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Morphological properties of hair and their variation when subjected to oxidation via chemical bleaching

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ABSTRACT

Four key morphological properties of hair were investigated in order to understand their variation in response to oxidation of the strand via bleaching. These were hair diameter, cuticle damage, porosity and fluorescence. Hair strands were oxidised by immersion in liquid and cream hydrogen peroxide of differing concentrations (3%, 6%, 9% and 12% w/v) and times, with changes to the structure recorded using three microscopic instruments: compound, scanning electron and fluorescence microscopy.

Results indicated a proportional correlation between bleaching time and cuticle damage, which was further linked to porosity. In relation to fluorescence, strands with a higher proportion of pheomelanin were more fluorescent compared to darker hairs containing eumelanin. An unanticipated result of this investigation was the discovery of a *dark point*, named as it represented the time at which fluorescence decreased to a point where the strand was barely perceptible from the dark background of the image. After this point, fluorescence was found to build back up to its original or increased level, termed *pseudo-fluorescence*.

Introduction

The hair strand is one of the most useful tools available to a forensic toxicologist, owing to its non-invasive collection procedure and its ability to hold information on drug use - particularly the concentration of a drug consumed over time.[1] Since hair can grow on average around 0.6-1.4cm in length each month[2] and its growing phase lasts approximately 90 days, the period of time drugs can be detected in hair can stretch back 3 months. As well as in science, hair also has important anthropological meaning within human culture and beauty. Many techniques exist for the alteration of hair by colour, texture and length, albeit with the potential for damage.

A popular chemical treatment, and arguably the most damaging, is colouring the hair which can be done either temporarily or permanently, depending on the oxidative effect of the treatment. Hydrogen peroxide acts as the oxidising agent and alongside ammonia and persulfate works to remove the natural colour of the hair by lightening and removing the pigment from the melanin. The addition of coloured chromophores and intermediates then produce the new colour within the strand.[3,4] For this process to happen however, the chemicals must pass through the cuticle layer, designed specifically to protect the hair from such stimuli. It is during this process that the greatest damage occurs, for example, hydrogen peroxide at high percentage concentrations can burn holes in the cuticle, damaging it beyond repair.[5] This damage caused by bleaching is well known and documented,[6–9] with many researchers concluding

the cause is a mixture of mechanical stresses and disruption to the chemical composition of the hair.

The hair strand consists of three concentric sections, the medulla, cortex and cuticle. The cuticle is the outermost layer, consisting of overlapping cells, similar in look to scales. Its main function is to protect the strand from outside stimuli, such as dirt or chemical oxidation; it also controls water content and hence the porosity of the hair. The scales can lift away from the strand to allow water and other chemicals to pass into the cortex, however too much manipulation of the cuticle can lead to permanent damage, as seen in Figure 1 below. In this instance, Hadjur et. al used confocal microscopy to study the hair from an untreated state (Figure 1a) to extreme repeated weathering over time. Figure 1c shows the partial degradation of the cuticle, exposing the cortex below.[10]

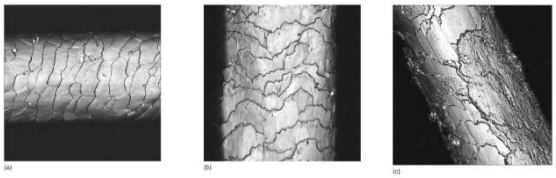


Figure 1: Change in cuticle structure seen over repeated weathering (shampooing and brushing/combing.[10]

The cortex is the thickest layer with its structure consisting of oval-shaped elongated cells, tightly packed and laying parallel to the medulla. Within these cells, α-helical keratin proteins (microfibrils) give the strand its mechanical properties,[11] such as tensile strength i.e. the ability of the hair to withstand strain without breakage. It also plays a major role in the colour of hair; as the hair grows, cells named melanocytes inject two types of melanin into the cortex of each hair as it grows. The concentrations of each: eumelanin (dark) and pheomelanin (light) determine the final hair colour.[12] Finally, the centre of the hair shaft, namely the medulla, is disorganised, amorphous and is not always present in hair strands; in fact, its presence can differ from strand to strand on a single person.

Properties of hair

Diameter

The diameter of the hair strand can give mechanical insights into the hair and its ethnic origin; Asian hair, particularly south Asian has been reported to be much thicker compared to hair from people of European descent.[13] This measurement has been proven to change when coming into contact with bleach and other cosmetic treatments, perhaps as a consequence of swelling via increased water retention. A study by Wolfram showed a 38% increase in moisture regain in bleached hair and 59% increase after a 'relaxing' treatment (chemical straightening)[14], which was explained by the damage caused to the structure of the hair via the treatments, as it allowed more water to penetrate the hair.

Cuticle damage

The surface architecture of the hair can change over time but can be exacerbated depending on the extent of the treatments and weathering it has endured. The most damaging treatments include bleaching and colouring; as discussed previously this is due to the alkaline component's function to lift the cuticle cells in order to allow for the passage of the dye molecules into the cortex, which leads to cuticle cells becoming loose or broken.

Porosity

Both diameter and cuticle damage are intrinsically linked to porosity. A property necessary to healthy hair, it determines how the hair absorbs and retains moisture; highly porous strands absorb moisture very easily, however, it is then lost just as easily leading to overall dry and frizzy hair. These hair types can undergo cosmetic treatments much more effectively compared to their lower porosity counterparts, because bleach and dye molecules are able to penetrate the cortex with ease. Less porous hair is likely to become greasy and suffer from product build up, due to molecules coating the outside of the cuticle and having nowhere further to go.

Multiple methods have been developed to qualify porosity due to its usefulness as a damage indicator.[15] Hill et. al. employed a simple methylene blue staining method to show the uptake of dye by the hair – the more porous the hair, the darker the blue stain.[16] Along similar lines, a less specific but equally effective method involves the use of water: here, a hair placed in a beaker of water sinks or floats depending on its porosity. Hessefort's gas sorption method is a rare instance in which the porosity of hair is being quantified as opposed to qualified.[17]

Fluorescence

Fluorescence refers to the emission of light after a substance absorbs electromagnetic radiation. This process occurs when electrons are excited to a higher energy state after absorbing a photon (quantum amount of energy). This energy leads to a temporary jump in states for the electron, where stability is reduced. The electron spends a few moments in its excited state and promptly relaxes back down to the ground state, a position which affords more stability. This relaxation emits a photon, usually of a lower energy which is due to some vibrational energy being lost. Whilst this process is occurring, the spin state of the electron is retained, meaning the overall activity is very fast. The path of the electron is shown diagrammatically in figure 2.

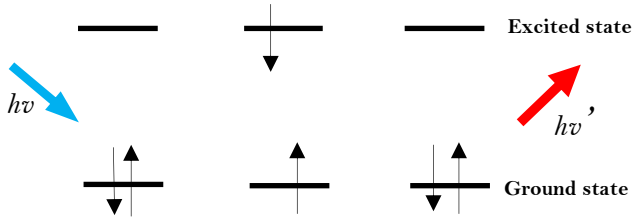


Figure 2: Illustrated diagram showing electron movement when undergoing fluorescence

Autofluorescence refers to the natural fluorescence occurring from certain biological molecules after being subjected to specific wavelengths, as opposed to manually adding fluorescence markers to generate emissions. Biological materials such as skin and hair are able to autofluoresce, which can be viewed using fluorescence microscopy.[18] This type of microscopy works in a very similar way to a simple light microscope, but instead of using only white light to illuminate the sample, the specimen can be subjected to light at a variety of wavelengths. Fluorophores in the sample then absorb this light and emit at a differing wavelength, which is visible through the instrument. In hair particularly, this ability to fluoresce is thought to originate from the melanin granules within the cortex, which become excited in the wavelength range 340 – 400cm^{-1} and emit light between 360 - 560cm^{-1}.[19] Fellner et. al. hypothesised that higher levels of autofluorescence in darker hairs was due to the eumelanin and its breakdown products.[20]

Fluorescence microscopy has been employed in the past to look into how fluorescence changes with regards to cosmetic treatments to the hair, most commonly oxidation - in these investigations, fluorescence was found to increase after oxidative treatments using a hydrogen peroxide medium.[21] Figure 3 compares a reddish-brown untreated hair with a dark blond hair which has undergone oxidation via bleaching (hydrogen peroxide coupled with an alkaline component).[12] A clear difference is visible in the level of fluorescence, however since the strands are not from the same sample, a direct comparison is scientifically inaccurate. The work can act as a baseline from which improvements to the methodology can be made as minimal literature has been published investigating the link between fluorescence and other kinds of cosmetic treatments such as thermal or chemical straightening.

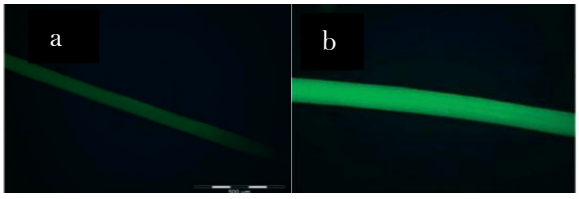


Figure 3: Comparison of fluorescence in two strands of hair which have undergone different levels of oxidation. (a) Untreated reddish-blonde (b) hydrogen peroxide treated dark blonde hair. Excitation/Emission filter: 450 - 490/515nm.[12]

Drug testing

Even though hair can act as a timeline for drug use, the literature available for the effect of hair morphology on drug concentration is limited. The theory that these factors can affect results was investigated as early as 1990 by the Uematsu research group, who published a series of papers investigating the haloperidol concentration in human scalp hair - a link between the pigmentation (melanin) and the concentration of the drug found was established.[22–24] Research in this area has remained of interest with papers being published as recently as 2017 discussing the role of pigmentation in hair analysis.[25] Problems in interpreting results gained by the medium of hair analysis are public, however the use of this method shows no signs of ceasing, especially in situations such as workplace testing and criminal cases due to its non-invasive and convenient nature.[26]

In any case, it is important to realise all the possible techniques in which results could be manipulated, hence this paper. A handful of articles have been published discussing the influence of such treatments on drug testing, however some papers have been found to be contradictory; for instance, a discussion on 'ethnic' hair-care products and their effect on false negative results in drug analysis was refuted by Hill et. al.[27,28] Another major example includes the difference in results found when analysing cocaine uptake in bleached and/or permed hair. Papers published by Pötsch and Skopp,[29] Yegles[30] and Jurado[31] all conclude a decrease in cocaine concentration when testing damaged hair, whereas Hill[16] and Gerace et. al[32] found a pronounced uptake in concentration. For this reason, it is extremely important to understand the fundamentals of how certain cosmetic treatments affect the morphology and properties of hair strands from all manner of samples including colour, age, gender and condition of hair. These results can then be collated, and any conclusions collected from hair analysis will be informed by the strand's history and condition and will therefore be as true as possible.

Experimental

Materials

Hair samples were sourced from 17 volunteers of differing ages, hair colour and condition of hair. All hair was used as supplied, with no further treatment or cleaning but samples were cut to 2±0.5cm lengths for ease of use.

Hydrogen peroxide at 3% w/v concentration was supplied from the University of Huddersfield Chemical Stores and kept in refrigerated conditions when not in use. Other concentrations (namely 6%, 9% and 12%) were diluted down from a stock solution of 30% w/v concentration (also supplied from the university) and then stored alongside the 3% solution. Cream hydrogen peroxide was purchased from Dennis Williams Hair & Beauty (Wakefield, England) at concentrations 3%, 6%, 9% and 12% w/v, and stored in a cool, dry area.

The major microscopic instruments used were a Leica DM500 microscope (magnifications x4, x10, x40 and x100 available), which was coupled to a Leica ICC50 HD camera with LAS EZ software used for width measurements. Jeol 6060LV and FEI Quanta FG 250 scanning electron microscopes were also used, with samples being coated in gold using a Quorum Instruments SC7620 Mini Splutter Coater before analysis. All images were collected as Back Scattered Electron Images (BSEI). Finally, the fluorescence of the hair was analysed by a Zeiss Axio Observer Z1 epi-fluorescence microscope with a filter cube eGFP Excitation BP 470/40 and Emission BP 525/50. Zen Pro software was also used for image taking and manipulation.

Glycerol, nail varnish, microscope slides and methylene blue solution were provided from the University of Huddersfield Chemical Stores.

Methodology

Bleaching

Hair samples were bleached by soaking them in the appropriate hydrogen peroxide (500µL) for the allotted time, for instance 3% liquid for 60 minutes. The solution was replaced every 30 minutes for fear of degradation whilst the same cream was used for the full amount of time. Post bleaching, the hair was washed - the washing procedure consisted of the sample being removed from the peroxide and immediately being transferred to 500µL deionised water for 5 minutes with some agitation. The hair was then dried by patting with paper and stored in a sealed, labelled bag at room temperature away from any light sources. The method for cream peroxide was identical, save for 1.5ml peroxide being used in lieu of the solution. The bleached hair was analysed and compared to its untreated counterpart by the methods set out below.

Hair diameter

After the bleaching and washing procedures, the sample was placed onto a microscope slide using a wet mount. Images were taken at magnification x40 and diameter measured in mm using the computer software.

Cuticle damage

A scanning electron microscope (SEM) was employed to look at the outer cuticle damage. Each strand was fixed to a metal plate and coated in gold using a splutter coater in order to see the sample. The sample was analysed in high vacuum and images recorded at magnification x1000.

Porosity

Porosity was examined by immersing the hair in methylene blue solution for 5 minutes. The uptake by the hair was then analysed under the compound microscope at magnification x40. For darker hairs where the subtle differences in brown/black and blue were not visible, a water test was used to complement the results. The strand was placed into a beaker of water and an approximation of the time taken for the hair to sink to the bottom was recorded.

Fluorescence

All samples were mounted onto microscope slides using a glycerol medium (10µL), with the cover slide being sealed with nail varnish in order to prevent leakage. They were then placed in the microscope and observed under brightfield with x63 magnification before using a green excitation-emission filter of 470/40nm-525/50nm to analyse fluorescence levels. Images were taken from the middle of the length of hair in order to achieve the best representation of the sample.

Results and Discussion

Diameter

A baseline was established by measuring the width of all the hair samples received, pre-treatment. This was done by measuring the width of the hair in three places along the middle of the strand, and with an average taken. The samples were also grouped into categories (age, hair colour, gender and treatment history of hair) to allow any patterns to be identified with ease. With regards to initial hair width, previous literature has shown a link between ethnicity and diameter,[13] with Asian hair purportedly being the thickest. Despite this, within the small sample size available this was not found to be the case. The thickest hair was in fact blonde, with mid-level treatment implemented upon it, and the thinnest hair was black (of South-Asian origin), although a child. This can be seen in Table 1 below. No notable links were apparent between genders, colours and cosmetic treatment history.

Sample Number	Gender	Colour	Cosmetic use	Width of untreated hair (mm)
5*	Female	Black	1	0.0627
11	Female	Blonde	5	0.0630
10	Female	Ginger	3	0.0635
15	Male	Brown	0	0.0644
13*	Female	Blonde	0	0.0670
4	Female	Black	4	0.0688
14	Female	Brown	0	0.0739
7	Male	Brown	0	0.0816
16	Female	Ginger	3	0.0827
1	Female	Black	0	0.0832
9*	Female	Ginger	0	0.0836
6	Female	Brown	1	0.0882
3	Male	Black	2	0.0977
8	Female	Brown	5	0.1050
2	Female	Grey	0	0.1055
12	Female	Blonde	3	0.1470

Table 1: Table showcasing the initial diameter of hair strands taken from all samples available to the research project.

To investigate how the diameter changed with bleaching, a single strand from each sample was treated with 3% liquid hydrogen peroxide (500µL) for 2, 4, 6, 8, and 10 minutes followed by the washing procedure; this amount and duration were decided upon after various trials. At first glance, no obvious patterns were apparent in any categories; all the hair underwent some change to its diameter, be it an initial increase followed by a decrease, or decreasing first before increasing. A rough 50:50 split was found in those samples who experienced an overall increase in diameter and in those who decreased, however in these two groups there were no linking variables. When observing the effect of cream peroxide as opposed to its liquid form, no links were found between bleaching and diameter in this medium.

Regarding the percentage change in diameter, the most treated hair available in the samples displayed the biggest change in width, dramatically increasing in the initial bleaching period 0-2 minutes by 129% from an initial diameter of 0.0630mm to 0.144mm as seen in figure 4 below. This powerful change could be due to the cortex and cuticle being damaged originally, due to the vast history of bleaching and colouring done to the hair.

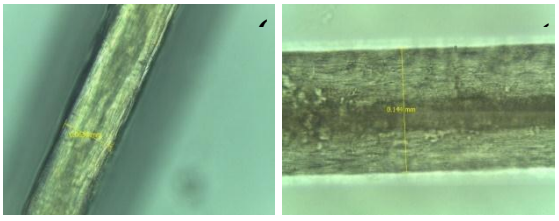


Figure 4: Side-by-side comparison of blonde sample (a) untreated vs. (b) bleached for 2 minutes in 3% liquid hydrogen peroxide. Magnification x40.

Cuticle damage

Strands were bleached at 3% concentration for 10 and 60 minutes, and photographed under SEM. Firstly, a sample of brown hair from a female with previous regular bleaching/colouring history, was analysed, as shown in figure 5. The untreated hair already exhibited signs of damage, which were exacerbated dramatically after 60 minutes of contact with the peroxide solution, where the cuticle layer was vastly depleted. Comparing this to hair from a female with zero treatment history i.e. unadulterated hair, it was observed that for those samples with a history of treatment, it is less likely that any change will occur in a short period of bleaching time. Conversely, those hairs with no

previous bleaching experience will change for the worse rapidly, as the unadulterated hair sample showed major signs of damage after only 10 minutes of immersion. This result was replicated with the other unadulterated versus heavily treated samples available, however due to the small sample size, caution must be applied. Male hair with the same characteristics as figure 5 (unadulterated and brown in colour) was also observed using the same variables. The SEM images showed similar results, with the cuticles cracking and peeling off the strand. In order to see if this is common in male vs female samples, a larger sample size is required.

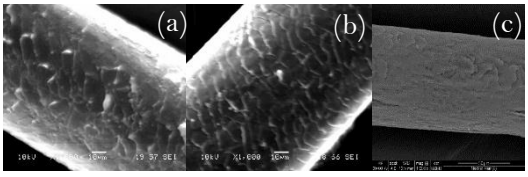


Figure 5: Brown female hair with substantial treatment history (a) Untreated (b) 3% 10 mins (c) 3% 60 mins. Magnification x1000.

The samples discussed above were of a Caucasian background – when comparing these results to samples of South Asian origin, the hairs showed minimal cuticle lifting. The main visual differences in the cuticles of both races includes the denser packing and greater overlap of cells in Asian hair. This could go some way in explaining why the breakage and lifting of cells is minimised. An example is shown below in Figure 6.

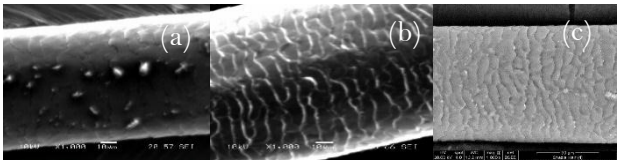


Figure 6: SEM image of hair of Asian origin (female, black in colour) (a) untreated (b) 3% 10 mins (c) 3% 60 mins. Magnification x1000.

Finally, hair from children can often display different characteristics compared to hair from adults. Even though the majority of children do not undergo cosmetic treatments of any kind during their formative years, hair is still much thinner and weaker, and hence more susceptible to weathering, from daily activities such as washing and combing. However, in this project bleaching was the primary focus and so it was found that the children's hair was much more susceptible to breakage by oxidation; figure 7 shows a whole cell 'peeling' off the strand, with many cells chipped and lifted.

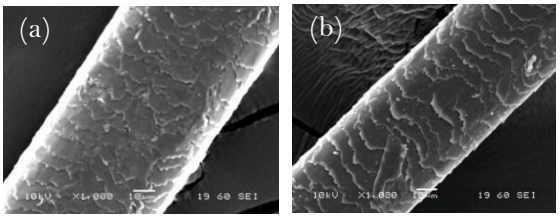


Figure 7: SEM image of hair (child, female, unadulterated, blonde hair) (a) Untreated (b) Bleached at 3% for 10 minutes. Magnification x1000.

Porosity

The porosity of hair has a direct link to the condition of hair via cuticle damage. figure 8 below illustrates the effect damage can have on a strand – both hairs were taken from the same volunteer, with the only difference being a tear in the cuticle – as highlighted. These tears are commonly accrued during everyday weathering such as brushing and even regular shampoo washes.[33] The uptake of the methylene blue is visibly different in the two strands, with the damaged hair presenting a darker shade of blue. The area marked with a red circle shows the damage, in this case a tear, to the hair. Here, the cuticle has been stripped away, allowing a greater proportion of methylene blue to be taken up by the strand.

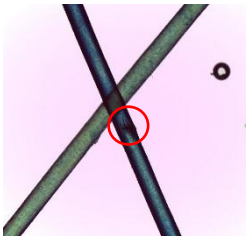


Figure 8: Two strands of hair (ginger, mild non-oxidative and thermal treatment history), after immersion in methylene blue. Magnification x40.

To investigate post bleaching porosity, 3% peroxide was used throughout, with time being the variable analysed. A single 10-minute cycle was carried out to begin with (10 minutes bleaching in 500µL hydrogen peroxide, followed by 5 minutes in distilled water to wash), however this was not enough time to see a significant change in the uptake of methylene blue in most samples. This cycle was then repeated for a total of three times (i.e. samples were bleached for 10, 20 and 30 minutes), which generated results. A higher porosity was observed across the board, with two samples being exemplified below (Figures 9 and 10). The grey hair (Figure 9) showed very clearly the uptake of the methylene blue solution at the cuticle, shown by the two thin blue lines at the edge of the strand. As the extent of bleaching increases, the solution begins to move further into the middle of the strand illustrated best in figure 9c i.e. the porosity is increasing with bleaching. Comparing this to the brown hair, the strand is porous before being treated with the bleach (the whole strand is tinged blue). However, less change was seen over the full bleaching period i.e. porosity did not have as much of a link to bleaching when compared with the images shown in figure 9. However, this may be due to the colour change being more apparent in the grey strand, due to it having no colour, with the difference in blue and brown not as visible.

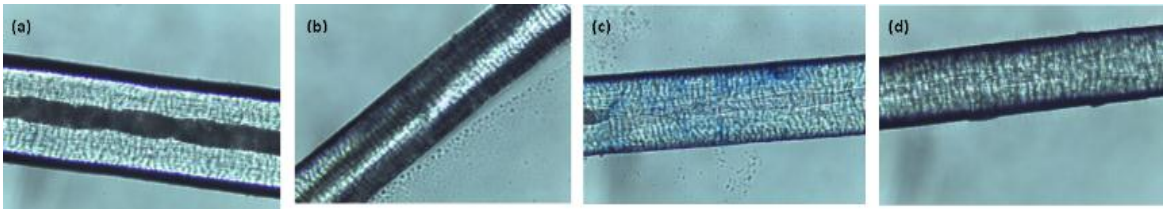


Figure 9: Grey unadulterated adult hair soaked in methylene blue (a) Untreated hair. (b) 3% hydrogen peroxide 10 mins (c) 3% H_2O_2 20 mins (d) 3% H_2O_2 30 mins. Magnification x40.

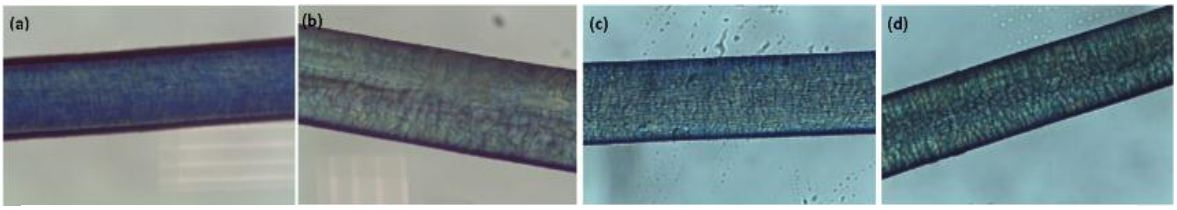


Figure 10: Brown adult hair (history of mild thermal treatment) soaked in methylene blue (a) Untreated hair. (b) 3% hydrogen peroxide 10 mins (c) 3% H_2O_2 20 mins (d) 3% H_2O_2 30 mins. Magnification x40.

In order to confirm these results, a water method was used to show a rough qualitative measure of porosity, especially in those hairs too dark to notice a colour difference, such as the brown or black hair samples. Results correlated with the methylene blue test in that hairs that are more porous sank (water travelled through the cuticle faster and weighted the hair) whilst lower porosity caused hairs to sink more slowly.

Fluorescence

Fluorescence levels in strands from different categories were observed whilst changing the time, concentration and medium of peroxide over samples of varying ages, hair colour and condition of hair. Beginning the experimental work, samples with seemly opposite properties were bleached and analysed – light blonde child hair and dark black adult hair – in order to gauge how the different melanin molecules reacted to the oxidation (Eumelanin in dark hair, pheomelanin in lighter hair). In the case of light blonde hair, the control sample showed very strong fluorescence that decreased over a 60 minute bleaching period (Figure 11). The control sample for the untreated black hair showed a much lower fluorescence output compared to that of the blonde (Figure 12), even when a stronger concentration of the peroxide was employed. It could be proposed that pheomelanin is much more fluorescent than eumelanin in its non-oxidised form.

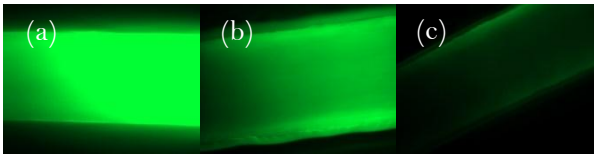


Figure 11: Fluorescence of hair shown from light blonde, female child hair (a) Untreated (b) 3% 30 mins (c) 3% 60 mins. Magnification x63. Excitation-Emission: 470/40nm-525/50nm.

Figure 12: Fluorescence of hair shown. Black unadulterated hair from female (a) Untreated (b) 9% 20 mins (c) 9% 40 mins (d) 9% 60 mins. Magnification x63. Excitation-Emission: 470/40nm-525/50nm.

These results informed the next stage of analysis - bleaching a range of hair colours over a longer time period in order to see how fluorescence levels changed. Keeping all other factors constant i.e. age, gender and treatment history, samples from four female children of differing hair colour (black, blonde, ginger and brown) were bleached at 3% in increasing 10-minute intervals for a total of 180 minutes. The theory informing this stemmed from the fact that it may be possible to conclude whether just the colour of the hair or the treatment history and age affect the fluorescence results received as well. Should all four samples act similarly, it would be reasonable to conclude that age and history are also contributing factors, with inconsistent results pointing to the colour of the strand solely.

In order to visualise the results a scale was created for measuring the fluorescence shown. A more accurate way of quantifying the results would have been to assign arbitrary units to the brightness of the fluorescence using the software linked to the microscope. However, in order to do this, multiple strands of hair would have to be bleached and numerous images taken, which due to time constraints was not possible. As an alternative, images of increasing fluorescence were scaled from 1 to 5 with 1 showing no or very minimal fluorescence and 5 showing the strongest, with the images seen below in figure 13 serving as a baseline to compare the results to. Importantly, in order to make sure images from each sample could be compared to others, the exposure of the camera was set as a constant.

Graphs 1 - 4 below show the change in fluorescence over 180 minutes of bleaching with 3% liquid peroxide. As seen, the darker hairs began at a lower fluorescent level before increasing, compared to the lighter hairs which naturally contain higher levels of fluorescence. However, all the hairs at some point within the 180 minutes showed a minimum level of fluorescence. This moment was termed the *dark point*, as the fluorescence of the hair was not visible against the dark background of the image. Both brown and black hairs, presumably with higher levels of eumelanin present, experienced their dark point around the 40-50 minute mark, compared to the blonde hair that saw two dark points at 80 and 120 minutes. The ginger hair, which most likely contained a mixture of both eumelanin and pheomelanin, decreased to a minimum very early on around the 20-minute mark, before increasing. From these results, a conclusion can be drawn that colour is the primary factor in fluorescence level as opposed to age and history since all the samples displayed a unique correlation between time and fluorescence.

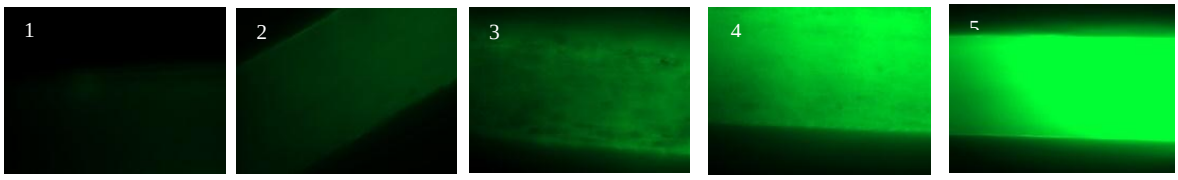
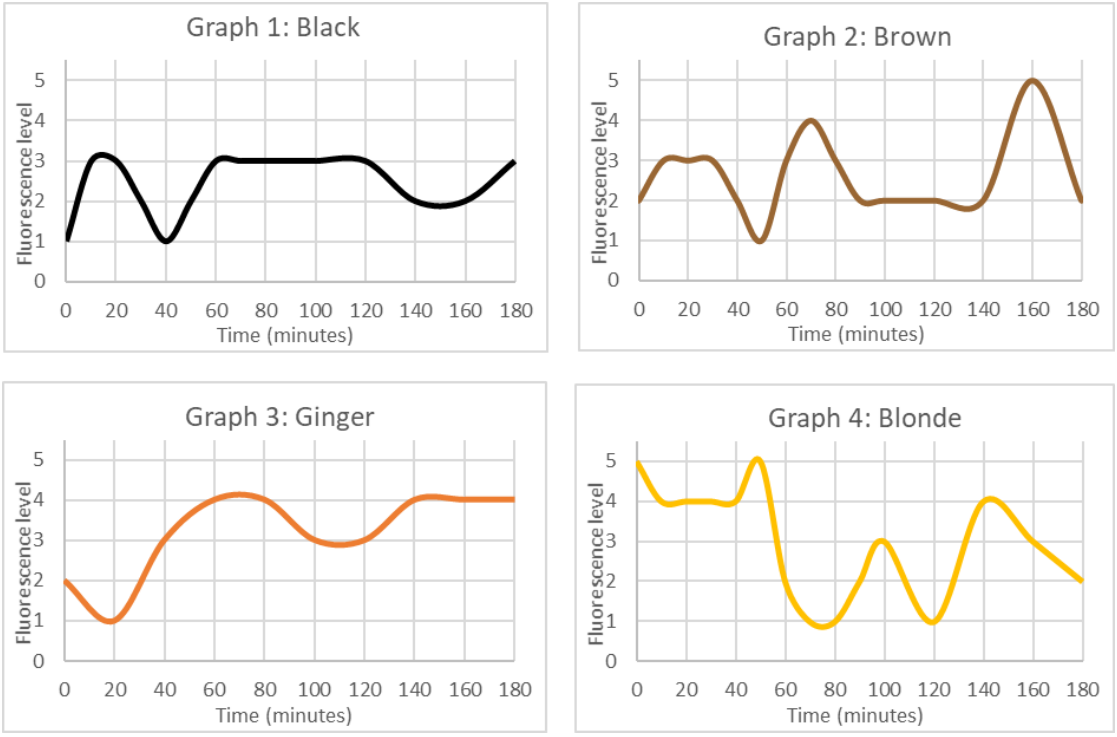


Figure 13: Images of increasing fluorescence numbered from 1-5, constituting a scale in order to graphically represent the results.



Graphs 1 – 4: Showing the scale of fluorescence vs time for children's bleached hair of varied colours.

Work in this vein could not be found in any literature, meaning results could not be used to support or contradict any existing claims, nor could any indication be given as to why this process occurs. I speculate that it may be due to the melanin granules in the hair being degraded by the bleach, with the pseudo-fluorescence originating from the degradants. Alternatively, a reaction not yet categorised could occur, affecting the fluorescent output, but this is speculation. Any fluorescence observed in the control sample was assumed to be the hair's natural, so-called auto-fluorescence, which has been discussed by previous researchers,[18,20] whereas any seen post the dark point was termed *pseudo-fluorescence.*

This term was chosen as the prefix 'pseudo-' denotes close or deceptive resemblance to the root word. In this case, what the author believes is happening is that the natural fluorescence of the hair strand is destroyed before a similar output is observed through the microscope. This development of the full decline of fluorescence before an increase back to normal or increased levels was thought to occur due to the melanin granules being damaged by the bleach, before regaining the ability to fluoresce again, by some unknown process, possibly due to the oxidation.

The above work was done in order to gain a greater understanding into the colour and melanin type, therefore liquid peroxide was used as a constant. As most treatments occur using cream products, cream hydrogen peroxide was also used and compared directly to liquid peroxide at the same concentrations and times. Four adult samples were used with a treatment history of 'none' to moderate, as can be expected in real world samples. The same four standard hair colours were used – black, brown, ginger and blonde.

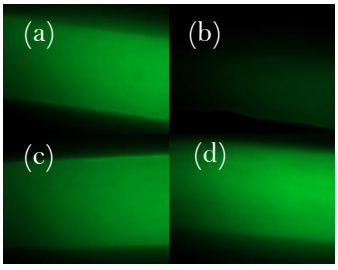


Figure 14: Ginger hair sample from female bleached at 3%. (a) 3% cream, 30 minutes (b) 3% cream, 60 minutes (c) 3% liquid, 30 minutes (d) 3% liquid, 60 minutes. Magnification x63. Excitation-Emission: 470/40nm-525/50nm.

Figure 14 above shows how the medium of peroxide can make a difference to fluorescence. Using cream as opposed to liquid on ginger hair proved that the cream bleaching for 60 minutes led to the hair reaching its dark point, but that using the liquid made no difference to the fluorescence level. It was postulated that this was due to the more efficient coating given to the hair by the cream compared to the liquid due to the higher viscosity, which led to results being observed in a shorter time period for this sample. This result was replicated by the black hair sample, but not with the blonde or the brown, with little knowledge gleaned as to why.

In my study, time had a considerable effect on the level of fluorescence observed on the strand. In real world scenarios, peroxides are available to purchase in a range in concentrations and are used at a hairdresser's discretion. In order to explore the effects of this, 3%, 6%, 9% and 12% w/v peroxide concentrations were applied to hair whilst keeping the time immersed constant. No difference in output was observed when increasing the concentration across all colours, concluding that time is a more effective transformer of hair. Finally, all the hair tested for fluorescence so far has been that of natural colouring. In order to confirm that it was in fact the melanin producing the fluorescence as the literature stated, grey hair was also examined. As expected, the control sample showed no fluorescence which only increased after 60 minutes of bleaching at 3% liquid peroxide as shown in figure 15, giving more credence to the theory that oxidation of the strand increases the fluorescence output in some way after the melanin is destroyed or damaged.

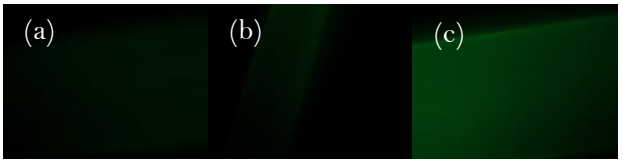


Figure 15: Grey hair bleached with 3% liquid peroxide (a) untreated (b) 30 minutes (c) 60 minutes of bleaching. Magnification x63. Excitation-Emission: 470/40nm-525/50nm.

Conclusion

No trend was found between hair diameter and its background both pre- and post-bleaching, but cuticle damage and increased porosity of all samples were proportionally linked to bleaching time. An apparently novel finding in this project was related to the fluorescence of hair. Published literature in this area of research is sparse and yet the results collected in this project found certain features related to bleaching and fluorescence which could be applied to commercial testing in the future. All hair tested over a variety of categories showed a decrease in fluorescence over time to a point where the output appeared to be 'switched off' – this was termed the *dark point*. After this minimum was reached (the dark point differed in time over varying hair colours, but nonetheless it always occurred) the fluorescence steadily increased again. All fluorescence occurring after the dark point was termed *pseudo-fluorescence*.

Several questions remain unanswered presently, particularly regarding the mechanism behind melanin fluorescence and its link to human hair. Abundant room for further progress remains in this field. Yet, it is the hope of this paper that the research amassed on these features will help aid the hair analysis discipline, particularly in cases of drug testing, where previous oxidative treatments can affect drug uptake into the hair.

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