair setting efficiency.

Introduction

The use of hot irons to straighten or curl hair is a common practice among people who often like to change the shape of their hair. Unfortunately, because of the high temperatures involved in this process (150 - 220 C) and the frequency of its application, hot irons produce a gradual accumulation of damage in hair that impact negatively its manageability, and its sensory, and visual properties. Whenever a hair bundle is exposed to a heat source such as a hot iron, a temperature gradient is set up across the shaft of each individual hair fiber. This temperature gradient creates heat transfer from the cuticle sheath surface to the center of the cortex. As heat flows from the surface of the hot iron to the center of the hair fiber, the internal temperature of the fiber cortex rises. Short contact times between hot iron and hair create steep temperature gradients while long contact times will lead to temperature equilibration between hot iron and hair.

Results summary

This paper will show that the types of physical and chemical processes responsible for hair straightening and hair damage depend on the temperature profile across the hair fibers. Among these processes the most important are severe fiber dehydration, hydrogen bond breakage and reformation, keratin denaturation, softening of hair proteins, disulfide bond breakage, explosive moisture evaporation, pore formation, cuticle cell lifting, protein oxidation, and protein melting and aggregation. Some of these processes are part of the mechanism for hair straightening and need to occur in order for the hair fiber to change its shape. However, some of them are not necessary and represent purely damaging and weakening processes that do not contribute to reshape the hair.

Crucial in the occurrence of these processes is moisture, hot iron temperature, and contact time between hot iron and hair. Moisture seems to play, depending on the temperature range, different roles in the mechanism of hair straightening and damage. The application of various combinations of hot iron temperatures, contact times, and moisture contents seems to indicate that it is possible to find the optimum conditions for hair straightening. For instance, it is possible to find the point at which if higher levels of heat transfer are allowed, the result will be higher levels of hair damage that are not worth the increments in, either, hair straightening efficiency or straightening persistence.

CATIONIC CASSIA POLYMERS AS EFFICIENT NATURALLY-DERIVED POLYMERS FOR PROVIDING ENHANCED DEPOSITION FROM SHAMPOO SYSTEMS

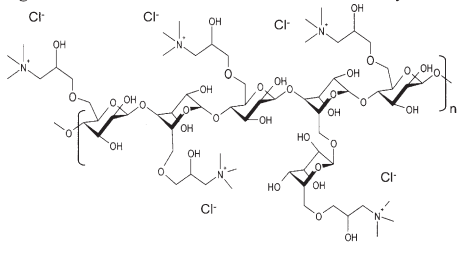
Carole Lepilleur, Ph.D., John Mullay, Ph.D., Wing Li and Duane Krzysik

Lubrizol Advanced Materials, Inc., Noveon® Consumer Specialties, 9911 Brecksville Road, Brecksville, OH 44141

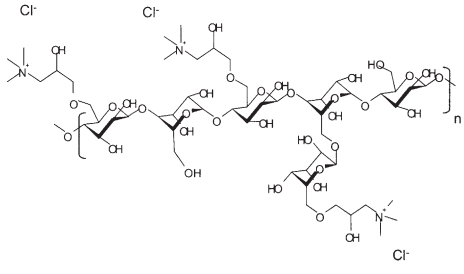
Introduction: New opportunities to improve hair conditioning are emerging due to the increased frequency of hair damage resulting from various grooming techniques, demographic changes in age and ethnicity and affluence in both developed and emerging markets. Silicones are commonly used in conditioning shampoos to improve wet and dry combing, hair feel, shine and manageability. Cationic conditioning polymers are used in combination with silicone in high performance formulations to enhance hair conditioning. One of the primary functions of cationic conditioning polymers is to increase the amount of silicone deposited on hair during the shampoo cycle via the formation of coacervates. Coacervates formed during shampoo dilution can effectively deposit silicone and other ingredients onto hair fibers (1).

Polysaccharide derivatives have a long history of use in personal care applications as thickeners, conditioning polymers, deposition aids and film formers. Cationic derivatives of guar gum, a galactomannan, are used in conditioning shampoos in combination with silicones to impart improved combing and sensory properties. Novel cationic cassia polymers are introduced here as new and efficient silicone deposition aids. Cassia gum is a natural, vegetable-based carbohydrate extracted from the endosperm of the seed of *Cassia tora* and *Cassia obtusifolia*. It is a member of the galactomannan family of polysaccharides with a ratio of mannose to galactose content of at least 5. Cassia plants grow wild in tropical zones around the world. Cassia gum can be modified to generate cationic polymers with various levels of cationic substitution (2). That modification produces two novel cationic cassia conditioning polymers, with the INCI classification of Cassia Hydroxypropyltrimonium Chloride having cationic charge density levels of 3.0 meq/g and 1.9 meq/g detailed below as Cat Cassia 3.0 and Cat Cassia 1.9, respectively. The chemical structures of Cat Cassia 3.0 and Cat Cassia 1.9 are represented in Figure 1.

Figure 1. Chemical Structure of Cationic Cassia Polymers



Cat Cassia 3.0



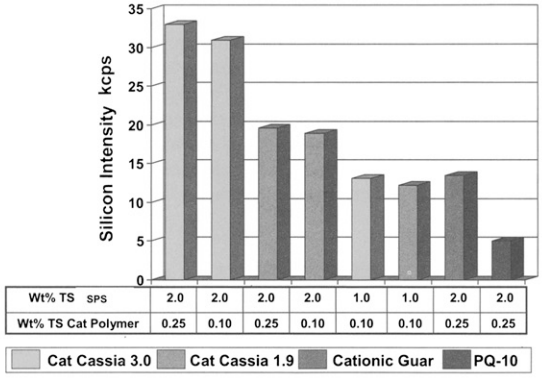
Cat Cassia 1.9

The performance of cationic polymers varies with respect to silicone and cationic polymer deposition efficiency, sensory and build-up potential. Efficacy also varies by hair type. In this study, European brown hair was treated twice with a surfactant formulation based on SLES-2 and CAPB, with varying levels of small particle size silicone emulsion and cationic polymer.

Methodology: Cationic polymer deposition is studied by a colorimetric method using Direct Red 80 dye (3). Silicone deposition is studied by X-Ray Fluorescence (4).

Results: Cationic cassia polymers deposit silicone much more efficiently than cationic guar or PQ-10 (Figure 2). Compared to the benchmarks, higher silicone deposition is achieved with both cationic cassia polymers in formulations containing significantly reduced levels of silicone emulsion and even reduced levels of cationic cassia polymers. Other results show that cationic polymer and silicone deposition levels are also influenced by the surfactant composition of the shampoo.

Figure 2. Silicone Deposition Study of Cationic Polymers on European Brown Hair



Formulation containing: 14 wt% TS SLES-2, 3.0 wt% TS CAPB, 1.5 wt% TS EGDS, 0.3 wt% TS NaCl, 0.10 or 0.25 wt% TS cationic polymer and 1.0 or 2.0 wt% TS Small Particle Size (SPS) (Dimethicone (and) Laureth-23 (and) C12-15 Pareth-3 (and) Sodium Laureth Sulfate).

Build-up of silicone on the surface of hair is assessed through the measurement of silicone deposition after repetitive shampoo cycles. At comparable use levels, deposition levels for cationic cassia polymers, as well as the benchmark polymers, reach a maximum deposition level after a fourth wash cycle. When compared to benchmark polymers, cationic cassia polymers are more efficient silicone deposition aids. The improved efficiency of cationic cassia polymers leads to higher levels of silicone deposition during wash cycles 1-3. Thus, the use of cationic cassia polymers allows formulators to use lower amounts of silicone and cationic polymer than formulations based on the benchmarks to achieve at least comparable if not better conditioning performance. Cationic cassia polymers also have the ability to offer a unique sensory experience with improved silicone and cationic polymer deposition.

References

(1) "Conditioning agents for hair and skin", edited by R. Schueller and P. Romanowski, *Cosmetic Sci. and Tech. Series*, 21, 28-30, 255-257, (*1999*).
(2) F. Utz et al., Cationic cassia derivatives and applications therefore, *US patent 7,262,157 B2,* (*2007*).
(3) R. J. Crawford and C. R. Robbins, A replacement for Rubine dye for detecting cationics on keratin, *J. of the Soc. of Cosmet. Chem.,* 31, 273-278, (*1980*).
(4) J. V. Gruber, B. R. Lamoureux, N. Joshi and L. Moral, The use of X-ray fluorescence spectroscopy to study the influences of cationic polymers on silicone oil deposition from shampoo, *J. Cosmet. Sci.,* 52, 131-136, (*2001*).

USING HUMAN GENOMIC MICROARRAYS IN PERSONAL CARE

James V. Gruber[1], Ph.D., Philip Ludwig[1], and Robert Holtz[2]
[1]*Arch Personal Care Products, 70 Tyler Place, South Plainfield, NJ 07080*
[2]*Bioinnovations Laboratories, 7220 W. Jefferson Avenue, Suite 112, Lakewood, CO 80235*

Introduction

The advent of the mapping of the human genome and the subsequent advances in testing of skin cells using human genomic microarrays has offered opportunities to examine the influence of skin ingredients on skin cells in ways not previously seen. Current microarrays from companies like Agilent can contain upwards of 25,000 genes from the human genome. Recently, we published two studies that were efforts to look at the role of skin antioxidants on human skin cells (fibroblasts and keratinocytes) and an opportunity to examine the influence of potent skin lighteners on melanocytes.[1,2] This presentation will review the results of these studies in a comprehensive way, looking at the types of information that can be gleaned from such studies and how this information might help to design newer ingredients intended for skin and hair care applications. In addition, recent studies conducted on the influences of a red rice meristem extract on epigenome methylation will be discussed.

Material and Methods

Human microarrays test kits including epigenomic test kits were purchased from Agilent Technologies and used per the manufacturer's instructions. Fibroblasts, keratinocytes and melanocytes were grown under standard conditions and treated with active ingredients at the highest non-cytotoxic concentrations determine via prior MTT assays. Treatment times were 24 hours for cellular arrays. Determination of genomic influences was made using the following criteria: Ratio of median values greater than 1.3 demonstrates a statistically significant upregulation of the gene, values less than 0.7 demonstrate a statistically significant down-regulation. The author's selection of 205 genes felt important for skin in the fibroblast and keratinocyte studies was made to cover a variety of important skin functions. Ingredients employed in the antioxidant studies were purchased from multiple sources as noted in reference 1. Ingredients for the melanocyte studies were purchased from Sigma Chemical (Milwaukee, WI) and were used as received. Studies on global and Type 1A epigenome methylation were conducted using an extract of Himalayan red rice meristem culture extract (INCI name: Ozonized *Oryza sativa* callus culture extract.

Results and Discussion

Antioxidant Studies

Results from the genomic examination of 205 skin important genes demonstrates that topical application of various well-know antioxidants on fibroblasts demonstrate influence on nine genes including ACLY, AQP1, COL1A1, COX1, GRN, NOS 3, PLOD3, RARA and TXN. The treatments downregulated six genes including: DSG3, HAS1, IL1A, KL, NOS2 and PGR. Topical application on keratinocytes demonstrated upregulation of 14 genes including: ACLY, AQP1, AQP3, CD44, CDH1, COX1, FGF1, GRN, HSPB1, KRT5, NOS3, PLOD3, TPT1 and TXN. The treatments downregulated 19 genes including: AR, CYGB, EGF, ESR2, FBN1, FBN2, IGF1, KL, MC1R, PGR, POMC, PPARG, PTGER1, RAD23A, RXRA, SOD3, SRD5A2, TERT and TYRP1.

Skin Lightening Studies

Topical treatment of three well known skin lighteners, Hydroquinone, Kojic Acid and Niacinamide on Melanocytes for 24 hours at the highest non-lethal dosage demonstrated a significant upregulation of the Tyrosinase genen (TYR) which was demonstrated in protein assays as well. In addition, a critical iron binding protein gene, Ferritin (FTH1), was significantly upregulated in the gene arrays as well as in protein assays suggesting a role for iron in melanogensis not previously appreciated.

Epigenomic Study

Studies conducted on intrinsically and extrinsically (i.e., UV)-aged fibroblasts treated with an extract taken from a meristem culture of Himalayan red rice demonstrated an overall reduction in CpG methylation at the promoter regions of the entire genome. In addition, Type 1A1 and Type 1A2 collagen showed reductions in epigenomic methylation at the 4 promoter regions of the gene which was further supported by increases in Type 1A collagen expression in the aged fibroblasts. The study results suggest that the extract has an ability to inhibit or reverse methylation of critical CpG islands in the promoter regions of the genome which causes the cells to behave more characteristically as young, non-aged cells.

References

1. Gruber JV et al., Examining the Genomic Influence of Skin Antioxidants *In Vitro*. *Mediators of Inflammation* Vol 2010, In Press.

2. Gruber JV et al., Examining the Impact of Potent Skin Lighteners on Melanocytes *In Vitro*. Society of Investigative Dermatology, Atlanta, GA, May 2010, Poster 860.

THE ROLE OF CLOCK AND SIRT-1 IN CHROMATIN REMODELING: A NEW CODE OF ENTRY FOR DNA REPAIR IN HUMAN SKIN

Isabelle Imbert, Ph.D., Jean-Marie Botto, Ph.D., Karine Cucumel, Ph.D., Claude Del Farra, Ph.D. and Nouha Domloge

ISP-Vincience, 655 Route du Pin Montard, BP212, Sofia-Antipolis, 06904, France

INTRODUCTION:

The link between chromatin remodeling, metabolism and circadian control in gene expression and DNA repair has been clearly established [1, 2]. Latest scientific data have shown that more than 20% of genes expressed in a given tissue were under circadian regulation, and evidenced the clock genes as modulators of cell metabolism, proliferation and survival at the epigenetic levels [2]. While reduced rythmicity has been linked with aging, there is now compelling evidences that defects in core clock components as well as repeated desynchronisations in humans induce premature aging and reduced lifespan [3]. These recent scientific data have allowed a better understanding of the molecular clockwork architecture and raised interests in chronobiology applied to cosmetics. In this concern, a new peptide (IV09.008) was designed to maintain expression of clock genes in conditions of stress-induced desynchronization by UVB or aging. This study provides new insights supporting the key role of clock and is tight modulation by sirt-1 in controlling cellular functions such as DNA repair in human skin.

MATERIAL AND METHODS:

Immunohistochemistry was used to evaluate and compare expression of clock, bmal-1 and sirt-1 on normal human skin versus "*in vitro* aged human skin". Comet assays were used to study the effects of clock genes modulation on DNA protection and repair in human keratinocytes and human fibroblasts treated or not with IV09.008 compound and irradiated by UVB at 60mJ/cm^2. Two models were developed including single treatment before or after irradiation and double treatment before and after irradiation by UVB at 60mJ/cm^2. Clock, Per1 and Bmal1 expression were evaluated on skin biopsies after irradiation with UVB at 100mJ/cm^2 in presence or absence of IV09.008. Hematoxylin and eosin staining was used to evaluate sunburn cells on skin biopsies treated or not with IV09.008 at 1% and irradiated by UVB at 100mJ/cm^2. Impact of clock genes expression on cellular synchronicity was assessed by measure of protein synthesis such as collagen III in presence of IV09.008 at 1%. A clinical double blind study was conducted on 12 volunteers to evaluate the effect of clock genes on skin renewal by skin DHA staining with or without application of a cream containing IV09.008 molecule at 1% for 17 days. *In vivo* confocal microscopy (Vivascope) was also used to evaluate effect of the treatment on *stratum corneum* and granular cells organization. The number of sunburn cells was estimated *in vivo* 24h after UV stress with 2 MED applied at day 16.

RESULTS:

Results obtained showed a reduced expression of clock genes on *in vitro*-aged human skin versus normal human skin (Figure 1).

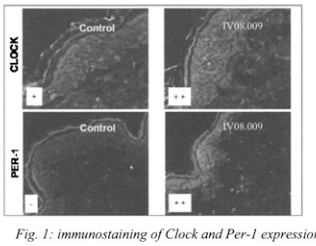


Fig. 1: immunostaining of Clock and Per-1 expression on in vitro-aged human skin

The ability of IV09.008 compound to modulate positively clock genes expression was shown *in vitro* (+54% by immunoblotting on normal human fibroblasts) and confirmed *ex vivo*. The impact of clock genes modulation by IV09.008 molecule on both DNA protection and repair was clearly demonstrated with a significant reduction of UVB-induced damage (respectively -78% and -81%) (Figures 2 and 3).

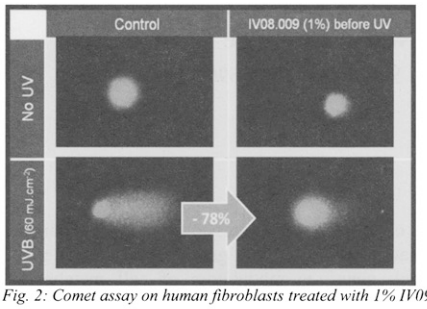


Fig. 2: Comet assay on human fibroblasts treated with 1% IV09.008 for 24h, and then irradiated with 60mJ/cm² UVB

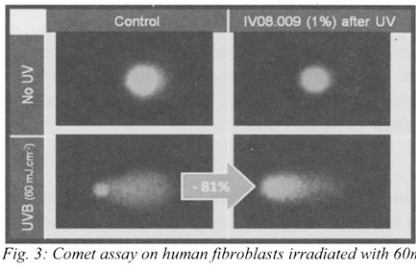


Fig. 3: Comet assay on human fibroblasts irradiated with 60mJ/cm² UVB and treated with 1% IV09.008 for 24h

The protective activity of IV09.008 against UVB-induced damages was also confirmed by a significant decrease in the number of sunburn cells observed on UVB-irradiated human skin biopsies (Figure 4).

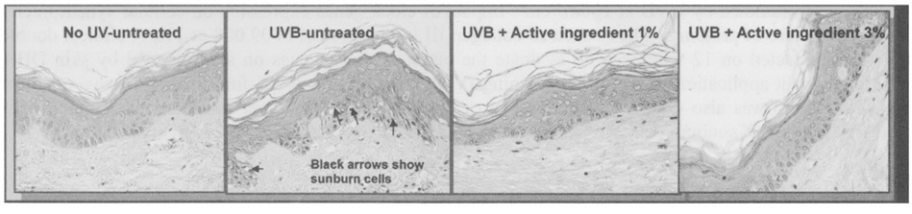


Fig. 4: Haematoxylin Eosin staining of human skin biopsies treated with IV09.008 for 24h, then UVB-irradiated

Positive modulation of clock genes expression leading to improved cellular synchronicity was shown to be linked to a global improvement of cellular metabolism as assessed by a 33% increase in collagen III production. Effect of IV09.008 on skin renewal was observed clinically after 4 days of treatment. This regenerating effect was confirmed by *in vivo* confocal microscopy observations showing a better organized granular layer with improved cohesion between cells. The protective activity of IV09.008 against UVB-induced skin damage was finally confirmed *in vivo* by a significant decrease (-38%) in the number of sunburn cells observed on human volunteers 24h after 2 MED exposure.

CONCLUSION:

These studies provide new insights supporting the key role of clock genes in controlling cellular functions such as DNA protection and repair. It highlights the importance to maintain cellular synchronicity in order to improve cellular metabolism. All together, these results strongly confirm the interest of a targeted modulation of clock genes for future cosmetic applications with innovative anti-aging approaches.

REFERENCES:

1) Nakahata Y, Sahar S, Astarita G, Kaluzova M, Sassone-Corsi P. *Science.* **324**(5):654-57, *2009*.
2) Reddy AB, O'Neill JS. *Cell.* **20**(1):36-44, *2009*.
3) Geyfman M, Andersen B. *J. Invest. Dermatol*. **129**:1063-66, *2009*.

GLOBAL METABOLOMICS AND ITS APPLICATION IN PRODUCT DEVELOPMENT FOR CONSUMER CARE

John A. Ryals, Ph.D.
Metabolon, Inc. Durham, NC 27713

Abstract The cosmetics and consumer health industries are increasingly challenged to provide convincing evidence of the effectiveness of their products. Biochemistry acts at the basal level of homeostasis and both essential and non-essential compounds ingested or applied to the body interact with a number of metabolic pathways and functions and often influence health and wellness beyond the target. Global metabolomics is a powerful technology that provides a relatively complete picture of metabolism in biological systems and can be used to identify biochemical changes associated with aging, inflammation, tissue damage and repair, wound healing and skin diseases and can be applied to cosmetic discovery and development in terms of elucidating mechanism of action, safety and support of competitive claims.

Introduction Metabolomic analysis and interpretation is based on small molecule biochemistry. Biochemistry is a mature, highly developed field of science and metabolomics takes advantage of this repository of biochemical pathway knowledge. Many biological effects of xenobiotics and disease result from a combination of the the overall health of an individual along with his or her environment, lifestyle and diet. While genetics can play an important part in predisposing an individual to xenobiotic effects or disease, the biochemistry of an individual is likely a more informative measurement of an individual's current state and condition. Many significant reports have been recently published that describe using metabolomics to analyze disease state, identify drug targets, evaluate the mechanism and safety of drugs and ingredients and select individuals likely to respond[1-5].

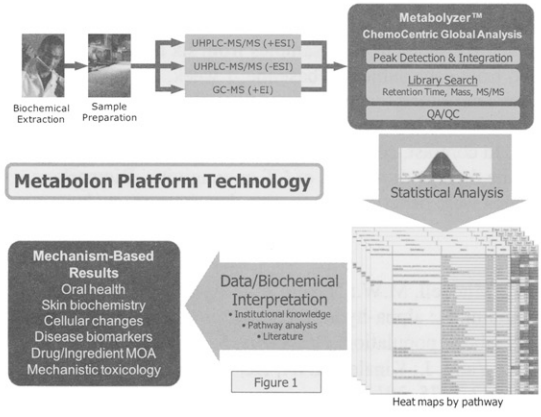


Figure 1

Metabolon has developed a robust metabolomics platform based on the combination of three independent chromatography methods: ultrahigh performance liquid chromatography/tandem mass spectrometry (UHPLC/MS/MS^2) optimized for basic species, UHPLC/MS/MS^2 optimized for acidic species, and gas chromatography/mass spectrometry (GC/MS)[6-8]. Following sample extraction, full scan mass spectrometry is carried out to record the retention time, molecular weight (m/z) and fragmentation spectra of all detectable ions/biochemicals present in the samples. Identification of the biochemicals in the experimental samples is achieved through automated comparison of the ion features in the experimental samples to a comprehensive and proprietary chemical reference library. After data generation and quality control, Metabolon combines its institutional knowledge with integrated tools including statistical analysis, pathway mapping, and data visualization to rapidly provide powerful insights into understanding biological systems.

Periodontal disease mechanism and biomarkers An unbiased metabolomic analysis of gingival crevicular fluid (GCF) collected from healthy, gingivitis and periodontitis sites in human subjects uncovered the complex host and bacterial interaction in biochemical pathways associated with inflammation, cellular defense, and tissue degradation[9]. Twenty-two persons with chronic periodontitis were selected from research volunteers at The Forsyth Institute Dental Clinic. GCF samples were obtained from 3 different site categories for each patient: healthy, gingivitis and periodontitis. Altered levels of approximately 50% of the 228 metabolites profiled were found among the three sites, thus providing an extensive pool of potential biomarkers for periodontal disease progression (Fig2A). An ensuing clinical study demonstrated that these disease associated biomarkers can be effectively suppressed by triclosan containing toothpaste (Colgate Total), thus providing further confirmation for Colgate Total's therapeutic effects on gingivitis.

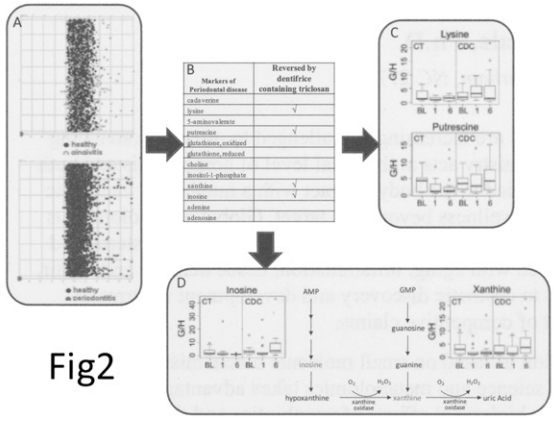


Fig2

One of the most striking results was the up-regulation of inosine, hypoxanthine, xanthine, guanosine, and guanine at the disease sites, which indicated accelerated metabolic flux of the purine degradation pathway (Fig 2B). This suggests that periodontal-disease-induced oxidative stress and inflammation are mediated through this pathway. The complex host-bacterial interaction was further highlighted by depletion of anti-oxidants, degradation of host cellular components, and accumulation of bacterial products in GCF (not shown).

Clinical Validation The top 10 most significantly changed metabolites from the previous study were evaluated for how these metabolites responded to a triclosan-containing dentifrice (Colgate Total, "CT") in a 6-week clinical study initiated to 1) validate the markers using a separate cohort of subjects; 2) to define which markers can offer the most predictive value of periodontal disease state based on their responses to a dentifrice with known clinical efficacy against gingivitis; and 3) to assess if biochemical confirmation of therapeutic benefits from the dentifrice can be established[10].

A panel of 10 markers was selected from the previous metabolomic study based on their statistical significance. Thirty-nine chronic periodontitis subjects were randomly assigned to a toothpaste regimen: control dentifrice (n = 21) or triclosan-containing dentifrice ([CT] n = 18). Subjects were instructed to use their assigned dentifrice twice daily for 6 weeks. Gingival crevicular fluid samples from six healthy, six gingivitis, and three periodontitis sites were collected from each subject at baseline, 1 week, and 6 weeks. The relative levels of the markers in the samples were determined by analysis at Metabolon. Statistical analysis indicated that CT significantly decreased the levels of inosine, lysine, putrescine, and xanthine at the gingivitis sites as early as week 1 (Fig 2C and D). In contrast, control dentifrice had little effect. The clinical study achieved biochemical confirmation of the therapeutic effects of CT on gingivitis. Biomarkers were significantly altered by CT before clinical changes were observed, suggesting that the markers have predicative value for disease state assessment.

References

(1) Gall, W.E.; Beebe, K.; Lawton, K.A.; Adam, K.P.; Mitchell, M.W.; Nakhle, P.J.; Ryals, J.A.; Milburn, M.V.; Nannipieri, M.; Camastra, S.; Natali, A.; Ferrannini, E.: alpha-hydroxybutyrate is an early biomarker of insulin resistance and glucose intolerance in a nondiabetic population. *PLoS One*, **5**: *e10883, 2010.*
(2) Reitman, Z.J.; Jin, G.; Karoly, E.D.; Spasojevic, I.; Yang, J.; Kinzler, K.W.; He, Y.; Bigner, D.D.; Vogelstein, B.; Yan, H. *Proc Natl Acad Sci USA*, **108**: *3270-75, 2011.*
(3) Sreekumar, A.; Poisson, L.M.; Rajendiran, T.M.; Khan, A.P.; Cao, Q.; Yu, J.; Laxman, B.; Mehra, R.; Lonigro, R.J.; Li, Y.; Nyati, M.K.; Ahsan, A.; Kalyana-Sundaram, S.; Han, B.; Cao, X.; Byun, J.; Omenn, G.S.; Ghosh, D.; Pennathur, S.; Alexander, D.C.; Berger, A.; Shuster, J.R.; Wei, J.T.; Varambally, S.; Beecher, C.; Chinnaiyan, A.M. *Nature*, **457**: *910-14, 2009.*
(4) Wetmore, D. R.; Joseloff, E.; Pilewski, J.; Lee, D. P.; Lawton, K. A.; Mitchell, M. W.; Milburn, M. V.; Ryals, J. A.; Guo, L. *J Biol Chem*, **285**: *30516-22, 2010.*
(5) Watson, M.; Roulston, A.; Belec, L.; Billot, X.; Marcellus, R.; Bedard, D.; Bernier, C.; Branchaud, S.; Chan, H.; Dairi, K.; Gilbert, K.; Goulet, D.; Gratton, M. O.; Isakau, H.; Jang, A.; Khadir, A.; Koch, E.; Lavoie, M.; Lawless, M.; Nguyen, M.; Paquette, D.; Turcotte, E.; Berger, A.; Mitchell, M.; Shore, G. C.; Beauparlant, P. *Mol Cell Biol*, **29**: *5872-88, 2009.*
(6) Dehaven, C.D.; Evans, A.M.; Dai, H.; Lawton, K.A. *J Cheminform,* **2**: *9, 2010.*
(7) Evans, A.M.; DeHaven, C.D.; Barrett, T.; Mitchell, M.; Milgram, E. *Anal Chem*, **81**: *6656-67, 2009.*
(8) Ryals, J.; Lawton, K.; Stevens, D.; Milburn, M. *Pharmacogenomics*, **8**: *863-6, 2007.*
(9) Barnes, V. M.; Teles, R.; Trivedi, H. M.; Devizio, W.; Xu, T.; Mitchell, M. W.; Milburn, M. V.; Guo, L. *J Dent Res,* **88**: *851-855, 2009*
(10) Barnes, V. M.; Teles, R.; Trivedi, H. M.; Devizio, W.; Xu, T.; Lee, D. P.; Mitchell, M. W.; Wulff, J. E.; Milburn, M. V.; Guo, L. *J Periodont*, **81**: *1273-9, 2010.*

ORIDONINE, DARUTOSIDE AND THE ANTIOXIDANT RESPONSE ELEMENTS (ARE): A STUDY OF THE COHERENCE BETWEEN GENOMICS, PROTEOMICS AND CLINICAL RESULTS

Karl Lintner[1], Ph.D., Phillippe Mondon[2], Ph.D., Nada Andre[2], Emmanuel Doridot[2] and Olga Gracioso[2]

[1]*Kal'Idees S.A.S., Paris, France*

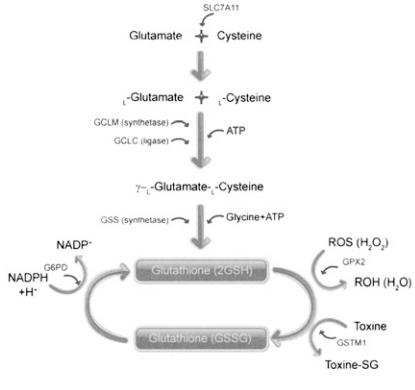
[2]*Sederma, S.A.S., Le Perray en Yvelines, France*

INTRODUCTION:

Aging gives rise, among other things, to the emergence of a heterogeneous appearance of the skin; redness, blemishes, age spots and local hyperpigmentation as well as wrinkles and roughness characterize the tone, hue and radiance of its surface for which oxidative stress is often evoked as a major cause.

In the cell, the production and elimination of free radicals is controlled by a complex defense system, among which the anti-oxidant responsive element (ARE) has been shown to be a major factor. ARE is a DNA sequence found in promoter regions of genes, which code for important anti-oxidant and detoxifying enzymes. But ARE needs to be activated itself in order to initiate downstream gene expression. One of the proteins activating ARE is called NRf2 which again needs upstream phosphorylation by PRKCD and **PRKCFBP**. Once ARE is thus activated, anti-oxidant enzymes such as **SLC7A11** (amino acid transporter), **GCLM** (g-glutamylcysteine synthetase), **GCLC** (glutamate cysteine ligase) and **GSS** (glutathione synthetase) are upregulated. This should lead to an increased concentration of reduced glutathione, one of the endogenous protective compounds produced by the cell; glutathione is a tripeptide, which enables control of oxygen free radicals, peroxides and NO°. Reduced glutathione acts as a detoxifying agent acting in conjunction with glutathione peroxidases (**GPX2**) and transferases **GSTMx** as well as with **G6PD** (glucose 6 phosphate dehydrogenase), which restores the GSH pool.

Other ARE mediated genes are **HMOX1** and **NOQ1** which combat inflammation and oxidative stress, as well as the **IL-11** gene which downregulates IL1b and IL8.



OBJECTIVE:

Oridonine (extracted from *Rabdosia rubescens*) reduces UV-B induced pro-inflammatory messengers PGE2, IL6 and IL8 in human dermal fibroblasts by up to 70% ($p<0.01$), and protects them significantly ($p<0.05$) against H_2O_2 stress (DCFH test). Darutoside (extracted from *Siegesbeckia orientalis)* is known for improved wound healing.

Based on these observations, we were interested to see if the protective effect of these natural substances could be explained in genetic and proteomic mechanistic terms way beyond simple, but inherent radical scavenging properties.

METHODS:

Human keratinocytes were incubated with either oridonine or darutoside for 24 and 48 hours; extracted mRNA was hybridized on full gene DNA array chips and gene modulation was analyzed by Predisearch™ software. Anti-oxidant activity of oridonine was measured by the ORAC and DCFH tests; protein synthesis was followed by ELISA protocols. Skin tone related experiments included melanin content determination in melanocytes, melanosome phagocytosis, VEGF assay by ELISA; *in vitro* anti-irritant effect was tested by a HET-CAM derived protocol with expert assessment of irritancy. Clinical studies on volunteers employed the SIAScope™, Visia™ system and Ultrasound echography on >25 panelists with mature skin. The cream used in the clinical study included as actives ingredients both Oridonine and Darutoside[1].

RESULTS:

The Whole Genome DNA array study revealed that all Nrf2 dependent ARE mediated genes (in **bold** mentioned above) were upregulated. This means, the entire cascade from Nrf2 activation by **PRKCFBP** to Glutathione pool restoration (**G6PD)** is enhanced by the incubation with oridonine. A clear genomic base for oridonine efficacy is thus established. Proteomic determination of the true **GSH** pool in both UV-

[1] *The patented combination of oridonine and darutoside is proposed as* ***CHROMOCARE™*** *by SEDERMA, France*

B irradiated or not irradiated keratinocytes after incubation with oridonine showed a dose dependent increase of up to 65% in GHS synthesis, confirming the gene study results. As is well known, inflammatory states, especially chronic ones, lead to hyperpigmentation. We therefore examined the potential of oridonine to influence this downstream phenomenon. Indeed, both melanin content in cultured human melanocytes and melanosome phagocytosis by keratinocytes are significantly decreased ($p<0.01$) by oridonine. The photographs above illustrate the phenomenon: the nuclei were Hoechst-counterstained; the melanosome models show as green (bright) dots.

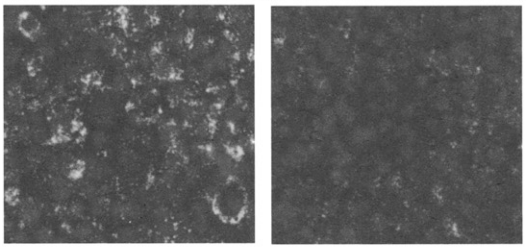


Figure 2:
bright: model melanosomes; dark: cell nucleus; LEFT: control; RIGHT: treated with oridonine

Oxidative stress-induced inflammatory symptoms also include increased VEGF synthesis, increased MMP1 production and increase in vascularization and blood flow. The gene array study on darutoside revealed downregulation of **MMP** genes, and increase of inhibin A (**INHBA)** as well as integrin **(ITGB8)** and **CTGF/CCN2** (connective tissue growth factors) upregulation. Not surprisingly, incubation of keratinocytes with the oridonine/darutoside blend then decreased VEGF synthesis by 44% ($p<0.01$), MMP1 production by 43% ($p<0.01$), and increased collagen I and III synthesis by up to 170% ($p<0.01$); a HETCAM score (an "*in vitro"* model for studying vascularization, blood flow and irritation) went from "10" to "4" ($p<0.01$) which compares favorably with aspirin (used as positive control, at a score of "2").

The *in vivo* clinical efficacy studies of the two ingredients were conducted with the aim of finding a connection between the described genetic and proteomic *in vitro* activities of oridonine/darutoside and the three chromophores determining skin tone: **melanin, hemoglobin and collagen**, using echography, SIAScope® and VISIA®. *Verum* and vehicle cream were applied twice daily for two months (half face study).

Fig. 3 : echography before and after 2 months treatment

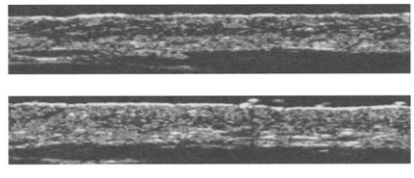
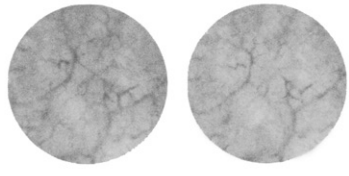


Figure 3 shows the increase in tissue density (echogenicity); SIAScope® data confirm the increase in dermal collagen, both results tying in well with the increase in collagen I and III as well as the INHBA increase, MMP decrease and overall tissue related results observed previously. The results obtained with the VISIA® equipment confirm the action of the oridonine component on the melanin chromophore (12% decrease in lentigines, $p<0.01$) and both VISIA® and SIAScope® allow the observation of a remarkable reduction in redness, vascularization and thus hemoglobin chromophore on the treated sites in the face of the panelists, with no significant effects being observed on the vehicle receiving sites.

Figure 4 : SIAScans before and after 2 months application of the oridonine/darutoside blend.



CONCLUSION

Aging is not just a matter of wrinkles, after all they are just a symptom. The consequences of oxidative aging are just as much visible and effectively perceived by the consumer: brown spots, redness and dermal decline.

The association of two plant extracts brings an effective solution for the treatment against the visible effects of oxidative aging thanks to its antioxidant mechanism of action based on the activation of ARE and the glutathione cascade, as demonstrated by the DNA Array techniques, proteomic quantification of gene products and anti-inflammatory activity with the reduction of the VEGF and the pro-inflammatory messengers (PGE-2, IL-6, IL-8).

The evidence for the efficacy of these ingredients is thus based on a coherent chain of genetic and proteinic activations, inhibitions, synthesis and degradation. Not only is it possible to claim that the plant molecules improve the skin's complexion, we also have a much better understanding why and how they do it. By rectifying the quantity and distribution of the collagen, they modify light scattering on the skin; by decreasing melanin transfer and vascularisation, they improve skin tone uniformity and luminosity, thus creating a younger and fresher looking face.

THE BEAUTY IN SYNCHROTRON LIGHT

Vivian Stojanoff, Ph.D.
Brookhaven National Laboratory, Upton, NY 11973

The development of cosmetic products requires in-depth assessment of their effectiveness and safety. Synchrotron sources worldwide have contributed to the better understanding of the effects of cosmetic products to skin, hair and nails. Synchrotron light is used today to characterize, analyze, and monitor chemical components, pigments, additives, formation of gels and emulsions, storage effects, etc.

Embracing a wide range of the electromagnetic spectrum, from infrared light to X-rays synchrotron radiation sources provide the researcher with a set of non-destructive methods and techniques. Although several of these methods, such as, Fourier transform infrared spectroscopy (FTIR) and small angle X-ray scattering (SAXS) are available in the laboratory synchrotron sources provide: tunability, choice of wavelength; higher signal-to-noise, the higher intensities of synchrotron sources allow the study of more dilute samples; better spatial resolution, synchrotron light sources are generally better collimated compared to sources found in the research laboratory; and faster analysis, due to the higher intensity of the source. Several examples can be cited taking advantage of these properties.

Taking advantage of the high flux, the high energy X-rays and the high energy resolution measurements achievable at synchrotron sources researchers from the Louvre and European Synchrotron Radiation Facility studied ancient Egyptian cosmetics using powder diffraction methods to identify the composition and origin of the minerals used in the preparation of Egyptian make-up. The research was further complemented with micro-X-ray tomography and X-ray spectroscopy methods. The studies allowed to identify two provenances of the mineral ingredients in the make-up and to distinguish between natural ores, black galena (PbS) and cerussite (PbCO3) and synthesised products laurionite (PbOHCl) and phosgenite (Pb2Cl2CO3)[1].

Another example is synchrotron infrared microspectroscopy. Widely employed to study the effects of chemical treatment on the structure of hair; the high spatial resolution attainable with synchrotron sources allows to study the three individual hair components, the medulla, the cortex, and the cuticle independently[2]. Ref3=Dumas Using X-ray tomography Guive Balooch[3] Ref4 revealed how relaxer treatments can compromise hair strength showing noticeable formation of cracks and voids. Ballooch suggests that a combination of X-ray tomography and X-ray absorption spectroscopy and FTIR could hint to ways and means to avoid weakening of the hair.

More recently Hatta and co-workers[4] Ref5 studied the uptake mechanism of hydrophilic and hydrophobic cosmetic ingredients by the skin, which are important for the moisture-retaining ability of the skin. Using time resolved synchrotron small- and wide- angle diffraction methods these authors found that ethanol, a typical hydrophilic molecule, penetrates the cell via a transcellular route while d-limone, a typical hydrophobic molecule, enters the cell via the intercellular route.

The beauty of synchrotron radiation lies in that a wide selection of non-destructive methods and techniques are particularly well adapted to the analysis of the molecular structure, chemical analysis, and cell and element distribution imaging.

[1] P. Walter, P. Martinetto, G. Tsoucaris, R. Bréniaux, M. A. Lefebvre, G. Richard, J. Talabot, E. Dooryhee, *Nature* **397,** 483-484, (*1999*).
[2] P. Dumas and L. Miller, *Vibrational Spectroscopy,* **32**, 3-21, (*2003*).
[3] K. Schaefer, *Cosmetics and Toiletries formulating results in cosmetics R&D* June 10 (*2009*).
[4] I. Hatta, H. Nakazawa, Y. Obata, N. Ohta, K. Inoue, N. Yagi, J. Phys.: Conf. Ser. **272** 012025, (*2011*).

EVALUATION OF PHOTO-TOXIC EFFECT OF FRACTIONATED MELANIN — A COMPARATIVE STUDY BETWEEN THREE DIFFERENT CELL LINES

Nava Dayan[1], Ph.D., Vishwas Rai[2] and Bozena Michniak-Kohn[2], Ph.D.

[1]*Lipo Chemicals, Inc., Paterson, NJ 07504*
[2]*Department of Pharmaceutics, Rutgers,*
The State University of New Jersey, Piscataway, NJ 08854

Objective:

The objective of the study was to investigate the *in vitro* phototoxic effect of fractionated melanin (FM, INCI name: Melanin) and chlorpromazine HCl (CPZ) (positive control) in three different cell lines: mouse embryonic fibroblast cell line (Balb/c 3T3) (the line recommended by Organization for Economic Cooperation and Development - OECD) [1], primary dermal fibroblasts (HDF) and primary human keratinocytes (HEKn). The human derived cell lines were selected in order to correlate data obtained from the mouse fibroblast cell line (Balb/c 3T3) and explore the possible replacement of animal with human derived cell lines.

Methodology:

Stock solutions of FM and CPZ and final dilutions were prepared. Cell were seeded and exposed to different concentrations of FM and CPZ in presence (HEV/UVA+ conditions) and absence of light (HEV/UVA- conditions). HBSS containing 1% DMSO served as a negative control. After addition of the solutions, the plates were incubated for 60 minutes. Than plates were exposed to solar radiation (SOL 500 lamp equipped with a H1 filter; Honle, Martinsried, Germany) of 4.08mW/cm^2 for 50 min through the plate lid leading to a cumulative dosage of 12.24 J/cm^2. Cellular viability was measured using Neutral Red Uptake assay and data obtained from spectroscopic analysis was then fed into Phototox software version 2.0 [1, 2]. The software generates graphs for cellular viability vs. concentration of test compounds under light and dark conditions thereby generating values of EC_{50}, Photo Irritation Factor (PIF) and Mean Photo Effect (MPE). Digital images of the cells were also taken at predetermined time intervals during the experiment.

Results:

The mean toxic concentration (MTC) for CPZ during HEV and UVA exposure conditions was found to be similar using Balb/c 3T3 (36.25 µg/ml) and HEKn (39.99 µg/ml) showing that cells exhibit similar responses at HEV/UVA- conditions (Figure 1a and 1b; Table 2). However, Balb/c 3T3 showed more sensitivity to CPZ at HEV/UVA+ conditions (MTC = 0.87 µg/ml; mean PIF= 55.33; MPE =0.395) than HEKn (MTC = 5.35 µg/ml; PIF= 7.61; MPE =0.276) suggesting that this should be the preferred cell line for photo-toxicity evaluations (Table 2). In HDF, the graphs for light and dark conditions due to exposure of CPZ and FM was found to be overlapping (showed little difference) resulting in low mean PIF of 1.03 and 0.54 respectively. The overall sensitivity of the HDF cells to both compounds was also found to be relatively lower (lower EC_{50} values) compared to Balb/c 3T3 and HEKn. Detailed HEKn and HDF data is not shown in this publication and can be found in the literature [3].

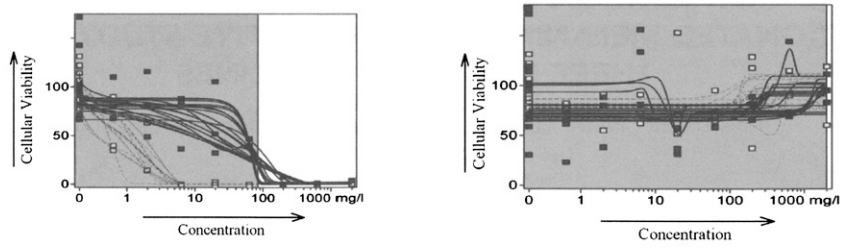


Figure 1: Concentration vs. cell viability data for (a) Chlorpromazine (b) FM, in Balb/c 3T3. The blue dots represent the raw data (under dark conditions) and the solid lines represent the corresponding statistical fit. Similarly, the yellow dots represent the raw data (under lighted conditions) conditions) and the dashed lines represent the corresponding statistical fit. Taken from [3].

Table 1: EC_{50} values for CPZ and FM in Balb/c 3T3 cells. Taken from [3].

Chemical	HEV/UVA light	EC_{50} mean	EC_{50} variance
CPZ	-	36.25	20.95
CPZ	+	0.87	0.42
Mel-HEV	-	-	-
Mel-HEV	+	-	-

+ HEV/UVA light = presence of light; - HEV/UVA light = absence of light.

Table 2: Photo Irritation Factor (PIF) and Mean Photo Effect (MPE) values for CPZ and FM generated in Balb/c 3T3 cells. Taken from [3].

Chemical	PIF Mean	Toxicity probability	MPE	Toxicity probability	Phototoxic potential
CPZ	55.33	1.0	0.395	1.0	Yes
Mel-HEV	C1235*	0	-0.050	0	No

C1235* equals no photo-toxicity potential [2, 4]

Table 3: Comparative chemo-toxicity and photo-toxicity sensitivity of cell lines
(+) represent sensitivity, (++) comparatively higher sensitivity, (-) lack of sensitivity to reaction.

Cell Line	Cytotoxicity	Photo-toxicity
Balb/c 3T3	+	++
HEKn	+	++
HDF	++	-

Conclusion:

It was concluded that cell lines – Balb/c 3T3 and HEKn showed sensitivity to photo-toxicity, while HDF showed little difference between light and dark conditions for positive control, CPZ (Table 3). FM did not show signs of cytotoxicity or photo-toxicity in both sensitive cell lines - Balb/c 3T3 and HEKn assuring its safety as a cosmetic ingredient.

References:

[1] OECD, Organization for Economic Cooperation and Development Guidelines for the Testing of Chemicals / Section 4: Health Effects Test No. 432: In Vitro 3T3 NRU Phototoxicity Test *2004*.

[2] B. Peters, H.-G. Holzhutter, In Vitro Phototoxicity Testing: Development and Validation of a New Concentration Response Analysis Software and Biostatistical Analyses Related to the Use of Various Prediction Models. *ALTA* **30**; 415-432 *(2002)*.

[3] V. Rai, N. Dayan, B. Michniak-Kohn, A comparative evaluation of photo-toxic effect of fractionated melanin and chlorpromazine hydrochloride on human (dermal fibroblasts and epidermal keratinocytes) and mouse cell line/s (fibroblast Balb/c 3T3). *Toxicology in Vitro* In Press, Corrected Proof.

[4] H. Spielmann, M. Balls, J. Dupuis, W.J.W. Pape, O.d. Silva, H.-G. Holzhütter, F. Gerberick, M. Liebsch, W.W. Lovell, U. Pfannenbecker, A Study on UV Filter Chemicals from Annex VII of European Union Directive 76/768/EEC, in the In Vitro 3T3 NRU Phototoxicity Test. *ALTA* **26**; 679-708 *(1998)*.

STIMULATION OF SKIN IMMUNITY AND LANGERHANS CELLS PROTECTION DRAMATICALLY REDUCES UV — INDUCED SKIN ERYTHEMA AND TEWL

Giorgio Dell'Acqua, Ph.D.
Induchem AG, Volketswil, Switzerland, 8604

Introduction

Skin defense and reactivity involve production of innate immunity proteins by keratinocytes. These proteins, also present in the skin's Langerhans cells, help trigger skin's natural response to environmental aggressors and to initiate its repair mechanism[1,2].

In order to identify natural molecules capable of stimulating skin's immunity and protecting Langerhans cells from UV damage, we isolated middle MW polysaccharides from Tamarindus Indica and combined them with glycoside stevioside from Stevia Rebaudiana. We then tested this combination (Ingredients Complex) to induce skin's immunity markers in human keratinocytes and to protect Langerhans cells from UVB irradiation. We finally correlated the data with erythema and trans-epidermal water loss (TEWL) analysis in a UV-stressed human panel treated with the Ingredients Complex.

Methods

Normal human epidermal keratinocytes (NHEK) were used to detect skin immunity markers (cathelicidin, defensin beta4, HO-1, Toll-like receptor 2, S100A7) by gene expression profiling using RT-qPCR technology. Full thickness human skin explants were used to quantify Langerhans cells protection from UV-induced depletion (UVB 1500 mJ/cm^2). Langerhans cells were detected by immuno fluorescence (CD1a-FITC antibody) and counted. A double blind clinical study on human volunteers (n=25) was run to compare a placebo gel to the gel containing 3% of the Ingredients Complex, after UV-induced erythema and TEWL at 24 hours and 48 hours after irradiation (1.25xMED). Statistical analysis of the data was performed.

Results

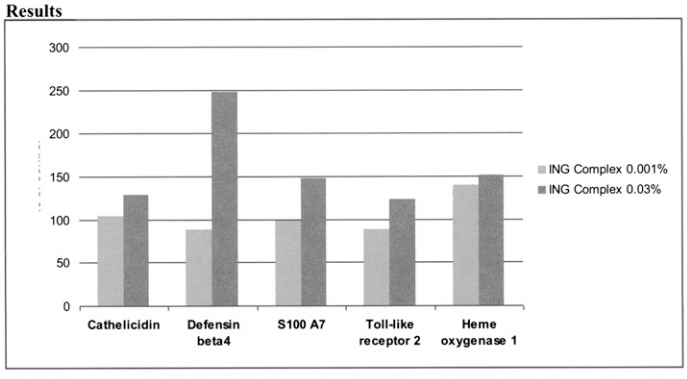


Fig.1. Treatment with the ingredients complex (ING Complex) stimulates immunity markers mRNA synthesis in NHEK.

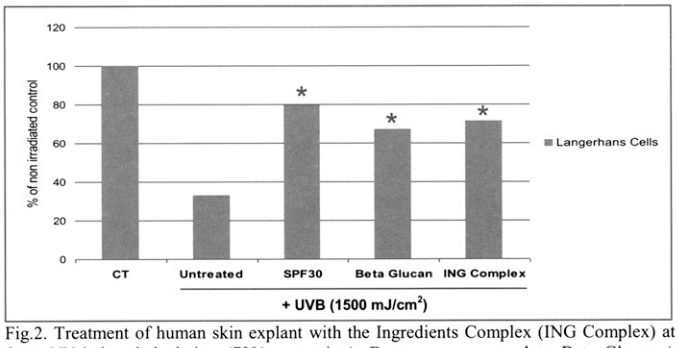


Fig.2. Treatment of human skin explant with the Ingredients Complex (ING Complex) at 0.04% protects Langerhans cells from UV-induced depletion (73% protection). Data were compared to Beta Glucan (used at 0.04%) and to a SPF30 formulation. *All treatments were statistically significant compared to irradiated untreated control ($p<0.001$, Student's t test).

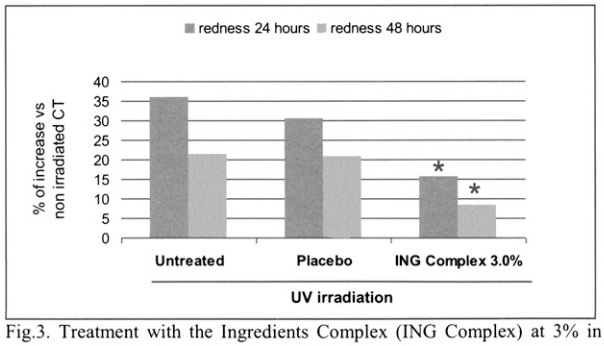


Fig.3. Treatment with the Ingredients Complex (ING Complex) at 3% in a gel reduces UV-induced erythema in human volunteers (-49% after 24 hours and -59% after 48 hours compared to Placebo, *p<0.001, Student's t test).

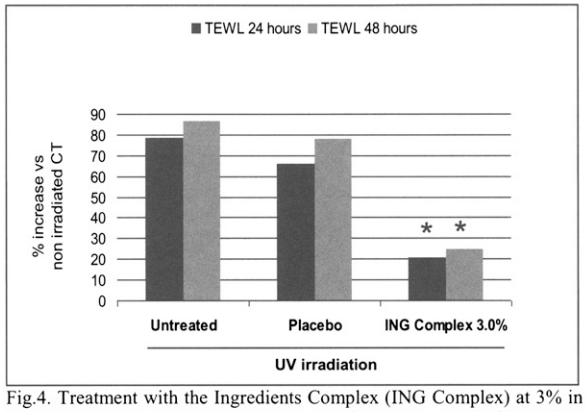


Fig.4. Treatment with the Ingredients Complex (ING Complex) at 3% in a gel reduces UV-induced Trans Epidermal Water Loss (TEWL) in human volunteers (-68% after 24 hours and after 48 hours compared to Placebo, *p<0.001, Student's t test).

Conclusion and Discussion

We demonstrated in this paper that by using an ingredients complex composed by middle MW polysaccharides from Tamarindus combined with glycoside Stevioside from Stevia, it is possible to boost skin's innate immunity markers and to protect Langerhans cells (LS) from UVB-induced damage significantly. These data confirm the role of Tamarindus[3,4] and Stevioside[5,6]as immune modulators. Interestingly a SPF 30 reference cream did not fully protect skin from UV-induced LS depletion, demonstrating that even a high SPF product does not completely protect UV-induced cellular damage. The data on skin models suggest the possibility to combine natural ingredients that boost skin immunity in modern SPF formulations for better protection of skin cells and especially LS. Our clinical data correlate the effect observed on skin models to the soothing capacity of the ingredients complex at 3% concentration, showing a significant and dramatic reduction in UV-induced erythema and TEWL in human volunteers. Since UV-irradiation is commonly associated with immuno suppression[7], it is deducted that the soothing effect observed is related to the immuno stimulating properties of the ingredients complex that bring an overall healing effect. The complex can therefore be suggested as a treatment for UV-stressed and irritated skin and as a potential adjuvant for modern sun and after sun care formulations.

References

1. Nestle FO, Di Meglio P, Qin JZ, Nickoloff BJ. *Nat Rev Immunol* **9**:679-91, *2009*
2. Goodarzi H, Trowbridge J, Gallo RL. *Clin Rev Allergy Immunol* **33**:15-26, *2007*
3. Strickland FM, Kuchel JM, Halliday GM. *Cutis* **74**:24-8, *2004*
4. Strickland FM, Darvill A, Albersheim P, Eberhard S, Pauly M, Pelley RP. *Photochem Photobiol* **69**:141-7, *1999*
5. Chatsudthipong V, Muanprasat C. *Pharmacol Ther* **121**:41-54, *2009*
6. Sehar I, Kaul A, Bani S, Pal HC, Saxena AK. *Chem Biol Interact* **173**:115-21, *2008*
7. Norval M. *Prog Biophys Mol Biol* **92**:108-18, *2006*

COMPLEMENTARY *IN VITRO* MODELS TO INVESTIGATE THE MODE OF ACTION OF ACTIVE INGREDIENTS ON THE PROTECTION OF EXTRACTED EPIDERMAL STEM CELLS AGAINST DIFFERENT TYPES OF STRESSES

Sandy Dumont, Ph.D., Laetitia Cattuzzato, Cindy Sanchez, Ambre De Pooter and Michael Puginier

SEPPIC Laboratoire de biologie, 127 chemin de la Poudrerie, 81105 Castres Cedex, France
sandy.dumont@airliquide.com

OBJECTIVE

Epidermal stem cells (ESC) are necessary for epidermis renewing. These cells are also known to be more or less resistant to different types of apoptosis, in comparison with their keratinocyte counterparts: UV-induced cell death (Marconi *et al.*, 1999), oxidative stress-induced apoptosis (Noblesse *et al.*, 2008) or anoikis (a particular apoptosis resulting from loss of adhesion to extracellular matrix) (Tiberio *et al.*, 2002). However, such a resistance can be impaired under a high level of stress. Thus, some models have been developed to extract ESC from epidermis, to culture them and to investigate their specific functions such as colony-forming capacity or their sensitivity to oxidative stress.

The purpose of this study was, first, to develop new *in vitro* models to investigate ESC response after these different types of stresses and, second, to investigate the preventive effect of active ingredients. We particularly focused on Cocoyl alanine (CA), an antioxidant and anti-wrinkle cosmetic product. Indeed, previously obtained results suggested that it was able to protect elderly skin explants from *ex vivo* culture-induced decrease in survivin expression within epidermis.

METHODOLOGY

First, to confirm the phenotype of extracted and cultured cells, expression of the different ESC-associated markers was investigated by immunofluorescence.

Then, influence of H_2O_2 (50 µM, 18h) on ESC-enriched cultures (ESCC) was investigated by measuring the size of colony after hematoxylin-eosin staining as well as the proportion of survivin-positive cells after immunocytochemistry experiments. The protective role of CA was compared with that of α-tocopherol, a well-known antioxidant molecule which had previously shown to be able to protect ESCC form oxidative stress.

The level of resistance of ESCC to UVB was compared to that of total keratinocyte cultures by measuring cell viability (MTT) in both cultures after UVB irradiation ranging from 25 to 200 mJ/cm^2, in presence or not of an antagonist of the NGF pathway, *i.e.* the tyrosine kinase inhibitor, K252a (200 nM).

Influence of UVB (200 mJ/cm^2) on ESCC was investigated by calculating the proportion of ΔNp63 (transcription factor)-positive cells after immunocytochemistry experiments. The protective role of CA was compared with that of interleukin (IL)-1β, which is known to prevent UVB-induced decrease in p63 expression.

Influence of anti-integrin β 1 blocking antibody-induced anoikis was investigated by calculating the proportion of survivin-positive cells and the protective role of CA was also investigated.

In addition, in the three aforementioned models, TUNEL assays (cytochemistry experiments) were performed to quantify the proportion of apoptotic cells. Finally, whether such protective roles were mediated by the Nerve growth factor (NGF)-signalling pathway or not was investigated by decreasing the level of UVB irradiation (50 mJ/cm^2), while adding K252a (200 nM).

RESULTS AND DISCUSSION

Investigation of ESCC phenotype

First, as expected, extracted and cultured cells expressed the ESC-related marker MCSP (*Melanoma Chondroitin Sulfate Proteoglycan*), ΔNp63, K15, survivin and showed a high level of integrin β1 expression (Figure 1 and data not shown). Conversely, as described in the literature, they did not express the gap-junction related marker Connexin 43. Thus, taken together these results showed that the extracted cell population was actually mainly composed of ESC.

Investigation of H_2O_2-induced apoptosis in ESCC

H_2O_2 treatment (50 µM) of cultures induced a significant decrease in the size of colonies, *i.e.* of 58% (Figure 1). It also induced a significant increase in the proportion of TUNEL-positivUNEL$^+$) cells, *i.e.* of 284%. Investigation of the proportion of survivin$^+$ positive cells also showed a decrease after H_2O_2 treatment (-65%).

The reference molecule α-tocopherol (10 µg.mL^{-1}) partially limited such effects, inducing a restoration effect of 94% and 68%, regarding the size of colonies (Figure 1) and the proportion of TUNEL$^+$ cells, respectively. It also induced a preventive effect (37%) against the H_2O_2-induced decrease in the proportion of survivin$^+$ positive cells.

CA tested at the two higher concentrations (0.0001% or 0.001%), also showed a restoration effect regarding both the size of colonies, respectively of 54% and 69% (Figure 1), and the proportion of TUNEL$^+$ cells, respectively of 32% and 54%. It also induced a preventive effect (from 37% to 52%) against the H_2O_2-induced decrease in the proportion of survivin$^+$ positive cells.

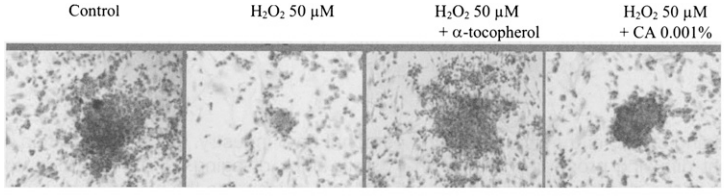


Figure 1: Investigation of colony size by standard HE staining in ESCC cultures after oxidative stress.

Comparison of ESCC and keratinocyte response to UVB

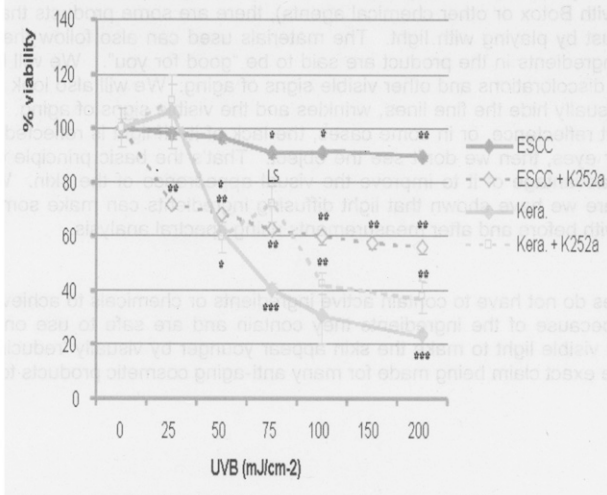


Figure 2: Investigation of ESCC and keratinocyte response to UVB.

Both NHK and ESCC were submitted to a range of UVB ray doses to evaluate their resistance to such a stress. Mortality induced by UVB did not exceed 11% in ESCC, even when the cells were exposed to the highest dose of UVB, *i.e.* 200 mJ.cm^{-2} (Figure 2).

On contrary, cell viability was highly impaired in NHK, as from the dose of 50 mJ.cm^{-2} (Figure 2). When pre-treated with K252a, ESCC viability was decreased to 70% as from the dose of 25 mJ.cm^{-2}, while the differences observed between untreated and K252a-treated NHK were not significant (Figure 2).

Thus, two UVB doses were chosen: 200 mJ.cm^{-2} for the investigation of ESCC resistance in basal conditions; and 50 mJ.cm^{-2} for the investigation of ESCC resistance in conditions of NGF pathway inhibition (*i.e.* treatment with K252a).

Investigation of UV-induced apoptosis in ESCC

UVB irradiation (200 mJ.cm^{-2}) of ESCC induced a significant decrease in the proportion of ΔNp63$^+$ cells, *i.e.* of 67%.

The reference molecule IL-1β (20 U.mL^{-1}) partially limited such effects, inducing a restoration effect, *i.e.* of 58%, regarding the proportion of ΔNp63$^+$ cells.

CA tested at the two higher concentrations (0.0001% or 0.001%), also showed a restoration effect regarding the proportion of ΔNp63$^+$ cells, of 29% and 48% respectively.

When ESCC were both treated by K252a and UVB, an increase in the proportion of TUNEL$^+$ cells was observed from an UVB dose of 50 mJ.cm^{-2} to 200 mJ.cm^{-2}. At 50 mJ.cm^{-2}, variations were of +116%.

CA tested at the two higher concentrations (0.0001% or 0.001%) showed a restoration effect regarding the proportion of TUNEL$^+$ cells of respectively 42% and 52%.

Investigation of the effect of anti-β1-integrin antibody on ESCC anoikis

AB1I treatment (1:250) of cultures induced a significant increase in the proportion of TUNEL$^+$ cells, *i.e.* of 203%. CA tested at the highest concentration (0.001%), showed a restoration effect regarding the proportion of TUNEL$^+$ cells, *i.e.* of 69%.

CONCLUSION

In conclusion, after confirmation of ESC phenotype (*i.e.* MCSP$^+$ β1-integrin^{high} ΔNp63$^+$ K15$^+$ Surv$^+$) and their functionality (UVB-resistance), three different kinds of models could be validated to evaluate the effect of oxidative stress, UV and loss of adhesion to extracellular matrix on ESCC apoptosis. Such models also enable the study of the protective ability of cosmetic active ingredients. More particularly, CA showed a protective effect in the three models, which is coherent with its antiradical properties and its clinically proven anti-age activity. In the future, intracellular signalling pathways regulating such effects could be more precisely investigated by studying phosphorylation events and transcription factor activities.

USING LIGHT DIFFUSION TO MAKING ANTI-AGING CLAIMS

Nick Morante

Nick Morante Cosmetic Consulting, 233 Union Avenue, Suite LL1, Holbrook, NY 11741
nmorante@optonline.net

Anti-Aging trends will always be running strong in the cosmetics and personal care industry. This presentation will focus on the use of light altering and light diffusing properties to hide the visible signs of aging. Many products today use ingredients that alter the appearance of the skin by using ingredients that plump or increase the skin volume by increasing blood circulation. Others increase the rate of cell turnover or other means in order for the skin to appear as though it looks and feels younger. Although some consumers may opt not to use products that contain ingredients that make these claims or use chemicals on (or 'in' their skin as with Botox or other chemical agents), there are some products that do a very good job at hiding fine lines and wrinkles just by playing with light. The materials used can also follow the same mineral-based marketing direction where these ingredients in the product are said to be "good for you". We will look at some of the products that are used to hide flaws, discolorations and other visible signs of aging. We will also look at light diffusion, a property of some ingredients which visually hide the fine lines, wrinkles and the visible signs of aging. These properties of hiding are actually a function of light reflectance, or in some cases, the lack of it. If light is reflected off an object but the light is not reflected directly to our eyes, then we don't see the object. That's the basic principle of light reflectance and we will show how we can take advantage of it to improve the visual appearance of the skin. We will examine some initial and very basic studies where we have shown that light diffusing ingredients can make some skin flaws "disappear", or appear visually diminished with before and after measurements using spectral analysis.

Simply put, products using light diffusing properties do not have to contain active ingredients or chemicals to achieve their goal. They don't have to be super-expensive because of the ingredients they contain and are safe to use on many different types of skin. These products *play* with visible light to make the skin appear younger by visually 'reducing' the appearance of fine lines and wrinkles, which is the exact claim being made for many anti-aging cosmetic products today.