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Damaged hair retrieval with ceramide-rich liposomes

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Synopsis

Lipids from human hair consist mainly of cholesterol esters, free fatty acids, cholesterol, ceramides, and cholesterol sulfate. They are structured as lipid bilayers in the cell membrane complex (CMC) and make a large contribution to diffusion, cell cohesion, and mechanical strength. The loss of these lipids could impair the integrity of the hair, leading to deterioration in its tensile properties. Internal wool lipids (IWL) resemble those of the membranes of other keratinic tissues such as human hair or stratum corneum. The application of IWL structured as liposomes on pretreated hair samples has been demonstrated to restore the natural properties of the fibers. This study seeks to apply IWL liposomes to untreated hair fibers and to hair fibers subjected to chemical treatment. Differences in the lipidic composition of all chemically treated hairs were found with respect to the untreated ones. Lipid recovery of damaged hair due to the application of IWL liposomes was corroborated by lipid analysis of the hair. A high resistance to break of hair samples post-treated with IWL liposomes was observed. An increase in hydrogen bonds and electrostatic forces and an improvement in the cohesion between matrix and filaments were detected, probably because of some lipid recovery.

INTRODUCTION

Human hair is a keratinized fiber 50–100 μm in diameter and is divided into three structural zones: medulla, cortex, and cuticle. The medulla, which is located in the central region of the hair, has a diameter of 5–10 μm and is composed of loosely packed cells leaving a series of vacuoles along the fiber axis. The cortex is made up of crystalline proteins (microfibrils) surrounded by a relatively amorphous matrix. The high-sulfur and high-glycine/high-tyrosine proteins are concentrated in the matrix. The microfibrils are relatively rich in low-sulfur proteins (these proteins are rich in amino acids that favor α-helix formation). The outermost layer of the hair is the cuticle, which is generally 5-μm thick. It is made up of nine to ten scales. Each scale has a laminar structure with an outer and inner layer, known as exo- and endocuticle, respectively (1,2). The three different kinds of cells (medullar, cortical, and cuticular) are separated by the cell membrane

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complex (3). This consists of a protein layer (4,5), termed δ-layer (~18 nm), which is surrounded by two lipid layers (each layer being 3-μm thick) known as β-layers (6).

Lipids from human hair consist mainly of cholesterol esters, free fatty acids, cholesterol, ceramides, and cholesterol sulfate. The loss of lipids along the fiber (7), may be attributed to repeated shampooing, grooming, and UV exposure. In some individuals, chlorine, hair dyes, and hair bleaches may also contribute to the loss of lipids. The loss of these lipids could impair the integrity of the cuticle. This loss of cuticle integrity would increase the susceptibility of the protein and lipid lamellae to degradation, leading to a decrease in the tensile properties of the hair (7). A number of studies suggest that hair lipids can contribute to physicochemical phenomena such as diffusion, cell cohesion, and mechanical strength, despite occurring in a considerably lower proportion than proteins (2,8–10).

Wool is another keratinous tissue, whose internal lipids have been extracted and analyzed in different works (11,12). These lipids are rich in cholesterol, free fatty acids, cholesterol sulfate, and ceramides, and they resemble those found in membranes of other keratinous tissues such as human hair or stratum corneum. The application of liposomes made up of internal wool lipids (IWL) on skin has been studied because of the similarity of composition of IWL and SCL (stratum corneum lipids of human skin) and because of their capacity to form stable bilayer structures. The beneficial effect of liposomes with ceramides extracted from wool fiber on intact skin in aging populations or in individuals with dry skin has been reported (13,14). Accordingly, internal wool lipids could be regarded as a new natural extract suitable for topical application and incorporation into pharmaceutical or cosmetic formulations for skin care (15).

The similarity in chemical and morphological structures of wool and hair fibers prompted us to study the effect of applying IWL on human hair. Natural properties of hair fiber were restored to a certain degree, especially when IWL structured as liposomes were applied (16). Consequently a new cosmetic application of these lipids on hair and nail repair was envisaged (17).

The study seeks to evaluate the effect of IWL liposome application on untreated hair fibers and on hair fibers subjected to chemical treatment. The effect of this lipid supplementation on the moisture regulation and mechanical strength of hair fiber was determined. The lipid absorption was evaluated in order to measure the recovery of these lipids in the treated fibers. Lipid modifications were related to the chemical and mechanical properties of hair fiber.

MATERIAL AND METHODS

CHEMICALS

The chemicals used in this study were acetone (Merck, Darmstadt, Germany), formic acid 85% (Probus S.A., Badalona, Barcelona), citric acid monohydrate (Merck, Darmstadt, Germany), chloroform (Merck, Darmstadt, Germany), diethyl ether (Merck, Darmstadt, Germany), hydrogen peroxide 30% (Merck, Schuchardt, Germany), methanol (Merck, Darmstadt, Germany), N-hexane (Merck, Darmstadt, Germany), sodium hydroxide (Carlo Erba Reagenti, Rodano), and thioglycolic acid (Merck, Darmstadt, Germany).

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HAIR CHEMICAL TREATMENTS

The hair sample used in this work was a virgin natural brown hair and was supplied by De Meo Brothers (New York). The hair was chemically damaged by different cosmetic treatments such as perming, bleaching, and relaxing:

Permed hair. Hair (0.5 g) was placed in a perming solution (8% thioglycollate, pH 8) for three hours on a rocking table. It was then rinsed with water and placed in a neutralizing solution (2.5% H_2O_2, pH 3) for 30 minutes. It was then rinsed again and dried in air.

Bleached hair. Hair (0.5 g) was placed in a bleaching solution (9% H_2O_2, pH 8.3, 1% ammonium persulfate) for three hours on a rocking table. It was then rinsed with water and dried in air.

Relaxed hair. Hair (0.5 g) was placed in a 2.5% NaOH solution for 30 minutes on a rocking table and then rinsed with water for five minutes. Next, it was placed in a 9.5% citric acid solution for five minutes and then rinsed with water for ten minutes.

IWL APPLICATION

Internal wool lipids obtained from Spanish Merino wool fibers were submitted to methanol extraction at 56°C as described in reference 11. The amount of lipids extracted was not very high (1.4% on wool fiber), but this may be sufficient for cosmetic utilization due to the high amount of wool processed for textile purposes. Aliquots were dissolved in chloroform/methanol 2:1 (v/v) and evaporated to dryness under a stream of dry nitrogen to form a thin film on the flask. The film was hydrated with 0.9% NaCl solution to give a final lipid concentration of 10 mg/ml. Liposomes were formed by sonication of the suspension in a sonicator, Labsonic 1510 (B. Braun, Melsungen, Germany), at 100W for about 15 minutes. The temperature was maintained at 60°C by a thermostatic bath, Ultraterm 6000383 (Selecta SA, Barcelona, Spain).

These IWL liposomes (1%) were applied to untreated and chemically treated hair as follows: 1-g samples of hair were soaked in a volume of 10 ml of 1% IWL liposomes for ten minutes at 40°C. Then the hair fibers were washed with distilled water and dried. This procedure was repeated ten times.

LIPID EXTRACTION

Lipids of untreated and chemically treated hair samples that were subjected to IWL liposome application and those that were not subjected were extracted. The extraction was made at room temperature for two hours with mixtures of chloroform-methanol (2:1, 1:1, and 1:2, v/v). These extractions were repeated for one hour with the same mixtures and with methanol overnight. The different extracts were then combined, concentrated, and dissolved in chloroform-methanol (2:1) prior to analysis. To evaluate the total amount of lipids extracted, 1 ml of each of the extracts was evaporated to dryness in a P_2O_5 desiccator and weighed to a constant weight.

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HAIR LIPID ANALYSES

Lipid analyses of the different extracts were performed by thin-layer chromatography coupled to an automated flame ionization detector (TLC-FID), Iatroscan MK-5 analyzer (Iatron, Tokyo, Japan), following the analysis methodology referred to in earlier works (12,18). Samples (15–20 μg) were spotted on Silica gel S-III Chromarods by means of a precision 2-μl Hamilton syringe coupled to an SES (Nieder-Olm, Germany) 3202/15-01 sample spotter.

An analysis of apolar and polar compounds was performed by developing the rods initially to a distance of 10 cm with n-hexane/diethyl ether/formic acid (53:17:0.3, by vol) to separate the apolar and polar lipids. After a partial scan of 72% to quantify and eliminate the apolar lipids, a second development, again to a distance of 10 cm, was performed with chloroform/n-hexane/ methanol/acetone (55:5:3:7, by vol) to separate the ceramides. Following a partial scan of 85% to quantify and eliminate the ceramides, a third development, again to a distance of 10 cm, was performed with chloroform/methanol/formic acid (57:12:0.3, by vol) to separate and quantify, after a total scan of 100%, the glycosilceramides and sterol sulfate. After each elution, the rods were heated for five minutes at 60°C to dry the remaining solvent. The experimental conditions were: air flow 2000 ml/min, hydrogen flow 160–180 ml/min, and scanning speed 2–3 mm/s. Data were processed with Boreal version 2.5 software.

These procedures were applied to the following standard compounds: palmitic acid and cholesterol from Fluka Chemicals (Buchs, Switzerland), and type II ceramides, cholesterol ester, galactoceramides, and sodium cholesteryl sulfate from Sigma (St. Louis, MO), to determine the corresponding calibration curves for quantification of each compound.

MOISTURE RETENTION BY THERMOGRAVIMETRIC ANALYSIS (TGA)

TGA provides a measurement of the weight loss of the sample as a function of time and temperature. Before measuring, the hair was kept in a humidity-controlled room (55% RH) for 24 hours. All investigations were performed on a TGA instrument (TG-50, Mettler Toledo, Spain). Samples consisted of short fiber snippets (approx. 2 mm in length). Approximately 6 mg of the hair samples were packed into a 70-μl TGA pan. Samples were transferred to an aluminum crucible, weighted, and sealed for an elapsed time of 30 seconds. The crucible was then placed in the TGA balance where it was pierced. The heating rate used in this study was 20°C/min, with a flow rate of nitrogen gas of 200 ml/min. The internal and external water contents were evaluated separately. The hair sample was heated from 25° to 65°C, and this temperature was maintained for 40 minutes, which is assumed to be the normal temperature used by a hair dryer, and the water content found corresponded to the external water content (19). Again the temperature was increased, from 65° to 180°C, and was kept for 30 minutes to measure all the water contained in the hair (internal water content). TGA curves showed the amount of water in the samples.

STRENGTH MEASUREMENTS

Stress-strain test. Five fibers were randomly taken from samples previously conditioned for 48 hours in a standard atmosphere (20°C, 65% RH) and centrally attached to a pair of

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cardboard frames with an internal rectangular cut frame of 50 × 25 mm following the longest direction. Fiber fineness along the 50 mm subjected to testing was examined by image analysis, and the minimum diameter was taken as fiber fineness because breakage is normally produced at the thinnest (weakest) point. Samples in the cardboard were attached to an Instron 5500R dynamometer with a gauge length of 50 mm. The two sides of the cardboard were cut before the beginning of the stress-strain test. The test was performed according to ASTM Standard D 3822 (1980) with some modifications. The gauge length was 50 mm, the rate of strain was 30 mm/min, and the breaking stress in MPa and the strain in % were recorded. The work necessary to break the hair was calculated as the product of the breaking stress and the percentage of deformation at break.

Stress-relaxation test. Five fibers were randomly taken following the same procedure as in the stress-strain test and were also attached to the Instron 5500R dynamometer to perform the stress-relaxation test. Fibers were strained 30% at the same rate as in the stress-strain test, and stresses at 0, 2, 5, 10, 15, 30, 45, 60, 120, and 180 seconds were recorded. The high-rate, the medium-rate, the low-rate, and the non-relaxed stresses were estimated by using the results and applying non-linear regression. The objective to strain 30% is to ensure that a great amount of links are under stress and, depending on their energy, they are broken at different rates. Low-energy links are broken first and high-energy links are broken later (20).

RESULTS AND DISCUSSION

The hair sample was subjected to the three most common cosmetic treatments: bleaching, perming, and relaxing. The permed hair involves the cleavage of cystine links with a reduction solution and their reformation in a new position with an oxidation solution to achieve a permanent deformation (21). The bleached hair is achieved by an oxidation of the melanin pigments (21). The relaxed hair involves breakage of the disulfide bonds in the keratin fibers and prevents the chemical reformation of these bonds (22). Hair was permed, bleached, and relaxed as discussed above in order to study its chemical and mechanical damage and its possible recovery owing to IWL application.

Internal wool lipids (IWL) structured as liposomes were applied to the four kinds of hair: untreated and three chemically treated hair samples. The composition of the IWL liposomes applied to these samples consisted mainly of cholesterol esters (4%), free fatty acids (18%), cholesterol (13%), ceramides (22%), glycosilceramides (9%), and cholesterol sulfate (8%) (11).

Evaluation of all the chemically pretreated and nontreated samples before and after application of IWL liposomes was performed. The lipid composition, the water content, and the mechanical properties of all these samples were evaluated.

Lipids were extracted from untreated and chemically treated hair samples (bleached, permed, and relaxed) before and after the application of the internal wool lipid (IWL). Quantitative and qualitative analyses were performed by TLC-FID (Table I).

The percentage of total lipids extracted from hair samples was always higher than that of the lipids analyzed. This could be due to the possible extraction or solubilization of other compounds such as proteins or peptides. Smaller amounts of lipids were always obtained in the pretreated samples with respect to the untreated ones. The bleached sample was

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Table I
Lipid Composition in % of Total Hair Fibers (untreated (UT), bleached (B), permed (P), and relaxed (R)) Obtained by TLC-FID

	UT	UT+IWL	B	B+IWL	P	P+IWL	R	R+IWL
Chol-Est	0.39	0.35	0.34	0.35	0.31	0.37	0.31	0.56
FFA	1.02	1.18	0.71	0.79	1.03	1.05	1.06	1.08
R-OH	0.14	0.14	0.09	0.09	0.08	0.06	0.07	0.13
Chol	0.11	0.13	0.06	0.10	0.05	0.07	0.08	0.17
Ceram.	0.20	0.34	0.18	0.16	0.15	0.19	0.10	0.18
GC	0.10	0.16	0.05	0.09	0.05	0.06	0.06	0.13
Chol-S	0.21	0.31	0.07	0.16	0.05	0.10	0.09	0.17
% Analyzed	2.17	2.61	1.50	1.74	1.72	1.90	1.77	2.42
% Extracted	2.81	3.33	2.34	2.79	2.52	1.82	3.09	2.76

Cholesterol ester (Chol-Est), free fatty acids (FFA), fatty alcohol (R-OH), cholesterol (Chol), ceramides (Ceram.), glycosyl ceramides (GC), and cholesterol sulfate (Chol-S).

the most affected. In particular, all the chemically treated hair samples showed a decrease mainly in cholesterol and cholesterol sulfate. The lipid composition of the permed sample bore the greatest resemblance to native hair. The bleached extract recorded lower amounts of free fatty acids, and the relaxed extract showed smaller amounts of ceramides.

In all the samples treated with IWL liposomes there was an increase in the total of lipid analyzed, which confirmed the absorption of IWL into the fiber. The absorption of IWL liposomes descended in the following order: relaxed, untreated, bleached, and permed. The relaxed sample (subjected to the most aggressive treatment) absorbed the highest amount of IWL, whereas the permed sample (whose composition most resembled that of the untreated sample) absorbed the lowest amount.

The selective absorption of the lipids by hair fibers should be pointed out. Although the FFA percentage remained unaltered after IWL application, the damaged and the undamaged fibers mainly absorbed the polar lipids: ceramides, glysosilceramides, and cholesterol sulfate. This selective absorption induced equilibrium in the lipid composition, which resembled that of the untreated fiber, resulting in lipid restoration (Table II). Based

Table II
Percentages of Lipid Extracted (%) from Hair Samples (untreated (UT), bleached (B), permed (P), and relaxed (R)) Obtained by TLC-FID

	UT	UT+IWL	B	B+IWL	P	P+IWL	R	R+IWL
Chol-Est	17.97	13.41	22.67	20.11	18.02	19.47	17.51	23.19
FFA	47.00	45.21	47.33	45.40	59.88	55.26	59.89	44.72
R-OH	6.45	5.36	6.00	5.17	4.65	3.16	3.95	5.38
Chol	5.07	4.98	4.00	5.75	2.91	3.68	4.52	7.04
Ceram.	9.22	13.03	12.00	9.20	8.72	10.00	5.65	7.45
GC	4.61	6.13	3.33	5.17	2.91	3.16	3.39	5.38
Chol-S	9.68	11.88	4.67	9.20	2.91	5.26	5.08	6.83

Cholesterol ester (Chol-Est), free fatty acids (FFA), fatty alcohol (R-OH), cholesterol (Chol), ceramides (Ceram.), glycosyl ceramides (GC), and cholesterol sulfate (Chol-S).

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on relative lipid percentages, there was a tendency for the IWL application to repair the changes induced previously in the pretreatments. Polar and charged lipids, which decreased in the treatments, tended to increase after IWL application. These lipids have been reported to play a role in maintaining the bilayer structure of the CMC of the hair fiber (23). Therefore, the fibers could have more affinity with the native hair lipids to form and stabilize lipid bilayers.

The effect of this lipid supplementation on the moisture regulation and mechanical strength of the hair fiber was determined, even though these properties are mostly representative of protein integrity. On the one hand, intercellular lipids of another keratinized tissue, such as the stratum corneum from the skin, are known to be fundamental to maintain the physiological water content (24). Therefore, a modification of the lipid intercellular layers of hair could play a similar role in water permeation. On the other hand, lipid extraction from wool has shown poorer transference on stresses, decreasing the elongation at break (25). The b-layers, which are generally believed to arise from the hydrophobic ends of a lipid bilayer, were shown by Rogers (26) to constitute regions of relative weakness in the fiber. Therefore, a modification of the lipid bilayer from the hair fiber could also have influence on tensile properties.

The maintenance of an optimal level of hydration by the SC is largely dependent on several factors. One of these factors is the intercellular lamellar lipids, which provide an effective barrier to the passage of water through the skin tissue (27). The water content of hair exerts an influence on the mechanical properties of the fiber (22), i.e., when hair is wet, the load required to extend the fiber or to break it is lower than in the case of dry hair because of the loss of hydrogen bonds and coulombic interactions. Therefore, the knowledge of water content could indicate chemical and morphological modifications of fibers subjected to the different treatments.

A thermogravimetric analysis was performed for the untreated and chemically treated hair before and after IWL liposome application. The water content of the hair was measured at internal and external levels. First, the hair sample was heated at 65°C, which is assumed to be the normal temperature when using a hair dryer (28), in order to measure the external water content. The internal water content is the amount of evaporated water at 180°C. The percentages of internal and external water content are shown in Table III.

There were no significant differences found in the internal and external water content between untreated and chemically treated hair. The lowest values of internal and external water content corresponded to the relaxed hair. This could be due to the maximum damage

Table III
Percentages of Total, Internal, and External Content of Untreated, Bleached, Permed, Relaxed, and the Same Hair Samples Treated with IWL Liposomes

	Internal water (%)		External water (%)		Total water (%)	
	Initial	IWL	Initial	IWL	Initial	IWL
Untreated	3.62 ± 0.12	3.70 ± 0.36	10.64 ± 1.09	10.49 ± 1.80	14.26 ± 0.98	14.19 ± 2.10
Bleached	3.81 ± 0.21	4.00 ± 0.16	10.54 ± 0.90	11.59 ± 0.66	14.35 ± 1.00	15.59 ± 0.82
Permed	3.92 ± 0.16	3.86 ± 0.16	10.51 ± 0.65	10.72 ± 0.45	14.43 ± 0.74	14.58 ± 0.60
Relaxed	3.56 ± 0.21	3.57 ± 0.13	10.42 ± 0.81	10.45 ± 0.43	13.98 ± 1.00	13.91 ± 0.52

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to the fiber as a result of the NaOH treatment. For the bleached and permed samples, a slight decrease in external water was observed with respect to the non-treated sample, and a slight increase in the internal water was found, resulting in a small increase in the total moisturization of the hair. The decrease in external water could be attributed to the aggressiveness of the different treatments: the more aggressive the treatment, the greater the damage to the hair surface. However, an increase in hydrophilicity of the hair fiber due to the oxidative treatment could lead to an increase in the internal water content.

Internal wool lipid application on untreated and pretreated hair did not significantly modify the internal or external water content of the samples. Moisture is slightly decreased in the untreated and relaxed hair and is increased in the permed hair, mainly because of the external water modification. However, the bleached hair is the most affected sample, owing to the application of IWL liposomes. The high absorption of IWL, which is rich in polar lipids with OH and NH groups, could facilitate the formation of hydrogen bonds, thereby increasing the water content of the fiber.

Strength and relaxation measurements of the untreated and chemically treated hair before and after IWL liposome application were performed to evaluate the influence of lipids on the mechanical properties of hair fibers. The mechanical properties of hair depend on two principal components: the fibrils, helically coiled molecules, and the amorphous matrix in which the fibrils are embedded (Figure 1).

The energy required to fracture hair (breaking stress) and the deformation of hair before fracturing (deformation at break) were evaluated in the strength measurement. The relationship between the load applied on a hair fiber and the elongation obtained is illustrated in Figure 2. Three main areas may be distinguished. Between 0 and 2% stretching there is the "Hookean region" or "pre-yield region," where the elongation is proportional to load. The area between 2% and 25-30% is known as the "yield region," where elongation increases rapidly without a notable increase in load. The last area is the "post-yield region" between 30% stretching and breakage of the fiber (21).

This stress-strain behavior can be attributed to the process of conversion of α-keratin, where the chains are arranged in compact patterns, to β-keratin, where the chains are completely unfolded. The "pre-yield" zone of the extension curve represents the α-form, which is homogeneously resistant to stretching. This resistance is mainly provided by

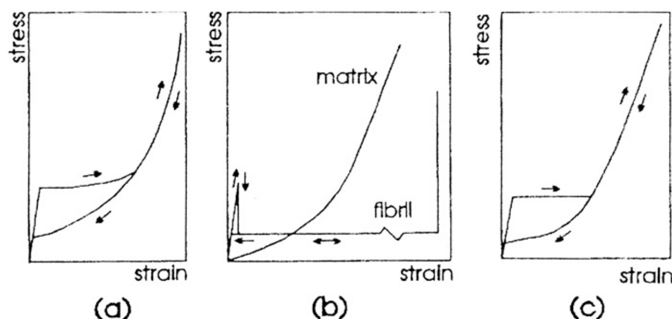

Figure 1. (a) The tensile properties of fiber. (b) The properties of the two components. (c) The predicted response of the composite structure.

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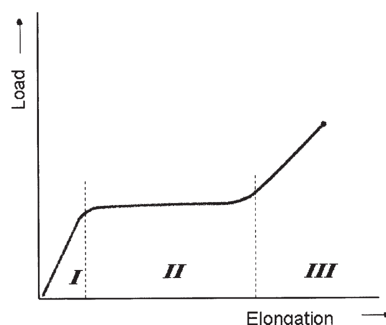

Figure 2. Schematic diagram for load-elongation curves for human hair fibers. I. Hookean region. II. Yield region. III. Post-yield region.

hydrogen bonds, which are present between turns and stabilize the α-helix. The "yield region" represents the transition from α-keratin to β-keratin, the chains unfolding without offering any resistance. The "post-yield region" shows the resistance of β configuration to stretching up to the disruption point.

Breaking stress, deformation at break, and work necessary to break the fiber were evaluated for all the fibers studied in the strength measurement. The results are shown in Table IV.

The highest value of the energy required to break hair (breaking stress) corresponded to the untreated sample. This value indicated a high hair reticulation proportional to the amount of crosslinks. The chemically treated hair revealed differences in the breaking stress. The more aggressive the treatment, the greater the damage to the reticular integrity of the hair: the relaxed treatment proved to be the most aggressive because of the facility of the hair to break, and the bleached treatment was the least aggressive. The deformation at break indicates the resistance of hair to break and is related to its internal lubricity. The high value of this deformation in the case of the bleached and permed samples with respect to the untreated one could be due to its higher water content in the previous thermogravimetric study. The water inside the fiber can act like a lubricant, rendering the hair less rigid and facilitating the displacement of the fibrils. The high value obtained in the case of the relaxed sample was not significant because of its low breaking stress value. The breaking work was calculated to combine the effect of the breaking stress and the deformation at break. Again the chemically treated fibers had lower values than the untreated ones, the relaxed samples being the most affected.

Table IV
Values Obtained in the Strength Measurements

	Breaking stress (MPa)		Deformation at break (%)		Breaking work	
	Initial	IWL	Initial	IWL	Initial	IWL
Untreated	1052.6 ± 146	1039.5 ± 241	47.9 ± 1.9	49.6 ± 2.3	50419.1 ± 8640	51559.2 ± 6043
Bleached	976.22 ± 161	965.58 ± 272	49.9 ± 7.5	55.1 ± 5.6	48713.4 ± 10297	53203.5 ± 10548
Permed	884.31 ± 234	869.52 ± 294	48.2 ± 9.8	49.7 ± 6.9	42623.7 ± 17711	43215.1 ± 11992
Relaxed	652.71 ± 170	606.1 ± 163	58.7 ± 4.0	56.4 ± 4.3	38314.1 ± 8015	34184.0 ± 16408

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After the application of IWL liposomes, the same strength measurements were performed. A notable increase in the breaking stress, deformation at break, and breaking work were observed in the case of the untreated sample. The IWL liposome application increased the deformation at break of the bleached and permed samples, although the breaking work was only slightly increased. Finally, no improvement was found in the relaxed fiber after IWL application. Indeed, the deformation at break, breaking stress, and breaking work were diminished.

The relaxation of the fiber is attributed to the breakage of various physical and chemical crosslinks and to their reformation with the passage of time. Longer relaxation time means that hair has more bonds to break and form again, with the result that its integrity is improved. Based on time, these bonds are divided into three main groups (29): weak, intermediate, and strong bonds. Weak bonds have a short relaxation time below ten seconds, and they include hydrogen bonds, salt linkages, and van der Waals and electrostatic forces. Intermediate bonds have relaxation times between ten seconds and ten minutes, and they correspond to bonds between matrix and filament components. Finally, strong bonds have a relaxation time exceeding ten minutes and include the mainly disulfide bonds.

The fibers were extended up to 30% of the length before the relaxation analysis. The low and intermediate relaxation times (t_{short} and t_{interm}), the relaxed stress at these times (σ_{short} and σ_{interm}), and the non-relaxed stress ($\sigma_{non\text{-}relaxed}$) were measured in the relaxation analysis (Table V). The percentage of σ_{short}, σ_{interm}, and $\sigma_{non\text{-}relaxed}$ indicate the proportion of weak, intermediate, and disulfide bonds, respectively, in the breaking stress, which is discussed above.

In the case of σ_{short} and the t_{short}, a decrease in the amount of weak bonds was observed in bleached and permed samples, where a reduction in the lipidic composition was also detected in this study. Depletion of hair lipids made up of charged groups and groups such as OH and NH can contribute to a decrease in the amount of hydrogen bonds and electrostatic forces. The relaxed hair showed a significant decrease in $\sigma_{non\text{-}relaxed}$, which indicated damage in the disulfide bonds after NaOH treatment. Subsequently, the percentage of weak bonds was even higher than in the untreated sample.

In all the hair samples, the application of IWL involved a marked change in the σ_{short}, indicating an increase in the weak bonds. The best results obtained in the weak bonds after applying IWL liposomes were found in the case of the untreated and bleached samples,

Table V
Values Obtained in the Relaxation Measurement

	σ_{short} (%)		t_{short} (s)		$\sigma_{interm.}$ (%)		$t_{interm.}$ (s)		$\sigma_{non\text{-}relaxed}$ (%)	
	Initial	IWL	Initial	IWL	Initial	IWL	Initial	IWL	Initial	IWL
Untreated	15.33	19.86	5.95	6.67	11.59	16.86	80.50	95.32	73.81	63.77
Bleached	11.71	18.49	5.32	6.34	8.69	15.98	59.80	84.33	79.83	66.22
Permed	11.87	15.04	5.56	5.55	7.66	8.90	52.99	76.90	80.82	76.28
Relaxed	17.63	22.13	4.23	3.12	12.61	19.37	62.66	48.24	69.90	58.43

Short relaxed stress (σ_{short}), short relaxation time (t_{short}), intermediate relaxed stress ($\sigma_{interm.}$), intermediate relaxation time ($t_{interm.}$), and non-relaxed stress ($\sigma_{non\text{-}relaxed}$).

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where an increase in the t_{short} was also observed. This could be related to the high lipid absorption of these two samples. As seen in the lipid analysis, the IWL liposomes triggered an increase mainly in the content of polar lipids (ceramides, glycosilceramides, and cholesterol sulfate). All these lipids have N-H and O-H groups, which can form hydrogen bonds, and some of them are charged, with the result that they can interact with other charged groups. All these lipids could account for an increase in the amount of weak bonds in hair fibers.

The σ_{interm} and the t_{interm} indicated that the amount of intermediate bonds decreased in the treated fibers as well as in the case of weak bonds. An increase in intermediate relaxed stress and intermediate relaxation time was observed when IWL liposomes were applied. Again, the IWL application improved the cohesion between the matrix and filaments, especially in the case of the untreated and bleached samples.

Some variations in non-relaxed stress caused modifications in the percentage of disulfide bonds of the protein structure. After the application of IWL liposomes, strong bonds normally decreased because of the increase in the percentage of weak bonds, which were generated by IWL liposomes. These new lipids (IWL) were not able to form or destroy disulfide bonds.

The strength and relaxation studies indicate a high resistance to break of the untreated, bleached, and permed samples post-treated with IWL liposomes. This was accompanied by an increase in the short and intermediate relaxed stress. This indicates an increase in hydrogen bonds and electrostatic forces and an improvement in cohesion between the matrix and the filaments, probably because of some lipid recovery.

CONCLUSIONS

Lipid analysis of chemically treated samples showed a reduction in the amount of lipids, cholesterol and cholesterol sulfate being the most affected. The permed sample had a lipid composition that most resembled native hair. The bleached extract showed lower amounts of free fatty acids, and the relaxed extract had smaller amounts of ceramides.

Lipid recovery of damaged human hair by the application of IWL liposomes can be corroborated by lipid analysis of the hair. An increase in the lipids analyzed confirms the absorption of IWL into the fiber. There is a selective absorption of polar lipids, ceramides, glycosilceramides, and cholesterol sulfate by the undamaged and damaged hair fibers. The role of cholesterol sulfate in maintaining the bilayer structure could be related to moisture retention, elasticity, and the strength of the fibers.

The thermogravimetric analyses showed few differences in the water content of the hair as a result of chemical pretreatments and IWL liposome application. There was a small decrease in the external water content owing to the pretreatments, whereas an increase was observed after IWL liposome application, especially in the case of bleached hair.

Lower breaking stresses were obtained in all the chemically treated hairs, which indicated lower hair reticulation. The application of IWL improved breaking stress and deformation at break and led to an increase in the breaking work of untreated hair. Little improvement was obtained in the case of the chemically treated hair. Only a slight increase in the resistance to break was observed in the bleached and permed samples. Moreover, the relaxation study showed a decrease in weak and intermediate bonds in the chemically

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treated hair samples, probably because of a decrease in their lipidic composition. IWL were able to improve the cohesion between the fibers, increasing the hydrogen bonds and electrostatic forces and improving the cohesion between the matrix and the filaments. This is especially true in the case of non-treated and bleached samples.

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REFERENCES

(1) S. B. Ruetsch and H. D. Weigmann, Mechanism of tensile stress release in the keratin fiber cuticle, *Proc. 9th Int. Wool Textile Research Conf.,* **2**, 44–55 (1995).
(2) J. D. Leeder, The cell membrane complex and its influence on the properties of the wool fibre, *Wool Sci. Rev.,* **63**, 3–35 (1986).
(3) J. A. Swift and A. W. Holmes, Degradation of human hair by papain. Part III. Some electron microscope observations, *Text. Res. J.,* **35**, 1014–1019 (1965).
(4) A. K. Allen, J. Ellis and, D. E. Rivett, The presence of glycoproteins in the cell membrane complex of a variety of keratin fibres, *Biochim. Biophys. Acta,* **1074**, 331 (1991).
(5) G. P. Mitchell, J. Mifsud, D. E. Rivett, and A. K. Allen, Characterization of formic acid-derived cell membrane complex protein from wool, *Biochem. Soc. Trans.,* **20**, 90S (1992).
(6) L. N. Jones and D. E. Rivett, The role of 18-methyleicosanoic acid in the structure and formation of mammalian hair fibres, *Micron.* **28**, 469–485 (1997).
(7) L. Duvel, D. Chun, D. Deppa, and P.. Wertz, Analysis of hair lipids and tensile properties as a function of distance from scalp, *Int. J. Cosmet. Sci.,* **27**, 193–197 (2005).
(8) K. Nishimura, M. Nishino, Y. Inaoka, Y. Kitada, and M. Fukushima, Interrelationship between the hair lipids and the hair moisture, *Nippon Koshohin Kagakkaishi,* **13**, 134–139 (1989).
(9) Y. Masukawa, H. Narita, and G. Imokawa, Characterization of the lipid composition at the proximal root regions of human hair, *J. Cosmet. Sci.,* **56**, 1–6 (2005).
(10) D. Braida, G. Dubief, G. Lang, and P. Hallegot, Ceramide: A new approach to hair protection and conditioning, *Cosmet. Toiletr.,* **109**, 49–57 (1994).
(11) R. Ramírez, M. Martí, A. Manich, J. L. Parra, and L. Coderch, Ceramides extracted from wool: Pilot plant solvent extraction, *Text. Res. J.,* **78**, 73–80 (2008).
(12) L. Coderch, J. Fonollosa, M. Martí, F. Garde, A. de la Maza, and J. L. Parra, Extraction and analysis of ceramides from internal wool lipids, *JAOCS,* **79**, 1215–1220 (2002).
(13) M. de Pera, L. Coderch, J. Fonollosa, A. de la Maza, and J. L. Parra, Effect of internal wool lipid liposomes on skin repair, *Skin Pharmacol. Appl. Physiol.,* **13**, 188–195 (2000).
(14) L. Coderch, M. de Pera, J. Fonollosa, A. de la Maza, and J. L. Parra, Efficacy of stratum corneum lipid supplementation on human skin, *Contact Dermatitis.,* **47**, 139–146 (2002).
(15) L. Coderch, J. Fonollosa, M. de Pera, A. de la Maza, J. L. Parra, and M. Martí, Compositions of internal lipid extract of wool and use thereof in the preparation of products for skin care and treatment, *Patent WO/2001/004244.*
(16) S. Méndez, C. Barba, A. Roddick-Lanzilotta, R. Kelly, J. L. Parra, and L. Coderch, Application of internal wool lipids to hair, *Skin Res. Technol.,* **14**, 448–453 (2008).
(17) R. Kelly, A. Roddick-Lanzilotta, S. Vorwerk, and L. Coderch, Treatement of hair or nails with internal wool lipids, *Patent WO/2007/098075.*
(18) R. Ramírez, M. Martí, A. Cavaco-Paulo, R. Silva, A. De la Maza, J. L. Parra, and L. Coderch, Liposome formation with wool lipid extracts rich in ceramides, *J. Liposom. Res.,* **19**, 77–83 (2009).
(19) N. Hayashi, A. Koyanagi, S. Daikai, N. Gotou, Y. Ueda, and K. Uehara, Effect to hair conditioner, an uniquely developed hybrid polymer consisted of hydrolyzed wheat protein, silicone and alkyl chain, *Proc. 24th IFSCC Congress, Osaka, Japan,* PE-216, 432–433 (2006).

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From: SCC Media Library & Resource Center (library.scconline.org)

(20) H. L. Liu, W. D. Yu, and H. B. Jin, Modeling the stress-relaxation behaviour of wool fibers, *J. Appl. Polym. Sci.,* **110,** 2078–2084 (2008).
(21) C. Zviak, Ed., *The Science of Hair Care,* (CRC Press, 1986).
(22) B. A. Etemesi, Impact of hair relaxers in women in Nakuru, Kenya, *Int. J. Dermatol.,* **46,** 23–25 (2007).
(23) A. Körner, S. Petrovic, and H. Höcker, Cell membrane lipids of wool and human hair form liposomes, *Textile Res. J.,* **65,** 56–58 (1995).
(24) P. M. Elias., Lipids and the epidermal permeability barrier, *Arch. Dermatol. Res.,* **270,** 95–117 (1981).
(25) M. Martí, A. M. Manich, M. H. Ussman, I. Bondia, J. L. Parra, and L. Coderch, Internal lipid content and viscoelastic behaviour of wool fibers, *J. Appl. Polym. Sci.,* **92,** 3252–3259 (2004).
(26) G. E. Rogers, Electron microscopy of wool, *J. Ultrastruct. Res.,* **2,** 309–330 (1959).
(27) A. V. Rawlings and C. R. Harding, Moisturization and skin barrier function, *Dermatol. Ther.,* **17,** 43–48 (2004).
(28) N. Hashimoto, N. Hayashi, A. Koyanagi, Y. Kasahara, T. Adachi, and K. Uehara, Characterization of a hydrolyzed pea protein, similar to hydrolyzed keratin, *Proc. 24th IFSCC Congress, Osaka, Japan,* PE-215, 430–431 (2006).
(29) V. B. Gupta and D. Rama Rao, Stress relaxation studies on wool fibers, *J. Appl. Polym. Sci.,* **45,** 253–263 (1992).

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