J. Cosmet. Sci., **61,** 381–420 (September/October 2010)

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

June 3–4, 2010
Long Island Marriott
Uniondale, New york

HENRY MASO KEYNOTE AWARD LECTURE SPONSORED BY THE HENRY MASO FAMILY AND SILTECH, LLC WHY GREEN? WHY NOW?

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The environment within which we operate has become more complicated each year. The formulating chemist and supplier have to consider a myriad of issues in order to get a product to market and still make a profit. For the supply chain, there is greater accountability than ever before. For the downstream user/finished goods manufacturer the spotlight is white, hot and relentless.

Central to the modern industry are the concepts of "greenness" and sustainability. These are no longer options that are "nice to have" or "important for marketing". These represent a tough, hard minded approach to formulation and production that requires considerable innovation, precision in design, and discipline in execution.

In this industry, suppliers and producers have always enjoyed close working relationships.
Now, we find these moving to even a higher level of coordination and information exchange. This is necessary in order to meet the needs of business sustainability.

The common sustainability/green program has four key pillars. These are centered on: human health, environmental impact, social responsibility and business optimization. Each pillar stands as an opportunity to optimize business gain and consumer acceptance. Effective sustainability programs are designed to optimize all four pillars to the greatest common denominator. Only then can the business hope to establish long term and lasting benefit.

Make no mistake about it, Darwin is at work.

MEASURING SUSTAINABILITY AND INTEGRATION INTO STRATEGIC DECISION MAKING

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Introduction:
Companies that have been able to effectively integrate sustainability into their organizations and more specifically their strategic decision making processes have been able create both distinct competitive advantage and long term business success. By prioritizing sustainability as a key focus and strategic guideline and managing it similar to other parts of their businesses, leading companies have been able to better identify and manage risks, enhance brand value and their corporate reputation, and more importantly facilitate clear, measurable value creation throughout their supply chain. The challenges facing today's companies are how to consistently and comprehensively define sustainability, how to develop the expertise and tools to measure sustainability throughout their value chain and finally how to develop the means to effectively communicate sustainability to their various stakeholders and in the markets they do business.

Methodology:
The purpose of the eco-efficiency analysis is to harmonize economy and ecology with the aim to spur product innovations in chemistry and to ensure sustainable development. The eco-efficiency analysis provides information about the relationship or balance between the economic benefits of a product and its impact on the environment. BASF has used the eco-efficiency analysis as a strategic tool since 1996, completing over 400 studies for a diverse range of products including chemical intermediates, consumer and personal care products, vitamins, packaging materials, adhesives and renewable based products. Not only does the analysis provide the necessary data to support strategic decisions related to new product development but it also provides our customers with a comprehensive comparison of products or processes that is both science based and comprehensive but presented in a way that is clear, easily understood and facilitates informed decision making about the sustainable attributes of products. The analysis has helped to increase the market success of products by clear and transparent differentiation from competing technologies.

The eco-efficiency analysis assesses the life cycle impacts of a product or manufacturing process from the "cradle to the grave" with equal importance to environmental and economic impacts. As a life cycle approach, the unit of comparison for the assessment is defined as the customer benefit and includes not only the impacts of the starting and intermediate raw materials but also takes into consideration the consumption behavior of the end consumers during the use phase of the product as well as the various end of life options such as recycling and various other disposal options. More specifically, the environmental impacts are measured for the alternatives being compared in six main categories as reflected in Figure 1.

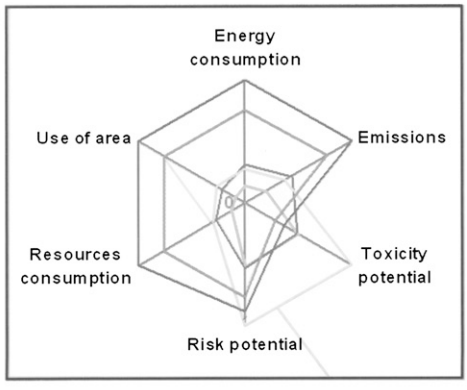

Figure 1: Environmental Fingerprint

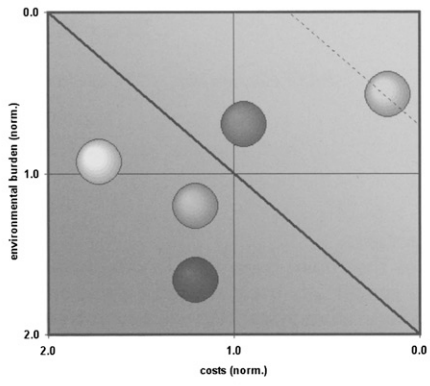

Figure 2: Eco-efficiency portfolio

Energy consumption includes both the cumulative energy utilized over the life cycle as well as the energy content remaining in the product. Resource consumption applies weightings to the basic raw materials consumed over the life cycle based on their current demand and exploitable reserves. This allows a higher weighting to be applied to materials that are either scarce or have a very high consumption rate. The emissions category is further sub-divided into emissions to air, water and solid waste with assessments that consider both the amount and potency of the specific emission. The risk category looks mainly at the physical hazards related to the products as well as the frequency of occupational illnesses, accidents and diseases. The toxicity potential category looks at the life cycle human health impact of all the materials utilized during the production, use and disposal phases. Finally, how our eco-systems have been impacted or impaired through achieving the defined unit of measure, or customer benefit, is measured in the land use category.

Economic data are also compiled for each alternative for their respective life cycles. All the various costs incurred in the manufacturing, use or disposal of the product are included in the calculation. Through the use of environmental relevance and social weighting factors, the six environmental impacts for each alternative are combined into a single environmental score. This allows for a clear understanding of the relative overall environmental impact of each alternative considered. Likewise, single life-cycle costs for each alternative are developed. These environmental and economic scores are plotted on a biaxial graph for each alternative (Figure 2). The costs are shown on the horizontal axis and the environmental impact is shown on the vertical axis. The graph reveals the eco-efficiency of a product or process compared to other products or processes. As both environmental impact and costs are equally important, the most eco-efficient alternative is the one with the largest perpendicular distance above the diagonal line in the direction of the upper right hand quadrant. Less eco-efficient alternatives are located in the lower left hand section and reflect an area of higher environmental burden and higher life cycle costs.

Case Study:

A relevant topic for many of us today is, "Are bio-based materials more sustainable than traditional petroleum based products?" A recent eco-efficiency analysis compared a conventional, petroleum based polyol production process vs. polyol manufacturing processes utilizing renewable or natural oils such as soy and castor. Figures 3 and 4 depict the environmental fingerprint and final eco-efficiency portfolio.

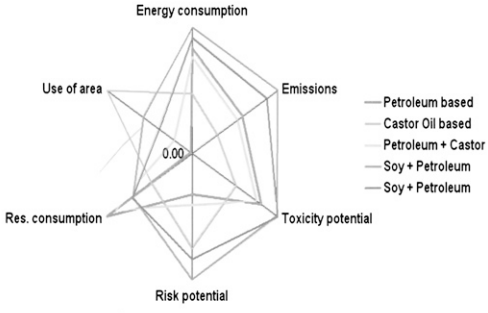

Figure 3: Environmental Fingerprint

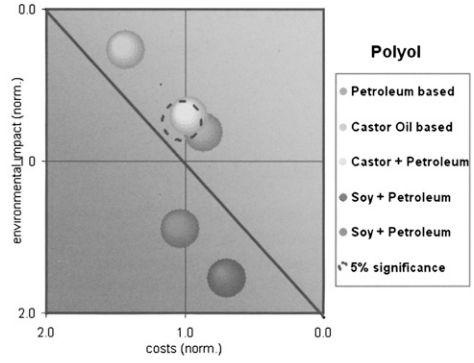

Figure 4: Eco-efficiency portfolio

As depicted in Figure 4, the petroleum and castor oil based alternatives are the most eco-efficient alternatives for this analysis. The castor oil alternative clearly has the lowest environmental impact and benefits from its low overall emissions score and low use of energy and resources. However, its environmental benefits are off-set by its higher life cycle cost. The petroleum based alternative balances both environmental impact with low life cycle costs. The soy based alternatives are the least eco-efficient due to their significantly higher environmental impact. This analysis demonstrates that renewable based products may not always be the more sustainable product or even have the lowest environmental impact when all the costs and environmental burdens over the life cycle are considered. The eco-efficiency analysis enables a rigorous scientific approach to assessing the relative sustainability of products or processes and presents the results in a way that facilitates expedient review and decision making at all business levels.

Conclusions:

The eco-efficiency analysis tool facilitates strategic decision making along the entire value chain. It enables companies to drive innovative product development focused on bringing more sustainable products to the market place. Though a very complex and comprehensive tool, the eco-efficiency analysis can help customers clearly understand the trade-offs between the environmental and cost impacts of different products or processes over their life cycle.

BEYOND SUSTAINABILITY: A PIONEERING TRIPARTITE APPROACH TO CORPORATE SOCIAL RESPONSIBILITY

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Cognis France (Division Laboratoires Serobiologiques)

Situation and aim of the project

Sustainable sourcing of new raw materials.

Global specialty chemicals supplier Cognis and its active ingredients business Laboratoires Sérobiologiques (LS) are continuously looking for new plants which can be used in the development of innovative actives for the cosmetic industry. In 1998, this search led LS to Morocco, where we began to study the local ecosystem, and in particular the Argan tree.

The Argan tree (Argania spinosa (L.) Skeels) is indigenous to southern Morocco. It is largely found in the south-east of Essaouira on the Souss plain. The Argan forest extends over approximately 800,000 hectares and contains more than 20 million trees. It acts as a natural barrier against the advance of the neighboring Sahara desert. The fruit of the tree contains a very hard shell holding between one and three kernels, and a valuable oil can be extracted from these.

The Argan forest is extremely important socio-economically, as it supports over three million people (out of a total Moroccan population of just over 30 million). However, unfortunately it is in decline, as a result of changes to rural lifestyles. Over-exploitation, soil erosion and desertification have all played a part, and as a result, Unesco classified the Argan forest as a Biosphere Reserve (RBA) in 1998.

To take into consideration traditional know-how and local expertise, a partnership was set up between LS and Zoubida Charrouf, a Professor at Rabat's Mohamed V University and the co-founder of the Targanine cooperative network. The long-term strategy was to combine scientific innovation, commercial development of the Argan supply chain in Morocco and specific sustainability principles. Key elements included sustainable diversification of local revenues, the protection of the Argan forest, and the support of local cooperative members.

This program resulted in three cosmetic actives developed by LS based on argan oil-cakes and argan leaves, including the cosmetic grade Argan oil which is certified 100% organic by Ecocert*, and labeled Ecocert Faire Trade (ESR)**.

(*)Raw material certified by Ecocert France according to the Ecocert Standard for Natural and Organic Cosmetics available at http://cosmetics.ecocert.com: 100% of the total ingredients are from Organic Farming.

(**)Raw material controlled by ECOCERT SA according to the Ecocert Fair Trade standard (ESR/EFT) available at www.ecocert.com

LS supplies oil and oil-cakes itself through an approved network of cooperatives that respect our sustainability strategy, and employ a mainly female workforce, thereby improving the integration of women in the Moroccan society.

A step further in the sustainability of the supply chain.

However, LS was committed to making the supply chain for its Argan products even more sustainable and equitable, exceeding the requirements of some organic certifications and fair trade labels. To this end, it implemented a comprehensive Corporate Social Responsibility (CSR) program, in collaboration with L'Oréal and Yamana, a NGO chosen as a partner in this innovative tripartite approach because of its unique position compared to most certifying bodies.

An 18-month long program was initiated in June 2008.The aims of this was to assess the strengths and weaknesses of the current processes in the supply chain, and to propose relevant and realistic improvement measures in the following areas: fair return, traceability, respect for traditional knowledge related to local biodiversity and local empowerment.

Measures taken to improve the situation

Fair return.

Fair price**:** LS's policy for setting the prices paid to cooperatives is guided by the sharing of monetary benefits. LS pays local producers directly, without intermediaries, at a non-negotiated price equal to or greater than the market price. This payment policy follows the fair trade guidelines, and incorporates payment in advance, a long-term contract, and a commitment to buy specified volumes.

Non-monetary benefits: in addition to the literacy classes and professional training, educational programs relating to environmental protection have also been introduced by local and international stakeholders. As a result, network members understand the importance of the Argan tree better, and are more involved in its conservation.

We and our partners are also involved in a pilot program that aims to set up and manage a social fund. This fund could be used to support social, medical or cultural programs that aim to improve living conditions for cooperative members. Increased financial autonomy and decision-making power for female workers has helped to raise the social status of cooperatives women.

Traceability.

A traceability guide has been written and translated into Arabic. Full traceability, from fruit origin to delivered product, is now being implemented in all cooperatives.

Protection of the biodiversity

To ensure the conservation of biodiversity, the following measures have been taken**:** strict guidelines are followed during the harvesting and exploitation of natural Argan resources. The leaves are collected on Argan parcels, which are managed according to an agreement with the Moroccan Water and Forests Authorities.

The supply chain safeguards that the Argan products are cultivated and harvested without using any chemical treatments, pesticides or fertilizers. Argan oil and Argan oil-cakes have been certified by Ecocert as 100% coming from organic farming.

Protection of traditional knowledge.

LS recognized its local partner as co-inventor in the patent applications for new uses that were identified from Argan fractions previously non-exploited in cosmetic applications - namely the protein-rich extract of the oil-cake, and flavonoid-rich extract of the leaves- , making sure that traditional knowledge and geographical origin would be documented and respected. Yamana was given the task of checking the impact of patents on the economic activity and general functioning of the sector, and on local people's perceptions of the patents' influence on their monetary and non-monetary returns. Interviews conducted locally did not identify any negative perceptions about LS's patents.

Local empowerment.

We and our partners were involved in initiating a Policies and Procedures manual for the cooperatives, translated in Arabic language. The manual sets clear rules for members, similar to a CSR code of conduct. This increases the cooperatives' autonomy and sense of social responsibility. It covers various aspects including: a democratic governance approach, transparency and equitability between cooperatives; monitoring of benefit allocation; supply conditions; quality guidelines; and social and non-monetary benefits.

Quality, safety and hygiene guidelines have been implemented in order to improve working conditions in the cooperatives. As a result, local people now better understand the importance of safety and hygiene practices, and have incorporated these into their daily work. LS also provides technical assistance on a regular basis. This empowers local workers in the areas of production, planning and quality assurance. The cooperatives also share best practices and learnings among themselves. This ensures continuous improvement.

Finally, Yamana has found that the cooperatives network is increasingly autonomous when it comes to economic development and quality assurance compared to the Argan sector as a whole. It believes that within a short period of time, the network will be capable of managing itself without external support.

IMPROVED SENSORIAL PERFORMANCE IN NATURAL FORMULATIONS USING POLYGLYCEROL MODIFIED PLANT WAXES

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Introduction

Natural formulators have available an increasing number of cosmetic ingredients that are derived from plant sources. In particular, liquid emollients obtained by esterification of natural fatty acids and alcohols have become almost common. However, the offer in truly moisturizing and texturizing emollients is still limited.

Plant waxes provide an interesting source of innovation for cosmetics. Abundant in nature, they play an essential role in preventing plants from dehydration and mechanical damage in frequently hostile climates. Such waxes can be found in many food products due to an excellent safety profile and are also useful in decorative cosmetics such as lipsticks. The properties required in the latter field, high melting point and mechanical strength, make the same ingredients rather incompatible with skincare products, in particular emulsions. Indeed, these hydrophobic and high melting point ingredients, in addition to being a challenge to solubilise, tend to recrystallize in emulsions causing instability and phase separation. The feel upon the skin is also unacceptably rough. Yet, the potential barrier properties of waxes in skincare have stimulated researchers to seek a solution. In this study, liquid and solid plant waxes have been reacted with vegetable polyglycerol to overcome the formulation drawbacks and allow the realization of new and more efficient textures.

Green chemistry

A selection of plant waxes have been chosen according to origin, composition, sensorial potential and commercial availability. Waxes issued from beeswax, sunflower seed, candelilla (CITES approved), mimosa flower, rice bran and jojoba plant were studied. Jojoba wax is liquid, due to the presence of unsaturated fatty acids and alcohols. Rice bran and sunflower, both hard waxes, are rich in mono-esters of long chain saturated fatty acids and alcohols and are excellent for building viscosity,. Mimosa and candelilla waxes combine esters and inert hydrocarbon chains that reinforce the barrier properties.

In a process that follows the principles of green chemistry, a blend of liquid and solid waxes was reacted, in absence of solvent, with vegetable polyglycerol. The reaction is based on alcoholysis, the replacement of an alcohol group by another. In the process , the liberated fatty alcohols are soluble in the medium so all atoms are conserved. Some hydroxyl groups of the polyglycerol are esterified with fatty acids from the plant waxes to create a number of new polyglycerol-3 wax esters. Exchange also occurs between the different fatty acids and alcohols of the original wax esters by transesterification, creating new esters with intermediate properties and softer feel. The reactions are equilibrium balanced, one-step and no bleaching or steam stripping is necessary on the resulting product.

Physicochemical properties

Gas chromatography analysis of the polyglycerol-wax blend shows that a number of new structures are obtained, each a combination of the original esters. Mixed wax esters that combine a fatty acid or alcohol of jojoba with those of sunflower or mimosa present semi-solid physical properties that are of strong texture interest. Esters of polyglycerol are also obtained, these being easy to solubilise and demonstrating amphiphilic properties. The melting profile of the blend is also significantly changed as shown in figure 1.

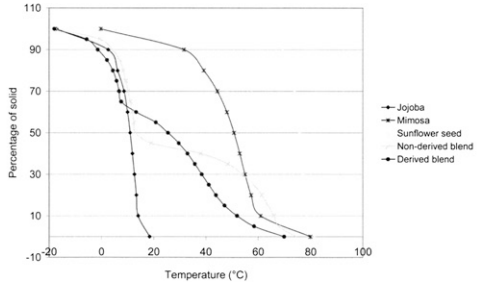

Figure 1: Graph showing the amount of solid compounds present in individual plant waxes and the wax blend before and after reaction with vegetable polyglycerol

The difficult-to-solubilise, high melting point entities have been reduced though combination with both polyglycerol and the liquid jojoba wax.
The derived wax blend melts more gradually and at around 55°C, the compound is largely fully melted.

The polyglycerol derived waxes are softer and easier to solubilise in oil phases in particular in vegetable oils. The crystallization of the modified waxes has a lower thickening effect than the original waxes and the crystals are smaller, more rounded and appear to be more compatible with emulsions.

Formulation

The polyglyceryl-3 wax esters bring an amphiphilic nature to the plant waxes. In presence of water, the wax blend swells, imprisoning the same water in a gel. In oil in water emulsions, the polyglyceryl-3 modified waxes no longer show the recrystallization issue of the native waxes. On the contrary, they improve compatibility between the oil and water phases and improve stability. The skin feel of emulsions is also improved. Results show that the feeling of softness is durably increased and that the formulations become more comfortable with an increased perception of moisturization.

Added to an oil phase, in absence of an emulsifier, the polyglyceryl-3 wax esters enable the introduction and stabilization of a water phase. A fine and homogeneous dispersion of water in lipstick formulations has been demonstrated. This is of interest in traditionally anhydrous color cosmetics such as lipsticks where hydrophilic actives such as Vitamin C can now be added. In other formulations, the wax complex allows the creation of stable innovative textures that resemble easy-spread butter.

Clinical efficacy

The influence of a polyglyceryl-3 modified blend of Acacia Decurrens (mimosa), jojoba and sunflower seed waxes on skin moisturization was carried out on a panel of 25 volunteers over 4 weeks[1] in comparison with a positive reference, lanolin. Corneometry shows a moisture increase of 23% and TEWL demonstrates that a two-fold improvement in water retention is obtained. The efficacy is at minimum equivalent to that of lanolin. As the sensorial properties are shown to be superior to those of lanolin, this new ingredient has an advantage for treating dry and dehydrated skins.

A study using microscopy of the skin's microtopography shows that the polyglyceryl wax esters improve the organization of the skin's surface (figure 2).

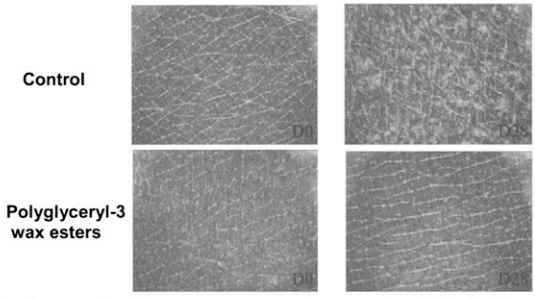

Figure 2: Microscopy photographs of the skin microtopography before and after treatment (28 days) with a formulation 5% polyglyceryl-3 wax esters

The furrows of the skin are well orientated, without appearance of any scales. This indicates improved moisturization. The microtopography of the skin is even slightly improved compared with lanolin.

Conclusion

The grafting of vegetable polyglycerol with a combination of solid and liquid plant waxes enables the preparation of new materials with highly interesting physical and clinical properties. The formulation difficulties of waxes in cosmetics, which include poor solubility and recrystallization in emulsion, are overcome. The polyglycerol modified waxes present excellent texturizing properties, bringing softness and comfort to skincare formulations. The amphiphilic nature of the new wax esters can stabilize oil-in-water emulsions and even emulsify a water phase in a water-in-oil emulsion. They can also boost moisturization of skincare formulations and improve skin microtopography.

References

1. Improving Skin Moisturization with Polyglycerol-derived plant waxes, P. Lennon & J-D. Rodier. Cosmetic& Toiletries, January 2010, volume 125.

PROCESS INNOVATIONS FOR SUSTAINABLE BIOCATALYTIC ESTERIFICATION

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Biocatalysis has gained much attention over the last years as it is generally regarded as a technology that yields high quality products such as emollient esters[1]. Furthermore, due to the intrinsic selectivity of the used enzymes and the low reaction temperatures necessary to achieve catalyst activation biocatalysis is usually considered to be an environmentally friendly process. However, the latter attribute is much dependent on the detailed reaction conditions and needs to be proven for the specific classes of reactions and products where this technology is used for.

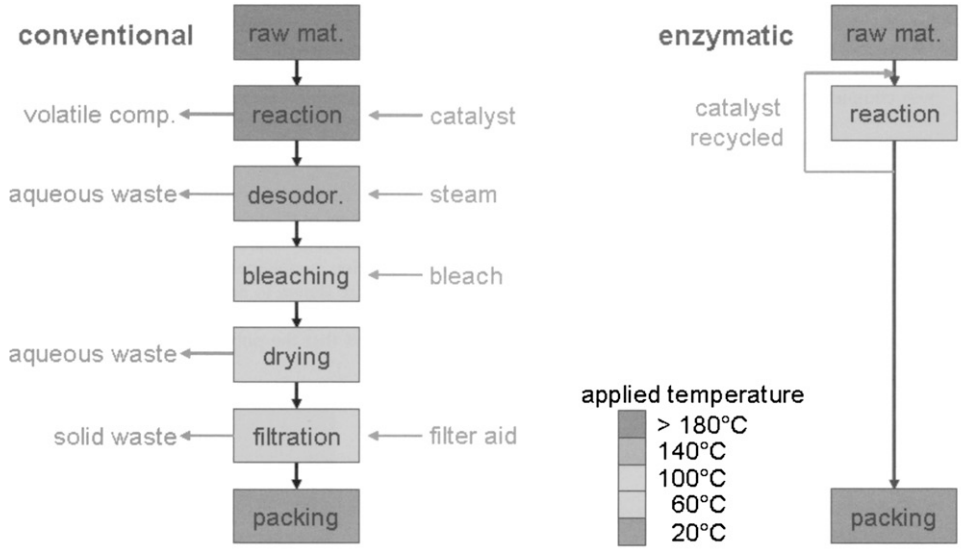

Figure 1: Comparison of the conventional (left) and enzymatic production process for fatty acid esterification

For the very first time the environmental benefits of enzymatic production of cosmetic esters compared to conventional processes (cf. Figure 1) have been proven and quantified by performing an environmental Life Cycle Assessment (LCA) according to ISO14040. The production of the frequently used emollient ester myristyl myristate served as a model reaction, but the results can be transferred to any other kind of ester-based product.

In the assessment, immobilized lipase B from the organism *Candida antarctica* was used to examine the industrial enzymatic process, including recovery of the enzyme, all the way to its deactivation. It was compared with the conventional chemical production process, which is carried out at elevated temperatures and uses tin oxalate as catalysts. Other parameters for this variant included the use of nitrogen as inert gas, and a refinement process consisting of bleaching, steam stripping, and filtration.

Individual Life Cycle Assessments of all the starting materials were used and the impact of all other consumables (energy, electricity, etc) was calculated. Afterwards both processes were evaluated on five standardized environmental categories: energy consumption, influence on global warming using greenhouse gas emissions, acidification of soil through noxious gases such as SO_2, the eutrophication of soil and water through the emission of nutrients such as phosphorous and nitrogen, as well as smog formation through volatile organic compounds.

The results speak loud and clear: Despite conservative assumptions, the biocatalytic manufacturing process for the emollient ester myristyl myristate can, on balance, save more than 60 percent energy while reducing the formation of environmentally damaging impurities by as much as 88 percent (cf. Figure 2). All these facts clearly support the sustainability of the biocatalytic process.

Results of the life cycle assessment 5 ton scale				
		Conventional	Enzymatic	Savings %
Energy	GJ	22.5	8.63	62
Global warming	kg CO_2 eq.	1,518	582	62
Acidification	kg SO_2 eq.	10.58	1.31	88
Nutrient enrichment	kg PO_4 eq.	0.86	0.24	74
Smog formation	kg C_2H_4 eq.	0.49	0.12	76

Figure 2: Results of the Life Cycle Assessment

This technology platform is currently used for the production of a whole family of high quality emollient esters on a scale of hundreds of tons:

- **Myristyl Myristate**, a cosmetic wax that imparts body to cosmetic formulations and leaves a pleasant soft skin feel
- **Decyl Cocoate**, a unique cosmetic oil with low viscosity and low spreadability
- **Cetyl Ricinoleate**, a cosmetic wax ester which gives a silky, but non-oily skin feel and that melts at skin temperature
- **Isocetyl Palmitate**, a cosmetic ester for dry skin application

Most recently, a new enzymatic product has been launched:

- **Oleyl Erucate**, a vegetable based emollient having a structure and properties similar to Jojoba Oil but showing significant better quality with regards to color and odor.

However, current process technologies and commercially available biocatalysts limit the broader application of this process: The mechanical instability of the enzyme carriers used forces the use of fixed bed reactors, thus limiting it to low viscous products, such as emollients. Furthermore, surfactant-like products cause desorption of the non-covalently bound enzymes, thus causing product contamination with enzymes as well as insufficient enzyme recyclability. To expand this technology platform to ester based surfactants these drawbacks of the current state-of-the-art had to be overcome:

First of all a universal and highly efficient bubble column reactor for enzymatic esterifications has been developed that allows the processing of raw materials with a broad viscosity range while reducing mechanical stress on the enzyme. To further improve the mechanical long-term stability of the used enzymes and to allow the use in surfactant-like systems an innovative coating technique has been developed that shows outstanding stability of the obtained enzyme preparations.

In conclusion, we have been able to show the ecoefficiency of biocatalytic esterifications and the sustainability of enzymatic emollients. By the combination of new process innovations this technology is now able to produce novel cosmetic ingredients, such as polyglycerol esters, in an economically feasible way, thus meeting the demand for new “green” cosmetic ingredients.

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[1] O. Thum, *Tenside Surf. Det.* **41**, 287-290 (*2004*)

NEW PRODUCT DESIGN STRATEGIES FOR COLORED HAIR

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Background:

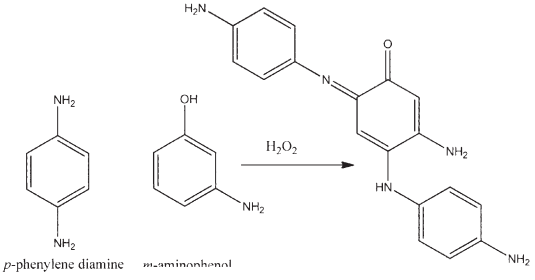
In the US over 60% of women regularly color their hair and the majority of these women use level 3 permanent hair colorants. These products enable these women to either change their natural hair color, for example to go to a lighter blonde or a vibrant red, or to maintain their natural color and cover gray. However, there are trade-offs that are made if the products are used on a regular basis. One of these trade-offs is the changes to the hair quality that the consumer notices over time, for example, a poor feel when the hair is both wet and dry, a decreased resilience to physical processes such as combing and blow drying and reduced shine. This talk will outline the changes that occur to the hair structure during coloring and the chemistry that is causing these changes. We propose that colored hair needs to be considered a different substrate to uncolored hair and thus we need to develop different product design strategies for this segment of consumers. This talk will also highlight several of these product strategies and the methods we have used to measure the benefits delivered.

Basics of Hair Colorants:

The majority of level 3 permanent hair colorants use similar chemistry to achieve the range of shades required. The product works by simultaneously lightening the underlying hair by bleaching the melanin and depositing color inside the hair that is resistant to washing. The lightening chemistry is done by hydrogen peroxide buffered at pH 10 with ammonia as the alkalizer thus forming the perhydroxyl anion (HOO^-) which is the key bleaching species. The color comes from the oxidative coupling of dye precursors inside the hair to form the chromophores. There is a range of dye precursor structures used but the majority are based on substituted aromatics such as *p*-phenylene diamine, *m*-aminophenol and resorcinol.

$$H_2O_2 \Leftrightarrow HOO^- + H^+$$

Formation of the perhydroxyl anion



Formation of the chromophores

Hair Structure Changes Caused by Colorants

Our approach to studying the hair structure changes has been to first identify the key reactive species that can interact with the hair and then identify the nature of this reactivity with the separate components of the hair. The aim is to measure this reactivity either directly or monitor the consequent change to a specified hair property.

(i) Key reactive species

The two reactive species that we have identified as important to hair structure changes are the perhydoxyl anion and the hydroxyl radical. The perhydoxyl anion is formed as shown above from the deprotonation of hydrogen peroxide at pH 10. The hydroxyl radical is formed from the catalytic reaction between hydrogen peroxide and a redox metal such as copper (Fenton Chemistry). The source of copper is generally from the tap water and is located predominantly in the cuticle.

(ii) Key reactions with the hair structure

The Chart 1 summarizes the key chemistries of the two reactive species with hair and a selection of the techniques used to measure the changes to the hair structure, either directly or indirectly. One significant change we observe is an increase in the hair's hydrophilicity caused by the perhydrolysis of the F-Layer. This change strongly influences the performance of our shampoo and conditioner products and increases the susceptibility of the hair to physical damage. When we have studied hair from women who regularly use colorants and those who do not we have seen these hair structure differences in vivo. These findings led us to define 'colored hair' as a separate substrate from

'uncolored hair' and to develop products specific for this group of women. We have also developed strategies to prevent the chemistry that causes these changes during the coloring process.

Reactive Species	*Chemistry*	*Direct Measures*	*Indirect Measures*
Perhydroxyl Anion HOO^-	Perhydrolysis of F-Layer	ToF SIMS, extraction	Contact angle, combing
	Cysteic acid formation from cystine	FT-IR	Dye penetration, tensile strength
Hydroxyl Radical HO*	Protein/Lipid degradation of cuticle	Chemiluminescence	SEM microscopy, shine, manageability

Chart 1 – Changes to the hair structure on coloring & methods to measure these changes

Product Design Strategies

(i) Shampoo Design

The strategy used here is to modify the surface energy of the hair during the shampoo use in order to increase the hydrophobicity of the surface. This increase in hydrophobicity increases the efficiency of deposition of the silicones from both the shampoo itself and also the conditioner. The approach was to use a liquid crystal (LC) colloidal structure, created with a high charge density cationic polymer, poly(diallyldimethyl ammonium chloride) and negatively-charged surfactants. It is proposed that the LC structure deposits from the shampoo onto the hair to provide "slip planes" along the hair surface for wet conditioning purposes, and form a hydrophobic layer which changes the surface energy of the fibers. The Table 1 below shows the increased silicone deposition achieved.

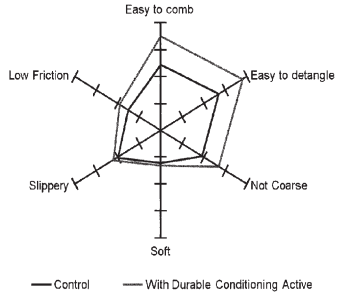
	Silicone Deposition (ppm)			
	Untreated	Permed	Color-treated	Bleached
Liquid-crystal shampoo	648 +/- 141	427 +/- 52	211 +/- 5	353 +/- 30
Non-polymer control	465 +/- 56	91 +/- 40	<10*	<10*

* Below detection limit of method (<10ppm)

Table 1 – Silicone deposition from liquid crystal shampoo vs control shampoo

(ii) Conditioner Design

The key strategy in this work was to identify a silicone material that more effectively deposited on colored hair. To do this the deposition of a wide range of silicone materials was first tested on colored hair to determine the key properties required to maximize efficiency. The first factor was viscosity and it was found that a minimum viscosity of ~600cps was required for the effective deposition of the silicone material. The second factor was the polarity of the silicone material as measured by its silicone-water interfacial tension. An optimum polarity was required which was close to the polarity of the colored hair. On formulation of this optimized silicone into a conditioner chassis the performance benefits of this approach were confirmed.



(iii) Colorant Technologies

The third strategy was to reduce the chemistry from the colorants that interact with the hair structure. The strategy developed was to identify an alternate chemistry for the hair colorant where the lightening and color performance is maintained but the fiber changes are minimized. This has been achieved with an oxidant consisting of ammonium carbonate, hydrogen peroxide and glycine at pH 9. One key benefit of this oxidant is the reduction in pH which has the consequence of a significantly lower loss of the F-Layer. In addition, the copper-induced radical chemistry at the hair surface has been reduced via the lower pH and the introduction of the glycine which acts as radical scavenger.

The consumer benefits of this technology will be presented during the talk.

STUDY OF MULTILAYER LAMELLA VESICLES IN HAIR STRAIGHTENING FORMULAS

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Introduction

Permanent hair straightening has become increasingly popular over the past years, especially in Asian region. Multilayer Lamella Vesicles (MLV), as a good active-delivery system, has been widely used in personal care products. MLV improves dispersion of difficult-to-solubilize compounds and enhances adhesion on the skin surface and sustained release of active ingredients. Phosphate esters have long been used in hair and skin care formulations as excellent emulsifiers and viscosity thickening agents. In our previous study, we reported that MLV structure formed in sunscreen formulas with a phosphate ester emulsifier played an important role enhancing the deposition of sunscreen oil on the skin surface, and therefore, improving the SPF water-wash resistance (1). Recently, we reported the coloring enhancement by MLV structure in permanent hair dye formulations (2-3). In this paper, we report our recent studies on formations of MLV structures in hair straightening formulas and the related hair straightening performance.

Experimental

Hair Straightening Formulations: Four hair straightening formulas containing different phosphate ester/non-ionic emulsifies were obtained from Croda Japan: Dicetyl Phosphate (and) Ceteth-10 Phosphate (CES); Ceteth-20 Phosphate (and) Dicetyl Phosphate (CS-20); Oleth-5 Phosphate (and) Dioleyl phosphate (HCE); and Ceteth-20.

Hair Samples: Natural curly hair was purchased from International Hair Importers, Inc., New York

Hair Straightening Steps

Reduction: 20 grams of a tested straightening base was applied to a respective tress for 20 minutes at room temperature, and then washed and blow-dried.

Heat Treatment: The reduced hair tress was continuously pressed for 3 seconds along hair fibers by using a special iron with a pair of flat heating plates at 180°C. Pressing was repeated three times before hair oxidation

Oxidation: The heat-treated hair tress was then immersed into 2% Hydrogen Peroxide solution (pH = 9.0) for 10 minutes at room temperature. Then washed and dried at room temperature.

Evaluation/Test Methods

MLV Phase Identification and Imaging: Digital images of straightening bases were captured using a Nikon ACT-2U Digital Camera System attached to a Nikon Optiphot-Pol Polarizing Microscope.

Hair Straightening Efficacy: Hair straightening efficacy was evaluated according to the method in reference (4) by comparing the length of hair fibers before and after immersion of the straightened hair into water for 5 minutes at 35°C. The retained percent of hair length was used as the straightening efficiency. 15 fibers were used for statistical analysis.

Single Fiber Stress Relaxation: Modified single-fiber stress decay test method from references (5-6) was used to measure stress relaxation.

***DSC Study of Hair*:** Denaturation enthalpy (ΔH_d) and RHC (Relative Helix Content) of hair samples with different treatments were determined using a Q100 DSC (TA Instrument, DE, USA) according to reference (7).

Results and Discussion

1. Different MLV Structure with Various Phosphate Esters

Figure 1 shows digital images of the straightening bases under the microscope with crossed polarizers. No typical liquid crystal pattern was observed in the hair straightening formula containing non-ionic Ceteth-20. Other hair straightening formulas containing phosphate esters showed clear liquid crystal patterns – well-defined Maltese crosses. It was found that sizes of the formed MLV particles were related to the molecular structure of the added phosphate ester. The determined average particle sizes of formed liquid crystals are: CS-20 – 5.0μm; CES – 8.5μm; HCE – 14.5μm (broad distribution). It is determined that smaller the hydrodynamic size of the phosphate ester molecule, less the average diameter of the formed MLV particles is.

2. Hair Straightening Efficacy

Figure 2 shows the straitening efficacy of different formulas. It is observed that hair straightening formulas containing phosphate esters showed statistically better straightening efficacy compared to the one with ceteth-20.

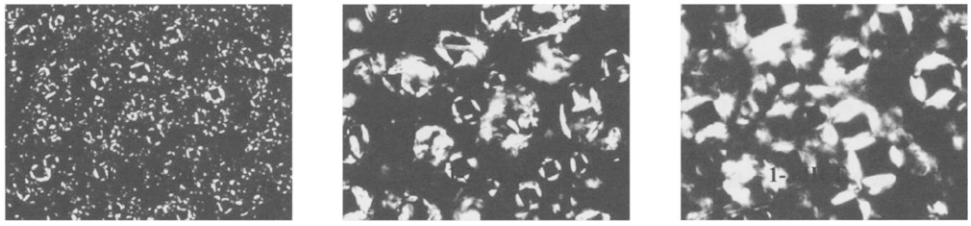

Figure 1 Different MLV structure with various phosphates in hair straightening bases

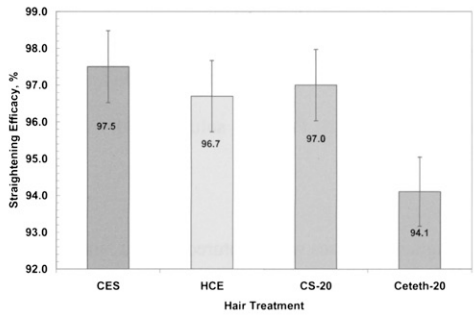

Figure 2 Hair straightening efficacy

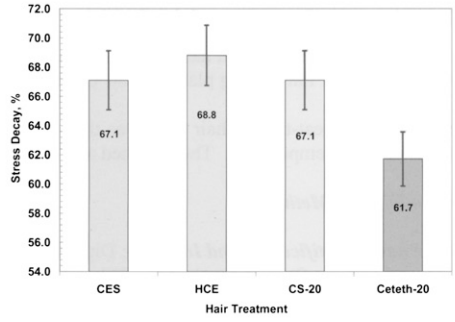

Figure 3 Stress decay

3. Stress Decay of Hair Fibers with Different Straightening Bases

Figure 3 demonstrated stress decay results of different formulas. It can be seen that hair straightening bases containing phosphate esters exhibited larger stress reduction at the same time period (20 min) compared to the one with non-ionic emulsifier. This may indicate that the penetrating rates of reducing agents into the hair cortex in formulas containing phosphate esters were faster than that in Ceteth-20 formula.

4. ΔH_d and RHC of Hair Samples with Different Treatments

Experimental data are summarized in Table 1. It can be seen that hair samples treated with straightening bases containing phosphate esters showed average smaller retaining RHC compared to the one treated with ceteth-20 formula. This may indicate that these hair samples underwent more reconstruction of helix, and therefore, may exhibit better straightening efficacy, which was validated by results in Sections 2 and 3

Table 1 Changes in Td and RHC of hair after different treatments

Formulas	CES		HCE		CS-20		Ceteth-20	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
ΔH_d (J/g)	6.17	5.00	6.18	5.12	4.90	3.90	5.45	4.74
Retaining RHC, %	100	81.0	100	82.8	100	79.6	100	87.0

Conclusion

- Replacement of non-ionic emulsifier with an equal amount of phosphate esters in hair straightening formulas enhanced formation of MLV structure.
- Smaller the hydrodynamic size of the phosphate ester molecule, less the average diameter of the formed MLV particles is.
- Hair straightened with formulas with MLV structure showed better straightening efficacy, faster and larger stress decay, and smaller retaining RHC compared to that treated with the formula without MLV.

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HAIR GROWTH MODULATION, UP OR DOWN: A REVIEW OF WAYS TO INFLUENCE THE BIOLOGICAL EVENTS IN THE HAIR FOLLICLE

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Key words: biotinyl-GHK, Minoxidyl™, collagen IV, laminine, Anagen / Telogen ratio

INTRODUCTION:

The biology of hair growth is, like most phenomena in the skin, characterized by a high level of complexity. From a cosmetic point of view, "hair care" is interested (beyond cleaning and conditioning the hair) in several items such as improving and/or maintaining the quality ("health") of the hair on the head, reducing excessive hair loss, reducing (or reversing) the loss of pigmentation of the hair, preventing dandruff; on the other hand, reducing/slowing the growth of unwanted body hair (beard, underarm, legs…), reducing its visibility are also fields of research.

OBJECTIVE OF THE STUDY:

To study cellular mechanisms of hair growth in order to find ways to either stimulate follicular activities (mitosis, anchoring proteins) or to inhibit them on purpose when so desired.

METHODS:

Isolated hair follicles are maintained in survival medium and incubated with Biotinylated peptide GHK compared to Minoxidyl. Hair growth is measured by image analysis. Collagen IV and Laminine 5 are measured by fluorescent tagging and image analysis. Mitosis rate is followed by quantifying Ki67 or BrdU incorporation. Gene expression in the 3D Skinethic™ model is followed by DNA array technology. The reduction of hair growth metabolism is followed by incubating hair bulb keratinocytes with plant derived molecules (NDGA, palmatine, protoberberines, chelidonine). Clinical studies over 4 resp. 2 months are conducted to measure improved hair anchoring (phototrichogram) for the former and to quantify reduced hair growth (image analysis) for the latter technologies.

RESULTS:

1. Hair growth up-modulation *in vitro*: Table 1 shows the major genes which are up-regulated by incubation of epidermis with Biotinylated GHK peptide, in presence of oleanolic acid. They reflect high growth activity with very strongly expressed cell metabolism enzymes. Antioxidant protective enzymes were also stimulated as well as markers of cell proliferation such as proliferating cell nuclear antigen (PCNA), steroid receptor co-activator and Cytokeratins 10, 14 and 16. This was accompanied by an increase in several adhesion proteins, those enabling cohesion between cells and the adhesion and the deployment of keratinocytes in cell layers (Desmogleins, Desmocollin) and those involved in cell attachment to the basal lamina (laminine binding protein, Vimentin, integrins α and β). Exposed to 2 ppm Biot-GHK peptide for 14 days, the hair shaft grew 58% more than the control hair, similar to that observed in the presence of 2 ppm Minoxidyl®. With 5 ppm Biotinyl-GHK the growth was 121% greater than that of the control (fig. 1).

Change in gene expression	%
Adhesion complex proteins	
Desmosomal proteins 1&3 (Desmogleins)	135% / 138%
Desmocollin 1	146%
Fibronectin receptor β-subunit	134%
Vimentin	138%
Laminine binding protein	146%
Integrins β1 & β2	134% / 144%
Antioxidant enzymes	
Thioredoxins peroxidases (TDPX2 & AO372)	152 and 174%
SOD (mitochondrial & cytosolic)	150 and 169%
Metallothioneins MTH & HMT	188 and 190%
CYP b-reductase	160%
Cell metabolism enzymes	
Acetyl CoA transferase	137%
Isocitrate dehydrogenase	189%
iNOS	143%
NADP Isocitrate dehydrogenase	189%
Proliferation / differentiation markers	
Proliferating cell nuclear antigen (PCNA)	191%
Cytokeratins 10, 14 and 16	154 / 150 / 144%
Steroid receptor co-activator	160%

Freezing microtome sections were made on D0 and D14 and exposed to peroxidase-bound anti-Ki67 antibody. All the cells showing Ki67 marker were counted.

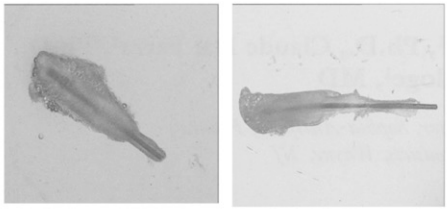
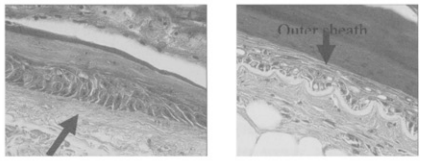


Fig.1: T0: Isolated hair follicle growth: T14

For the control bulb, the results showed a decrease in mitotic keratinocytes on day 14 of culture, reflecting cell aging. Minoxidyl® maintained proliferative activity as did 0.3 µM biotinyl-GHK (2 ppm) and approximately 1 µM (5 ppm) biotinyl-GHK.

The quality of the dermoepidermal junction depends on the formation of a very dense basal lamina rich in laminine 5 and collagen IV, on which the keratinocytes of the first basement layer rest and to which they adhere.

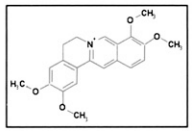


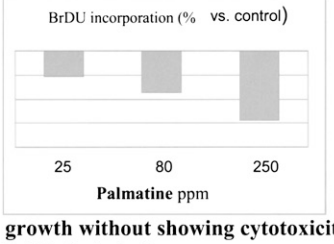
Control: 14 days Treated: 14 days

Morphological observation after 14 days of culturing showed in the control a dermoepidermal junction on the outer sheath side that was flattened and had lost its basal lamina. In contrast, when the hair follicle was incubated with biotinyl-GHK for 14 days, the basal lamina persisted and was clearly drawn showing its sinusoidal character.

These two findings reflect a strongly adherent and living dermo-epidermal junction.

2. Hair growth down-modulation *in vitro* and *in vivo*: Cultured keratinocytes actively divide. Mitotic activity is assessed by the incorporation of BrdU. Keratinocytes cultured with 10% FCS were incubated in the presence or absence of palmatine at various concentrations for 48 hours. Fig. 2 shows the concentration dependent decrease in proliferative rate of keratinocytes incubated with palmatine (-21%, -35%, -58%). A very similar effect is obtained with nordihydroguaiaretic acid (NDGA), with protoberberines and with chelidonine. **All these structures are able to inhibit cell growth without showing cytotoxicity at these use levels.**





3. Clinical studies:

The efficacy of a product slowing the multiplication of the proliferative keratinocytes at the base of the hair bulb may be determined *in vivo* by the reduction in the hair growth rate. The mean growth rate of hair is 250 µm per 24 hours. If the hair is shaved at T0 and its length is determined after 48 hours, its growth may then be compared to that after 2 months of product application using the same type of measurement: shaving at T56 days and length measured at T56 + 48 hours. Table 2 summarizes the results. It was observed that the subjects tending to have the fastest growth rate responded best to treatment. This finding is concordant with the action mechanism of palmatine which exerts its activity on actively proliferating keratinocytes. An increase in the number of days between necessary shaving of the legs was thus achieved. Visibly, hair also was less hirsute and apparent.

Population of 25 volunteers	**30 ppm palmatine**		**PLACEBO**	
	T0	T56	T0	T56
Mean re-growth rate (µm/day) on legs	239	202	247	226
Variation	-	-15.4%	-	-8.5%
Significance *vs.* placebo	**p <0.05**			

A four month clinical trial on 35 male panelists, conducted to measure improvement in hair anchoring (Anagen/Telogen ratio) showed that treatment of the scalp with the Biotinyl-GHK peptide (together with oleanolic acid as DHT inhibitor) has significant, perceptible, benefits, with 67% responders and A/T ratios increasing by up to 46%, whereas the placebo group showed no effects.

CONCLUSION:

Although no miracle hair-regrowth product has yet been found, it is clear from these results that safe and efficacious substances (a natural biomimetic peptide of tissue repair reputation, purified plant derived non-steroidal structures) can be used to selectively modulate hair growth and/or anchoring in the skin, on various body sites. Improved genomic, proteomic and metabolomic research tools will further our understanding of these processes.

ENERGIZING THE HAIR FOLLICLE: MODULATION OF HAIR FOLLICLE COMPONENTS

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Objective

Understanding the roles of different hair follicle components and the possibilities for acting on them are key challenges in hair biology. This study investigated the effect of actives previously described as cell energizers on the modulation of hair follicle markers.

Methodology

This study was carried out on human scalp skin grafts obtained from face-lift surgery. 6 mm diameter punch biopsies were performed on freshly collected skin samples. The biopsies were maintained in emersion on a porous membrane and fed by a serum-free medium [1].

In order to test the reactivity of this model and to study the modulation of key hair follicle components, we applied two molecules, designed to boost cell energy level, on the top of scalp biopsies for 48 hours. At the end of the incubation, scalp grafts were formalin-fixed and paraffin-embedded or frozen in OCT, to perform immuno-histochemical analyses. The level of fluorescence intensity was evaluated in treated biopsies in comparison with control biopsies receiving placebo (PBS). Hair follicle proteins such as keratins from the outer root sheath (ORS), proteins implicated in cell-cell and cell-matrix adhesion, as well as markers of cellular activity (Ki-67 and p63) were investigated.

Results

Studies showed that stimulating cAMP induced an increase in keratins 14, 15 and 16 in the ORS. These keratins play a role in the different stages of ORS cell differentiation. K14 and K15 are associated with early stage of differentiation [2, 3], whereas K16, also present in the companion layer, is a marker of intermediate stage of differentiation [4]. The modulation of these keratins could be relevant for molding of the inner layers of hair follicle, improving the quality of hair anchoring, as well as contributing to the guidance and shape determination of the growing hair.

Beta-1 integrin, fibronectin, Ki-67, and p63 were modulated by ATP-inducing active. Beta-1 integrin appears to be a key integrin subunit for the communication between basal layer of ORS and the dermal sheath. Moreover, this integrin is also associated with hair growth cycle and described as one of the stem cell markers [5, 6]. Fibronectin has also strong implication in hair follicle development [7]. These results suggest an optimized cell-matrix adhesion and enhanced cell signaling in the hair follicle.

Fig.1: Immunostaining of β1 integrin on human scalp skin biopsies treated with active ingredient at 1% for 48h

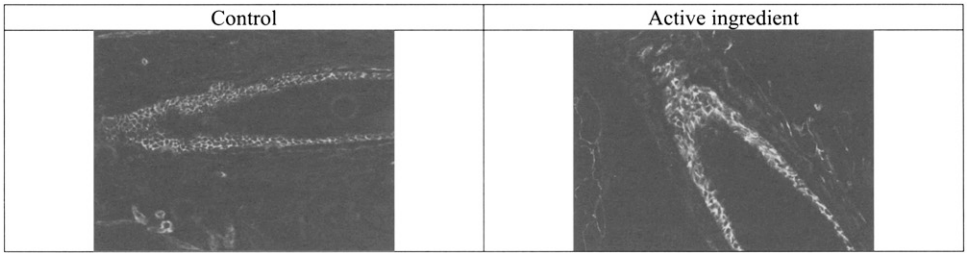
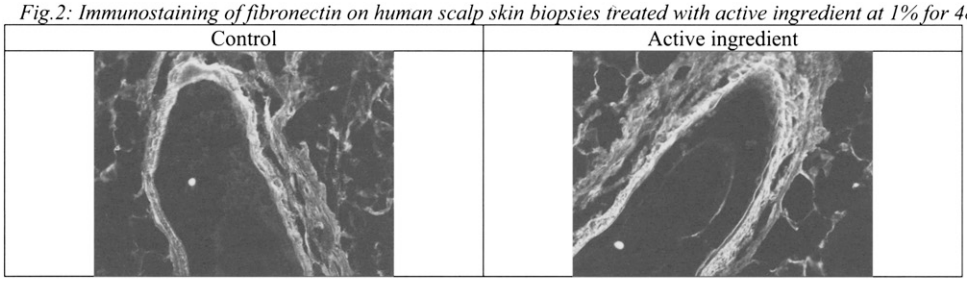
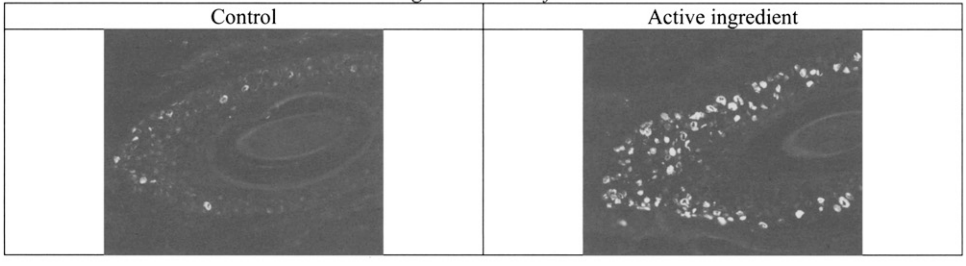


Fig.2: Immunostaining of fibronectin on human scalp skin biopsies treated with active ingredient at 1% for 48h



Ki-67 is used as a nuclear marker of cell proliferating activity and is closely related to state of alopecia [8]. p63 is a marker of keratinocyte stem cells, expressed in the basal and suprabasal layer of epidermis, ORS and hair matrix. p63 expression has been shown to be deficient in areas affected by alopecia [9].

Fig.3: Double-immunostaining of Ki-67(green) and p63 (red) on human scalp skin biopsies treated with active ingredient at 1% for 48h



Conclusion

This model of human scalp skin graft maintained in culture has been used to characterize key components of the hair follicle and examine their modulation by previously described cell energizer actives. In this model, communication is maintained between the hair follicle and its cutaneous environment. Energizing the hair follicle could be instrumental for improving hair vitality and growth.

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QUANTIFYING AND MODELING THE PROPENSITY FOR HAIR BREAKAGE

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Introduction

Breakage is a major issue in the attempt to create and maintain attractive looking hair styles. Unsightly split ends arise from fraying of broken fibers, while their presence also hinders fiber alignment – often leading to the perception of frizziness, changing the flowing motion of hair and diminishing shine qualities. As such there is a need to better understand factors that contribute to breakage. The most common approach of measuring "hair strength" involves generating stress-strain curves via stretching individual fibers at a constant elongation rate. However, it may be argued that such experiments are not a particularly accurate simulation of how consumers make judgment. Accordingly, some have suggested that repeated grooming experiments may represent a better simulation of how consumers assess the strength of their hair. In these experiments hair tresses are repeatedly brushed or combed a given number of times, with subsequent counting of the number of broken fibers that result. This testing dramatically demonstrates benefits associated with conventional conditioning products, in that surface lubrication reduces grooming forces, snagging and tangling – thus leading to considerably less breakage. In mechanical testing terms, the grooming of hair represents a fatiguing process where individual strands experience repeated exposure to an external stimulus. Therefore, in accordance with fatiguing principles, one expects a gradual propagation of flaws within individual fibers, until ultimately catastrophic failure (breakage) occurs. Previously[1,2,] we have described the modeling of single fiber fatigue data using the Weibull approach – and, in so doing, introduced the idea of treating fiber breakage as a statistical variable. Here, a grouped Weibull methodology was used to analyze breakage data from repeated grooming experiments.

Methodology

Repeated grooming experiments are performed using a custom designed apparatus where brushes/combs are mounted on a rotating drum which allows for reproducible grooming of hair tresses. Our equipment allows for four tresses to be groomed simultaneously, while broken fibers are collected in catch-pans located under each tress. The tresses are groomed in repeated blocks of cycles (for example, 1,000 strokes) with an evaluation of the number of broken fibers at the end of each block. This way, a cumulative distribution curve can be generated to show the total number of broken fibers as a function of the number of grooming strokes. This data can then be treated by grouped Weibull analysis to model the propensity for breakage under various conditions[3].

Results:

Figure 1 shows such a cumulative distribution curve representing the total number of broken fibers for 8 virgin Caucasian hair tresses as a function of the number of grooming strokes. Reference 4 shows how all Weibull calculations can conveniently be performed using an Excel spreadsheet. At a top level, generation of the two Weibull parameters provides a means of quantifying these experiments. However, the real strength of the approach involves the ability to generate Survival Probability plots (see Figure 2) that provide predictions as to the likelihood of fiber breakage under different conditions. Therefore, assuming these laboratory experiments are a reasonable representation of real-life conditions, it becomes possible to predict breakage rates on actual heads as a function of different habits and practices. Figure 3 also shows how the two Weibull parameters, together with information about the number of fibers in the

test tresses, allows for the modeling of repeated grooming testing and allows anyone to re-create the experimental outcome for comparison to their own experiences.

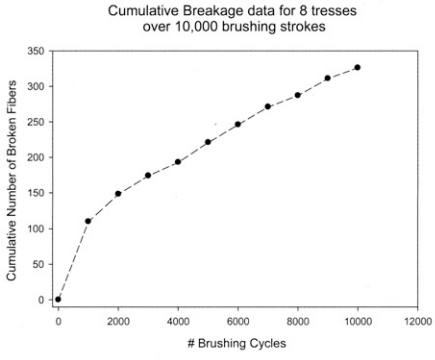

Figure 1

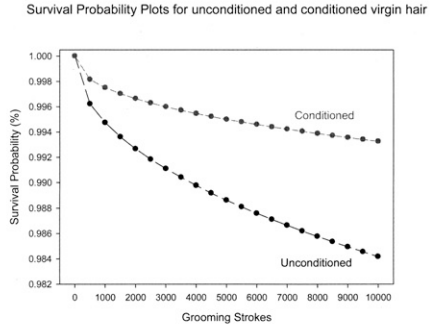

Figure 2

Results dramatically demonstrate the ability for conditioning products to retard hair breakage and provide a significant level of protection. Results from similar experiments will be shown to illustrate the influence of chemical damage and the relative humidity on the propensity for breakage.

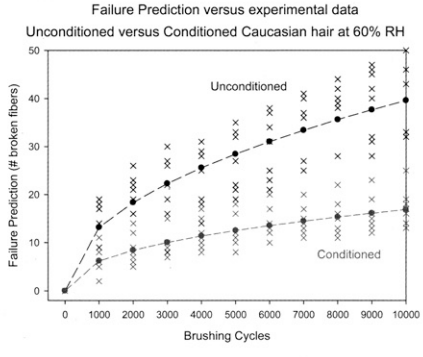

Figure 3

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REMIX. REFORMULATE. RENEW. REDO. THE NEW CHEMISTRY OF SUCCESS

Betsy Schmalz Ferguson

American Flavors and Fragrances

Wow. What a year it's been. We've all seen a lot, been through a lot, and discovered a lot about our industry. …Reflecting on the past year, I can't help but remember what Winston Churchill advised: When you are going through hell…keep going." And we have.

Let's take a look at what happened and where the color segment is going. As chemists, we always look at the big picture as well as under the microscopic for the subtle changes that sometimes have profound impact on what we do and create.

…We know that makeup, especially color, does something for a woman that very few consumer products can do. It has a beneficial psychological effect on her well being that is unique and powerful. Utilizing this proven fact more to reinforce the intangible psychological benefits as well as the visible or experiential benefits of our products may be an important direction to go in, in the years to come.

…Just a couple of the successful launches last year illustrate the trends – we saw ever more sophisticated foundation formulas that blend seamlessly with natural skintones resonate strongly with customers. Mineral makeups went from niche to mainstream and became ever more user-friendly with better formulas and delivery systems. Both were evidence of a strong trend towards "naturalness," or the effect thereof, in makeup. While the general makeup category entered the holiday '09 period in decline, new foundation and concealer products rose over 30%.

We also saw new mascaras electrify an overcrowded market with battery-operated designs which just goes to show that if you build a better mousetrap the world will still beat a path to your door.

In keeping with the cautious mood of the country, many of the most successful new launches weren't flashy nor were they designed to change the world. Companies simply took new product ideas or proven winners in categories that had always been successful --– and made them better, smarter, easier.

For many consumers, beauty has become just another option among many competing for her attention and discretionary spending. Studies recently have shown that many women spent less on beauty than in previous years. And the trend is growing. With the economic climate being what it is today, more consumers are asking themselves "how important is it" to get the latest lipstick or

yet another skincare cream. According to Karen Grant, NPD's Senior Global Analyst, in the past year, 2.3 million women, ages 18 to 64, reported not using beauty products.

How can we bring the x-beauty customer back into the fold? I think we have to examine why today's customers buy beauty in the first place. Is it just for appearance's sake or are customers looking for something deeper than that? As I mentioned before, color has immense psychological power to enhance a woman's self-esteem and confidence. Color is one of our customer's tools of reinvention. The world is full of lipsticks. Increasingly, the customer is looking for a product that enhances her view of herself and even her life, not just the novelty of buying a new shade.

Our advertising, formulations and customer service skills all need to answer those subtle inner needs in order to win back loyalty and trust. We need to re-establish our value by providing products that feature bolder innovation, higher quality, superior performance and impressive benefits. Product excellence has never been more important than it is today.

…A major bright spot, despite all the gloom and doom, was the stunning growth of the online market. Expanding as if it were on steroids, online buying is beginning to make a trip to the department store cosmetic counter seem positively, well, quaint. Small, specialty and niche brands have become a powerful and permanent presence in the global marketplace, thanks to the internet. And that means new clients for us in every sector of the market.

…As we reach the midpoint of 2010, we know we still have many challenges ahead.

The thrill isn't gone in buying makeup. It's just that our customer sometimes forgot it, especially in the past year and a half, in the noise of the crashing economy, the rush of accelerating time pressures, the chatter of social networks and the changing buying and social behaviors of society at large.

But we also know that consumers still love beauty. And, like any great partnership, no matter what we face together, our relationship with the customer needs constant attention, nurturance and flexibility. It demands nothing less than our commitment to giving her our best in everything we do.

COMPLEX EFFECT PIGMENTS: THE CHALLENGE OF FORMULATING WITH PIGMENTS OF DIVERSE OPTICAL AND PHYSICAL PROPERTIES

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1. Introduction

The objective of this paper is to study and understand the impact of different effect pigments on the formulation of several major cosmetic products. The study presented here is intended as an overview of a large pigment system and the data is presented as a guideline only. Each effect pigment presents its own characteristics and it is impossible to cover them all in within the scope of this study. Therefore, we have chosen to examine two of the more common pigment systems: titanium dioxide and mica, as well as borosilicate and mica. In each system we examined three coating thicknesses and three particle size distributions, leading us to a matrix of 18 experimental conditions. We tried to cover a diverse range of cosmetic products and therefore we introduced these pigments into formulations for a pressed powder, a lipstick, a cream and a shampoo.

2. Experimental Procedure

a. Formulations

Standard formulations for all the products were used and modified as given later in the text. The formulations contained a concentration of effect pigments shown in Table 1.

b. Pigments used

Eighteen pigments were used in this study. Two different substrates were chosen: mica and borosilicate. In each system three particle size distributions were chosen, small, medium and large. The particle sizes are shown in Table 2. The coating system for each substrate is titanium dioxide, to limit the amount of pigments needed. For each particle size, three different colors were selected, pearl, red and green. This represents an increasing coating thickness of the titania layer on the substrate. In addition to mica and borosilicate based bonded pigments, an uncoated bismuth oxychloride pigment was used in all studies as well. Its average particle size was approximately 6 – 15 μm.

Table 1: Pigment loading

Product	Pigment load [wt.%]
Pressed powder	60
Lipstick	15
Cream	5
Shampoo	0.1

Table 2: Particle size distributions

Pigment	Particle size [μm] D_{10} - D_{50} - D_{90}
Small	10 – 22 – 37
Medium	16 – 37 – 68
Large	32 – 68 – 128

c. Testing procedure

Hardness testing was conducted using a Zwick DuroTest 3100 with a 50N load. Seven hardness measurements were conducted with the highest and lowest results being discarded. Viscosity measurements were conducted using a Brookfield Engineering Digital Viscometer DV-II with a smooth #4 spindle in case of the shampoo and a #F heliopath attachment for the creams. The breaking points of the lipsticks were measured with a Chatillion DFM10 force gauge and a Cavalla Inc. LS/BC 97 movable lab stand at a speed of 25 mm/min. Four data points were taken. Oil absorptions were measured using linseed oil (ASTM D-281) and each experiment was conducted twice.

3. Results and Discussion

a. Shampoo

The addition of different effect pigments had a minor effect on the viscosity of the shampoo formulation as can be seen from Figure 1. The viscosity however, did not change more than ±2000 cps, which was a change too small to affect flow properties.

b. Cream

In all cases, the addition of effect pigment lowered the viscosity of the cream by roughly 45% (Figure 2). The viscosity differences between the different pigments are small. However, the viscosity of the creams is decreasing slightly with increasing particle size.

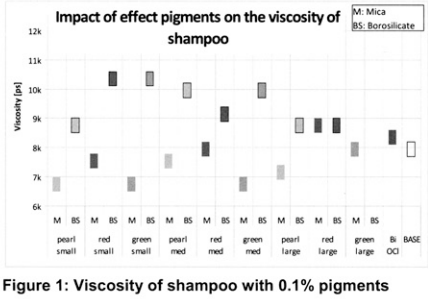

Figure 1: Viscosity of shampoo with 0.1% pigments

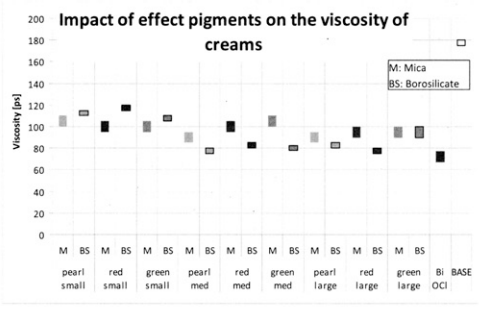

Figure 2: Viscosity of creams with 5% pigment

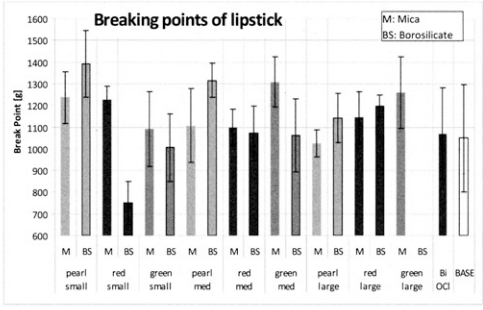

Figure 3: Breaking points of lipstick with 15% pigment

c. Lipstick

The breaking points of lipsticks were measured and are displayed in Figure 3. It is clear that the pigment can have a major effect on the strength of a lipstick. But the data also indicates that each batch of lipstick was highly variable by itself. The high standard deviations and the fact that no larger trends can be discerned leads to the conclusion that the major influence on the breaking point of a lipstick results from formulation and processing variations of the base, and not the effect pigment.

d. Pressed powder

The hardness of the pressed powder formulations is shown in Figures 4 and 5. The oil absorption of the effect pigments alone is shown in Figure 6. With the pressed powders the formulation had to be adjusted significantly to allow for a good product to be made. With the borosilicate-based pigment, a 25% reduction of the pressing pressure was all that was needed to obtain acceptable performance using the small and medium pigments. The large borosilicate pigments required a lowering of the wet binder content by 25% in addition to the reduction in press pressure. The mica-based pigments presented a much larger challenge. The small mica fraction could be pressed resulting in a good product by increasing the wet binder content by 25% to account for the lower oil absorption of most micas compared to borosilicate. In the medium and large fractions of mica, the ideal pigment loading for the selected formulation was set to 45% in order to produce the best product. Other possible formulation strategies to achieve a higher pigment loading include manipulations of the wet and dry binder content.

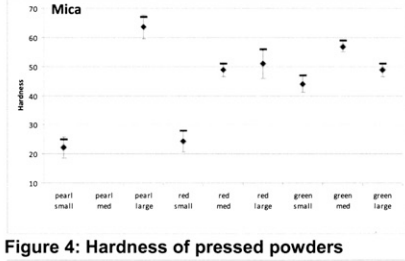

Figure 4: Hardness of pressed powders

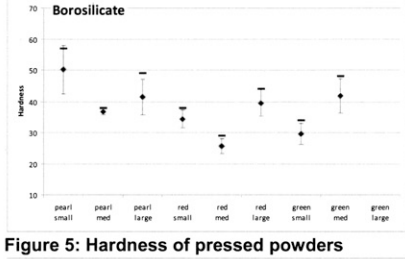

Figure 5: Hardness of pressed powders

4. Conclusions

Each effect pigment presents its own unique challenges to the formulator. It is evident that a formulator must carefully consider the impact each pigment has on the physical properties of a formulation in order to achieve a stable and aesthetically acceptable product. Effect pigments, especially when used at high concentrations as commonly occurs in powder formulations, should be considered as an integral part of the formula rather than simple colorants. We hope to have given the reader a broad overview of three common pigment systems used in four common cosmetic applications. We have also presented some information regarding what kinds of adjustments should be considered by the formulator in order to produce an acceptable cosmetic product.

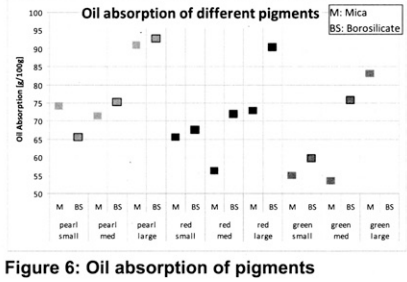

Figure 6: Oil absorption of pigments

SURFACE TREATMENTS TO IMPROVE PIGMENT DISPERSION IN AQUEOUS MEDIA

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OBJECTIVE

Study of hydrophilic silanes as a means to improve pigment wetting and dispersion in water based color cosmetics. Correlation of methodology to measure performance.

MATERIALS AND METHODS

Silanes: Triethoxysilanes having the following hydrophilic "R" groups: PEG_{6-9}, carboxyl, aminopropyl, and acetyl hydroxyprolyl were synthesized.

Pigments treated : Titanium Dioxide (anatase), Red Iron Oxide, Yellow Iron Oxide, Black Iron Oxide

Tests performed:

Aqueous dispersion (visual): 0.3 grams of pigment was added to 15 ml deionized water. Behavior was observed without stirring.

Sedimentation: Ultrasound used to disperse treated pigments in water. 10 ml aliquots were introduced into graduated cylinders, sealed and allowed to stand for one month. Sediment density (mg/ml was measured)

Dispersion viscosity (Butylene Glycol): Dispersions of the pigments were prepared by wetting in butylene glycol with stirring for one hour, followed by three passes over a three roll mill. Viscosity was measured using a Brookfield viscometer, standard spindles @ 20 RPM.

Formulation: Anionic oil in water emulsion concealer containing 20% pigment: Parameters observed were: ease of pigment wetting, presence or absence of color flotation during processing and in final product, pigment dispersion.

RESULTS

Visual Evaluation

	Yellow Iron Oxide	Red Iron Oxide	Black Iron Oxide	Titanium Dioxide
control (untreated)	wets, falls to bottom	wets, falls to bottom	wets, falls to bottom	wets, falls to bottom
PEG_{6-9} Silane	self disperses	self disperses	dispersion, settles	self disperses
Aminopropyl Silane	wets slowly, disperses	disperses 50%	sl dispersion; settles	wets slowly; sl dispersion
Na Carboxyethylsilanetriol	self disperses	self disperses	dispersion; settles	self disperses
Acetyl Hydroxyprolyl Silane	self disperses	self disperses	sl dispersion; settles	self disperses

.All of the pigments with hydrophilic modification deagglomerate and disperse without stirring to some extent. PEG_{6-9} Silane and Sodium Carboxyethylsilanetriol treated pigments dispersed completely, some particles remaining in suspension for over one month.

Sedimentation

Sedimentation study is another means to compare multiple surface treatments in a vehicle. Sediment volume is affected by dielectric/wetting characteristics of the vehicle and the presence or absence of surface treatment . Relative changes in performance among surface treatments and levels of treatment can be quantified. Higher sediment volume is due to flocculation, indicating poor wetting and dispersion, whereas low sediment volume is the result of deflocculation in a system having good particle wetting.

Sediment Density (g/ml)

	Pigment			
Treatment	Yellow Iron Oxide	Red Iron Oxide	Black Iron Oxide	Titanium Dioxide
control (untreated)	0.11	0.42	0.38	1.00
PEG_{6-9} Silane	dense, incomplete	dense, incomplete	0.63	0.45
Aminopropyl Silane	0.21	0.83	0.40	0.37
Na Carboxyethylsilanetriol	1.25	2.00	0.83	1.25
Acetyl Hydroxyprolyl Silane	0.33	1.00	0.56	0.48

All of the hydrophilic treatments deflocculate the iron oxides. Once dispersed by ultrasound, the untreated titanium dioxide wets well. Only Sodium Carboxyethylsilanetriol treatment further reduces the sediment volume.

Dispersion Viscosity

Multiple surface treatments can be evaluated on a given pigment substrate by preparing pigment grinds. Lower viscosity at equal concentration and degree of dispersion (particle size) indicates better wetting.

Dispersion Viscosity (cps)

	Pigment , % in Butylene Glycol			
Treatment	Yellow Iron Oxide 45%	Red Iron Oxide 50%	Black Iron Oxide 50%	Titanium Dioxide 50%
control (untreated)	73,400 cps (spindle #7)	24,500 cps (spindle #6)	10,950 cps (spindle #6)	11,150 cps (spindle #6)
PEG_{6-9} Silane	1,610 cps (spindle #3)	2,100 cps (spindle #4)	3,970 cps (spindle #4)	12,150 cps (spindle #6)
Aminopropyl Silane	675 cps (spindle #3)	4,100 cps (spindle #4)	9,000 cps (spindle #4,5)	4,700 cps (spindle #4)
Na Carboxyethyl-silanetriol	540 cps (spindle #3)	2,690 cps (spindle #4)	6,500 cps (spindle #4,5)	415 cps (spindle #3)
Acetyl Hydroxy-prolyl Silane	520 cps (spindle #3)	3,120 cps (spindle #4)	5,940 cps (spindle #4,5)	2,870 cps) (spindle #3)

All of the hydrophilic treatments pigments improved wetting and dispersion in butylene glycol, except, surprisingly, PEG_{6-9} Silane on titanium dioxide. Comparison of the silane treatments showed the dispersions of carboxylated silane treated pigments have the smaller agglomerate size and higher degree of color development.

Formulation

o/w concealer

	Parameter			
Treatment	Dispersion	Color development (1 is lowest)	Wetting	Flotation
control (untreated)	undispersed pigment	1	slow	white
PEG_{6-9} Silane	complete	4	rapid	white/yellow/black
Aminopropyl Silane	undispersed TiO_2	2	slow	white
Na Carboxyethylsilanetriol	complete	5	rapid	yellow > 60°C
Acetyl Hydroxyprolyl Silane	complete	3	intermediate	none

DISCUSSION

Deposition of the polar compounds alone on the pigment surfaces did not produce the instant dispersion effect that results from surface treatment with silanes having the polar compounds as a functional group. The dry treated pigments are deagglomerated to some extent due to the milling steps in the treatment process, but the effect on the tests was negated by particle size reduction steps of all samples used for dispersion testing. The greater surface area actually can appear to slow the wetting process, but the results of the viscosity and sedimentation tests show that wetting of the treated particles improved compared to that of the untreated pigments.

The dramatic dispersion of the hydrophilic treatments seen in visual evaluation is confirmed by the quantitative measurements:

The sedimentation test shows effect of the treatments on wetting of the particles. Of the silanes evaluated, PEG_{6-9} Silane and Sodium Carboxyethylsilanetriol produced the densest sediments and the most uniform improvements over the untreated oxides. Whether complete deflocculation is desirable in a given formulation is a separate issue.

Dispersion viscosity measurements indicated that PEG_{6-9} Silane and Sodium Carboxyethylsilanetriol treated iron oxides exhibited the best wetting. Sodium Carboxyethylsilanetriol treatment is the most effective treatment for titanium dioxide.

Interaction of the treated surfaces with other raw materials has an effect on performance in actual formulation. Surface activity of the PEG group of the PEG_{6-9} Silane may affect the formation of the emulsion, causing excessive pigment flotation and appears to influence wetting of some thickening agents. The anionic nature of Sodium Carboxyethylsilanetriol does stabilize pigment dispersions, but the effect of the added electrolyte on other raw materials must be considered. Similarly, the amino group of aminopropyl silane reacts with formulation ingredients as would any other amine.

LUSTER MEASUREMENTS OF LIPS TREATED WITH LIPSTICK FORMULATIONS

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Introduction

Lipsticks are described as dispersions of coloring matter in bases made of oils, fats and waxes. They have been used to impart an attractive color and appearance to the lips. In this article we discuss the use of digital photography and image analysis for measuring luster from lipsticks applied on a model mannequin. The interpretation of the data is based on the shape of the light scattering curves, calculated luster parameters, and visual examination of the digital images of the lips. The data could help formulators refine or make unique claims about their formulations.

Experimental

Formulations

In this study six formulations containing varying amounts and types of polymers were compared to a control. Formulations A, B, C, D, E, and F, contained 11% VP/Hexadecene Copolymer, 11% VP/Eicosene Copolymer, 7% VP/Hexadecene Copolymer plus 4% VP/Eicosene Copolymer, 5% Polybutene, 5% VP/Eicosene Copolymer and 5% VP/Hexadecene Copolymer, respectively.

Digital Photography

A white light source (14 W) was placed at a distance of 24 inches from the mannequin head. A Cannon EOS 20D digital camera with a resolution of 8.2 MP and with EFS 17-55 mm f/3.5-7.1 lens was employed as the image collection device for all studies presented in this article.

Calculations

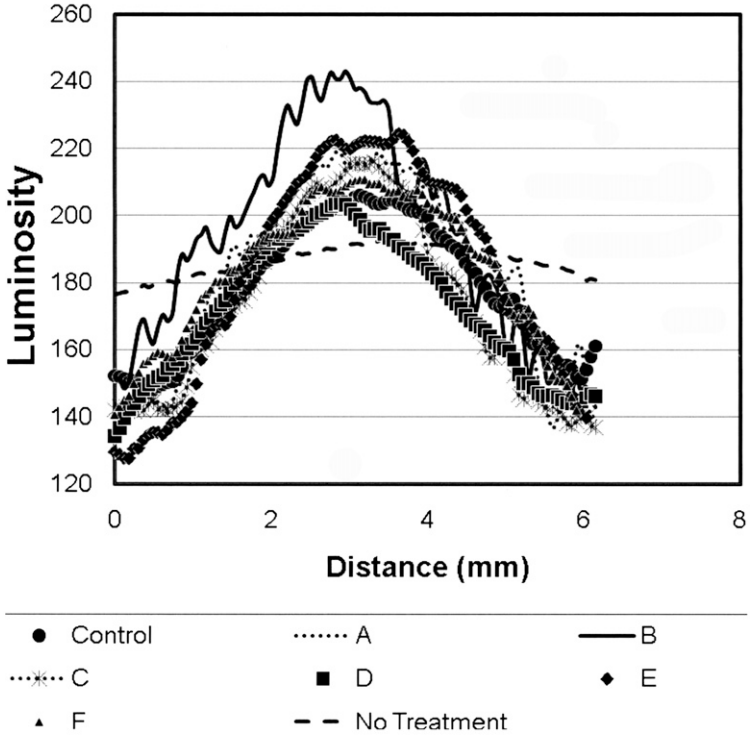
The equations used to calculate luster were adopted from Stamm *et al.,* (1) and Reich and Robbins (2). Statistical analysis was performed using Sigma Plot statistical software. Dunnett's test was performed to compare multiple treatments to a control

Results

Image analysis was performed on the shine bands obtained from various treatments and the results are plotted in Figure 1. Formulation B (11% VP/Eicosene Copolymer) has the highest peak, in other words, it has the highest shine. Treatments C (7% VP/Hexadecene Copolymer and 4% VP/Eicosene Copolymer) and E (5% VP/Eicosene Copolymer) are slightly lower, and treatment D (5% Polybutene) came closest to the control. The results seem to be in agreement with the visual differences depicted among treatments but a more rigorous analysis was needed. Luster parameters for the various formulations evaluated were calculated. The results for the peak height were 197.54, 242.25, 214.07, 217.01, 197.89, 230.63, and 213.96 for the control and formulations A, B, C, D, E, and F, respectively. The two treatments that had the highest peaks and were statistically different from the control were treatments B (11% VP/Eicosene Copolymer) and E (5% VP/Eicosene Copolymer). The rest of the treatments, although visually different, they were not statistically different from the control. Both Luster parameters were calculated: L_{Stamm} and $L_{R\text{-}R}$. The values for L_{Stamm} were 0.18, 0.27, 0.23, 0.21, 0.18, 0.25, and 0.24 for the control and formulations A, B, C, D, E, and F, respectively. The values for $L_{R\text{-}R}$ were 0.36, 0.45, 0.37, 0.43, 0.38, 0.39, and 0.36 for the control and formulations A, B, C, D, E, and F, respectively. The only treatment that was significantly different from the control in both Luster parameters was treatment B (11% VP/Eicosene Copolymer).

Figure 1

Light distribution curve of various lipsticks



Conclusions

It appears from the results obtained thus far that the addition of VP/Eicosene Copolymer to a lipstick formulation increases its shine. The incremental increase in shine is concentration dependant as the lipstick containing 11% had more shine than the one containing only 5%. The addition of Polybutene had a very small contribution to the shine of the lipstick as the peak shine was closest to the control, however the addition of VP/Hexadecene Copolymer contributed to the overall shine, but was not as significant as the addition of VP/Eicosene Copolymer.

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THE SHIFTING LINE DIVIDING DRUGS AND COSMETICS

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The increasing sophistication of cosmetic formulations and new insights into skin physiology has succeeded in blurring the line dividing cosmetics and drugs. Traditionally, cosmetics were products intended to color and scent the skin, but the new concept of "skin care" has led to the marketing of moisturizers, cleansers, and serums that modify the structure and function of the skin, a realm delegated to drugs. Consumers are in part driving the "therapeutic cosmetic realm" by demanding products that reverse aging, improve skin appearance, minimize pigmentation irregularities, and soothe sensitive skin. Conversely, the introduction of "prescription moisturizers" approved through the FDA 510K route has created further confusion. Thus, it is worthwhile to ponder the shifting line dividing drugs and cosmetics.

While there are legal definitions of cosmetics and drugs, Table 1 contains a practical working definition of these concepts as viewed from the medical perspective.

Table 1
Working Definition of Cosmetics vs. Drugs

COSMETICS	DRUGS
Does not affect the structure or function of the body	Changes the structure and function of the body
Does not treat any condition	Treats a condition
Induces only temporary change	Requires a prescription from a medical professional
Improves "appearance"	Dangerous
Does nothing medically meaningful	Must be carefully used with supervision

While Table 1 presents the pertinent working concepts for drugs vs. cosmetics, in practicality drugs and cosmetics are divided based on claims. If you make drug claims, you are a drug. Conversely, if you make cosmetic claims, you are a cosmetic. For example, a product claiming to get rid of wrinkles after one minute of use that treats the five signs aging and is suitable for men and women of all ages is a drug. Why? Any product that "gets rid of wrinkles" is a drug, since cosmetics can only "improve the appearance" of wrinkles. Eradicating a finding is a drug claim while improving appearance is a cosmetic claim.

Beyond the realm of claims, cosmetics can have a profound effect on the skin. Water, glycerin, and petrolatum are some of the most skin structure modifying substances known to dermatology. Water, petrolatum, and glycerin are the mainstay of treatment in

complex skin diseases, such as atopic dermatitis, xerotic eczema, and psoriasis. In part, these common ingredients are classified as cosmetic ingredients because it was not until recently their structure modifying effect on the skin was understood. Both water and glycerin are transported via aquaporin channels in the skin, which also control cellular differentiation. Most physicians would consider cellular differentiation modification within the drug realm, but it is clear that water and glycerin are cosmetic ingredients.

Water, glycerin, and petrolatum also form the basis for the prescription device moisturizers approved through the 510K route. While the device route was typically reserved for machines that plug into the wall, creams were approved due to their physical effects based on increased skin hydration. There are no major formulation differences between cosmetic moisturizers and prescription moisturizers and both function to increase skin barrier function.

The dividing line between drugs and cosmetics follows the "don't ask, don't tell" rule of legal conduct. Don't ask what the cosmetic is doing to the skin and don't tell what is really known about the skin effects of the cosmetic. In summary, the rules governing cosmetics are know a lot regarding the effect of your formulation, but tell little. Stay away from functional claims that indicate change and focus on appearance claims.

The drug vs. cosmetic controversy becomes even more complex when an analysis is performed on the vehicle in prescription skin formulations. Most topical drugs are carried to the skin in a moisturizing vehicle similar to those marketed as cosmetics. The profound effect of the vehicle on the skin can be seen in the large placebo effect observed in vehicle-controlled studies. In acne studies, the placebo effect can be as high as 40%. While some of this effect is due to natural fluctuation in acne, some of it is due to the effect of standardized hygiene required in many acne studies and the vehicle. Cosmetics could also be viewed as moisturizing vehicles with an active, very similar to the construction of drugs.

There is no doubt that the dividing line between cosmetics and drugs is blurring. Cosmetics and drugs were defined in an era when the skin was thought to be a nonliving structure with little functionality. It now recognized that the skin is an immunologic, photoprotective, thermoregulatory, hormonally active structure that is affected by everything it contacts. Many cosmetics are moving closer to drugs by incorporating active ingredients to improve functionality while many drugs are moving closer to cosmetics by placing the drug a cosmetically elegant vehicle.

STIMULATION OF EXTRACELLULAR MATRIX PROTEINS BY UV-LIGHT IN THE PRESENCE OF OPTICALLY-RESPONSIVE POWDERS

Vito Cataldo, James V. Gruber, Ph.D., Francesca Muia, and Lisa Bouldin

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Introduction

It is well established that the skin both relies on light energy and is damaged by light energy, in particular UV-light. People work extensively to protect their skin from the damaging effects of UV radiation and in doing so they may inadvertently diminish the skin's ability to perform critical biochemical processes dependent on light. The skin also responds to light through daily fluctuations in body chemistry brought on by the presence or absence of sunlight.

Methodology, *In Vitro*

Upon arrival, the tissues were stored at 4°C until used. For use, the tissues were removed from the agarose-shipping tray and placed into a 6-well plate containing 4ml of assay medium and incubated at 37+/-2 °C and 5+/-1% CO_2 overnight. On the following day the assay medium was replaced with fresh media for the start of the experiment.

On Day 1 of the study, 100 µl of fresh test material was applied to the surface of the tissues. After application of the test materials the test the tissues were they either treated with an additional application of 25 µl of sunscreen blend (7.5% Ethylhexyl Methoxycinnamate, 1.5% Benzophenone-3 prepared in 30% ethanol, 70% propylene glycol) and exposed to UVB (20 mj/cm^2), or treated with the test materials and sunscreen alone with no additional light exposure.

The surface area of the tissues is 1.0 cm^2, so the volume of sunscreen applied was 0.025 ml/cm^2. It was estimated, based on *in situ* measurements that the approximate SPF is 15. After exposure, the UVB treated tissues were returned to the incubator until the next treatment while the tissues treated with the test materials and sunscreens were returned immediately to the incubator.

On Days 2, 3 and 4 of the study the tissues were removed from the incubator and washed with PBS to remove the test materials from the previous day. After washing, fresh test material was applied to the tissues, and the tissues were again treated as described above and then returned to the incubator. On Day 3 of the study, in addition to the fresh application of the test material and treatment, the tissue culture media was also changed. On Day 5, the tissue culture media was collected and stored at -75°C until analyzed for elastin.

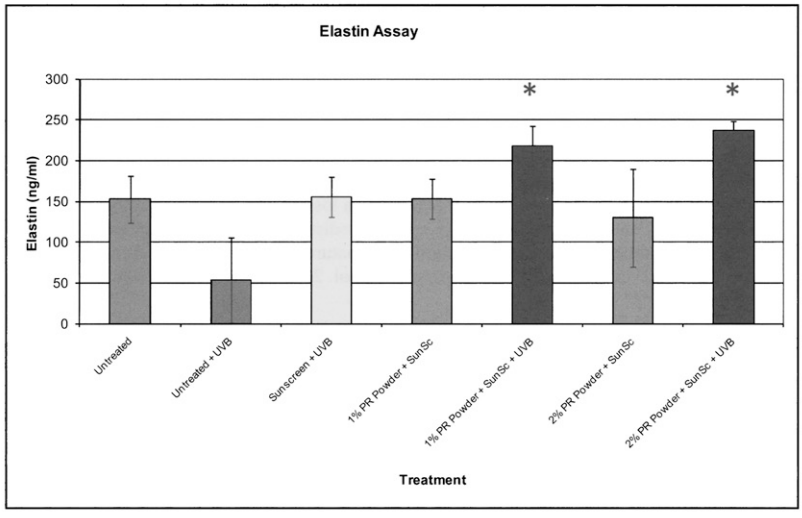
Methodology, *In vivo*

A 60-Day, placebo-controlled, double-blind, full face study was conducted with 86 volunteer subjects broken into four groups: 1) Placebo vehicle group, 2) 2% Photoresponsive Powder, 3) 5% Photoresponsive Powder, 4) 2% Photoresponsive Powder at night. The participants in the first three groups were required to spend at least 2 hours of the day in the sun and the study was run in Arizona to assure that adequate sunlight was available. The vehicle contained a sunscreen blend offering an SPF of 8 to the volunteers. The participants were brought into the testing lab on days 15, 30 and 60 and their skin was analyzed for total elastic response using a MPA 580 Cutometer.

Results

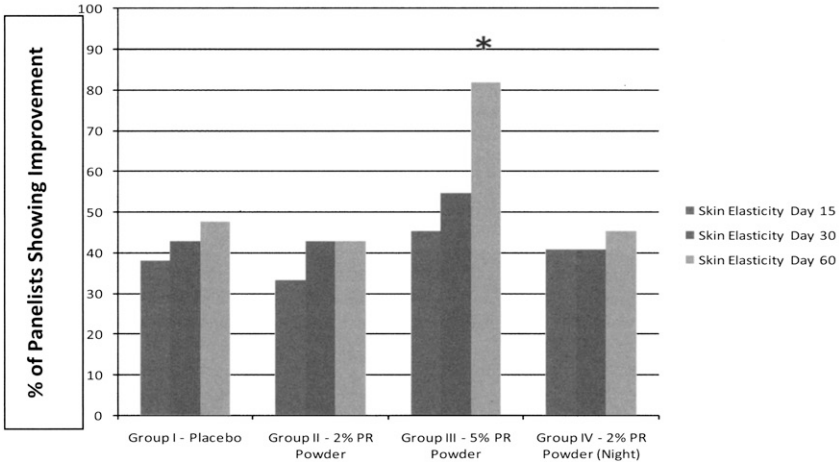
The results of the *in vitro* assays looking at the effects of UVB radiation on full thickness tissues in the presence or absence of the Photoresponsive powder mixture are shown in Figure 1.

Figure 1. Total Elastin expression from treated tissues.



The results of the *in vivo* assay looking at skin elasticity are shown below in Figure 2.

Figure 2. Results of elasticity measurements on skin.



A NEW LIPOAMINOACID ABLE TO RESTORE AGING-INDUCED MODIFICATIONS IN BIOMECHANICAL PROPERTIES OF HUMAN DERMAL FIBROBLASTS AND IN FUNCTIONAL PROPERTIES OF PREADIPOCYTES

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OBJECTIVE

The aim of this study was to investigate the anti-ageing efficacy of a new lipoaminoacid, Palmitoyl isoleucine (P-ISO) and to determine its mode of action, by studying *in vitro* and *in vivo* the following biological properties: restoration of biomechanical properties of young fibroblasts, promotion of adipocyte differentiation, prevention of Mesenchymal Adipocyte-like Default cell (MAD cell) generation, anti-wrinkle and anti-ptosis efficiency. Indeed, in addition to the well described consequences on extracellular matrix production, epidermal differentiation, *etc*., skin aging is able to modify the biomechanical strengths developed by dermal fibroblasts *in vivo*. Similarly, hypodermis is impacted by ageing, which leads to a loss of fat tissue in elderly people, which can be explained by different physiologic phenomena, such as fat redistribution, decrease in the size of mature adipocytes and alteration of preadipocyte differentiation into mature adipocytes, these last ones being less functional and becoming "MAD cells" [Kirkland *et al*. Exp Gerontol. 2002 June;37(6):757-67].

METHODOLOGY

For this purpose, contractile forces developed by normal fibroblasts extracted from either a young (30 y-old) or an aged (65y-old) skin were measured in the P-ISO-treated, TGF-β_1-treated (reference molecule), or untreated (control) GlaSbox® lattices (*i.e*. a device enabling the measure of the contractile forces developed by fibroblasts within a restrained lattice, Bioexigence, France).

Investigation of P-ISO ability to promote adipocytes differentiation was performed on normal human preadipocytes (NHP) cultured in a diluted differentiation medium. This test was based on the detection of lipid droplets by using a fluorescent fatty acid tracer, Bodipy FL, and the differentiation level was compared to that observed on P-ISO-treated cells and on non diluted medium-cultured untreated cells. To investigate P-ISO ability to prevent MAD cell generation, senescent normal human preadipocytes were cultured in the non diluted medium and compared with younger preadipocytes, the level of differentiation being evaluated by measuring the production of neutral triglycerides and lipids (Red-Oil stainer).

Finally, clinical trials were conducted on Caucasian female volunteers to confirm P-ISO anti-age ability (Dermscan, France). In the first trial (42d-treatment, mean age: 55y-old), measure of wrinkles was performed by skin printing and image analysis, in comparison with a marketed reference anti-age lipopeptide, while in the second one (56d-treatment, mean age: 58y-old), skin biomechanical properties were assessed using Ballistometer® and check volume by fringe projection (Primos Body®), in comparison with a placebo formulation.

RESULTS

In the GlaSbox® model, a decrease in elderly fibroblast contractile forces in comparison with those of younger fibroblasts was observed (Figure 1; ≈-20%). Treatment of lattices with either TGF-β_1 (2.5 ng/mL) or P-Iso (0.005%) totally restored the level of contractile forces (Figure 1: respectively of 66% and 56% at 5h and 94% and 94% at 48h).

Preadipocyes induced to differentiate into the incomplete differentiation medium showed a statistically significant reduction in lipid droplet quantity (Figure 2: 95%), compared with those cultured in the complete medium. P-ISO (0.001%) induced a statistical significant increase in adipocyte differentiation, leading to a restoration of adipocyte differentiation of 75% (Figure 2).

Senescent preadipocytes induced to differentiate showed a statistically significant reduction in triglyceride/lipid reserves (mean effect: ≈62%, example illustrations on Figure 3), compared with young cells, while P-ISO (0.0005%) induced a mean restoration effect of 41% (example illustrations on Figure 3).

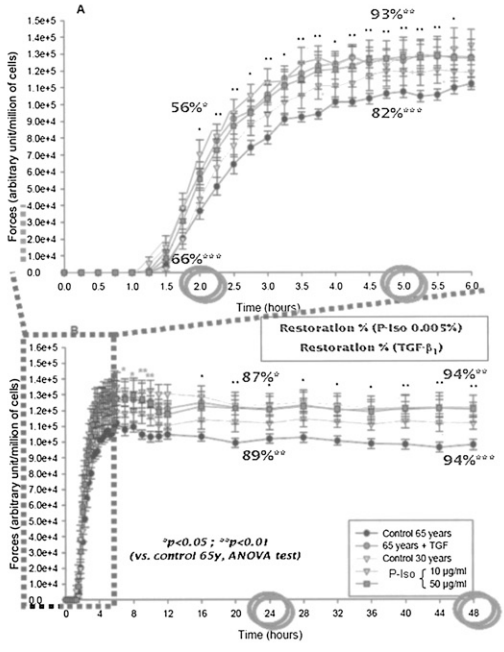

Figure 1: Contractile forces (arbitrary unit/million of cells) developed by 30 years old fibroblasts or by 65 years old-fibroblasts treated or not by TGF-β_1 or P-ISO

Finally, anti-wrinkle efficacy (vs. lipopetide), improvement os skin ekasticity and "repulping" efficacy (*i.e.* increase in the check volume; both *vs.* placebo) could be demonstrated on Caucasian women after twice-a day-topical applications of a 1% P-ISO formulation on the face.

CONCLUSION

Taken together, these results confirm the anti-age efficacy of P-ISO and suggest that this ability is strongly correlated to its ability to act on both the dermal compartment and the hypodermis (action at the epidermal level was also demonstrated in another study). Indeed, in our experimental conditions, P-ISO was able to partially restore fibroblast biomechanical forces as well as preadipocyte differentiation capacity. In the future, signalling pathways regulating such aging-induced and P-ISO-restored biological processes could be investigated.

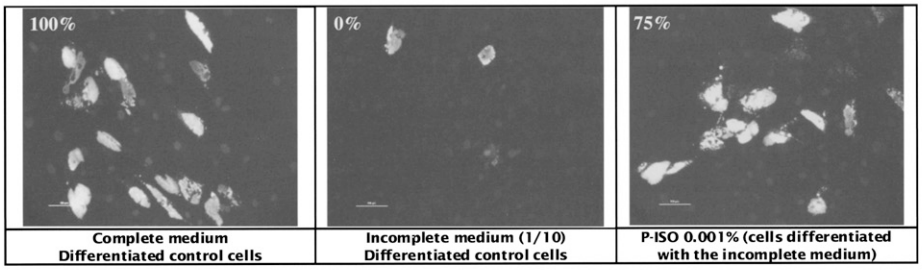

Figure 2: Visualization of the differentiation state of human preadipocytes (lipid droplet staining)
green: bodipy FL staining (lipid droplet tracer), blue: Hoechst reagent (nuclei), X20 objective. Results are expressed as mean differentiation restoration percentages

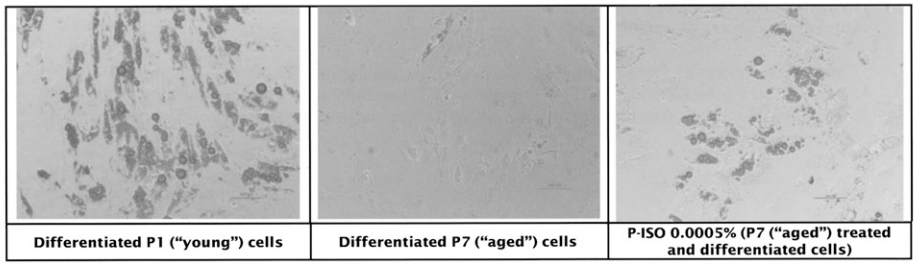

Figure 3: Visualization of the effect of Voluform on female senescent preadipocytes differentiation (lipid and triglyceride staining with Red-Oil) red: lipids and triglycerids revalation, phase contrast, X20 objective.

SKINCARE APPLICATION FOR A NOVEL CYCLOPEPTIDE

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INTRODUCTION

Specific amino acid sequences are responsible for modulating chemical, biological and regulatory processes in nature. A series of amino acids bonded together form a peptide. The ability to create synthesized peptides has opened up a new field of modulating peptides available to the skin care industry. Most commercially available peptides have an "open chain" conformation peptide structure. Disadvantages of the open chain structure are reduced efficacy related to a rigid conformational structure and ease of peptide digestion by proteases at the site of intended action. The cyclic form makes the peptide more selective to the desired target and is believed to provide enhanced efficacy by promoting normalization of the extracellular matrix components of skin. We have designed a smart and highly selective peptide with an arginine-glycine-aspartic acid (RGD) amino acid sequence (Figure 1- Cyclopeptide-5). The RGD motif promotes repair of the extracellular matrix components (ECM) at the dermoepidermal junction zone (1). Located at the interface of the epidermis and dermis, the region contains collagen proteins, laminin, fibronectin, elastins and other components responsible for structural support of skin. Chronological aging is associated with decreased production of ECM-derived collagen (2, 3). The RGD sequence signals epidermal keratinocytes through integrins, which are cell surface receptors. The objective of this research was to conduct various *in vitro* and *in vivo* studies to confirm the hypothesis that the cyclic structure with the RDG motif can provide enhanced skin care benefits.

METHODOLOGY:

Peptide binding to integrins, an in house study: Integrins are a large family of proteins responsible for cell migration, cell-cell communication and ECM interactions. They are composed of α- and β- chains with large extracellular domains and short cytoplasmic tails. Fibronectin, an ECM protein binds to the integrins resulting in intra-/extra-cellular signaling, ultimately causing a conformational change of the integrins to generate the cell signal. Results of competition studies with isolated integrins αvβ3, αvβ5 and αvβ6 are shown in Table 1. Biotinylated human native ligand vitronectin in the presence of serially diluted RGD cyclopeptide was added to a 96-well plate. After 3 hours at 37° C, bound ligand was detected using alkaline-phosphatase labeled anti-biotin MAbs. Detection instrument; ELISA-Reader at 405 nm. For αvβ3, tissue gene was αvβ3 integrin ΔTM, for αvβ5, tissue gene was αvβ5 integrin ΔTM, for αvβ6, tissue gene was αvβ6 integrin ΔTM. For each tissue, human baculovirus was used, Table 1 shows data obtained.

Effect on skin surface topography, *in vivo* study: 20 healthy female volunteers with normal skin were evaluated during a 28 day study for improvement in skin roughness. PRIMOS was used to measure increase in skin smoothness. ANOVA and Tukey were used for statistical analysis of resulting data. Observed results are shown in Figure 2.

Conclusion: Analysis of the referenced studies help to confirm the beneficial effects of our cyclic homodetic peptide based on the RGD binding site of the extracellular matrix protein modeling. The specific ligand-receptor interaction promotes activation of certain signaling pathways and targets involved in skin aging,

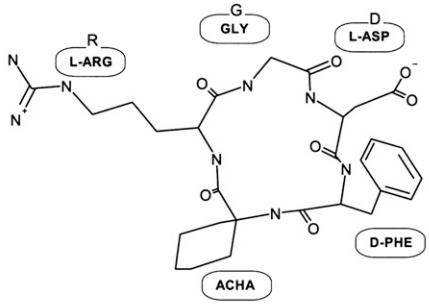

Figure1:Cyclopeptide-5 consists of five amino acids. RGD is the letter code for arginine, glycine and aspartate respectively. D-PHE is D-phenylalanineand ACHA is the abbreviation for aminocyclohexane carboxylic acid, a synthetic amino acid.

Integrin	αvβ3	αvβ5	αvβ6
IC_{50}	2.3 +/- 0.8 nM	700 +/- 0.8 nM	1.2 uM

Table 1: Results of integrin competition studies. ECM proteins bind to specific integrins resulting in cell signaling

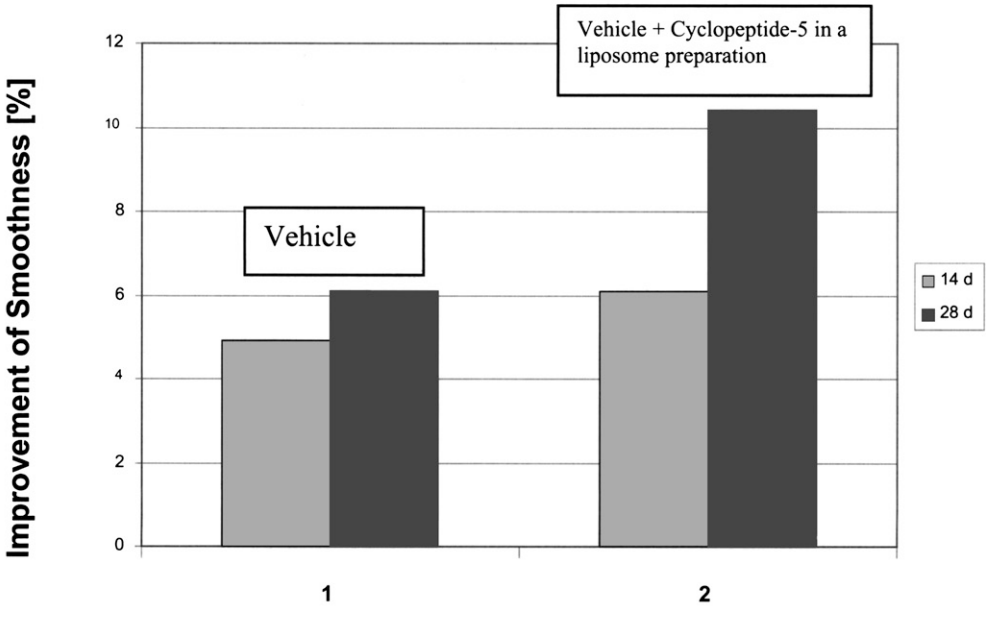

Figure 2: Improvement of Smoothness [%]. *In vivo* 28 day skin study using PRIMOS. ANOVA analysis indicated a statistically significant improvement in skin smoothness compared to untreated controls.

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BENEFICIAL REGULATION OF EXPRESSION OF EXTRACELLULAR MATRIX PROTEINS AND TRANSFORMING GROWTH FACTOR-β IN DERMAL FIBRBLASTS, AND EPIDERMAL KERATINOCYTES BY *POLYPODIUM LEUCOTOMOS*

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Skin Aging:

Ultraviolet (UV) radiation and intrinsic aging are the major reasons for skin aging. The clinical manifestations of skin aging such as wrinkles, sagging, and laxity are largely due to alterations in extracellular matrix (ECM). The primary structural extracellular matrix proteins are collagen and elastin, remodelled by matrixmetalloproteinases (MMP) and elastases. The fibrillar collagens, in order of predominance types I, III and V, provide structure whereas elastin forms elastic fibers with fibrillin to give skin structural firmness and elasticity. The atrophy of collagen and elastin fibers in skin aging is from reduced synthesis, and the increased expression or activity of MMPs. In addition, there is reduced expression of the cellular MMP inhibitors, tissue inhibitor of metalloproteinases (TIMPs) in skin aging. A primary beneficial regulator of the ECM is transforming growth factor-β (TGF-β). The effects of TGF-β are down regulated by UV radiation. The ECM structural or proteolytic proteins and TGF-β are produced by epidermal keratinocytes and dermal fibroblasts. Products that directly or cellularly inhibit MMPs and elastase while simultaneously stimulating TIMPs, collagen, elastin, fibrillin and TGF-β are ideal in counteracting skin aging.

P. leucotomos:

Polypodium leucotomos L or calaguala (Polypodianceae) is a tropical fern plant typical of Central America, which is rich in polyphenols. Its antioxidant and anitinflammatory properties lend to photoprotection. *P. leucotomos* is marketed as Difur® for the treatment for psoriasis and as Fernblock® for photoprotection. Our initial research on *P. leucotomos* demonstrated predominant stimulation of elastin and inhibition of MMP-1, lipid peroxidation and membrane damage in fibroblasts and keratinocytes[1].

Research Objectives and Methods:

We have extended *P. leucotomos's* photoprotective mechanism by determining the efficacy of *P. leucotomos* extract to (a) directly inhibit MMP-1, 2, 3, 9 and elastase activities by enzyme inhibition kinetics with respective substrates following incubation with or without *P. leucotomos*, (b) inhibit MMP-2, and stimulate TIMPs, fibrillar collagens and TGF-β in non-irradiated or UV radiated fibroblasts, and (c) stimulate expression of fibrillins (-1, -2), TIMPs (-1, -2), and TGF-β in non-irradiated or UVB radiated keratinocytes[2,3]. For experiments with

fibroblasts or keratinocytes (b, c), cells were non-irradiated or exposed to UV radiation and subsequently dosed without (control) or with *P. leucotomos* extract for 24 hours. The cells were examined for cell viability, and the media for the expression of collagen, fibrillins, TIMPs and/or TGF-β protein level. Fibroblasts were transfected with type I collagen-promoter plasmids to examine transcriptional regulation by *P. leucotomos* in non-irradiated or UV radiated cells.

Results and Discussion:

P. leucotomos directly inhibited the activities of MMPs and elastase[2,3]. It inhibited the expression of MMPs while stimulating the expression of TIMPs in fibroblasts and keratinocytes[2,3]. *P. leucotomos* stimulated types I, III and V collagen in non-irradiated fibroblasts, and types I and V collagen in UV radiated fibroblasts[2]. *P. leucotomos* had predominant stimulatory effects on TGF-β expression in non-irradiated or UV radiated fibroblasts and keratinocytes[2,3]. Further, it stimulated fibrillin expression in keratinocytes[3]. The effects of *P. leucotomos* were similar to vitamins C or E except in the regulation of TGF-β, which was not regulated by the vitamins[2,3].

P. leucotomos demonstrates dual protective effects on the extracellular matrix via its direct or cellular inhibition of MMPs, and the stimulation of the collagens, TIMPs, fibrillin and TGF-β. The effects of *P. leucotomos* may be partly via its regulation of TGF-β expression and partly via its antioxidant property. The intake or topical application of *P. leucotomos* may be beneficial to skin aging prevention or treatment.

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