Study of cocaine incorporation in hair damaged by cosmetic treatments

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Abstract

The present study investigated the alleged relationship occurring between possible hair damage resulting from repeated cosmetic treatments and the uptake of cocaine from a soaking solution into the hair matrix, simulating external contamination. Different types of drug-free hair were submitted to either bleaching, dyeing, or straightening. Untreated and treated hair were then soaked in a cocaine solution for 60 min. The analytical procedure included a common washing and decontamination step, followed by GC-MS detection. Morphological changes of hair submitted to cosmetic treatments were assessed by scanning electron microscopy (SEM). Minor damage was observed at the surface of thermally straightened hair, whereas substantial morphological changes of the hair structure was observed after bleaching and dyeing. Accordingly, untreated and straightened hair did not exhibit any significant uptake of cocaine upon 60 min soaking, whereas bleached and dyed hair exhibited considerable cocaine uptake, yielding final concentrations above the 0.5 ng/mg cut-off value.

Keywords	hair; cocaine; incorporation; SEM; cosmetic treatments TRL 2
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Corresponding Author	Enrico Gerace
Corresponding Author's Institution	Centro Regionale Antidoping "A. Bertinaria"
Order of Authors	Enrico Gerace, Agnese Veronesi, Gianmario Martra, Alberto Salomone, Marco Vincenti
Suggested reviewers	Luca Zamengo, donna cave, Elena Lendoiro

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AUTHOR DECLARATION

We wish to confirm that there are no known conflicts of interest associated with thispublication and there has been no significant financial support for this work that could haveinfluenced its outcome.

We confirm that the manuscript has been read and approved by all named authors and thatthere are no other persons who satisfied the criteria for authorship but are not listed. Wefurther confirm that the order of authors listed in the manuscript has been approved by all ofus.

We confirm that we have given due consideration to the protection of intellectual propertyassociated with this work and that there are no impediments to publication, including thetiming of publication, with respect to intellectual property. In so doing we confirm that wehave followed the regulations of our institutions concerning intellectual property.

We further confirm that any aspect of the work covered in this manuscript that has involvedeither experimental animals or human patients has been conducted with the ethical approvalof all relevant bodies and that such approvals are acknowledged within the manuscript.

We understand that the Corresponding Author is the sole contact for the Editorial process(including Editorial Manager and direct communications with the office). He/she isresponsible for communicating with the other authors about progress, submissions ofrevisions and final approval of proofs. We confirm that we have provided a current, correctemail address which is accessible by the Corresponding Author and which has beenconfigured to accept email from ForensicChemistry@elsevier.com.

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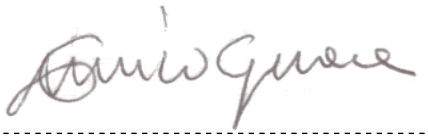
Enrico Gerace	14.11.2016	
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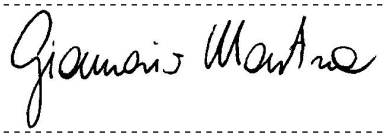
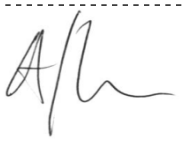
Gianmario Martra	14.11.2016	
Alberto Salomone	14.11.2016	
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Centro Regionale Antidoping
Alessandro Bertinaria
LABORATORIO REGIONALE DI TOSSICOLOGIA

Torino, January 2nd, 2017

Dear Editor,

I am pleased to submit the revised version of the manuscript **FORC_2016_95** entitled " Study of cocaine incorporation in hair damaged by cosmetic treatments" for publication on *Forensic Chemistry*.

The manuscript was reviewed following your recommendations and all the Referees' comments.

All revisions in the text were marked using a yellow highlighting. The answers to the Referees' comments are listed below:

Reviewer #1 comments:

In vitro cosmetic treatments

Page 5, Section 2.1.: In previous research articles bleaching has showed an increase in hair damage compared to dyeing. However, in the present work, hair samples were submitted to dye only once, while they were submitted to bleach 2 or 5 times. Why this protocol (dye once and bleach 2 or 5 times) was chosen? Is it realistic to compare hair damage after dye to hair damage after bleach in these conditions?

Authors' response: different cycles of bleaching were used in order to reproduce different grades of damage on the hair structure. In most real cases, bleaching is periodically used on regrown hair (quite often before dyeing), but part of the hair previously bleached is inevitably involved in the new treatment. This is the reason for testing double and also five-times bleaching. On the other hand, single dyeing was tested to simulate another condition typically found in reality, namely the use of an occasional treatment for the sake of experimenting a new hair color. In the range of treatments tested, the latter was selected to represent a mild condition.

In vitro hair contamination

Page 5, Section 2.2.: It is possible to find in the literature a range of aqueous cocaine solution between 0.05-10 µg/mL to simulate external contamination by soaking. Why a concentration of 1 µg/mL was selected in this paper? It is a representative amount of real external contamination?

Authors' response: In our work, hair strands (approximately 300 mg) were placed into 30 mL of a 1 µg/mL cocaine hydrochloride solution. This corresponds to 0.1 mg cocaine per gram of hair. Approximately the same conditions were tested in the paper entitled "*Removing and identifying drug contamination in the analysis of human hair*" (Cairns et al.; Forensic Science International 145 (2004) 97–108), where 12-15 mg of hair were contaminated by soaking for 60 min in 2 mL of a solution containing several drugs, including cocaine, at 1 µg/mL concentration each. In real cases, the amount of cocaine involved in contamination processes may vary considerably. In our study, an average condition was adopted because our primary goal was to study the possible correlation occurring between the hair damage observed after repeated in vitro cosmetic treatments and the exchange of cocaine between a soaking solution and the inner part of the hair structure. To this purpose, 0.1 mg cocaine per gram of hair simulated a realistic contamination.

Sample preparation

Page 5-6, Section 2.3.: It would be interested the analysis of the last wash solvent (DCM) to achieve if part of the drug incorporated into the hair strand after soaking could be eliminated with a normal wash procedure. These solvents were analysed?
Moreover, a ratio between concentrations found in the wash residue (W) and the levels detected in hair (H) is frequently used to distinguish between drug intake and external contamination. The inclusion of this ratio in the manuscript could be interested.

Authors' response: as reported in the "Results and discussion" section 2.2, all samples collected after contamination with cocaine were washed five times and the last washing solution was analyzed, turning out to be negative for any residual presence of cocaine and benzoylecgonine (in all cases). This confirms the complete removal of any residual presence of cocaine and benzoylecgonine from the hair surface before the procedure of extraction and analysis took place

(section 2.3). Consequently, the W/H ratio requested by the Reviewer was always equal to zero, taking into account the limit imposed by the LOD of the procedure

Discussion

The discussion of the results is adequate, but a comparison with other research articles should be performed.

Authors' response: direct comparison of the present manuscript with other published articles is not straightforward because the experimental conditions adopted were quite dissimilar. In particular, several papers illustrated the alleged correlation occurring between repeated cosmetic treatments, hair damage and drug loss (release) from the hair structure of drug-positive samples, but very few articles examined the reverse phenomenon, namely the incorporation of drugs into cosmetically treated hair arising from external contamination. The few of them that examined drug incorporation by external contamination did not use SEM images to investigate the correlation between different cosmetic treatments and the grade of hair damage.

Only recently, Kaliyadan and co-workers published SEM images of damaged hair after cosmetic treatments (Int J Trichology. 2016; 8: 94–98.). In that study, no correlation between different cosmetic treatments and grade of hair damage was found (see table 1). In our opinion, the effect of these treatments on the hair structure has to be verified for each case with electron microscopy.

In our study, SEM images were extensively used to evaluate the grade of damage of the hair structure after cosmetic treatments. This information was consistently used to discuss our experimental results, supporting a possible correlation between hair degradation and cocaine incorporation. In this sense, a direct comparison with other articles, not reporting the evaluation of the hair structure damage after cosmetic treatments appears improper.

Minor Comments

- *"Cocaine chloridrate" appears in the manuscript and Table 1, but in my opinion "cocaine hydrochloride" is more accurate.*
- *Page 8, Section 3, line 22: misspelling of "an overall".*

Authors' response: the text was modified as suggested by the Reviewer.

Reviewer #2 comments:

Highlights

The third point in the highlights: bleached and dyed hair retained more cocaine than untreated and straightened hair - this is not accurate as it is well known that chemically treated hair loses drugs and metabolites and thus does not retain more than untreated hair in real life situations. Your paper shows that chemically treated hair has a larger uptake then non treated hair when soaked in a cocaine solution.

Authors' response: we agree with the Reviewer. In fact, we reported in the introduction that "cosmetic treatments like bleaching or dyeing may damage the cuticle, change the molecular structure of hair melanin, or decompose the incorporated drugs, leading to a decrease of drug hair content". However, other studies demonstrated that the water content of perming and bleaching treatments may open the hair cuticles, allegedly facilitating the incorporation of drugs from sweat, sebum or external sources. Our results demonstrated that treated hair, when soaked in a cocaine solution, retain more drug than untreated hair. Following the referee's comment, the third point of the highlight was modified as follows: "Contaminated bleached and dyed hair exhibited a larger uptake of cocaine than untreated and straightened hair".

Conclusions

I would maybe add in the conclusion that no BZE was detected therefore a false interpretation of use would not be given when interpreting results.

Authors' response: the text was modified as suggested by the Reviewer.

I would also mention that although the study and its findings is useful that soaking hair in cocaine solution for an hour is not realistic in terms of live case samples and how hair may be exposed in real life.

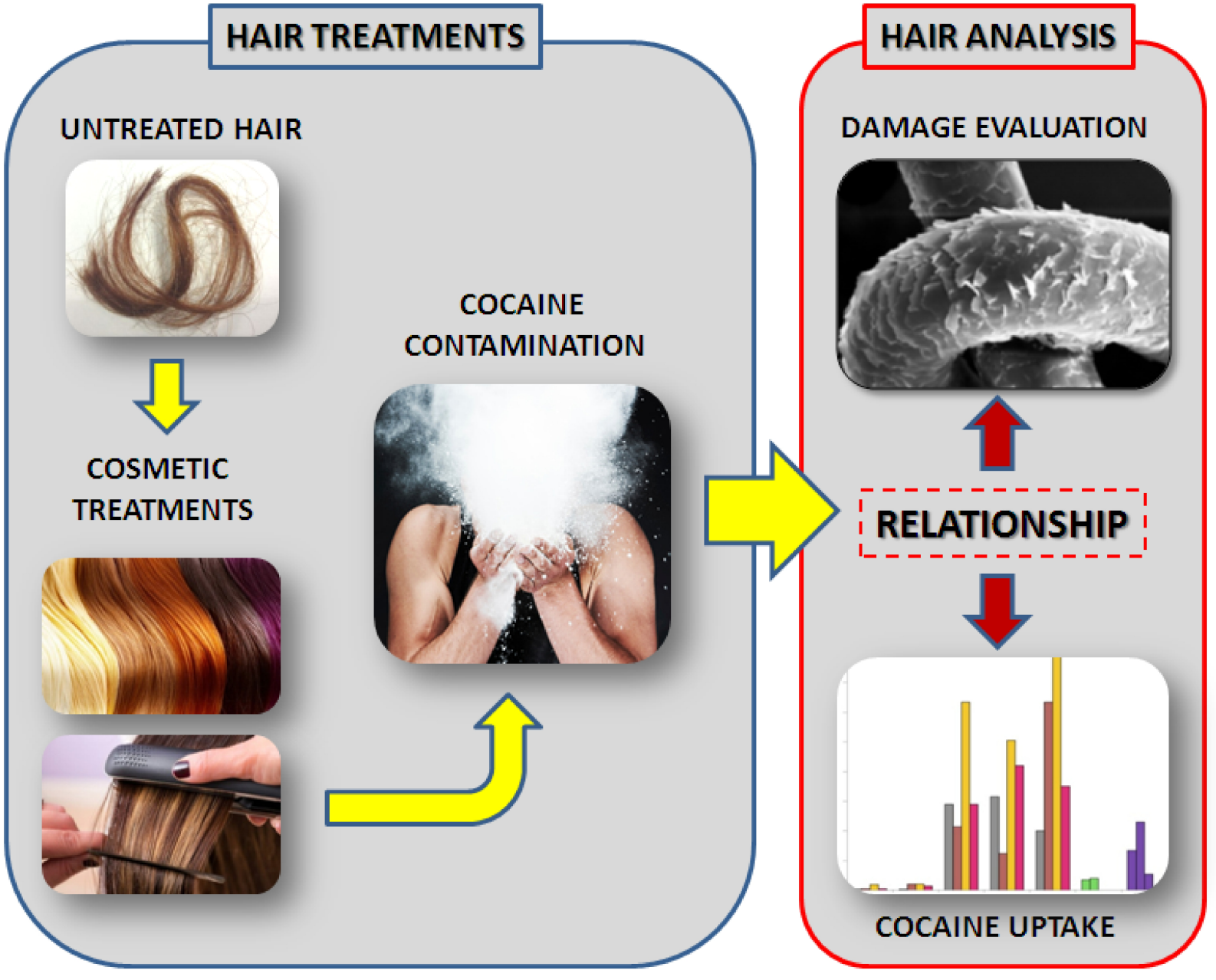
Authors' response: Our aim was to test extensive but still realistic contamination conditions, in order to evaluate up to what level the detection of drugs in hair samples can be influenced by

external factors (see previous comment). In real cases, the exposure and amount of cocaine involved in contamination may vary considerably. In order to simulate real contamination conditions, several Authors suggested different procedures. Among these, contamination by soaking is extensively used. In their study, Cuypers et al. produced contamination by soaking the hair in a cocaine solution for 5 min or 5 hours. In the paper entitled "*Removing and identifying drug contamination in the analysis of human hair*" (Cairns et al.; Forensic Science International 145 (2004) 97–108), hair samples were contaminated with several drug, including cocaine, by soaking them for 60 min. This condition (60 min soaking) was tested also in our study, taking into account that also sweat may persist on the scalp for quite a long time, especially during physical exercise. This consideration was added to the "Results and Discussion" section.

Minor Comments

When talking about the figures, the paragraph that starts figure 1 needs 'a' changing to 'an' in the second sentence. In particular an overall general slight cuticle break and scales is observed.....

Authors' response: the text was modified as suggested by the Reviewer.


HAIR TREATMENTS
UNTREATED HAIR
COSMETIC TREATMENTS
COCAINE CONTAMINATION
HAIR ANALYSIS
DAMAGE EVALUATION
RELATIONSHIP
COCAINE UPTAKE

Study of cocaine incorporation in hair damaged by cosmetic treatments

Enrico Gerace[1,*], Agnese Veronesi[2], Gianmario Martra[2], Alberto Salomone[1] and Marco Vincenti[1,2]

[1]*Centro Regionale Antidoping e di Tossicologia "A. Bertinaria", Orbassano, Torino, Italy*

[2]*Dipartimento di Chimica, Università di Torino, Torino, Italy*

[*]**Corresponding author**: Enrico Gerace

Centro Regionale Antidoping "A. Bertinaria", Regione Gonzole 10/1 - 10043 Orbassano, Torino, Italy

Tel.: +39.011 90224249; FAX.: +39.011 90224242;

E-mail: enrico.gerace@antidoping.piemonte.it

Abstract

The present study investigated the alleged relationship occurring between possible hair damage resulting from repeated cosmetic treatments and the uptake of cocaine from a soaking solution into the hair matrix, simulating external contamination. Different types of drug-free hair were submitted to either bleaching, dyeing, or straightening. Untreated and treated hair were then soaked in a cocaine solution for 60 min. The analytical procedure included a common washing and decontamination step, followed by GC-MS detection. Morphological changes of hair submitted to cosmetic treatments were assessed by scanning electron microscopy (SEM). Minor damage was observed at the surface of thermally straightened hair, whereas substantial morphological changes of the hair structure was observed after bleaching and dyeing. Accordingly, untreated and straightened hair did not exhibit any significant uptake of cocaine upon 60 min soaking, whereas bleached and dyed hair exhibited considerable cocaine uptake, yielding final concentrations above the 0.5 ng/mg cut-off value.

Keywords: hair, cocaine, incorporation, SEM, cosmetic treatments

1. Introduction

Hair analysis currently represents a reliable and well-established means of clinical and forensic investigation [1]. The determination of common psychotropic drugs at low concentration is increasingly requested in hair samples for the retrospective investigation of habitual drug abuse and dependence, as well as in other toxicological investigations [2–5]. One of the most critical issues in hair testing is the interpretation of the analytical results, that may be affected by several sources of bias. Individual metabolic variability, hair pigmentation and thickness, keratin permeability, environmental and self-contamination, longitudinal diffusion, effects of cosmetic treatments, the hair decontamination strategy and many other behavioral factors have to be taken into account in the interpretation of hair testing results [1,6–17].

Among these, chemical and physical cosmetic hair treatments, such as the use of oxidants, highly basic colouring and perming agents, and thermal straightening may lead to morphological changes of the hair structure ultimately influencing either the mechanism of drug incorporation into the hair or promoting the drug release from the keratin structure [18,19]. Indeed, cosmetic treatments like bleaching or dyeing may damage the cuticle, change the molecular structure of hair melanin, or decompose the incorporated drugs, leading to a decrease of drug hair content [20]. On the other hand, perming or bleaching are generally water-based treatments. As previously demonstrated, water can open the cuticles allegedly facilitating the incorporation of drugs from sweat, sebum or external sources [19,21,22]. In particular, both chemical reactions with various treatment agents and physical transport phenomena, induced by morphological changes of the hair structure, are expected to produce biased results. Therefore, it is commonly assumed that drug concentrations in hair may be significantly affected by cosmetic treatments [21].

Several authors reported a significant decrease of drugs concentration in cosmetically treated hair, possibly related to the degree of hair damage produced by the treatment [18,19,23–25].

In the present study, we evaluated the possible relationship occurring between the hair damage observed after *in vitro* cosmetic treatments and the uptake of cocaine from a soaking solution into the inner part of the hair structure. Scanning electron microscopy (SEM) was used in order to verify potential morphological changes occurring to the hair structure after various cosmetic treatments and make a morphological comparison between untreated and treated hair.

2. Materials and methods.

Hair samples were collected from the posterior vertex as close as possible to the skin. For the incorporation study, brown, blonde, red and grizzled head hair were collected from a total of 7 volunteers. Hair samples were untreated and free from cocaine. From each type of sample, the proximal 5 cm portion was selected, treated, and analyzed.

The possible occurrence of cocaine incorporation after a variety of cosmetic treatments and its extent was investigated by means of quantitative GC-MS analysis, in comparison with a corresponding negative hair sample used as a reference control. Studied cosmetic treatments included straightening, dyeing, and 2 or 5 cycles of bleaching.

The morphological changes of hair submitted to cosmetic treatments were evaluated by scanning electron microscopy (SEM) and compared with untreated hair strands.

2.1 In vitro cosmetic treatments

Bleaching of the hair strands was carried out by applying a commercially available bleaching product (Testanera Nordic-blonde, Henkel-Italia, Milan, Italy) consisting of an aqueous hydrogen peroxide/ammonium and sodium persulfate solution. Following the manufacturer's

instruction, the treatment was maintained for 30 min. Then, the hair strands were extensively rinsed with tap water and allowed to dry at room temperature. The whole procedure was then repeated 2 or 5 times on two different sets of samples.

Dyeing of the hair strands was achieved by applying a dyeing product (Garnier Color Intense, Garnier, Paris, France) consisting of a mixture of hydrogen peroxide, pigment precursors (diaminobenzenes and phenylenediamines), resorcinol and ammonia. Treatment time and subsequent washing and drying was as for bleaching.

Straightening of the hair strands was executed by applying a flat iron (IMETEC Bellissima B100, Tenacta Group, Azzano S. Paolo (BG), Italy) heated at 180°C for 5s. Then, the hair was allowed to cool down. The heating-cooling cycle was repeated for 50 times on each hair strand.

2.2 In vitro hair contamination

Before exposure to cocaine, the cut ends of all hair fibers were sealed with nail polish to avoid any drug uptake from their cross-sections [26]. Hair strands were exposed to an aqueous solution of cocaine hydrochloride at 1 µg/mL concentration (30 mL) within a test tube for 60 min. Then, the hair samples were allowed to dry at room temperature on absorbing paper overnight. The hair strands were subsequently washed with deionized water (25 mL each, vortex mixed for 1 min) and the washing was repeated five times. The last washing solution was collected and analyzed in order to ascertain the complete removal of any residual presence of cocaine and benzoylecgonine from the hair surface.

2.3 Sample preparation

Approximately 100 mg of hair was twice-washed with dichloromethane and methanol (3 mL each, vortex mixed for 3 min). After complete removal of solvent wash, the hair was dried at

room temperature by a gentle nitrogen flow and subsequently cut with scissors into 1-2 mm segments. For cocaine detection, hair samples were fortified with 5 µL of an internal standards mixture yielding a final concentration of 0.5 ng/mg in cocaine-D_3 and benzoylecgonine-D_3. After the addition of 2 mL of methanol, the samples were incubated at 55 °C for 15 h without stirring. Lastly, the organic phase was collected in a new test tube and dried at 70°C under a nitrogen stream. The dry residue was derivatized with 75 µL of a PFPA/PFPOH mixture (2:1 v/v) for 30 min at 70°C. The resulting residue was evaporated to dryness at room temperature under a stream of nitrogen and subsequently reconstituted with 50 µL of ethylacetate. Lastly, an aliquot of 1 µL was injected (split ratio of 5:1) into the GC/MS system operating in the SIM (selected ion monitoring) mode.

2.4 Apparatus and methods

Cocaine and benzoylecgonine were determined with a GC/MS system consisting of a 6890N gas chromatograph interfaced with a 5975 mass spectrometer both from Agilent Technologies (Milan, Italy). The separation was carried out with a J&W HP-5 capillary column, 17m × 0.200 mm × 0.33 µm. Helium was employed as the carrier gas at a constant pressure of 20.16 psi. The GC oven temperature was set at 150°C for 1 min and then raised to 200°C with a 30°C/min heating rate. The oven temperature was then raised to 270°C with a 10°C/min heating rate and lastly to 310°C with a 50°C/min heating rate. The total run time was 11.47 min. The GC injector and transfer line were maintained at 270°C and 280°C respectively. The mass spectrometer was operated in EI at 70 eV and SIM acquisition mode at dwell times of 30 ms. The fragment ions monitored for cocaine were m/z 198 (target ion), 303 and 182 (qualifiers), while for benzoylecgonine (as PFPA-derivative) the diagnostic ions were at m/z 421 (target ion), 316 and 300 (qualifiers).

The method was fully validated according to national and international guidelines [27,28]. Linearity was verified in the interval 0.05–5.0 ng/mg. Whenever the real samples concentrations were found to exceed the highest calibration point, the final extracts were diluted with methanol and re-injected into the system. Limit of Detection (LOD) and Limit of Quantitation (LOQ) for cocaine and benzoylecgonine were, respectively, 0.02 and 0.01 ng/mg (LODs) and 0.05 and 0.03 ng/mg (LOQs). Interday precision and accuracy were tested at 0.1 ng/mg, showing that all experimental values were below the acceptable CV and bias limits of 10%. Laboratory performances are constantly monitored through regular participation to inter-laboratory proficiency tests.

For scanning electron microscopy (SEM) observations, the intermediate portion (segment from 2 to 3 cm) from a single 5 cm hair was deposited on an aluminium stub covered with a bi-adhesive conductive carbon tape, and sputtered with Au, to form a surface-conductive layer ca. 30 nm thick (Balzers BAL-TEC SCD-050 Sputter-Coater, Balzers Union, Liechtenstein). Images were obtained with a Leica Stereoscan 420 microscope 20 kV (Leica Microsystems, Wetzlar, Germany) by collecting secondary electrons (E-T detector) emerging from the samples under the following operational conditions: acceleration potential, 20 kV; beam current, 60 μA; I probe, 80 pA.

3. Results and discussion

Table 1 reports the quantitative results for cocaine obtained from seven hair samples after they had been soaked for one hour into an aqueous cocaine hydrochloride solution. In real cases, the exposure and amount of cocaine involved in contamination may vary considerably, but the conditions adopted in the present study [29] were intended to simulate abundant sweating, as it may occur during physical exercise.

For all samples tested, the analysis of the last washing solution collected after the contamination procedure was negative for the presence of cocaine and benzoylecgonine. This result confirms the complete removal of any residual presence of cocaine and benzoylecgonine from the hair surface. The presence of cocaine was detected in all samples, whereas its major metabolite, benzoylecgonine, was never detected. Thus, incorporation of cocaine inside the keratin structure was observed to some extent from both treated and untreated hair. In the cases of untreated and straightened hair, the concentration of cocaine detected was below the commonly used cut-off of 0.5 ng/mg [30,31], with the exception of sample 3 that showed cocaine concentrations slightly above 0.5 ng/mg. On the other hand, in all hair samples contaminated after dyeing and bleaching treatments a much higher amount of cocaine was retained. These treatments are generally water-based so it is likely that water can open the cuticles making hair more sensitive to incorporation. For example, in dyed hair samples the cocaine concentrations detected after the washing procedure ranged between 2.53 and 14.1 ng/mg. Even more extensive cocaine incorporation was observed in the bleached hair, with concentrations ranging from 3.21 to 12.7 ng/mg (2 bleaching treatments). By increasing the number of bleaching treatments up to 5, the hair contamination from cocaine appears to increase further, even considerably (32.2 ng/mg in blonde hair), with the notable exception of grizzled hair.

The amount of cocaine incorporated in the hair after the soaking does not appear to depend on their melanin content. In fact, blonde and brown untreated and straightened hair exhibit similar results, whereas cocaine incorporation appears to be even higher in blonde hair than in brown hair, despite its reduced melanin content.

Figure 1 compares the SEM images of brown hair specimens before and after thermal treatment as for hair straightening conditions: minimal damage was observed at the hair surface of the treated hair, in agreement with the irrelevant change of cocaine incorporation

observed between untreated and straightened hair. In particular, an overall general slight cuticle break and scales raise is observed, on account of the thermal treatment (compare panels A1/2 and B1/2), and only few localized and punctual hole-shaped damages occurred (compare panels A3 and B3).

Figure 2 depicts the SEM images from four hair samples of different color after 5 cycles of bleaching treatment. These images reveal that the same chemical treatment produce significantly dissimilar damages on the cuticles of different hair, possibly depending on their structure, density and cross-section. In particular, the blond hair used in the SEM analysis reported in Figure 2c was more deeply damaged than the brown, grizzled, and red hair considered in the SEM comparison. The comparison between Figure 1 and 2 stresses the various modifications that the bleaching treatment had produced. Not only most of the surface cuticles appear to be broken and scarcely overlying to one another, but also holes of considerable size were produced in the cortex of blond hair depicted in Figure 2c. Indeed, the blonde hair considered in the present study incorporated more cocaine than brown, grizzled and red hair both before and after any chemical treatment (see Table 1). This effect can allegedly be attributed to the more fragile hair structure and more extensive damages induced by the treatments, in turn promoting an extensive cocaine incorporation inside the keratin matrix.

The latter conclusions cannot be generalized, since several other intrinsic factors, such as hair thickness and porosity, should be taken into account. Hairs that apparently exhibit similar damage (Figure 2a, 2b, 2d) yield significantly different cocaine incorporation (last column of Table 1). Moreover, the intensification of cosmetic treatments, such as five bleaching instead of two, definitely results in more extensive damage of the keratin matrix, but the enlarged morphological alteration of the keratin structure does not necessarily correspond to an increase of the cocaine uptake, as is evident for the grizzled hair considered in this study.

Dyed brown and blonde hairs absorb less cocaine than bleached hair, while, on the opposite, grizzled and red hairs submitted to a dyeing treatment apparently incorporate more cocaine than when they are bleached. These opposite observations may be explained with the coexistence of several competing effects, including hair damage, density, and thickness. Moreover, a more extensive cuticle damage is likely to increase the hair porosity and, consequently, to promote both a large drug intake and also its plentiful release, during the washing and decontamination procedures. Lastly, highly porous hair samples definitely give exhaustive extraction of the incorporated drugs, whereas the extraction yields from untreated hair are not necessarily complete.

4. Conclusions

In hair testing, the occurrence of external contamination has been demonstrated for several drugs of abuse, particularly cocaine, making the interpretation of analytical results susceptible of criticism. Within the consideration of potential biasing circumstances, the results of this study demonstrated that the hair samples collected from several individuals bind cocaine inside their inner keratin structure, when they are subjected to various cosmetic treatments and then soaked into a cocaine solution. In contrast, the contamination from cocaine proved to be limited, whenever the same hair samples were not previously treated with either strong oxidants or bases. The effect of cosmetic treatments on cocaine uptake are so pronounced to cover any other possible influencing factors (for example, hair color).

Scanning electron microscopy provided high resolution images of both treated and untreated hair, that allowed us to distinguish specific treatment-related damages, that appear to be localized (punctual) for thermal treatments, but extended to the entire hair surface when chemical treatments were applied. The latter created damages of variable scale, but a substantial increase of hair porosity can be recognized.

The quantitative determinations demonstrated a widely increased uptake and incorporation of cocaine in damaged keratin matrices with respect to untreated and straightened hair. However, the progressive increase of cocaine uptake seems not to be directly reflected into the morphological alteration of the keratin structure, possibly because the augmented hair porosity facilitates any drug exchange, i.e. both incorporation during soaking and release during washing procedures. In all cases, the major metabolite of cocaine, benzoylecgonine, was never detected, excluding a false interpretation of cocaine use. Nevertheless, further studies are needed with different substances, metabolites or testing different contamination conditions, in order to simulate as much as possible real cases and verify if similar phenomena may occur with other drugs of abuse or their metabolites. In general, it is confirmed that cosmetic treatments such as bleaching and dyeing can strongly enhance the cocaine uptake in the treated hair. Therefore, the effect of these treatments should be taken into account when hair analysis results for drug abuse have to be interpreted for forensic purposes.

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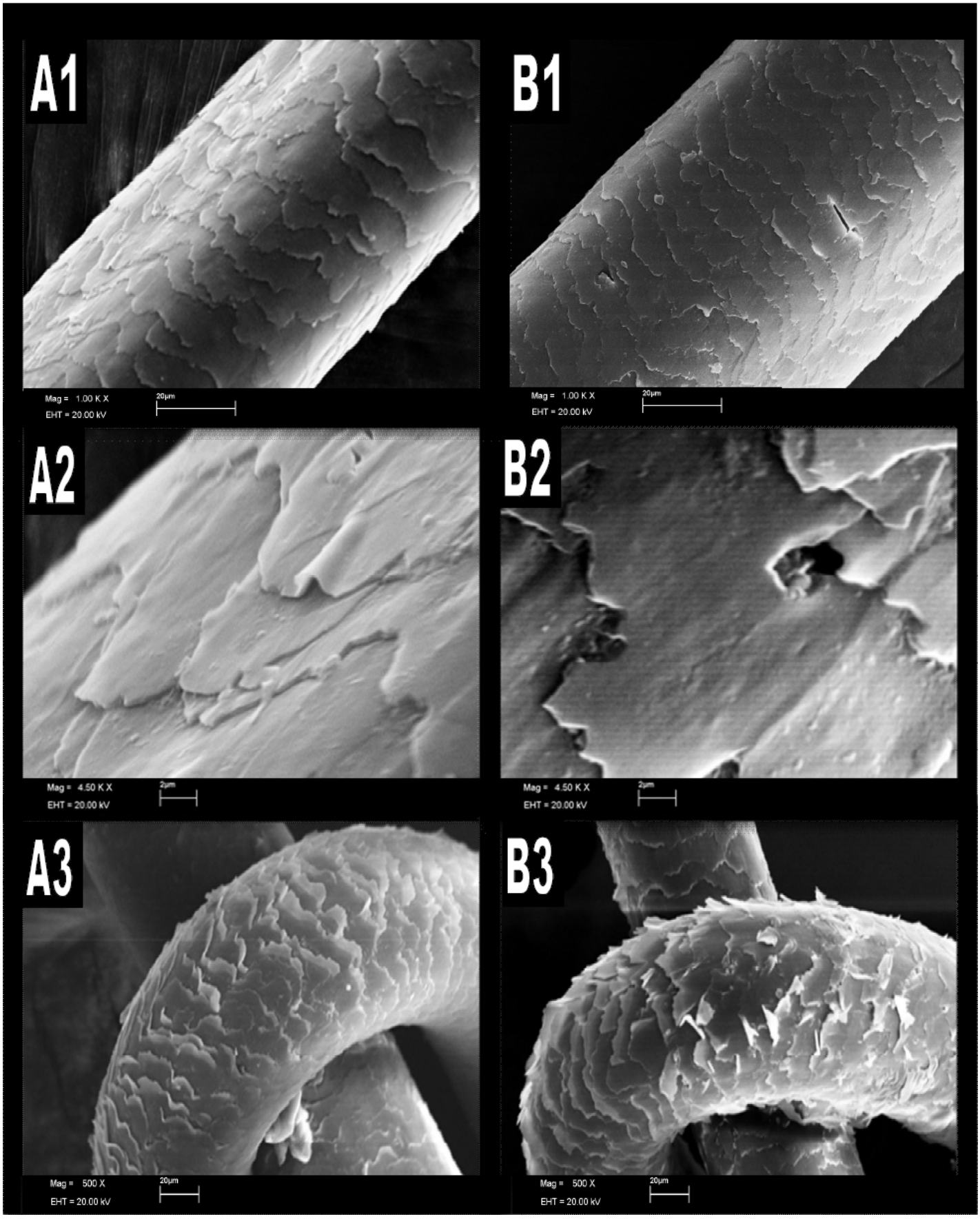

Figure 1. SEM images of a brown hair sample before (panels A) and after (panels B) straightening recorded at 1000× (A1-B1), 4500× (A2-B2) and 500× (stressed, A3-B3).

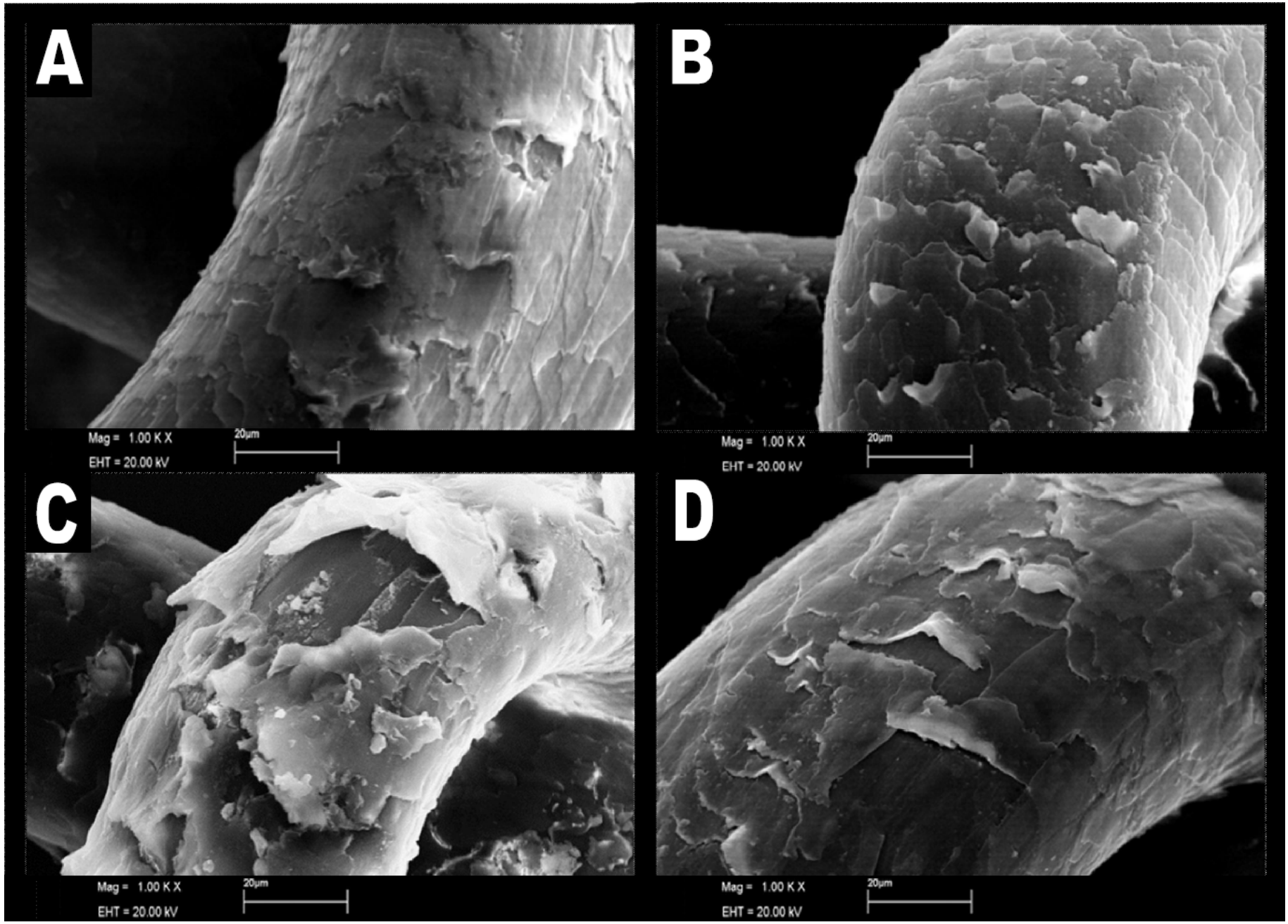

Figure 2. SEM images (1000×) of brown (panel A), grizzled (panel B), blonde (panel C) and red (panel D) hair after 5 cycles of bleaching treatment.

Table 1. Cocaine concentration (ng/mg) in untreated and cosmetically treated hair samples (n=5) after *in vitro* contamination with an aqueous solution of cocaine hydrochloride at a 1 µg/mL concentration

		Cosmetic treatment									
		Untreated		**Straightening**		**Dyeing**		**2×Bleaching**		**5×Bleaching**	
Sample	**Hair type**	**mean**	**σ**	**mean**	**σ**	**mean**	**σ**	**mean**	**σ**	**mean**	**σ**
1	*Brown*	0.13	*0.05*	0.43	*0.10*	2.53	*0.73*	4.28	*1.46*	12.6	*4.6*
2	*Brown*	0.13	*0.07*	0.26	*0.05*	3.31	*0.10*	5.55	*0.19*	7.98	*0.47*
3	*Brown*	0.71	*0.09*	0.69	*0.17*	2.55	*0.14*	3.21	*0.20*	6.03	*0.04*
4	*Grizzled*	0.09	*0.02*	0.09	*0.02*	6.28	*0.26*	5.81	*0.12*	4.03	*0.22*
5	*Blonde*	0.40	*0.03*	0.42	*0.01*	10.1	*0.10*	12.7	*0.41*	32.2	*0.47*
6	*Blonde*	0.44	*0.02*	0.48	*0.10*	14.1	*0.20*	11.7	*0.49*	12.0	*0.29*
7	*Red*	0.13	*0.02*	0.28	*0.02*	8.36	*0.46*	5.81	*0.44*	7.05	*0.04*

Highlights

- Cosmetic treatments can influence drug incorporation/release to/from the hair.
- Morphological changes of cosmetically treated hair were assessed by SEM images.
- Contaminated bleached and dyed hair exhibited a larger uptake of cocaine than untreated and straightened hair.
- Cosmetic treatments should be considered for hair results interpretation.