Scanning Electron Microscopy Assay

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Tradename: AC Keratin Hydrolysate 30 PF

Code: 20586PF

CAS #: 69430-36-0

Test Request Form #: 14309

Lot #: S210318I

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Maureen Danaher*
Principal Investigator: *Jennifer Goodman*

Test Performed:
Scanning Electron Microscopy (SEM) Assay

Introduction

Chemical treatments such as coloring, relaxing, or perming obtain desired results by utilizing harsh chemicals that alter the internal structures in hair fibers. These treatments lead to extensive damage of hair fibers resulting in breakage, thinning, and split ends. Despite this, chemical treatments are still popular and demand for less damaging products is widespread. Therefore, cosmetic applications designed to mitigate damage and promote healthier chemically processed hair are critical.

Visual damage of hair fiber cuticles can be examined with Scanning Electron Microscopy (SEM) imaging. Damage caused by chemical treatments is observed as irregular textures, flaking, and fragmenting whereas healthy hair appears smooth with no fragmenting and flakes. A product that provides protection against chemical treatments will maintain visible structures similar to untreated hair.

Accordingly, a Scanning Electron Microscopy (SEM) assay was conducted to visually assess the ability of **AC Keratin Hydrolysate 30 PF** to protect hair against chemical treatments.

Assay Principle

Virgin human hair tresses were tested with a 40 V bleach chemical treatment to understand the protective capability of a cosmetic product. After treatment, tresses were treated with cosmetic products and examined using scanning electron microscopy examination to visualize the extent of damage. Images provide qualitative damage of the hair fibers.

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Materials

A. **Hair Samples:** Human Virgin Brazilian Hair Tresses
B. **Products:** Paul Mitchell The Color Cream Developer 40 V*

*Or suitable alternatives, subject to change without notice based off vendor availability

Methods

Three virgin hair tresses were collected and assigned to each condition described in Table 1. 40 V developers were mixed with bleach to a "butter cream" consistency. Two tresses were treated with bleach according to product directions, after which the tresses were thoroughly rinsed. Next, tresses were treated with 2.0% **AC Keratin Hydrolysate 30 PF** or left untreated, then rinsed again and allowed to air dry fully. The third tress was left unbleached and treated as the Untreated Virgin Control.

Table 1. Descriptions of the Conditions for each 40 V Brunette Hair Tresses.

Conditions
Untreated Virgin Control
40 V Bleach
40 V + 2.0% **AC Keratin Hydrolysate 30 PF**

Microscopic examination of the hair tresses was conducted on a Zeiss DSM 962 at 20.0 kV with a magnification range from 200x-800x. The electron microscope produces images of hair fibers by scanning them with focused beams of electrons. The electrons interact with the atoms of the hair sample to provide images of the hair's surface topography and surface composition. Ten individual fibers were selected at random from each sample for imaging.

Longitudinal images were further analyzed to determine hair integrity. After normalizing image brightness/contrast, standardized regions of interest (ROI) were set and placed along the strands of hair, hair integrity was measured using the Raw Integrated Density of each image calculated using ImageJ software (NIH). Raw integrated density is the sum of all the pixel intensities in the ROI, with lighter and darker regions retaining higher and lower pixel intensity, respectively. Accordingly, Raw Integrated Density values correspond to hair integrity.

Results

The data obtained met criteria for a valid assay and the controls performed as anticipated. Compared to the Untreated Virgin Control, hair treated with 40 V Bleach experienced visual fiber damage. Conversely, the tress treated with **AC Keratin Hydrolysate 30 PF** after chemical treatments demonstrated less visible fiber damage compared to the chemically treated controls.

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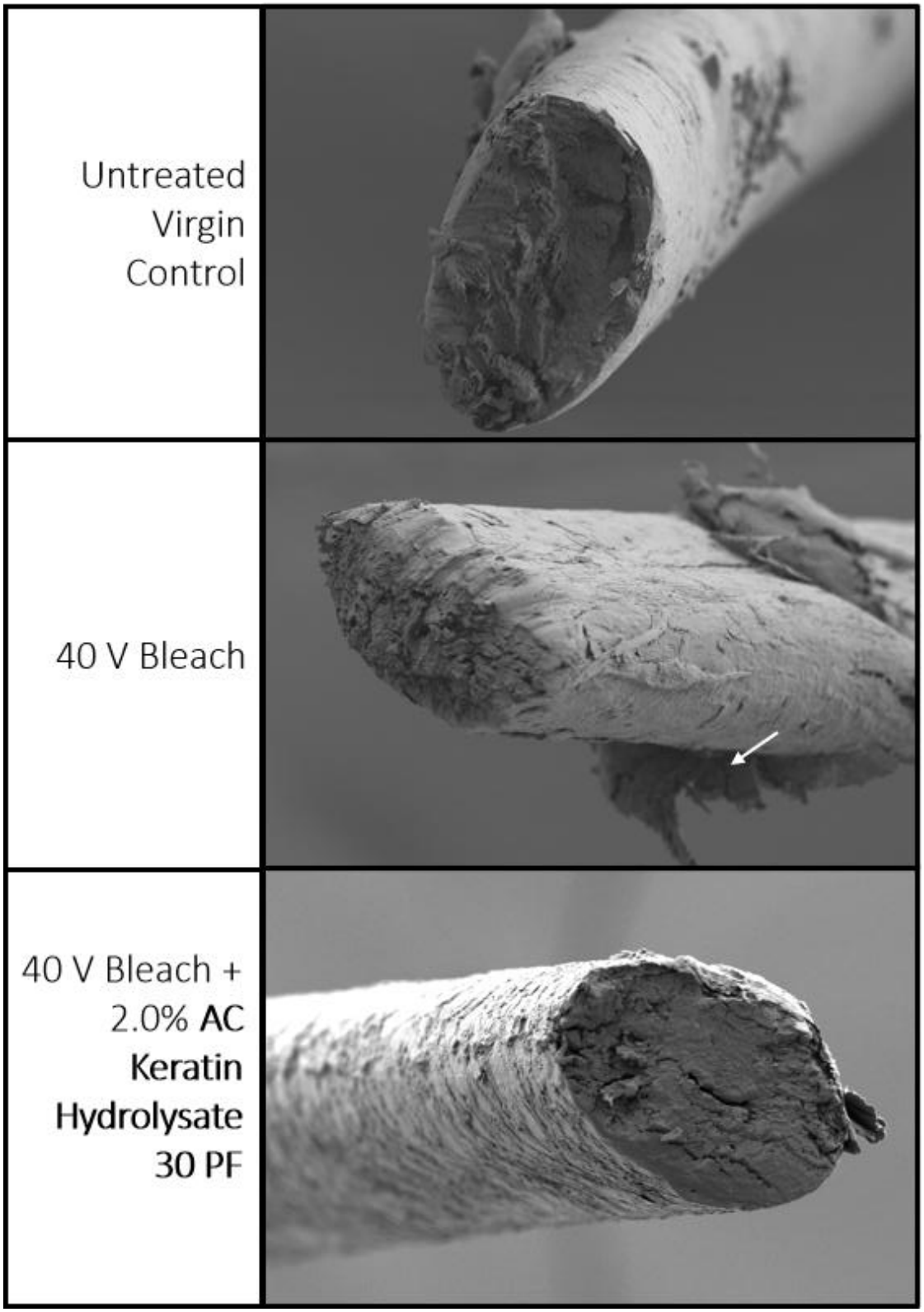

Figure 1. Representative SEM Cross Section Images of Hair Fibers Treated with 40 V Bleach. White arrow highlights major damage of the hair fiber.

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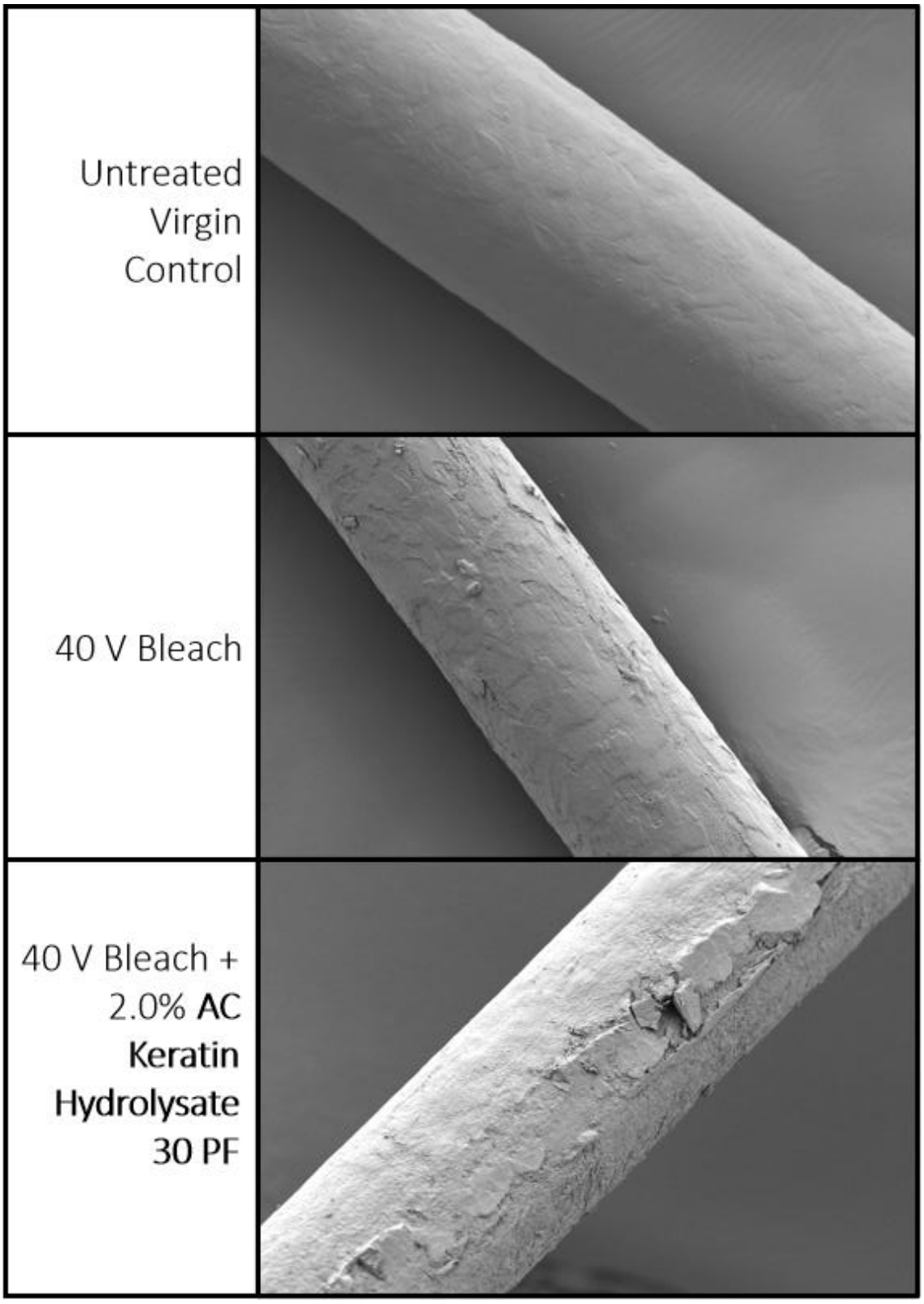

Figure 2. Representative SEM Longitudinal Images of Hair Fibers Treated with 40 V Bleach

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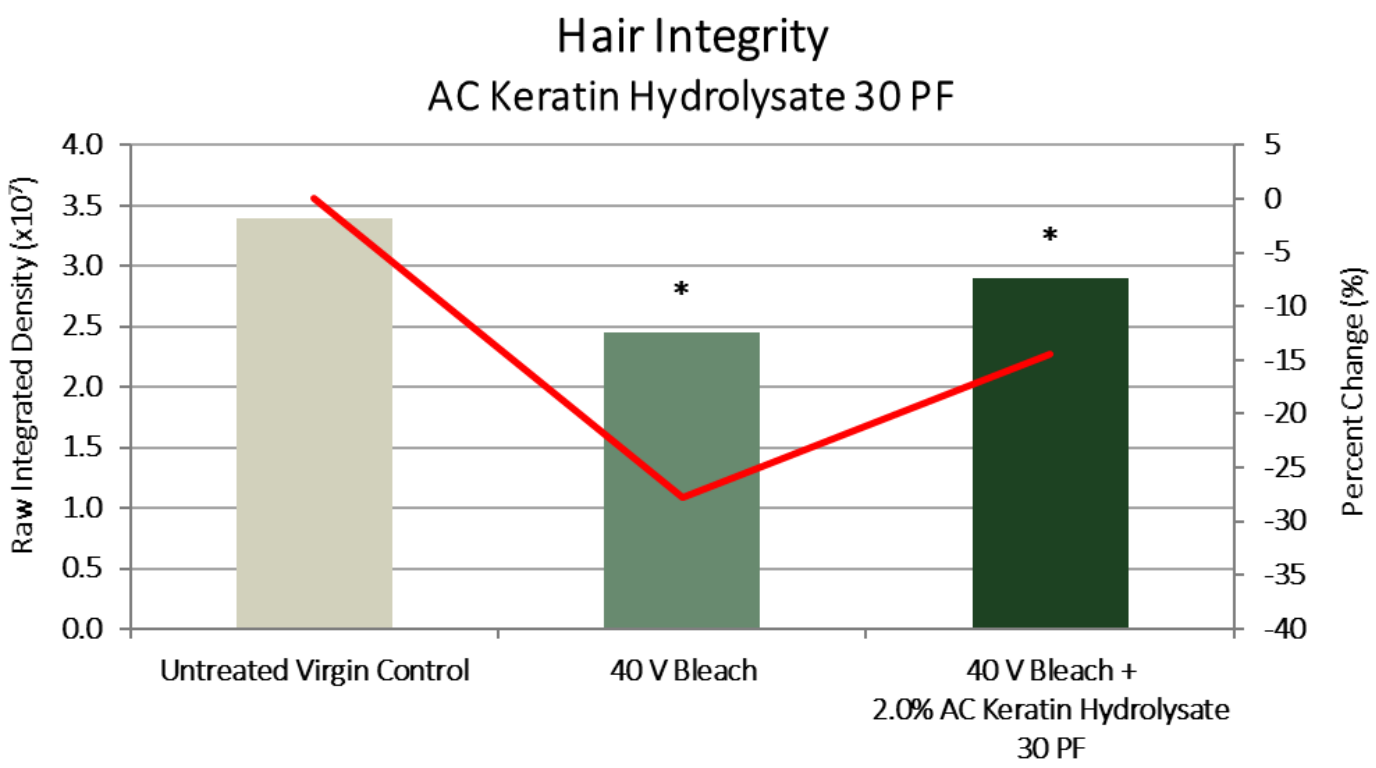

Figure 3. Integrity of Hair Fibers Treated with 40 V Bleach. * indicates significance ($p \leq 0.05$) compared to Untreated Virgin Control.

Table 2. Results from one-way ANOVA Statistical Analysis for Hair Integrity. Results represent p-values compared to Untreated Virgin Control. * indicates significance ($p \leq 0.05$) compared to Untreated Virgin Control.

	40 V Bleach	2.0% AC Keratin Hydrolysate 30 PF
P-value	< 0.001*	0.039*

Discussion

As demonstrated in Figures 1 and 2, the 40 V Bleach caused visible damage to the hair tresses. Compared to the Untreated Control, the 40 V Bleach tress experienced major fragmenting and cracking of the cuticle. Furthermore, the 40 V Bleach tress experienced a significant 28% decrease in hair integrity compared to the untreated control (Figure 3, Table 2). This data demonstrates that bleach treatments on hair cause significant damage to the hair fiber cuticle.

Alternatively, 2.0% AC Keratin Hydrolysate 30 PF applied after bleaching relieved the visible damage from the chemical treatment (Figures 1 and 2). Compared to the 40 V Bleach, 2.0% AC Keratin Hydrolysate 30 PF visually improved texture of the hair fibers and only experienced a 14% decrease in hair integrity compared to untreated virgin hair indicating protective properties (Figure 3, Table 2). This data indicates AC Keratin Hydrolysate 30 PF protects hair against 40 V bleach developer induced damage.

Collectively, these results indicate AC Keratin Hydrolysate 30 PF provides protective capabilities when used after hair chemical hair treatments by maintaining cuticle structure. In summary, AC Keratin Hydrolysate 30 PF protects hair from harsh bleaching without influencing the desired results when used at recommended use levels.