

Prevention of hair surface aging

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Synopsis

The hydrophobic character of the surface of human hair is particularly attributed to the lipid components of the epicuticle and to a layer of covalently bound fatty acids. This outer f-layer mainly consists of 18-methyl eicosanoic acid (18-MEA), which is covalently bound to the underlying protein matrix, forming the epicuticle as composite surface structure. Daily weathering and chemical treatments, specifically oxidative bleaching, decrease the hydrophobicity of the outer hair surface drastically.

Multiple daily stress, simulated by an automatic test device including shampooing, blow drying and sun light exposure, changed the lipid composition of hair significantly. A marked loss of 18-MEA was observed. Decreasing contact angles are the direct consequence. A new method to determine the “pseudo-static” contact angle on hair was developed. The results correlate with the corresponding data obtained by dynamic contact angle measurements according to Wilhelmy. Besides that, the resorption time of water droplets by the hair surface provides additional information about the intactness of the outer f-layer.

Specific proteolipids, which are lipid-modified keratins, are able to reconstruct the surface layer of damaged hair by creating renewed surface hydrophobicity and extending the water resorption time by the hair surface.

INTRODUCTION

Human hair is protected against extrinsic aging stress by a hydrophobic outer layer. The intactness of which is essential for the consumer's perception of healthy and shiny hair (1,2). This outer layer, generally referred to as epicuticle, is a thin, chemically resistant layer, which is estimated to contain a high proportion of lipids ranging from approx. 22% up to 44% depending upon the method of removal and subsequent treatments (3). In addition to solvent extractable lipids there are furthermore covalently bound lipids at the surface forming the so called f-layer. It mainly consists of anteiso (+)-18-methyleicosanoic acid (18-MEA), bound as a thioester (5–7) to the cysteine residues in the underlying protein layer (4). This arrangement imparts pronounced hydrophobicity to the hair surface, which is in marked contrast to the overall hydrophilicity of the hair bulk.

Contact angle measurements of undamaged hair, taken near the scalp, show consistently contact angles with water of 100°–110° (8,9). Due to daily aging and cosmetic influences

the hydrophobicity of the surface decreases. Hydrophilic regions are induced on the fiber surface through the oxidation of the lipid end groups and the exposure of protein patches. Thus hydrophilic effects increase along a hair towards the fiber tip due to removal of lipids on the surface, leading to contact angles typically around 70° or 80° at the fiber tip (9). These effects are mainly caused by UV light exposure, intensive shampooing, mechanical abrasion and chemical processes, e.g., oxidative bleaching. Oxidative bleaching might decrease the contact angles even further to values around 40° (10,11).

In order to investigate effects of daily aging on hair more systematically a recently established multiple day-by-day stress simulation was applied (12). This automatic test device includes shampooing, blow drying and sun light exposure. The test procedure was carried out by a robot, which offers a high degree of reproducibility with respect to the induced hair damage profiles. The impact of different oxidative bleaching treatments was studied in comparison to the natural aging effects.

The contact angle of hair with water is an excellent parameter to indicate the general damage constitution of the hair surface. It is easily measurable by means of the dynamic principle according to Wilhelmy, a broadly published method for the determination of contact angle on single hair fibers (9–11,13). Determination of the wettability of a hair by the Wilhelmy balance principle involves the measurement of the vertical force on hair fiber when contact with the liquid is established (9). The forces F are recorded while the fiber is immersed into the liquid. The contact angle Θ (Figure 1) is now accessible by knowing the fiber perimeter L and the surface tension γ of water according to

$$F_o = L\gamma_{LV} \cos \Theta \quad (1)$$

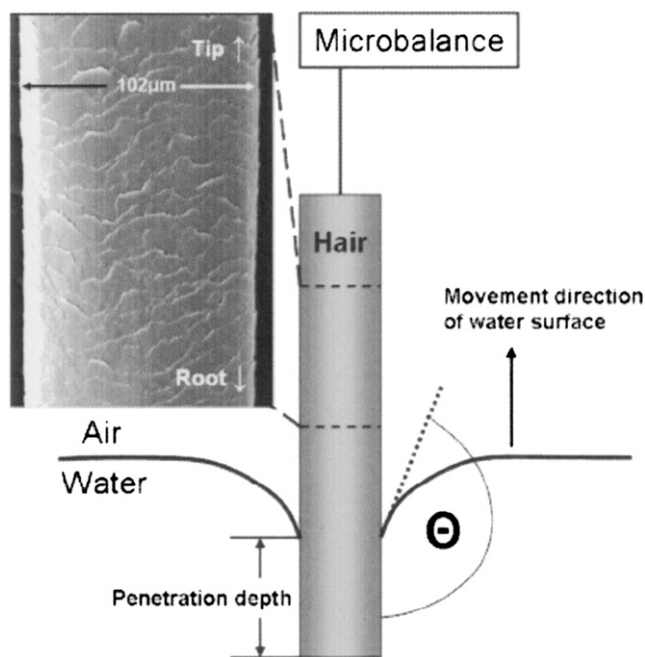


Figure 1. Determination of the dynamic contact angle Θ of single hair fibers in contact with water (13).

The resulting microbalance reading F includes the buoyancy force F_b on the fiber, the weight of the fiber F_g , and the wetting force F_w :

$$F = F_w - F_b + F_g \quad (2)$$

For a single human hair fiber the buoyancy force is negligible versus the wetting force F_w . The weight of the hair is zeroed before the hair touches the surface of the liquid. Thus, the resulting force can be considered as equivalent to the wetting force.

In addition to the dynamic single fiber approach a new method was developed to determine the "pseudo static" contact angle formed by water droplets on parallel aligned hair collectives. In order to reach the "pseudo-static" stage, hair should deliver contact angles above approx. 80° . If contact angles are lower, the water resorption by hair takes place too fast. The shape of the droplet was recorded from a perpendicular positioned directly after deposition (approx. 1s). The evaluation is based on the asymptotical fitting of a tangent to the segment where the water contacts hair (Figure 2).

The resorption time of the water droplet by the hair can be considered as an additional parameter to evaluate the damage degree of the hair surface. Thus the droplet was monitored over time and the elapsed time until the water was totally resorped by the hair was determined, as shown in Figure 3. The resorption kinetic differs from a spontaneous resorption for severely damaged hair to no detectable resorption for virgin hair. Water droplets remain for hours on virgin hair strands. They slowly shrink with evaporation. In agreement with these observations medium bleached hair was used for this study. The determined resorption times ranged from 10 to 100 s.

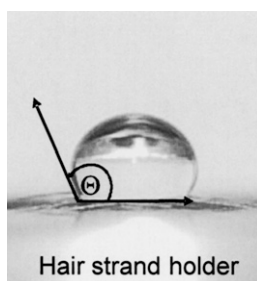


Figure 2. Determination of the "pseudo-static" contact angle of a droplet of water deposited on an aligned hair strand.

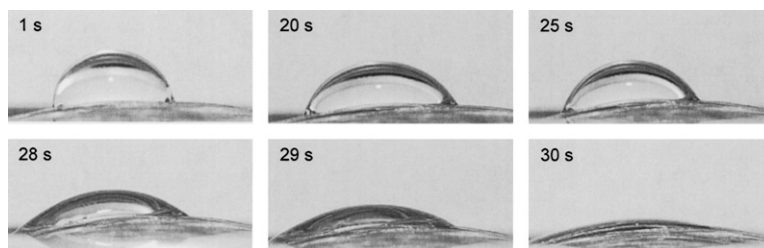


Figure 3. Resorption process of a droplet of water on an aligned hair strand recorded from a perpendicular camera angle.

RESTORATION OF THE DAMAGED HAIR SURFACE

The objective of this study was to restore the natural properties of the virgin hair surface. Considering the morphology of the outer hair surface, where lipids are covalently bound to a protein matrix, lipid-modified proteins, the so called proteolipids, were tested as potential surface restoring agents.

Assuming that the adhesion and organization of proteolipids on the hair surface is controlled by electrostatic and polar forces (protein-protein) as well as apolar interactions (hydrophobic forces), a lipophilic surface layer should be specifically formed, where protein patches are exposed (Figure 4). The incorporation of proteolipids should increase the hydrophobicity of damaged hair, leading to higher contact angles.

MATERIALS

HAIR

For all tests a mixture of light brown Caucasian hair (Color Code 7/0) from Kerling International Haarfabrik GmbH in Backnang (Germany) was used. The tests were performed on hair strands (width 4 cm, length 13 cm, weight 250 mg) and on single fibers (length 30 mm).

STANDARD HAIR CLEANSING

An aqueous solution (pH 5.5) of 12.5% (w/v) sodium laureth sulfate was applied on hair (0.25 g / 1 g). The fibers were rubbed against each other for 1 min. Afterwards it was washed out with tap water at approx. 30 °C for 1 min.

Shampoo A. Aqua, sodium laureth sulfate, disodium cocoamphodiacetate, citric acid, sodium chloride, sodium benzoate, salicylic acid.

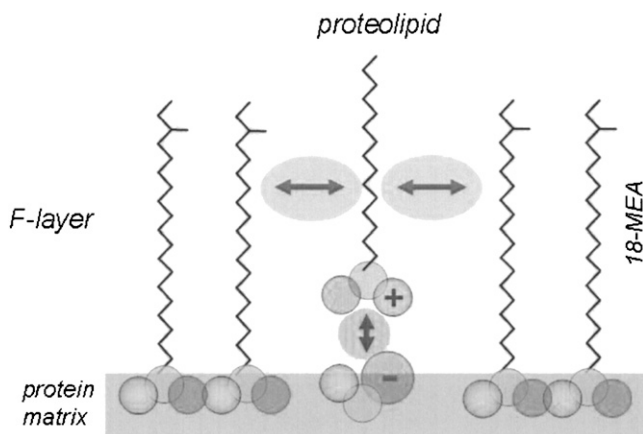


Figure 4. Schematic diagram of the hypothesis of the adhesion principle of proteolipids on damaged hair surfaces.

Shampoo B. Aqua, sodium laureth sulfate, disodium cocoamphodiacetate, citric acid, sodium chloride, sodium benzoate, proteolipid SR, salicylic acid.

Proteolipid SR. All investigations were performed with proteolipid CR which consists of an alkyl chain derived from coco palm oil (C₈–C₁₈), a hydrolyzed protein present in wool keratin and a quaternized adapter group (cocodimonium hydroxypropyl hydrolyzed keratin). Proteolipid SR is water soluble.

METHODS

MULTIPLE DAILY STRESS SIMULATION

The stress simulation, consisting of shampooing, drying and sun exposure, was automatically run by the RV E2 robot from Mitsubishi Electric Corporation, Tokyo (Japan). Firstly all hair samples were immersed into 160 ml of a solution of shampoo A for 3 min at a temperature of 38°C. Afterwards the hair samples were removed from the vessel and rinsed with 20 liter tap water at a temperature of 38°C for 2 min and dried with an air heater (LE 10000S, Leister Process Technologies, Kaegiswil, Switzerland) at 80 °C for 30 min. Sun exposure was simulated for 60 min at 764 W with an ATLAS Suntest (CPS+, Chicago, USA). The time for one cycle was approx. 2.5 h. Fifty cycles in total are run automatically within 5 days (12).

ULTRA BLEACHING

Hair strands were treated for 45 min with a mixture of 6% hydrogen peroxide (Hyprox 500®, Evonik) and 3% potassium persulfate (KPS-5®, Evonik) at pH 10. This mixture was spread by using a brush and washed out with tap water at approx. 30 °C for 3 min.

MEDIUM BLEACHING (I)

Hair strands were treated for 50 min with 7% hydrogen peroxide (Hyprox 500®, Evonik) at pH 8.8. This mixture was spread by using a brush and washed out with tap water at approx. 30 °C for 3 min.

MEDIUM BLEACHING (II)

Hair strands were treated for 25 min with 3% hydrogen peroxide (Hyprox 500®, Evonik) at pH 9.5. This mixture was spread by using a brush and washed out with tap water at approx. 30 °C for 3 min.

QUANTIFICATION OF COVALENTLY BOUND LIPIDS

The hair samples were subjected to a 16 h extraction chloroform/methanol azeotrope in a Soxhlet apparatus. Alkaline catalyzed total hydrolysis of the hair material was performed with 2M alcoholic potassium hydroxide solution and the lipids were recovered by repeated

hexane extraction. Aliquots of the lipid extract were reacted with a silylation (MSHFBA, Macherey & Nagel) reagent to form their trimethylsilylester derivatives and the fatty acids, namely 18-methylsanoic acid, were analyzed by gas-liquid chromatography/mass-spectrometry coupling (GC-MS). Tridecanoic acid was applied as internal standard for quantitative measurements. Duplicate sub-samples of each lipid extract were prepared and analyzed by GC-MS.

FLUORESCENCE MICROSCOPY

The fluorescence microscopic investigation was performed with a scanning photometer microscope MPM03, Zeiss, Jena, Germany). The fluorescence labeling of the proteins was carried out with fluorescein isothiocyanate (FITC) isomer I (Sigma, St. Louis, USA) (14,15).

SCANNING FORCE MICROSCOPY

Scanning force microscopy (SFM) studies were conducted using a Nanoscope IIIa from Digital Instruments operating in the tapping mode. Standard silicon cantilevers were used (PPP-NCH from Nanosensors) with a spring constant $k \approx 42$ N/m and an oscillation frequency $f_0 \approx 330$ kHz. Height and phase images were recorded simultaneously at a scan rate of 1 Hz. All measurements were performed at amplitude A_0 of the freely oscillating cantilever of 30–50 nm. Set-point amplitudes A_{sp} were in the range of 0.85–0.95 A_0 and 0.35–0.45 A_0 , corresponding to light-tapping and hard-tapping conditions, respectively (16).

DYNAMIC CONTACT ANGLE MEASUREMENTS

The determination of the dynamic contact angles was performed by the Tensiometer K14 (Krüss GmbH, Hamburg, Germany). The hair perimeter L was determined by immersing the hair fiber in n-heptan (99%), which shows total wettability ($\Theta=0$). Advancing contact angle was measured in distilled water at 20°C. The immersion rate was 1mm/min in root-tip orientation at the maximum immersion depth of 1mm.

PSEUDO-STATIC CONTACT ANGLE MEASUREMENTS AND DETERMINATION OF DROPLET RESORPTION TIME

A droplet of 25 μ l of distilled water was placed by using a micro-pipette on a parallel aligned and fixed hair strand of 2 g weight and 18 cm length. The side of the droplet is recorded by digital camera (D40 F/3.5-5.6, Nikon co., Japan). Camera perspective is a perpendicular to the hair fiber axes on level with the hair strand.

RESULTS AND DISCUSSION

AGING OF OUTER HAIR SURFACE

After simulation of approximately 100 Middle European summer days (equivalent to 50 cycles of shampooing, blow drying and sun light exposure) significant changes both in

the lipid composition and in the contact angle were detected. The contact angle dropped from 106° to 68° and the content of 18-MEA decreased by approximately 24%. The ultra bleaching process removed significantly more surface lipids and showed smaller contact angles shown in Figures 5 and 6.

DETECTION OF SURFACE CHANGES RELATED TO PROTEOLIPIDS

A high affinity to the hair surface for the proteolipid SR was confirmed by means of fluorescence microscopy. Hair was immersed into an aqueous solution of 0.15 % FITC-labelled proteolipid for 5 min and rinsed out under tap water for 3 min. An intensive and widespread surface coverage was detected for the ultra bleached hair (Figure 7a). Areas of cuticle edges show the highest fluorescence intensity. "Aged" hair shows a much more inhomogeneous light distribution. Here the fluorescence intensity is mainly located at cuticle edges (Figure 7b). These differences in the fluorescence intensity correspond well to the obtained differences in contact angles and the 18-MEA losses reported in Figures 5 and 6.

In addition to fluorescence microscopy SFM investigations were performed to visualize the cuticle surface topography and possible lipid organization at the hair surface before

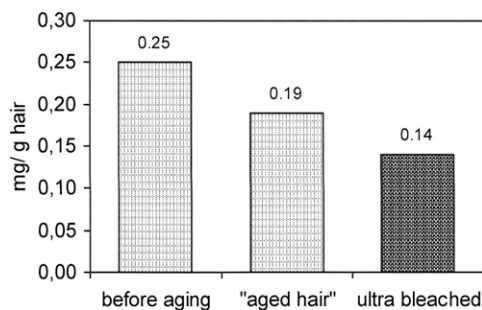


Figure 5. Amount of 18-MEA extracted from hair before (untreated) and after aging simulation ("aged hair") in comparison to the amount of 18-MEA extracted after ultra bleaching (ultra bleached).

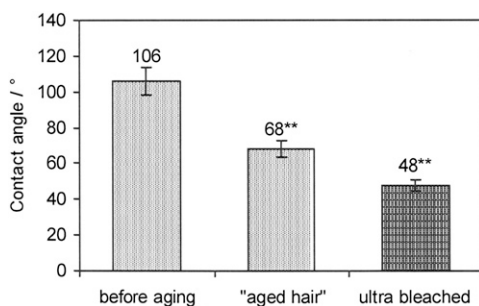


Figure 6. Dynamic contact angles determined according to Wilhelmy before (untreated) and after aging simulation ("aged hair") in comparison to the contact angle of ultra bleached hair (ultra bleached) (** $p < 0.01$ calculated between "before aging" and the samples).

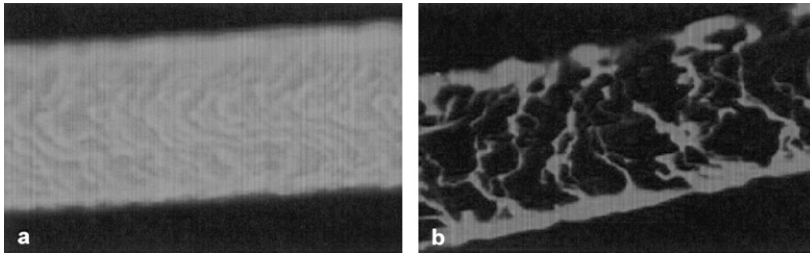


Figure 7. Fluorescence micrograph of ultra bleached (a) and “aged” hair (b) treated (for 5 min) with FITC-labelled proteolipid SR (0.15%, m/m).

and after bleaching and after treatment with the lipid-modified keratins respectively. The upper graph of Figure 8 shows the surface of the virgin hair after cosmetic relevant standard cleansing; it is covered by a “mosaic structure” formed from stacks and terraces of lipids showing a well-defined, reproducible height of approximately 3 nm (green arrows in figure 8a) and widths between 20 nm and 70 nm (pink arrows in Figure 8a).

This nano-scaled lipid multilayer structure is completely removed by oxidative bleaching as demonstrated in Figure 8b. Instead a surface topography showing streaks oriented

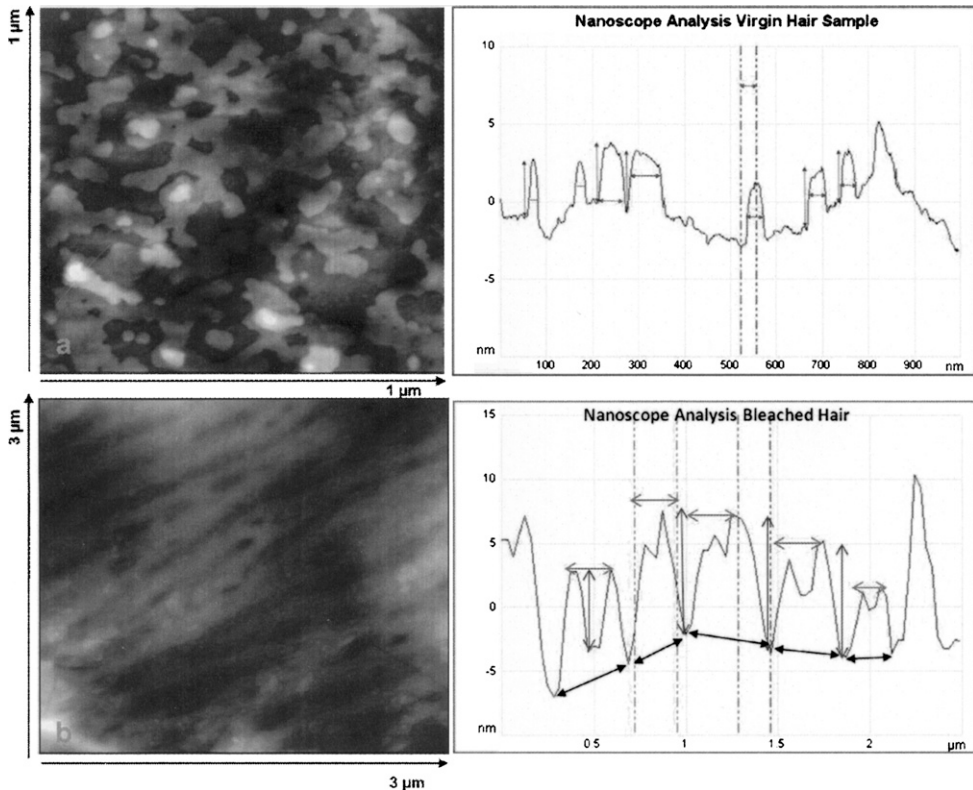


Figure 8. SEM images (topography) of virgin hair with cosmetic relevant standard cleansing (a) and of ultra bleached hair (b).

parallel to the longitudinal axis of the hair is observed; a width of approx. 350 nm (in a range between 200 nm and 500 nm) at the basis of the streaks (black arrows in Figure 8b) is determined by the nanoscope analysis while at their top ends a width of approx. 100 to 200 nm is measured (pink arrows in Figure 8b). The indentations arranged parallel to the longitudinal fibre axis have depths of approx. 7 nm to 10 nm (green arrows in Figure 8b); as a consequence of the high resolution in this study, smaller cavities of approx. 3 nm depths are observed on top of these striations, some of them round, some more oblong.

The phenomenon of longitudinal “striations” has already been described for mammalian hair by J. Smith (17) in 1997 and he assigned this “woodgrain appearance” to exocuticle material exposed after the removal of epicuticle and A-layer due to damaging effects. Later Swift and Smith (7) detected this striation phenomenon on the outer surface of a wide range of mammalian keratin fibers afore subjected to a thorough cleansing procedure by either sonication in sodium dodecyl sulphate or Soxhlet extraction with chloroform/methanol. They determined the striations “to have a lateral spacing of 350 nm, to be of convex profile, and rising to a height of approx. 9 nm”. These dimensions fit very well to those detailed above for the cuticle of bleached hair.

The rinse-off treatment of bleached hair with an aqueous solution of 0.15% proteolipid SR changes the surface structure of the damaged hair significantly. Figure 9 shows in direct comparison the SFM images of the virgin hair (a), the ultra bleached hair (b) and the ultra bleached hair treated with an aqueous solution of proteolipid SR (c). In consequence of the proteolipid treatment the striations are no longer visible and substituted by patterns which rather resemble the multi layered structures observed for the virgin hair surface (a).

EFFECTS OF PROTEOLIPIDS ON THE PHYSICAL PROPERTIES OF THE HAIR SURFACE

The effects of proteolipid SR were investigated by means of dynamic contact angle measurements according to Wilhelmy on ultra damaged hair. The application of a 0.03% solution of the proteolipid increases the contact angle significantly up to 54°. The effect of hydrophobization increases linearly with the concentration up to a level of 0.15%. At this stage a saturation concentration seems to be reached (Figure 10).

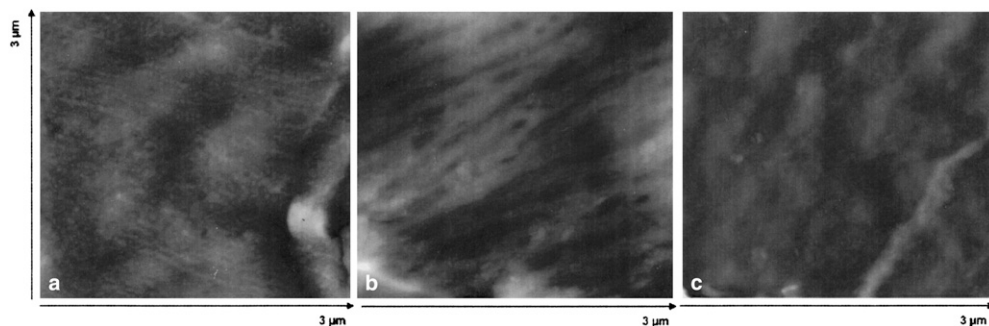


Figure 9. SFM image (topography) of a) virgin hair, b) ultra bleached hair, and c) ultra bleached hair treated with an aqueous solution of proteolipid SR (0.15%, m/m).

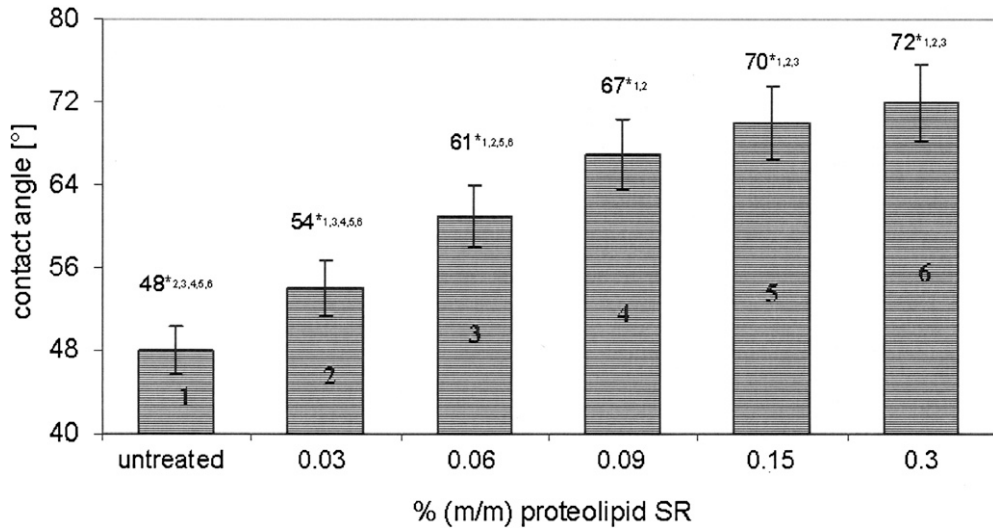


Figure 10. Dynamic contact angles of ultra bleached hair and treated hair with different concentrations of proteolipid SR (* $p < 0.05$. Numbers indicate references for calculation).

The effect of a rinse-off treatment consisting of an aqueous solution containing 0.15% proteolipid SR strongly depends on the damage degree of the hair surface, as shown in Figure 11. The contact angle of ultra bleached hair increases more than 40% (from 48° to 69°). The surface properties of medium bleached hair do not significantly change and the contact angle of the virgin hair surface slightly decreases. The latter effect may be explained by hydrophobically controlled surface adhesion of proteolipids at the intact hydrophobic hair surface.

Investigations by means of the droplet method delivered similar results for the medium bleached hair (II) and the virgin hair. No significant difference between the “pseudo-static”

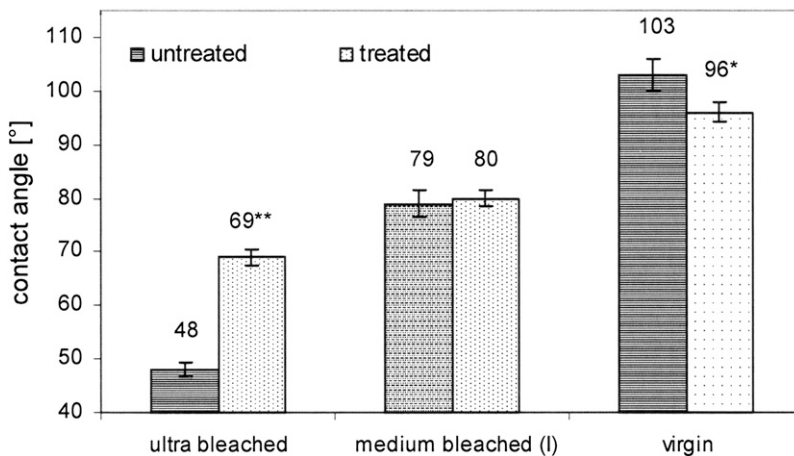


Figure 11. Dynamic contact angle of ultra bleached, medium bleached and virgin hair before and after treatment with an aqueous solution of proteolipid SR (0.15%, m/m) (* $p < 0.05$; ** $p < 0.01$ calculated “untreated” vs. “treated,” respectively).

and the “dynamic” contact angle were found for untreated hair. In contrast to the dynamic contact angle measurements, significant hydrophobization effects for proteolipid SR were observed even on medium bleached hair by using the pseudo-static contact angle measurement, as shown in Figure 12. The application of the droplet method on ultra bleached hair is not feasible due to the quick water resorption by hair.

RESORPTION TIME OF WATER BY HAIR

The resorption time of the water droplet by the medium bleached hair (II) before and after the treatment of a basic shampoo B containing 0.15% proteolipid SR was determined (Figure 13). The resorption time was significantly extended from 79 s to 140 s ($p < 0.05$).

PREVENTION OF HAIR SURFACE AGING

The multiple usage of shampoo B containing 0.15% proteolipid SR during the simulation of approximately 100 Middle European summer days reduced the degree of aging damage significantly. The decrease of the contact angle due to the simulated aging, as shown in Figure 6, significantly lowers by 11.4% ($p < 0.05$).

SUMMARY AND CONCLUSIONS

Simulated day-by-day aging causes a significant reduction of the natural hydrophobic properties of virgin hair. Contact angles strongly decrease due to a significant loss of 18-MEA. Ultra bleaching shows significantly stronger damage on the hair surface than the simulated aging. The contact angle of hair with water is an accurate parameter to evaluate

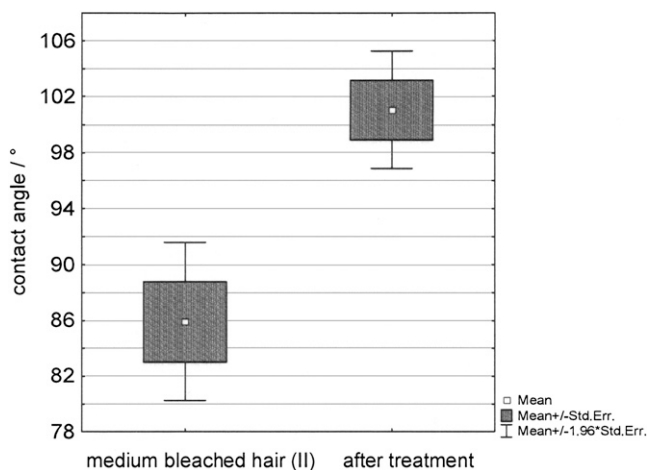


Figure 12. Pseudo-static contact angle on medium bleached hair (II) before and after application of basic shampoo B containing proteolipid SR (0.15%, m/m).

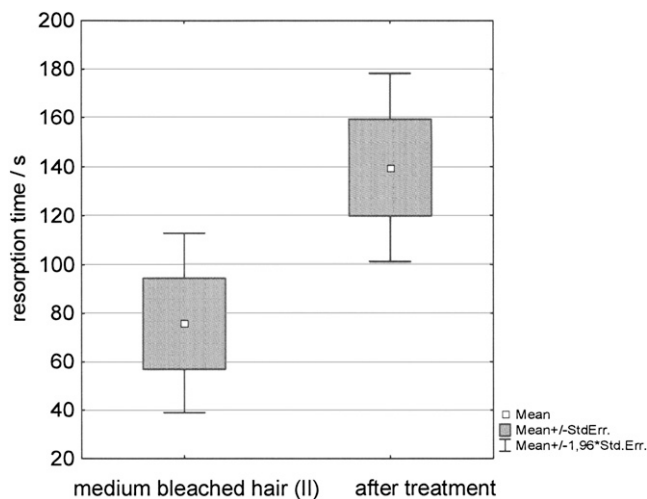


Figure 13. Resorption time of water droplets by medium bleached hair (II) before and after application of basic shampoo B containing proteolipid SR (0.15%, m/m).

the damage degree of the hair surface and the intactness of the F-layer. Contact angles can be determined on single hair fibers indirectly by means of dynamic wetting force measurements according to Wilhelmy and directly by evaluation of water droplets on hair strands under “pseudo-static” conditions. For the latter approach a new method was established. The results of this method correlate well for untreated hair to the corresponding Wilhelmy-data. The determination of the resorption time of the water droplets by hair offers an additional test parameter to characterize the constitution of the hair surface.

Specific proteolipids, which are lipid modified proteins, show a very high affinity to damaged areas on the hair surface. This was confirmed by fluorescence microscopy and scanning force microscopy studies. The distribution strongly depends on the damage degree of outer hair surface. Due to the high surface deposition on damaged hair, proteolipids are able to restore the natural hydrophobic properties of virgin hair. This surface restoration leads to an extended resorption time of water by hair.

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