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Nanoindentation of Hair Cortex and Medulla Regions

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Abstract: Reports related to cuticle damage and hair's mechanical properties are of interest to dermatologists in treatment of hair disorders. Apart from tensile test, few studies were reported on mechanical properties of hair, especially nanomechanical properties of cortex and medulla regions. Inner diameter of hair fiber (8-12 μm) is considered as medulla and it exists in hair at the beginning of the anagen phase. This study is aimed for quantitative comparison of nanomechanical properties between cortex and medulla and also with respect to donor age by performing nanoindentation on fiber cross sections using two different instruments. The medulla and cortex regions revealed hardness values of 70-170 MPa and 200-312 MPa, respectively. Similarly lower values of indentation modulus were observed for medulla compared to cortex because medulla has poorer orientation of microfibrils compared to the surrounding cortex. This study also found a linear correlation between hardness and indentation modulus especially for medulla region. This could be due to the fact that organization of the microfibrils increases from the medulla center to medulla periphery. It is also found that donor age has no influence on cortex modulus and hardness. The findings of this study will be useful for dermatologists in estimating the hair disorders and for estimating dye uptake of keratin fibers in textiles.

Keywords: Nanoindentation, Hair medulla, Hardness, Age

Introduction

Human hair and wool are made up of keratin proteins. Microscopically hair is divided into three parts namely cuticle, cortex, and medulla. The outer surface of hair fiber consists of 6 to 10 layers of cuticles which are glued together by cell membrane complex (CMC). The cortex is the thicker part in hair fiber and it consists of elongated cortical cells and each cortical cell consists of macrofibrils of 0.4-0.5 μm diameter. Macrofibrils are made up of microfibrils which are embedded in a strong matrix having around 21 % cystine content (high in S-S linkages) [2]. CMC is present between the cortical cells, but macrofibrils are separated from each other by intermacrofibrillar matrix. Microfibril is the same as the intermediate filament of 7-10 nm diameter and microfibrils overall are low in cystine (6 %) content (low in S-S linkages). The medulla is a thin cylindrical layer in the center of the hair fiber with one or more loosely packed porous regions and it also made of microfibrils. Medulla can be continuous or fragmented or absent in human hair fibers, but it exists in most of the animal hair fibers [3]. Medulla occupies around 22-25 % of the whole hair fiber at the beginning of the anagen phase and slowly it disappears by the end of the anagen phase which normally lasts for 2-5 years [4]. Medulla has high lipid content and low cystine content compared to the rest of fiber. Among the hairs, beard hair has lower number of S-S linkages due to the extensive medullation compared to scalp hair [5]. Medulla's function is not yet completely elucidated since it is difficult to isolate for further characterization. Recently, Wagner *et al*. [6] studied the medulla region using cryofractured samples and observed three subunits of medulla (globular structures, unorganized microfibrils, and smooth covering layer). From the TEM images, they have concluded that thin medulla has a sharp interface with cortex, whereas thick medulla shows gradual organization of the microfibrils from the medulla center to medulla periphery, i.e. interface with cortex. So it can be said that medulla region has weak nanocomposite structure on the inner side and shows progressive strengthening of the composite structure towards the outer side resembling plant fiber cross sections [7]. But no studies were carried out to quantify the weak nanocomposite structure of medulla region because of its low diameter (8-12 μm).

Nanoindentation has emerged as an important method for the evaluation of the mechanical response of small regions (2 to 10 μm width and 0.04 to 1.0 μm depths) of hair and cellulose fibers [2,7-12]. Here loads in the range of μN-mN and indentation depths in the nanometer range are continuously measured while testing. Finally hardness of the small region is measured by taking the maximum load and indenter depth (corresponding indenter area) unlike in Vickers hardness wherein maximum load and area of residual impression are used to determine hardness. In nanoindentation while unloading, both load and depths are recorded which allows in measurement of indentation modulus from the initial zone of the unloading curve. It is reported that hardness and modulus normally decrease with respect to increase in loads (0.1, 1, 10, 100 mN) or depths [2,10,11]. Wei *et al*. [2] reported higher hardness and modulus for cortex followed by medulla, but the study was restricted to three hair samples (Caucasian, African, and Asian hair) of unknown age. Samanta *et al*. [11] studied the nanomechanical properties of cortex and medulla and found higher hardness, modulus, and high surface roughness for medulla compared to cortex region. Both studies were focused on the effect of treatment of virgin hair samples on nanomechanical properties, but

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neither focused on the effect of donor age on nanomechanical properties. Differences between medulla and cortex with respect to indentation modulus and hardness were also not reported in detail. In this paper, an attempt has been made to study the nanomechanical properties of untreated human hair samples considering cortex, medulla regions separately and also to study the effect of donor age by taking hair samples from males and females between the ages of 5-64.

Experimental

Materials and Methods

Hair samples were collected from scalp (vertex position) of 16 healthy people of age ranging from 5-64 years old living in Hyderabad, India, after getting approval from Ethics Committee (Caucasian ethnicity). Samples were washed with mild shampoo and rinsed in water and dried at room temperature and kept in zip lock covers. Video of sample preparation for nanoindentation test was provided in our previous paper [9]. Specimens are prepared for nanoindentation by placing hair samples in molding clay (one side round clay ball and other side clay suspension pole) as shown in Figure 1. Inner diameter of 6 mm and length of 30 mm silicone pipes were used as molds and sealed from one end using clay. 2-3 hair fibers of about 30 mm length are placed in silicone pipe by filling the liquid epoxy resin (agar 100-R1140A) and cured at 60 ^{o}C for 12 h. From our single fiber testing it is found that conditioning at 60 ^{o}C for 12 h has no effect on tensile properties. The agar epoxy resin did not retain any air bubbles and no color was imparted from the clay to the hardened resin. Hence, a transparent resin block was obtained which allows the observer to check the alignment of the embedded hair fibers using stereomicroscope.

After separating resin blocks from the silicone mold as shown in Figure 1, polishing was carried out using abrasive papers on both faces to expose the cross section of the hair and microtomed using a Leica RM2255 microtome to obtain smooth surface. The epoxy blocks with hair embedded in them can be cut with microtome in such a way that the modified block has hair fibers oriented longitudinally. Sections of 10 μm are removed initially followed by sections of 5, 2, 1, and 0.5 μm to avoid the surface cracks and to minimize the residual stresses. Recently one study [13] reported 20.3 GPa of indentation modulus for Kevlar fibers which is relatively low compared to its tensile modulus

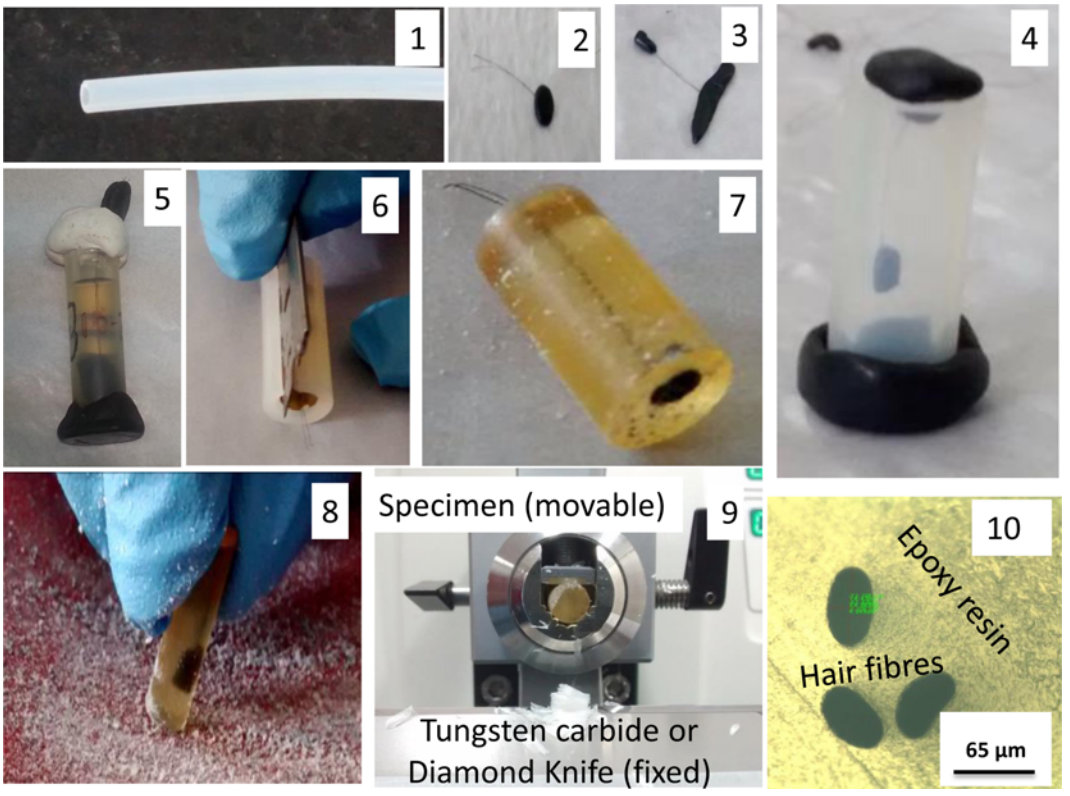

Figure 1. Images (1-9) correspond to sample preparation for nanoindentation test. Hair samples are embedded in agar epoxy resin using silicone mold (1) and clay (2-4). After curing of liquid epoxy resin (5), epoxy block was released from the mold using a razor blade (6) followed by smooth polishing (8, 9) using a rotary microtome (thin slices). Image (10) shows such a smooth epoxy block in which three fiber cross-sections are visible.

(85 GPa) and felt that surface preparation technique underestimated the modulus data because surface was not microtomed. But from the author's perspective, anisotropy of Kevlar fiber is also contributing for such difference in modulus values. In the current study, specimen surfaces were microtomed initially using tungsten carbide and later with diamond knives until smooth cross sections were achieved. Smoothness was checked by determining the surface roughness of few samples using optical profilometry (Zygo) for which Ra values were found to be below 150 nm.

After sectioning, the specimen blocks were fixed to aluminum disks vertically using adhesive and mounted on the sample holder of a nanoindenter and tests were performed using two different nanoindentation instruments equipped with a Berkovich diamond indenter. Micro Materials Nanoindenter (NanoTest TM) known for its high loads and depths is equipped with an open platform and a powerful optical microscope. Hysitron TriboIndenter is a popular nanomechanical testing instrument in the category of low loads and depths and it is equipped with optical microscope as well as in-situ scanning probe microscope (SPM) for selection of precise test locations (Figure 2). The in-situ SPM images are obtained by raster scanning the indenter probe over the sample surface to allow for pre- and post-test observation of the material surface. Post-test imaging is useful in protein fiber testing which have medulla of 8-12 μm (few fibers reach 20 μm) diameter and cortex of 30-45 μm thickness. The projected contact area (A) at peak load is usually determined from a measure of the contact depth of penetration (h_c) such that the projected contact area is given by equation (1).

$$A = 24.494h_c^2 \tag{1}$$

The tip area function defined in equation (1) is for perfect Berkovich tip without any tip defect. At nm level depths, it may be assumed that the real shape of the indenter is going to differ from an ideal pyramid and subsequently it may alter the nanoindentation results. So, the tip has been calibrated with two term area function of the form $A_c = C_1h_c^2 + C_2h_c$ on laboratory grade fused silica to obtain the calibrated elastic modulus in the depth ranges of present measurements (depths for determination of moduli were in the range of 1000 to 2000 nm).

The parameters peak load (P_{max}), depth at peak load (h_{max}) and the initial slope of the unloading curve (S) are determined [14] from the representative load-depth curves shown in Figure 3. The hardness is obtained by dividing P_{max} by A and the reduced elastic modulus E_r is obtained from equation (2).

$$E_r = \frac{1}{2}\sqrt{\pi}\frac{S}{\sqrt{A}} \tag{2}$$

The reduced elastic modulus E_r values can be used for comparison in case of anisotropic fibers such as hair fibers [10] and it takes into account the compliance of the indenter tip by the relation shown in equation (3)

$$\frac{1}{E_r} = \frac{1-\nu^2}{E} + \frac{1-\nu_0^2}{E_0} \tag{3}$$

where E, ν and E_0, ν_0 are the elastic modulus and Poisson's ratio of the sample and indenter, respectively. For soft and flexible materials like hair fibers, where $E_0 >> E$, the difference between E_r and E is minimal, but the difference in Poisson's ratio may slightly change the sample modulus E. In this study, 4-5 indents were placed on cortex region and one indent on medulla region as shown in Figure 2. Separate indents were placed on medulla to avoid the influence of interface effects. For all tests, Berkovich indenter was loaded to a peak load of 10 mN in 15 s, held for 30 s and unloaded at a speed of 0.66 mN/s.

Results and Discussion

The residual imprints of nanoindentation captured by optical microscopy and scanning probe microscope are shown in Figure 2. Representative load – depth curves obtained from nanoindentation test are shown in Figure 3.

The conclusion from Figure 3 is that medulla is softer and weaker compared to cortex and epoxy resin. Here medulla diameter is around 16 μm (higher side) and it is highly porous (random network of microfibrils). Looking at Figure 3 for medulla part, the maximum penetration depth is 2 μm resulting in a diagonal imprint of about 14 μm. In our studies, we used the criteria of diagonal imprint lower than diameter of medulla for acceptable indents. The indents which are influenced by surrounding material (cortex) are rejected. Epoxy infiltrations can modify the mechanical response of cortical cells. From the resulted load-depth plots for cortex, medulla, and epoxy regions, it is assumed that

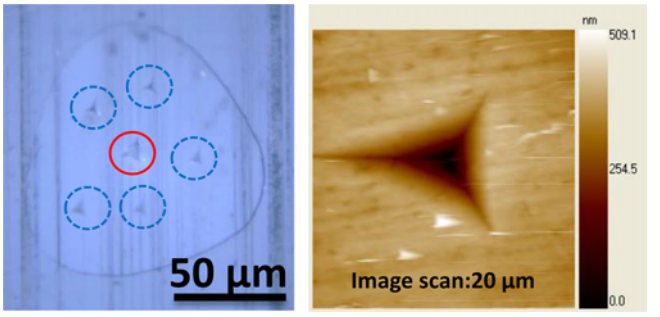

Figure 2. Left image shows five residual indentation impressions (dotted circle) on cortex region and one residual indentation imprint (solid circle) on medulla. Implanted knife markers due to sectioning are also visible on hair cross section. Right image shows one residual indentation imprint of Berkovich diamond indenter on cortex region which was taken using a scanning probe microscope.

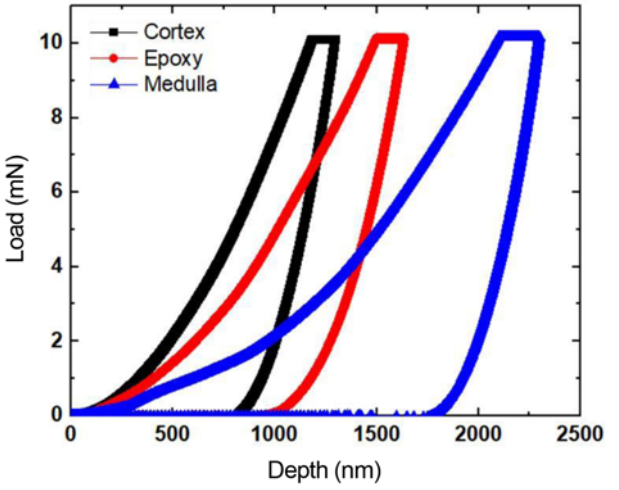

Figure 3. Typical load-depth graphs of nanoindentation test performed on hair fiber cortex and medulla regions. Since hair samples were embedded in epoxy resin, indents were also placed on surrounding epoxy.

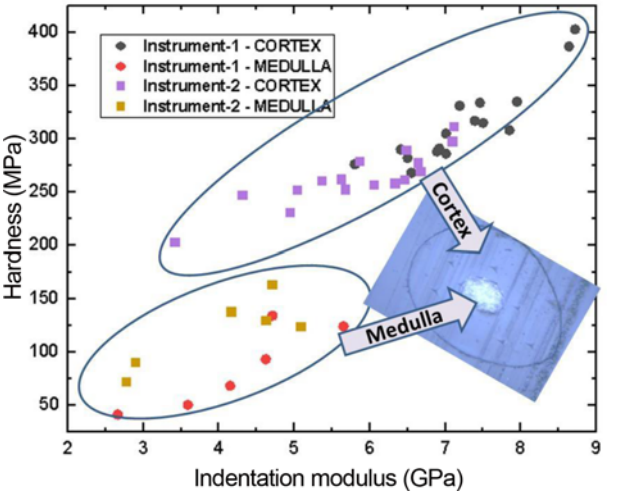

Figure 4. Variation of hardness and indentation modulus of hair samples with respect to cortex and medulla. Here nanoindentation on hair cross sections was performed using two instruments (Instrument-1 & Instrument-2) to validate the results.

epoxy did not infiltrate in to the medulla region. From the measured values, it is found that medulla region of hair shows lower indentation modulus and hardness compared to cortex region of hair (Figure 4). However, absence of medulla resulted in equal or higher indentation modulus and hardness compared to cortex region. Recently Chegdani *et al*. [12] reported 1 GPa of indentation modulus for flax fibers (Berkovich indenter with tip radius of 40 nm) which is relatively low compared to its tensile modulus (40-70 GPa). Tests were repeated on the same samples with MTS Nanoindenter (Berkovich indenter with tip radius of 400 nm) and obtained the indentation modulus of 20 GPa indicating the influence of tip radius and variability in surface roughness on indentation modulus. In this study, indentation tests have been performed at high loads (meaning at large depths) to reduce the influence of roughness on the contact area determination using Hysitron (indenter moves in vertical position) and Micromaterials (indenter moves horizontally) indenters. In Figure 4, Micromaterials is named as instrument-1 and Hysitron is named as instrument-2 and in both cases maximum load of 10 mN was applied. Both instruments resulted in lower values of hardness and indentation modulus which are in the range of 70-170 MPa and 2.7-5.1 GPa, respectively, for medulla region. Slightly higher hardness (200-312 MPa) and indentation modulus were reported (3.4-7.1 GPa) for cortex region. The lower values of hardness for medulla could be due to the weak nanocomposite structure of cortical cells especially poorer orientation of microfibrils and weak amorphous matrix (low cystine content) surrounded by larger cavities [6]. Similarly, smaller values of modulus could be due to the poorer alignment of the microfibrils in medulla. This can be further explained by assuming macrofibrils are not oriented parallel to fiber direction.

As reported by Gindl *et al*. [10], small amount of load is also applied in the transverse direction of macrofibril despite doing indents in the fiber direction due to the tip geometry of the Berkovich indenter. The relation between composite constituents and nanomechanical properties is well described [8-10] because it can be used to measure the responses of matrix and microfibrils individually in a quantitative manner. The response of the matrix would be indicated by value of hardness (so greater the resistance of the matrix due to the increase in cross-linking of sulfur rich amorphous matrix, higher the hardness value). Similarly the response of microfibrils would be indicated by indentation modulus (better arrangement of microfibrils in cortical cells, higher indentation modulus). This hypothesis seems to be valid for cells having high dense composite structure like cell wall in wood or cortical cell in hair/wool, but it needs further discussion if cells have a loose network structure such as cortical cells in medulla region. But the increase in the orientation of microfibrils in the cortical cells (cortex or medulla) corresponds to increase in indentation modulus and hardness. Recently Bourmaud and Baley [7] also reported lower modulus values for xylem side of flax stem and lumen side of flax fiber cross section due to the late thickening of the cell walls. Here late thickening corresponds to poorer alignment of microfibrils resulting in lower values of indentation modulus.

Wei *et al*. [2] studied the nanomechanical properties of cuticle in lateral direction for Caucasian, Asian, and African hairs and found higher hardness and modulus compared to the values of hardness and indentation modulus of cortex

and medulla obtained during longitudinal indentation. They also reported slightly higher hardness and indentation modulus for the hair cortex compared to hair medulla which is in full agreement with the results presented in this study. But Samanta *et al*. [11] reported slightly higher hardness and modulus for medulla region compared to cortex region. In our analysis, few samples also revealed higher hardness and modulus when indents were performed on center of the hair fiber assuming it as a medulla, but medulla region was not observed when viewed under microscopy. From this study it is evident that one can measure the mechanical properties (hardness and modulus values) of hair cortex and medulla regions separately by using nanoindentation. It is possible to estimate the orientation of microfibrils in medulla region from the indentation modulus, which is not reported earlier. Lower the orientation, lower the modulus/hardness and it indicates loose network of microfibrils which is of interest in forensic science [15] and in textiles (better dyeing of textile wool fibers).

In order to get a better understanding of medulla's properties and characteristics, more indentations were performed in medulla region of different donors. Figure 5 shows linear correlation between hardness and indentation modulus of medulla region of different hair samples with correlation factor (R^2) of 0.89. In fact such correlation was also found between hardness and modulus values of cortex (Figure 4). It should be stressed here that microfibrils present in cortex and medulla regions are essentially the same, but cortex and medulla differ in microfibril arrangement and matrix content. Several studies were reported about microfibrillar arrangement in cortical cell of cortex region [1,16,17]. No reports were found regarding variation of microfibril arrangement within medulla region, but it was clearly reported by Wagner *et al*. [6] that organization of microfibrils of medulla decreases from periphery to center. Lower modulus values for medulla as reported in the correlation could be due to the higher degree of anisotropy (poorer arrangement of microfibrils) that exists in the center of the medulla region (Figure 5). But the corresponding lower values of hardness could be due to the random orientation of microfibrils (low density of microfibrils) and not due to the amorphous matrix unlike previously reported [9]. It can be said from the correlation shown in Figures 4 and 5 that different levels of strengthening of nanocomposite structure (i.e arrangement of the microfibrils with respect to fiber direction) occurred across the medulla regions of the hair fiber. Such correlation between two nanomechanical properties was not reported earlier though it may exist in wood and plant fibers. The correlation of the linear variance of hardness and indentation modulus can be used to map the nanomechanical properties which have different degrees of microfibril orientation. In fact Gindl *et al*. [10] reported higher hardness and lower modulus for viscose fibers compared to lyocell fibers due to reduced lateral bonding in

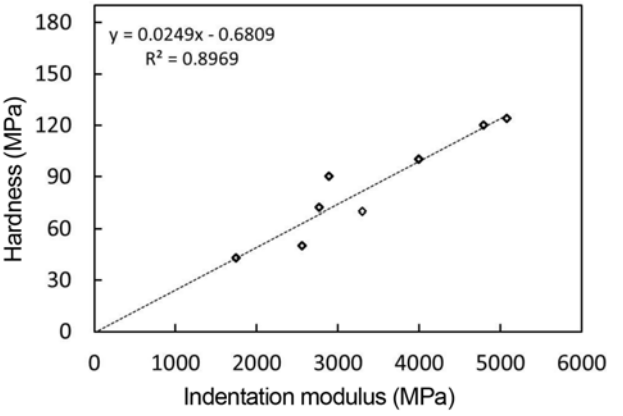

Figure 5. Correlation between hardness and indentation modulus found for medulla data indicating the variation in nanocomposite structure of macrofibrils especially orientation of helical intermediate filaments (microfibrils) in medulla.

lyocell. But lyocell and viscose fibers have only cellulose microfibrils arranged in fiber direction whereas in hair fiber, macrofibrils are arranged in fiber direction, where in each macrofibril consists of micro fibrils (low in cystine ~6 %) and the matrix (rich in cystine ~21 %).

We have measured medulla diameter for the all the hair samples having medulla and found that donor age has no correlation to medulla index which is the ratio of medulla diameter to fiber diameter (Figure 6). Srettabunjong *et al*. [15] found that gender has no influence on medulla index. Consistent value of medulla index between 0.10-0.25 indicates that medulla index is not dependent on hair fiber diameter. The shape of the medulla cross section was also found to be less elliptical than the whole fiber cross section [4].

The results of nanoindentation test on cortex with respect to donor age and gender are shown in Table 1. For all 16 donors, hairs cross sectional diameters are in the range of 60-110 μm with coefficient of variation (ratio of the standard deviation to the mean×100) between 5-19 %. Among 16 donors, one of the 5 year old male donor has the least diameter (69 μm) and 41 year old female donor has the highest diameter (101 μm). As reported in our previous publication [9], donor age is not influencing the diameter of the hair fiber (either longitudinal or cross sectional diameter) but some reports [15,18] discussed a correlation between age and diameter. The hardness and indentation modulus of cortex region are in the range of 200-320 MPa and 4-7 GPa, respectively. The coefficient of variation for hardness and indentation modulus is between 2-9 % indicating the significance of the nanoindentation test and this is primarily due to the small regions used for indentation tests. Indentation modulus and hardness with respect to donor age are displayed in Figure 7 for cortex regions of all hair samples. It is observed that both hardness and modulus show the same

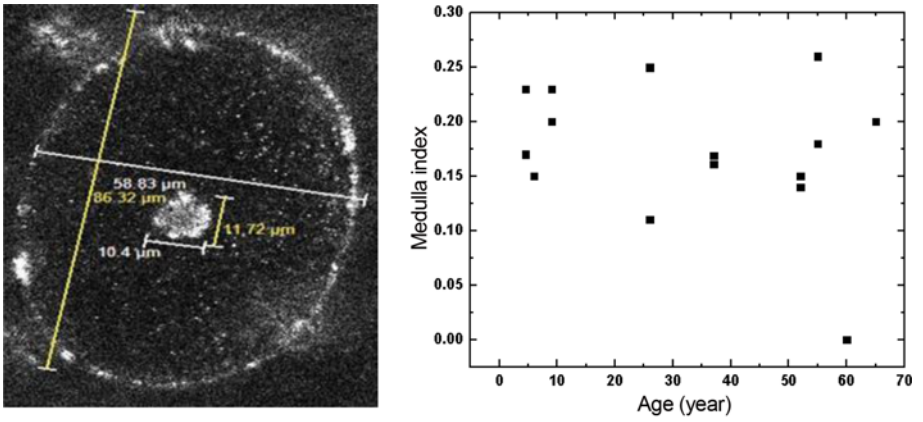

Figure 6. Confocal laser scanning microscopy images of hair with visible medulla of around 10 µm. Medulla index (ratio of medulla diameter to fiber diameter) of zero indicates the absence of medulla.

trend reaffirming the nanocomposite structure of hair and overall packing and stability of helical intermediate filaments embedded in cysteine matrix. No significant variation in hardness and indentation modulus can be seen when data is compared with respect to donor age except F32-2 donor who is found to be vegetarian. However, one donor of same age and gender shows different modulus and hardness compared to other donor of same age and gender (F32-1 & F32-2, M5-1 & M5-2) indicating that nanostructural heterogeneities exists in hair cortex regions of those two donors. But this is not true in other donors of same age and gender (M11-1 & M11-2) in which no significant variation was found between two hair fibers when modulus and hardness were compared. Similar trend was seen when hair from donors of same age and different gender (M41-F41) were compared indicating strong and uniform structure of the nanocomposites exists in these hair cortex regions. It can be concluded that donor age has no significant influence on indentation modulus and hardness values. This result was also reported in our earlier publication using different set of samples [9].

The results of age dependence is also shown for medulla region in Figure 8 no age dependence was found for cortex and medulla regions (Figures 7 and 8). When hardness and indentation modulus values were compared with respect to

Table 1. Indentation modulus and hardness of single hair fiber cross sections obtained from instrument-2

S. No.	Sample (gender, age)	Cross sectional diameter (μm)	Coefficient of variation in diameter	Hardness of cortex region (MPa)	Coefficient of variation in hardness	Indentation modulus of cortex region (GPa)	Coefficient of variation in indentation modulus
1	M5-1	85±11.7	14	312±09	3	7.1±0.3	4
2	M5-2	69±7.1	10	280±05	2	5.9±0.2	3
3	M6	73±9.2	12	294±12	4	6.6±0.2	3
4	M11-1	89±13.0	15	250±20	8	5.6±0.5	8
5	M11-2	86±16.1	19	261±13	5	5.4±0.2	4
6	F14	77±12.9	17	270±15	6	6.7±0.2	3
7	M15	83±8.3	10	257±07	3	6.1±0.1	2
8	F23	76±8.6	11	248±23	9	5.0±0.2	4
9	F29	87±9.4	18	232±08	3	4.9±0.1	2
10	F32-1	98±17.9	11	298±12	4	7.1±0.2	3
11	F32-2	82±6.6	8	204±14	7	3.4±0.2	7
12	F35	79±8.4	11	262±16	6	6.5±0.3	5
13	M36	89±10.3	12	255±16	6	4.4±0.1	3
14	M41	84±13.9	17	263±08	3	5.7±0.1	2
15	F41	101±7.9	8	267±22	8	6.5±0.4	7
16	F64	79±4.0	5	278±14	5	6.6±0.2	3

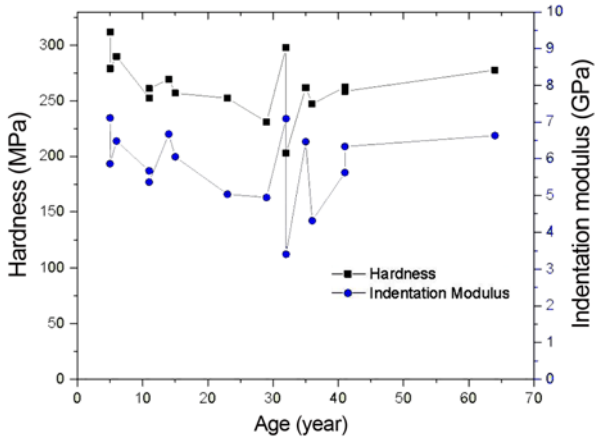

Figure 7. Variation of hardness and indentation modulus of hair samples (cortex) with respect to age. Note that all data points are average of 10 indents and age 5, 11, 32, and 41 years corresponds to two people each.

age, the individual variation is quite low for cortex data, but it is significant for medulla data. In both cases, composite structure of cortical cells with nanostructural heterogeneities contributes in little variation, but for medulla data the additional variation could be due to the large variation existing in the orientation of microfibrils from medulla center to medulla periphery.

Microfibrils are highly oriented in cortex region and poorly oriented in medulla region. Optical microscopy and SEM are used to view the medulla region. TEM is used to check the orientation of microfibrils (qualitative). First time nanoindentation technique is used to quantify the orientation of microfibrils especially in medulla region in terms of hardness and indentation modulus. Higher the microfibril orientation, higher the hardness and indentation modulus. Wood consists of cellulose microfibrils acting as reinforcement

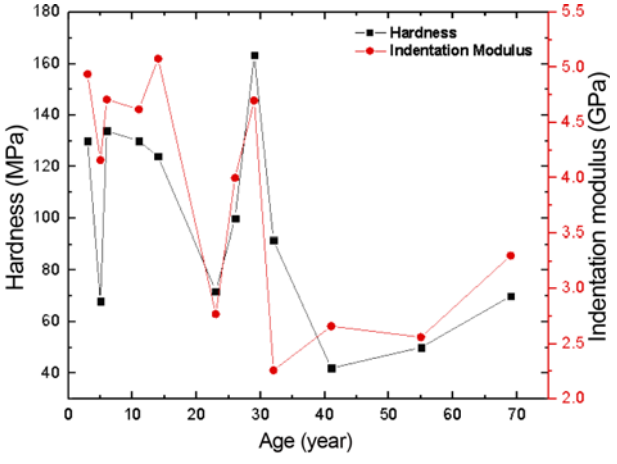

Figure 8. Variation of hardness and indentation modulus of hair samples (medulla) with respect to age.

and lignin acting as matrix. The hardness is apparently matrix-dominated and indentation modulus is sensitive to the arrangement of the cellulose microfibrils [19,20]. Here cellulose microfibrils (non helical) corresponds to keratin microfibrils (helical) and lignin corresponds to cystine rich matrix (~21 %) in hair. Microfibril is the same as the intermediate filament of 7-10 nm diameter and microfibrils overall are low in cystine (6 %) content. So the increase in the orientation of microfibrils in the cortical cells (cortex or medulla) corresponds to increase in indentation modulus and hardness.

The results from nanoindentation of hair can be used in three fields.

- They can be used as a diagnostic tool in estimating structural differences in damaged hair caused by physical handling interventions such as combing, drying, curling, and straightening. Since root side of the hair has good number of cuticles and tip side hair has damaged or fewer number of cuticles [2] there is high probability that dye uptake can cause damage to tip side cortical cells.
- It can be used in forensic science to quantify the medulla in hair and wool fibers.
- This study can be extended to understand the core-skin structural differences that exist in other fibers such as Kevlar where core is less aligned than skin [13] region.

Conclusion

1. Nanoindentation tests were performed on cortex and medulla regions of hair fiber. Hardness and indentation modulus of cortex region were almost same for 15 donors (excluding one donor of female 32-2 years) indicating the age has negligible effect on nanomechanical properties.
2. From the nanoindentation test, it is found that medulla has less organized microfibrils and weak matrix resulting in lower values of modulus and hardness compared to the cortex region of the same hair fiber. For one male sample, it was found that medulla revealed modulus of 2.2 GPa and hardness of 61 MPa in comparison to 7.3 GPa and 310 MPa, respectively, measured for the cortex region of the same hair fiber. Hair samples without any visually discernible medulla showed no significant difference in properties between cortex and medulla regions.
3. The linear correlation between hardness and modulus data of medulla and cortex regions indicates the different degree of microfibril orientation that exists in cortical cells.
4. Hair fiber cross sectional diameter is found to be between 70-100 μm. Medulla index (medulla to fiber diameter ratio) is found to be between 0.10-0.25 and it is not influenced by age.
5. The quantitative values of nanoindentation hardness and modulus of cortex and medulla regions of single hair fiber are of interest for dermatologists in treatment of hair

disorders including the negative effects of hair dye penetration. Since the individual tests are performed on 100 μm^2 area, the observed differences between cortex and medulla will help in developing new insulation materials [21] or textile dyes.

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