

**Papers Presented at the 2003 Annual
Scientific Meeting and Technology Showcase
(Thursday's Program)**

**December 4–5, 2003
New York Hilton
New York, NY**

EVIDENCE AND UTILITY OF MELANIN DEGRADING ENZYMES

Thomas Mammone, Ph.D., Ken Marenus, Ph.D., Neelam Muizzuddin and Daniel Maes, Ph.D.

Estee Lauder Research Laboratories, 125 Pinelawn Road, Melville, NY 11747

Abstract:

The biochemical synthesis of human melanin is understood in some detail. However, little is known about melanin degradation and catabolism of melanin. We hypothesize that human skin contains enzymes that degrade melanin and these enzymes can be used to reduce skin color.

To test this hypothesis, HaCaT keratinocytes and normal human keratinocytes in culture were pulse labeled for one hour with radiolabeled synthetic melanin. This melanin was synthesized in vitro using tyrosinase enzyme from mushrooms and using radiolabeled [^{14}C] 3,4-dihydroxyphenylalanine (DOPA) as a substrate. After the initial pulse labeling, samples of both the cells and media were taken at 2, 4, 6 and 18 hours. Over these time periods the counts remaining in the media and cell fraction were significantly decreased. This data suggests the need for new protein synthesis and the lysosome organelle function for the degradation.

Melanin degrading extracts isolated from *Aspergillus fumigatus* and *Saccharomyces cerevisiae* were applied to human skin. These extracts cause significant reduction in UVB induced pigmentation. These extracts may be useful in developing new whitening products to even skin color and tone.

INTRODUCTION:

Previously, Ohtali and Seiji [1] investigated the in vitro degradation of melanosomes and melanin in mouse liver. These authors discovered that melanosomes are degraded by the lysosomes, but that melanin is not appreciably degraded.

These experiments were conducted with B16 mouse melanoma derived melanosomes, which may be more difficult to digest. Also mouse liver lysosomes were used and this may not be representative of all tissues. In addition, the duration of incubation with lysosomes was 60 minutes in these experiments and may not have been entirely relevant in the case of melanin in the skin. We have investigated human skin keratinocytes, which would be a physiologically relevant site for a melanin-degrading enzyme.

Later investigators reported that lysosomal extracts from human skin were unable to degrade natural melanin [2] and therefore it must be shed intact from the epidermis in squames. These authors also did not observe any changes in melanin injected into guinea pig skin. Although melanosomal proteins and lipids did appear to decrease with time in the guinea pig skin.

However, we have observed that synthetic radiolabeled melanin, when placed in cultures of human keratinocytes, was lost with increasing time after the initial labeling period.

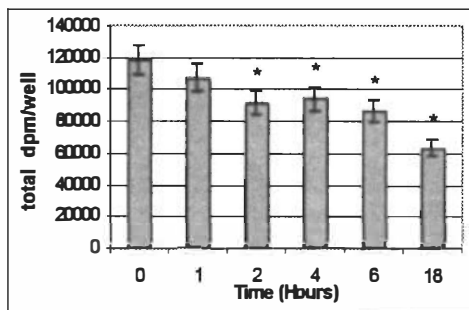


Figure 1. Pulse labeling of HaCaT cells with [^{14}C]-Melanin. Total labeling in dpm per well at times 0, 1, 2, 4, 6, and 18 hours post label. Data is the average dpm/well of three wells and representative of three experiments. * $p < 0.05$ compared to time zero.

In addition, we have used ^{14}C DOPA labeled melanin isolated from melanocyte for the pulse labeling of HaCaT keratinocytes and observed similar decreases in counts over time (data not shown). However, natural melanin is also known to contain various proteins and these may still be protease sensitive sites.

Since melanosomes are known to be engulfed by keratinocytes and partitioned into lysosomal bodies, we hypothesized that lysosomal inhibitors would suppress this degradation. Chloroquine, one such lysosomal inhibitor was observed to suppress the degradation and incorporation of synthetic melanin. This process requires new protein synthesis since cycloheximide was found to inhibit it. This further supports the theory that melanin is degraded in lysosomes

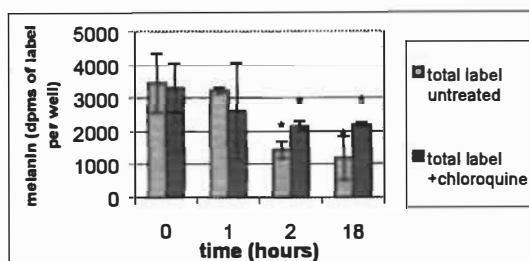
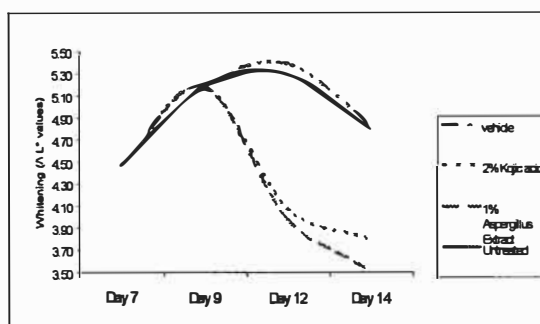


Figure 2. Effects of cycloheximide on radiolabeled melanin content in HaCaT cells. HaCaT cells were treated with 0, 1, 2, and 4 μ g/well of cycloheximide. Total counts in cells and media counted at time zero and 24 hours. Data is the average dpm/well of three wells and representative of three experiments.

Microsomal membrane prepared fractions from HaCaT cell cultures demonstrated the ability to solubilize synthetic melanin. This enzyme appears to localize to the lysosomal fraction and have a pH optimum of pH 4 (data not shown). The protein nature of this enzyme is further supported by the demonstrated heat inactivates this extract.



To test for the effects of a melanin degrading enzyme on skin color, we investigated the topical application of a fungal melanase. *Aspergillus fumigatus* extracts were applied to human skin that had previously been irradiated with UVB. On kojic acid, a known inhibitor of tyrosinase, and the *Aspergillus* extract both reduce enhanced skin color loss after UVB. The above data supports the hypothesis that human skin degrades melanin and this process contributes to skin color.

References:

1. Ohtaki, N., Seiji, M. (1971) Degradation of melanosomes by lysosomes. *J. Invest. Dermatol.* 57:1-5.
2. Saito, N., Seiji, M., (1976) Degradation of melanosomes in vitro and in vivo. *Pigment Cell.* 3:384-392.

EFFECT OF DEOXYARBUTIN ON MELANOGENESIS: IN VIVO COMPARISON WITH OTHER MELANOGENESIS INHIBITOR

Saja H. Hamed, Penkanok Sriwiiyanont, R. Randall Wickett, Ph.D. and Raymond Boissy, Ph.D.

University of Cincinnati College of Pharmacy and Department of Dermatology, Cincinnati, Ohio

Introduction:

Skin color is mainly determined by the amount of melanin in the epidermis. Melanin biosynthesis occurs in specialized organelles called melanosomes, which are synthesized within the melanocytes; dendritic cells of neural crest origin that resides in the basal layer of the epidermis (1). Through dendritic processes, melanocytes transfer pigmented melanosomes into neighboring keratinocytes.

Tyrosinase, an enzyme present within melanosomes catalyses the initial step in the conversion of tyrosine to melanin (1). Competitive inhibitors of tyrosinase (hydroquinone, kojic acid, and arbutin) are currently being used in drugs to ameliorate hyperpigmented lesions and in cosmetics to lighten skin complexion. Although these skin lightening products have been available for several decades, their success has been somewhat limited for two basic reasons: safety or overall effectiveness.

Therefore, to create a better and non-irritating tyrosinase inhibitor, a rational design approach was used that evaluated structure-function relationships of tyrosinase inhibitors. One designed compound, deoxyarbutin (dA), demonstrated effective skin depigmenting properties using a black guinea pig model.

The research project in this report seeks to ascertain the effectiveness of deoxyarbutin in inhibiting melanin synthesis within human melanocytes and its effectiveness in inhibiting hyperpigmentation of human skin grafted to athymic mice. Human cutaneous xenografts placed on athymic mice survive permanently (2). Thus athymic animals are an excellent model for studying various aspects of human skin including hyperpigmentation of grafted skin on burn patients.

Methodology:

Xenografting:

Female ICR-SCID (Taconic, NY) kept under pathogen-free condition (Children's Hospital Research Foundation, Cincinnati, OH) were shaved with an electric clipper to remove the dorsal hair. The mice were anesthetized by isoflurane/oxygen (3%/0.8 liter). The dorsal site was cut to produce a wound bed of approximately 2.0-3.0 in diameter. Fresh split-thickness cadaveric skin (Ohio Valley Tissue and Skin Center, OH) from a caucasian donor was sutured in place with a reversed cutting precision monofilament PS-3, 6-0 (Moore medical, CT). Grafts were left untreated for two months during which time hyperpigmentation occurred. Stability of subsequent hyperpigmentation was assessed on weekly intervals using Charmview system. Treatment was initiated when no further increase in pigmentation was observed. Animals were randomized according to their L value among four treatment groups (deoxyarbutin (dA), hydroquinone (HQ), 4-tertry butyl phenol (4-TBP) and control group). Each group had 4 mice.

Treatment was applied at 5% concentration 5 days per week for 8 weeks. Treatment sites were assessed on biweekly basis for degree of pigmentation using Charmview system. This system takes enlarged digital photos for the treatment sites and then the color parameters for these images (L, a, b) were obtained by using Photoshop software (Adobe Systems Inc., San Jose, Calif.). The L* a* b system is recommended by the CIE (Commission Internationale de l'Eclairage) for skin color assessment. In this project the L value which represents the Luminance was used to assess the lightening effect of the applied treatments.

Cytotoxicity: Cultures of normal human melanocytes was established from individual neonatal foreskins that were obtained from the nursery of University Hospital after routine circumcision. Established melanocytes from a light and a dark skin donor were treated with fresh growth media containing various dosages of test compounds for 5 days. On the 6th day, cell number was determined by direct counting using the Coulter Counter.

In Situ (Intact) Tyrosine Hydroxylase Assay: Cultured human melanocytes were treated in triplicate with fresh growth media containing various dosages of test compounds for 5 days. On the 5th day cells were fed with fresh media containing 1μCi/ml of H³-tyrosine (Amersham Pharmacia Biotech, Piscataway, NJ). Radioactivity of the tritium water in the elute was counted in a Packard 1600 CA liquid scintillation analyzer. Tyrosinase activity was expressed as DPM/24 hours/μg protein.

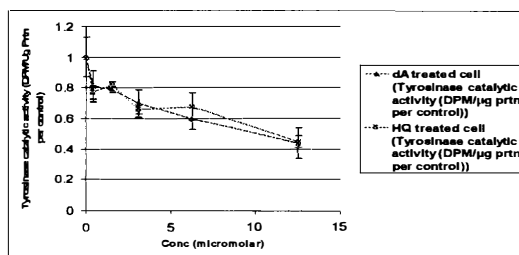
Results:

Deoxyarbutin was less cytotoxic than hydroquinone by 4 fold on both light and dark human melanocytes (Table 1). Further more deoxyarbutin was as effective as hydroquinone in inhibiting *In situ* tyrosinase activity of dark human melanocytes at equimolar concentrations (Figure 2).

Table 1. Concentration (μM) of each test compound that lead to 95% viability of dark and light human melanocytes.

Compound	Dark human melanocytes	Light human melanocytes
hydroquinone	0.391	1.5625
deoxyarbutin	1.5625	6.25
4-tertry butyl phenol	50	50
kojic acid	50	50
arbutin	50	50

Figure 2. Effect of 5 days of treatment with deoxyarbutin and hydroquinone on *In situ* tyrosinase activity of dark human melanocytes.



Animal study showed that both deoxyarbutin and hydroquinone are able to reverse graft hyperpigmentation (Figure 3). However the mean value for Change in L from the baseline was higher for deoxyarbutin treated group at the 2 weeks treatment point compared to hydroquinone. Brown discoloration of the hair in hydroquinone treated mice was observed. Such brown discoloration was not observed in the deoxyarbutin treated mice.

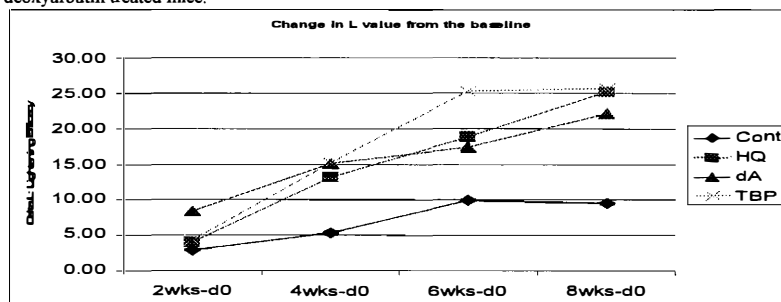


Figure 3. Time course of change in L value from treatment with Deoxyarbutin, Hydroquinone and 4-TBP

Conclusion: The ability of deoxyarbutin to inhibit pigmentation with reduced cytotoxicity relative to hydroquinone establishes deoxyarbutin as an excellent skin depigmentation agent.

References:

- Boissy RE and Nordlund JJ: Biology of Melanocytes, In: Arndt KA, LeBoit PE, Robinson JK, Wintrob BU (eds): *Cutaneous Medicine and Surgery: An Integrated Program in Dermatology*. Philadelphia, WB Saunders Co, pp. 1203-1209 (1995)
- Manning DD, Reed ND, Schaffer CF: Maintenance of skin xenografts of widely divergent phylogenetic origin of congenitally athymic (nude) mice. *J Exp Med* 133: 488-494 (1973)
- U.S patent no. 6,068,834 (5/30/00)

**FRONTIERS OF SCIENCE AWARD LECTURE
SPONSORED BY COSMETICS AND TOILETRIES®**

The Enigma of Beauty

Jodi Cobb

National Geographic Magazine, Washington, DC

What exactly is beauty and why are we so obsessed by it?

Evolutionary scientists claim that recognition of beauty is an ancient, innate and universal biological means to ensure the survival of the species. They say beauty is an advertisement for your value as a mate, for the quality of your genes. Not everyone agrees. But we all believe we know it when we see it.

Standards of attractiveness are surprisingly universal. All cultures value symmetry, clear skin and thick shiny hair—markers of youth, health and fertility. But that's where the fun begins. From the exorbitant hair and makeup of the Huli wigmen of Papua New to the bizarre tradition of footbinding in China, humans have gone to obsessive, humorous and absurd lengths to achieve beauty, or prolong it or fake it. Or exploit it. Jodi Cobb, a staff photographer at National Geographic for over 20 years who has worked in more than 55 countries, will take you on a trip around the world and through the looking glass in search of answers to the enigma of beauty.

EFFECT OF HAIR COLOR ON LUSTER

Karin Keis, Ph.D., Ram Ramaprasad, Ph.D. and Yash Kamath, Ph.D.

TRI/Princeton, Princeton, NJ 08542

Introduction

The luster of hair is an essential quality for hair beauty. It is an optical phenomenon resulting from the specular reflection of light at the air-cuticle interface. So far many models of light reflection at air-cuticle interface have been described in the literature, however, there is no simple relationship between luster and the light reflected from hair (1-6). The large interest in luster has resulted in a large number of studies on the effect of various cosmetic products on luster and development of variety of methods for measuring it (1-12). For natural hair colors, such as blond, brown and black, the luster is the highest for the one with the darkest hue, explained by the low intensity of the diffusely scattered light, while the intensity of the specularly reflected light remains the same. In this work, the interest is in the instrumental evaluation of luster of hair dyed with different colors and depths of shade, to demonstrate the effect of artificial color on luster. Also, we make an attempt to study the effect of hair color on subjective evaluation of luster.

Materials and Methods

The naturally unpigmented Piedmont hair was dyed for 5, 20 and 45 minutes with commercially available semipermanent dyes. The colors were chosen to cover the extremes and middle of the visible spectrum. Also, the pure single-component dyes were used to saturate Piedmont hair fibers by dyeing under specific conditions. Using a UltraScan XE spectrophotometer equipped with integrating sphere, the total reflectance spectra and CIELAB color parameters were obtained. From measured L^* , a^* and b^* parameters the changes in chroma, total color, lightness and hue were calculated. Luster was measured by using a modified Brice-Phoenix goniophotometer (GP) to record the intensity of scattered light as a function of angle. Measurements were carried out on 30 randomly chosen single hair fibers, for each color and dyeing time. He-Ne laser with a wavelength of 632 nm and white light from the Quartz Tungsten Halogen lamp was used as light sources. The dye penetration profiles on cross-sections of hair fibers were examined by optical microscope.

Results and Discussion

The largest change in reflectance curves was found to occur between 0 and 5 minutes of dyeing time. Thereafter the decrease in total reflectance is small indicating that additional dye uptake is small. Correspondingly, most change in luster occurred between 0 and 5 minutes dyeing time (see Figure 1). Even though the dye penetrates the fiber completely during 45 minutes, the dye concentration in cuticular sheath is much higher compared to fiber interior. The low color uptake is related to weak polar and van der Waals interactions of semipermanent dyes with hair.

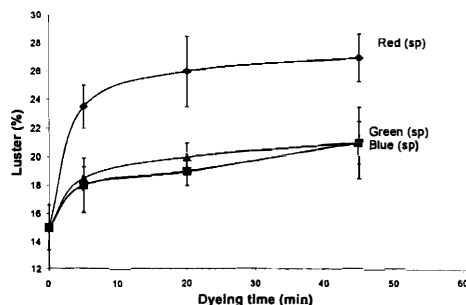


Figure 1. Luster for various dyeing times for different semipermanent colors (illumination $\lambda=632$ nm).

Luster by instrumental detection depends on spectral reflectance of the hair, spectral power distribution of light source and eventually on spectral response of the detector. Goniophotometric measurements when using a monochromatic illumination source serve as a sensitive probe of fiber absorptive and scattering properties. GP curves in Figure 2 show that the diffuse components for Piedmont hair colored with semipermanent and pure dyes to various colors are different. Thus, the luster values are different for hair colored to different colors. Results show the complexity when comparing the luster of colored hair from goniophotometric curves, arising from variations in dye composition, concentration and its penetration depth into the fiber. Semipermanent dyes namely consist of combination of various dyes (aromatic amines, amino nitrobenzenes and anthraquinone derivatives) with different affinities. On the other hand, pure dyes serve as an example of single component dyes with homogeneous dye distribution throughout the fiber. In order to analyze the effect of above mentioned parameters on scattering and absorptive processes, the expression for the dependence of diffuse reflectance on dye absorbance and the path length of the dyed region within the fiber has been derived. According to this equation, diffuse reflectance is reduced by dyes having higher extinction coefficient and are capable of penetrating the fiber completely. Such hair colors will increase luster.

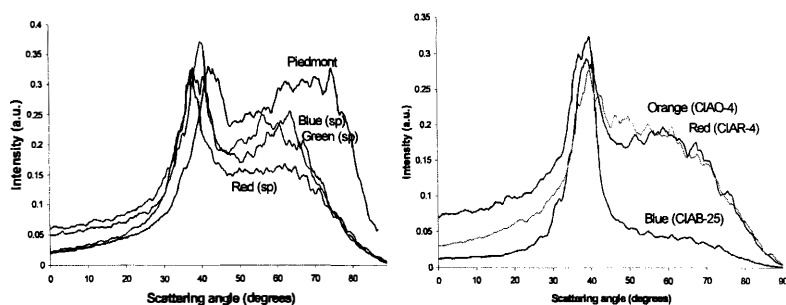


Figure 2. GP curves for Piedmont hair dyed with multi-component semipermanent (left) and single-component dyes (right) to various hues (illumination wavelength 632 nm).

Ideally, under the illumination with a broad homogeneous spectral power distribution light source, the luster by instrumental detection should not depend on hair color as long as the dyeing level and dye distribution within the fiber is the same. Beside the spectral power distribution of the light source and reflectance of the object, the perceptual description of color depends on spectral sensitivity of the eye. Taking into account that the eye of a person with normal vision is not equally sensitive over the entire visible spectrum, the expression for perceived luster is derived. From the theoretical considerations under given assumptions, the luster from hair of chromatic colors is perceived differently by the human eye.

References

- (1) R. F. Stamm, M. L. Garcia and J. J. Fuchs, *J. Soc. Cosmet. Chem.*, **28**, 571-599 (1977).
- (2) W. Czepluch, G. Hohm and K. Tolkiehn, *J. Soc. Cosmet. Chem.*, **44**, 299-317 (1993).
- (3) H. K. Bustard and R. W. Smith, *Applied Optics*, **30**, 3485-3491 (1991).
- (4) A. Guiolet, J. C. Garson, and J. L. Levecque, *Int. J. Cosmet. Sci.*, **9**, 111-124 (1987).
- (5) C. Reich and C. R. Robbins, *J. Soc. Cosmet. Chem.*, **44**, 221-234 (1993).
- (6) J. H. S. Rennie, S. E. Bedford and J. D. Hague, *Int. J. Cosmet. Sci.*, **19**, 131-140 (1997).
- (7) C. Scanavez, M. Zoega, A. Barbosa, and I. Joekes, *J. Cosmet. Sci.*, **51**, 289-302 (2000).
- (8) Y. Tango and K. Shimamoto, *J. Cosmet. Sci.*, **52**, 237-250 (2001).
- (9) S. Nagase, S. Shibuichi, K. Ando, E. Kariya and N. Satoh, *J. Cosmet. Sci.*, **53**, 89-100 (2002).
- (10) M. Okamoto, R. Yakawa, A. Mamada, S. Inoue, S. Nagase, S. Shibuichi, E. Kariya and N. Satoh, *J. Cosmet. Sci.*, **54**, 353-366 (2003).
- (11) F.J. Wortmann, E. Schulze zur Wiesche and A. Bierbaum, *J. Cosmet. Sci.*, **54**, 301-316 (2003).
- (12) R. McMullen and J. Jachowicz, *J. Cosmet. Sci.*, **54**, 335-351 (2003).

SYNERGISTIC EFFECTS OF HIGH MOLECULAR WEIGHT POLYETHYLENE OXIDE (PEO) AND CATIONIC CELLULOSIC POLYMERS ON CONDITIONING PROPERTIES OF HAIR CARE PRODUCTS

Wing Li, Susan L.P. Jordan, Xiaodong Zhang, Jennifer Amos and Cal Davis

Amerchol Corporation, A Subsidiary of The Dow Chemical Company

Summary:

A hair cleansing composition containing both high molecular weight PEO and cationic hydroxyethyl cellulose (HEC) was found to provide superior conditioning performance. Hair treated with a formulation containing both cationic HEC and high molecular weight PEO showed 30% better wet combing reduction than the formulation containing cationic HEC only. In conjunction with PEO, cationic HEC-dependent deposition of silicone oil and octyl methoxycinnamate (OMC) onto hair was enhanced 27% and 25%, respectively. When examined with a polarized microscope, the appearance of the polymer-surfactant complex (coacervate) of the diluted formulation differed in the presence of PEO. In particular, the particle size of the coacervate in the formulation containing both PEO and cationic HEC was smaller. This result indicates PEO reduces the size of the deposition precipitate by preventing the coacervate from agglomerating. Surface analysis also showed that the presence of PEO in formulations containing cationic HEC deposited insoluble actives more evenly on the hair surface.

Background

A conditioning shampoo should provide suitable cosmetic properties to hair, such as making it soft, lustrous, and easy to comb. Polymers and various types of benefit agents contribute to cosmetic properties. For example, silicones are insoluble actives that are commonly used as conditioning agents for hair. Silicones can effectively reduce combing friction of hair and provide a soft, smooth feel and a shiny appearance. UV inhibitors are becoming more commonly used in shampoo formulations for preventing hair color fading and damage. Cationic polymers are known to aid the deposition of such insoluble actives. They are believed to form a coacervate (polymer-surfactant complex) phase or be in a coacervate phase upon application and rinsing of the formulation (1,2). The formation and properties of the coacervate affect the combined deposition of insoluble actives and cationic polymers onto keratinous substrates (3,4). In addition, foam properties are important component of the consumer's perception of product performance.

High molecular weight polyethylene oxide (PEO) is a linear, nonionic, water-soluble homopolymer with molecular weight from 100K to 4M. These polymers have been increasingly used in cleansing systems such as shampoos and body washes because they are known to enhance foam properties such as foam volume and density. They also impart a soft feel to the cleansed skin and hair. This study was undertaken to examine the effect of PEO on cleansing formulations that contain cationic HEC. The interactions of high molecular weight PEO with high molecular weight cationic HEC were studied in two different cleansing systems: (A) 4% ammonium lauryl sulfate (ALS) / 13.5% ammonium laureth sulfate (ALES) / 2.6% cocamidopropylbetaine (CAPB) / 1% sodium chloride; and (2) 15.5% sodium laureth sulfate (ES-2) / 2.6% disodium cocamphodiacetate (DSCADA). Nonionic emulsion of high molecular weight polydimethylsiloxane and OMC were used for deposition study.

Experimental Results:

Foam volume and density measurement - Foam volume and density were quantitatively measured by washing hand and hair with prototype formulations. The formulation containing PEO increased the foam volume by 70% and produced denser and creamier foam than the formulation without PEO.

Wet combability - The wet combing force was measured by using the load cell of a Dia-Stron Miniature Tensile Tester (MTT). When a comb is pulled through a wet hair tress, reduction in total work done is associated with the removal of entanglements of the hair fiber. Commercial bleached blond hair was treated with formulations containing 0.3% high molecular weight, low charge (HL) cationic HEC with and without 0.1% PEO in both surfactant A and B. The hair treated with the formulation containing cationic HEC and PEO showed 30% better combing reduction force compared to the formulation containing HEC only.

Silicone deposition on hair - The total amount of silicone deposited on hair treated with a shampoo formulation containing 0.25% high molecular weight, high charge (HH) cationic HEC with and without 0.1% PEO was measured. Virgin brown hair was treated five times with the prototype formulation. The

silicone was extracted from the hair by a 50/50 (v/v) methyl butyl ketone / toluene solution, then measured using an atomic absorption spectrophotometer. The result shows cationic HEC aids in silicone deposition. The incorporation of PEO with cationic HEC demonstrates a 27% enhancement of silicone deposition in the same system over the formulation without PEO.

UV absorber deposition – European brown hair was treated with a prototype formulation comprised of 1% octyl methoxycinnamate (OMC), HH cationic HEC and PEO(300K) in surfactant B. The amount of OMC deposited was quantified by extracting the hair with isopropanol (IPA) and measuring the absorbance at 290 nm. The result showed that PEO increased OMC deposition from a prototype formulation by 25% over the regular shampoo system.

Coacervate study – Coacervate formation in surfactant B with 10X dilution was examined using a polarized microscope with the aid of Red Dye 80. The particle size of coacervate from the formulation containing HL cationic HEC and high MW PEO(600K) was significantly smaller (Figure B) than the coacervate from cationic HEC only (Figure A).

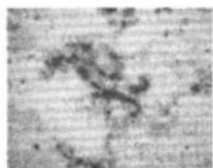


Figure A: Cationic HEC

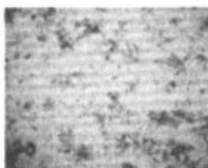


Figure B: Cationic HEC/PEO(600K)

Hair surface characterization – Kruss Tensiometer K-12 was used to measure the wetting force of a single hair fiber that had been treated with the shampoo formulation in surfactant B. The hair fiber was also examined by Scanning Electron Microscope (SEM). The wetting force in advancing mode of hair showed that the hair treated with shampoo containing cationic HEC, PEO and silicone had a smoother surface. This indicates the polymer complex is uniformly deposited on the hair surface. The SEM micrographs confirmed that PEO aids in more uniform deposition of the polymer complex onto the hair surface.

The results clearly show that PEO affects coacervate formation and reduces the size of the deposition precipitate by preventing the coacervate from agglomerating, thereby aiding uniform deposition of polymer and actives onto the hair surface. The highly lubricious film formed on the hair surface translates into very low combing resistance and to elimination of hair tangles during combing. In addition, PEO with cationic HEC also enhances the deposition of silicone and an UV absorber onto the keratinous substrates and improves the overall conditioning performance.

Conclusion:

High molecular weight PEO is widely recognized for its ability to improve foam quality and wet feel in cleansing systems. This study demonstrated the synergistic effect of high molecular weight PEO with cationic HEC in surfactant systems that enhance overall product performance. The combination of cationic polymer and PEO increased the deposition of insoluble actives, such as silicone oil and octyl methoxycinnamate onto both hair and skin leading to more efficient, uniform deposition.

References:

- (1) R.Y. Lockhead, *Conditioning Shampoo, Soap/Cosmetics/Chemical Specialties*, PP 42-49, October 1992
- (2) E.D. Goddard, *Polymer/Surfactant Interaction in Applied Systems*, in *Principles of Polymer Science and Technology in Cosmetics and Personal Care*, PP-181, Eds. E.D. Goddard and J.V. Grubber, Marcel Dekker, New York, 1999
- (3) M. Gamez-Garcia, *Polycation Substantivity to Hair*, IFSCC, Vol. 4, PP 99-107 (2001)
- (4) Wing Li and Susan L.P. Jordan, *Cationic Cellulosic Polymers with Multifunctional and Outstanding Performance for Personal Care*, Cosmetics and Toiletries Manufacture Worldwide, PP 1-4 (2003)

CATIONIC CONDITIONING-POLYMER DEPOSITS ON HAIR

Paquita E. Erazo-Majewicz, Ph.D. and Shu-Chun Su, Ph.D.

Hercules Incorporated, Wilmington, DE

Introduction - Microscopic and chemical methods, including SEM [1], ESCA [2], and AFM [3,4] have been used to study the deposition of conditioning polymers on hair. Recent developments with environmental SEM (ESEM)[5] have allowed hair samples to be examined under lower vacuum conditions, without desiccation or coating, making this technique overall less destructive than standard SEM. Confocal laser scanning microscopy (CLSM) offers the advantage of viewing the hair fiber surface under ambient conditions [6]. In addition, CLSM surpasses these techniques with the enhanced capabilities of optical sectioning and a fluorescent channel for viewing fluorescent polymer deposits and fluorescent probes applied to hair [6,7,8,9]. This fluorescence capability has been used on hair to create detailed graphical images of conditioning polymer deposits on human hair [9,10,11].

Objective - In this study, we characterized the deposits left on hair by the cationic conditioning polymers in Table 1 using 1) Natural Scanning Electron Microscopy (NSEM), 2) Confocal Laser Scanning Microscopy (CLSM), and 3) CLS Fluorescent Microscopy (probe: 5-carboxyfluorescein dye, Figure 1).

Table 1. Conditioning Polymers

Polymer	INCI	Backbone	Viscosity ^a	Mw ^b / kDaTon	Charge Density ^c mEq/g
A	guar hydroxypropyltrimonium chloride	galactomannan	3200 - 4200 cps @ 1% solids	High	1.5
B	guar hydroxypropyltrimonium chloride	galactomannan	4000 - 5000cps @ 1% solids	High	1.1
C	guar hydroxypropyltrimonium chloride	galactomannan	3000 - 4000 cps @ 1% solids	High	1.2
D	guar hydroxypropyltrimonium chloride	galactomannan	< 100 cps @ 10wt% solids	Low	1.2
E	Polyquaternium-10	hydroxyethylcellulose	300-500 cps @ 2% solids	Medium	1.5
F	Polyquaternium-10	hydroxyethylcellulose	300 - 500 cps @ 2% solids	Medium	1.1
G	Polyquaternium-7	diallyldimethylammonium chloride-acrylamide copolymer	7500 - 15000 cps @ 8.1-9.1% solids	High	1.2
H	Polyquaternium-44	quaternized vinylimidazole-vinylpyrrolidone copolymer	30000 cps @ 7.0% solids	High	1
I	No Polymer Control	-	-	-	-

a Viscosity information from supplier brochures correlates with molecular weight.

b Low Mw is less than 100,000; Medium Mw is 400,000-700,000; High Mw is greater than 1,000,000.

c Charge density measured by turbidimetric titration, except Polyquaternium-44 was taken from supplier literature.

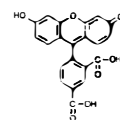


Figure 1.
5-carboxyfluorescein (5-FAM)

Experimental - Bleached European medium brown hair and virgin European medium brown hair were supplied as 12 inch tresses from De Meo Bros., New York. Tresses were washed with a 4.5% sodium lauryl sulfate solution prior to applying one of the following treatments:

- A) *Shampoo A*: 0.2wt% polymer in 12wt% SLES (sodium laureth sulfate (3EO)) + 3wt% CAPB (cocamidopropyl betaine), was used in the studies with bleached hair tresses.
Shampoo B: 0.4wt% polymer in 12wt% ALS (ammonium lauryl sulfate) + 3.9wt% ALES (ammonium laureth sulfate (3EO)) + 1.2wt% CAPB, was used in the studies with virgin European brown hair. To assess removal of polymer from the hair, second shampoo treatments were performed using Shampoo A, without polymer. Tresses were combed while wet and were then air dried at 72 deg. C and 50% relative humidity.
- B) *Conditioning treatment*: 0.2wt% polymer in deionized water for 4 minutes, rinsing for 1min @ 40 deg. C; deionized water rinse.

Sample Prep - For all microscopic analyses, hair samples were mounted on a clean glass slide with double-sided tape. For CLSM fluorescence imaging, hair samples that were treated according to hair treatment A or B were subsequently treated with a 15 μ M solution of 5-FAM in deionized water, at pH 6.5, for twenty minutes. Samples were then rinsed with deionized water and air-dried overnight, prior to analysis.

Microscopy - Natural scanning electron microscopy was carried out on a Hitachi S2460N Variable pressure Scanning Electron Microscope (NSEM). All confocal microscopy data was acquired on a Zeiss inverted 200M Axioskop equipped with a Zeiss 510 LSM NLO confocal microscope (Jena, Germany) using AIM software release v3.2.

Results and Conclusions

NSEM - The NSEM micrographs of bleached hair tresses in Figure 2 show the surface texture of the hair fibers treated with shampoos containing polymers A, D, E, and F to be visibly smoother than the control hair fibers in I. Samples treated with formulation I have a jagged, roughened cuticle structure. The surface of the hair samples treated with formulations containing polymers A, D, E, or F have a smooth, coated appearance, the edges of the cuticle visibly less jagged. At higher magnification, the coating appears to be a textured deposit, with larger concentrations near the cuticle edges. The deposit visible on the control sample I is spread over the cuticle surface with less concentration at the cuticle edge, suggesting that these textured deposits represent the surfactant or polymer+surfactant deposits left on the hair. The surface of the hair sample treated with polymer E appears to be more heavily coated, with thread-like trails of deposit remaining on the hair. Note that this polymer has the highest cationic charge density of all the polymers in Table 1. Estimated coating thickness increases in the order polymer D<A<E<F and apparent ease of removal by a second shampoo treatment shows the opposite trend, E>A>F>D. These results are consistent with charge density and polymer backbone type being the dominant factor in polymer deposition with molecular weight and charge density playing a role in buildup of this deposit from anionic shampoo.

CLSM - CLSM was used to study bleached hair fibers from the same tresses examined with NSEM. As with NSEM, the reflection image in Figure 3 of hair fibers from the control sample, I, shows a roughened cuticle surface. The reflection images for all hair fibers in Figure 3 treated with a shampoo containing a cationic polymer show an improvement in the smoothness of the cuticle surface, reflecting the conditioning performance of these cationic polymers. The level of coating between samples can only be estimated from this technique as following the order: polymer D<H<G<A<E. Ease of removal of the surface coating by a second shampoo treatment is shown by the reflectance images in Figure 4.

CLSM Fluorescence Images - Bleached and virgin European medium brown hair tresses were treated with 0.2wt% aqueous solutions of the polymers in Table 1. Tresses were then treated with 5-FAM, an anionic dye, intended to bind to cationic polymer deposits on the hair fibers. All tresses in Figure 5 show varying levels of fluorescence located mainly at the cuticle edges of the tresses, with the exception of the tress treated with polymer E. The fluorescence image for polymer E is a nonuniform deposit trailing down the hair fibers. A similar image was reported for polyquaternium-10 containing a covalently bound fluorescent chromophore [12]. The location of the fluorescence at the edges of the cuticle for polymers A-G correlate with the NSEM results, showing a concentration of a deposit at the cuticle edges. The results for polymer E correlate with both the NSEM and CLSM images, showing a deposit along the hair fiber for polymer E. In a second study with virgin European brown hair and shampoo formulation B, concentration of the fluorescence at the cuticle edges was again observed for polymers A, B, D, F, and G. These results support the interpretation that the deposits at the cuticle edges observed in NSEM and in CLS fluorescent microscopy are derived from cationic polymer and surfactant.

References

1. S. B. Ruetsch, Y. K. Kamath, H.-D. Weigmann, *J. Cosmet. Sci.*, **54**, 63-83, 2003
2. E. D. Goddard and W. C. Harris, *J. Soc. Cosmet. Chem.*, **38**, 233-246, 1987.
3. P. Hossel, R. Dieing, R. Norenberg, A. Fau, R. Sander, *International Journal of Cosmetic Science*, **22**, 1-10, 2000
4. V. F. Monteiro, A.S. Pinheiro, E. R. Leite, J. A. M. Agnelli, M. A. Pereira-Da-Silva, E. Longo, *J. Cosmet. Sci.*, **54**, 271-281, 2003
5. J. S. Dalton, G. C. Allen, P. J. Heard, K. R. Hallam, N. J. Elton, M. J. Walker, G. Matz, *J. Cosmet. Sci.*, **51**, 275-287, 2000
6. C. Hadjur, G. Daty, G. Madry, P. Corcuff, *Scanning*, **24**(2), 59-64, 2002
7. S. T. A. Regismond, Y.-M. Heng, E. D. Goddard, F. M. Winnick, *Langmuir*, **15**, 3007-3010, 1999

8. J. V. Gruber, F. M. Winnick, A. Lapierre, N. D. Khaloo, N. Joshi, P. N. Konish, *J. Cosmet. Sci.*, **52**, 119-129, 2001
9. H.-D. Weigmann, Y. K. Kamath, S. B. Ruetsch, P. Busch, H. Tesmann, *J. Soc. Cosmet. Chem.*, **41**, 379-390, 1990
10. M. L. Tate, Y. K. Kamath, S. B. Ruetsch, H.-D. Weigmann, *J. Soc. Cosmet. Chem.*, **44**, 347-371, 1993
11. S. Jindal, Presentation at the *HBA Global 2002 Product Development Conference*, 2002
12. M. Gamez-Garcia, J. V. Gruber, F. M. Winnick, B. R. Lamoureux, *Proceedings XXIst IFSCC International Congress 2000*, Berlin, 176-183

Fig 2 Natural Scanning Electron Microscopy Images of Bleached European Medium Brown Hair after Shampoo Treatment

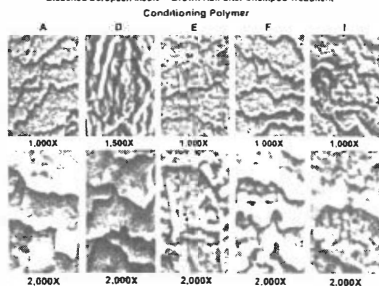


Fig 3 Confocal Laser Scanning Microscopy Reflected Light Images of Bleached European Medium Brown Hair after Shampoo Treatment

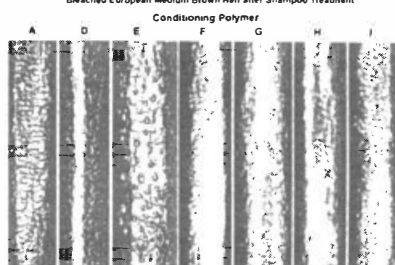


Fig 4 Confocal Laser Scanning Microscopy Reflected Light Images of Shampooed, Bleached European Medium Brown Hair after 2nd Shampoo

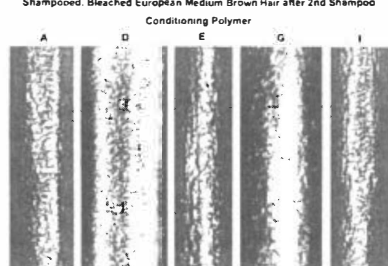


Fig 5 Confocal Laser Scanning Microscopy Fluorescence Images of Bleached European Medium Brown Hair after Polymer and S-AM Treatment

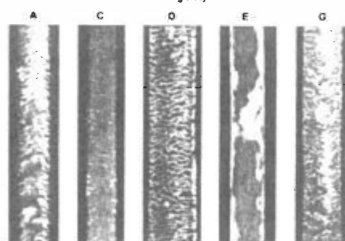


IMAGE ANALYSIS OF HAIR TREATED WITH STYLING PRODUCTS

Roger McMullen and Janusz Jachowicz, Ph.D.

International Speciality Products, Wayne, New Jersey

Introduction

In a previous report [1], we described the measurements of luster for various types of hair by quantifying the intensity of light reflected from hair fibers spread over a cylindrical surface and illuminated with a collimated beam of light. Such an experimental set-up allows one to obtain reflected light distribution curves similar to those typically generated by goniophotometers. By analyzing the shape of the reflection curves and by calculating luster parameters we were able to investigate the effect of polymer and oil treatments on the luster of hair. In this paper we have explored the application of Image Analysis to analyze hair treated with complete cosmetic formulations such as hair gels, hair creams, hair sprays, and hair conditioners which were applied to hair in a manner similar to that employed by an average consumer. We have performed measurements on both idealized geometrical arrangements of straight hair spread over a cylindrical surface as well as on a free hanging hair tresses illuminated by a collimated light. Image analysis also allowed us to quantitatively characterize the luster of tresses with hair types characterized as frizzy, curly, and very curly hair before and after treatment with hair care products. Curly and very curly hair was studied by measuring light reflection profiles, which allowed for the calculation of specular/diffuse light intensities and luster parameters. All three types of hair were also studied using an image threshold technique, which permitted ranking them according to the absolute and relative content of specular reflections.

We have also used images of hair fibers spread over a cylindrical surface and illuminated with a collimated light to analyze subtle color changes in hair treated with colorless styling products. This was achieved using the image processing package, Adobe PhotoShop. Using such an approach, it is possible to obtain histograms of the various components of light (blue, red, and green as well as the total luminance) in an image obtained under strictly defined and reproducible illumination and geometrical conditions.

Methods

Hair Samples

Luster analysis was performed on straight, natural light brown and dark brown hair of Caucasian origin purchased from IHI & Products, Inc. (Valhalla, NY). In addition to this, we utilized frizzy, curly, and very curly hair of Caucasian origin supplied by IHI & Products, Inc. (Valhalla, NY). Hair samples were pre-cleaned with a 3% ALS solution and thoroughly rinsed prior to experimentation.

Luster Measurements

The luster evaluation apparatus and procedures used in this study were described previously [1]. An Olympus Camedia E10 digital camera with a front-element auxiliary macro lens was employed to collect images of illuminated hair, typically one or two tresses, from a distance of 10 inches. We scanned the light intensity parallel to the fiber axes of a hair tress by employing Image Analysis software (Sigma Scan Pro 5.0).

Image Color Measurements

Analysis of image histograms was accomplished by importing the images obtained for luster analysis into Adobe Photoshop 6.0. Histograms for luminosity, red, green, and blue were obtained and the positions of peak maxima were recorded.

Results

We sought to characterize the changes in luster and color produced by applying to hair a series of products which included a gel, hair spray, cream lotion, and a conditioner. The products caused a change in the intrinsic luster of hair as measured by a method involving the use of straight hair in circular configuration. This change was quantified by determining two well known luster parameters, L_{Stamm} and $L_{\text{Robbins-Reich}}$, on both dark brown and light brown hair. The results suggested that the use of light brown hair can underscore the luster enhancing effect of films, deposited by cosmetic products, on the surface of hair.

Luster can be also affected by hair style geometry. Thus, we have also investigated the luster of hair in its natural configuration i.e. in the form of free hanging tresses before and after treatments with styling products. The hair types examined consisted of Caucasian frizzy, Caucasian very curly, and Caucasian curly hair. Using the products mentioned above, the luster of curly hair, for example, can be increased by reducing the number of frizzies and increasing the concave and convex specular reflection which is intrinsic to the tress due to the curliness of the hair. Figure 1 provides an example of luster analysis performed on very curly hair.

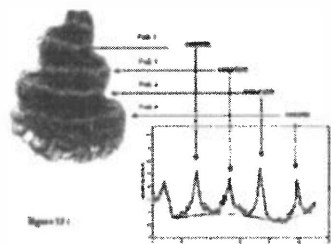


Figure 1. Plot of luminance as function of distance for very curly hair, where the outlined area in each image was used as the sampling area.

Figure 2, on the other hand, illustrates the color analysis of a light brown hair image obtained under controlled illumination and geometry for straight hair spread on a cylinder. Histograms (plots of pixel quantity as a function of color value, which scale from 0 to 255) for red, green, and blue tones as well as luminosity were generated and the positions of peak maxima were recorded. It is demonstrated that different types of styling products can produce small optical effects on the surface hair leading to differences in hair coloration.

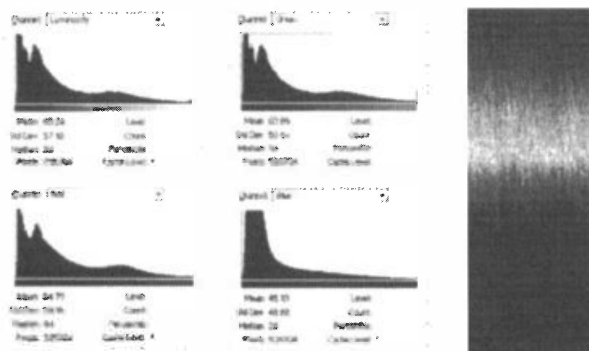


Figure 2. Image histograms for untreated light brown hair.

References

- [1] R. McMullen and J. Jachowicz, "Optical properties of hair: Effect of treatments on luster as quantified by image analysis," *J. Cosmet. Sci.*, **54**, 335-351 (2003).
- [2] R. McMullen and J. Jachowicz, "Optical Properties of Hair – Detailed Examination of Specular Reflection Patterns in Various Hair Types", *J. Cosmet. Sci.*, accepted.

THE USE OF SILICONES AS A COLOR-LOCK AID IN RINSE-OFF HAIR CONDITIONERS

Sabrina Marchioretto*

Dow Corning S.A., Seneffe, Belgium

* This presentation is being made by Todd Ostergaard on behalf of Sabrina Marchioretto.

Synopsis

Based on a market study of existing hair care products and claims, a study was conducted to determine the efficacy of several relatively new silicone technologies in hair color preservation when delivered from rinse-off conditioner formulations.

Testing methodology was developed which evaluated the effect of intense UV radiation exposure, shampoo washing and rinse-off product treatments along with their contributions to the removal of permanent hair colorants. Color evaluation was completed using a spectrophotometer-colorimeter which measures L*, a* and b* values. The washing process was found to be the most significant factor in the removal of hair color. While UV exposure had a significant impact only after 90 hours of intense irradiation.

Results of the study concluded that the use of amino functional silicones along with other functional silicone materials provide enhanced color retention when delivered from a rinse-off conditioner formulation. It was also confirmed that silicone materials provide additional benefits beyond color retention including, shine, wet combing, reduced drying time, etc.

Introduction

There has been significant amount of work in the area of prevention of fading of natural and artificial hair dyes, as this is a stated market need from consumers. There are several published studies that address this subject (1-3). There have been several products introduced on the market that claim efficacy in color preservation.

This study focused on the contribution of silicones, when incorporated into rinse-off conditioner formulations, and the effect they have in preventing color loss of permanent colorants from hair fibers. In this study, a three-step approach was taken to determine the most appropriate methodology for evaluating the efficacy of silicones to prevent color loss from hair. The three phases consisted of 1) a market study of formulated hair care products that claimed color protection, 2) color loss methodology development and, 3) finally an investigation of silicone efficacy in hair color protection.

Market Study

A market study was completed which looked at current market products, product form penetration, product claims, and formulation ingredients that could possibly promote hair color longevity. The results of this investigation showed that the use of silicones is very high in both rinse-off and leave on hair care products with many including two or more silicones in the formulation. Products claiming color protection, however, focused on other dominating benefits such as shine and softness more than the color protection claim, which led the researchers to conclude that, the benefit of color protection has not been completely satisfied.

Method Development

Sample Preparation

Samples of partially bleached Caucasian hair, obtained from International Hair Importers were used for this work. Flat hair tresses of 15 cm in length and 4 ± 0.5 g were prepared for this investigation. Each tress was dyed using a leading commercial permanent hair colorant to help ensure color reproducibility. The particular shade used was a red hair color. A commercial non-silicone containing shampoo (0.4 g/gram of hair) was used for washing the tresses each washing procedure. The rinse-off conditioner was a base formulation that included a UV filter. This formulation was also applied at a level of 0.4g / g of hair.

Methods

An initial study was completed to determine the most significant effect for color loss from treated hair. Four different trials were conducted to determine the effect of 1) shampoo washing alone, 2) Intense UV exposure, 3) Shampoo Washing + UV exposure and, 4) Shampoo + Rinse-off Conditioner with UV filter included + UV exposure.

UV exposure was completed using a QUV Accelerated Weathering Tester at an exposure intensity of 0.89 W/m²/nm. The color measurement was conducted using a Spectrophotometer Colorimeter BYK Gardner. When testing silicone efficacy, each hair tress received a similar procedure using the commercial shampoo and rinsing, application and rinsing of the rinse-off conditioner formulation, a drying procedure and seven hours of intense UV light exposure. Each tress had a control zone that was protected from UV light exposure.

Results and Discussion

Initial methodology development showed that the most significant factor in color loss was from shampoo treatments of the hair tresses. The greatest discoloration occurred during the first shampoo wash after coloration in both L^* and b^* measurements. Investigation of hair color loss due to UV exposure showed that visual discoloration occurred after 90 hours of intense UV exposure. A combination of shampooing and UV exposure again affected the hair color after one shampoo treatment and 56 hours of UV exposure. A combination of shampoo, rinse-off conditioner with added UV filter and UV exposure showed color loss after the first wash but no color loss due to the UV exposure. This suggests that the UV filter may have prevented the color from fading.

The effect of silicone in preventing color degradation was investigated by incorporating various silicones at 2% active silicone into a rinse-off conditioner formulation and applying to tresses following a shampoo wash. Results of the study are depicted in Figures 1-3. The figures show a relationship between a tress treated with a non-silicone control formulation and a similar base formulation containing various silicones. The mathematical relationship shown is used to quantify the contribution the silicone has in each measured attribute associated with hair colorant degradation (L^* , b^* , a^*). These figures show the positive impact that various silicones have on preventing the loss of hair color as compared to a non-silicone control when delivered from a rinse-off hair conditioner.

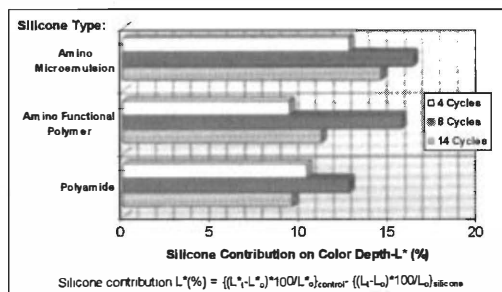


Figure 1 - Contribution of various silicone on color darkness

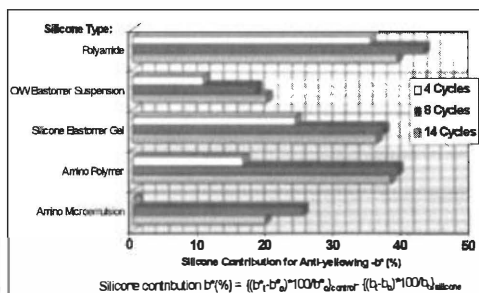


Figure 2 - Contribution of various silicones to prevent yellowing

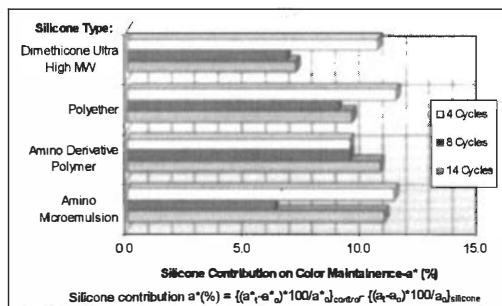


Figure 3 - Contribution of various silicones to maintain redness

Conclusions

Based on a market study of several commercial hair care products, color maintenance is still an unmet need in the market place. Washing the hair was shown to be the most significant factor in degrading hair color. UV exposure was also shown to degrade hair color but to a lesser extent. Results of this study also confirmed the ability of various silicones, including Amodimethicone, to help maintain permanent hair color when delivered from a rinse-off hair conditioner.

- (1) J.L. Wolfram and L. Albrecht, *J.Soc.Cosmet.Chem.*, **82**, 179 (1987).
- (2) B. Locke and J. Jachowicz, *J.Soc.Cosmet.Chem.*, **54**, 212 (2003).
- (3) M.V. Bhambhan, A. Chan, and G.R. Hawkins, *Revlon*, **6**, 143, 286.

BASICS OF SKIN STRUCTURE

R. Randall Wickett, Ph.D.

University of Cincinnati College of Pharmacy, Cincinnati, Ohio

Introduction to the skin

The skin is the largest organ of the human body accounting for about 16% of total body weight. Its vital role is to keep us in and the world out. The skin also has immune functions and helps to regulate body temperature. Understanding the skin especially the Stratum Corneum is important to the cosmetic scientists as cosmetic products are bound to come in contact with the skin.

Layers of the skin

The main structural components of the skin are the **dermis**, the **epidermis**. The dermis is divided into two layers, the papillary dermis and the reticular dermis. The dermis provides the bulk of the mechanical strength to the skin. The **hypodermis** which contains the subcutaneous fat is beneath the dermis. Growing hair follicles are rooted in the hypodermis. The epidermis is subdivided into five strata, basal, spinous, granular, lucid and corneum. The top layer, the **Stratum Corneum (SC)** is the primary barrier to transport of water and other molecules across the skin. This lecture will focus on the formation and structure of the Stratum Corneum.

Nomenclature for layers of the Epidermis

English	Latin	Alternative
Basal cell layer	<i>Stratum Basale</i>	<i>S. Germinativum</i> , Malpighian layer
Prickle Layer	<i>S. Spinosum</i>	Malpighian layer
Granular Layer	<i>S. Granulosum</i>	Malpighian layer
Clear Layer	<i>S. Lucidum</i>	
Horny Layer	<i>S. Corneum</i>	

The major cell type of the epidermis is the keratinocyte which makes keratin proteins. Keratins are fibrous proteins of the epidermis that are the major structural proteins of SC hair and nail. Keratins are in class of proteins called intermediate filaments which form part of the cytoskeleton of all nucleated cells.

Formation of the SC barrier

The epidermis continually renews. Slow cycling stem cells at the basal layer divide and one daughter cell remains as a slow cycling stem cell and the other becomes a transient amplifying cell. T.A. cells continue to divide until they become post mitotic and terminally differentiate. Specific keratins (k1 and k10) are expressed as markers of the transition from proliferative TA cells to terminally differentiated keratinocytes that will be committed to forming SC.

At the *Stratum Granulosum* (SG) keratohyalin granules full of protein and lamellar bodies appear in the cells. Then the cells are transformed to squames. The nucleus is digested, the cytoplasm disappears, lipids are dumped into the intercellular space, the keratin filaments aggregate to microfibrils and the cell membranes is replaced by a cell envelope.

As a result of transformations at the SG the SC barrier is formed.

15 – 20 layers of cells

Flattened cells (corneocytes or squames) with resistant cell envelope and attached lipids
Content is keratin microfibrils
Squames are joined by desmosomes.

Intercellular lipids

Multiple layers between cells, polar but relatively hydrophobic
Ceramides, cholesterol, cholesterol esters and long chain fatty acids

The Bricks and Mortar Model

The permeability barrier of the SC is sometimes modeled as a brick wall with the bricks being the corneocytes with the resistant cell envelopes and keratin microfibrils and the mortar being the intercellular lipids. The mortar is the main barrier to water passing through the SC and lipid soluble molecules are modeled as winding their way through the mortar.

The bricks (corneocytes) are 70-80% keratin by dry weight. The keratin is condensed in the form of microfibrils. The keratinocyte cell membrane is replaced by a tough new structure of cross-linked protein called the cell envelope. There are lipids covalently attached to the cell envelope on the outer surface and the corneocytes are joined by desmosomes.

The keratins in keratinocytes below the SG are in the form of coiled-coiled coils of α -helix. As the SG cells are transformed into squames the coiled coils aggregate to form 32 chain structures called microfibrils which lay parallel to the surface of the skin and restrict in plane swelling of the squames. Two proteins from the keratohyalin granules, filaggrin and loricrin play key roles in the formation of the "bricks". Filaggrin is an acronym for filament aggregating protein. Filaggrin contain a high level of positively charge amino acids and participates in the aggregation of the negatively charged keratin coiled coils.

Loricrin is a globular protein that is rich in hydrophobic amino acids and cysteine. It is released from the granules and is crossed-linked to the protein involucrin which is already in the cytoplasm of the cell. The two proteins are cross-linked by the membrane bound enzyme transglutaminase to begin forming the cell envelope. The crosslink is formed between lysine and glutamic acid side chains to form what is known as the isopeptide bond. Eventually the entire cell membrane is replaced by cross-linked protein and lipids (ceramides) are covalently attached to the outer surface of the this new structure which is known as the resistant cell envelope. Keratin microfibrils on the inside are also cross-linked to the envelope.

The lamellar bodies that appear at the SG contain lipids which are released into the intercellular space as the SC forms. These lipids are glucosyl ceramides, cholesterol, cholesterol esters and long chain fatty acids. In the intercellular space the glucosyl ceramides are converted to ceramides and phospholipids from the original cell membrane are degraded to fatty acids. The SC contains no phospholipids. The lipids in the intercellular spaces arrange themselves into multiple layers. These multilamellar lipids are the mortar of the bricks and mortar model of SC barrier function.

References:

Marekov and Steinert, Ceramides are bound to structural proteins of the human foreskin epidermal cornified cell envelope, *J. Biol Chem.* **273**, 17763-17770 (1998)

Steinert, P.M., Marekov, L.N., Fraser, R.D.B. and Parry, D.A.D. Keratin intermediate filament structure: Crosslinking studies yield quantitative information on molecular dimensions and mechanics of assembly. *J Mol Bio* **230**, 436-452. (1993)

Steinert and Marekov, Direct evidence that involucrin is a major early isopeptide cross-linked component of the keratinocyte cornified cell envelope. *J. Biol Chem.* **272**:2021-2030(1997)

Swartzendruber, D. C., Wertz, P. W., Kitko, D. J., Madison, K. C., & Downing, D. T Molecular models of the intercellular lipid lamellae in mammalian stratum corneum. *J Invest Dermatol*, 92(2): 251-257. (1989).

Wertz, P. W. & Downing, D. T. Covalently bound omega-hydroxyacylsphingosine in the stratum corneum. *Biochim Biophys Acta* , 917(1): 108-111. (1987).

THE INS AND OUTS OF SKIN PROTECTION TECHNOLOGIES

**Daniel Maes, Ph.D., D. Collins, H. Corstjens, Ph.D., L. Declercq, Ph.D.,
R. Foyouzi-Youssefi, Ph.D., D. Gan, L. Hellemans, Ph.D.,
M. Ingrassia, T. Mammone, Ph.D., M. Matsui, Ph.D., E. Pelle, Ph.D. and I. Sente**

Estee Lauder Companies, Inc., 125 Pinelawn Road, Melville, NY 11747

It is now well accepted that most of the visible signs of aging which appear on the face, are caused by the various factors we are exposed to on a continual basis.

As Cosmetic Scientists, we used to believe that most if not all of this damage, visible in the form of lines and wrinkles, abnormal skin pigmentation, and skin sagging, were the clinical manifestations of damage resulting from an excessive exposure to the sun.

However, it has now become obvious that exposure to solar ultraviolet (UV) and infrared (IR) rays is only partly responsible for the progression of cutaneous damage. Recent evidence suggests that other factors such as smoke from cigarettes, industrial pollution, Ozone which accumulates during the day at ground level, irritants and sensitizers which we get in contact with, and even the psychological stress which we endure in our daily lives play an important role in promoting additional damages to the skin. Clearly, from such an exhaustive list of challenges, it seems likely that the more classical way of protecting the skin with **chemical sunscreens** will not suffice.

Indeed they have the great merit to reduce significantly for some time the damages caused by UVB and UVA to essential bio-molecules, such as DNA lesions as well as oxidized proteins and lipids. But over the past 15 years substantial progress has been made by marrying the effect of sunscreens with the activity of topical **antioxidants**, in order to provide broader and longer lasting protection benefits.

During that time period, a few long-term clinical studies have demonstrated the benefits one can expect from the regular treatment of the skin with such molecules. They do however present some challenges to the formulator, as their lack of stability in an emulsion, obviously due to their antioxidant activity, limits sometimes their usage to low concentrations that do not allow for the complete control of the oxidative damage taking place in the skin. As a consequence, a fairly complex and devastating cascade of events is taking place:

First, the oxidative damage to the cell membrane will induce the release of proinflammatory mediators that will ultimately lead to the activation of metalloproteases, capable of gradually degrading the extracellular matrix. The end result will be a loss of integrity, firmness and elasticity of the skin.

A second, even more dreadful impact of the oxidative damage to our cells is certainly linked to protein oxidation. Indeed, this process will not only affect the function of essential structural proteins such as collagen, elastin and keratins, but in addition it can lead to a loss of activity for key enzymes in our skin. Such deactivation can have an impact at various levels. It can affect barrier integrity by perturbing the cellular desquamation process, since the stratum corneum chymotryptic enzyme (SCCE) is susceptible to UV-induced oxidative deactivation. It can also compromise the internal antioxidant defense mechanism since catalase, responsible for the reduction of hydrogen peroxide into water, is known to be deactivated by UVA exposure.

More recent developments in technology allow us to **compensate** for such deactivation of the cells' own ability to protect themselves from these oxidative damages. Specific molecules have been synthesized or extracted from plants to reactivate the deficient antioxidant mechanism such as N-Acetyl-Cysteine to replenish the pool of intra-cellular glutathione, or the UVA stable catalase mimetic EUK-134 (Fig. 1), to compensate for the UVA-induced loss of catalase activity.

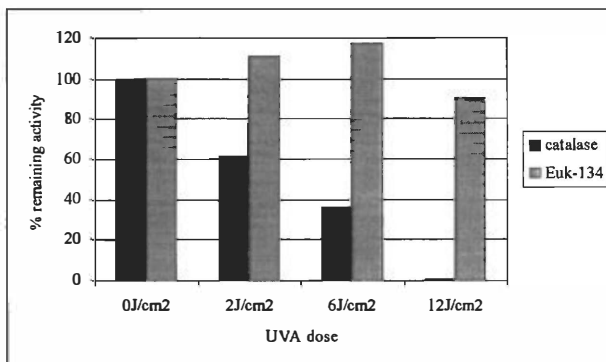


Fig. 1: Effect of UVA exposure on the *in vitro* catalase activity of EUK-134 (Eukarion, Inc) and catalase from bovine liver (Sigma)

Another interesting avenue for the enhancement of the cellular protection mechanisms is to induce the expression of various Heat Shock Proteins which have the properties not only to protect specific UV sensitive enzymes such as catalase, but have been shown to refold the proper tertiary structure of damaged proteins, resulting in a restoration of the activity.

Finally, increasing the cell's own energy reserve via topical application of Creatine has been shown to enhance cellular protection mechanisms in UV exposed skin cells, resulting in a significant reduction of both mitochondrial and nuclear DNA damage as well as a reduction of sunburn cell formation. These results clearly demonstrate the relevance of increasing the overall cellular metabolism to improve the overall defense capacity against the oxidative stress generated by exposure to the environment.

Conclusion

These observations strongly support the need for development of a multi-branched technology to provide the necessary protection to cells exposed to the oxidative damages generated by an environment which has evolved dramatically over the last 100 years and which is creating a significant burden to mammalian cells. The development of an optimal protection technology cannot be based exclusively on a simple combination of efficacious sunscreens alone, but should provide a well balanced combination of sunscreens, antioxidants, anti-inflammatory agents, together with ingredients that will enhance the cells' own ability to protect themselves from various environmental insults.

We do believe that the cosmetic industry is playing an important role in providing consumers with the best protection available today, as clearly mammalian cells have not been able to adapt their protective mechanisms to the additional oxidative stress which results from the increase in environmental pollution all over the world.

THE EXTRACELLULAR MATRIX- FROM STRUCTURAL RESILIENCE TO MODULATION OF CELL FUNCTIONS

Sanford R. Simon, Ph.D.

State University of New York at Stony Brook, Stony Brook, NY 11794-8691

The extracellular matrix (ECM) is classically regarded as having a role in maintaining structural integrity of connective tissue. Two components of the interstitial ECM, collagen and elastin, play especially prominent roles in establishing the balance between rigidity and elasticity which characterizes normal connective tissues. When these proteins are serving as structural elements in the interstitium, they exist predominantly as fibers and are characterized by insolubility and resistance to many proteolytic enzymes. Infiltration of the interstitium by inflammatory cells, especially neutrophils and mononuclear phagocytes (monocytes and macrophages) is traditionally implicated as a critical step in establishing a milieu in which specialized proteinases are released, leading to degradation of collagen and elastin fibers. A net loss of elastic fibers due to chronic degradation is considered to be characteristic of normal ageing of skin, while UV damage to the skin is associated with an accumulation of elastin-like material which has been postulated to result from exaggerated biosynthetic activity triggered by peptides released from inflammatory destruction of normal collagen and elastin fibers. Thus, proteolytic degradation of collagen and elastin has been considered to lead to undesirable changes in the characteristics of skin, resulting either in abnormal thinning or thickening. The proteinases implicated in these changes have been purified and extensively studied as soluble enzymes, and considerable efforts have been directed towards characterizing their endogenous inhibitors as well as developing synthetic inhibitors to supplement the endogenous antiproteinases. This model of degradation of purely structural proteins by soluble enzymes released by inflammatory cells has proved to be oversimplified. In this presentation, we consider some of the results which have emerged from many laboratories indicating that the functional roles of components of the ECM go beyond maintenance of rigidity and elasticity. Moreover, inflammatory proteinases may function in highly specialized microenvironments, such as the inflammatory cell surface, and may have physiologically relevant targets beyond the principal structural elements. Thus, attempts to modulate the activity of these proteinases may have consequences beyond changes in collagen and elastin fiber structure.

The Roles of ECM-Associated Matricellular Proteins

The ECM which separates epithelial and stromal components, as seen at the dermal-epidermal junction, is referred to as basement membrane (BM), and has unique components, including nonfibrillar collagens (types IV, XV, and XVIII) as well as a variety of bridging elements such as laminin, entactin (nidogen), and heparan sulfate proteoglycans. These components interact not only with each other but also with epithelial and stromal cells. More recently, a number of additional proteins have been characterized which appear to function primarily as cell regulatory molecules rather than structural components. These molecules include thrombospondins, SPARC or osteonectin, osteopontin, and tenascins. With the notable exception of osteopontin, which enhances adhesion of cells to BM through integrins, these so-called matricellular proteins typically reduce the adhesion of cells to BM and may facilitate their migration. Moreover, thrombospondin-2 appears to play an important role in clearing the BM of one of the important matrix-degrading proteinases, MMP-2 or gelatinase A, by binding to the enzyme and facilitating its internalization. Several matricellular proteins regulate such cellular events as proliferation and responsiveness to growth factors, apoptosis, and angiogenesis. Virtually all the matricellular proteins are targets of leukocyte elastase, the serine proteinase released from the azurophilic granules of neutrophils. In addition, the combined activities of elastase and the matrix metalloproteinases (MMPs) released by neutrophils and macrophages can degrade types IV and XVIII collagen to liberate proteolytic fragments such as endostatin and tumstatin, which bind to integrins on cell surfaces, resulting in significant anti-angiogenic activities. The consequences of pathologically elevated levels of the inflammatory cell-derived proteinases or of abrogation of proteolytic activity by injudicious use of exogenous antiproteinases on the multiple functional roles of the matricellular proteins have not yet been fully explored.

Proteinase-Antiproteinase Imbalance in ECM Damage

One of the more challenging aspects of attempting to intervene in preventing excessive damage to ECM through the use of proteinase inhibitors relates to the interactions between the most common proteinases and their endogenous inhibitors. The serine proteinase, leukocyte elastase, is targeted by the endogenous antiproteinases, alpha-1-Proteinase Inhibitor (alpha-1-PI), secretory leukoprotease inhibitor

(SLPI), and elafin. The MMPs are, in turn, targeted by the Tissue Inhibitors of Metalloproteinases (TIMPs). It has been appreciated that a number of the members of the family of MMPs, several of which are classically referred to as collagenases or gelatinases based on their ability to degrade collagen fibers or the unstable fragments formed by limited proteolysis of the fibers, have other physiologically relevant targets. In particular, several MMPs are capable of inactivating α -1-PI and SLPI. In fact, α -1-PI as well as other members of the so-called class of "serpins," or serine proteinase inhibitors, are the only known targets of one of the MMPs. SLPI and elafin are two-domain molecules, anchored or tightly bound to ECM components. This feature greatly enhances their anti-elastolytic activity, but when they are cleaved by MMPs, their association with the ECM is disrupted and their potencies as inhibitors of elastase-mediated ECM degradation are diminished. Meanwhile, unopposed leukocyte elastase can cleave the TIMPs, markedly diminishing their inhibitory activity towards the MMPs. Thus, the two families of proteinases released by inflammatory cells can degrade each other's endogenous antiproteinases. This synergy is suspected as an underlying mechanism for the catastrophic inflammatory injury seen in such conditions as Acute Respiratory Syndrome, or ARDS. However, the cross-inactivation mechanism may be turned to advantage by judicious introduction of exogenous proteinase inhibitors. Rather than attempting to achieve complete inhibition of both classes of proteinases, it may be adequate to employ exogenous agents to inhibit the proteinases only to the point at which their unopposed activities are diminished so that they do not destroy each other's endogenous antiproteinases. An example of the use of one such group of synthetic proteinase inhibitors, which are based on the tetracycline ring structure but are distinctive for their lack of antimicrobial activity, will be presented. The most promising of these agents inhibits MMPs as well as leukocyte elastase, blocks neutrophil-mediated ECM degradation, and prevents inactivation of α -1-PI. This so-called "chemically modified tetracycline" also inhibits angiogenic activity associated with cell-BM interactions, as evidenced by the capacity of the agent to diminish release of VEGF from monocytes and to reduce formation of tube-like structures from endothelial cells plated on a reconstituted BM. This anti-angiogenic activity is currently being evaluated in clinical trials of patients with Kaposi's sarcoma. In animal models of ARDS, the tetracycline derivative reduces morbidity and mortality dramatically. The pleiotropic effects of nonantimicrobial tetracycline derivatives may reflect biological activities beyond direct inhibition of inflammatory proteinases, but may also illustrate how the actions of proteinases on targets (e.g. matricellular proteins) other than structural elements of the ECM may elicit complex physiological responses.

Cell Surface-Bound Proteinases in ECM Damage

The use of low molecular weight proteinase inhibitors to modulate ECM degradation in an inflammatory milieu has been assumed to confer the advantages of greater bioavailability and stability, but recent studies on the properties of cell-bound proteinases have revealed a somewhat different set of challenges which may be addressed by even more selective antiproteinase design strategies. While neutrophil elastase and many of the MMPs are found as soluble enzymes in the extracellular environment, both of these classes of proteinases can also be present at high levels bound to the surfaces of neutrophils and macrophages. Several forms of metalloproteinases which are true integral membrane proteins have been recently characterized and have been shown to have unique activities associated with their location on the cell surface. In their cell surface-bound state, the serine proteinases and the metalloproteinases display a very different profile of sensitivity to endogenous antiproteinases and exogenous proteinase inhibitors than the soluble enzymes. It has been suggested that surface-bound proteinases on infiltrating inflammatory cells may account for a significant fraction of the ECM damage which occurs even in the presence of high levels of endogenous antiproteinases. Some examples of markedly different sensitivities of cell-bound and soluble proteinases to natural and synthetic proteinase inhibitors, using low molecular weight substrates as well as experimental ECM models to evaluate inhibitory potencies, will be presented.