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The effects of lipid penetration and removal from subsurface microcavities and cracks at the human cuticle sheath

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Synopsis

An analysis of the light interference patterns produced by the penetration and removal of lipids from the cuticle sheath has shown that cuticle cells in their virgin state have a few intrinsic imperfections in the form of voids and cracks. The experiments also showed that when the cuticle sheath undergoes friction, extension, torsion, and thermal stresses additional microvoids, cavities, and gaps are created at the cuticle cells subsurface. Because of the activity of the sebaceous glands it is quite normal to find these cavities filled with exogenous lipids. Cuticle sheath dehydration, lipid addition, and lipid removal indicate that the viscoelastic deformations giving rise to microcavities can be reversible or irreversible. The presence of exogenous lipids in these cavities was found to be critical in maintaining the mechanical integrity of the cuticle cells. Regions presenting microcavities and cracks produced by reversible deformations were seen to fully recover and heal with the onset of a plasticization effect produced by the synergy of lipids and water. In contrast, microcavities produced by irreversible deformations were always filled with lipids. In both cases the lipids acted as weak adhesives, in particular, in those cavities and gaps opened in the cuticle cell interfaces.

INTRODUCTION

The cuticle sheath of human hair is a composite structure formed by hundreds of cuticle cells that overlap continuously in stacks of 5 to 10 units like shingles on a roof (1–2). This part of the human hair is considered to be essential for maintaining the physical integrity of the whole fiber as it acts as a protective shield against environmental stresses that may otherwise damage the cortical cells. Unfortunately, modern grooming practices impose harsh mechanical and thermal stresses to the hair fibers (3). Consequently, early signs of damage in relation to the life span of a hair fiber appear in the cuticle sheath compromising its protective role.

One type of damage that is prone to appear within the cuticle sheath due to its multilayer character is the formation of gaps and cavities at the junctions of the various internal layers. Once these defects form they coalesce under the application of further stresses causing decementation and delamination of large portions of cuticle cells. As a result, breakage of these cuticle cell portions rapidly ensues even under the action of slight friction (1–4). This paper discusses the formation of gaps and cavities at the cuticle sheath subsurface and analyzes how water and lipid penetration affects their development.

EXPERIMENTAL

PROTOCOLS

The method used to study the formation of gaps and cavities within the cuticle sheath consisted in analyzing the light interference patterns (LIPs) produced by cuticle cells when seen under a powerful light by optical microscopy (4). As it has already been shown (4) cuticle cell decementation causes disruptions in the continuity of the cuticle sheath producing the inclusion of air in the formed cavities. Light interference patterns are then produced by a mismatch in the indexes of refraction between the cuticle cell layers and the air cavities as the light passes through. The presence of these defects within the cuticle sheath is thus revealed by the appearance of colorful patterns appearing at the hair surface. A similar phenomenon has been reported to occur with pores within the medulla of hair (5).

MATERIALS AND INSTRUMENTS

The light interference patterns were analyzed by microscopic analysis using a Hi-Scope KH-3000 equipped with a metal halide lamp. The hair used for testing thermal and mechanical damage was European virgin hair from International Hair Importers. For experiments requiring analysis of hair close to the root, virgin hair from 4 female donors was obtained from areas close the scalp. Hair from the same source was used for lipid extraction. Solvents used for oil and lipid extraction from hair were IPA and Hexane GC grade. Extraction of lipids from hair was made by immersing 1 g bundles of hair fibers in 100 ml of solvent at room temperature conditions for periods of time ranging from minutes to months. Analysis of the lipids extracted from hair was made by GC-MS after methylation.

During solvent immersion single hair fibers were selected and taken for microscopic analysis at various time intervals. When needed, cycles of mechanical and thermal stresses were applied to single hair fibers using a blow dryer or curling iron according to methods described elsewhere (6–7). The number of gaps and cavities appearing at the cuticle sheath of hair fibers, either treated with solvents or damaged mechanically or thermally, were counted and plotted as a function of hair length. All defects producing patterns of light interference ranging in size from 0.1 to 5 microns were considered as gaps or microcavities. The oil used for deposition into these cavities was mainly Jojoba oil. The deposition method consisted in immersing the hair fibers in a solution of oil at 0.1% in IPA at RTC for three minutes.

RESULTS

GAPS AND CAVITIES FORMED BY MECHANICAL AND THERMAL STRESSES

Microscopic analysis of hair fibers taken from the various donors showed that the presence of gaps and cavities in people's hair was pervasive. Figure 1 shows, for instance, that the average count of gaps and microcavities increases in regions of the fiber distant from the root, while in areas close to the root the count is nil. Also, it was observed that the average

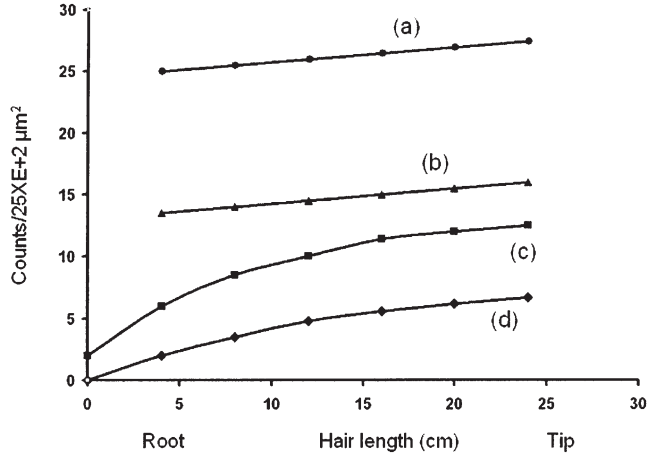

Figure 1. Average count of gaps and microcavities (15% SD) in a section of 50×50 µm as a function of hair length obtained from four groups of ten hair fibers each after various treatments as follows: (a) After 20 cycles of 15% extension and retraction. (b) After 20 cycles of 10-s blow-drying at 65°C followed by 10 s of immersion in water. (c) After immersion in IPA for 3 min. (d) With no treatment.

count of gaps and micro-cavities increases substantially when the hair fibers are subjected to any of the following treatments, namely: (i) Cycles of mechanical stresses, (ii) cycles of thermal stresses, or (iii) immersion in solvents (see Figure 1). It should be mentioned here that the average number of gaps and cavities found along the various hair fibers was seen to vary strongly from subject to subject.

The shape and size of defects giving rise to the appearance of patterns of light interference was observed to be dependent on the type of stresses applied to the hair fibers. Cycles of mechanical extension and retraction usually led to the formation of extended gaps or large open cavities between the two top cuticle cells (see Figure 2). In most cases the top cuticle cells appeared deformed, buckled, and separated from the second one that remained cemented. A few cuticle cells, however, showed de-cementation and gap formation but did not appear deformed or buckled. Furthermore, it was noted that gaps also formed after the second, third, or fourth cuticle cell. In these instances stacks of two or three cuticle cells appeared cemented but separated from the underlying adjacent cuticle cell. Most gaps and openings created by mechanical extension were seen to extend from the tips of the cuticle cells towards the cortex and in most cases they were filled with air as indicated by the appearance of patterns of light interference after stress application.

It is worth mentioning that in certain cases cuticle cells presenting gaps and microcavities though always apparent by light interference analysis were not often detected by scanning electron microscopy (SEM). The main reason for this is that light interference analysis is sensitive to the presence of gaps and cavities deep underneath the cuticle sheath surface regardless whether the cuticle cells are buckled or not. Previous research has already shown that these types of extended gaps involving cuticle cell decementation are formed by Poisson contraction stresses acting on the cuticle sheath as the hair fibers are subjected to cycles of elongation and retraction (6). The cyclic radial contraction and extension of the hair fibers during elongation produces cuticle cell cement breakage by fatigue, and also induces viscoelastic deformations on the whole cuticle cell, therefore, causing buckling and creating extended gaps or cavities filled with air.

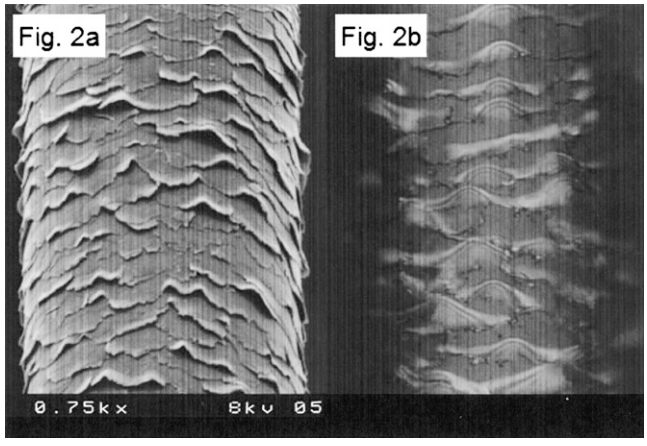

Figure 2. Micrographs showing typical gaps and cavities formed by buckled cuticle cells after 20 cycles of 15% extension and retraction obtained as follows: (2a) by SEM, and (2b) by optical microscopy using light interference analysis.

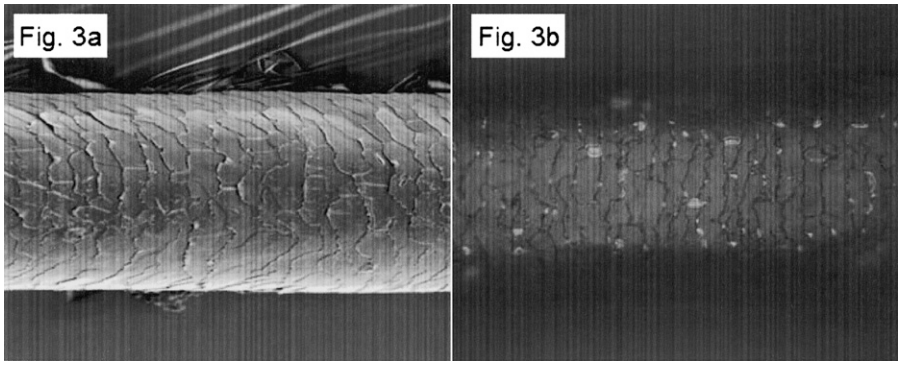

Figure 3. Micrographs (250×) of hair showing thermal cracks and narrow channel-like deformations after exposure to 15 cycles of 30-s blow-drying at 75°C followed by 30 s of immersion in water. Micrograph 3a was obtained by SEM. Micrograph 3b, displaying patterns of light interference, was obtained by optical microscopy.

The shape and size of gaps and microcavities created by thermal stresses varied depending on the source of high temperature and number of cycles, and on whether the fibers were immersed in water after each temperature cycle. Figure 3a,b shows SEM and optical micrographs of elongated cavities forming channel-like patterns ~3 to 4 um long protruding away from the cuticle cell surface with and without cracks. Crack formation by thermal stresses has already been reported in the past (7), however, the results described here indicate that channel like deformations may form before cracking occurs. Furthermore, the fact that these protrusions produce patterns of light interference indicates that they consist of hollow cavities filled with air. It is worth to note that these types of cavities are not caused by decementation and deformation of the whole cuticle cell as previously seen in the case of extended cavities produced by mechanical stresses. The thermal cavities appear rather to be produced by more localized deformations.

Yet other types of localized defects appear at the cuticle cells sub-surface when the hair fibers are exposed to cyclic contact with hot surfaces, i.e. curling or flat irons. These types of

defects approximately 1–3 μm in diameter form cavities filled with air as evidenced by their associated punctual bright colored spots due to light interference (see Figure 4a,b,c). An inspection of the SEM micrographs and the light interference patterns shown in Figures 4a and 4b indicates that these types of defects are of two types, namely, (i) cavities forming bulges and blisters protruding away from the surface, and (ii) cavities buried deep beneath the surface with no bulging. The formation of such disruptions in the continuity of the cuticle cell certainly constitutes a loss in the cross-linking density of the protein structure due to a rapid expansion of water within the cuticle cell (see Figure 4c).

GAPS AND CAVITIES FORMED BY SOLVENT EXTRACTION

The immersion of hair fibers in solvents increased substantially the average number of micro-cavities (see Figure 1, line c); although, not all cuticle cells were affected by the immersion and only a few cuticle cells underwent cavity formation. Furthermore, it was observed that immersion in IPA always led to higher increments in the number of micro-cavity and gap formation than did fiber immersion in hexane. Increments in the number of gaps and cavities appearing in regions closer to the root after solvent immersion were low, while in regions away from the root, the increments were quite large. These observations suggest that increments in the number of micro-cavity and gap formation observed after solvent immersion are related to damage that has been done to hair prior to solvent immersion. If this were not the case solvent immersion would produce equal increments of defects all along the fiber from root to tip.

However, before adopting this hypothesis as conclusive, we should analyze other alternative explanations. One possible hypothesis is that gaps and cavities formed after solvent immersion is due to solvent swelling. For instance, it is known that IPA has the ability to swell the hair fiber. However, hexane does not swell the keratin fiber and yet it causes gap

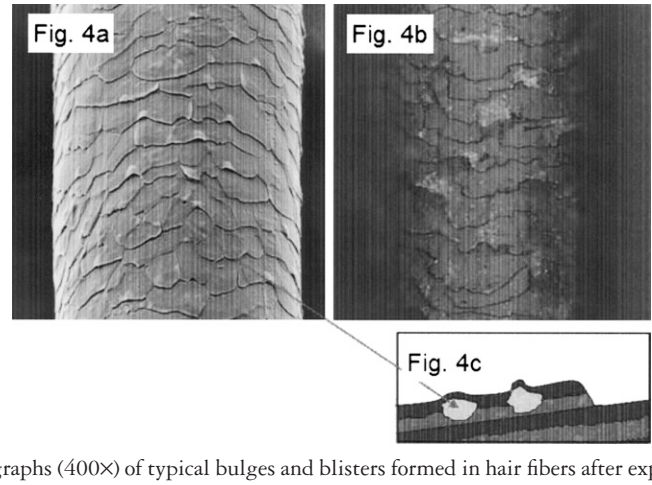

Figure 4. Micrographs (400×) of typical bulges and blisters formed in hair fibers after exposure to five cycles of 30 s in contact with a hot iron surface at 120°C followed by 30-s immersion in water. Figure 4a was obtained by SEM. Figure 4b, showing patterns of light interference, was obtained by optical microscopy. Figure 4c is a schematic representation of loss of continuity within the cuticle cell, leading to cavity formation by rapid water evaporation.

and cavity formation, so the cause for their appearance must be found elsewhere. The other hypothesis is that the cavities may be caused by removal of endogenous lipids from cuticle cells since both solvents are known to extract lipids from hair. The nature of endogenous and exogenous lipids in hair has already been reported in the past (8). This latter hypothesis does not explain, however, why lipid extraction from cuticle cells only leads to the formation of gaps and cavities in a few cuticle cells and not in all cuticle cells. The endogenous lipids of hair exist only in the cell membrane complex and it has been reported that their removal requires high temperatures and long extraction times.

One last hypothesis that might satisfactorily explain gap and cavity formation after solvent immersion is that these defects were already formed prior to solvent immersion and were later gradually filled with exogenous lipids, either from scalp sebum or from cosmetic formulations. In fact, GC-MS analysis of the material extracted from hair by solvent immersion indicated that it was mainly composed of exogenous lipids characteristic of sebum. The following fatty acids were found in the extracted material after methylation: methyl caprylate, methyl laurate, methyl myristate, methyl palmitate, methyl linoleate, and methyl stearate. These observations strongly suggest that gaps and cavities formed after solvent immersion were pre-existent to the analysis.

The above described observations indicate that hair from common people contain gaps and cavities that can be classified according to two main groups, namely: (a) gaps and cavities present in the cuticle sheath caused by damage done prior to the analysis that are visible by light interference, and (b) gaps and cavities that were also present before the analysis but that are not visible by light interference or by any other microscopic technique due to the fact that they are filled with sebum and oils. These later types of gaps and cavities can be divided in turn into defects of natural origin, and defects produced by mechanical or thermal damage done to hair prior to the analysis.

It should be mentioned here that gaps and cavities of natural origin were considered as those appearing in hair cut 2 or 3 mm away from the root and after solvent immersion. Because of their proximity to the scalp it is most likely that they were natural imperfections in hair that were not caused by damage due to grooming stresses. They only appeared after exogenous lipids such as sebum were extracted from cuticle cells by solvent immersion. Therefore, their impregnation with sebum concealed their detection by both scanning electron microscopy and analysis of patterns of light interference. Incidentally, their count was extremely low and it was observed that not all fibers had these natural defects. In contrast, the number of gaps and cavities that were concealed by sebum and appeared in hair sections away from the root after solvent immersion was always higher.

It appears, thus, that sebum is able to wet and spread over the hair surface after it has been secreted from the sebaceous glands near the hair follicle. As the sebum spreads over the hair surface it is also able to penetrate and impregnate gaps and micro-cavities present in the cuticle sheath. This will explain why in many cases when hair fibers obtained from common people were analyzed by optical microscopy, gaps and cavities were not detected until after the hair fibers had been immersed in solvents. This explanation also implies that sebum diffused into gaps and cavities may not easily be removed by normal shampooing.

Another observation confirming that gaps and cavities were concealed by sebum inclusion and had formed prior to solvent immersion was that they vary in shape and size. Some of the gaps and cavities were of the extended type produced by extension and retraction stresses

while others were of the punctual type produced by thermal stresses. Figure 5 shows, for instance, agglomeration of micro-cavities at the cuticle cell edges appearing after solvent immersion. These gaps, although, somewhat irregular in shape are similar to those shown in Figure 2. Figure 6 shows punctual cavities that appeared also after solvent immersion. These cavities have a striking similarity to those produced by thermal stresses.

EFFECTS OF WATER PENETRATION INTO GAPS AND CAVITIES

In most cases immersion of hair fibers in water led to the closing of both types of defects namely, the extended gaps and the punctual cavities; although, not all these defects were always seen to close even after long periods of water immersion (see Figure 7a,b). In fact, it was observed that a large number of cavities, in particular, those associated to buckled cuticle cells of hair fibers subjected to large cyclic extensional deformations (>25%) were unaffected by water immersion. Since keratin fibers can be considered as biopolymers that display viscoelastic or plastic behavior (9) depending on the strain level, it is expected that gaps and cavities will close when their associated deformations fall within the protein viscoelastic range (see Figure 7b). Water immersion will, thus, have the ability to accelerate the recovery process within this range as it plasticizes and relaxes the stressed proteins of the cuticle cells.

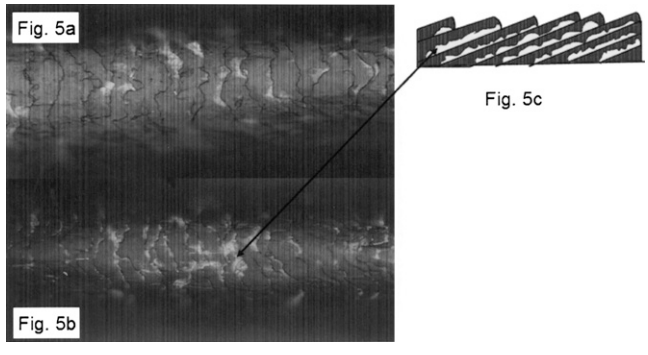

Figure 5. (a,b) Micrographs (300×) of cavity agglomeration produced in hair fibers after immersion of 3 min in IPA. The images were obtained by optical microscopy and show patterns of light interference due to the presence of cavities filled with air under the cuticle cell surface. Figure 5c is a schematic representation of cavities filled with air after extraction of exogenous lipids.

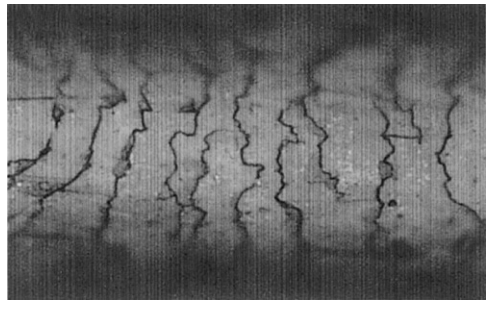

Figure 6. Micrograph (600×) of punctual cavities ($\Phi \sim 1.0$ μm) without bulge formation produced in hair fibers after immersion in IPA for 3 min. The micrograph, taken by optical microscopy, shows the presence of colorful light interference dots produced by the presence of air cavities under the cuticle cell surface.

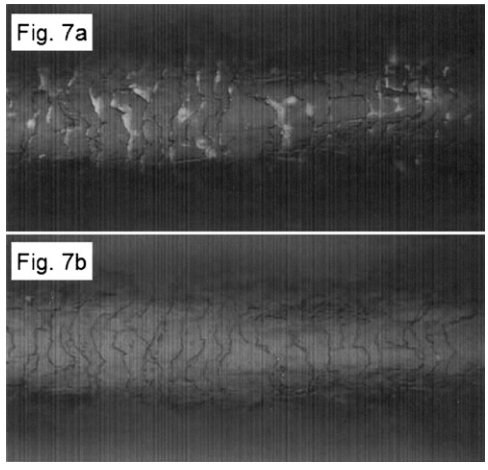

Figure 7. Micrographs (300×) of a hair fiber before (a) and after (b) it was immersed in water for 3 min. Observe the disappearance of the light interference patterns in Figure 7b due to viscoelastic recovery of deformations sustaining the cavities. The hair fiber had previously been immersed in IPA for 3 min.

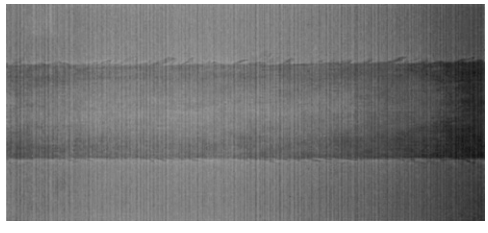

Figure 8. Micrograph (250×) of a hair fiber showing cuticle lifting while immersed in water at pH 10.0. The cuticle cells of this fiber had been previously decemented by applying 10 cycles of 15% extension and retraction. Subsequently, before immersion is alkaline water, the deformed cuticle cells were allowed to recover in water at neutral pH for 5 min. and the fiber was allowed to dry at room temperature for 24 h.

Gaps and cavities are, thus, the result of harsh mechanical and thermal grooming stresses that deform the protein structure by excessive extension or contraction. In many cases these deformations may be accompanied, either, by a loss in the cross-linking density of the protein structure or by cement breakage at the cuticle cell membrane complex. If the deformations are within the range of viscoelastic reversibility, the cuticle cells will recover by water plasticization. However, if the damaging stresses are severe, the cuticle cells undergo creeping and plastic deformation and gaps and cavities are recovered by water immersion.

It is interesting to note that, while buckled cuticle cells forming gaps were able to recover from their deformed state, their cementing didn't recover once it was broken. This phenomenon could be confirmed by the following simple experiment: First, hair fibers with decemented and buckled cuticle cells were allowed to recover by water immersion. Subsequently, the hair fibers were dried for 24 h at room temperature. Later, when the dried hair fibers were re-immersed in water again the decemented cuticle cells reopened and buckled while in water (see Figure 8); this phenomenon was, in particular, stronger at alkaline pHs ranging between 9 and 11. Hair fibers whose cuticle cells were not decemented didn't display this behavior. These observations indicate that once the cuticle

cells are decemented, they can buckle and deform in water by swelling stresses even if their deformations were previously recovered.

EFFECTS OF OIL PENETRATION INTO GAPS AND CAVITIES

When Jojoba oil or any other oil was applied to lifted cuticle cells presenting gaps or cavities it did not lead to deformation recovery. Thus, oils did not induce plasticization of the cuticle cell proteins. The oils, however, were seen to penetrate into gaps and cavities destroying the light interference patterns. Gaps and cavities protruding away from the hair surface were still visible by optical microscopy after oil penetration even when their associated patterns of light interference were destroyed. This is as expected since air in the cavities has been substituted by oil; a material with a higher index of refraction than air. In Figure 9 it can be seen, for instance, that cavities and gaps produced by lifted and buckled cuticle cells are still visible even when their patterns of light interference have been destroyed by the presence of oil.

In contrast, oil penetration concealed gaps and cavities existing deep inside the cuticle sheath with no associated surface protrusions. Figure 10a,b shows, for instance, that gaps and cavities not involving cuticle cell protrusions were not longer visible by optical microscopy after oil penetration. Thus, oil impregnation of these cavities gives the appearance that the hair surface doesn't have any damage or any defect at all (see Figure 10b). These observations confirm the previous hypothesis proposing that damage by grooming stresses produces gaps and cavities that later are filled and concealed by the penetration of lipids, either, from sebum or from cosmetic formulations. It is worth to mention here that other researchers have found that Jojoba oil does not penetrate into the cortex although the experiments here show that it can penetrate in cavities formed at the cuticle sheath (10).

EFFECTS OF WATER AND OIL PENETRATION INTO GAPS AND CAVITIES

As shown in previous paragraphs water induces a rapid mechanical recovery of the deformed protein structure within the cuticle sheath, while oil penetrates into these defects.

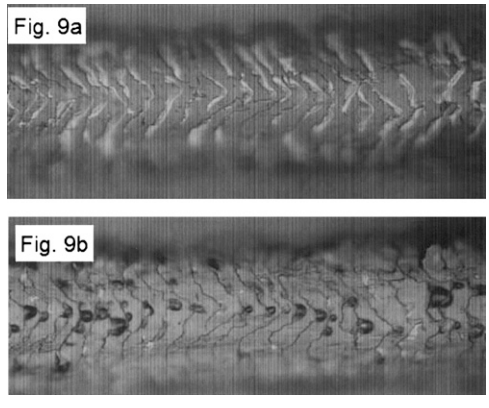

Figure 9. Micrographs (300×) of a hair fiber with decemented and buckled cuticle cells before (9a) and after (9b) immersion in a solution of 0.1% Jojoba oil for 3 min. Observe that the oil has penetrated underneath the buckles or cavities but that the cuticle cell deformations remain.

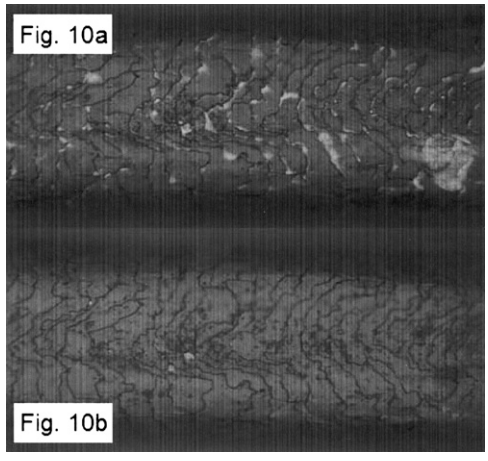

Figure 10. Micrographs (300×) of a hair fiber showing agglomerations of cavities without bulging before (a) and after (b) it was immersed in a solution of 0.1% Jojoba oil for 3 min. Observe that the oil has penetrated and impregnated the air cavities, concealing them and destroying the light interference phenomena.

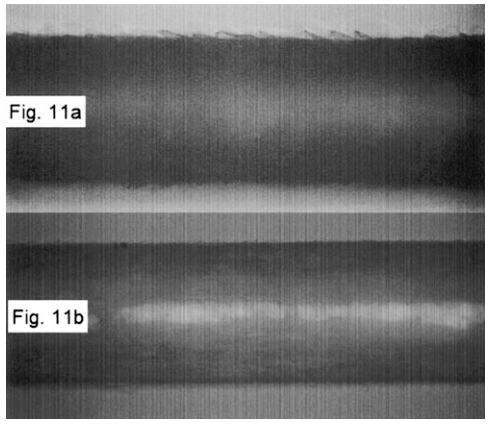

Figure 11. Micrograph (250×) of two hair fibers with buckled and recovered cuticle cells showing degree of cuticle lifting while immersed in water at pH 10.0. One fiber was immersed into the alkaline water without oil treatment (a), while the other (b) was treated with oil before the buckled cuticle cells recovered.

Both phenomena were seen to lead to the disappearance of gaps and cavities; water by deformation recovery while oil by cavity impregnation. Possible benefits and effects resulting from the occurrence of both phenomena are analyzed in the following sections. As it was mentioned before cavities formed by cuticle cell buckling close once recovered by water immersion. The cavities could, however, be reopened again after the hair fibers were dried and immersed in water at alkaline pHs.

This phenomenon of lifting and reopening took place while the hair fibers were immersed in water and was ascribed to the action of unbalanced water swelling stresses in decemented cuticle cells. Further, analysis showed, however, that the degree of cuticle cell lifting and reopening at alkaline pHs was substantially reduced if the cavities were impregnated with oil previous to their recovery process with water (see Figure 11a,b). In the absence of oil treatment the degree of cuticle cell reopening was higher.

These observations suggest that the presence of oil in recovered cavities prevents the occurrence of cuticle cell lifting at alkaline pHs by the combined action of the two following mechanisms, namely: (a) by the oil acting as a hydrophobic barrier that decreases rapid swelling stresses as water diffuses into the cuticle cells, and (b) by the oil acting as a weak adhesive cement between two separate cuticle cells. The action of both mechanisms seems necessary to explain the reduction in cuticle cell lifting at alkaline pHs. For instance, the presence of oil in the recovered cavities or gaps acts as a barrier that decreases steep gradients of swelling. However, eventually the cuticle cells swell and reopen again if the oil does not provide enough adhesion between cuticle cells.

CONCLUSIONS

The experimental results discussed in the previous paragraphs indicate that the analysis of gap and microcavity formation in cuticle cells by light interference techniques can yield important information when water and oil are allowed to penetrate into these defects. Gaps and microcavities produced by reversible deformations were seen to close by a plasticization effect induced by water. However, the phenomenon of gap and cavity closing alone did not prevent the cuticle cells from reopening again when the hair fibers were immersed in water at alkaline pHs. In contrast, when the phenomenon of cavity closing by water plasticization was combined with oil penetration, the degree of cuticle cell reopening was substantially reduced. Water penetrates into the cuticle cells and allows for recovery of the protein structure by plasticization. However, water penetration also induces swelling stresses that buckle the cuticle cells if they are decemented. The protective role of oils or lipids may arise, thus, from their capacity to act as a barrier preventing rapid diffusion of water and swelling, and also from their ability to produce a weak adhesion at cuticle cell junctions.

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