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Study of cocaine incorporation in hair damaged by cosmetic treatments

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(Article begins on next page)

Manuscript Details

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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
Manuscript category	Technology Readiness Level 2
Corresponding Author	Enrico Gerace
Corresponding Author's Institution	Centro Regionale Antidoping "A. Bertinaria"
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Suggested reviewers	Luca Zamengo, donna cave, Elena Lendoiro

Submission Files Included in this PDF

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Figure 1.doc [Figure]

Figure 2.doc [Figure]

Table 1.doc [Table]

Highlights.doc [Highlights]

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AUTHOR DECLARATION

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


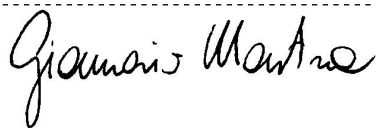
We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

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

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

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Signed by all authors as follows:

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Alberto Salomone	14.11.2016	
Marco Vincenti	14.11.2016	

In case more space is needed, please attach a second page.

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 than 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.....

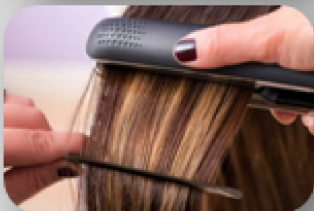
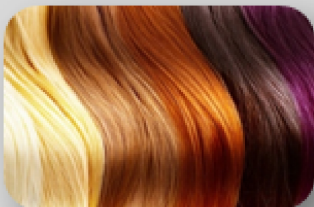
Authors’ response: the text was modified as suggested by the Reviewer.

HAIR TREATMENTS

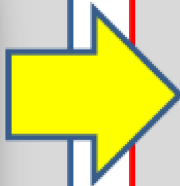
UNTREATED HAIR



COSMETIC TREATMENTS

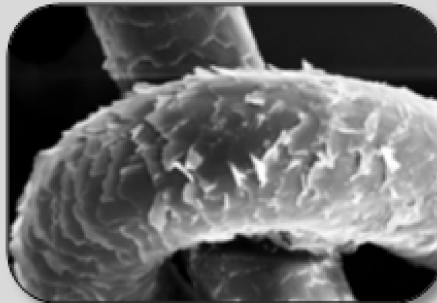


COCAINE CONTAMINATION

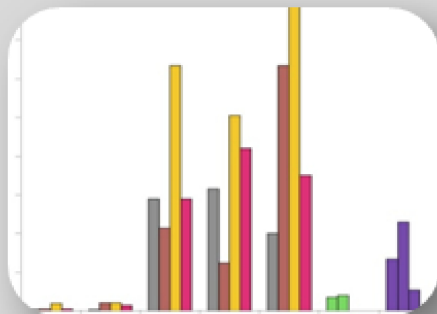


HAIR ANALYSIS

DAMAGE EVALUATION



RELATIONSHIP



COCAINE UPTAKE

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4 **Study of cocaine incorporation in hair damaged by cosmetic**
5 **treatments**
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9
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11 Vincenti^{1,2}
12

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62 **Abstract**
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64 The present study investigated the alleged relationship occurring between possible hair
65 damage resulting from repeated cosmetic treatments and the uptake of cocaine from a soaking
66 solution into the hair matrix, simulating external contamination. Different types of drug-free
67 hair were submitted to either bleaching, dyeing, or straightening. Untreated and treated hair
68 were then soaked in a cocaine solution for 60 min. The analytical procedure included a
69 common washing and decontamination step, followed by GC-MS detection. Morphological
70 changes of hair submitted to cosmetic treatments were assessed by scanning electron
71 microscopy (SEM). Minor damage was observed at the surface of thermally straightened hair,
72 whereas substantial morphological changes of the hair structure was observed after bleaching
73 and dyeing. Accordingly, untreated and straightened hair did not exhibit any significant
74 uptake of cocaine upon 60 min soaking, whereas bleached and dyed hair exhibited
75 considerable cocaine uptake, yielding final concentrations above the 0.5 ng/mg cut-off value.
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91 **Keywords:** hair, cocaine, incorporation, SEM, cosmetic treatments
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121 **1. Introduction**
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123 Hair analysis currently represents a reliable and well-established means of clinical and
124 forensic investigation [1]. The determination of common psychotropic drugs at low
125 concentration is increasingly requested in hair samples for the retrospective investigation of
126 habitual drug abuse and dependence, as well as in other toxicological investigations [2–5].
127
128 One of the most critical issues in hair testing is the interpretation of the analytical results, that
129 may be affected by several sources of bias. Individual metabolic variability, hair pigmentation
130 and thickness, keratin permeability, environmental and self-contamination, longitudinal
131 diffusion, effects of cosmetic treatments, the hair decontamination strategy and many other
132 behavioral factors have to be taken into account in the interpretation of hair testing results
133 [1,6–17].
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135 Among these, chemical and physical cosmetic hair treatments, such as the use of oxidants,
136 highly basic colouring and perming agents, and thermal straightening may lead to
137 morphological changes of the hair structure ultimately influencing either the mechanism of
138 drug incorporation into the hair or promoting the drug release from the keratin structure
139 [18,19]. Indeed, cosmetic treatments like bleaching or dyeing may damage the cuticle, change
140 the molecular structure of hair melanin, or decompose the incorporated drugs, leading to a
141 decrease of drug hair content [20]. On the other hand, perming or bleaching are generally
142 water-based treatments. As previously demonstrated, water can open the cuticles allegedly
143 facilitating the incorporation of drugs from sweat, sebum or external sources [19,21,22]. In
144 particular, both chemical reactions with various treatment agents and physical transport
145 phenomena, induced by morphological changes of the hair structure, are expected to produce
146 biased results. Therefore, it is commonly assumed that drug concentrations in hair may be
147 significantly affected by cosmetic treatments [21].
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180 Several authors reported a significant decrease of drugs concentration in cosmetically treated
181 hair, possibly related to the degree of hair damage produced by the treatment [18,19,23–25].
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184 In the present study, we evaluated the possible relationship occurring between the hair
185 damage observed after *in vitro* cosmetic treatments and the uptake of cocaine from a soaking
186 solution into the inner part of the hair structure. Scanning electron microscopy (SEM) was
187 used in order to verify potential morphological changes occurring to the hair structure after
188 various cosmetic treatments and make a morphological comparison between untreated and
189 treated hair.
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199 **2. Materials and methods.**

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201 Hair samples were collected from the posterior vertex as close as possible to the skin. For the
202 incorporation study, brown, blonde, red and grizzled head hair were collected from a total of 7
203 volunteers. Hair samples were untreated and free from cocaine. From each type of sample, the
204 proximal 5 cm portion was selected, treated, and analyzed.
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210 The possible occurrence of cocaine incorporation after a variety of cosmetic treatments and its
211 extent was investigated by means of quantitative GC-MS analysis, in comparison with a
212 corresponding negative hair sample used as a reference control. Studied cosmetic treatments
213 included straightening, dyeing, and 2 or 5 cycles of bleaching.
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218 The morphological changes of hair submitted to cosmetic treatments were evaluated by
219 scanning electron microscopy (SEM) and compared with untreated hair strands.
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225 2.1 *In vitro* cosmetic treatments

226
227 Bleaching of the hair strands was carried out by applying a commercially available bleaching
228 product (Testanera Nordic-blonde, Henkel-Italia, Milan, Italy) consisting of an aqueous
229 hydrogen peroxide/ammonium and sodium persulfate solution. Following the manufacturer's
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239 instruction, the treatment was maintained for 30 min. Then, the hair strands were extensively
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241 rinsed with tap water and allowed to dry at room temperature. The whole procedure was then
242
243 repeated 2 or 5 times on two different sets of samples.
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245 Dyeing of the hair strands was achieved by applying a dyeing product (Garnier Color Intense,
246
247 Garnier, Paris, France) consisting of a mixture of hydrogen peroxide, pigment precursors
248
249 (diaminobenzenes and phenylenediamines), resorcinol and ammonia. Treatment time and
250
251 subsequent washing and drying was as for bleaching.
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254 Straightening of the hair strands was executed by applying a flat iron (IMETEC Bellissima
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256 B100, Tenacta Group, Azzano S. Paolo (BG), Italy) heated at 180°C for 5s. Then, the hair
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258 was allowed to cool down. The heating-cooling cycle was repeated for 50 times on each hair
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260 strand.
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262 263 264 2.2 *In vitro* hair contamination

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266 Before exposure to cocaine, the cut ends of all hair fibers were sealed with nail polish to avoid
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268 any drug uptake from their cross-sections [26]. Hair strands were exposed to an aqueous
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270 solution of cocaine **hydrochloride** at 1 µg/mL concentration (30 mL) within a test tube for 60
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272 min. Then, the hair samples were allowed to dry at room temperature on absorbing paper
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274 overnight. The hair strands were subsequently washed with deionized water (25 mL each,
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276 vortex mixed for 1 min) and the washing was repeated five times. The last washing solution
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278 was collected and analyzed in order to ascertain the complete removal of any residual
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280 presence of cocaine and benzoylecgonine from the hair surface.
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285 286 2.3 Sample preparation

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288 Approximately 100 mg of hair was twice-washed with dichloromethane and methanol (3 mL
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290 each, vortex mixed for 3 min). After complete removal of solvent wash, the hair was dried at
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298 room temperature by a gentle nitrogen flow and subsequently cut with scissors into 1-2 mm
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300 segments. For cocaine detection, hair samples were fortified with 5 μL of an internal
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302 standards mixture yielding a final concentration of 0.5 ng/mg in cocaine- D_3 and
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304 benzoylecgonine- D_3 . After the addition of 2 mL of methanol, the samples were incubated at
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306 55 $^\circ\text{C}$ for 15 h without stirring. Lastly, the organic phase was collected in a new test tube and
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308 dried at 70 $^\circ\text{C}$ under a nitrogen stream. The dry residue was derivatized with 75 μL of a
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310 PFPA/PFPOH mixture (2:1 v/v) for 30 min at 70 $^\circ\text{C}$. The resulting residue was evaporated to
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312 dryness at room temperature under a stream of nitrogen and subsequently reconstituted with
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314 50 μL of ethylacetate. Lastly, an aliquot of 1 μL was injected (split ratio of 5:1) into the
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316 GC/MS system operating in the SIM (selected ion monitoring) mode.
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322 2.4 Apparatus and methods

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324 Cocaine and benzoylecgonine were determined with a GC/MS system consisting of a 6890N
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326 gas chromatograph interfaced with a 5975 mass spectrometer both from Agilent Technologies
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328 (Milan, Italy). The separation was carried out with a J&W HP-5 capillary column, 17m \times
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330 0.200 mm \times 0.33 μm . Helium was employed as **the** carrier gas at a constant pressure of 20.16
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332 psi. The GC oven temperature was set at 150 $^\circ\text{C}$ for 1 min and then raised to 200 $^\circ\text{C}$ with a
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334 30 $^\circ\text{C}/\text{min}$ heating rate. The oven temperature was then raised to 270 $^\circ\text{C}$ with a 10 $^\circ\text{C}/\text{min}$
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336 heating rate and lastly to 310 $^\circ\text{C}$ with a 50 $^\circ\text{C}/\text{min}$ heating rate. The total run time was 11.47
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338 min. The GC injector and transfer line were maintained at 270 $^\circ\text{C}$ and 280 $^\circ\text{C}$ respectively. The
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340 mass spectrometer was operated in EI at 70 eV and SIM acquisition mode at dwell times of
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342 30 ms. The fragment ions monitored for cocaine were m/z 198 (target ion), 303 and 182
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344 (qualifiers), while for benzoylecgonine (as PFPA-derivative) the diagnostic ions were at m/z
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346 421 (target ion), 316 and 300 (qualifiers).
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357 The method was fully validated according to national and international guidelines [27,28].
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359 Linearity was verified in the interval 0.05–5.0 ng/mg. Whenever the real samples
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361 concentrations were found to exceed the highest calibration point, the final extracts were
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363 diluted with methanol and re-injected into the system. Limit of Detection (LOD) and Limit of
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365 Quantitation (LOQ) for cocaine and benzoylecgonine were, respectively, 0.02 and 0.01 ng/mg
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367 (LODs) and 0.05 and 0.03 ng/mg (LOQs). Interday precision and accuracy were tested at 0.1
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369 ng/mg, showing that all experimental values were below the acceptable CV and bias limits of
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371 10%. Laboratory performances are constantly monitored through regular participation to
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373 inter-laboratory proficiency tests.
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376 For scanning electron microscopy (SEM) observations, the intermediate portion (segment
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378 from 2 to 3 cm) from a single 5 cm hair was deposited on an aluminium stub covered with a
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380 bi-adhesive conductive carbon tape, and sputtered with Au, to form a surface-conductive layer
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382 ca. 30 nm thick (Balzers BAL-TEC SCD-050 Sputter-Coater, Balzers Union, Liechtenstein).
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384 Images were obtained with a Leica Stereoscan 420 microscope 20 kV (Leica Microsystems,
385
386 Wetzlar, Germany) by collecting secondary electrons (E-T detector) emerging from the
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388 samples under the following operational conditions: acceleration potential, 20 kV; beam
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390 current, 60 μ A; I probe, 80 pA.
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395 **3. Results and discussion**

396

397 Table 1 reports the quantitative results for cocaine obtained from seven hair samples after
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399 they had been soaked for one hour into an aqueous cocaine hydrochloride solution. In real
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401 cases, the exposure and amount of cocaine involved in contamination may vary considerably,
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403 but the conditions adopted in the present study [29] were intended to simulate abundant
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405 sweating, as it may occur during physical exercise.
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416 For all samples tested, the analysis of the last washing solution collected after the
417 contamination procedure was negative for the presence of cocaine and benzoylecgonine. This
418 result confirms the complete removal of any residual presence of cocaine and
419 benzoylecgonine from the hair surface. The presence of cocaine was detected in all samples,
420 whereas its major metabolite, benzoylecgonine, was never detected. Thus, incorporation of
421 cocaine inside the keratin structure was observed to some extent from both treated and
422 untreated hair. In the cases of untreated and straightened hair, the concentration of cocaine
423 detected was below the commonly used cut-off of 0.5 ng/mg [30,31], with the exception of
424 sample 3 that showed cocaine concentrations slightly above 0.5 ng/mg. On the other hand, in
425 all hair samples contaminated after dyeing and bleaching treatments a much higher amount of
426 cocaine was retained. These treatments are generally water-based so it is likely that water can
427 open the cuticles making hair more sensitive to incorporation. For example, in dyed hair
428 samples the cocaine concentrations detected after the washing procedure ranged between 2.53
429 and 14.1 ng/mg. Even more extensive cocaine incorporation was observed in the bleached
430 hair, with concentrations ranging from 3.21 to 12.7 ng/mg (2 bleaching treatments). By
431 increasing the number of bleaching treatments up to 5, the hair contamination from cocaine
432 appears to increase further, even considerably (32.2 ng/mg in blonde hair), with the notable
433 exception of grizzled hair.

434
435 The amount of cocaine incorporated in the hair after the soaking does not appear to depend on
436 their melanin content. In fact, blonde and brown untreated and straightened hair exhibit
437 similar results, whereas cocaine incorporation appears to be even higher in blonde hair than in
438 brown hair, despite its reduced melanin content.

439
440 Figure 1 compares the SEM images of brown hair specimens before and after thermal
441 treatment as for hair straightening conditions: minimal damage was observed at the hair
442 surface of the treated hair, in agreement with the irrelevant change of cocaine incorporation

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475 observed between untreated and straightened hair. In particular, **an** overall general slight
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477 cuticle break and scales raise is observed, on account of the thermal treatment (compare
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479 panels A1/2 and B1/2), and only few localized and punctual hole-shaped damages occurred
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481 (compare panels A3 and B3).
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483
484 Figure 2 depicts the SEM images from four hair samples of different color after 5 cycles of
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486 bleaching treatment. These images reveal that the same chemical treatment produce
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488 significantly dissimilar damages on the cuticles of different hair, possibly depending on their
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490 structure, density and cross-section. In particular, the blond hair used in the SEM analysis
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492 reported in Figure 2c was more deeply damaged than the brown, grizzled, and red hair
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494 considered in the SEM comparison. The comparison between Figure 1 and 2 stresses the
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496 various modifications that the bleaching treatment had produced. Not only most of the surface
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498 cuticles appear to be broken and scarcely overlying to one another, but also holes of
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500 considerable size were produced in the cortex of blond hair depicted in Figure 2c. Indeed, the
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502 blonde hair considered in the present study incorporated more cocaine than brown, grizzled
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504 and red hair both before and after any chemical treatment (see Table 1). This effect can
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506 allegedly be attributed to the more fragile hair structure and more extensive damages induced
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508 by the treatments, in turn promoting an extensive cocaine incorporation inside the keratin
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510 matrix.
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513 The latter conclusions cannot be generalized, since several other intrinsic factors, such as hair
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515 thickness and porosity, should be taken into account. Hairs that apparently exhibit similar
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517 damage (Figure 2a, 2b, 2d) yield significantly different cocaine incorporation (last column of
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519 Table 1). Moreover, the intensification of cosmetic treatments, such as five bleaching instead
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521 of two, definitely results in more extensive damage of the keratin matrix, but the enlarged
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523 morphological alteration of the keratin structure does not necessarily correspond to an
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525 increase of the cocaine uptake, as is evident for the grizzled hair considered in this study.
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534 Dyed brown and blonde hairs absorb less cocaine than bleached hair, while, on the opposite,
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536 grizzled and red hairs submitted to a dyeing treatment apparently incorporate more cocaine
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538 than when they are bleached. These opposite observations may be explained with the
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540 coexistence of several competing effects, including hair damage, density, and thickness.
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542 Moreover, a more extensive cuticle damage is likely to increase the hair porosity and,
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544 consequently, to promote both a large drug intake and also its plentiful release, during the
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546 washing and decontamination procedures. Lastly, highly porous hair samples definitely give
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548 exhaustive extraction of the incorporated drugs, whereas the extraction yields from untreated
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550 hair are not necessarily complete.
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553 554 555 **4. Conclusions** 556

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558 In hair testing, the occurrence of external contamination has been demonstrated for several
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560 drugs of abuse, particularly cocaine, making the interpretation of analytical results susceptible
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562 of criticism. Within the consideration of potential biasing circumstances, the results of this
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564 study demonstrated that the hair samples collected from several individuals bind cocaine
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566 inside their inner keratin structure, when they are subjected to various cosmetic treatments
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568 and then soaked into a cocaine solution. In contrast, the contamination from cocaine proved to
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570 be limited, whenever the same hair samples were not previously treated with either strong
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572 oxidants or bases. The effect of cosmetic treatments on cocaine uptake are so pronounced to
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574 cover any other possible influencing factors (for example, hair color).
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578 Scanning electron microscopy provided high resolution images of both treated and untreated
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580 hair, that allowed us to distinguish specific treatment-related damages, that appear to be
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582 localized (punctual) for thermal treatments, but extended to the entire hair surface when
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584 chemical treatments were applied. The latter created damages of variable scale, but a
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586 substantial increase of hair porosity can be recognized.
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593 The quantitative determinations demonstrated a widely increased uptake and incorporation of
594 cocaine in damaged keratin matrices with respect to untreated and straightened hair. However,
595 the progressive increase of cocaine uptake seems not to be directly reflected into the
596 morphological alteration of the keratin structure, possibly because the augmented hair
597 porosity facilitates any drug exchange, i.e. both incorporation during soaking and release
598 during washing procedures. In all cases, the major metabolite of cocaine, benzoylecgonine,
599 was never detected, excluding a false interpretation of cocaine use. Nevertheless, further
600 studies are needed with different substances, metabolites or testing different contamination
601 conditions, in order to simulate as much as possible real cases and verify if similar
602 phenomena may occur with other drugs of abuse or their metabolites. In general, it is
603 confirmed that cosmetic treatments such as bleaching and dyeing can strongly enhance the
604 cocaine uptake in the treated hair. Therefore, the effect of these treatments should be taken
605 into account when hair analysis results for drug abuse have to be interpreted for forensic
606 purposes.
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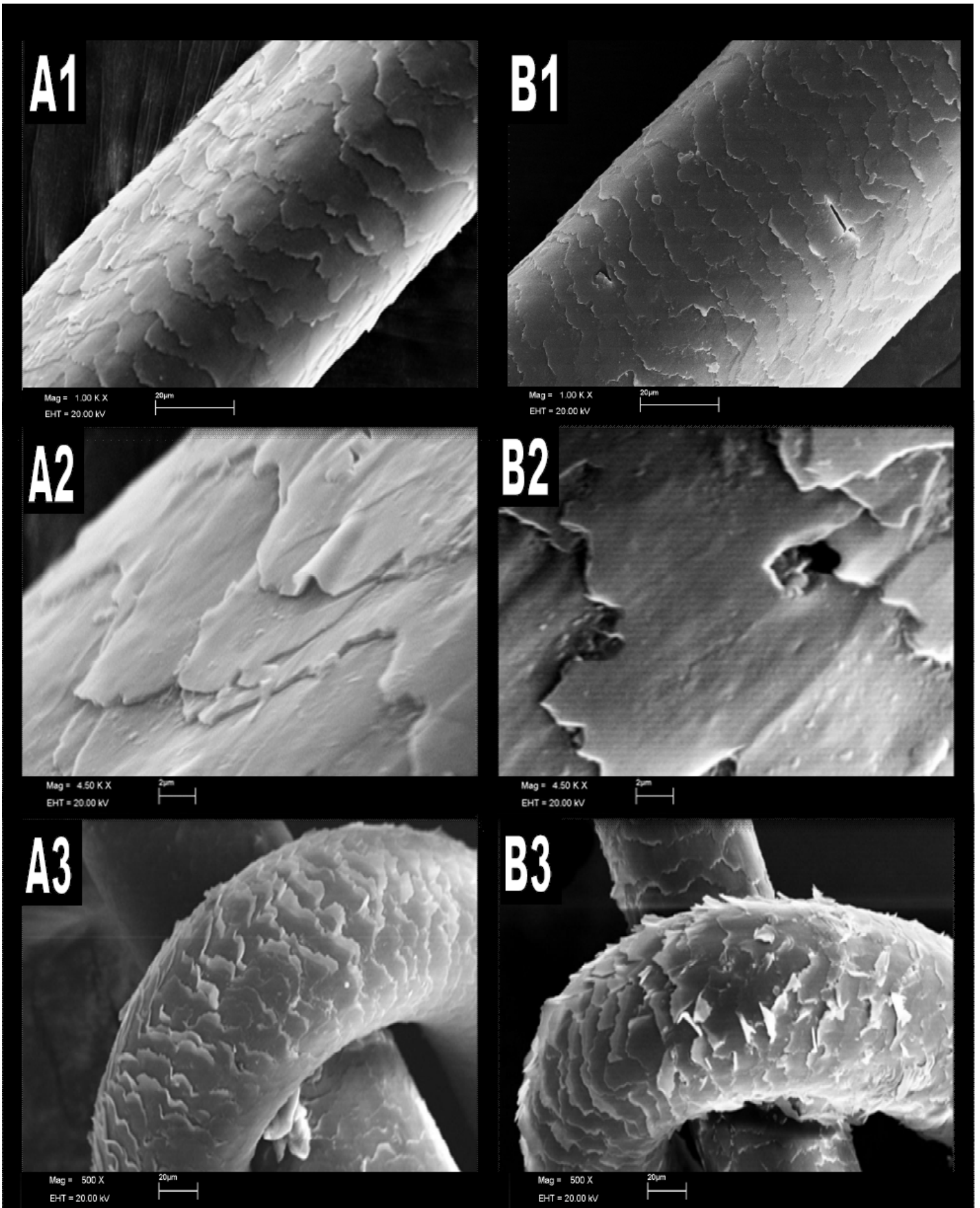


Figure 1. SEM images of a brown hair sample before (panels A) and after (panels B) straightening recorded at 1000× (A1-B1), 4500× (A2-B2) and 500× (stressed, A3-B3).

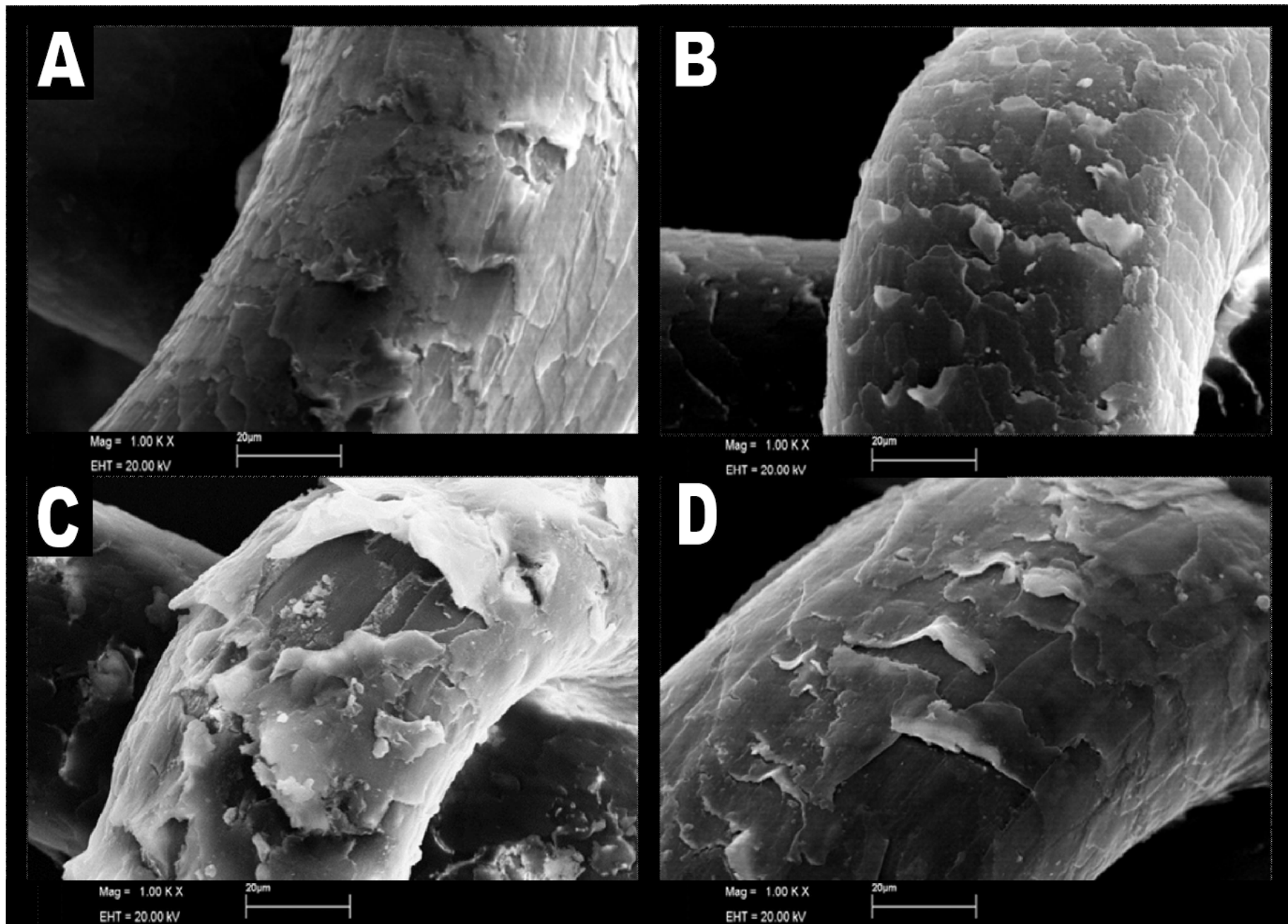


Figure 2. SEM images (1000×) of brown (panel A), grizzled (panel B), blonde (panel C) and red (panel D) hair after 5 cycles of bleaching treatment.

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

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