Physiology

Adhesion Failure of External Hair Cuticles Caused by Prussian Blue: Possible Electrochemical Roles of Sulfur and Cystine

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Background: The effect of Prussian Blue on the hair fiber is unknown. In this report a new optical microscopy technique tailored to evaluate the effect of chemicals in solution on the hair shaft is introduced. The hair is a natural fiber consisting of keratin, a protein containing high concentration of disulfide derivative cystine. Prussian Blue (PB) is the main ingredient used to stain iron particles in tissue and advertised as ingredient in some hair coloring products. The effects on *ex vivo* human hairs are herein reported.

Methods: A working solution of 2,5% Potassium Ferrocyanide was prepared by mixing the crystals with deionized water. Working solution, 2.5% Aqueous Solution of Potassium Ferrocyanide: Potassium ferrocyanide, Trihydrate (K4Fe(CN)6.3H2O (2.5 g); Deionized water (100 ml). Mix to dissolve. Freshly plucked human hairs were placed in the center of a 25x75x1mm glass slide. Three small drops of the PB solution were delivered via a micropipette surrounding the hair. Then a second slide was placed covering the first and secured by thin masking tape strips placed at the slides edge. At this point the solution spreads interstitially between the slides. Care should be taken to avoid air pockets. This preparation will be referred as a sandwich (SDW) throughout the manuscript. The solution was then allowed to evaporate (average time 4 hours) and images were documented by optical microscopy and recorded n=10. After drying, the same hair was sequentially remounted in similar fashion x4 times. Ancillary testing: Hairs stored for 20 months in dry cardboard slide boxes were also evaluated. Equipment used: All specimens were viewed and digitally recorded at different magnifications via a video microscope interfaced with a computer system.

Results: In both, freshly harvested and old hairs when in contact with Prussian Blue, exo-cuticles separation was observed.

Conclusions: Exposure of human hair to Prussian Blue causes large sections of imbricate pattern cuticles segments to separate from the shaft. These segmental adhesion failures were observed intermittently around the length of the shaft. The observed cuticle segments detachment failure caused by PB is hypothesized to be caused by a reaction between PB and the intrinsic sulfur and cystine present in the hair fiber. This reaction is hypothesized to be of an electromagnetic nature.

Cuticles Detachment | Hair Cuticles Harvesting | Prussian Blue

Introduction

The aims of the present report are twofold: First to introduce an optical microscopy technique to evaluate the effect of chemicals in solution on the human hair. Secondly, To evaluate the effect of Prussian Blue (PB) alone on the human hair fiber..

Numerous studies have focused on the hair cuticle function, chemical composition and health [1,2,3]. Previous report introduced an optical microscopy technique for imaging electromagnetic energy in plant and animal tissues [4]. Drops of Prussian Blue mixed with 2,5% HCl and 2000 nanometers in diameter iron particles (PBS Fe2K) were trapped between two glass slides and dubbed a sandwich (SDW). In this manuscript the effect of Prussian Blue proper (minus the iron particles) in solution (PB) in SDW is introduced.

In previous experiments when using the iron particles (PBS Fe2K) it was hypothesized that the phenomenon of separating cuticles from the hair shaft could have been as result of a combination of egress images (caused by electromagnetic forces) and imprints that occur when the hair shaft moved inside the SDW. In this report, is demonstrated that after evaporation, PB alone also separates the cuticles from the hair shaft and are visualized in different depth of focus. The hair shaft is seen shifting away from the hair cuticles (Video not shown), instead still microphotographs of the video-recording demonstrate the shifting of the hair shaft leaving the cuticles behind (Fig 1).

Materials and Methods

A Prussian Blue solution (PB) consisting of 2.5% of Potassium Ferrocyanide Dihydrate (K4Fe(CN)6.2H2O was prepared as follows:
2.5% Aqueous Solution of Potassium Ferrocyanide:
Potassium ferrocyanide, Dihydrate
(K4Fe(CN)6.3H2O, FW 422.4, Sigma, Cat# P-3289) -----2.5 g,
Deionized water ----------100 ml
Mix to dissolve

The Glass Slide Sandwich (SDW)
Using a transfer pipette, three drops of the Prussian Blue (PB) solution were placed in the center of a 25x75x1mm glass slide. Human peri-umbilicar hairs from (from author) were plucked by tweezers and carefully placed in the center of a clean glass slide. A second identical glass slide covered the first, thus trapping the hair and PBS in a sandwich (SDW) n=6. This preparation will be referred as a sandwich SDW in the manuscript. Since it contains PB then was dubbed a PB SDW preparation. The PB SDW was allowed to stand for at least 4 hours or until the liquid had dried. Microphotographs and video-recordings of the hair images were obtained at X4, x10 and x40 magnifications via a video microscope (Celestron, LCD Digital Microscope II model # 44341 Torrance California, USA).

Repeated PB SDW experiments on same hair
All samples were subjected to sequential four (4 times) individual PB SDW immersions.

Ancillary testing
Ruling out metabolic activity as the cause of the separation was shown in 20 months old hairs (stored in an empty slide box). These old samples n=3 also submitted to PB SDW depicted the same cuticle separations phenomenon (Image not shown) as freshly plucked hairs.

Conflict of interest: No conflicts declared.
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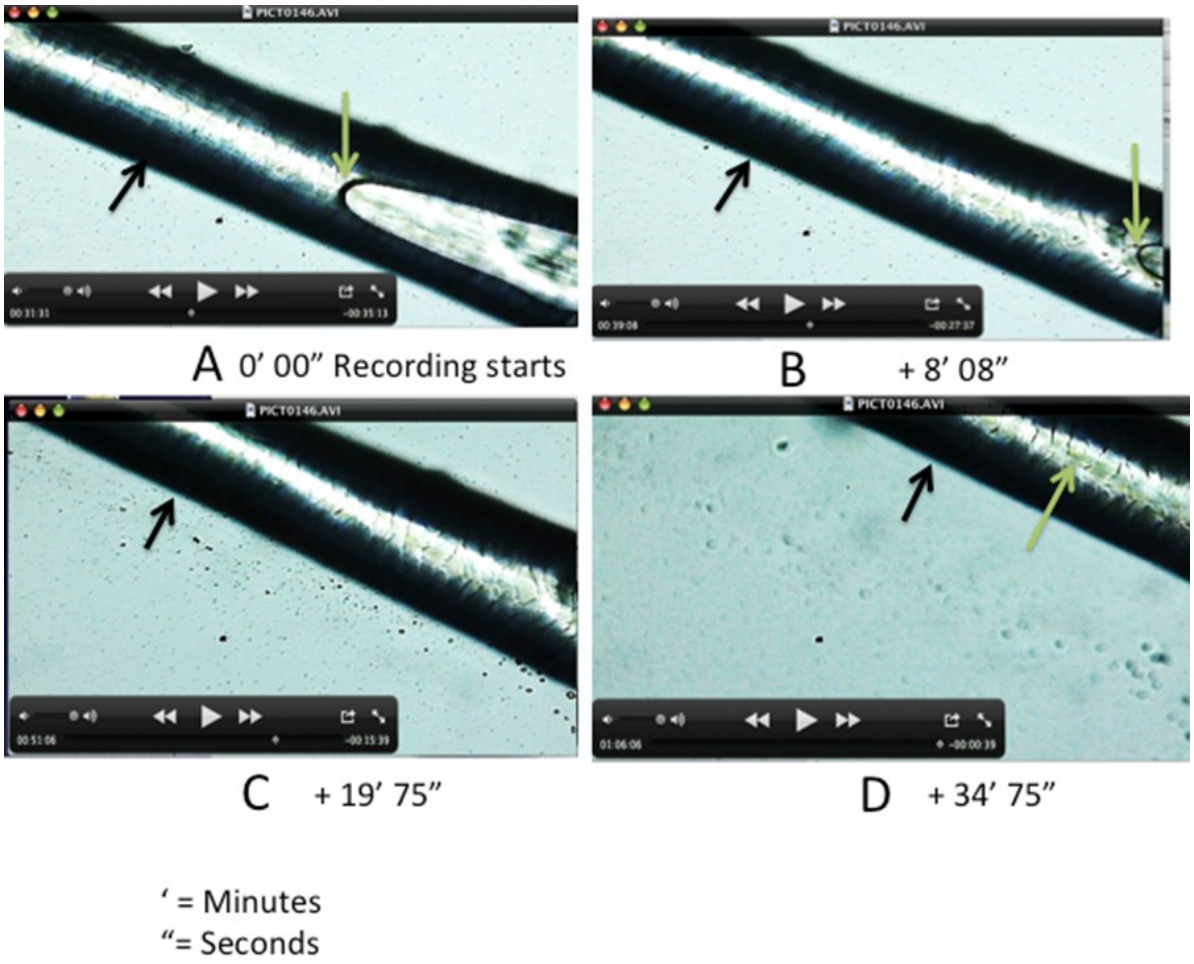

Figure 1. Panel of selective successive frames of video-recording depicting the effect of Prussian Blue solution evaporation on the human hair in a slide sandwich (SDW). Notice as the evaporation progresses, the hair shaft shifts leaving the cuticles behind. In panels A and B, Red arrows points at the moving evaporation line. Black arrows indicate edge of hair shaft. In panel D the color arrow shows enhanced details of hair medulla seen after evaporation.

Results

The first time around, extensive segmental detachment was observed as the shaft shifted positions.[Figs 2,3,4]. Subsequent exposures to PB SDWs of same hair (up to 4 times) showed minimal sporadic detachments (Fig 4). These observed cuticle segments detachment failures caused by PB are hypothesized to be caused by a reaction between PB and the intrinsic sulfur and cystine present in the hair fiber. Upon drying, the cuticles are seen adhering to the top and bottom inner surface of the SDW. On occasions single cuticles were also seen separated from the shaft. Is worth noticing that under the microscope viewing field there was evidence of a two dimensional detachment. It was apparent that the segmental outermost layer cuticles separation was detached from an anterior and a posterior plane. The detachments occurred throughout the entire length of the hair shaft surface (Image not shown). Notably it was demonstrated that only the outermost layer detached n=10. Samples subjected to repeat PB SDW testing showed a drastic reduction in cuticle separation when compared to the first time.

Single layer imbricate sections cuticle detachment

Optical microscopy relies on light penetrating through a given material for display. To ascertain that only one layer of cuticles were detached, microphotographs were compared on each side of the SDW. In other words, an image was recorded, then the slide flipped and a mirror image was obtained from the opposite flat plane.

Limitations

The author recognizes the lack of quantitation of cuticles detachments in this publication, although the qualitative images at different stages are strikingly different. Further experiments and additional equipment may be necessary for a complete quantitated report.

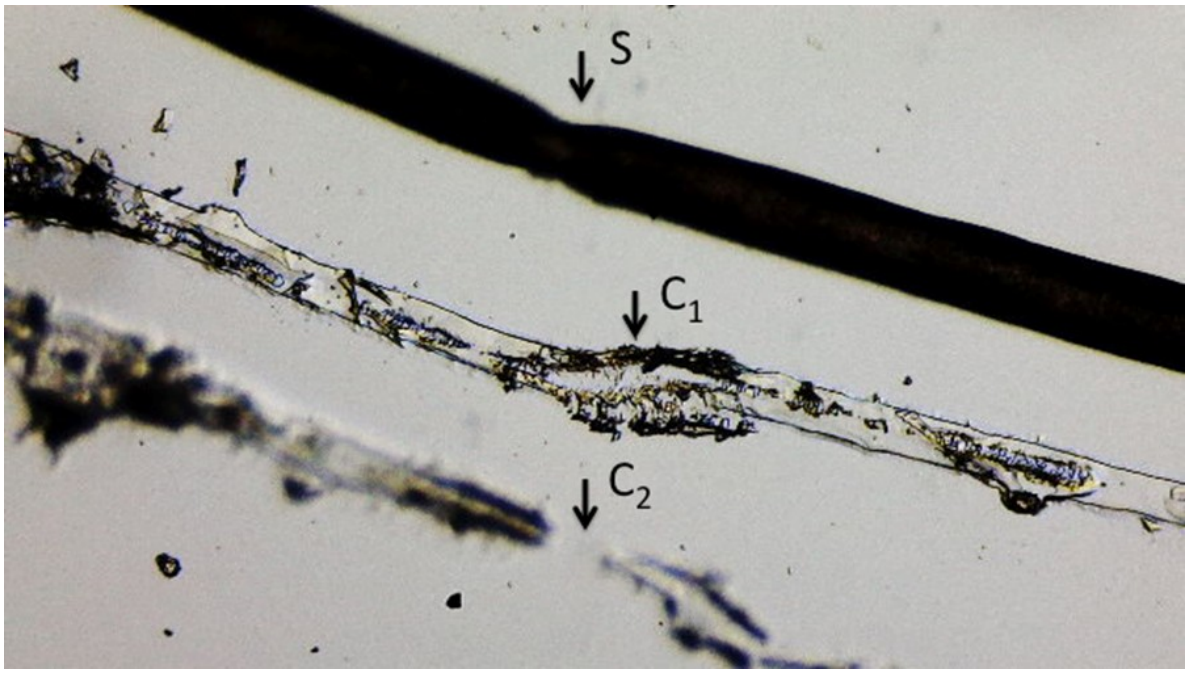

Figure 2. Microphotograph of hair in contact with Prussian Blue (PBS) between two glass slides (SDW) after evaration, showing: S= Shifting shaft showing damage caused by tweezers, C_1 = In focus damaged anterior level cuticles, C_2 = Out of focus damaged posterior level cuticles (out of focus). X4 Magnification.

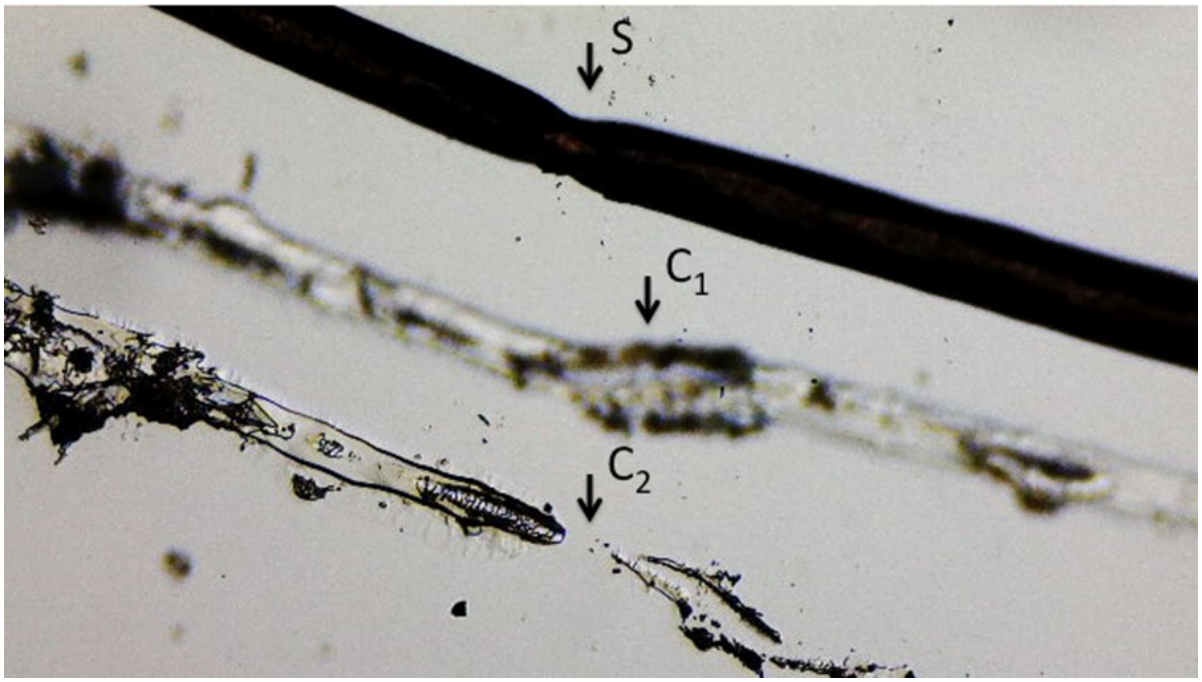

Figure 3. (Same area as in Figure 1) Microphotograph of hair in contact with Prussian Blue (PB) between two glass slides (SDW) after evaporation, showing: S= Shifting shaft showing damage caused by tweezers, C_1 = Out of focus damaged anterior level cuticles, C_2 = In focus posterior level cuticles (out of focus). X 4 Magnification.

Figure 4. Large segmental cuticle detachment after initial PB SDW. C= Cuticles

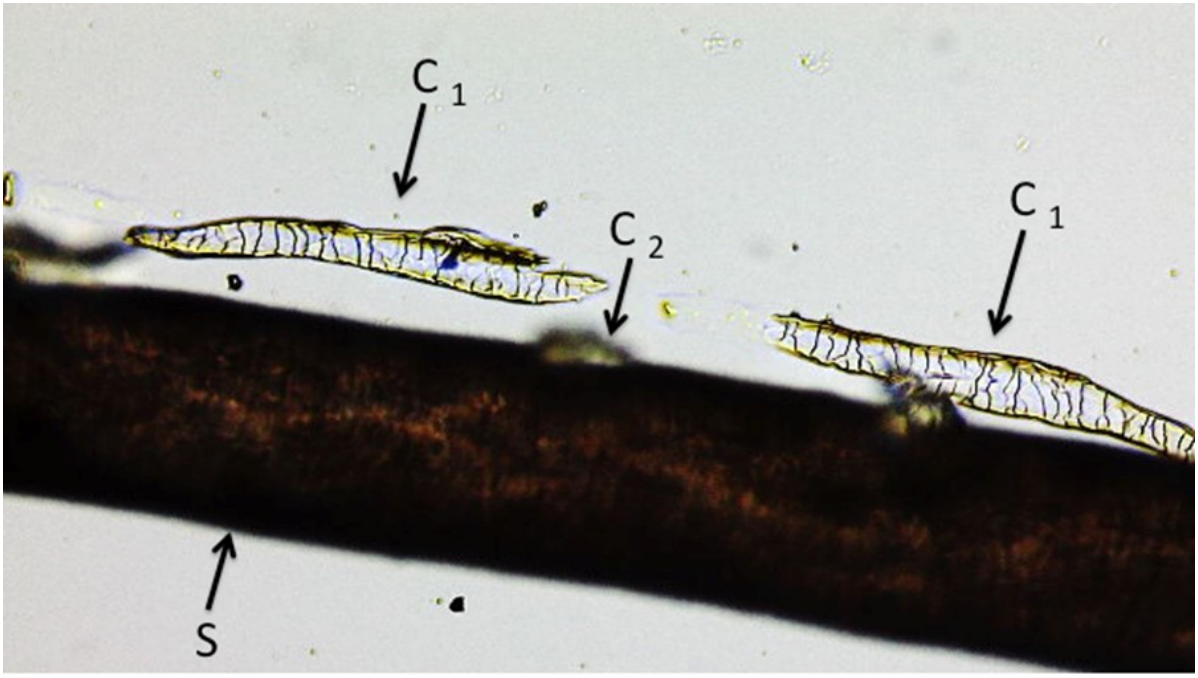

Figure 5. Reduced segmental detachment after fourth PB SDW experiment. C_1= Cuticle level 1. C2= Cuticle level 2, out of focus showing different spatial plane. Please compare with Figures (1,2 & 3).

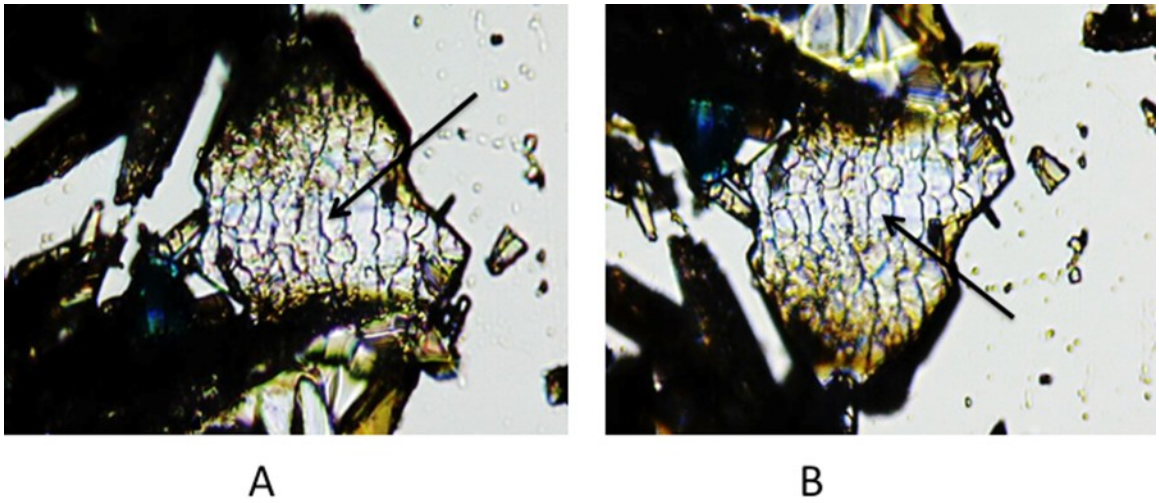

Figure 6. Panel shows detached cuticle fragment. Panels A and B. Anterior and posterior views showing mirror images, thus proving a single cuticle layer adhesion failure due to exposure to Prussian Blue.

Discussion

Ruling out metabolic activity [5,6] as the cause of the separation was shown in 20 months old hairs (stored in an empty slide box). These old samples n=3 also submitted to PB SDW depicted the same cuticle separations phenomenon as freshly plucked hairs (Image not shown).

The question arises: Why does Prussian Blue (PB) cause such a high detachment prevalence of the outermost cuticles?

The Prussian Blue/Sulfur/Cystine factor

Cuticle detachment failures are caused by mechanical, chemical or radiation (ultraviolet) amongst others [6]. It has been documented that "The hair thread is a natural fiber formed by keratin, a protein containing high concentration of sulfur coming from the amino acid cystine."[7]. The electrochemical properties of PB are backed by several publications such as: Nano composite of Prussian blue based sensor for l-cysteine have been recently developed [8], and notably half a century ago, the electro histochemistry of cysteine was introduced in the literature [9,10,11]. The author of the latter reference mixed human hairs with silver nitrate and metallic particles were produced in the exocuticles; furthermore PB's affinity for sulfur has been shown in removing organic sulfur from coal [12]. Since the separation of the exo-cuticle (A Layer) is readily done by Prussian Blue, it is noteworthy that when cuticles were exposed to enzymatic digestion "The fractions obtained after digestion for 45 and 90 min evidently contain mainly A layer and cell membrane complex" [13], in other words, the A- layer was proven to be different when compared to the endo-cuticles.

Conclusions

- Hair shaft segments when in contact with PB SDW for the first time exhibit segmental hair exo-cuticles separation.
- In the experiments, this occurs when the fluid evaporates in the SDW causing movement of the hair shaft.
- That said segments consist of the outermost cuticle layer.
- That the first PB SDW separation always showed a much greater number of segmental detachments.
- Also shown was that subsequent immersions in PB SDW showed minimal detachments.
- It was documented that at least in one instance only the top (outermost) layer of cuticles was detached throughout the length of the hair fiber.
- Referenced publications in this manuscript support a possible electrochemical (electromagnetic) reaction of PB with the intrinsic sulfur and derived cystine present in the fibers of the human hair. This could very well trigger the significant first cuticle layer adhesion failure as demonstrated in this manuscript.

Acknowledgments

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