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Understanding Hair Repair Mechanism by Hybrid SIMS Technique

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Abstract

Understanding the spatiotemporal dynamic behavior of cosmetic ingredients in hair matrix remains one of the key challenges at the intersection of cosmetic formulation and bio-matrix. In this study, we used a Cryogenic Hybrid SIMS platform that integrated high spatial resolution ToF-SIMS imaging and ultra-high mass resolution Orbitrap detector, enabling the in-situ molecular localization of key markers inside hair fiber. This study reveals spatiotemporal interactions among ingredient actives, hair matrix and metal compounds through chelation, ion exchange and structural restoration via active ingredient-induced bond rearrangement. The experimental approach contributes to understand hair repair mechanism through identifying specific damage characteristics, validating targeted functional effects, investigating deposition and penetration dynamics of ingredient actives, for fundamental hair science and haircare product innovation.

Key Words: Hybrid SIMS, Orbitrap, Hair repair dynamics

1. Introduction

It is well known that human hair can be damaged by chemical treatment and various daily factors [1]. The interaction between haircare products and hair is the key to uncovering the repair mechanism, which is a cornerstone for fundamental hair science and haircare product innovation.

The application of ToF-SIMS technology in hair research has leaped from elemental distribution analysis to molecular dynamic characterization [2]. However, it has been limited by the trade-off between mass resolution and spatial resolution for in-situ characterization. Many previous published articles utilized ToF-SIMS depth profiling and 3D imaging in fundamental structural analysis and interaction between cosmetic ingredients and bio-matrix, in both hair and skin research field [3-5]. While the low mass resolution of traditional TOF detectors ($m/\Delta m <$

10,000) makes it difficult to distinguish complex molecules, resulting in the inability to accurately analyze the diffusion heterogeneity of cosmetic ingredients [6]. Although multi-mass spectrometry can locate the depth distribution of exogenous substances [7], its single-dimensional analysis mode cannot reconstruct three-dimensional penetration dynamics [8]. In response to the above bottlenecks, this study used Hybrid SIMS, integrated Orbitrap ultra-high resolution mass spectrometry (m/Δm > 240,000) and ToF-SIMS platform, to investigate the damaged hair fibers before and after treatment with hair care products under cryogenic conditions (-100℃).

This study visualized the surface accumulation and differential diffusion patterns of active ingredients (such as citric acid, behentrimonium chloride) in damaged hair [9,10]. Combining GCIB sputtering with dual analyzer 3D imaging, the chemical composition and normalized signal intensity of key markers in hair fibers were obtained [11,12], interpreting the potential active ingredients - hair matrix - metal compounds interaction. This study echoed some of instrumental test results which showed significant improvement of the hair physical properties after this treatment [13]. Compared with previous studies, this integrated experimental approach further establishes a method for identifying specific damage characteristics, validating targeted functional effects, investigating actives deposition and penetration dynamics, to explore hair microstructure and power haircare product development.

2. Materials

The original straight Chinese medium bleached hair swatches (27cm x 2.5cm x 6g, free 24.5cm) were purchased from Shanghai Canyu Hair Commercial Co., Ltd (Shanghai, China), which mimics the damaged hair fibers after chemical treatments. The bleached level was controlled by alkaline solubility measurement. All the original hair swatches were cleaned by the simplex shampoo formula (without silicone) to wash off the unintended stain on hair surface before treatment.

Divide all the original hair swatches into three groups randomly with different treatment, shown as follow.

(1) Untreated-Reference Sample. Original hair swatch without any further treatment, to mimic the bleached damaged hair without any haircare treatment.
(2) 5-Minute-Treated Sample. Original hair swatch treated with the haircare product, under normal application procedures, to mimic the standard consumer usage time of haircare products. Firstly, on the wet hair swatch, applied 0.4 grams of the test haircare product for each gram of hair. Then massaged the product into the hair and leave each swatch for 5 minutes. Finally, rinsed, combed and dried the swathes. This group primarily served to investigate the immediate effects of the hair repair treatment on hair surface and its initial molecular distribution.
(3) 3-Day-Treated Sample. Original hair swatch treated with the haircare product, under extreme conditions, to explore the penetration dynamics and deep repair mechanisms under prolonged exposure. An extended 3-day treatment condition was implemented by directly immersing the hair swatch into the haircare product for 3 days. After that, rinsed, combed and dried. This group aimed to reveal the long-term structural and compositional effects of prolonged exposure, with a particular focus on the spatial distribution of treatment agents within the cuticle and cortex.

The haircare product used in this work was already launched on the market. It is a hair conditioner type product, with well-designed citric acid, sodium citrate, behentrimonium chloride, cetearyl alcohol and other active ingredients composition.

3. Methods

3.1 The Instrument Setup

This study employed Hybrid SIMS (IONTOF GmbH M6), which is equipped by Orbitrap ultra-high resolution mass spectrometry (Q Exactive, Thermo Fisher). The hair strands were pre-cooled in a nitrogen atmosphere (-80°C) and then analyzed at cryogenic conditions (-100°C) to maintain their near-natural state. Initially, Bi_3^+ secondary ion by ToF detector was conducted to acquire molecular spectra and high-resolution spatial distributions of ions across untreated, 5-minute-treated, and 3-day-treated samples. Subsequently, 10KV Gas Cluster Ion Beam (GCIB) sputtering (sputtering time: 2000 s, depth: ~2 μm [4]), equipped with orbitrap detector, enabled depth profiling of key markers to investigate the penetration of active ingredients and their interactions with hair matrix components, with high mass resolution. Both positive and negative ion signal acquisition modes were employed to detect metal compounds, hair matrix (e.g., amino acids [4], protein backbone), and active ingredients (e.g., citric acid, behentrimonium chloride) markers, shown in Table I.

To ensure data reliability and reproducibility, all ToF-SIMS and Orbitrap mass spectrometry data were normalized (by total ion count or hair protein backbone signals) and systematically compared against untreated reference samples. Reproducibility assessments and statistical analyses were conducted to validate the consistency of the findings.

Table I. Some of key markers chemical information

Key Markers	Fragment Ions	Origin
Hair Protein (+)	CH_4N^+	Protein (Backbone)
Hair Protein (-)	CNO^-	
Behentrimonium Chloride	$C_{25}H_{54}N^+/C_3H_8N^+$	Active Ingredient
Citric Acid	C_2HO^-	
Cysteine/Cystine	$C_2H_6SN^+$	Protein (Amino Acid Fragment)
Proline	$C_4H_6N^+$	
Serine	$C_2H_6NO^+$	
Glutamic Acid	$C_4H_6NO^+$	
Valine	$C_4H_{10}N^+$	

3.2 Effect of Cryogenic Condition on Surface Detection Sensitivity

To evaluate the impact of cryogenic condition on detection sensitivity, we compared depth profiles of representative ions acquired under room temperature (RT) and -100°C cryogenic (Cryo) condition across different treatment states. As shown in Figure 1, the normalized intensities of small molecular fragments (C_2HO^-/CNO^-) increased significantly during initial surface sputtering (<200 s) compared to RT conditions, indicating a significant reduction in vacuum-induced sublimation and superior retention of thermally labile acids such as citric acid.

3.3 Assess the High Mass Resolution Capability of Orbitrap Analyzer

To assess the Hybrid SIMS platform's capability in resolving high-mass species, we analyzed the $C_{25}H_{54}N^+$ molecular ion (theoretical isotope m/z: 368.4256 Da), as a characteristic ion for behentrimonium chloride, a common active ingredient in haircare product, under Orbitrap and ToF detector configurations. As shown in Figure 2, the Orbitrap mode precisely captured the

mono-isotopic peak (tested isotope m/z: 368.4251 Da), while ToF mode exhibited broadened signals, indicating lower mass accuracy and potential overlapping of unintended interfering ions. Importantly, the Dual Beam-Orbitrap mode combines ultra-high mass fidelity with spatial resolution, highlighting the platform's advantage in localizing high-mass species within the hair matrix.

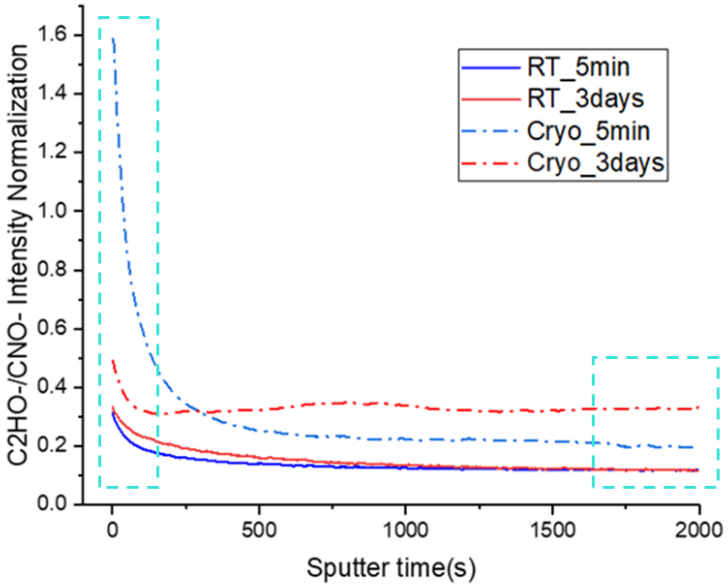

Figure 1. The surface sensitivity enhancement of cryogenic condition (Cryo, in dashed line), compared with room temperature condition (RT, in solid line), for 5min and 3days treated sample.

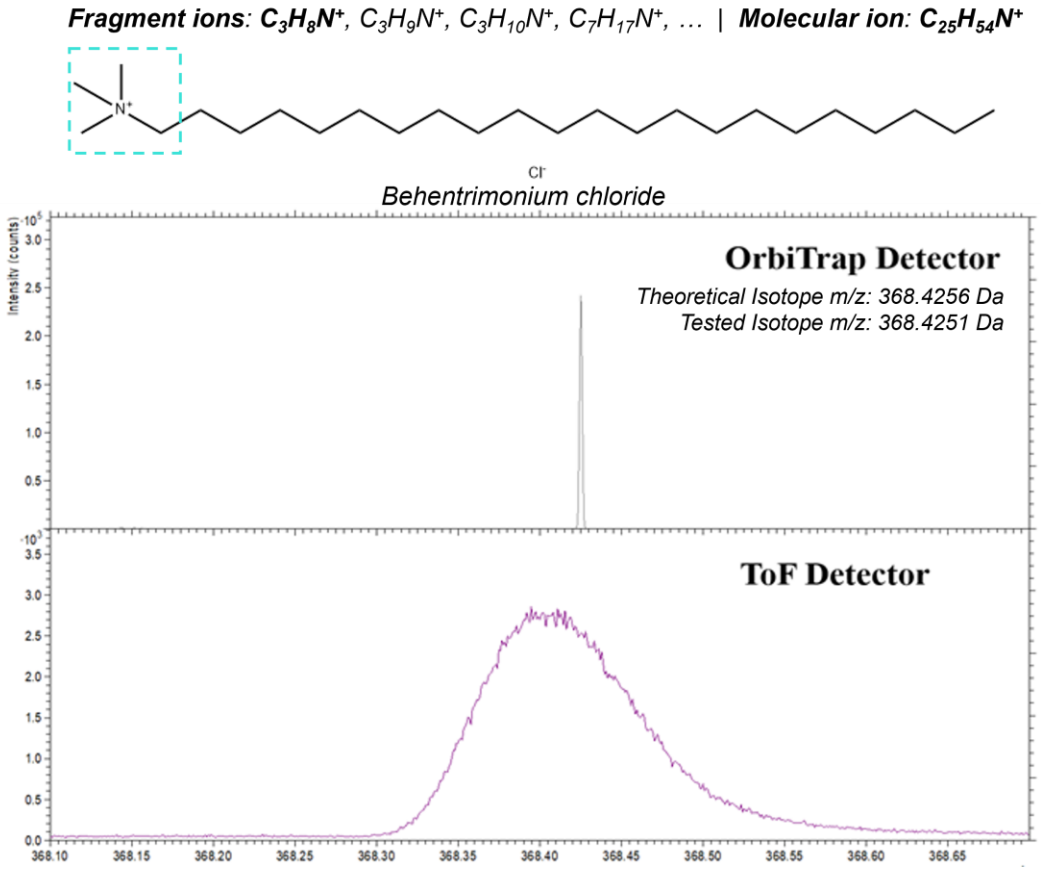

Figure 2. The MS spectrum of behentrimonium chloride using different detectors

4. Results and Discussion

4.1 Deposition of Active Ingredients on Hair Surface

In Figure 3, the spatial distribution of key treatment markers (C_2HO^-, $C_{25}H_{54}N^+$) and hair protein backbone ions (C_2HO^-, CH_4N^+) on damaged hair surfaces revealed different deposition distribution across treatment durations. Untreated hair exhibited homogeneous but low-intensity distributions of treatment-related ions. After 5-minute treatment, C_2HO^- signal preferentially accumulated in cuticle layer and its boundaries, complementary to the matrix ion (CNO^-) distribution. Prolonging treatment to 3 days intensified this localized accumulation, particularly in regions with severe cuticle defects, while $C_{25}H_{54}N^+$ maintained homogeneous surface coverage.

However, as the sputtering depth increased to 2000 seconds, C_2HO^- transitioned from surface-accumulation deposition (targeting damaged regions) to homogeneous distribution at deeper layers (~2 μm). This observation suggests that small molecules within the treatment, such as citric acid, can be preferential adsorbed in damaged cuticle regions (filling inter-cuticular voids and coating exposed proteins) followed by time-dependent homogeneous diffusion into the cortex, with penetration increasing along the treatment duration. For behentrimonium chloride, $C_{25}H_{54}N^+$ signal was attenuated fast and will be further investigated in section 4.2.

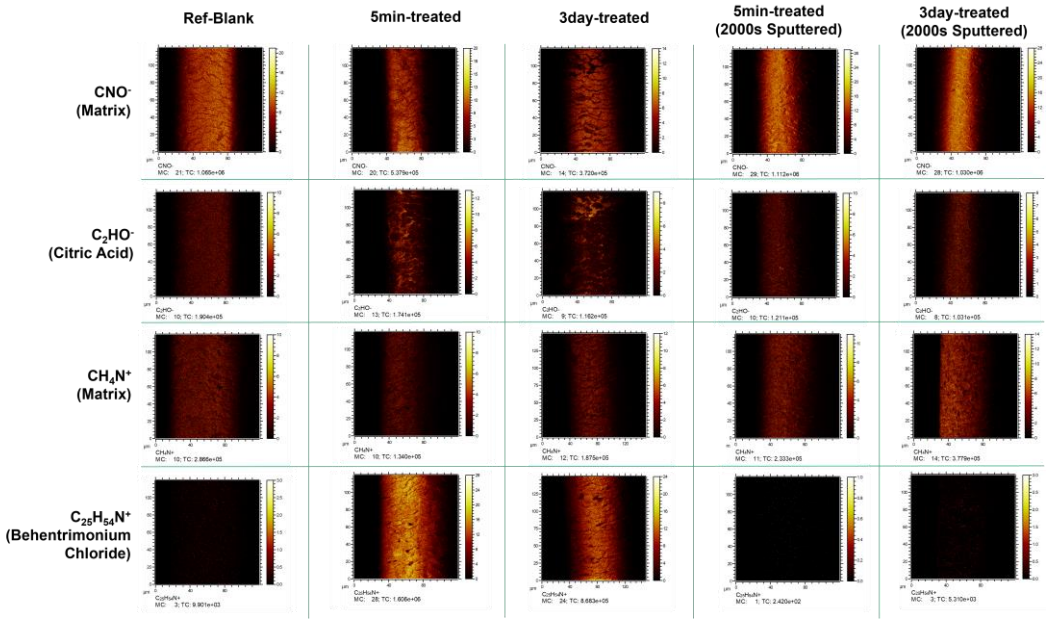

Figure 3. Citric acid and behentrimonium chloride deposition and penetration

4.2 Spatial Response of Hair to Repair Treatment

In this section, we studied diffusion dynamics of behentrimonium chloride ($C_{25}H_{54}N^+$) within the multilayered cuticle, investigated through ToF-SIMS depth profiling (0–2 μm) and 3D reconstruction. The oscillatory variations in amino acid signals reflect the multilayers structure of cuticle tissue [4]. As compared to Figure 4. (a-b), the short-term treatment (5 min) led to surface-dominated accumulation. This initial deposition is predominantly governed by physical adsorption, electric charge interaction [14] or other interaction shown in section 4.3. While prolonged exposure (3 days) enabled $C_{25}H_{54}N^+$ penetration through CMC layers, evidenced by oscillatory signal patterns aligning with low cystine regions (near endocuticle) [15]. In Figure 4. (c), Keratin-based hair matrix and behentrimonium chloride exhibited the spatial

complementarity and periodic distribution along the depth axis, indicating that behentrimonium chloride interacts with hair matrix and preferentially accumulates at specific structural layers within the hair. Combined with Figure 4. (b), the behentrimonium chloride signal peak is localized in the vicinity of the low cystine regions, spatially matching the lipid-rich CMC layer [15]. This suggests that behentrimonium chloride tends to accumulate and diffuse through the thin fat-rich cuticle-cuticle CMC layers next to endocuticles in cuticle layers, due to their hydrophobic long-chain alkane tail and its quaternary ammonium cationic head with synergistic mechanisms (electrostatic adsorption, non-polar interaction, and other unknown mechanisms) [5, 16-17]). This structural accumulation of behentrimonium chloride within multilayered CMC structure in cuticle is likely to enhance hair surface properties, consistent with prior observations of cationic surfactants modifying surface charge distribution [2,6].

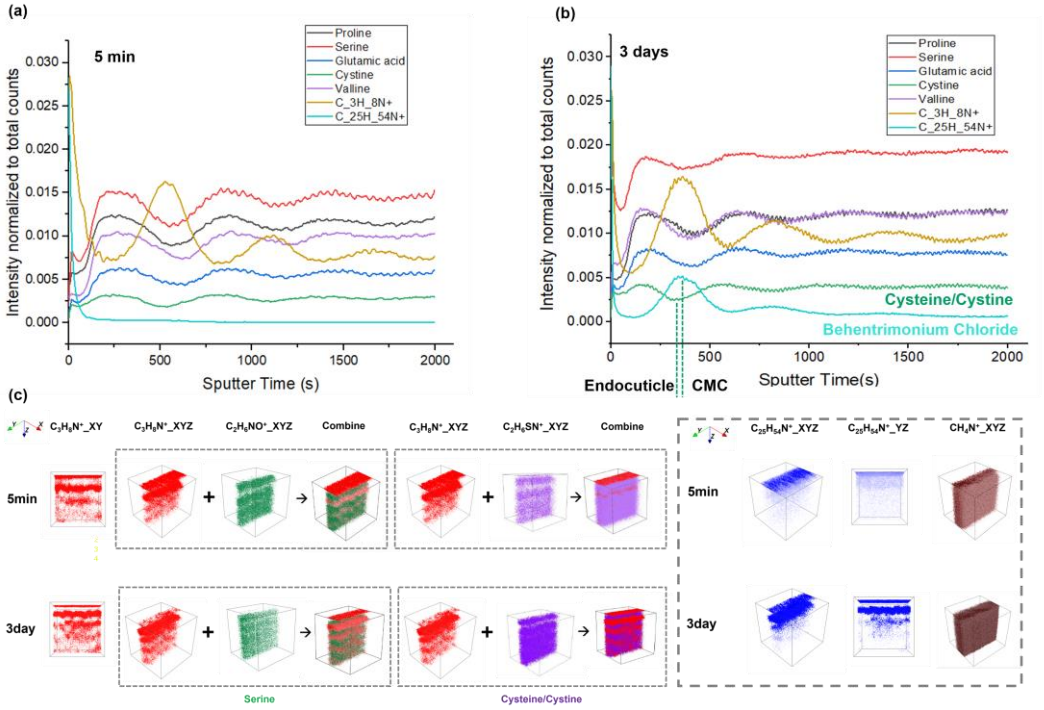

Figure 4. Spatial Respond of Hair Treatments: (a-b) Profile for keratin matrix and active ingredients; (c) 3D reconstruction for keratin and active ions: behentrimonium chloride ($C_{25}H_{54}N^+/C_3H_8N^+$); Serine ($C_2H_6NO^+$); Cysteine/Cystine ($C_2H_6SN^+$); Protein backbone ($CH_4N^+$).

4.3 Active ingredients - hair matrix – metal compounds interactions Dynamics

Here, we systematically analyzed the repair mechanisms of the treatment on metal ion migration, chelation, and matrix reconstruction over short-term and long-term durations.

Metal compounds, including heavy metals (Fe, Cd, Pb) and calcium species, can be accumulated in hair through environmental exposure (e.g., different source of water, air pollution) and lead to disruption of hair internal week bonds. As shown in Figure 5(a–c), within a short duration (5 min), the signal intensity for metal oxides (M_xO_y) and hydrated cluster forms ($M_xO_yH_z$, such as $Ca_3(OH)_5^+$) decreased on the hair surface, especially for calcium compounds. Moreover, the prolonged treatment (3 days) reduced normalized signals intensities of heavy metals and calcium compounds throughout entire analyzed region. The deep-fixed metal ions were gradually displaced, likely due to the chelation of undissolved metal compounds by citric acid, causing its transformation to its dissolved ion state, and thus leading the migration toward the surface and eventual removal.

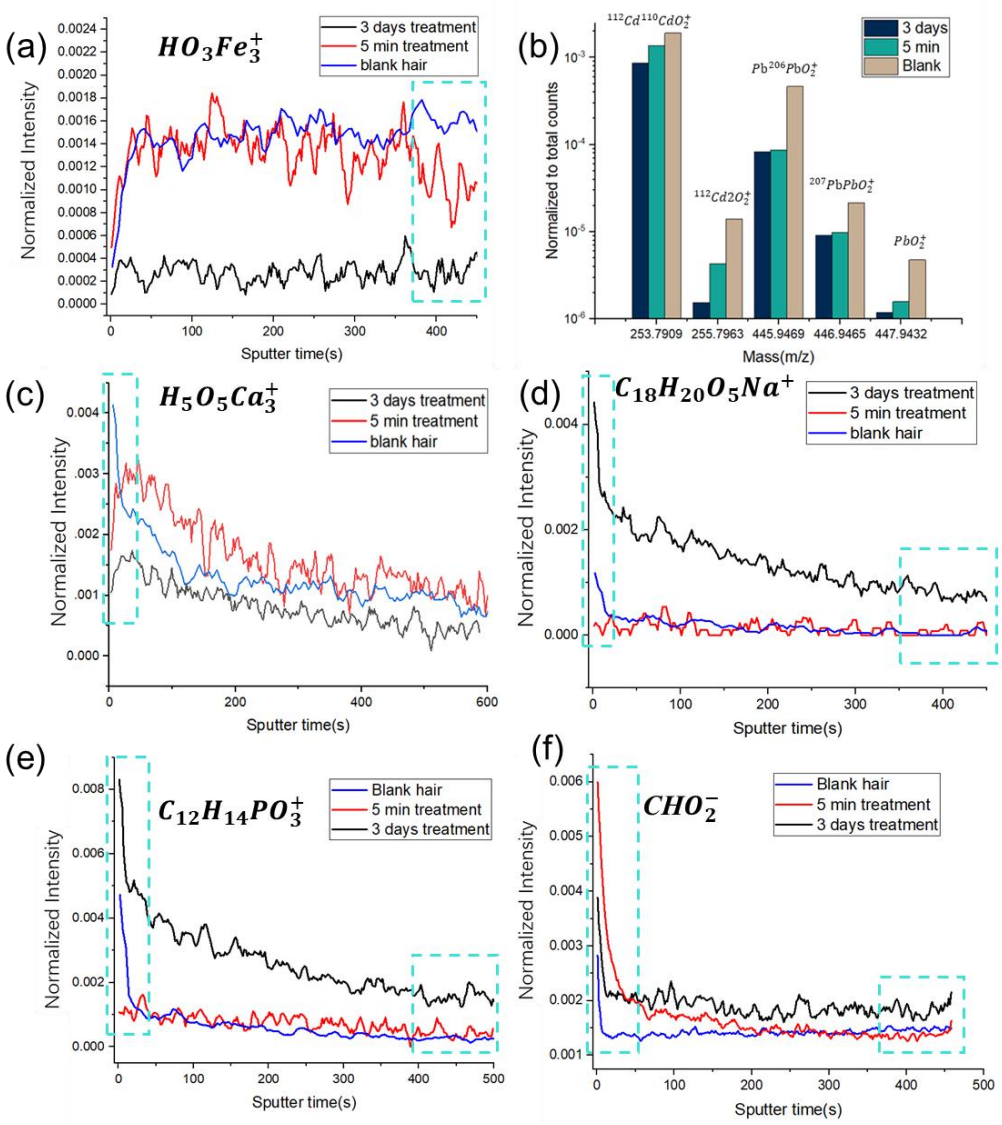

Figure 5. Normalized signal intensity for key markers in analyzed region to investigate active ingredients - hair matrix – metal compounds interactions dynamics: depth profile of (a) iron oxide ion; (b) total normalized signal Cd and Pd oxide, not showing the depth profiling graph due to its very low signal intensity; (c) hydrated calcium ion cluster; (d) sodium adduction component; (e) phosphorylated Component; (f) CHO_2^- ion.

Meanwhile, as shown in Figure 5(d), an increase in sodium ion signals in the corresponding region suggests that sodium ion, originated from sodium citrate, may rebind to the hair matrix through ion-exchange mechanisms, potentially acting as a new stabilizing factor within the hair structure. This process not only significantly reduces the residual levels of metals but also facilitates the reassociation of sodium ion with the hair matrix, forming new metal-matrix interaction patterns, thereby revealing a re-equilibration effect of citric acid and its sodium salt within hair.

As shown in Figure 5(e), the phosphorylated components in the hair matrix exhibited a distinct treatment-dependent variation following haircare treatment. For short-term treatment (5 min), surface cleaning effect is dominant and along with the repair agent rapidly removes surface

bound metal compounds, the surface signal intensity decreases fast. In the long term (3 days), as citric acid gradually permeates into inner hair structure, the overall level of “free” phosphorylated groups/species increases, which implies that the treatment may affect the existing state of phosphorylated groups/species, leading to metal-dissociation and may transform from metal-bonded state back to its free state.

As shown in Figure 5(f), short-term treatment (5 minutes) significantly enhanced the CHO_2^- signal (carboxyl group) on the hair surface, while its intensity further increased throughout the deeper analyzed region after 3 days of treatment. This indicates that citric acid may further promote CHO_2^- formation inside hair, such as transform carboxyl group from metal-bonded stated to its free state, acid hydrolysis or other deeper structural changes. This process may be related to protein sidechain rearrangement, pH changing, and structural transformation inside hair.

5. Conclusion

This study revealed the dynamics of hair repair under the treatment of haircare products. For the first time using Hybrid SIMS technology in hair analysis, the depth profile/3D distribution of key markers showed the complementary distribution of active ingredients and keratin. Behentrimonium chloride diffused non-uniformly and accumulated in CMC layer, while citric acid showed a more uniform diffusion into the hair. The treatment synergistically restored the disruptive bonds network and created new bonds through metal chelation, sodium ion replenishment and matrix reconstruction (phosphorylation and CHO_2^- changes) from the surface to the deep layer.

Based on this, future research will focus on establishing a strategy to identify unknown species using Orbitrap MS^n functions, numerical-resolved analysis (such as PCA tools) and chemical transformation-repair mechanism correlations. We hope this work can provide a new paradigm for molecular-level dynamic analysis for the interaction between cosmetic product and biomatrix, and thus promote the leap from phenomenon description to fundamental mechanism.

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7. Conflict of Interest Statement

Zidu TANG and Yan YU are employees of L’Oréal Research & Innovation (Shanghai, China), which is the company provided the financial support for this study. Yuxiao HOU, Jiawen QIU and Lu-Tao WENG are employees of The Hong Kong University of Science and Technology (Guangzhou, China), which is a Public University having received financial support from L’Oréal for this study.

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