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## ***Impact of Hydrogen Peroxide on Hair Fiber and Follicle Integrity: A Study Combining FTIR-ATR, Mechanical Strength and Immunostaining of Proteins Related to Melanogenesis***

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### **1. Introduction**

Hydrogen peroxide ( $H_2O_2$ ) is extensively used in cosmetic formulations, particularly for hair bleaching and dyeing, due to its high oxidative potential. However, its application leads to significant structural and biochemical alterations in hair fibers. A primary target of oxidative damage is the keratin matrix, where  $H_2O_2$  cleaves disulfide bonds ( $-S-S-$ ), converting them into cysteic acid residues ( $-SO_3H$ ), thereby compromising the fiber's mechanical strength and tensile properties [1,2]. Fourier-transform infrared spectroscopy (FTIR) studies have revealed an increased absorbance at  $1040\text{ cm}^{-1}$  in treated samples, consistent with cysteic acid formation, confirming extensive oxidation of cystine residues [3].

In addition to structural degradation,  $H_2O_2$  induces damage at the follicular level, affecting melanocytes and the melanogenesis process. Accumulated  $H_2O_2$  in hair follicles disrupts the function of tyrosinase, the rate-limiting enzyme in melanin biosynthesis, through methionine sulfoxide formation at its active site. This oxidative mechanism has been directly linked to follicular dysfunction [4]. Moreover, a lack of catalase and methionine sulfoxide reductase in gray hair follicles exacerbates oxidative stress, leading to

melanocyte apoptosis and reduced pigment production [5,6]. Markers such as MITF and C-KIT, critical for melanocyte survival and signaling, have also been shown to decrease following oxidative insult, further contributing to pigmentary loss [7].

Melanin degradation products, such as pyrrole-2,3,5-tricarboxylic acid (PTCA), have been identified as specific biomarkers of oxidative cosmetic treatment. Their detection via chromatographic and mass spectrometric methods enables discrimination between untreated and bleached hair, with PTCA and related compounds appearing exclusively post-H<sub>2</sub>O<sub>2</sub> exposure [8,9]. These markers are critical in both cosmetic science and forensic toxicology for confirming chemical alterations in hair pigmentation.

Advanced imaging and spectroscopic techniques, including scanning electron microscopy (SEM), FTIR-ATR, and Raman spectroscopy, provide non-destructive evaluation of protein and pigment damage in hair. SEM analyses have revealed scale lifting and surface erosion in bleached fibers, while FTIR confirms biochemical modifications in the cortex and cuticle layers [1,10]. Furthermore, FT-Raman spectroscopy has proven valuable for *in situ* monitoring of disulfide bond cleavage and peptide backbone damage, enhancing our understanding of hair photodamage and chemical treatment effects [11].

This study aims to evaluate the oxidative degradation induced by hydrogen peroxide in both the fiber matrix and hair follicles using a multidisciplinary approach involving FTIR-ATR spectroscopy, mechanical resistance testing, and immunolabeling of melanogenesis-associated proteins. By correlating chemical, structural, and molecular parameters, we seek to provide comprehensive insight into the oxidative impact of H<sub>2</sub>O<sub>2</sub> on hair health.

## 2. Materials and Methods

### 2.1. Hair samples preparation and treatment

Natural Asian, Caucasian, and Curly hair strands were prepared (three replicates; 2.5g; 25cm). The strands were exposed to 3.5% hydrogen peroxide for 1 hour. The measurements were obtained before and after the oxidative damage exposing.

## 2.2. Infrared Spectroscopy – FTIR-ATR

The cystine-to-amide area ratio ( $1040\text{cm}^{-1}/1650\text{cm}^{-1}$ ) and disulfide bond ( $525\text{cm}^{-1}$ ) content were quantified using FTIR-ATR spectroscopy. Measurements were conducted 5 cm above the segment immersed in peroxide, in order to assess the extent of capillary diffusion and the propagation of oxidative damage along the fiber.

## 2.3. Mechanical Resistance

Mechanical properties were evaluated from 50 hair fibers by measuring Young's modulus and the force required at 20% deformation. Measurements were conducted 5 cm above the segment immersed in peroxide.

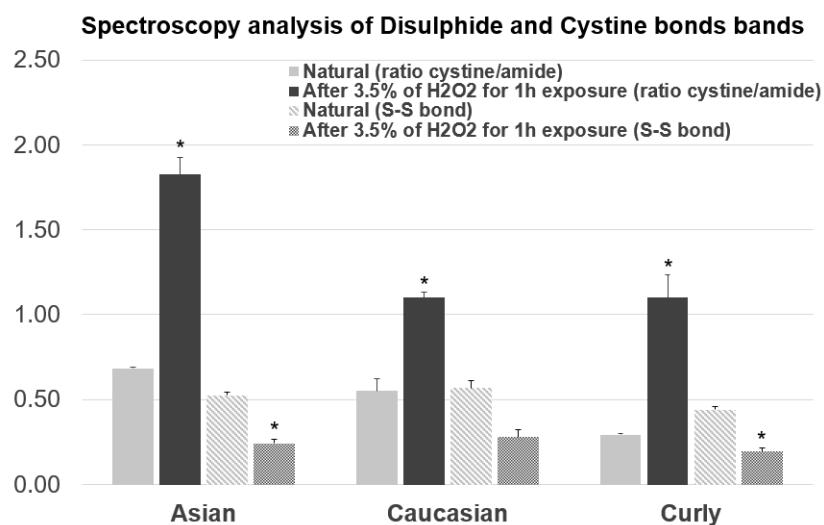
## 2.4. Histological sectioning and Immunofluorescence Marking

Hair follicles were exposed to 3.5% hydrogen peroxide for 3 hours and subsequently embedded in a cryoprotective medium for sectioning. Cryosections of standard thickness were obtained using a cryostat, and individual slides were prepared for each target protein. Sections were incubated overnight with primary antibodies targeting melanogenesis-associated markers, including MITF and C-KIT. Following PBS washes, a fluorescent secondary antibody was applied. Nuclear staining was performed using DAPI (4',6-diamidino-2-phenylindole), and final washes were conducted before imaging and analysis.

## 3. Results

### 3.1 Infrared Spectroscopy – FTIR-ATR

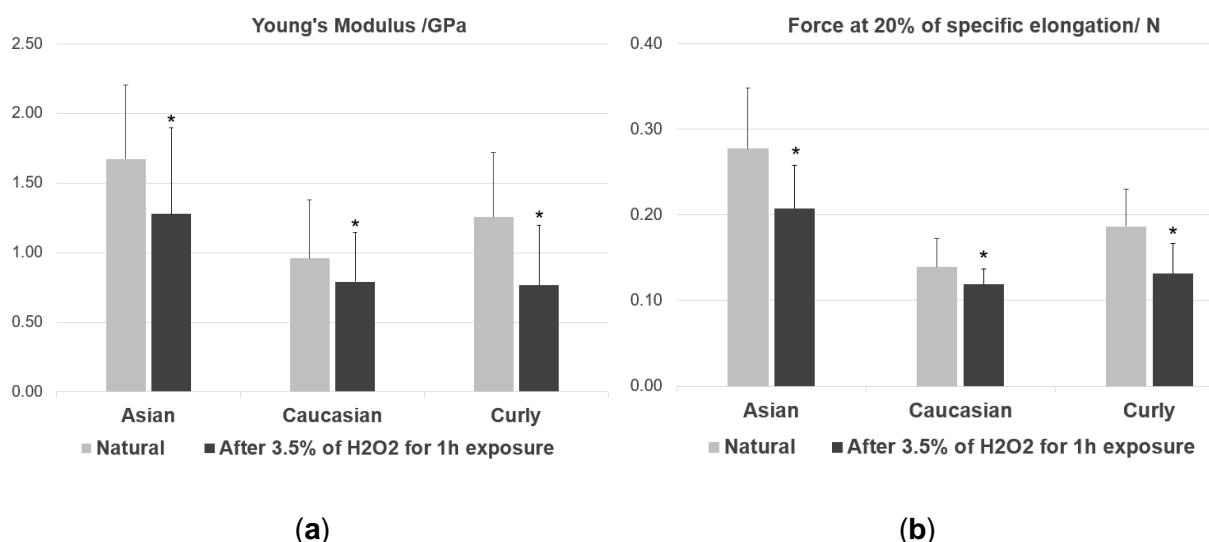
Fibers of Asian, Caucasian and Curly hair exposed to 3.5% hydrogen peroxide for 1 hour showed a significant decrease of disulfide bond area ( $525\text{cm}^{-1}$ ) when compared to respective natural controls. Also, an increase of oxidation band (ratio cysteine/amide) is shown. Both results are complementary, and reveal the hair's structure degradation due to oxidative process.



**Figure 1.** FTIR-ATR results – disulfide and cystine bonds mean areas. \* $p < 0.0001$  in comparison to respective Natural hair.

### 3.2 Mechanical Properties

Similar to the results of infrared spectroscopy, mechanical properties of Asian, Caucasian and Curly hair fibers were significantly affected by the oxidative damage. The measurements revealed a decrease of Young's Modulus and also on Force at 20% of specific elongation, in comparison to respective natural fibers.

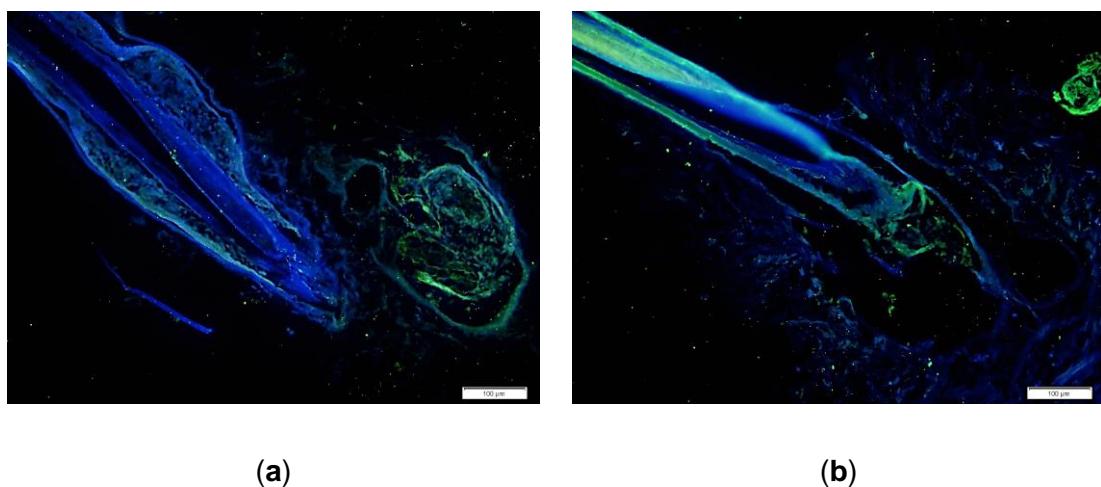


**Figure 2.** Mechanical resistance results – (a) Young's Modulus (GPa); (b) force required at 20% deformation (N). \*  $p<0.0001$  in comparison to respective Natural hair.

### 3.3. Immunostaining of MIT-F

Hair follicles exposed to 3.5% hydrogen peroxide for 3 hours showed a slight decrease in MITF immunolabeling when compared to untreated controls. Fluorescence microscopy revealed reduced nuclear staining intensity for MITF in treated samples, indicating lower protein expression. The reduction in fluorescence was observed across multiple follicles, with a noticeable decrease in labeling density within melanocyte regions of the outer root sheath.

Control samples exhibited well-defined nuclear MITF labeling, while treated follicles showed diminished signal, although still detectable. Quantitative analysis of fluorescence intensity confirmed a modest decrease in MITF levels post-treatment.

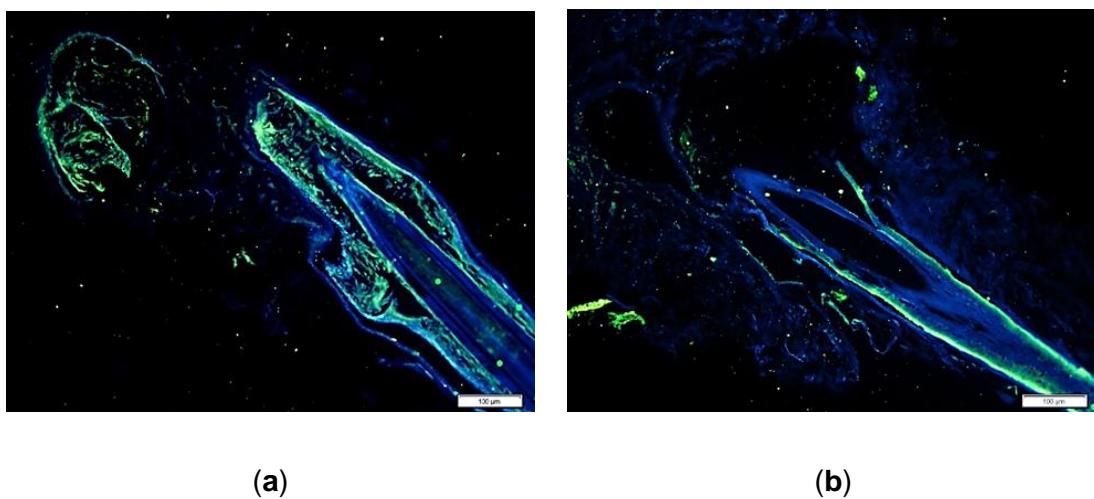


**Figure 3.** The evaluation of fluorescent micrograph of the MIT-F synthesis hair follicles: (a) Un-exposed hair follicles (Basal Control);(b) Hair follicles treated with H<sub>2</sub>O<sub>2</sub>. MIT-F is marked in green mainly in the dermis and the blue marking represents the nucleus of the cell (DNA; DAPI). The reference bar corresponds to 100  $\mu$ m.

### 3.4 Immunostaining of C-KIT

Similar to the observations for MITF, treatment of hair follicles with 3.5% hydrogen peroxide for 3 hours resulted in a modest decrease in C-KIT immunolabeling. Fluorescence microscopy showed visibly reduced signal intensity in regions corresponding to melanocyte populations within the follicular epithelium. Untreated control samples exhibited strong and well-distributed C-KIT labeling, while H<sub>2</sub>O<sub>2</sub>-treated follicles presented with weaker fluorescence, indicating lower protein expression.

This decrease in labeling was observed across biological replicates, with the reduction in C-KIT signal being especially evident in the basal and suprabasal layers of the outer root sheath. Qualitative analysis confirmed a slight decrease in C-KIT expression following oxidative treatment.



(a)

(b)

**Figure 4.** The evaluation of fluorescent micrograph of the C-KIT synthesis hair follicles: (a) Un-exposed hair follicles (Basal Control); (b) Hair follicles treated with H<sub>2</sub>O<sub>2</sub>. C-KIT is marked in green mainly in the dermis and the blue marking represents the nucleus of the cell (DNA; DAPI). The reference bar corresponds to 100 μm.

#### 4. Discussion

The modest reduction in MITF and C-KIT expression following treatment with 3.5% hydrogen peroxide indicates that oxidative stress can disrupt key regulators of melanogenesis. The downregulation of both markers suggests impaired melanocyte function and reduced pigment synthesis. These findings are consistent with previous studies showing that H<sub>2</sub>O<sub>2</sub> accumulation in hair follicles contributes to graying by interfering with melanocyte enzymes and antioxidant systems [4]. Our results expand on this by demonstrating early molecular alterations at the level of transcriptional and receptor regulation, even after short-term exposure. Overall, the data suggest that cosmetic exposure to hydrogen peroxide, even at low concentrations, can compromise pigmentation-related pathways, reinforcing the sensitivity of follicular melanocytes to oxidative damage.

The cystine-to-amide area ratio serves as an indicator of disulfide bond oxidation in the hair fiber, with elevated values corresponding to increased oxidative damage. Concurrently, reduced disulfide bond peak areas further confirm the degradation of structural protein cross-links. These results demonstrate that hydrogen peroxide-induced damage

is not confined to the directly exposed segment but propagates along the hair shaft, potentially affecting deeper structures such as the follicle and bulb. This is corroborated by the observed decline in mechanical resistance, indicative of cortical matrix deterioration, with damage severity following the order: Caucasian < Asian < Curly hair.

## 5. Conclusion

The results obtained from mechanical properties and spectroscopic analysis on hair fibers revealed that the damage cause by the use of hydrogen peroxide is not restrict to the directly exposed segment, but can immigrate by capillarity along the hair shaft. Thus, its use can potentially affect important hair structures such as the follicle and bulb, directly related to hair growth and health. Additionally, immunohistochemical analyses revealed decreased expression of MITF and C-KIT, key regulatory proteins in melanogenesis, suggesting that oxidative stress impairs melanocyte function by disrupting critical signaling pathways and transcriptional control.

## 6. References

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