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ORIGINAL PAPER

Criminalistics

Effects of crime scene contaminants on surface-enhanced Raman analysis of hair

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Abstract

Forensic analysis of hair is important as hair is one of the most commonly examined forms of trace evidence found at crime scenes. A growing body of evidence suggests that surface-enhanced Raman spectroscopy (SERS), a label-free and non-destructive analytical technique, can be used to detect and identify artificial colorants present on hair. However, hair collected at crime scenes is often contaminated by substances of biological and non-biological origin present at such locations. In this study, we investigate the extent to which four contaminants, saliva, blood, dirt, and bleach can alter the accuracy of SERS-based detection and identification of both permanent and semi-permanent colorants present on hair. Our findings show that saliva and dirt reduce the intensity of the colorants' signals but do not obscure their detection and identification. At the same time, an exposure of the colored hair to bleach or the presence of blood eliminates SERS-based analysis of artificial dyes present on such samples. We identified the procedure that can be used to remove blood contamination, which, in turn, enables identification of the hair colorants on such pre-cleaned samples. However, bleach treatment irreversibly eliminates SERS-based detection of artificial colorants on hair. These findings expand our understandings about the potential of SERS in forensic investigation of colorants on trace hair evidence.

KEYWORDS
colorants, contaminants, hair, partial least-squares discriminant analysis, PLS-DA, SERS, surface-enhanced Raman spectroscopy

Highlights

- SERS identifies dyes on hair when contaminated with saliva or dirt but not with blood or bleach.
- PLS-DA can distinguish contaminated spectra from each other with over 95% accuracy.
- Rinsing blood-contaminated hair allows for signal identification.

1 | INTRODUCTION

Hair evidence is one of the most commonly studied types of trace evidence in forensic crime scene analysis. Once the hair has been collected from a crime scene, it is analyzed by a forensic expert to (i) determine the biological origin of hair and to (ii) identify the area of the body from which the hair originates [1]. Next, a pattern-recognition process is employed to establish a connection between the hair found at a crime scene and hair of a suspect. In the pattern-recognition process, hair geometry, size, and its natural coloration are considered to establish a connection between the suspect and a crime scene or to demonstrate the absence of such connections. However, due to the subjective nature of the pattern-recognition approach, this comparison method is largely unreliable [2]. This conclusion was

made by the National Academy of Sciences based on a review of over 500 FBI cases in which a pattern-recognition approach was used. It was found that at least 90% of the reviewed cases contained errors in testimony. These errors resulted in 33 cases that wrongly received the death penalty [3]. Polymerase chain reaction (PCR) offers a good alternative to the subjective visual inspection of hair. PCR allows for a direct analysis of DNA material present in hair if tissue attached to the root end contains nuclear DNA. Unique palindromic sequences present in human and animal DNA make PCR-based phenotypical analysis of hair highly robust and reliable [4]. However, PCR is costly and laborious. These limitations often make suspects wait for months in prison before the results of the PCR analyses may become available. High-performance liquid chromatography (HPLC) coupled to mass spectrometry is also often used for forensic analysis of hair analysis. It allows for the detection of steroids, illicit drugs, and explosives on hair [5, 6]. However, similar to PCR, HPLC-MS is destructive and laborious, which limits its applicability in the forensic analysis of hair [7]. Absorption spectroscopy can be used to identify whether hair possesses artificial colorants. However, this approach is heavily affected by the underlying natural hair pigments, which drastically lowers its reliability [8]. These limitations of currently available forensic methods catalyzed a search for a fast, minimally invasive, and reliable technique that can be used to enhance the value of the forensic analysis of hair.

Several years ago, Kurouski and Van Duyne, demonstrated that surface-enhanced Raman spectroscopy (SERS) could be used to detect and identify artificial colorants present on hair. Using this technique, the researchers were able to identify whether the hair had been dyed or not, and if so, whether permanent or semi-permanent colorant was used [9]. SERS is based on localized surface plasmon resonances (LSPRs), coherent oscillations of conductive electrons, that are generated on the surface of noble metal nanostructures by light [10, 11]. LSPRs enable 10^6–10^8 amplification of Raman scattering from molecules present on the surfaces of noble metal nanostructures [12, 13]. Thus, if noble metal nanostructures are placed on colored hair, they will enhance Raman scattering from the molecules of a dye present on the hair surface [9]. Kurouski and Van Duyne also showed that noble metal nanostructures quench fluorescence, which further facilitates forensic analysis of fluorescent dyes that are used to color hair [14]. Building upon this in 2019, the Kurouski group found that SERS could be used to detect and identify the original colorant if the hair was re-colored afterward [15]. Based on these pieces of experimental evidence, SERS can be considered as a robust and reliable tool that can be used for a forensic examination of the artificially colored hair.

In addition to SERS, Infrared spectroscopy could be used to differentiate between colored and un-colored hair and differentiate between permanent and semi-permanent colorants present on hair. Furthermore, Contreras and co-workers showed that IR spectroscopy could be used to determine whether hair was exposed to bleach prior to coloration [16, 17].

One can expect that a broad spectrum of contaminants can be present at a crime scene, including substances of biological and non-biological origin. The most common biological contaminants are body fluids such as blood, semen, saliva, and vaginal secretions [18].

The Lednev group previously demonstrated that Raman and Infrared spectroscopies could be used to identify different body fluids, as well as determine species origin of such samples [19–21]. Furthermore, it was demonstrated that Raman spectroscopy could be used to determine the race, age, and sex of the donor based on the spectroscopic signature of blood [22–24].

In addition to biological contaminants, there are substances of non-biological origin, such as dirt or cleaning solutions that can contaminate hair. Consequently, it is important to determine the extent to which presence of such biological and non-biological contaminants can alter the efficiency of SERS-based analysis of hair colorants. Therefore, in this study, we investigate the effect of two of the most common biological contaminants, blood, and saliva, along with dirt and bleach, the non-biological contaminants, on SERS-based analysis of hair colored with both permanent and semi-permanent dyes.

2 | MATERIALS AND METHODS

2.1 | Materials

The two hair dyes, ion Color Brilliance™ Semi-Permanent Sky Blue (BLU-SP) and ion Color Brilliance™ Liquid Permanent Jet Black (BLK-P) were purchased from Sally Beauty Supply. The hair was obtained from anonymous donors in a local barbershop.

2.2 | Coloring procedure

All hair was first rinsed with Millipore DI water and dried under ambient conditions. Next, the hair was colored using both BLU-SP and BLK-P. For this, BLU-SP was first mixed with DI water. After that, hair was exposed to the dye solution for 20 min. The BLK-P was first mixed with ion Color Brilliance™ 20 vol. Crème Developer in a 1:1 ratio. After that, hair was exposed to this solution for 35 min. The dyed hair was then rinsed with Millipore DI water until no suds remained.

2.3 | Contamination

Four contaminants were used: saliva, blood, dirt, and bleach. Saliva and blood were acquired from anonymous donors and used as received. The dirt used was autoclaved before applied on hair to exclude the presence of bacteria, fungi, and other species of biological origin. The bleach was Clorox diluted with DI water at 1:10. These contaminants were applied on the colored hair to resemble the appearance of these substances on hair found at a crime scene. Specifically, 80 µl of saliva and 80 µl of blood were deposited onto previously colored hair samples and dried under ambient conditions.

For dirt, the hair was shaken with the autoclaved dirt in a closed Petri dish. Diluted bleach (100 µl) was dropped on colored hair and left on the sample for 5 min. After that, residual bleach was rinsed with DI water. All analyzed hair samples were transferred to clean microscope slides. Both contaminated and uncontaminated dyed hair strands then had 80 µl of gold nanorods (AuNRs) added to them directly before the acquisition of SERS spectra.

2.4 | Plasmonic materials

AuNRs were synthesized in the laboratory according to a procedure reported by Esparza and co-workers [15]. These nanoparticles do not oxidize on air preserving their high enhancement factor over long period of time. AuNRs have dimensions of 5×30 nm. Prior to their application on hair, AuNRs were purified from the detergent that was used upon their synthesis. For this, a solution of AuNRs was centrifuged at 12,000*g* for 10 min. After the supernatant was removed, the pellet, which was composed of AuNRs, was resuspended in DI water.

2.5 | Spectroscopy

SERS spectra were acquired for each hair sample using a confocal inverted microscope (Nikon, Model TE-300) with 20× dry Nikon objective (NA = 0.45). A 785 nm solid-state laser was used for excitation. The signal was collected in a backscattering geometry and sent to a spectrometer (IsoPlane-320, Princeton Instruments) equipped with a 600 groove/mm grating. Prior to entering the spectrograph, the Rayleigh scattering was filtered with a long-pass filter (Semrock, LP03-785RS-25). The dispersed light was then sent to the CCD (PIX-400BR, Princeton Instruments). At least 50 spectra were collected for each sample. The collected spectra were then processed first using PLS_toolbox by Eigenvector and MATLAB®. The averaged spectra then had the background subtracted using GRAMS/AI 7.0 (Thermo Galactic, Salem, NH). We built two PLS-DA models: one for BLU-SP-colored and one for BLK-P-colored hair, Figures S1 and S2. These models revealed the accuracy with which spectra from 5 different classes (uncontaminated, saliva, dirt, blood, and bleach) can be identified correctly. If the accuracy was lower than 100%, the models also showed classes to which spectra were misassigned to as a result of PLD-DA.

3 | RESULTS AND DISCUSSION

SERS spectra collected from uncontaminated hair colored with BLU-SP exhibited peaks at 463, 576, 703, 925, 974, 1047, 1158, 1234, 1320, 1348, 1396, 1510, and 1621 cm^{-1}, Figure 1. The same peaks were observed in SERS spectra collected from BLU-SP-colored hair exposed to both saliva and dirt. Based on this evidence, we can conclude that saliva and dirt do not affect SERS-based analysis of hair.

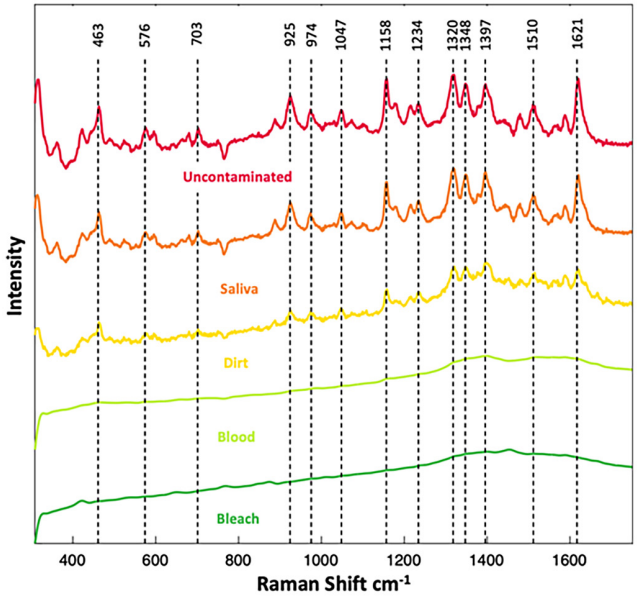

FIGURE 1 Average SERS spectra collected from hair colored by Sky Blue Semi-Permanent (BLU-SP) (uncontaminated), as well as colored hair exposed to saliva, dirt, blood, and bleach

The most prominent peaks in the BLU-SP uncontaminated, saliva, and dirt spectra (Figure 1) were at 463, 1158, 1396, and 1621 cm^{-1}.

At the same time, we found that exposition of BLU-SP colored hair to blood eliminates any initial analysis and exposition to bleach destroys all potential analysis, Figure 1. In the SERS spectra collected from blood- and bleach-treated hair, we observed only broad background that likely originated from the glass coverslip that was used to hold the hair sample during spectroscopic analysis.

Next, we utilized PLS-DA to investigate whether SERS could be used to identify the presence of both biological and non-biological contaminants on BLU-SP-colored hair. PLS-DA is a broadly used supervised chemometric method that allows for the identification of the accuracy of prediction of spectra to a certain class of spectra [25]. The PLS-DA algorithm was provided with 50 spectra for each contaminant group and then used pattern recognition to learn each group of spectra. Next, it "tested" itself by looking at all spectra combined and sorting them into contaminant groups. Our results show that SERS coupled to PLS-DA enables 100% and 98% accurate identification of the presence of saliva and dirt on hair, Table 1. Specifically, all 50 spectra collected from BLU-SP-colored hair contaminated with saliva were predicted correctly. At the same time, 49 out of 50 spectra collected from BLU-SP-colored hair contaminated with dirt were correctly predicted by our model with only one spectrum misclassified as "blood contamination." Furthermore, presence of blood on hair can be identified with 96% accuracy, whereas exposition of hair to bleach can be revealed with 100% accuracy. Specifically, 48 out of 50 spectra collected from BLU-SP-colored hair contaminated with blood were identified correctly, whereas only 2 spectra were misclassified as "dirt." It should be noted that acquired SERS spectra had no signatures of hemoglobin and other

TABLE 1 PLS-DA Confusion matrix of SERS spectra collected from BLU-SP-colored uncontaminated and contaminated hair

	Actual Class >>	Accuracy	Uncontaminated	Saliva	Dirt	Blood	Bleach
Predicted Class	Uncontaminated	96%	48	0	0	0	0
	Saliva	100%	2	50	0	0	0
	Dirt	98%	0	0	49	2	0
	Blood	96%	0	0	1	48	0
	Bleach	100%	0	0	0	0	50

blood components. One can expect that this could be due to the low concentration of nanoparticles on hair. Alternatively, we dealt with a very thin layer of blood present on the hair was not enough to detect hemoglobin. Finally, all 50 spectra collected from BLU-SP-colored hair exposed to bleach were identified correctly. It should be noted that SERS coupled to PLS-DA enables 96% accurate identification of the absence of any of these contaminants on the hair. Specifically, 48 out of 50 spectra were assigned correctly by the model with only 2 misassigned as "saliva." These findings show that SERS can be used to determine the chemical nature of the contaminants present on hair. Consequently, one may expect that not only the colorants but also an exposition of hair to the substances of biological and non-biological origin can be revealed using SERS. Such PLS-DA-based prediction of contaminants is possible because presence of substances of biological and non-biological origin causes small changes to the spectra that are utilized by machine learning for their identification.

SERS spectra collected from hair colored by BLK-P exhibited vibrational bands at 448, 506, 582, 661, 733, 827, 948, 1082, 1162, 1318, 1430, and 1515 cm^{-1}, Figure 2. The same spectral pattern can be observed in the SERS spectra collected from hair colored by BLK-P that was exposed to both saliva and dirt. We also found that the information about BLK-P could not be revealed if the colored hair was exposed to either blood or bleach. Such SERS spectra exhibit only broad fluorescence background that likely originated from the underlying glass coverslip.

We also found that SERS spectra collected from bleach-treated hair previously exposed to both dyes exhibit two vibrational bands that were present neither in the spectra of intact colored hair nor in the spectra collected from the colored hair exposed to dirt, blood, and saliva. This finding suggest that bleach induces chemical changes in keratin molecules of hair. This observation is in a good agreement with the previously reported results by Contreras et al. [18]. Specifically, using Infrared spectroscopy Contreras and co-workers showed that bleaching induced irreversible oxidation of disulfides in keratin to sulfites. This chemical transformation of biological material results in appearance of novel vibrational bands in the corresponding Infrared spectra of hair [18].

We used PLS-DA to examine the accuracy of identification of contaminants present on hair, Table 2. Our results show that SERS coupled to PLS-DA enables 100% and 98% accurate identification of the presence of saliva and dirt on hair, Table 2. Specifically, all 50 spectra collected from BLK-P-colored hair contaminated with saliva were predicted correctly. At the same time, 49 out of 50 spectra

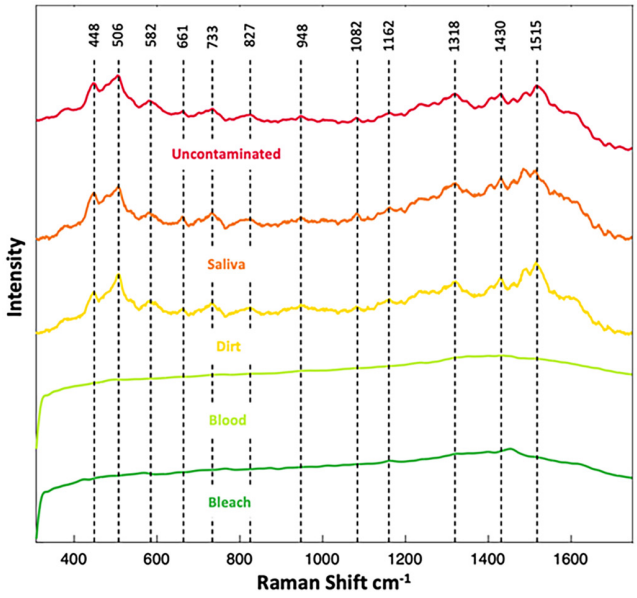

FIGURE 2 Average SERS spectra collected from hair colored by Jet Black Permanent (BLK-P) (uncontaminated), as well as colored hair exposed to saliva, dirt, blood, and bleach

collected from BLK-P-colored hair contaminated with dirt were correctly predicted by our model with only one spectrum misclassified as "blood contamination." Furthermore, presence of blood on hair can be identified with 98% accuracy, whereas exposition of BLK-P-colored hair to bleach can be revealed with 100% accuracy. Specifically, 49 out of 50 spectra collected from BLU-SP-colored hair contaminated with blood were identified correctly, whereas only 1 spectrum was misclassified as "bleach." We also found that all 50 spectra collected from BLK-P-colored hair exposed to bleach were identified correctly. Finally, SERS coupled to PLS-DA enables 100% accurate identification of the absence of any of these contaminants on the BLK-P-colored hair. Specifically, all 50 spectra collected from BLK-P-colored hair exposed to bleach were identified correctly.

One can expect that if a blood-contaminated sample can be washed from the contaminant, SERS can be used to detect and identify the colorant present on such hair [26]. To test this hypothesis, we washed BLU-SP- and BLK-P-colored blood-contaminated hair with multiple copies of DI water. Next, AuNRs were applied on the cleaned colored hair and SERS spectra were collected. We found that both BLU-SP and BLK-P can be detected and identified on the cleaned hair, Figure 3. These results show that blood can be washed

TABLE 2 PLS-DA confusion matrix of SERS spectra collected from BLK-P-colored uncontaminated and contaminated hair

	Actual Class >>	Accuracy	Uncontaminated	Saliva	Dirt	Blood	Bleach
Predicted Class	Uncontaminated	100%	50	0	0	0	0
	Saliva	100%	0	50	1	0	0
	Dirt	98%	0	0	49	0	0
	Blood	98%	0	0	0	49	0
	Bleach	100%	0	0	0	1	50

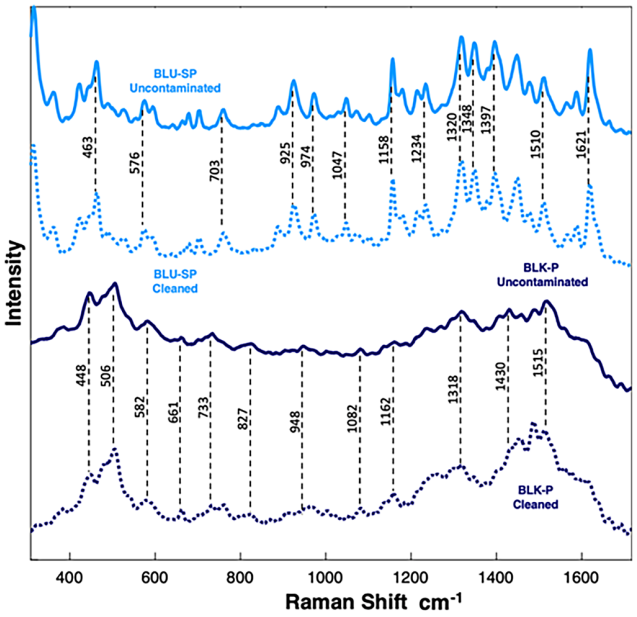

FIGURE 3 Average SERS spectra collected from BLU-SP-colored uncontaminated and cleaned from blood hair, as well as BLK-P-colored uncontaminated and cleaned from blood hair

from the colored hair with very little if any damage to the dye present on hair.

It is important to note that in this study we focused on the elucidation of the factors of pure biological and pure non-biological origin on SERS-based analysis of hair. One can expect that samples found at a crime scene may be exposed to both types of contaminants simultaneously. Therefore, it becomes important to test the effect of such mixed (biological and non-biological) contaminants on the SERS-based analysis of hair in future studies.

4 | CONCLUSION

Our findings revealed the extent to which biological and non-biological contaminants can alter SERS-based analysis of artificial colorants on hair. We found that saliva and dirt did not obscure SERS-based identification of both semi-permanent and permanent colorants on hair. If colored hair was exposed to blood, SERS-based analysis could not be directly performed on such hair samples. However, blood could be easily cleaned from the hair by DI water. Such cleaned hair could be used for the SERS-based identification of the colorants. Finally, our results showed that bleach irreversibly prevents SERS-based analysis of the artificial colorants on hair. Finally, we found that PLS-DA allows for the identification of contaminants on hair, as well as for identification of the absence of both biological and non-biological contaminants. These results demonstrate the strength of SERS in the forensic analysis of the hair colorants.

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CONFLICT OF INTEREST

The authors declare no competing financial interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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