Microstyling of Biofluorescence in Human Hair as Sustainable and Functional Waste

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Human hair is a huge untapped waste resource whose useful compounds require toxic and environmentally harmful chemicals to extract. Herein, the optical properties of human hair without using such chemicals, turning waste into a site-selective multicolored display, and a chemical sensor with a visual indicator through tunable fluorescence are transformed. The tunable fluorescence color, which includes both visible light and infrared components, is achieved using a scanning laser beam (microscopic) and hotplate heating at a low temperature of 360 °C for 3 min (macroscopic and large-scale production). These fluorescing hair readily detects methylene blue molecules within a concentration range of $10^{-12}$–$10^{-21}$ M due to the formation of tryptophan byproducts and electron contributing pyrrolic nitrogen. This work’s simple yet impactful consequence lays the foundation on which the industrial applicability of the functionalized human hair waste can be achieved, realizing a possible cyclical economy through sustainable resources.

1. Introduction

Human hair is a fascinating, sturdy material shed from its human subject every day. With one person likely to shed between 50 and 100 strands of hair per day,[21] this material readily accumulates into a sizable waste. This hair waste takes many years to decompose into its natural form in natural rural settings,[2] comprising mainly carbon, nitrogen, and sulfur.[2] With urbanization, this hair waste often ends up in incinerators or waste streams and dumps.[3] In bulk, this human waste can become a huge source of pollution, serving as a breeding ground for the pathogen and producing toxic fumes such as ammonia, sulfur dioxide,[4] etc. Leachate from hair can increase the nitrogen content in water bodies, leading to excessive algae growth detrimental to other life forms sharing the water source. Not only so with cosmetic industries driving the use of artificial chemicals for hair treatments and beautification, this chemically treated hair is now an even greater threat as a form of pollutant to our environment.

Given the catastrophic impact and environmental pollution on Earth’s living conditions, the drive toward sustainability through repurposing different waste materials is now one of the most promising ways to alleviate the current situation. Researchers have risen to the occasion, using their expertise in various fields to upcycle this human waste – the hair. Thus far, human hair has been implemented as an absorbent of the oil spill and other toxic chemicals such as palladium (II)[5] and Cr (VI) ions.[6] Carbon flakes[7] and microfibers[8] derived from human hair have also displayed promising performance in electrochemical supercapacitors and photocatalysts. One recent work has also shown that by controlling microalgal cells on human hair, this composite material can be used in wastewater treatment or even be implemented as a template for coating soluble drug molecules with slow-release capability in a physiological pH environment.[9] While achieving these amazing applications, some processes might inevitably require using toxic and environmentally harmful chemicals such as KOH[10] or O-chlorophenol to extract useful compounds from hair samples.[9] Thus, efforts to transform human hair into useful material via more environmentally friendly approaches continue to merit our attention.

Pristine human hair generally forms a multilayered structure comprising the outer layer—the cuticle, the middle section—cortex, and the core—medulla.[2,10,11] Contained within these layers are various chemical compounds, such as keratin-associated proteins (KAPs): 1-tryptophan[12] that exists mainly in the cortex, 1-tyrosine,[13] 1-tyrsoine[14] which have a strong presence in the cuticle and cortex,[15] and calcium carbonate in the medulla.[16] Human hair has been known to emit mild blue fluorescence under a wood lamp or ultraviolet (UV) light excitation.[17] Yet, there is no conclusive report to pinpoint the root
cause of such fluorescence in hair to the best of our knowledge. Despite a limited understanding of its fluorescence properties, human hair has already found application as an indicator. In forensic studies, traces of arsenic detected in human hair determine possible arsenic poisoning cases.\cite{18} In the medical field, changes to the optical property of human hair due to the invasion of hyphae into a hair follicle resulted in the use of a wood lamp to detect the presence of fungus.\cite{19} We anticipate that through engineering strong fluorescence emission from the human hair, the fundamental insight into the mechanism achieved will enable human hair to be developed into a chemical/biological sensor with a visual indicator. Developing human hair into a visual indicator will allow shorter diagnostic time, enabling corrective measures to be promptly implemented.

This work demonstrates that interesting physical properties can be engineered upon human hair in its most pristine form using two facile techniques without any chemical treatments. First, using a simple scanning focused laser beam (FLB), visible or hidden micropatterns are engineered on a single strand of human hair. Such micropatterns exhibit bright and distinct fluorescence colors when the hair is excited by UV light. The emitted fluorescence light comprises both the visible and infrared (IR) ranges. As a result, fluorescence microscopy or IR microscopy images of these human hair strands present clear and distinct patterns embedded. Second, single strands of human hair are transformed into fluorescence strands using a simple hotplate treatment process. Comparing heated hair with pristine hair, a fivefold increase in fluorescence intensity can be achieved, and the fluorescence peak wavelength can be tailored.

Through systematic studies and detailed characterization, we gained further insights into the transformative mechanisms in the heated hair that account for the observed enhancement in the fluorescence properties of the heated hair. Namely, 1) induced formation of tryptophan’s photo-oxidation byproducts such as L-tryptophan, N-formylkynurenine (N-FK), L-kynurenine, and 3-hydroxykynurenine; 2) denaturation of other KAP, L-cystine which could contribute to the hybridization of a carbon backbone with the possible presence of carboxylic and carbonyl surface states; 3) heat-induced decomposition of calcium carbonate resulting in the formation of calcium carbonate; 4) formation of midbandgap states due to (i) possible transformation of pyrrolic N to pyridinic N and graphitic N and (ii) the creation of oxidative surface states, which in turn serve as near-infrared (NIR) emission centers. Among the few identified contributors to the enhanced fluorescence in heated hair, we have tryptophan. Tryptophan is a naturally occurring fluorescent amino acid, recently developed as a template for developing fluorophores with emission in the visible light range. This identification allows us to further develop human hair into a sensitive sensor of methylene blue (MB), with a visible intensity change in the fluorescence of human hair under UV excitation.

MB is a common dye pollutant often discharged untreated into water bodies in developing nations. When ingested, this dye becomes a potent neurotoxin, acting as a monoamine oxidase A inhibitor, resulting in serotonin toxicity.\cite{20} MB in an aqueous medium exhibits weak emission between 600 and 700 nm and a strong emission peak at 293 nm,\cite{21} making it difficult to detect, especially in a very low concentration with a colorless appearance. There is much investigation about materials that can serve as MB detectors. However, most compounds used as MB detectors are lab synthesized\cite{22} and are expensive to mass produce. Human hair, being a natural and easily accessible material, would be cheaper to produce as an MB detector and is also eco-friendly and contributes to waste management by being a biodegradable option.

2. Results and Discussion

2.1. Focused Laser-Induced Biofluorescence in Human Hair

The first facile technique developed to engineer fluorescence in human hair is a serendipitous discovery. We utilize a scanning focused laser beam (FLB) to fabricate a micropattern on the surface of a single strand of black pristine (BP) human hair. When the human hair with a micropattern is examined under a fluorescence microscope (FM), the micropattern is found to exhibit remarkable fluorescence emission when it is excited by UV light. Such site-selective generation of hair fluorescence is achieved through localized and regulated thermal annealing. With a more delicate control of the laser intensity imparted onto the human hair, such fluorescence enhancement is also achievable in the absence of any apparent physical destruction of the human hair, revealing an unexpected steganographic attribute of the engineered human hair.

Figure 1a shows a schematic of the scanning FLB system utilized for heating in a controlled environment. In this setup, the monochromatic laser beam is reflected by a system of two mirrors into a customized microscope and focused onto the BP hair sample through a 100× lens with a working distance of 7.6 mm. The wavelength of the laser beam is 532 nm, and the focused laser spot is ≈1 μm in size. The BP hair sample rests on a scanning computer-controlled stage. This stage moves relative to the FLB to create interesting micropatterns on the sample. Such laser modification experiments can be carried out with the hair sample in ambient conditions. Alternatively, the hair sample can be housed inside a transparent vacuum box where we can carry out the laser modification with the hair under vacuum conditions or in a helium-filled box.

Figure 1b depicts the photoluminescence (PL) spectra of the pristine hair sample and the hair modified by the laser beam. The common features among the two spectra are the two fluorescence bands. The visible light band centers around 580 nm, whereas the other fluorescence band falls into the IR range centering around 900 nm. The PL spectrum for the hair sample modified by the FLB exhibits significantly enhanced fluorescence intensity. The enhancement stands at ≈400% for the visible light band and ≈350% for the IR band. The inset of Figure 1b shows an FM image of the cross-sectional view of a hair sample modified by a FLB at 0.45 MW cm⁻² laser power density. The laser created a narrow channel on the hair structure’s surface. We shall denote this mode of laser modification as P-mode where the hair sample has clear physical damage. Figure S1, Supporting Information shows the results of the systematic studies of the effect of focused laser modification on the human hair by gradually reducing laser power density from 0.46 to 0.38 MW cm⁻². Figure S1a, Supporting Information shows the bright-field (BF) and FM (UV) images of a single strand of human hair.
A few letters “A” created on it. Close-up views of the letters created using laser power density of 0.38 MW cm\(^{-2}\) (Figure S1b, Supporting Information), 0.39 MW cm\(^{-2}\) (Figure S1c, Supporting Information), 0.41 MW cm\(^{-2}\) (Figure S1d, Supporting Information), 0.42, and 0.43 MW cm\(^{-2}\) (Figure S1e, Supporting Information), 0.45 MW cm\(^{-2}\) (Figure S1f, Supporting Information), and 0.46 MW cm\(^{-2}\) (Figure S1g, Supporting Information), respectively, are presented. We find that when the power of the laser beam is reduced to 0.38 MW cm\(^{-2}\), we can still achieve laser modification of the human hair by way of localized fluorescence enhancement but remarkably, there is no apparent physical damage. We shall denote this mode of laser modification as the S-mode.

Figure 1c shows the effect of P- and S-mode laser modification on a strand of human hair. For this hair sample, P-mode (0.45 MW cm\(^{-2}\)) laser micropatterning is carried out on the left portion of the hair, whereas S-mode (0.38 MW cm\(^{-2}\)) laser micropatterning is carried out on the right portion of the hair. The two patterns combine to form an ancient character representing “dragon”. As shown in the BF optical microscope image of the hair sample, one can see the effect of the P-mode modification on the left, but there is no apparent physical damage to the hair on the right. However, when the sample is imaged using an FM under UV light excitation, the fluorescence emissions from both micropatterns are visible. In addition, the patterned region also shows strong IR emission as captured by an IR camera (Figure 1c).

A variety of micropatterned structures can be created using the P- and S-mode processes mentioned above, examples of which are found in Figure 2. Figure 2a,d demonstrates the effect of S-mode FLB micropatterning on hair, whereas Figure 2b,c demonstrates the effect of P-mode micropatterning. We have shown that a simple scanning FLB can be utilized to embed a hidden message in a single strand of human hair via S-mode. The process is akin to a form of steganography at a microscopic scale.

2.2. Thermal-Activated Fluorescence Enhancement of Human Hair

Considering that the laser patterning process is controlled by localized heating, a natural way to upscale the creation of such an inexpensive fluorescence material with wavelength tunability is through monitored annealing. Macroscopic thermal heating provides a means to upscale the production capability for the fabrication of human hair with enhanced fluorescence, boosting the industrial applicability of the functionalized human hair. Figure 3a shows a photocollage comprising a cross-sectional view of pristine black (BP) human hair taken under UV (left) and BF (right) illumination with labels highlighting locations of the cuticle, cortex, and medulla. Systematic studies of the effect of heating on the fluorescence of the BP hair sample are carried out by cutting the sample into 1 in. strands and placing them in a ceramic boat before heating on a hotplate at ambient for 3 min. In these studies, the heating temperatures are set at 300–400 °C at an increasing interval of 20 °C. Figure 3b shows FM images (under UV excitation) of the cross-sectional view of the black human hair after undergoing different degrees of thermal annealing in the ambient. Figure 3c shows the corresponding planar view of the same strand of hair, as presented in Figure 3b. Figure 3d shows the respective PL spectrum of
the thermally treated hair presented in Figure 3b,c. At 300 °C, no significant change to the hair’s fluorescence is observed. As the temperature increases in intervals of 20 °C, an enhancement to the fluorescence intensity is observed. It is coupled with a redshift in the fluorescence when the temperature reaches 380 °C and beyond. All fitted PL graphs are presented in the supplementary information, Figure S2, Supporting Information. Before thermally activated fluorescence enhancement occurs at 320 °C, fitted PL spectra of pristine and samples heated at 300 °C show similar peak positions at ≈426, ≈527, and ≈608 nm. Once activated, the peak fluctuating between 574 and 578 nm becomes the dominating peak. At 360 °C, the PL intensity is ≈3 times higher than that from the pristine sample. At 380 °C and beyond, the entire PL spectrum undergoes

Figure 2. A variety of micropatterned structures can easily be created using P-mode and S-mode. a) S-mode and b) P-mode crochet note. c) P-mode Chinese characters symbolize “glowing hair” or “invention” when taken as individual characters. d) An ancient character representing “dragon” is similar to that presented in Figure 1c, but the entire structure is created in S-mode.

Figure 3. a) Collage of cross-sectional view of a BP human hair. The right half is taken under BF, while the left half is obtained under UV excitation. Fluorescence microscope images are obtained under UV light excitation for b) series of a cross section of black human hair after different degrees of thermal annealing in ambient, and c) the corresponding planar view of the same strand of hair as presented in (b). d) The respective PL spectrum of the thermally treated hair is presented in (b) and (c). The dotted red line indicates a redshift in emission as heating temperature increases. e) Macroscopic “NUSHS” structure is made of an assembly of many strands of human hair. f) UV illumination highlights distinct fluorescence intensity and emission color between thermally treated and BP human hair.
≈100 nm redshift, with the dominating peak now ranging between 668 and 678 nm.

The cross-sectional images (Figure 3b) show that both the cuticle and medulla are intact, exhibiting increased fluorescence intensity after being heated at 360, 380, and 400 °C. The cortex, however, starts to degrade at 380 °C with an observable redshift in the fluorescence. At 400 °C, much of it has disintegrated, and the fluorescence changes from blue to orange. Interestingly, samples heated at 360 °C (Figure 3b,c) exhibit bright fluorescence under UV excitation, much like those achieved on BP hair samples along the laser path. The enhanced fluorescence appears uniformly across the entire cross section of the human hair.

Furthermore, the ability to achieve fluorescence tuning in both macro and microscopic aspects also expands this economical material’s applicability across a wider range of industries, potentially serving as counterfeit micromarkers to light indicators. As a proof of concept, we create macroscopic structures in the form of an array of letters formed by using many strands of pristine and heated black human hair. This array is shown in Figure 3e. The first three letters “NUS” are made of heated black human hair, while the remaining two letters “HS” are made of pristine black hair. Figure 3f shows that when the letter arrays are illuminated using a UV lamp, the enhanced fluorescence in the first three letters makes them stand out. This demonstration highlights how selected fluorescence enhancement can highlight specific alphabets, revealing a message within a text.

2.3. Proposed Mechanisms and Probing Experiments

2.3.1. Melanin Oxidation

The establishment of controlled functionalization of human hair to enhance the fluorescence effect allows the root cause of the enhancement to be determined. With all hair samples being heated in an oxygen-rich environment, ambient, it is natural to hypothesize that oxidation of melanin could contribute to the intense fluorescence due to oxidative stress.23 The redshift further supports the hypothesis in emitted fluorescence (Figure 3b,c) as the cortex (mainly comprises melanin and tryptophan) undergoes thermal-induced degradation. Testing of this hypothesis is carried out by 1) FLB heating of BP in a vacuum and helium environment to restrict the exposure of the sample to oxygen. In addition, we perform 2) heating of white hair (WH), which lacks melanin, in ambient on a hot plate.

Oxidation Effect: FLB of BP in a Controlled Environment: The BP sample (Figure 4a) is placed in a vacuum chamber connected to a gas pump and helium tank for environmental control. The chamber with the BP sample is then placed on the stage for FLB treatment. Figure 4 shows the results after the samples are laser treated under (Figure 4b) ambient conditions, (Figure 4c) within a vacuum chamber under vacuum (0.06 Torr), and (Figure 4d) helium conditions (10 Torr). In both cases shown in Figure 4c,d, despite the depletion of oxygen content in the environment, enhanced fluorescence is still observed, both at cut regions (i.e., P-mode) and at the regions that are modified but not physically damaged (i.e., S-mode). Using the same laser power density of 0.48 MW cm⁻² for localized heating (Figure 4b–d), it is noteworthy that it is easier to pattern hair in the vacuum environment but harder in the helium environment when compared with laser treatment in the ambient, as our vacuum environment has fewer air molecules, so the rate of heat loss to these air particles is decreased, allowing faster heating, and helium conducts heat more rapidly than air. Figure S3 and S4, Supporting Information, shows the detailed, systematic power-dependent studies of laser-treated BP in vacuum and helium environments.

Role of Melanin: Thermal Heating of WH: Figure 4e–h shows the effect of heating BH and WH on a hotplate. The FM images (Figure 4e–h) and PL spectra (Figure 4i) show that under UV light excitation, the fluorescence intensity of pristine WH and heated WH is ≈4 times stronger than that of BP hair and heated BH, respectively. WH exhibits superior fluorescence though the lack of melanin is a strong indication that melanin is not vital in the emergence of fluorescence properties in hair.

2.3.2. Photochemical Reaction

Human hair contains many forms of amino acids.24 With the bulk of human hair comprising keratin, KAPs with the ability to absorb UV excitation and undergo photochemical reactions are highly relevant to the phenomenon observed in this work. Out of these KAPs, L-tryptophan12 that exists mainly in the cortex, and L-cystine and L-tyrosine14 which have a strong presence in the cuticle and cortex, are of particular interest to us. It is important to note that these proteins are not independently isolated to the cuticle, cortex, and medulla. For example, L-cystine is present in both the cuticle and cortex, although a higher concentration exists in the cuticle.15 In addition, the photochemical reaction of thermally decomposed calcium carbonate, which makes up the bulk of the medulla structure,16 is also an element to be considered as we narrow down the possible contributions to the observed biofluorescence enhancement in thermally treated human hair.

Tryptophan Photo-Oxidation: L-Tryptophan has a chemical structure shown in Figure 4k. When oxidized in light, tryptophan side-chain oxidation induces redshifted tryptophan’s natural fluorescence into the visible range.24,25 At the same time, photo-initiated decomposition of tryptophan also results in primary compounds, N-FK and secondary compounds, l-kynurenine and 3-formylkynurenine.25 Deconvoluting the PL graph of WH (Figure 4l), 11 individual Gaussian graphs are required to achieve a similar global plot as the original spectrum. Of these eleven graphs, the first four peaks correspond to l-tryptophan ((404 ± 1) nm), N-FK ((438 ± 1) nm), l-kynurenine ((463 ± 1) nm), and 3-hydroxykynurenine ((490 ± 1) nm).25,26 The observed enhancement in fluorescence of hair within the visible range likely comes from tryptophan’s photo-oxidation byproducts, which can increase the concentration of fluorophores with emission in the visible range.25 The above analysis leaves the origin of the remaining peaks at (523 ± 1), (551 ± 1), (682 ± 1), (776 ± 1), (829 ± 1), (903 ± 1), and (969 ± 1) nm to be further investigated and identified in the subsequent discussion.

L-Cystine and L-Tyrosine Photochemical Reaction: Although high concentrations of l-cystine and l-tyrosine are found in the cuticle and cortex, l-tyrosine exhibits two absorption peaks.
at ≈220 and ≈275 nm.[14] These values are beyond the excitation range in this study; hence, the contribution from these KAP is likely to be insignificant. While l-cystine does not show significant absorbance above 240 nm,[13] we believe that l-cystine, a cysteine residue, can form disulfide bonds with other sulfur-containing amino acids, and depending on the number of disulfide bonds and the cysteine content, which determines the strength of the hair structure,[27] they can contribute to the observed fluorescence due to heat or dryness induced denaturation of cysteine.

Figure 4. a) BF and FM (UV excitation) images of pristine black hair. b–d) BF and FM images of laser-modified hair under (b) ambient, (c) vacuum, and (d) helium environment. BF and FM imaging of pristine and heated e,f) black hair and g,h) WH. i) PL spectra of pristine and heated black and WH. j) Molecular structure of keratin monomer. k) Tryptophan photo-oxidation process.[22] l) Deconvoluted PL spectrum of heated white hair. m–o) FTIR comparison between (m) BP, (n) BP and FLB-treated BP, and (o) BP and WH hair samples.
When heated, the hair's peptide backbone breaks apart (Figure 4j), exposing the C=O bond of the carboxylic acid. With high carbon content in human hair,[28] hybridizing a carbon backbone with the possible presence of carboxylic and carbonyl surface states likely contributes to green fluorescence. Such contribution agrees with similar green fluorescence observed in other carbon-based compounds[29,30] and hair-derived carbon dots.[30] As such, denaturation of keratin is likely to be the reason behind the peaks mentioned above at 523 ± 1 and 551 ± 1 nm (Figure 4).

**Dried and Damaged (Frizzy) Hair:** Dried or “frizzy” hair (based on its structure of many curls) experiences a change in structure due to moisture loss. Figure S5a, Supporting Information shows that “frizzy” hair experiences physical damage due to this structure change. When the same heating (Figure S5b, Supporting Information) and FLB (Figure S5c, Supporting Information), procedures are conducted on a strand of “frizzy” hair; enhanced fluorescence similar to those from treated BP hair is observed under UV light. From the PL spectra shown in Figure S5e, Supporting Information, fluorescence peak intensity from pristine and heated “frizzy” pristine is comparable with the corresponding pristine and heated BP hair. The results suggest that structural damage to hair is not likely linked significantly to the observed fluorescence enhancement.

**Thermal Decomposition of Calcium Carbonate in Medulla:** It is revealed through synchrotron Fourier-transform infrared (FTIR) microspectroscopy that medulla carbonate are coordinated to calcium due to a strong calcium carbonate signature.[31] As the human hair undergoes thermal treatment, heat-induced decomposition of calcium carbonate takes place, resulting in the formation of calcium carbonate,[31] whose energy bandgap was reported to be 2.46 eV.[32] The emitted wavelength thus coincides with the greenish fluorescence observed.

**Bleached Hair:** Hair bleaching can remove the outer layers of the hair to reveal the interior component of the hair. BP samples are bleached for varying periods (1, 5, 10, and 15 min, Figure S6a, Supporting Information) to remove the outer layers. After bleaching for more than 10 min, both the cuticle and cortex layers of the hair samples (containing melanin and tryptophan) are removed largely via the bleaching process. Looking at the UV FM imaging of BP hair bleached for 15 min and then followed by heat treatment, the fluorescence becomes greener, paralleling the peak shift observed with higher temperatures. The main contributing factor to the observed greenish fluorescence is found in the medulla only. PL analysis (Figure S6b, Supporting Information) quantifies the observed greenish fluorescence, identifying two strong peaks at 531 ± 2 and 599 ± 2 nm. These are akin to the most intense peaks (within the visible wavelength) identified in Figure 4l. The result suggests that the thermal decomposition of calcium carbonate in the medulla also contributes to the observed enhancement in hair fluorescence. From PL analysis, we also observe a very weak peak at 431 ± 1 nm, corresponding to the tryptophan oxidation byproduct.[25]

Even without bleaching the hair chemically, site-selective thermal heating of the cross section of a BP sample (at the cuticle, cortex, and medulla region) achieved through the FLB process (Figure S7, Supporting Information) exhibits biofluorescence enhancement across all three regions. With the same laser density (0.32 MW cm$^{-2}$), the strongest enhancement comes from the medulla (rich in calcium carbonate), followed by the cuticle (abundant in l-Cystine) and the cortex (presence of l-Tryptophan). The data presented in Figure S7, Supporting Information thus supported the above proposal that all three elements, l-Tryptophan, l-Cystine, and calcium carbonate, contributed to the observed optical phenomenon.

**2.3.3. FTIR Analysis**

FTIR analysis (for a range of 4000–600 cm$^{-1}$) is performed on BP, FLB-treated BP, and WH hair samples (Figure 4m–o) to determine the chemical composition on the surface of the respective samples. This process will sieve out the contributing factors to the observed fluorescence enhancement. In general, peaks above 3500 cm$^{-1}$ are attributed to H$_2$O from atmospheric absorption onto the sample surface.[33] and the peak at 2348 cm$^{-1}$ is due to the stretching of C≡N bonds.[34] Peaks of keratin, melanin, and tryptophan nature are assigned by the triangle, circle, and hexagon symbols, respectively, in Figure 4m–o. Regarding BP hair, the symbols are filled with blue color. For FLB-treated and WH samples, the orange triangles symbolize the increase in the keratin signal. In contrast, the open symbols of circles, hexagons, and triangles represent a decrease in the contribution from melanin, tryptophan, and keratin, respectively.

In the BP sample (Figure 4m), keratin-related peaks at 1702, 1562, and 1294 cm$^{-1}$ likely result from amide I: C=O stretching with in-plane bending from N–H and C–N stretching mode, amide II: C–N stretching mode with N–H in-plane bending, and amide III: C–N stretching. N–H in-plane bend is coupled to C–C stretching and CO in-plane bending.[35,36] Melanin-linked peaks at 3116 and 2967 cm$^{-1}$ are due to the vibration of O–H and N–H groups belonging to the amine, amide, carboxylic acid, phenolic, and aromatic amino acid function and the vibration of aliphatic C–H groups.[37,38] Finally, peaks at 3442, 1396, and 1106 cm$^{-1}$ come from N–H stretching in amine, C–N stretching in alylamines, and C–H bending in the aromatic ring aligned with peaks associated with tryptophan.[38]

With these in mind, comparisons are made to FLB-treated BP and WH samples. In general, thermal annealing reduces tryptophan-related N–H stretching and C–N bending. C–N stretching and N–H bending together with C–C stretching and CO in-plane bending related to keratin structures are also much reduced. However, orange triangles highlight significant enhancement of keratin-linked C–N stretching, and new keratin-related peaks at 962 cm$^{-1}$ and 885 cm$^{-1}$ are highlighted in Figure 4n. The two new peaks are amine IV: O=C–N bending and amine V: N–H out of plane bending.[36] In addition, stretching of the C≡N bond is now more prominently displayed after thermal annealing. Not surprisingly, the main distinction between FLB-treated BP and WH FTIR spectrums is the loss of melanin-related peaks.

**XPS Analysis:** Changes to surface states on carbon-based materials are longstanding as a great influencer to detect PL emission.[39] and one of the best ways to probe the presence and changes to these surface states is none other than X-Ray photoelectron spectroscopy (XPS). Coupling XPS and FTIR,
the combinatory results will enable us to decipher the root cause of the red and NIR PL detected in Figure 4l.

XPS analysis, high-resolution N1s, O1s, and C1s scans (Figure 5) show a general increase in binding energy (BE) from thermal annealing of BP and WP samples. C1s scan from BP can be deconvoluted into three Gaussian curves centered at 288.4, 286.3, and 285.0 eV. These are attributed to C═O/O─C─O, C─O, and C─C/C─H. O1s scan shows two deconvoluted peaks at 533.2 and 531.8 eV associated with O─C and O─Si, whereas the N1s scan identified a single peak at 400.1 eV from N─H. Upon heating, the BH sample now hosts O─C═O bonds (288.6 eV) and a 0.2 eV shift in the C─O bond toward the higher BE, while the WH sample also sees similar O─C═O bonds at a higher BE of 289.0 eV. Peak from C─O bonds shows a significant 0.3 eV increase in BE compared with the BP sample. C─C/C─H peak remains the same for all three samples, although the atomic percentage of this peak decreases from 65% (BP) to 53.3% (BH) and, finally, 49% (WH). O1s scans show a relative increase in BE of BH and WH by 0.2 and 0.6 eV for O─C associated peak and a similar shift of 0.2 and 0.7 eV for the O─Si peak. While the presence of Si in the hair samples can be attributed to the commercial hair product used in hair washing, an additional XPS scan of infant hair that has not interacted with Si-containing hair products (Figure S8, Supporting Information) suggested that Si is one of the many minerals present in human hair. Figure S9, Supporting Information shows the Scanning Electron Microscopy–Energy Dispersive X-ray Spectroscopy spectra obtained for both BH and WH, and the presence of Si in the sample is evident. These changes agree with FTIR results and are likely due to surface oxidative effect as the annealing process is conducted in the ambient. From the above results, we propose that the formation of C═O is a contributing factor to the observed green fluorescence from thermally treated hair.

Not surprisingly, although C─N and C≡N bonds are identified through FTIR, these peaks are not detected from XPS. The discrepancy is likely due to XPS probing only a less than 8 nm depth. Hence, C≡N in the subsurface layer will not be readily detected. However, XPS readily detects N─H surface states with 0.2 and 0.3 eV shift toward higher BE with an observable reduction in atomic percentage from 4.1% (BP) to 3.7% (BH) and 1.1% (WH). The presence of pyrrolic nitrogen could contribute to the strong PL signal as it could donate one pair of electrons to the conductivity of the material’s π-system.42 Due to their thermal instability, reducing pyrrolic nitrogen with annealing, coupled with an increase in C≡N and C─N bonds (from FTIR), indicates the possible transformation of pyrrolic N to pyridinic N and graphitic N.43 Graphitic N is a common contributor to a redshift of PL signal in nitrogen-doped carbon dots due to their ability to create midbandgap states by injecting one excess electron to the unoccupied π* orbital.42 These midbandgap states then facilitate low-energy transitions, which contributed to PL peaks detected at 682 ± 1 and 776 ± 1 nm (Figure 4l).

The concept of midbandgap states also helps to explain the enhancement of NIR PL peaks from heated hair samples. In silicon-rich oxynitride nanostructures, Si─O─N bond formation on the surface of Si nanocrystals was identified as luminescent centers with NIR emission.44 Furthermore, in oxygen-rich silicon nitride, the presence of this oxide induces a gap state in the bandgap of silicon nitride.45 Detailed XPS Si2p scans from BP, BH, and WH hair samples show a significant increase in the

Figure 5. High-resolution XPS scan of N1s, O1s, C1s, and Si2p from BP, BH, and WH samples.
formation of Si—O—N bond (from 1.8 to 3.0 to 19.4 At%), respectively. As the annealing process is conducted in the ambient, the process promotes the formation of oxidative surface states, which in turn serves as NIR emission centers, emitting wavelengths at (829 ± 1), (903 ± 1), and (969 ± 1) nm under 325 nm excitation.

With stronger PL enhancement coming from WH samples, the results from FTIR and XPS analyses reiterate that melanin's role in this observed phenomenon is inconsequential. Instead, the root cause is likely due to combinatorial contribution from the denaturation of keratin, which leads to the exposure of the C=O bond and, in turn, allows more hybridization of a carbon backbone with the possible presence of carboxylic and carbonyl surface states to take place, together with the byproducts of tryptophan, enhancement of C—N and C═N bonds, and increased formation of Si—O—N surface states.

2.4. Application: Methylene Blue (MB) Detection

MB has a blue appearance in its oxidized form that turns colorless (leucomethylene blue) upon reduction. As a common redox indicator, MB is reportedly used as a sensitized photo-oxidation of tryptophan in water, resulting in $\text{N-FK}$ and 3a-hydroxy-1, 2, 3 3a, 8, 8a-hexahydropyrrolo[2, 3-b]indole-2-carboxylic acid.\[46\] At a concentration of $10^{-4}$ M, MB has a blue appearance while exhibiting red (688 ± 1 and 725 ± 1) nm and NIR (774 ± 1) nm emission under 325 nm excitation (inset of Figure 6a). Diluting it to $10^{-21}$ M results in a colorless appearance and weak PL at (774 ± 1) nm (PL in Figure 6a). At both concentrations, no visible fluorescence is detected microscopically under UV excitation. Weak fluorescence in the NIR range and a colorless appearance highlight the challenges of detecting low-concentration MB. With MB commonly used as a dye in textile industries and discharged untreated into water bodies in developing nations, it can become a neurotoxin when ingested. Thus, it is prudent to develop a means to detect a low concentration of MB. Furthermore, a visual indicator would greatly enhance the detector's ease of use, making it more affordable without complicated and expensive analytical tools.

Since denaturation of keratin, byproducts of tryptophan, enhancement of C—N and C═N bonds, and increased Si—O—N surface states are critical in enhancing the fluorescence of human hair, WH is thus a natural candidate to use as an MB detector. This is done by immersing a single strand of WH into $10^{-4}$ M of MB solution for 5 min and then taken out to dry before examining the WH using FM or PL. At high MB concentration (blue color, $>10^{-9}$ M), fluorescence weaker than the pristine WH sample is detected (Figure 6b). The loss of fluorescence yield is largely due to obstruction from MB trapped in the overlapping cuticle cells. This phenomenon is highlighted by the orange arrow in Figure 6b, where blue patches are seen blocking the intrinsic fluorescence of WH under UV and blue excitation.

Figure 6. a) PL of $10^{-21}$ and $10^{-4}$ M (inset) of MB on a Si wafer. Right panel: corresponding BF and UV excited images. b) PL and FM images (under BF, UV, and blue (B) excitation) from WH immersed in $10^{-4}$–$10^{-9}$ M of MB. Orange arrows indicate the presence of MB. c) PL and FM from WH after being immersed in different concentrations of MB. Inset shows fluorescence from a single strand of WH with half of it immersed in $10^{-21}$ M of MB. d) XPS N1s of pristine and MB on WH.
Figure 6c shows the PL spectra of the WH after being submerged in MB solution with an even lower concentration from 10^{-12} to 10^{-22} M for 5 min. A significant enhancement in the fluorescence intensity is observed with a small amount of MB coating onto the WH. This attribute of WH shows that we can easily use WH as a visual chemical sensor to detect the presence of a tiny amount of MB in the solution. Below the concentration of <10^{-22} M, the concentration is too low to be successfully detected. Corresponding FM images of MB detection limit are shown in the right panel of Figure 6c. It is important to note that the exposure of FM images in Figure 6c is lower to avoid image saturation with 10^{-21} M of MB. The change in fluorescence of WH from blue to cyan is visible as the concentration of MB reduces from 10^{-12} to 10^{-21} M. The observation thus provided concrete evidence that WH can indeed be implemented as a visual indicator of low concentrations of MB, with a detection range between 10^{-12} and 10^{-21} M. The distinct difference in fluorescence is depicted in Figure 6c’s inset. The picture shows an FM image of a single strand of WH where the left half of the WH has been immersed in 10^{-21} M of MB for 5 min.

We propose that the enhancement of fluorescence is a combinatorial consequence of the following processes: 1) under UV illumination in the presence of MB, photo-oxidation of tryptophan in WH takes place, resulting in the formation of more fluorescence contributing byproducts of tryptophan such as N-FK and 2) high-resolution XPS N1s scan (Figure 6d) highlights the formation of more pyrrolic nitrogen on WH after being immersed in 10^{-21} M of MB for 5 min. As pyrrolic nitrogen can contribute more electrons to the π-system of WH, it is reasonable to observe a greater fluorescence yield after MB treatment. Furthermore, the reduction of MB concentration (within the detection limit) increases the participation rate of the MB molecules as a majority of them would be in contact with the WH for the reaction to occur. Having a higher concentration of MB can result in an undesired competition of oxygen and hydrogen molecules within the matrix and, as observed, does not necessarily enhance fluorescence detected.

3. Conclusion

Thermally activated human hair waste has been successfully functionalized to serve as a rapid detector (5 min) of low-concentration (10^{-21} M) MB molecules with a visual indicator via a change in the fluorescence emitted under UV excitation. Due to denaturation of keratin, byproducts of tryptophan, enhancement of C=N and C≡N bonds, and increased formation of Si–O–N surface states, low-temperature thermal heating, and/or site-selective laser annealing processes cause human hair to display significant fluorescence enhancement both macroscopically and/or microscopically. The MB detection limit of thermally treated human hair ranged between 10^{-12} and 10^{-21} M. The increase in fluorescence yield from immersing WH in MB solution is attributed to the formation of tryptophan byproducts such as N-FK through the photo-oxidation process and more electron-contributing pyrrolic nitrogen. This work’s simplicity yet impactful consequence lays the foundation on which the industrial applicability of the functionalized human hair can be readily achieved, realizing a possible cyclical economy through sustainable resources.

4. Experimental Section

**Human Hair:** The hair samples used were strands of cut hair collected from three volunteers of Asian origin from the same ancestry – one for pristine BH experimentation, one for WH comparison, and one for damaged (frizzy) hair comparison. All hair samples were precleaned with clear antidandruff nourishing shampoo before further analyses.

**Methylene Blue:** MB hydrate purchased from Merck was dissolved and diluted in deionized water to the required concentrations.

**XPS:** XPS analysis used Thermo Fisher Scientific Theta Probe XPS with monochromatic Al Ka X-Ray (1486.7 eV). Charge correction for BE was based on C1s from adventitious carbon at 285.0 eV.

**Fluorescence Microscope:** (FM) (Olympus BX51) with filter cubes U-MWU2 (300–390 nm) and U-MWB2 (450–500 nm) were used to illuminate the samples with UV and blue light. Samples under UV illumination were captured using the same exposure (2s). Exposure for the FM images captured under UV illumination for MB detection was lower than other UV images presented in this manuscript to avoid overexposing the images.

**Further Characterizations:** Raman and PL were carried out using a Renishaw Micro Raman System (Renishaw inVia Qontor) coupled with a 532 and 325 nm laser. FTIR analysis was measured using Bruker Vertex 70 attached to an upright microscope.

**Supporting Information**

Supporting Information is available from the Wiley Online Library or from the author.

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**Conflict of Interest**

The authors declare no conflict of interest.

**Data Availability Statement**

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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fluorescence, hair, lasers, methylene blue, thermal

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