J. Cosmet. Sci., **62,** 61–83 (January/February 2011)

Papers Presented at the 2010 Annual Scientific Meeting and Technology Showcase (Thursday's Program)

December 9–10, 2010
New York Hilton
New York, NY

Nothing to See but Also Nothing to Hide: An Evaluation of the Skin Penetration Enhancing Capability of Nanoparticles Used in Sunscreens

Johann W. Wiechers, Ph.D.

JW Solutions, Gasthuispolderweg 30, 2807LL Gouda, The Netherlands

Introduction:

To minimize the visibility of the inorganic sun filter, nanoparticles in the 35-50nm range with a narrow size distribution have been employed for more than 10 to 20 years, depending on the filter. While 'nano' stood for something exceptionally good and active about a decade ago, the public opinion on anything 'nano' has drastically changed. Reports by self-declared experts on the internet claim that nanoparticles in sun care products penetrate human skin with dangerous consequences for human health. Regulatory bodies in Europe, despite decisive statements on the suitability of such particles in cosmetics, even make the labeling of the presence of nano-sized materials compulsory from 2013 onwards, so that the consumer can make a well-informed choice whether or not to use such products. This suggests that there is indeed an issue with nano-sized particles in cosmetics. This is strengthened by the fact that sunscreen manufacturers are not all helpful in providing information on whether their products do indeed contain nanoparticles.

It is therefore time to look independently at all the scientific data to assess whether or not nanoparticles do penetrate into and through human skin from cosmetic formulations under normal in-use conditions. This analysis should lead to either a ban on these materials or an agreement that there is nothing to hide if there is no evidence of skin penetration. Not only do we need transparency for our transparent sunscreen formulations but also for our customers.

Literature investigations:

The year 2007 marked a turning point in the studies exploring the skin delivery of nanoparticles from sun care products. Until 2007, many studies were performed under widely varying experimental circumstances, culminating in the already now classic review paper by Nohynek *et al.* (1). They concluded that nanoparticles (NPs) did not penetrate beyond the human stratum corneum, *i.e.*, they would penetrate into the stratum corneum but not reach the living layers of the human epidermis and dermis. But a few articles were published that demonstrated skin penetration into the living layers of pig skin. A pivotal publication in this sense was the paper by Ryman-Rasmussen *et al.* (2). This study showed that quantum dots of different sizes, shapes, and surface coatings could penetrate intact skin at an occupationally relevant dose within the span of an average-length work day. These results suggested that "skin is surprisingly permeable to nanomaterials with diverse physicochemical properties and may serve as a portal of entry for localized, and possibly systemic, exposure of humans to quantum dots and other engineered nanoscale materials" (2). This concern was not completely hypothetical when one considers that (i) skin is nanoporous at the nanoscale, (ii) orifices of hair follicles and glands open on skin surface, providing alternative routes of entrance, and (iii) in everyday life skin may be damaged by detergent exposure, scratches, hydration or dryness, sunburn, or pathological states (3). The outside world reacted that even scientists did not know what was going on and therefore, what guarantee did the consumer have that the sun care products they used were safe?

The discrepancy on the internet on the safety of NPs originates from an incorrect extrapolation of the obtained scientific findings for skin penetration of NPs in general to the probability of their penetration following application of sunscreens under real-life conditions. The results could not be extrapolated because (i) the size of the investigated skin-penetrating NPs was significantly smaller than those used in cosmetics (*i.e.*, titanium dioxide and zinc oxide); (ii) all nanoparticles investigated were metallic, the penetrating nanoparticles used elements other than titanium or zinc, namely iron and silver; (iii) the formulations of these iron and silver nanoparticles were radically different from cosmetic formulations and were demonstrated to have an effect on skin barrier function. But before that could be studied, one important discovery was made by the same group that assessed the skin penetration of NPs, namely that while NPs did penetrate pig skin (the preferred model for human skin), they did not penetrate rat and human skin (4)! Results obtained on pig skin can therefore not be extrapolated to human skin.

Post 2007 research revealed that smaller NPs penetrate indeed better, especially those under 10 nm, and that their penetration was influenced by their surface charge (anionic NPs deposit and penetrate less than cationic NPs). With this knowledge, research attention could now focus on real-life application conditions of sunscreen products. Such conditions are characterized by UV radiation, flexing (think of sport activities on the beach) or damaged skin. Mortensen *et al.* (5), aimed to mimic real-life conditions as much as possible (although they used mouse skin!) and

found small but enhanced skin penetration of quantum dots with UV radiation. This was followed within two weeks by a letter from the Nanotechnology Industries Association (6), claiming that the mouse model used was incorrect, the particles were significantly smaller (both factors contributing to enhanced NP penetration) and that despite all that, skin penetration of NPs was still minute. Rouse *et al.* (7) found flexing for 60 to 90 minutes to enhance the penetration of NPs, but here it can be argued that this study was performed on pig skin which is now known not to be an appropriate model. Abrasion but not tape-stripping was shown to enhance the skin penetration of NPs (8). But the number of formulations used during these studies ranged from absolute ethanol and synthetic sweat to non-described commercial formulations and the statement of Bianca Baroli must be kept in the back of our minds: "Nevertheless, several articles on particle penetration have been recently published, suggesting that processes governing the penetration of chemicals and particles might not be the same (9)."

Evaluation and Conclusion

Where does that leave you and me in our desire to know whether NPs incorporated in sunscreen products do penetrate human skin under normal *in-use* conditions? If we look at the influence of the chemical, we see that while penetration of some metal NPs and fullerenes has been observed, this has not been seen for other metals and never for the metal oxides ZnO and TiO_2. If we look at the influence of particle size, we see that particles below 10nm and in the 400-600nm range show increased skin (or transfollicular) penetration. If we look at the influence of the coating on skin penetration of NPs, we see that cationic NPs deposit and penetrate better than anionic NPs. When comparing this to the potential penetration of NPs from sunscreen products, we see that the ZnO and TiO_2 agglomerates are much bigger than 10 nm, that their anionic coating does not favor skin penetration, that they are intended to be applied to human skin and not pig skin, so that therefore chances of skin penetration of NPs in sunscreen products are marginal. In fact, flexing and abrasion are the only two situations where we might anticipate a higher than normal penetration.

Until now, I have not seen evidence for fundamentally different rules applying to the skin penetration of NPs. While pig skin is normally a good model for human skin, it is not in the case of NPs. Pigs don't have eccrine glands whereas humans have and typically apply sunscreen under conditions of enhanced sweating.

It is clear that future research should concentrate on the skin delivery of NPs from commercial formulation structures under relevant dosing conditions only, but that the penetration is likely to be that low that it cannot be shown. Until that time, the cosmetic industry needs to be transparent in what it knows and not hide available information. After all, if there is nothing to see, there is also nothing to hide…

References:

1. G. J. Nohynek, J. Lademann, C. Ribaud and M. S. Roberts, Grey Goo on the skin? Nanotechnology, cosmetic and sunscreen safety, *Crit. Rev. Toxicol.*, **37**, 251-277 (2007).
2. J. P. Ryman-Rasmussen, J. E. Riviere and N. A. Monteiro-Riviere, Penetration of intact skin by quantum dots with diverse physicochemical parameters, *Toxicol. Sci.*, **91**, 159-165 (2006).
3. B. Baroli, Nanoparticles and skin penetration. Are there any potential toxicological risks? *Verbr. Lebensm.* **3**, 330-331 (2008).
4. N. A. Monteiro-Riviere and J. E. Riviere, Interaction of nanomaterials with skin: Aspects of absorption and distribution, *Nanotoxicol.*, **3** 188-193 (2009).
5. L. J. Mortensen, G. Oberdörster, A. P. Pentland and Lisa A. DeLouise, In vivo skin penetration of quantum dot nanoparticles in the murine model: The effect of UVR, *Nanoletters*, **8**, 2779-2787 (2008).
6. Anonymous, NIA comment on "In vivo skin penetration of quantum dot nanoparticles in the murine model: The effect of UVR", available at: http://www.nanotechia.org/lib/tmp/cmsfiles/File/NIA_Press_FAQs_Opinions/NIA_SunscreenSkinPenetration_Comment_FINAL.pdf
7. J. G. Rouse, J. Yang, J. P. Ryman-Rasmussen, A. R. Barron and N. A. Monteiro-Riviere, Effects of mechanical flexion on the penetration of fullerene amino acid-derivatized peptide nanoparticles through skin, *Nanoletters*, **7**, 155-160 (2007).
8. L. W. Zhang and N. A. Monteiro-Riviere, Assessment of quantum dot penetration into intact, tape-stripped, abraded and flexed rat skin, *Skin Pharmacol. Physiol.*, **21**, 166-180 (2008).
9. B. Baroli, M. G. Ennas, F. Loffredo, M. Isola, R. Pinna and M. A. López-Quintela, Penetration of metallic nanoparticles in human full-thickness skin, *J. Invest. Dermatol.*, **127**, 1701-1712 (2007).

Inorganic UV Filter Composite for Better Safety and Performance

David Schlossman, Yun Shao, Ph.D., Pascal Delrieu, Ph.D. and Carl Orr

Kobo Products, Inc.

Introduction

Despite their legacy, consumer activist groups have recently challenged the use of Inorganic UV Filters in cosmetic products, because their size may be in the nano range. Furthermore, the potential risks to human health when particles are inhaled or concerns that they may be carcinogenic are hot topics for debate. A recent study made in Sweden concluded that nano zinc oxide could be toxic to the environment. Because of these developments, regulators are likely to require labeling to identify products that contain nano particles in the future.

The European Union Cosmetic Directive, published in March 2009, defined a nano particle as an insoluble or bioresistant and intentionally manufactured material with one or more external dimensions, or an internal structure, on the scale from 1 to 100 nm. Techniques for measuring the particle size of Inorganic UV Filters include light scattering, TEM, and SEM. (1) Companies are screening their pigments for nanoparticles, and what is certain is that many popular filters may no longer be considered, because they may contain particles with primary particles or agglomerates less than 100 nm.

Composite PA-12 Powder technology is a strategy based on compounding traditional nano scale pigments with a polymer greater than several microns to attenuate UV light. (2) These new particles are non-nano, but attenuate UV light according to the same principles, and with similar optical properties as traditional attenuation grade pigments.

History of Inorganic UV Filters

Inorganic UV filters have been available for approximately the past forty years. JP Patent 45-32730, titled Sunscreen Cosmetic, was published in 1970 by Shiseido Company Ltd. The patent application discloses titanium dioxide of a primary particle size between 30 nm. and 40 nm. as a suitable pigment for the attenuation of UV light. (3) Another patent, JP 60-231607, assigned to Kobayashi Kose Co., cites using zinc oxide particles with a size of 10-60 nm. Kose provided an example formulation that combines a 50 nm. zinc oxide (15%) with 30 nm. titanium dioxide (5%) particles to protect against wavelengths as high as 385 nm. The skin whitening is less than conventional formulas. (4)

During subsequent years, the technology to manufacture sunscreen particles was continuously improved and finer particles with better inorganic and organic coatings were introduced, principally in Japan at the leading cosmetic Companies. These improvements to the particles likewise cited in patents and publications, included smaller particles, particles with improved physical and chemical stability, increased UV attenuation, and reduced skin whitening. (5) (6)

Thus, within industry and consumers, there came a greater recognition of the benefits of using sunscreen particles to provide safe and effective protection against UVA/B without skin whitening. (7) Products developed for babies and children typically contain attenuation grade pigments. They are also used to decrease the amount of organic sunscreens contained in sunscreen formulations. Titanium dioxide and zinc oxide particles are formulated in Ecocert sunscreen formulations. (8) There are no known allergies attributable to inorganic sunscreens.

Composite Powder Technology

Composite powders enable a formulator to work with traditional nano size pigments, since the pigments in their agglomerated form are restrained in a matrix greater than several microns in size.

The principle of dispersing a pigment into a matrix capable of attenuating UV light was published in 1989 in US Patent No. 4,882,143 titled "Lamina and a Cosmetic Comprising the Same," assigned to Sumitomo Chemical Company, Osaka Japan. The lamina is a matrix comprising a finely divided metal compound dispersed in a laminar substance where the refractive index difference between the pigment and the laminar is greater than 0.1. The matrix claimed in the invention has excellent UV attenuation and transparency. (9)

Composite PA-12 Powders

Spherical polyamide 12 ("PA-12") is a popular cosmetic filler, because of its smooth surface and creamy texture. It was the polymer chosen in the present study. The process to make the Composite Powder is to compound PA-12, a sugar, and an inorganic UV filter at the desired ratio in a twin-screw mixer to the desired particle size. Strands are going to be extruded. The next step is to wash the strands in a solvent to remove the sugar. The filtered solids are then dried and sieved to obtain a fine powder, which is spherical and contains the attenuation grade pigment.

The preferred UV filter may contain hydrophobic coatings such as fatty acids or silicones to improve their wetting and stability. The pigments used for the Composite PA-12 Powders presented herein, their primary particle size, surface treatment, and percent solids are as follows:

Attenuation Grade Pigment	Primary Particle Size	Surface Treatment	Percent solids
Titanium Dioxide	15 nm.	Aluminum hydroxide and stearic acid	50
Zinc Oxide	30 nm.	Methicone/dimethicone copolyol	50

Sunscreen pigments are hard particles, which typically feel coarse, because of their morphology (acicular or granular). However, Composite PA-12 Powders have soft and smooth textures. Accordingly, more than 50% may be contained in products requiring sun protection. It was found that the pigments are agglomerated further in the composite causing a significant increase in UVA protection. Combining the Composite PA-12 Titanium Dioxide Powder at 20% with organic sunscreens resulted in an in-vivo PA score greater than 20. When comparing transmittance curves between wavelengths of 290 nm. and 400 nm. The attenuation grade pigments alone are more efficient to attenuate UV light than the composites. We also evaluated finished sunscreen lotions (containing about 6% titanium dioxide), but between the attenuation grade titanium dioxide and Composites PA-12 Titanium Dioxide Powder, the same in-vivo SPF and PA scores were measured. Accordingly, the composites did not agglomerate further in the formula, unlike the attenuation grade pigments.

The titanium dioxide contained in the Composite PA-12 Titanium Dioxide powder we studied was homogeneously dispersed in the composite. However, it is possible to produce composites that are heterogeneous and contain more titanium dioxide on their surface. Combinations of titanium dioxide and zinc oxide of various particle sizes are also possible, and can be compounded with PA-12 to design composites with different UVA/B ratios. (10)

It was also found that formulations containing Composite PA-12 powders were not greasy or sticky.

Conclusions

Composite Powder technology offers Companies who are risk adverse to nano technology the opportunity to continue to formulate with traditional attenuation grade pigments. PA-12 is an acceptable polymer to make a composite powder, because it has a very desirable skin feel. Other Composite Powders available or under development at the time of this publication may contain PMMA, polyesters, silica, and biopolymers. (11)

The future demand for Composite Powders are likely to depend on factors including their cost, percent solids, and competing technologies to provide UV protection.

References

1. Non-Nano Zinc Oxide, Pascal Delrieu, Yun Shao, and David Schlossman, Kobo Products, Inc., Society of Cosmetic Chemists Annual Meeting, December 2009.

2. Hisayoshi Ito (Daicel Chemical Industies, Ltd.) Composition Having Disperse System and Process for Producing Colored Organic Solid Particle, US Patent No. 7,442,730 B2

3. Yoshio Fuziyama, Kiyoko Koshiba (Shiseido Company Ltd.), Sunscreen Cosmetic, Application filing JP 45-32730 (1970)

4. Kobayashi Kose Co., Sunburn Preventive Cosmetic Material, JP 60-231607

5. Nihihara, Iwane, and Imai (Teikoku Kako Co. Ltd.), Properties of Super Fine Grain Titanium Oxide and Application to Cosmetic Foundation, Fragrance Journal, No. 73, 43 (1985)

6. Katsumasa Iwaya, Hajime Hotta (Kao Coporation), Anti-Suntan Cosmetic Composition, US Patent No. 5,032,390 (1991)

7. Curtis A. Cole, Martin K.O. Lindemann, Elvin R. Lukenbach, Ralph C. Stutzman (Johnson & Johnson Consumer Products, Inc.), Sunscreen Compositions, US Patent No. 5,340,567 (1994).

8. Lovea solaire (Laboratoire Bicos), http:/www.lovea.fr

9. Hidekimi Kadokura, Kunio Saegusa (Sumitomo Chemical Company, Ltd.), Lamina and a Cosmetic Comprising the Same, US Patent No. 4,882,143

10. Daicel-Evonik, Company presentation (June, 2009)

11. Daicel-Evonik, Company Presentation (September, 2010)

Oxothiazolidine: A Safe and Bioavailable Antioxidant Opposes to the Damaging Effects of Deep-Penetrating Solar Radiations

Jean-Francois Nicolay[1], Ph.D., L. Valenti[2], M. Frechet[1] and P. Lafitte[1]

[1]Exsymol S.A.M., 4 Avenue Albert II, MC-98000, Monaco
[2]Sedifa Laboratoire, 4 Avenue Albert II. MC-98000, Monaco

Introduction

Several sun radiations are responsible for skin photoaging , e.g. sunlight-accelerated aging. Ultraviolet A (UVA: 320-400 nm) and ultraviolet B radiations (UVB: 290-320 nm) have long been in the focus, but recent findings have emphasized the role of near infrared radiations (IRA: 760-1440 nm). IRA induce stress signaling, collagen-degrading matrix metallo proteinase (MMP) overproduction, apoptosis, and modulation of intra-cellular calcium signaling [1,2]. ROS generation is a common feature for UVA, UVB and IRA, but ROS distribution is different (Figure 1), since skin penetration is wavelength-dependent. UVB mainly challenge the epidermis, while UVA and IRA are deep-penetrating radiations generating ROS in the dermis, IRA being capable to reach subcutaneous tissues.

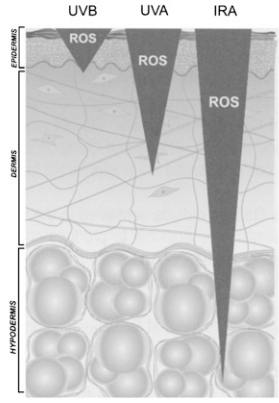

Figure 1: sun radiations penetration in human skin is wavelength-dependent

Topical antioxidant design

It comes that topical antioxidant protection, in particular against UVA and IRA, requires ROS scavengers with a good bioavailability. To design an antioxidant able to provide some protection against deep-penetrating radiations, we have used a predictive expert system (ADME and TOX boxes softwares from Pharma Algorithms) and the Potts and Guy equation [3]. Experimental bioavailability was determined *ex vivo* with human skin explants mounted on an automated Franz cell system.

Oxothiazolidine bioavailability and antioxidant properties

Oxothiazolidine (usual name OTZ) is a small molecule (Figure 2) that enters into the epidermis and the dermis. Its experimental permeability constant Kp is approximately 6 fold higher than caffeine Kp. OTZ has two reactive sites, the sulphur moiety being a ROS scavenger, and the amine a "Reactive Carbonyl Species" (RCS) scavenger. Thus, OTZ is not a highly reactive compound, but it has a large antioxidant spectrum, scavenging ROS (hydroxyl radical, hydrogen peroxide), RNS (peroxynitrite), and RCS, including the highly cytotoxic lipid peroxidation end-product 4-hydroxynonenal. Besides, we have shown that complete sulphur oxidation upon ROS/RNS scavenging leads to ring opening and formation of taurine, a natural protective compound present in the skin [4].

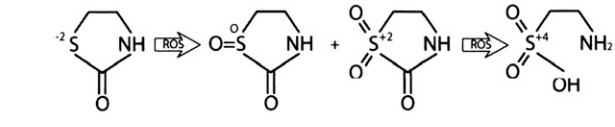

Figure 2: Oxothiazolidine (OTZ) chemical structure, and stepwise ROS-induced transformation into taurine

Protection against UVA

Protection of cutaneous cells was demonstrated *in vitro* and *ex vivo*. OTZ treatment (curative / preventive) of V79 fibroblasts exposed to UVA limits apoptosis and DNA damages. We have shown that OTZ opposes to UVA-induced intracellular oxidative stress, and that OTZ intracellular accumulation and ROS scavenging within the cells account, at least partly, for this result.

Demonstration of deep-sited protection against UVA was achieved *ex vivo* with human skin explants (epidermis + dermis). Expression and localisation of several proteic markers were analysed by

immunofluorescence. These studies were carried out on skin pieces 24h after irradiation. Treatments were either "systemic" (compound added in the culture medium) or "topical" (compound loaded in nitrocellulose patches deposited on the skin). N-acetyl-cysteine (NAC) was used as a reference antioxidant. Three dermal endpoints (MMP1, COX-2, and decorin), and one dermal epidermal junction endpoint (collagen VII) were monitored. Quantitative results obtained from computer-assisted image analysis (Cell F software, Olympus) show that systemic and topical OTZ can protect deep-sited targets of UVA (figure 3).

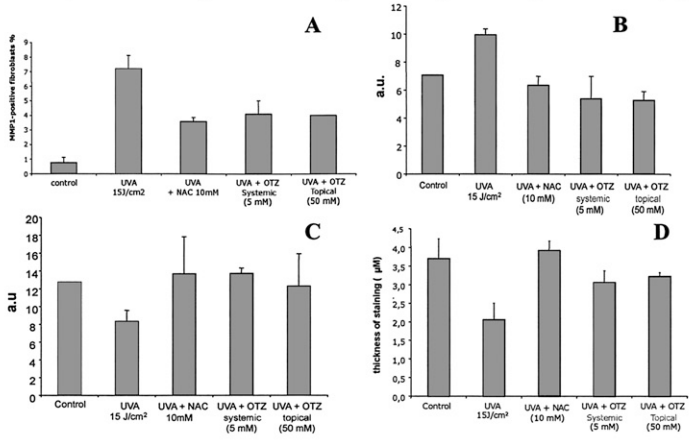

Figure 3: Quantitative analysis of staining intensities (a.u. = arbitrary units) in UVA-irradiated (15 J.cm^{-2}) and non irradiated (control) human skin explants. (A) MMP1expression in fibroblasts, (B) COX-2 expression, (C) decorin expression, (D) collagen VII expression. N-acetyl-cysteine (NAC) is tested in "systemic condition".

Protection against IRA

Near-infrared, the dominant infrared wavelength, is mainly absorbed by the dermis (approximately 50%). Increased mitochondrial ROS production in fibroblasts following IRA exposure was described as the upstream event modulating several signaling pathways, including MAP kinases, intracellular calcium, IP3 and IL-6 pathways [2]. One major biological response to IRA is MMP1 over-expression, without comparable increase of its specific natural inhibitor TIMP-1 [1]. So, as a first approach experiment, cultured human dermal fibroblasts (HDF) were incubated with OTZ (preventive mode to enable intracellular accumulation), and exposed to IRA. MMP1 and TIMP1 gene expression were assessed by semi-quantitative realtime PCR (four independent experiments with HDF isolated from different donors). OTZ dose-dependently inhibited the IRA-induced MMP1 overexpression. Interestingly, MMP1 basal expression in control fibroblasts was also dose-dependently reduced by OTZ. Lower MMP1 basal expression upon treatment with OTZ may reflect a cellular antioxidant status improvement, independently from an external challenge.

Conclusion

Taken together, our results strongly support OTZ usefulness for anti-photo-aging. OTZ specific features make it a bioavailable and safe product (taurine formation upon ROS scavenging), providing deep-sited protection of the skin.

References

1. Schroeder, P., Lademann, J., Darvin, M.E., Stege, Marks, C., and Krutmann, J. *J. Invest. Dermatol.*, **128**, 2491-2497 *(2008)*
2. Calles, C., Schneider, M., Macaluso, F., Krutmann, J. and Schroeder P. *J. Invest. Dermatol.*, **130**, 1524-1536 *(2010)*
3. Potts, R.O., Guy, R.H., Pharm. Res., **9** , 663-669 *(1992)*
4. Lafitte, P., Valenti, L., Casciani, L., Hoarau, T., Lomonte, E., Maccario, F., Nicolaÿ, J-F. *IFSCC Magazine*, **11**, 105-112 *(2008)*

FRONTIERS OF SCIENCE AWARD LECTURE

SPONSORED BY

COSMETICS AND TOILETRIES®

Stratum Corneum Lipid Composition and Structure in Relation to Barrier Function

Philip W. Wertz, Ph.D.
University of Iowa, Iowa City, IA 52242

Lipids in the stratum corneum provide barriers against the penetration of molecules and particles into the skin and participate in protection against microbial colonization. The structural lipids of the stratum corneum consist mainly of ceramides, cholesterol and fatty acids. The fatty acids of stratum corneum are mostly straight-chained saturated species with 20 – 28 carbons. The ceramides represent a structurally diverse group of sphingolipids (Figure 1). The base components contain sphingosine/dihydrosphingosine (S), phytosphingosine (P) and 6-hydroxysphingosine (H), and the amide-linked fatty acids include normal fatty acids (N), α-hydroxyacids (A) and ω-hydroxyacids (O). In the free ω-hydroxyacid-containing ceramides, linoleate is found ester-linked to the ω-hydroxyl group (prefix E). The normal fatty acids in the ceramides are mostly 20 – 28 carbons long and lack double bonds or methyl branches. The α-hydroxyacids are also straight and saturated and range from 16 – 28 carbons in length. The ω-hydroxyacids are 30 – 34 carbons long. The linoleate-containing acylceramides, EOS, EOP and EOH, are responsible for organization of the free lipids into trilaminar units. The long alkyl chains and cylindrical shapes of the stratum corneum free fatty acids and ceramides makes them well suited to form highly ordered and, thereby, impermeable membranes. The cholesterol may provide a degree of fluidity to these membrane systems. These membrane structures determine the diffusional resistance of the stratum corneum to small molecules. In addition to the free lipids, the stratum corneum also contains covalently bound ceramides (Figure 2). These consist of ceramides OS, OP and OH, and they are covalently attached through ester linkages between the ω-hydroxyl group and acidic groups on the outer surface of the corneocyte envelope. The covalently bound lipid layer contributes to the impermeability of the corneocytes and may provide a template upon which the lamellae formed from the free lipids form. In transmission electron micrographs with ruthenium tetroxide post-fixation one trilaminar unit with a 13 nm overall width and broad-narrow-broad lucent bands is seen between the edges of adjacent corneocytes in the same layer of stratum corneum (Figure 3). Between the broad flat surfaces of corneocytes in adjacent layers of stratum corneum contain multiple trilamellar units, again with a 13 nm width (Figure 3). The 13 nm units between the edges of corneocytes are thought to represent interdigitated covalently bound ceramides with some free lipids filling in some of the space (Figure 4). This imposes a strict limit on the physical size of particles that could penetrate through the stratum corneum intercellular space. The follicular route of penetration provides an alternative pathway that can accommodate much larger particles but is of limited importance on most regions of skin. The face and scalp are exceptions to this. In addition to the major structural lipids, the stratum corneum also contains free sphingosine/dihydrosphingosine and is coated with sebaceous lipids including lauric acid (C12:0) and sapienic acid (C16:1Δ6). These lipids are potent antimicrobials and contribute to an antimicrobial barrier.

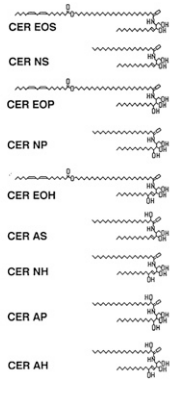

Figure 1. Free ceramides from human stratum corneum.

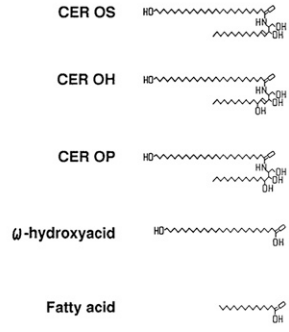

Figure 2. Covalently bound lipids from human strtatum corneum.

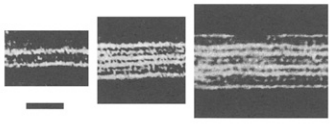

Figure 3. Intercellular lamellae in the intercellular spaces of stratum corneum.

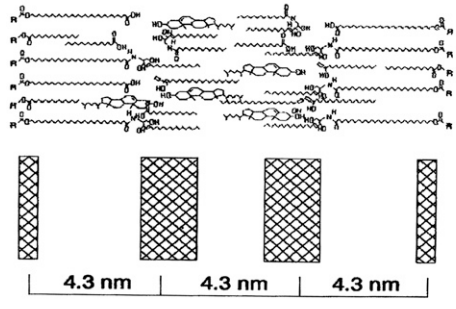

Figure 4. Molecular model of lipid lamellae between the ends of adjacent corneocytes.

Correlation Between Cell Viability and DNA Damage: Studying Chromosomal Effect of Cosmetic Substances

Nava Dayan[1], Ph.D., Vatsal Shah[2] and Tamara Minko[2], Ph.D.,
[1]*Lipo Chemicals, Inc., Paterson, NJ 07054*
[2]*Department of Pharmaceutics, Rutgers University, Piscataway, NJ 08854*

Introduction

While the use of cosmetic and personal care products has been demonstrated to be relatively safe there are a few factors that present challenges in terms of toxicity assessment. These are the concern associated with chronic life time exposure to a variety of chemicals, occupational exposure (such as with hairdressers) and contaminants (such as heavy metals). One of the important end points in safety assessment is the effect of the chemicals on the cellular genetic material, the DNA. In healthy proliferating tissue, when the cell divides, fragments of the chromosome are incorporated into one of the daughter cell nuclei. When this process is interfered with a chemical, the dividing cell forms a body called a micronucleus. The formation of micronuclei is a good reflection of the frequency of DNA aberrations, and therefore is used for rapid assessment of chromosomal damage.

The formation of micronuclei correlates with the dose of exposure to a certain chemical (1). With elevated concentrations of exposure, cells reach a plateau of micronuclei formation and at this point start expressing apoptotic morphological changes that lead to cell death. Therefore, testing for cell viability in conjunction with formation of micronuclei is not only relevant but a key in the understanding of safety of exposure. In our talk we will discuss the different paths for cell death, its possible correlation to DNA damage, testing for cell viability and induction of micronuclei formation.

Methodology

Chinese hamster ovary cells (CHO-K1) where exposed to test compounds with and without metabolic activation. After incubation, cells were fixed and observed under the microscope for micronuclei counting. Cell viability was examined by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay and statistical significance was analyzed using ANOVA. Genotoxicity of studied compounds was evaluated using Aroclor-1254 induced S9 mix. Two positive controls were tested; cyclophosphamide that requires S9 activation and ethyl methanesulfonate that does not. Cells were cultured and then incubated with the test compounds and controls. After 24 hours of incubation the media from all the flasks were removed, cells were fixed and the nuclei were stained. The cells were then observed under a microscope for detecting the presence of micronuclei (2, 3, 4).

Results

The two positive controls tested, cyclophosphamide with metabolic activation and ethyl methanesulfonate, induced substantial genetic damage leading to the formation of micronuclei. In contrast, cellular nuclei appeared intact after the incubation with test substance. Cellular viability measurements indicated that the test compounds and the two positive controls did not inhibit the growth of cells (Fig. 1).

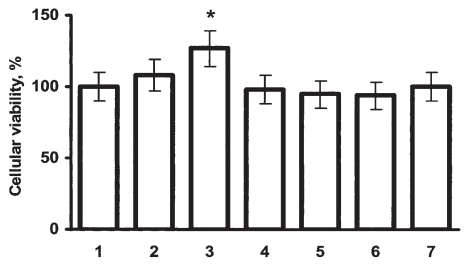

Fig. 1. Cellular viability values: CHO-K1 cells were incubated for 24 hours with the following substances: 1 – DMSO (Negative Control); 2 – Cyclophosphamide + S9 mix (Positive Control); 3 – Ethyl Methanesulfonate (Positive Control); 4 – Test Substance (TS) 0.2 mg/ml in DMSO; 5 – TS 0.3 mg/ml in DMSO; 6 – TS 0.2 mg/ml in DMSO + S9 mix; 7 – TS 0.3 mg/ml in DMSO + S9 mix. Means ± S.D. are shown. *$P < 0.05$ when compared with negative control (2).

Conclusions

While our industry is actively seeking compounds that will possibly regulate the expression of specific genes, we should employ safety assessment studies to ensure DNA integrity. This study demonstrates that conducting cell viability assessment concurrently with observation of chromosomal aberrations allows differentiating between cell death related to metabolic dysfunction and death that is caused by mutations of the DNA. It can assist in the identification of substances that may not promote cell death but can induce significant chromosomal changes that can potentially lead to carcinogenicity. Understanding the paths for cell death and its association with changes in DNA and its expression is a key to unraveling the effect of acute as well as chronic exposure to chemicals.

References

1. Akudugu J.M., Slabbert J.P., Serafin A., Bohm L. Frequency of radiation induced micronuclei in neuronal cells does not correlate with clonogenic survival. *Rad. Res.* **153(1)** 62-67 (*2000*).
2. Kirsche-Vilders M., Sofuni, M. Aardema T., Albertini S., Eastmond D., Fenech M., Ishidate M., Jr., Kirchner S., Lorge E., Morita T., H., Norppa J., Surralles A. Vanhauwaert, Wakata A., Report from the in vitro micronucleus assay working group, *Mutat Res*, **540** 153-163 (*2003*).
3. R.I. Pakunlu, Y. Wang, W. Tsao, V. Pozharov, T.J. Cook, T. Minko, Enhancement of the efficacy of chemotherapy for lung cancer by simultaneous suppression of multidrug resistance and antiapoptotic cellular defense: novel multicomponent delivery system, *Cancer Res*., **64** 6214-6224 (*2004*).
4. OECD guideline for testing of chemicals: In vitro Mammalian Cell Gene Mutation test http://www.oecd.org/dataoecd/18/32/1948426.pdf (Date accessed: May 28th, *2010*).

A Review of Current Analytical Genomic Techniques Used for Gene Expression Profiling of the Skin

Remona Gopaul

Nu Skin Enterprises, Provo, UT 84601

DNA (deoxyribonucleic acid) is a nucleic acid that contains the genetic information needed for the development and functioning of all living organisms.[1] Segments of DNA carrying this genetic information are known as genes. Information can travel from DNA to mRNA (messenger ribonucleic acid) through a process called "transcription", from mRNA to cDNA (complementary DNA) via "reverse transcription" and from mRNA to protein via 'translation".[1] By identifying which mRNAs are present in the cell; one can understand which genes are active in the specific cell type.

Recently, cosmetic companies have been using genomic techniques to understand the expression of specific genes and their relationship to particular skin attributes as well as to test topical ingredients and formulations. The most common techniques currently being used by researchers in the cosmetic field are microarray, SAGE, RNA-Sequencing, RT-PCR and northern blot. This review will evaluate and compare the efficacy, protocol designs, benefits, limitations as well as data interpretation tools for each of the methods.

Small scale Gene Techniques

Northern Blot

Historically, northern blot is one of the most standardized methods for detecting and quantifying RNA sequences. It is simple to run and can efficiently quantify RNA levels at a very low cost.[2] However the processing time is consuming, radioactivity is often used in most experiments and the technique is not as sensitive as most modern techniques. Because of these limitations, this method is not often the method of choice for gene expression and is consequently being replaced by more modern techniques.

Reverse transcription-polymerase chain reaction (RT-PCR)

RT-PCR works by amplifying cDNA made for a specific mRNA transcript. [3] There are two types of real-time PCR: 1) using dyes that fluoresce when bound to double stranded DNA (SYBR Green Method) and 2) using DNA-based fluorescent probes (Taqman Method), which measures the accumulation of fluorescent signals against a preset threshold. Taqman RT-PCR is the method that's most widely used in the cosmetic industry as it gives more comprehensive quantitative data than other techniques. Real-time PCR is considered one of the most sensitive techniques for gene expression and is highly quantitative and reproducible. This method can also be very expensive, it only analyses a few hundred genes at a time and can pick up RNA carryover which may lead to false positives. Nevertheless, real-time PCR is emerging as one of the best techniques used for gene expression.

Global Scale Techniques

Microarrays

Microarrays are constructed by spotting cDNA or oligonucleotides of known gene transcripts in a gridded pattern on a glass slide or chip. There are two main microarray platforms: slide based arrays and oligonucleotide arrays (DNAChips). These two platforms are based on similar probe hybridization principles with the main difference being the number of samples that can tested in each experiment. Oligonucleotide arrays measure the activity of one sample in each experiment instead of two, as with the glass slide method. Oligonucleotide arrays also often utilize full genome chips, where transcripts from the entire genome of an organism are placed on the chips. This allows for the measurement of gene expression of all genes in one experiment. This is one of the advantages over the glass slide method, where the expression of only specific genes is measured. Oligonucleotide arrays being of a larger scale and more technologically advanced are significantly more expensive than the glass slide method. They also produce less error since only one sample is tested per experiment, preventing cross hybridization. [3]

Serial Analysis for Gene Expression (SAGE)

SAGE is a genome-wide technique that identifies and quantifies new gene transcripts, instead of relying on pre-existing information about a specific sequence.[4] SAGE measures a short nucleotide sequence (tag), of a particular transcript located on the 3' end of each cDNA. Since the number of short sequences is proportional to the amount of mRNA in the original sample, the quantification and identification of specific gene transcripts can be obtained with this technique.[4] SAGE can be very useful in the discovery of new biomarkers for skin disease and aging since it does not require prior sequencing knowledge of gene transcripts like other gene expression techniques. Although SAGE is a novel approach to understanding gene expression, the technique can be time consuming.

RNA-Sequencing (RNA-Seq)

Similarly to SAGE, RNA-Seq is a genome wide technique that generates the sequences for the RNA in a specific sample. The assay provides a sensitive, digital count of all expressed mRNA in the sample. Since the signals are based on DNA sequencing and not hybridization as with microarray, the false positives are few, making this method statistically better than most other large scale techniques.[5]As with microarrays, RNA-Seq produces extremely large data sets, requiring specialized expertise and complex, expensive software programs to analyze and interpret results.

Conclusions

The method of choice in conducting genomic experiments on skin tissue is based on individual study objectives and hypotheses. Certain techniques such as microarray may be best if the objective of the experiment is to get a global understanding of gene expression under specific conditions. On the other hand, if the objective is to validate specific expression of targeted genes, then a small scale method such as RT-PCR may be the method of choice. In addition, since proteins are the end products of genes and are responsible for biological functions in the cell, it may also be essential to conduct protein assays to validate the expression of specific genes.

Although this is an exciting time for cosmetic scientists and formulators, who are looking for new stories and validation techniques to test and substantiate topical applications, these techniques, as with all new approaches, should be studied and reviewed extensively before being established as an industry standard.

REFERENCES

1. Lewin, B. (2006). *Essential Genes*. Upper Saddle River, NJ: Pearson Education, Inc.
2. Alberts, B., et al. (2008). *Molecular Biology of the Cell, 5th ed.* (pp. 538-539). New York: Taylor & Francis Group.
3. Avison, B.M. (2007). *Measuring Gene Expression*. New York, NY: Taylor and Fransis Group.
4. Velculescu, E.V., Zhang, L., Vogelstein, B., Kinzler, W.K. (1995). Serial Analysis of Gene Expression. *Science,* 270, 484-487
5. Wang, W., Gerstein, M., Synder, M. (2009). RNA-Seq: a revolutionary tool for transcriptomics. *Nature Review Genetics,* 10(1), 57–63

Achillea Millefolium Extract: An Innovative Anti-Aging Neuro-Cosmetic Ingredient?

Sabine Pain, Ph.D., Cecile Altobelli, Lysiane Cittadini, Magali Favre-Mercuret, Boris Vogelgesang and Valerie Andre-Frei

BASF Beauty Care Solutions France SAS, 32 rue Saint-Jean-de-Dieu, 69366 Lyon Cedex 07, France
sabine.pain@basf.com

So far, neurocosmetics have primarily been linked to well-being and sensitive skin related claims. Although the skin is a major interface in sensorial perception, the relations between skin and nervous system appear to be much more complex. The role of neuropeptides notably in epidermal homeostasis is of great importance.

POMC is the acronym for pro-opiomelanocortin. POMC is a pro-peptide originally described in the pituitary that was later shown to be expressed in many organs, including the skin. After enzymatic cleavage, POMC pro-peptide gives rise to multiple neuro-peptides including ACTH and β-endorphin. These latter were shown to have significant implications in epidermal function: β-endorphin induces keratinocyte differentiation and epidermal thickening. ACTH stimulates keratinocyte proliferation.

Since POMC-derived peptides normally participate in the normal functioning of the epidermis, and since epidermal functioning is disturbed with aging, we studied the age related variations of POMC as well as the related-receptors in aged epidermis. We discovered that POMC significantly increased with aging whereas MC-2R and MOR-1, the respective receptors for ACTH and β-endorphin continuously decreased with a dramatic drop after the age of 50. This appears as the hallmark of an age-disturbed balance between neuropeptide (POMC) and receptors (MC-2R and MOR-1) that could have implications in age-related epidermal dysfunction.

Looking for a cosmetic ingredient that would boost expressions of MC-2R and MOR-1 in cultured normal human epidermal keratinocytes (NHEK) from old donors, we identified *Achillea millefolium extract* as the most potent one. Indeed, a water extract of *Achillea millefolium extract* at concentrations ranging from 0.1% to 2% dose-dependently increased the mRNA expression of MC-2R and MOR-1 in monolayer cultures of NHEK as measured *in vitro* using quantitative RT-PCR methods (Fig. 1).

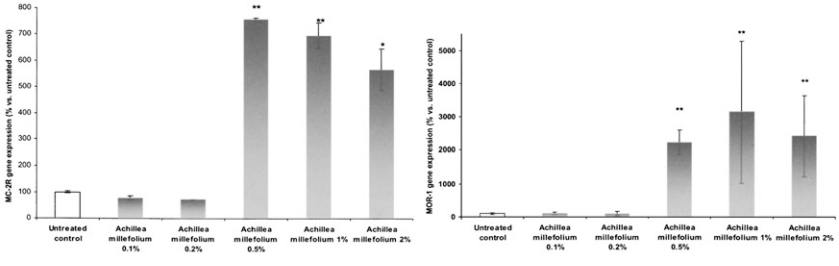

Figure 1: Dose-related effect of Achillea millefolium on MC-2R and MOR1 gene expression levels in cultured NHEK. *, **: *statistically significant vs. untreated control, $p<0.05$ and $p<0.01$*

Moreover, MOR-1 and MC-2R protein syntheses were significantly increased by x2.8 and x19 respectively vs. untreated control after a 48h incubation in the presence of *Achillea millefolium extract* at 0.5% as quantified using Western-blot methods (Fig. 2).

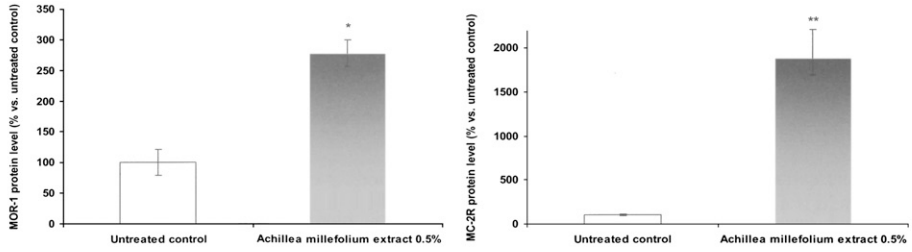

Figure 2: Effect of Achillea millefolium on MC-2R and MOR-1 proteins as measured using Western-blot in normal human keratinocytes. **, **: statistically significant vs. untreated control, $p<0.05$ and $p<0.01$ respectively*

The ability of a 72h hour incubation with *Achillea millefolium extract* at 0.5% to improve the expression pattern of epidermal differentiation markers was next assessed in full-thickness skin biopsies from a 60-year old donor. Transglutaminase-1, filaggrin and cytokeratin 10 expressions were thus measured using image analysis after specific immuno-staining. All three markers were significantly increased and/or their expression profile improved compared to untreated biopsies incubated in the culture medium alone (Fig. 3). Finally, as measured using automated image analysis, the epidermal thickness of biopsies was also significantly increased by 10% compared to untreated biopsies after 72h of incubation with *Achillea millefolium extract* at 0.5%.

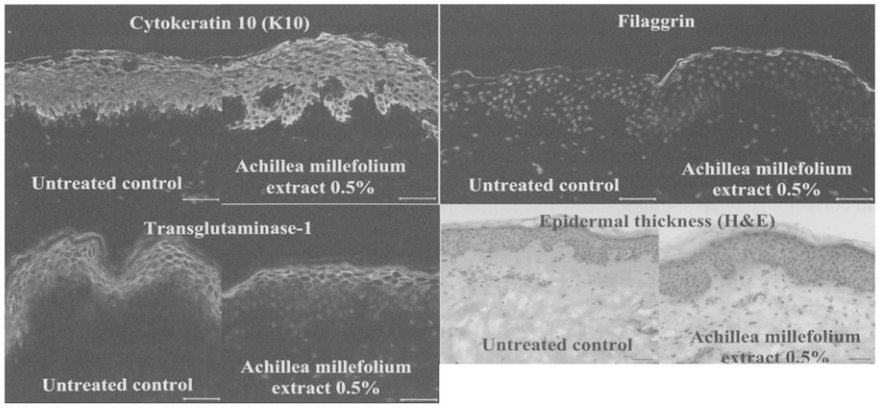

Figure 3: Microscopy observations of skin biopsies cultured with Achillea millefolium extract at 0.5%as compared to untreated biopsies. Scale bar: 50 µm.

The efficacy of a cosmetic formula containing 2% of *Achillea millefolium extract* to improve the appearance of aging skin was also evaluated in a double-blind placebo controlled split-face study and compared to that of glycolic acid used as reference re-surfacing anti-aging ingredient. As noted by the clinical expert who graded the appearance of wrinkles and pores, the formula containing Achillea millefolium extract, the appearance of wrinkles and pores was significantly reduced from 1 month of treatment and significantly reduced compared to placebo after 2 months, with results directionally better than those obtained with glycolic acid.

Conclusion

The reported results show that Achillea millefolium extract increases the expressions of MC-2R and MOR-1 in vitro in cultured keratinocytes, improves the epidermal differentiation pattern ex vivo and improves the appearance of two of the most visible signs of aging skin in vivo.

Comprehensive Exploration of Efficacy through Vector Analysis

Erica Babson, Barbara Brockway, Ph.D., Peter Dow and Durant Scholz

Active Concepts, LLC, Lincolnton, NC

DNA microarray technology has become an invaluable method for examining gene activity and cell function. It is a vital tool in many spheres, including academia, pharmacology and biotechnology. It has been especially useful in researching genetic diseases and has allowed for huge advances in cancer classification and customized treatment.[1]

DNA microarrays allow users to examine the activity and expression of thousands of genes at once. Activation of a gene prompts the cell to copy certain gene segments, resulting in mRNA, from which the body creates proteins. To perform a DNA microarray, the mRNA molecules are tagged with fluorescent dyes, then placed onto a slide. The mRNA binds to its complementary DNA and stamps its fluorescent marking. The array is scanned, and fluorescent markings are measured. A bright fluorescent tag symbolizes a very active gene; as brightness decreases, so does activity. No fluorescent tag means that the corresponding gene is inactive.[2]

These microarrays are now commonly utilized in the healthcare and pharmaceutical fields, especially in researching heart disease, infectious diseases, cancer, and mental illness.[1] They are steadily becoming more widely used in the cosmetic industry to indicate the specific pathways of action for certain ingredients. However, data analysis is still the major obstacle in making DNA microarrays a standard testing procedure for cosmetic and personal care products.

As a rule, traditional microarray analysis methods yield noisy results that are difficult to interpret and analyze. In general, only a few genes display significant expression changes, and patterns are difficult to identify.[3] The groundbreaking technology that DNA microarrays employ is less significant when users are unable to correctly analyze output and categorize important patterns.

Several methods have been developed to try to make the information more organized and easier to use, including self-organizing maps, hierarchical clustering, and principal components analysis.[4]

One of the most widely used methods involves KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways.[5] Microarray data can be evaluated by entering up- and down-regulated gene lists into the Internet software program DAVID (Database for Annotation, Visualization, and Integrated Discovery). The software identifies significant KEGG pathways that are affected by the experiment in question. While this method is extremely useful and has allowed users to make sense of gene lists that have no apparent meaning on their own, it does not allow for comparisons among different experimental factors.

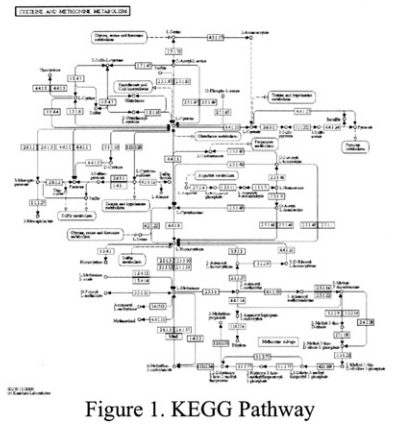

Figure 1. KEGG Pathway

The main benefit of using vector analysis techniques is that users can simultaneously compare how cells respond to different stimuli without having to reevaluate data for each experimental set. Data that is gathered from experiments using varied treatments, treatment times, and tissue types, among other parameters, can be assessed at the same time, which gives the user a clearer understanding of how responses vary based on variables involved. This is a clear improvement to previous analysis methods, which directly compare expression profiles of one set of regulated genes at a time. Using the gene response information, vectors are created as visual representations of changes in regulatory mechanisms.[6]

A vector is defined for each gene and mapped by assigning axes to different experimental variables and backgrounds. The vector's geometric coordinates represent the genes' expression values in each background. By using this method, all information about the gene in question is represented geometrically.[7]

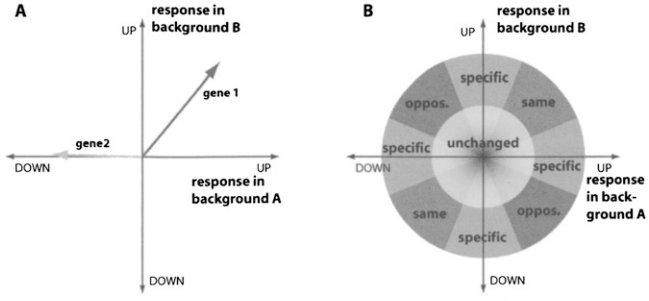

Figure 2. Principle of Vector Analysis.[6]

Vectors themselves are functions of length and direction. The vector's length represents the average strength of the response, and the direction is indicative of which typical behavior is the closest match to the particular gene's behavior. Calculating the angle between the vector and the several possible prototype vectors allows the analyst to assign a gene to a response prototype. The length of the sum vector tells how consistent the gene's behavior is with the given behavior type. Highly consistent gene behavior patterns result in a long sum vector, while more inconsistent patterns form shorter sum vectors. By looking at a vector analysis graph, one can easily tell if the observed gene behaviors were fairly steady or noisy. [6]

As DNA microarrays become a more common tool across various industries, data sets are growing larger and scientists' need for more efficient analysis methods are greater than ever. While KEGG pathways and other pattern identification techniques are useful for eliminating irrelevant data and supplying hypotheses for potential mechanisms of action, no current method compares to vector analysis in terms of scope and efficiency. Allowing users to compare and contrast gene responses among various experimental parameters opens up a new realm of possibility for the future of microarrays.

Works Cited

1. Morrow, John Jr., Ph.D. "Utilization of DNA Microarrays Increasing". *Genetic Engineering & Biotechnology News* 29.1 (*2009*).
2. "DNA Microarray Technology". *National Human Genome Research Institute*. *www.genome.gov.* 3 August 2010.
3: Agarwa, Pankaj and Tanner, Stephen W. "Gene Vector Analysis (Geneva): A unified method to detect differentially-regulated gene sets and similar microarray experiments." *BMC Bioinformatics* 9:348 *2008*).
4. Kuruvilla, FG, et. al. "Vector Algebra in the analysis of genome-wide expression data". *Genome Biology* 3(3): Research0011. (*2002*).
5. KEGG Pathway Database. *www.kegg.jp*. 2 August 2010.
6. Breitling, Rainer. Et al. "Vector analysis as a fast and easy method to compare gene expression responses between different environmental backgrounds". *BMC Bioinformatics* 6:181 (*2005*).
7. Quackenbush, J. "Computational Approaches to Analysis of DNA Microarray Data". *IMA Yearbook of Medical Informatics* (*2006*).

Hair Color Vibrance Factor: A New Parameter/Claim Combining Hair Shine and Color Strength

Timothy Gao, Ph.D., Sam Zhu, Ph.D. and Peter Landa

Croda, Inc., 300-A Columbus Circle, Edison, NJ 08837

INTRODUCTION

Hair luster (shine) is an important feature of hair appearance, and this attractive visual effect is a key consumer objective in the hair care market. Hair color strength is another important attribute for colored hair. Consumers always want their colored hair to be looked bright and striking, or Vibrant. But until now, neither a parameter, nor a test method, has been defined and developed to make quantified vibrancy claims. Lefaudeus and his colleagues have published principles for measuring hair luster (shine) using a SAMBA system (1-2). They have indicated that there are two bands on captured colored hair images: the "Shine" band (first reflection, no color) and the "Chroma" band (second reflection with color). They defined an "Overlapping Coefficient" to describe overlapping degrees between "Shine" and "Chroma". We observed that the overlapping coefficient increased after cosmetic treatments and that hair possessed correspondingly more shine and stronger color.

Based on these observations, we have defined a new parameter – Hair Color Vibrance Factor (HCVF) to describe simultaneously the comprehensive effects of hair Shine plus the overlapped portion of hair color strength (Chroma):

$$\mathbf{HCVF = L\ (1 + O_c) = L\ (shine\ factor) + L^*\ O_c\ (the\ factor\ of\ Chroma\ overlapped\ with\ shine)}$$

where, L is the shine index determined by SAMBA system using the BNT equation; $\mathbf{O_c}$ is the determined Overlapping Coefficient between hair shine and chroma. In our previous study (3), we have established experimental procedures and parameters to quantitatively determine hair shine by using SAMBA as the polarimetric imaging system. In this paper, we report our recent studies on Hair Color Vibrance Factor and its applications on colored hair.

EXPERIMENTAL

- ***Materials:*** Virgin dark brown, Oriental black, blond, white, and regular bleached hairs were purchased from International Hair Importers, Inc. (NY). Commercial Auburn hair dye from L'Oreal (Superior Preference) and deep red hair dye from Clairol (Herbal Essence) were used to dye the white hair.

A hair shine spray made from Croda Inc was used to treat these hair samples.

- ***Instruments:*** A SAMBA hair system from Bossa Nova Technologies, Venice, CA has been used for measurements of hair Luster (L_{BNT}), Chroma (C), and Overlapping Coefficient (O_c).

RESULTS AND DISCUSSION

1. Effect of Hair Color Shade on Shine, Overlapping Coefficient, and HCVF

Clean natural white, blonde, dark brown, black hair, bleached hair, and dyed auburn and deep red hair were tested W/O any cosmetic treatments. Their shine indexes (L), Overlapping Coefficients (O_c), and HCVF were determined or calculated. Data are summarized in Table 1.

Table 1 Value of L, O_c, and HCVF of Different Colored Hair

Hair Color	White	Blond	Bleached	Brown	Black	Auburn	Deep Red
L	1.69	8.45	6.46	31.5	34.6	7.68	13.4
O_c	99.0	80.8	86.1	77.8	89.5	70.9	60.2
HCVF	3.36	15.3	12.0	52.9	65.6	13.1	21.5

It can be seen:

- Hair shine value increases with hair color darkness. This may be attributed to the higher amount of light absorption and less scattering in dark colored hair and larger scattering and less reflection in light colored hair
- Dyed Auburn and Red colored hair showed relatively small shine and overlapping values compared to the natural brown and black hair due to the damage from coloring process.

- White Hair gave us the most unique results. It possessed the lowest shine value, but the highest overlapping coefficient. This can be attributed to the high amount of scattering light (on all wavelengths) and very broad bands of both shine and chroma; even the chroma value was very low.

2. The Effect of Hair Shine Spray Treatment on Shine, Overlapping Coefficient, and HCVF of Hair with Different Color Shades

A hair shine spray was applied to these hair samples. Percent changes in hair shine, overlapping coefficients, and HCVF before and after respective treatments are presented in Table 2. Typical hair images and light spectrum of auburn-colored hair are presented in Figure 1.

Table 2 Percentage Changes in L, O_c, and HCVF of Different Hair Color Shades after Spraying

Hair Color	White	Blonde	Bleached	Black	Auburn	Deep Red
ΔL, %	45.0	98.8	157	3.47	70.6	45.5
ΔOc, %	0.1	4.58	0	4.47	23.3	24.8
Δ HCVF, %	44.9	103	157	5.65	87.0	59.1

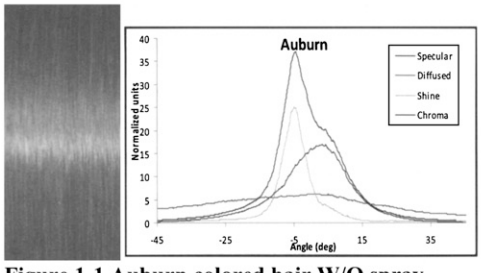

Figure 1-1 Auburn colored hair W/O spray

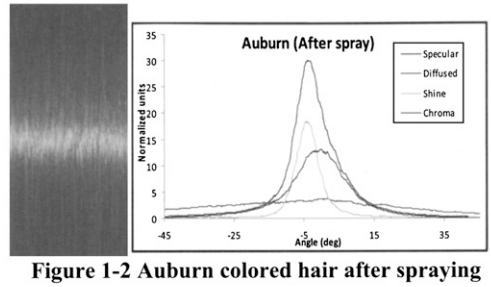

Figure 1-2 Auburn colored hair after spraying

It is observed:

- The hair shine spray greatly improved hair shine on blond and bleached hair, moderately enhancing hair shine for white and dyed hair, but had less effect on black hair.
- The hair shine spray especially showed great improvement in shine/chroma overlapping coefficient on dyed hair – Auburn and Deep Red, and made the colored hair more vibrant.

Internal subjective evaluations were conducted on auburn-dyed hair tresses with and W/O the spray treatment (blind samples). Results from subjective evaluations were in good agreement with those obtained from instrumental measurements: the auburn-colored hair samples with the treatment generated higher HCVF values and looked more vibrant – brighter and more striking color (Figure 1-2 compared to 1-1).

CONCLUSIONS

- A new parameter of Hair Color Vibrance Factor has been established and defined, combining hair shine with color intensity (Chroma) together to make a new claim for colored hair
- A new test technology has been developed and validated to determine HCVF of hair. It is convenient to use only one SAMBA system to obtain all three measurements simultaneously – Hair Shine Index, Chroma Maximum, and Overlapping Coefficient.
- Subjective evaluations showed good agreement with objective measurements.
- HCVF can be used to make a new claim – the higher the HCVF value, the more vibrant the hair color looks.

References

1. N. Lefaudeux, N. Lechocinski, O. Clemenceau, and S. Breugnot, "New luster formula for the characterization of hair tresses using polarization imaging", *J.Cosm. Sci,* 60(2), 153 – 169, 2009
2. SAMBA Hair System Manual, Bossa Nova Technologies, 2008
3. T. Gao, A. Pereira, and S. Zhu, "Study of hair shine and hair surface smoothness", *J.Cosm. Sc,* 60(2), 187 – 197, 2009

Penetration and Substantivity of Materials in 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, oils and even proteins. However, due to their size, the activity of such materials is likely restricted to the hair surface - or at least the very outer regions – as diffusion rates would be expected to be extremely slow. In order for a material to significantly alter the bulk properties, one would presume that penetration into the hair should readily occur – and preferably with some retention. However, surprisingly, the scientific literature in this area is rather sparse.

Undoubtedly, the molecule which has the biggest effect on the properties of hair is water. The properties of wet and dry hair are clearly very different; while intermittent states relating to different relative humidity also produce very noticeable changes. There is already a sizable literature relating to the penetration and interactions of water and hair, and this existing knowledge provides a spring-board for studies involving the penetration and adsorption of other small molecules.

This work has involved performing adsorption experiments on hair using a variety of volatile materials. These include commonly-used formulation ingredients (solvents, oils, fragrance) and also some model compounds intended to yield fundamental learning. Results involving both the amount and the rate of adsorption lead to contemplation of potential adsorption pathways and adsorption sites. Furthermore, as understanding develops, it becomes possible to postulate and pursue pathways for specifically tailoring the bulk properties of hair.

Methodology

Adsorption isotherms for volatile materials were generated by a gravimetric approach involving commercially-available Dynamic Vapor Sorption (DVS) equipment from Surface Measurement Systems. Additional complimentary mechanistic information was obtained from swelling experiments (performed using a laser micrometer) and from conventional Dia-stron tensile testing.

Results:

While water readily penetrates into hair quite quickly, Figure 1 shows how methanol and ethanol adsorption occurs considerably more slowly. This may be somewhat unexpected, given their small size and hydrogen bonding capability – which subsequently leads to questions involving any penetrating ability of substantially larger molecules. Indeed, given the very different adsorption behavior, one may speculate that these molecules access different locations within the hair structure. Additional mechanical testing studies suggest that ethanol adsorption does indeed provide a plasticization of fibers in a manner similar to water - but again, prolonged exposure times are necessary to produce these differences in hair properties.

Figure 2 shows adsorption data for cyclomethicone on bleached hair. Despite the larger size of this molecule, results suggest relatively rapid initial adsorption (taken to be surface adsorption), followed by a gradual further increase in the amount adsorbed (taken to represent slow penetration into the bulk). Moreover, this process is seen to reverse when the atmosphere is clear of the adsorbing species. Similar

behavior is being observed with a variety of larger, predominantly non-polar molecules - including typical fragrance components.

Data has also been generated to suggest how adsorption of certain foreign species appears to "cap" water adsorption sites within the hair and significantly reduce the water adsorbing capability. Figure 3 shows adsorption and desorption isotherms for hair that was soaked in a 5% resorcinol solution for 1 hour at room temperature. Results suggest an approximate 25% reduction in water content at elevated humidity. However, it is again noted that some time is required for these molecules to penetrate from aqueous solutions

Conclusion:

Studies are on-going in an attempt to better understand the penetration of small molecules into hair and their subsequent affect on hair properties. Evidence suggests penetration from a variety of molecules; but in most instances substantial exposure times appear to be necessary to induce significant effects.

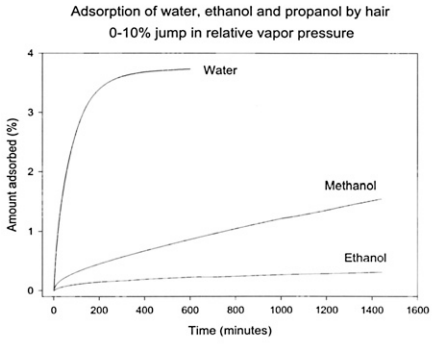

Figure 1

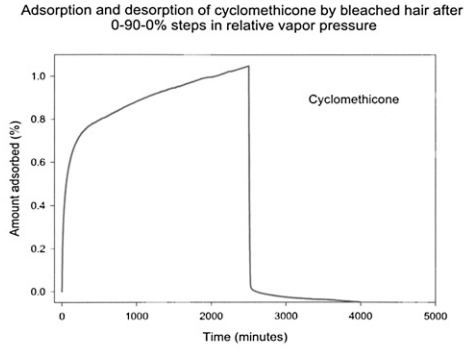

Figure 2

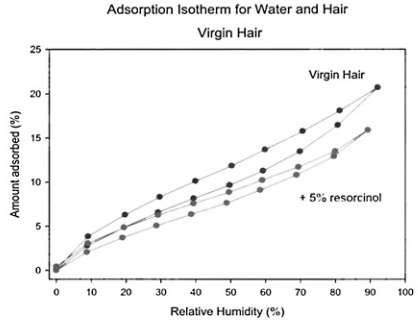

Figure 3

Hair Health: Effect of Diameter, Density and Age

Thomas L. Dawson, Ph.D., R. Scott Youngquist, Brian Fisher and Clarence Robbins, Ph.D.

Procter & Gamble

It is well known that women lose satisfaction with hair quality as they age. For centuries, humans have fought this decline in hair health and appearance through changes in habits and practices such as teasing, dying, permanent waves and straightening.

While several small studies implicate multiple scalp hair parameters with advancing age, including decreased shaft diameter and terminal hair density, few comprehensive data sets exist. The paucity of scalp related hair data is the result of the extreme cost, vast number of hairs, enormous variability between individual hairs and individuals, impact of life and environmental factors, and lack of robust, reproducible methods.

This presentation will summarize changes in hair diameter and density across several recent studies with current methods. In the reviewed studies, diameter and density were measured among over 1000 normal (without diagnosed hair loss) women ages 18-65, with or without self-perceived thinning. The role of menopause was also investigated.

It was found that hair density decreased with age after the twenties, with increasing rate after age 40. Hair diameter increased until the 4th decade, then dropped. Changes in hair density were similar across the scalp, while the changes in diameter were accentuated on frontal scalp versus occipital scalp. Hair shaft diameter was more influenced by menopause than density.

In conclusion, multiple factors contribute to the drop in satisfaction with hair quality associated with intrinsic aging. While decreasing number density is an obvious and important factor, decreasing hair diameter is also a key contributor. To fully address aging related concerns with hair, products must address multiple issues.

Innovations in Hair Styling

Colleen Rocafort

BASF Corporation, Florham Park, NJ

One of the latest trends in the hair styling product category is the importance of multi-functional products. The Hair Styling sub-category, in fact the whole Hair Products market, is becoming increasingly segmented with an array of choices and claims which go above and beyond simply holding a hair style in place. While products which provide several benefits are appealing on an economical, added value and convenient level, companies need to ensure that their products deliver what they claim.

Hair Styling products are offering more than just keeping a style in place; such as moisturizing, conditioning, adding shine, protecting from UV rays and the environment. All natural, organic and no additives/preservative type products are also being seen on the Market shelves. Long-lasting hold remains the top claim. Its dominance is no surprise given that most consumers want to maintain their hair styles for as long as possible throughout the day or night, without having to re-apply gel, hairspray, wax etc. The use of Hair Styling products is often reliant on what hair style the individual is sporting, not to mention their gender and age. Fashion is also influential.[1]

Typically the polymer or resin provides many of the attributes during the styling process, as well as the holding and texture characteristics. However the total formulation determines the functionality of the styling product such as ease of application, distribution of polymer, absence from flaking, shine, longevity of hold, restylability, and removability. The final hair style and styling technique will also determine the type and level of ingredients used in the styling products you develop.

During the wet phase, the hair is softened and susceptible to stretching and abrasion damage. Therefore, protection during the styling process is another key need that the styling product must offer. Added slip, detangling, or reduced friction between the hair and the styling devices during the entire process from very wet to the drying stage. Thermal damage has been quantified, and subsequently protection from thermal damage has been attributed to some polymers and conditioning agents. Styles can be retouched and refreshed throughout the day or daily between shampoos using thermal styling implements (curling irons/flat irons). Certain subsections of the hair may be curled repeatedly thus creating damage at the ends.

This paper will review the global market hair styling trends, the new hair styling products introduced into the marketplace to address these trends, and the new innovations in Hair Styling which have been developed to address these trends.

[1] Mintel "Hair Styling Trends" Report, July 2010