J. Soc. Cosmet. Chem., 24, 609-622 (September 16, 1973)

Innovative Scanning Electron Microscopic Techniques for Evaluating Hair Care Products

SAL P. DiBIANCA, M.A.*

Synopsis—The utilization of microscopy in studying human hair is briefly reviewed. Reasons for selecting the SCANNING ELECTRON MICROSCOPE (SEM) over the transmission electron microscope and the optical microscope are discussed. The use of the SEM in evaluating HAIR CARE PRODUCTS is then described. A new technique employing a ROTATING HAIR STAGE, specially designed and fabricated for this study, is presented. The procedure devised allows one to view hair in the SEM while still attached to the panelist's head. The technique is nondestructible to the hair, permitting the study of sequential treatments on the same hair. For example, the evaluation of a shampoo on the hair after 0, 5, 10, and 20 treatments is now possible. The hair is removable from the SEM as many times as required for treatment without the necessity of cutting the hair from the scalp. In addition, the apparatus allows for complete axial rotation of the hair in the SEM.

The functionality of two hair care products (a shampoo and a conditioner) is demonstrated using this technique. MICROGRAPHS of hair damages before and after treatment are categorized and numerically rated. The difference ratio was devised as an index to measure the degree of improvement of damaged sites.

INTRODUCTION

In the past, evaluation of hair care products centered around subjective beauty salon studies. Recently, however, the value of the scanning electron microscope (SEM) in revealing the effects of hair preparations has come to the forefront. This report summarizes part of our investigations into this area and reveals how to employ the scanning electron microscope as a tool to demonstrate the functionality of a hair product. Before giving the details of this

*The Mennen Co., Morristown, N.J. 07960.

investigation it is pertinent to briefly describe the capabilities and limitations of the three microscopic techniques available and demonstrate why the SEM was chosen.

Microscopy of Human Hair

The scanning electron microscope has overcome many limitations of optical microscopy and conventional transmission electron microscopy in elucidating the structure of human hair.

Using the optical microscope, one views a pattern of light and dark areas produced by the reflection or passage through a thin slice of the specimen. Although hair can be viewed without interferences, while maintaining its natural colors, only those parts that lie in the same plane can be reproduced sharply. Also, since light in the visible range of the spectrum is the energy source, the diameter of each part reproduced must be larger than the wavelength of light. Only at low magnifications (below 200 diameters) is the light microscope useful for showing the shape and depth of hair.

In order to avoid this problem, early studies of hair involved the viewing of very thin hair disks. Only limited information can be drawn from this technique, for only an extremely thin section of the specimen can be viewed.

The conventional transmission electron microscope (TEM) far exceeds the magnification of the optical microscope. This enormous magnification (up to 200,000×) allows the study of details which never appeared in the optical microscope. The TEM image is produced by monochromatic electrons that have illuminated a specimen which transmits or scatters the electrons. Once past the specimen, the electrons are then focused on a screen or sheet of film magnifying the image. What one sees is a two-dimensional pattern of light and dark areas produced by passage of electrons through the ultrathin specimen. The use of ultrathin specimens results in an extremely low definition of depth. Since the transmission electron microscope no longer works with light but electrons, the images produced are not colored.

Sample preparation for the TEM is complex and time consuming. Most biological samples are replicated. Acetyl cellulose or a similar material is placed over the specimen which is wetted with methyl acetate. After solvent volatilization the replica is carefully peeled off. This replica, or a second replica, is now shadowed. In this process a heavy metal such as platinum or gold is evaporated in vacuum on the sample surface. What we see then is not the sample but a two-dimensional silhouette of the metal deposits on the hair.

Eliminating the optical microscopy problem of narrow depth of field and the transmission electron microscope limitations of extensive specimen preparations, the SEM has gained preference in today's research endeavors. It is an extremely versatile instrument revealing the exact topographical structure of the specimen. In normal operation, the magnification range extends from 30× to 200,000×. The high depth of focus, a bonus characteristic of

the instrument, reveals extreme architectural detail. Sample preparation is relatively simple. If the sample is conductive it need only to be fastened to the sample mount. Our experience has shown that micrographs of hair up to 1000× may be obtained by this simple mounting procedure. For greater definition of features and magnifications to over 10,000×, the hair is coated with a thin layer of metal, usually a gold palladium alloy.

The SEM is fundamentally different from its TEM counterpart in that the electrons used to produce the image normally are not those from the electron source but low-energy (secondary) electrons released from the surface of the sample. Although the signal is typically produced by these secondary electrons, an image can be produced by any signal resulting from the interaction of the high electron source with the sample. Such interaction produces X-rays, uv radiation, deflected (backscattered) electrons, ir radiation, etc., all of which with the proper detection system could produce an appropriate signal.

The high-energy beam, usually originating from a heated tungsten source, is accelerated, demagnified, and focused to produce a beam spot of approximately 50 Å. Deflection coils placed between the last lens provide means for *X-Y* scanning of the specimen in a rectangular raster. When the electron source strikes the specimen, low-energy electrons are released from the surface. These secondary electrons are drawn to a collector and phototube. The instrument electronics are such as to produce a synchronism between the electron beam and a spot on a cathode ray tube, resulting in a one-to-one correspondence between the position of the electron beam on the specimen and that of the spot on the cathode ray tube. The result is an image produced on a television screen allowing the viewer to infer a three-dimensional structure from a two-dimensional screen.

The SEM was chosen for this study because the micrographs produced contain much more topographical information than other microscopic techniques. The images produced reveal the true surface structure over a wide range of magnifications. It is obvious that for even relatively low magnifications, the SEM has distinct advantages over a standard optical microscope; for example, only the SEM could reveal cuticle uplift or fiber flyaway as shown on Figs. 1, 2, and 3.

History

Electron microscopy has greatly extended our insights into the structure of hair. The first application of the instrument to this field was initiated by Zahn in Germany in the early 1940's (1). This work was continued by others in the United States, Netherlands, and Australia, and by 1948 various methods of replicating the surface of hair were devised (2). However, because of instrument limitations and the nonconductiveness of hair, little work was performed directly on hair itself. Most of the work involved the use of a metallic conductive coating.

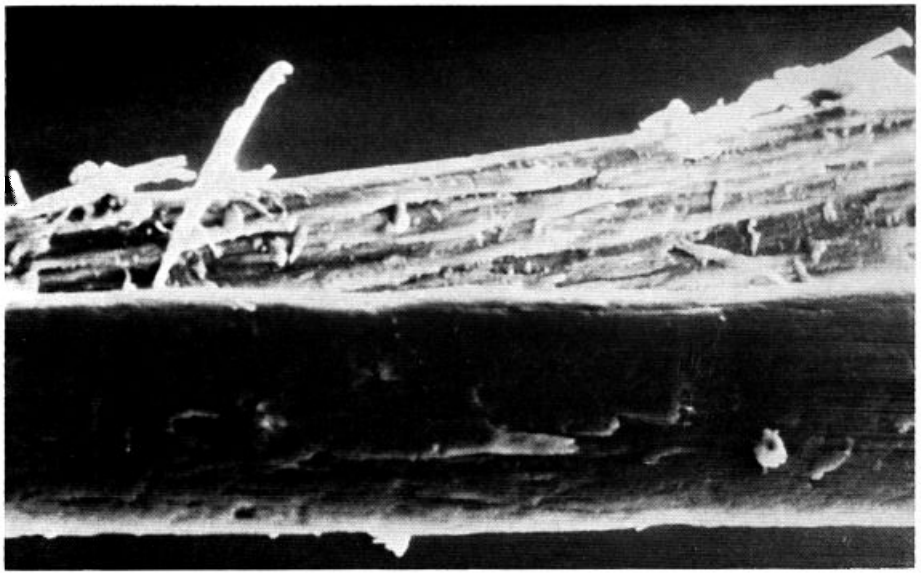

Figure 1. Micrograph of damaged hair fiber (250X) showing exposed cortex

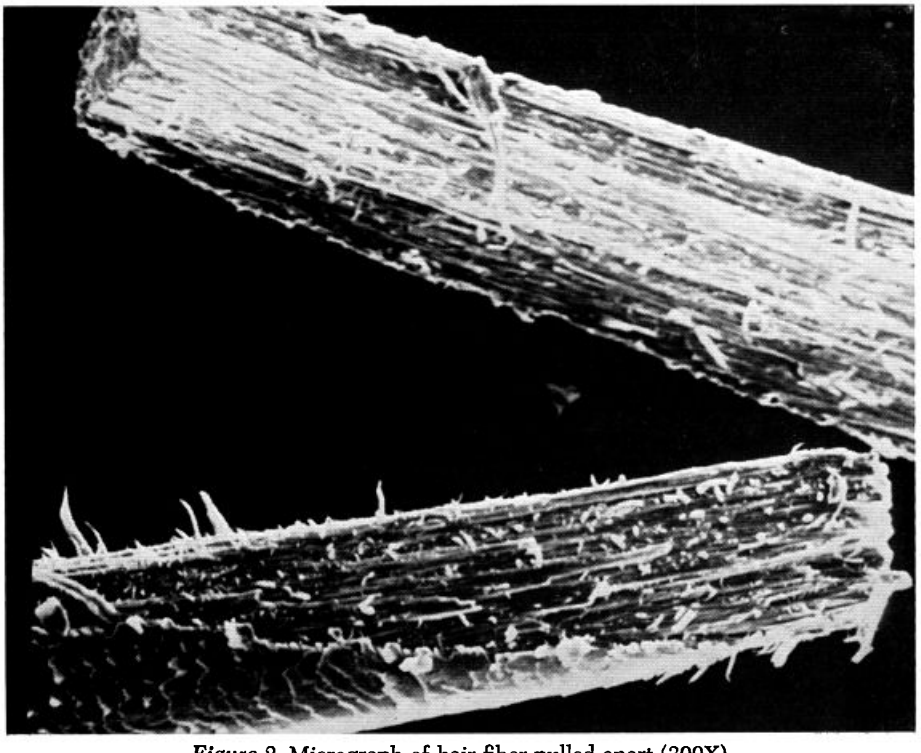

Figure 2. Micrograph of hair fiber pulled apart (300X)

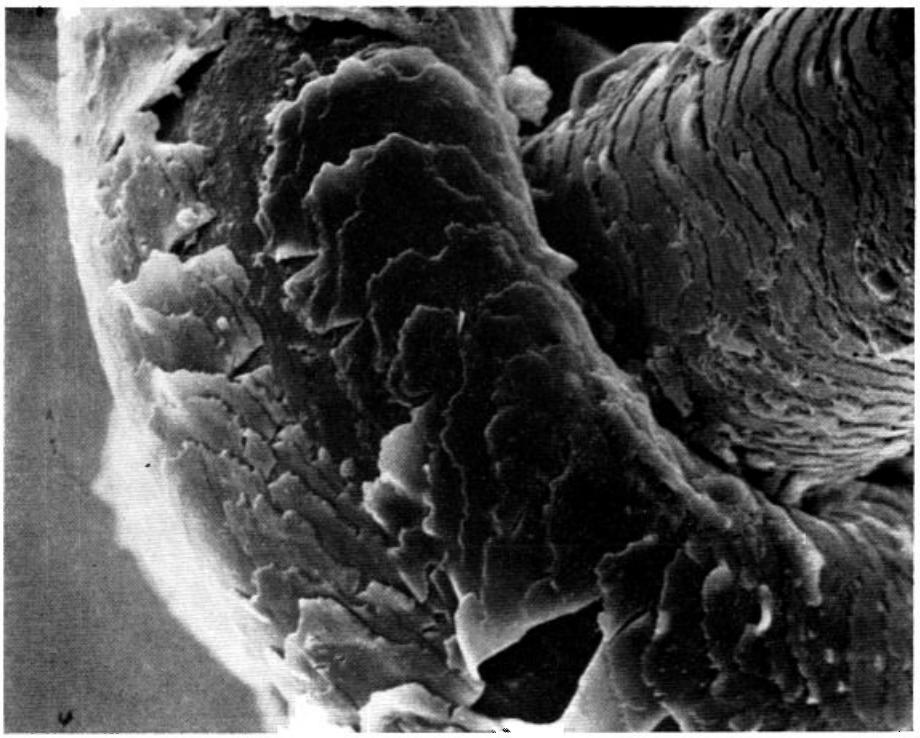

Figure 3. Micrograph of hair fiber pulled into a knot (800X) (note severe cuticle uplift)

Wolfram and Lindemann employed the SEM to reveal the morphological characteristics of hair (3). They pointed out that the cuticle, often neglected when considering the stress-strain properties of hair, may in reality have a substantial effect. The concept of cuticle-cortex ratio was proposed to explain the supercontraction properties of hair. It was found that the level of supercontraction decreases with increasing relative cuticle (decreasing fiber diameter).

Swift investigated the architectural changes of hair surfaces resulting from simple toiletry treatments (4). Although this study gives some insights in how to choose those products with optimum characteristics (e.g., cleaning by shampooing), it still implies that many hairs must be evaluated.

Ayer and Thompson (5) utilized the SEM to study the coatability of hair spray films on individual and small groups of hairs. They investigated the use of several surfactants to improve the coatability and improve the properties of hair spray formulations such as luster and flaking. The technique involved spraying a hair swatch, drying, and gluing it to a mount. The sample was then shadowed with a gold film, placed in the SEM, and viewed.

Experimental

Rotating Hair Stage

All previous SEM studies of hair suffer from the primary experimental difficulty that hair exhibits considerable heterogeneity not only from person to person but also between hairs from the same person and even different sections along the same hair shaft. Consequently, when studying the effect of a product it is extremely difficult to conclude that a particular feature resulted from the treatment and did not already exist prior to the treatment.

In order to overcome these drawbacks that have in the past been taken for granted as being impossible to overcome, a rotating hair stage (RHS)* was designed and fabricated (Figs. 4 and 5). The RHS provides for mounting four different hair shafts, each of which could be rotated around its axis by controls external to the microscope vacuum system. After several attempts, a satisfactory seal was developed consisting of a brass screw-on nut, beveled on the inside, into which fit a carefully slit beveled rubber grommet. When the hairs were placed in the slit, the pressure produced by the beveled screw-on nut was sufficient to produce an excellent high vacuum seal.

The rotating hair stage allowed us to make a completely valid evaluation of hair before and after treatment. The RHS and the SEM parameters used resulted in the ability to perform the following:

1. The identical area on the same hair was evaluated in the before and after micrographs. In order to ensure this, complete axial rotation in addition to the normal *X*-*Y* rotation of the SEM was performed, revealing the entire circumference of the hair.
2. The hair remained on the panelist's head when applying the product. The RHS allowed taking the sample in and out of the SEM without cutting the hair from the scalp.
3. The SEM instrument parameters were adjusted to obtain micrographs of hair without applying a conductive metallic coating. In other words, no special treatment was given to the various hair samples. The charging problems, normally encountered, were minimized by operating the SEM at reduced accelerating voltages (10 kv rather than 30 kv), low beam currents, and very specific settings for the condenser lens and detector power supply.

Test Protocol

Only girls with hair longer than 18 in. in length were selected; no other qualifications were used. Eighteen inches was our estimate of the minimum length required for getting the hairs into and out of the microscope without breaking.

*Designed jointly by the Mennen Co., Morristown, N.J., and Structure Probe Inc., West Chester, Pa.

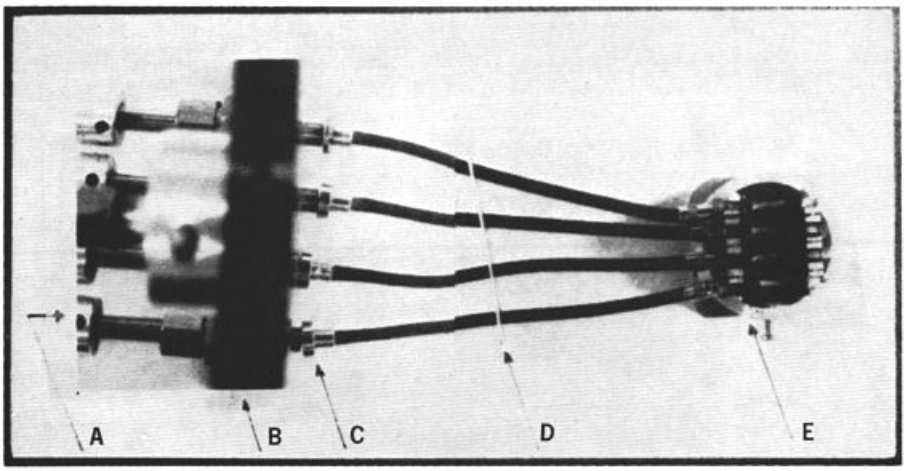

Figure 4. Rotating hair stage
A. External knobs for rotating hair shafts
B. Face plate for bolting into microscope
C. Vacuum seals for cables
D. Cable stabilizer
E. 4 mounting slots for mounting 4 hair shafts

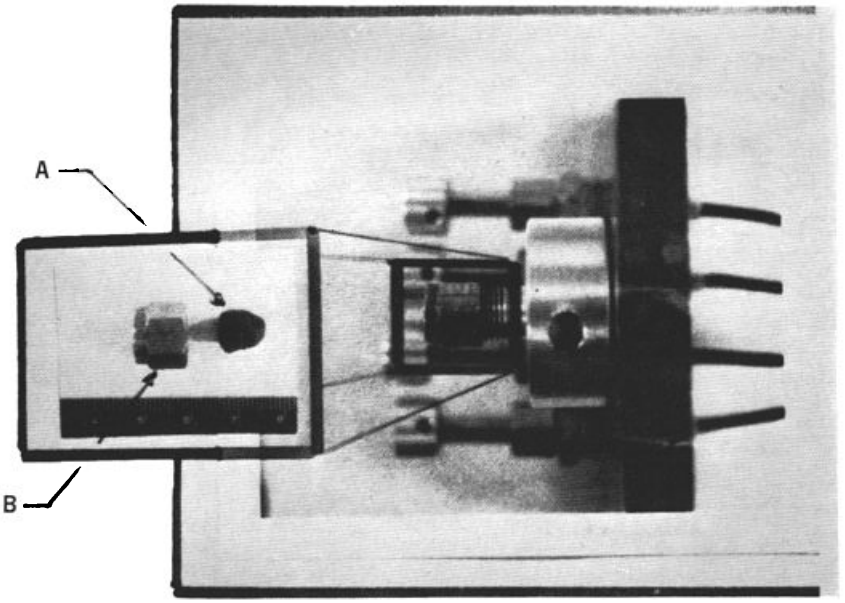

Figure 5. Rotating hair stage showing rubber grommet vacuum seal
A. Slit rubber grommet
B. Beveled nut

No effort was made to use panelists with or without any particular hair types (virgin, bleached, dyed, etc.), textures (fine, medium, coarse), amounts (thin, average, thick), and condition (oily, normal, dry). Table I shows the percentages of different categories finally selected.

Table I
Statistical Breakdown of Panelists' Hair Used in Testing[a]

Types		Amounts	
Virgin	67	Thin	12
Bleached	25	Thick	41
Dyed	8	Average	47
Textures		**Condition**	
Fine	46	Oily	47
Medium	50	Dry	2
Coarse	4	Normal	51

[a]Figures given as percentages.

Each of four selected hairs was color tagged near the scalp, then carefully fed through the rotating hair stage. The hairs were not conditioned or treated in any way. They were held in place with silver-plated wooden toothpick ends. After providing sufficient slack in the device, the rubber grommet was put in place, with the four hairs fitting within the slit. The apparatus was then bolted into the open side post of the SEM.

Using the television scan mode of the instrument, the damaged areas of each hair were located. In some cases the damaged areas were located at considerable distances along the hair shaft. To photograph such damages, montages consisting of as many as 8–10 individual photographs were made. Also, high magnification micrographs were made of selected damaged areas. The panelist's hair was removed from the instrument after a sufficient number of "before" treatment micrographs were taken.

The hairs were removed from the rotating hair stage and allowed to fall back into place. The hairs were now randomly distributed and for all practical purposes were similar to all others on the head. That is, when the product was applied, these hairs received no special treatment. For the shampoo product, the hair was shampooed (two latherings) six times. After each shampooing, the hair was thoroughly rinsed and dried with an electric drier. The hair conditioner product, a leave-on type, was applied to slightly wet hair and electrically dried. After treatment the panelist's tagged hairs were returned to the instrument and the "after" micrographs taken.

By viewing the hair by means of the TV mode and rotating it, we were able to find the exact area of the hair shaft and photograph the repaired area. In addition to the photographs, videotape data were also collected. This ability to view the scanning of a hair shaft while rotating it allows one to visually

appreciate the improvement of each hair shaft. The videotape data contain, for several reasons, a considerably greater amount of information than could ever be recorded on Polaroid film. Of these reasons, perhaps the most important is the fact that considerably higher magnification information can be recorded on videotape. This is possible because any image drift, which would ruin a 50-sec photographic exposure, does not have this catastrophic effect when videotaping. All videotaping is accompanied by narration which also documents the panelist's number, and time and place the data were recorded.

Discussion and Results

Healthy hair is composed of three proteinaceous layers: the medulla, which is the central core—rarely found throughout the entire shaft; the cortex, extremely long fibrils comprising most of the hairs volume; and the cuticle, the outer layer of overlapping plates. After enduring several years of exposure, abrasion, and styling, even well-treated hair becomes damaged.

After viewing many hairs under the SEM, it was found that, with some overlapping, the damage areas could be classified into four general categories (i.e., flyaway fibers, exposed cortex, split ends, and general shaft damage).

Figure 6. Damage category—flyaway fibers

Examples of each category are shown in Figs. 6 through 9. Also, a dramatic pair of before and after micrographs from an actual panelist, showing the improvement after treatment, is shown in Figs. 10 and 11.

To evaluate the degree of improvement resulting from the product treatment a numerical system was required. The difference ratio (DR), which is an index to measure the improvement of each damaged site, was devised and found to be a meaningful system to communicate the repair without being misleading.

Each "before" and "after" pair of micrographs was classified into one of the four mentioned damage types, and each damage site assigned a damage point from 0 to 4. (The higher the number the more severe the damage.) The DR for each site may be calculated by:

$$DR = \frac{\text{damage points after treatment}}{\text{damage points before treatment}}$$

A value of 1.0 indicates no change with treatment. DR values below 1.0 show improvement of a damage site and those above 1.0 indicate additional damage resulting from the treatment. A summary of the results is listed in Table II.

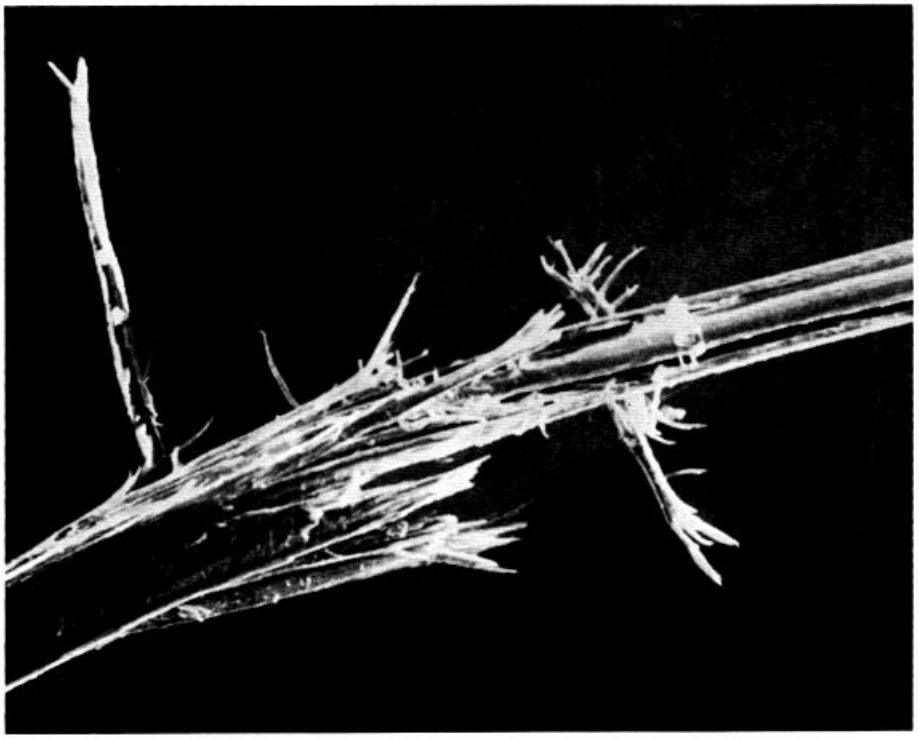

Figure 7. Damage category—exposed cortex

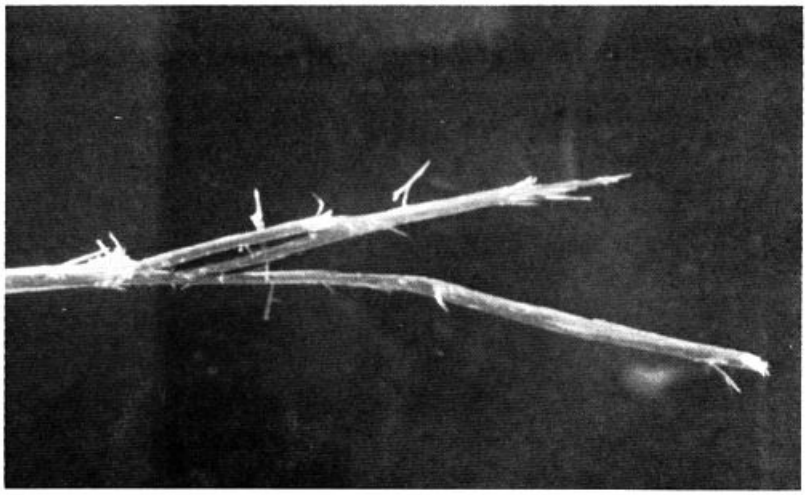

Figure 8. Damage category—split end

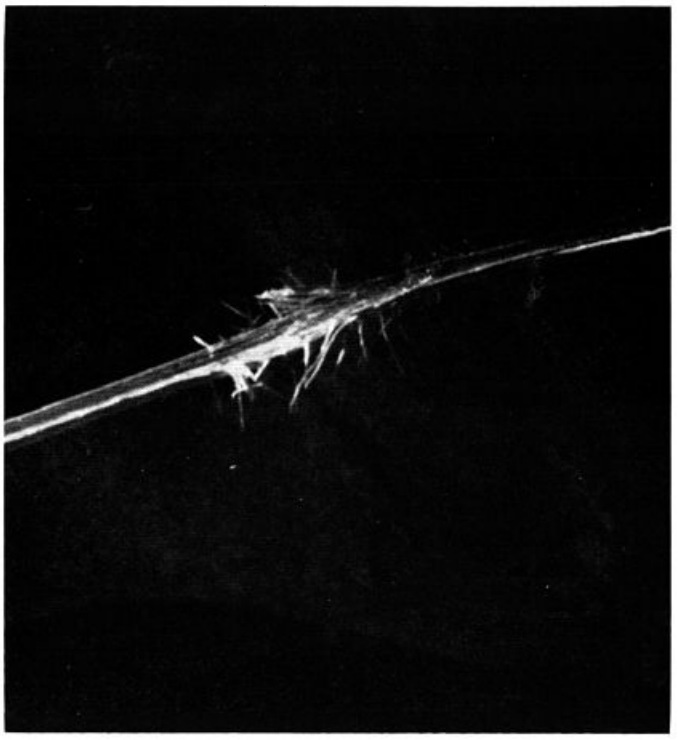

Figure 9. Damage category—general shaft damage

Figure 10. Micrograph of general shaft damage before treatment with conditioning formulation

Table II
Difference Ratio (DR) Values

	Conditioner[a]	Shampoo[a]
Total number of panelists	50	15
Total number of hairs evaluated	192	59
Total number of damage sites evaluated	442	127
Average difference ratio calculated	0.26	0.47
DR range per panelist	0.03–0.66	0.28–0.71

[a]Shampoo and leave-on conditioner supplied by the Mennen Co., Morristown, N.J. Both products are protein formulations.

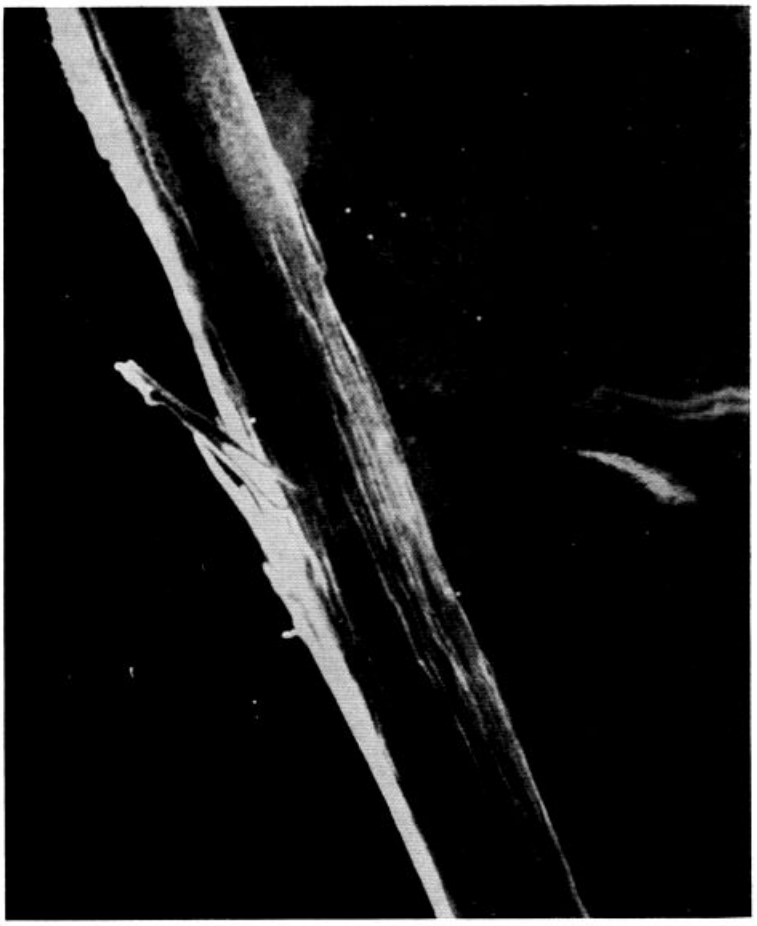

Figure 11. Micrograph of same area as in Fig. 10 showing repair of shaft damage with one application of conditioner

A closer look at the damage categories revealed that split ends account for 35% of all hair damage studies (Tables III and IV). Flyaway fibers and general shaft damage were found in 32 and 25% of the damaged sites, respectively.

Table III
Results of Shampoo Treatment

Damage Category	Category Per Cent	Per Cent Improved
Flyaway fibers	35	93
Exposed cortex	7	88
Split ends	35	60
Shaft damage	23	93

Table IV
Results of Conditioner Treatment

Damage Category	Category Per Cent	Per Cent Improved
Flyaway fibers from cuticle	28	94
Exposed cortex	8	93
Split ends	35	95
General shaft damage	26	92
Miscellaneous	3	80

This study demonstrates improvement of all damage types after treatment. For example, based on a minimum reduction of one damage point in our rating system, over 90% of the damaged site improved after treatment with the hair conditioning formulation. In fact, the conditioner actually closed 60% of the split ends (improved to zero column). The shampoo formulation tested improved all types of hair damage (Table III). This study not only demonstrates improvement of damage sites but reveals the products' ability to repair different types of damages.

Summary

A systematic approach to evaluate hair care products using the SEM has been developed. The technique, employing a rotating hair stage (RHS) allows for maximum rotational freedom of the hair in the SEM. The hair, while still attached to the scalp, may be removed and re-introduced into the SEM as often as necessary.

The difference ratio (DR), a numerical system showing the degree of change, revealed significant improvement of damaged sites after treatment with a hair conditioner and shampoo.

Acknowledgment

The author is grateful to Dr. C. Garber, President of Structure Probe Inc., for his continuing efforts and suggestions. All micrographs shown were taken at Structure Probe Inc.

(Received January 29, 1973)

References

(1) Fraser, R. D. B., and Rogers, G. E., *Aust. J. Biol. Sci.*, **8**, 129 (1955).
(2) Barnes, R. B., Burton, C. J., and Scott, R. G., Electron microscopical replica technique for the study of organic surfaces, *J. Appl. Phys.*, **16**, 730 (1945).
(3) Wolfram, J. L., and Lindemann, M. K. O., Some observations on the hair cuticle, *J. Soc. Cosmet. Chem.*, **22**, 839 (1971).
(4) Swift, J. A., New developments in electron microscopy, *Ibid.*, **22**, 477 (1971).
(5) Ayer, P. A., and Thompson, J. A., Scanning electron microscopy and other new approaches to hair spray evaluation, *Ibid.*, **23**, 617 (1972).