INTRODUCTION

The hair fiber is composed of three parts, namely, cuticle, cortex, and medulla [Figure 1b]. A cortical cell, major contributor to the hair tensile properties, consists of macrofibrils and matrix resembling composite structure. Each macrofibril contains intermediate filaments or microfibrils [Figure 1a]. Mechanical properties of α-keratin fibers are primarily dependent on the matrix in which intermediate filaments are embedded.[1] Tensile properties of twisted hair samples revealed that twisting followed by untwisting and giving 10 min time for relaxation has resulted in the properties same as of virgin hairs. However, twisting alone reduced the properties of hair such as moduli, strength, and strain values.[2] Nikiforidis et al.[3,4] studied hair tensile properties of two age groups considering one scalp position and also studied the properties of one age group (15–20 years) considering two scalp positions (P1-frontal and P4-occipital [Figure 2a]). His group reported the need to consider the viscoelastic behavior of hairs and the importance of linking the modulus ratio and energy ratio to the elastic and viscous behavior of hair, respectively. The physical and tensile properties of hair of pigs with different breed and position (neck and back) were studied and found to be almost similar to human hair and wool[5] despite reporting a lower value of ultimate strain (32%). Slight variation in tensile properties[5] with respect to breed and body position was also reported. Erik et al.[6] reported...
that the tensile breaking force of hair has not shown any significant dependency on gender, age, and presence of dyes. In another study, no significant differences were observed when tensile properties of hair were compared between vegetarian and nonvegetarian and pigmented and nonpigmented, but slight differences in elongation were observed when hair from young (<12 years) and old (>60 years) samples were compared. It is also reported that beard hair has less tensile modulus compared to scalp hair due to a lower percentage of disulfide bonds. Srinivasan et al. has reported that hardness of water has no influence on tensile properties of hair. Sayahi et al. reported that modulus shows a significant dependency on age, whereas cross-sectional area and maximum load are influenced by “age” and “curl type.” None of the above studies focused on a mechanical characterization of hair from different positions of the human scalp, despite scalp positions being subjected to varying magnitudes of environmental, physical, and hormonal stimuli and correspondingly different hair balding patterns are being observed. Commonly observed baldness patterns reveal low hair density at positions P1 and P3 for men and at position P3 for women [Figure 2a]. In this paper, an attempt has been made to find the tensile properties of human hair collected from four positions of the scalp namely frontal-P1, temporal-P2, parietal-P3, and occipital-P4 [Figure 2a]. The primary objective of this study is to quantify the differences among hair from four different scalp positions based on single fiber tensile tests followed by statistical analysis. Since the tensile properties are obtained due to responses from various structural units of hair such as, crystalline α-keratin, amorphous keratin, α + β keratin, and β keratin, the second objective is aimed to study the stress-strain curves in order to investigate structure–property relationship in regards to hair obtained from various scalp positions.

**MATERIALS AND METHODS**

Hair samples from people of five different families with ages ranging from 5 to 41 years were collected from four positions of the scalp. Hair samples were collected from 15 healthy people comprising 9 males and 6 females of Hyderabad, India (Caucasian origin), with their consent, documenting their age and gender anonymously. To reduce the thickness heterogeneity, samples were cut to 2 cm above the scalp from...
all four positions [Figure 2] and washed with mild shampoo, then with water and dried at room temperature. Minimum of 45 fibers per scalp position were used for tensile testing.

For tensile testing of single hair fibers, samples were prepared using paper frame technique as per the ASTM D3379–75 [Figure 2b–d]. Detail description, including video of tensile test is provided in our earlier publication.[13] The prepared samples were conditioned at 22°C and 55% relative humidity for a duration of 72 h because relative humidity has a large influence on hair tensile properties.[14,15] The prepared samples were then subjected to tensile test (TA-XT Plus, Stable microsystems, UK) using 500 N load cell. Instrument is located in a room maintained in a controlled temperature of 22°C ± 2°C and relative humidity of 50% ± 6%. Gauge length and test speed were 20 mm and 20 mm/min, respectively. Post tensile test, two matching fracture pieces were observed in the stereomicroscope (Olympus SZX7) to see any jaw breaks and slippage from the glue. For successful tests, load (mN)–depth (mm) plots were converted to stress (MPa)–strain (%) plots using fiber diameter and initial length. Diameter of samples prepared for the tensile test was measured using an optical microscope (Metavis U400) in transmission mode. Since the difference in hair diameters between longitudinal (average of three values) and cross-sectional view measurements (after embedding and microtoming) is between 5% and 15%, it was concluded that longitudinal view was acceptable and appropriate. From the stress–strain plots, accurate values for yield stress, modulus, maximum stress, work of elongation, and slope in the postyield region were obtained using Origin software. The fractured hair samples were also studied using optical microscopy and scanning electron microscopy (SEM) (Hitachi S-4300 SE/N) to understand the influence of incremental modulus on hair fracture. SEM was used after sputter coating and macrofibrib diameters were measured using Image J software (http://imagej.net/Help#Ways_to_get_help).[16]

Two different statistical methods were used to conclude the influence of scalp position on hair tensile properties, that is, yield stress, modulus, maximum stress, and work of elongation because these are impacted significantly by hair structure and dynamics. The first method is relative rating [Table 1], which is based on rating the four properties for one position individually (for 15 people) followed by cumulative rating, which involves summation of individual ratings of four properties. In individual rating, a scale of 1–5 was used with 1 and 5 being the lowest and highest set of values, respectively, as shown in Table 1. Finally “sum of the rating” is obtained for each position by summation of cumulative ratings. The second method called grey relational analysis (GRA) is used mainly in solving interrelationships among the multiple responses.[17] In GRA method, position, age, and gender are considered as factors (input parameters) with levels of 4, 3, and 2, respectively [Table 2a]. Similarly, yield stress, modulus, maximum stress, and work of elongation are considered as responses (output parameters). After determining the number of experiments with full factorial method using Minitab software which was obtained from Qsutra, U/o Cubic Computing (P) Ltd, Bangalore, Karnataka, India,[18] [Table 2b], GRA was applied to find the optimum factor-level combinations by measuring the grey relational grade for all 24 (levels of 4 × 3 × 2) combinations using four-step procedure described in the literature.[19] Finally “grey relational grade” is obtained for each position, gender, and age group [Table 3]. ANOVA was also used to compare the tensile properties between P1 and P4 positions, but we compared one property at a time such as yield stress with positions. But in GRA, the four properties together (as grey relational grade) can be compared with positions as explained in Table 3. Similarly, we can also do such comparison in relative rating method. The major advantages of the GRA method compared to relative rating method are that all four tensile properties can be compared with respect to position, gender, and age simultaneously as shown in Tables 2b–4. In relative rating, comparison needs to be done separately.

**RESULTS**

Figure 3 represents the stress–strain data with respect to positions P1–P4. For clarity, the data is restricted to two samples (male 15 years, female 29 years) and only representative plots are shown. From the plots obtained, it can be said that stress–strain curves show four characteristic regions (R1–R4) [Figures 3 and 4]. The first region is a Hookean region from 0% to ~3% strain. The second region is a plateau of constant stress (yield region) in which strain increases significantly without much increase in stress (between 4% and ~18% strain). The third region is a postyield region 1 in which strain increases with stress till about 33% strain followed by fourth region also known as postyield region 2 in which yielding and an ultimate failure occur between ~33% and 45% strain. In the third region, α to β transition continues followed by strain-induced alignment of the β-sheets and continuous increases of postyield modulus until ~33% strain wherein it exhibits maximum slope of 500–700 MPa [Figure 4]. As soon as the slope, that is, modulus in the postyield region starts to decrease; amorphous matrix starts to yield. Although 290–420 MPa of postyield modulus was reported for wool fibers,[19] the reduction of postyield modulus (i.e., change
of slope) at around 33% strain was not discussed earlier. In fact, we have seen kinks in few plots at around 33% strain, so we measured slope of the stress–strain plots and correlated with SEM fractographs for almost 20 samples and found that slope decreases consistently at around 33% strain. Full description regarding the fourth region, that is, ~33%–45% strain, is provided in discussion part.

The tensile properties of all 15 samples with respect to positions P1 to P4 are accurately measured using origin software and mean values were compared (not shown). In addition to mean values, Box-and-Whisker plots were also constructed [Figure 5] to compare the yield stress, maximum stress, modulus, and work of elongation with respect to the scalp positions. The reason for choosing these four properties is that they represent the four regions of the stress–strain curves previously described. As shown in Figure 5, Box-and-Whisker plots show no significant differences between positions when mean yield stress values are considered, though hair from P2 position revealed lowest yield stress. Mean values of the modulus were almost same for P1–P4 positions, but the value of the P1 position is dominated by three samples (F41:8223; M15:6701; F32:6195 MPa). A similar trend was observed in the case of maximum stress and work of elongation. Although significant variations in overall range and interquartile range are seen [Figure 5], minimal mean variations between the positions lead to data optimization using two different statistical methods, relative rating and GRA.

The two statistical parameters, namely, “sum of the rating” and “grey relational grade” with respect to scalp positions P1–P4 are shown in Figure 6. When the “sum of the rating” of the four positions are compared [Figure 6a], it resulted in slightly higher values for P1, P3 (140, 142) compared to P2, P4 (129, 128) positions. It is found that no significant difference exists between P1 and P4 positions if the limits (60–300) of the “sum of the rating” are considered.
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Table 2a: Factors and their assigned levels considered in Grey Relational Analysis (GRA)

| Control factor | Level 1 | Level 2 | Level 3 | Level 4 |
|----------------|---------|---------|---------|---------|
| Gender         | M-Male  | F-Female|         |         |
| Age (years)    | L1 (0-14)| L2 (15-29)| L3 (30-44)|         |
| Position on scalp | P1 (front) | P2 (side) | P3 (center) | P4 (back) |

Table 2b. Experimental layout following a full factorial design L24 that is gender with 2 levels, age with 3 levels and position with 4 levels (2*3*4) considered in testing and analysis (MINITAB software)

| Expt. no | Gender | Age | Position |
|---------|--------|-----|---------|
| 1       | F      | L1  | P3      |
| 2       | F      | L1  | P2      |
| 3       | M      | L3  | P3      |
| 4       | M      | L2  | P3      |
| 5       | F      | L2  | P2      |
| 6       | F      | L1  | P4      |
| 7       | F      | L3  | P4      |
| 8       | F      | L3  | P1      |
| 9       | M      | L1  | P1      |
| 10      | M      | L1  | P4      |
| 11      | F      | L2  | P1      |
| 12      | M      | L2  | P1      |
| 13      | M      | L2  | P3      |
| 14      | F      | L2  | P4      |
| 15      | M      | L2  | P4      |
| 16      | M      | L3  | P2      |
| 17      | F      | L1  | P1      |
| 18      | F      | L3  | P2      |
| 19      | M      | L2  | P2      |
| 20      | F      | L2  | P3      |
| 21      | M      | L1  | P2      |
| 22      | M      | L3  | P4      |
| 23      | M      | L1  | P3      |
| 24      | F      | L3  | P3      |

From Figure 7, hair diameters of positions P1–P4 were found to be in the range of 65–90 μm excluding few outliers. One literature\(^{[24]}\) reported that aging causes increase in the hair diameter until 20–30 years of life and after which it causes a decrease in diameter, but such trend was not observed in this study. Here, a difference of 10–20 μm in diameter was observed when individual hair diameters were compared between scalp positions (P1 and P4). But average values revealed lowest hair diameter for P4 position (76 μm) and highest hair diameter for P1 and P2 positions (81 μm). The fact is that age is not correlating with diameter [Figure 7 and also our previous publication ref. 13\(^{[24]}\)] due to variation of medulla index and presence of structural heterogeneities. As mentioned in experimental section, stress values (load/area) were considered in analysis instead of load values to avoid the influence of diameter variation. From Figure 7, it was also found that diameter is reduced by approximately 2 μm irrespective of the position as a result of tensile stretching and it could be due to high Poisson's ratio ~0.8 reported for keratin fiber.\(^{[21]}\)

**DISCUSSION**

**Tensile properties and statistical analysis**

Based on tensile property values [Figure 5] and statistical observations [Figure 6], slightly higher mean values were observed for the P1 position. Relative rating method has resulted slightly higher values for P1 and P3 positions [Figure 6a]. Although the difference is not significant, hair from position P4 has lower tensile values compared to other three scalp positions and it is true from experimental as well as statistical observations. Despite having higher diameter for the P1 position compared to other three positions, the slight increase in tensile values for position P1 could be due to the strengthening of the hair composite structure, that is, orientation of the microfibrils and decrease in medulla diameter. From Figures 5 and 6, it can be concluded that the proposed relative rating method and GRA are in agreement with observational data despite severe heterogeneity and variability in human scalp hair. The single observation from all three methods (Box-and-Whisker plot, relative rating, and GRA) is that scalp position values are almost same irrespective of “mean value data” or “cumulative sum of the rating” or “grey relational grade” are considered [Figures 5 and 6]. To support the “mean value data,” we have also carried one-way ANOVA in which P values are found to be 0.76, 0.88, 0.85, and 0.88 when scalp position is considered as source of variation for yield stress, modulus, maximum stress, and work of elongation,
Table 3: Grey relational analysis for single fibre tensile properties of hair. Grey relational coefficient of each response (YS, M, MS, WE) and “Grey relational grade” calculated from them for each experimental run is shown in the last column of the table

| Exp. no | Experimental data (response) | S/N ratios for original response data | Grey Relation Coefficient (GRC) | Grey Relational Grade |
|---------|------------------------------|-------------------------------------|---------------------------------|----------------------|
|         | YS (MPa) | M (MPa) | MS (MPa) | WE (MJ/m²) | YS (MPa) | M (MPa) | MS (MPa) | WE (MJ/m²) |       |       |       |       |       |       |       |       |
| 1       | 37.4273 | 69.1456 | 43.7009 | 34.3955 | 0.3713 | 0.3465 | 0.3397 | 0.4487 | 0.3765 |
| 2       | 39.9152 | 72.4236 | 46.1127 | 34.8259 | 0.4731 | 0.5000 | 0.4715 | 0.4761 | 0.4802 |
| 3       | 40.7226 | 74.4834 | 46.6266 | 35.5120 | 0.5193 | 0.5027 | 0.5140 | 0.5179 | 0.5353 |
| 4       | 41.9691 | 73.6997 | 47.4270 | 36.5286 | 0.6115 | 0.6046 | 0.5978 | 0.5981 | 0.6030 |
| 5       | 36.8836 | 69.8349 | 43.5359 | 34.5922 | 0.3546 | 0.3702 | 0.3333 | 0.3410 | 0.3498 |
| 6       | 42.7164 | 74.7042 | 47.3661 | 35.5637 | 0.6844 | 0.7236 | 0.5905 | 0.5213 | 0.6299 |
| 7       | 40.2083 | 72.3756 | 45.3126 | 34.9143 | 0.4889 | 0.4968 | 0.4777 | 0.4811 | 0.4711 |
| 8       | 41.3859 | 74.5118 | 47.2084 | 36.9726 | 0.5646 | 0.6793 | 0.5233 | 0.6376 | 0.6180 |
| 9       | 39.9386 | 71.4133 | 46.8802 | 35.6909 | 0.4743 | 0.4398 | 0.5379 | 0.5301 | 0.4955 |
| 10      | 38.9130 | 72.0376 | 45.9989 | 32.5582 | 0.4260 | 0.4752 | 0.4690 | 0.3783 | 0.4350 |
| 11      | 36.1109 | 68.7328 | 43.8191 | 32.2682 | 0.3333 | 0.3333 | 0.3444 | 0.3660 | 0.3443 |
| 12      | 44.6691 | 76.1126 | 49.3990 | 39.2783 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 13      | 44.0406 | 73.1738 | 45.0091 | 33.5837 | 0.4315 | 0.4378 | 0.4004 | 0.4513 | 0.4212 |
| 14      | 38.5330 | 71.2462 | 44.6155 | 33.6248 | 0.4206 | 0.4312 | 0.3800 | 0.4472 | 0.4097 |
| 15      | 40.8050 | 71.9629 | 46.6874 | 35.6104 | 0.5245 | 0.4706 | 0.5195 | 0.5245 | 0.5098 |
| 16      | 39.9629 | 72.4153 | 46.6705 | 35.5230 | 0.4756 | 0.4995 | 0.5179 | 0.5187 | 0.5029 |
| 17      | 39.8955 | 71.7707 | 43.6947 | 31.1850 | 0.4721 | 0.4594 | 0.3395 | 0.3333 | 0.4011 |
| 18      | 40.0121 | 73.3588 | 46.1140 | 35.5764 | 0.4782 | 0.4957 | 0.4716 | 0.5222 | 0.4919 |
| 19      | 43.5741 | 73.5195 | 48.1757 | 38.2973 | 0.7928 | 0.5872 | 0.7056 | 0.8069 | 0.7226 |
| 20      | 38.2711 | 70.4461 | 44.2841 | 33.2891 | 0.4005 | 0.3944 | 0.3643 | 0.4032 | 0.3906 |
| 21      | 38.3207 | 72.1236 | 45.7543 | 33.9225 | 0.4024 | 0.4811 | 0.4458 | 0.3549 | 0.4210 |
| 22      | 39.5903 | 71.4878 | 45.7607 | 35.0855 | 0.4524 | 0.4438 | 0.4462 | 0.4911 | 0.4584 |
| 23      | 38.6684 | 72.6345 | 46.1294 | 33.1999 | 0.4159 | 0.5147 | 0.4727 | 0.3997 | 0.4508 |
| 24      | 41.2361 | 74.1101 | 46.5242 | 35.7786 | 0.5537 | 0.6481 | 0.5049 | 0.5362 | 0.5607 |

**Table 4: Grey relational grade (mean) of all factor-level combinations**

| Control factor | Level 1 | Level 2 | Level 3 | Level 4 | Δ(maximum−minimum) |
|----------------|---------|---------|---------|---------|---------------------|
| Gender         | 0.5445  | 0.4603  | -       | -       | 0.0842              |
| Age (years)    | 0.463   | 0.5412  | 0.5047  | -       | 0.0800              |
| Position on scalp | 0.5467 | 0.4947  | 0.4825  | 0.4857  | 0.0642              |

Bold figures represent the higher value (optimal value). Gender, age, and position have negligible influence on tensile properties because of smaller values of Δ were found.

respectively. This indicates that scalp position is not able to influence any of the tensile property because \( P > 0.05 \).

Lack of significant differences in tensile properties between the positions could be due to the presence of micro and nanoscale structural heterogeneities that exists in the hair shaft. This can be explained by assuming hair cortex as nanocomposite in which different alignment structure of rigid intermediate filaments (including the degree of orientation of \( \alpha \) helices) could lead to different structural heterogeneities in cortical cells. It can also be due to the negation of data because of heterogeneity and variability due to age, gender, diet, and lifestyle among the samples considered. One study[8] also reported no difference in age and gender when single fiber properties were considered, but no information was given regarding scalp positions. In other literature,[9] slight variation in tensile properties with respect to “position on pig body” was reported, but the study was not restricted to a single area of the body like scalp in humans.

Since grey relational grade is considered as multiobjective performance index, mean grey relational grades were also compared for gender and age [Table 4]. No significant difference between the levels was observed, indicating that age and gender are not able to influence the tensile properties like scalp position. From one-way ANOVA analysis, \( P = 0.73, 0.76, 0.96, \) and 0.33 when age (between individuals) is considered as source of variation for yield stress, modulus, maximum stress, and work of elongation, respectively. However, the
P = 0.037 (<0.05) was obtained for maximum stress when gender was considered as source of variation. As shown in Table 3, grey relational grade value of 1 was observed for “Male-Position P1-age group 15–29 years” indicating that hair collected from this combination can have better tensile properties compared to other combinations shown in Table 2.
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Structure–property correlation

The following discussion describes the investigation of structure–property relationship with respect to the tensile properties obtained from hair in order to obtain a better understanding of the heterogeneous and composite structure of hair. Stress–strain plot of a human hair is normally split into three regions,\(^{[21,22]}\) but the plots are split into four regions in this study [Figures 3 and 4]. Postyield incremental modulus until \(\sim 33\%\) strain is reported in the literature\(^{[1,19,23,24]}\) as the third region, but the fourth region, that is, decrease of postyield modulus between \(\sim 33\%\) and \(45\%\) strain is not discussed earlier. A brief structure–property correlation is provided below considering the cortical cell as a composite comprising of crystalline and amorphous domains\(^{[23]}\) for detailed understanding of all four regions and four tensile properties described in this study.

1. The elastic modulus or the position of linearity qualitatively provides information regarding the packing, overall rigidity and stability of \(\alpha\)-keratin crystals which are embedded in the cross-linked amorphous keratin matrix. The crystalline domain stability arises from the degree of elastic response due to its intra and inter packing and the stability of the interfaces between the crystalline and amorphous matrix.

2. The yield stress qualitatively provides information regarding the percentage of crystallinity. In combination with vibrational modes, the effective elastic stress transfer or resistance to yield occurs due to predominantly shear modes within \(\alpha\) helix molecule which are elastic in nature. It has been reported in the literature\(^{[23]}\) that the onset of yield corresponds to significant conformational rearrangement, wherein the helix molecules begin to

\[\text{Figure 5: Box-and-Whisker plot of hair tensile properties of 15 people considered in this study. Data is shown with respect to the position on the scalp (P1–P4). Each Box-and-Whisker plot represents 45 values (15 hair samples with 3 replicates).}\]

\[\text{Figure 6: Sum of the rating (a) and grey relational grade (b) are shown with respect to scalp positions P1–P4. Note that no significant difference exists between positions when tensile properties are compared though P1 has slightly better tensile properties than P2, P3, and P4.}\]
unravel to form random coils. Hence, the resistance to such conformational changes would relate to the amount and stability of the helices that form the crystalline domains indicating that the yield stress value corresponds to the percentage of crystallinity.

3. The percentage of strain in the plateau stress region qualitatively provides information regarding the degree and kinetics of conformational events. The conformational events correspond to predominantly segmental motions in the amorphous network, which are entropic in nature. The higher the plateau stress region, the higher the degree of conformational motions in the soft and elastomeric amorphous matrix. However, if the plateau stress region existed beyond a strain value of 15%–20% based on relative humidity, it would correspond to additional contributions from α-helical keratin microfibrils being unraveled to form random coils.

4. The incremental change in slope of the postyield region qualitatively provides information regarding the degree of the continued unraveling of the α-keratin microfibril followed by repacking of the random coils to form β-sheets until which the random coils disappear completely to form recrystallized β-sheet structure. Further, there is also an additional contribution arising from the strain induced alignment of the formed β-sheets and its stability in the amorphous matrix. Correspondingly, this results in increased resistance to deformation until the β-sheet macrofibril bundles are pulled out from the supporting amorphous keratin matrix of variable mechanical integrity. In case a highly networked, elastic, and mechanically integrated amorphous matrix is present, it is primarily due to the formation of disulfide linkages as a result of cysteine-cysteine covalent interaction.

5. The second yield point (f) as shown in the stress–strain plot is an indication of the yield occurring in the amorphous matrix until some bonds are permanently broken. The failure point corresponds to the ultimate failure at higher strain, wherein the bonds of the amorphous matrix are permanently broken along with severe straining and pullout of the crystalline β-sheets.

For specific structure–property description, the stress–strain curves of M15 and F29 are taken as representative plots and the discussion is as follows. For M15, from the curve at the P1 position, it can be seen that the elastic modulus and yield stress values are the highest and this is an indication that the α keratin is relatively stable and is embedded well in the amorphous matrix. In addition, the curve also indicates...
that the percentage of crystallinity is highest among the tested samples. For P1, the percentage of strain in the plateau stress region is the lowest and this is followed by a significant increase in the postyield modulus (maximum slope 800 MPa at around 32% strain). This indicates that the degree of entropic response of the amorphous network is low and possibly the amorphous network is a hard elastomer. Postyield, the increased resistance to deformation as observed by the postyield modulus change for all positions (P1–P4) is due to the unraveling of the α keratin to random coil followed by repacking to β-sheet structure and the strain-induced orientation of the same. Further, an additional contribution to resistance due to effective stress transfer between the stable α and β fibrils and the amorphous keratin matrix is possible. In the case of P4 position, the curve indicates lower stability of α keratin, a lower degree of crystallinity, a relatively soft amorphous network resulting in decreased postyield properties (maximum slope is 500 MPa at around 32% strain). In contrast to M15, for F29, the curves for all four regions exhibit a very low stability of α keratin, a low degree of crystallinity, and a very soft amorphous network resulting in highly reduced postyield properties (maximum slope <500 MPa at around 32% strain for all four regions).

SEM studies were carried out as only a representation for fiber pullout and second yield (postyield modulus decreases at around 33% strain) and does not contain any statistical value. SEM fractograph of M38 hair with step fracture shows severe macrofibril pullout and fibrillation on the lateral surface [Figure 4b and c]. Bundle of macrofibrils that have been pulled out as a result of step fracture [Figure 4c] have diameter between 400 nm - 2 μm and lengths between 6 and 15 μm. The step fracture may be influenced by the defects close to the center or medulla. SEM fractograph of M5 sample with smooth fracture [Figure 4e and f] shows layer pullout and propagation of cracks in the radial direction indicating that defects in the center or medulla region have initiated the fracture. Similar values of postyield modulus (maximum of 600 MPa) were obtained in both cases despite one being step and other is smooth fracture mode because crack initiation happened from the medulla region which is rich in β-pleated sheets.

**CONCLUSION**

The four tensile properties of stress–strain diagram, Young’s modulus, yield stress, maximum stress and work of elongation were considered and statistical ratings were given separately for all four scalp positions P1–P4 using two different methods. In both methods, the overall rating with respect to scalp positions did not show significant quantitative differences and it could be due to the presence of micro and nanoscale structural heterogeneities.

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**Conflicts of interest**

There are no conflicts of interest.

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