

Alkaline peroxide treatment induces acquired unruly hair by apparently affecting distinct macrofibrils

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Synopsis

Individual hairs can be inherently curly; however, bleach treatment can cause damaged hairs to acquire a curl, a phenomenon we term acquired unintentional unruly hair. Because there have been no reports concerning acquired unintentional unruly hair, the influence of bleach treatment with alkaline peroxide that produce this phenomenon was investigated. First, it was validated that the radius of curvature in many curly hairs is reduced upon bleach treatment. Next, the influence of bleach treatment on the mechanical properties of inner components was studied by the force curve method using atomic force microscopy. This measurement revealed four types of macrofibrils—on the orthocortex- or the paracortex-like structure, and on the concave or the convex side—have different mechanical properties. Macrofibrils on the orthocortex-like structure on the convex side were especially influenced by alkaline peroxide treatment, and may be particularly important to acquired unintentional unruly hair.

INTRODUCTION

The term “curly hair” is generally applied to both inherently and posteriori unruly hair. The former is seen naturally in Caucasian or African persons (1). On the other hand, the latter can be acquired due to the influence of the ambient environment or chemical treatments. Furthermore, the acquired unruly hairs involved intentional unruly hair with permanent wave treatment and unintentional unruly hair with bleach treatment. People, who want straight hair but have unintentional unruly hair, worry about a decrease in hair luster and difficulty in setting their hairstyle (2). To meet the need for effective solutions to these problems, several studies on hair have been performed. Inherent curliness in

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human hair (3,4) and wool (5) is related to the spatial distribution of orthocortex-like and paracortex-like structures. Furthermore, it has been reported that keratin protein, hHa8, is generated in paracortex-like structures and is distributed on the concave side of curly hairs (6). While the influence of bleach treatments with alkaline peroxide on hair has been investigated (7–10), there has been no report of the relationship between acquired unintentional unruly hair and alkaline peroxide treatment. Therefore, we decided to research acquired unintentional unruly hair. Recently, we developed a force curve method using atomic force microscopy (AFM) (11–13) that enabled us to evaluate the mechanical properties and fine structure of hairs simultaneously at the nanometer scale without pretreatment (14). The force curve method showed that the orthocortex- and paracortex-like structures of bleached hair have different Young's moduli, suggesting that the active agent in bleach has a greater impact on the orthocortex-like structure (15). Therefore, it was speculated that the biased influence of bleach treatment on the inner components of hair is one of the factors that cause acquired unintentional unruly hair. In this report, the influences of bleach treatment with alkaline peroxide on the macroscopic shapes of hairs and the mechanical properties of inner components of both inherently curly and straight hairs were described.

MATERIALS AND METHODS

SAMPLE OF HAIRS

For all experiments, virgin hairs from Japanese donors, the hairs had not been previously chemically treated, were used. The curly hair used in this study was defined as hair that curled like the letter C with a radius of curvature (ROC) of less than 3 cm. Hair bleaching was performed using an aqueous solution of 1% ammonia and 3% hydrogen peroxide for 40 minutes at 30 °C. Ammonia solution and hydrogen peroxide (laboratory reagent grade) were bought from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Dipping in water was followed by drying at 25 °C under 50% humidity. ROC was measured at a random point under 50% humidity (16).

AFM MEASUREMENT

Each hair sample was cut in half, and one half was used as the untreated sample. The other half was treated with bleach and used as the bleach treated sample. The root versus the tip end was randomly selected for treated and untreated sample. Each sample was cut into small pieces by using a diamond knife equipped on the ultra microtome EM-Ultracut UCT (Leica Microsystems GmbH, Wetzlar, Germany) to make an extremely smooth cross section of hair; the resulting cross sections were placed between celluloid plates. The AFM measurements were performed using MultiMode and EnviroScope on a NanoScopeIVa and NanoScopeV controller (Bruker AXS Inc., Madison, Wisconsin), respectively. A cantilever made of silicone was used (SEIHR NanoWorld, Neuchâtel, Switzerland; length, 225 μm). The spring constant of the cantilever was measured in thermal tune mode on the NanoScopeV controller (17). To evaluate Young's modulus of macrofibrils on cortical cells, we performed force curve measurements in a two-dimensional array of 64×64 points in force-volume measurement mode. An in-house script within the IGOR

Pro software (WaveMetrics, Inc., Lake Oswego, Oregon) was used to calculate the Young's modulus from the force curve (18). AFM measurements were performed 10 times with both straight and curly hairs, and representative data were used for subsequent analyses.

RESULTS AND DISCUSSION

CHANGES OF APPEARANCE IN UNTREATED AND BLEACHED HAIRS

Straight hairs and curly hairs were bleached and their change in shape was observed (Figures 1A and B). In the case of bleached straight hairs, changes in the length or width of the hairs were not observed, and most straight hairs remained unaltered (Figure 1A). On the other hand, in the case of bleached curly hairs, it was observed that the ROC became smaller than the ROC of corresponding untreated curly hairs (Figure 1B). The ratio of measured ROC in bleached curly hairs vs. corresponding untreated hairs was 0.94 ± 0.09 (mean \pm S.D.), clearly demonstrating that bleach treatments decrease the ROC.

COMPARISON WITH THE SPATIAL DISTRIBUTION OF TWO TYPES OF CORTICAL CELLS

The differences in the distribution of inner components of straight and curly hairs were investigated by AFM measurement. Cross sections of virgin straight, bleached straight, virgin curly, and bleached curly hairs were scanned, using height and phase measurements commonly employed in topography observation. The measurements were scanned sequentially, starting at the cuticle and proceeding through the other end, with a scan area of $10 \times 10 \mu\text{m}$. A representative AFM image of virgin straight hair is shown in Figure 2 (for other hair types, see Supplementary Figures 1–3). Height, phase, and processed phase images are shown in Figures 2A–C, respectively. Measurements were performed in water because this condition made it possible to distinguish the cell membrane complex from the intermacrofibrillar matrix (14). For reference, Figure 2D contains a zoomed image of a $5 \times 5 \mu\text{m}$ region of the image in Figure 2B. Phase images painted in white by an image processor correspond to the distribution of the paracortex-like structure; the remaining region is orthocortex-like structure (Figure 2C). Furthermore, the distributions of the two types of cortical cells (black: orthocortex-like structure; white: paracortex-like structure) were estimated, which is shown in Figure 3. In curly hairs, cortical cells on the concave side

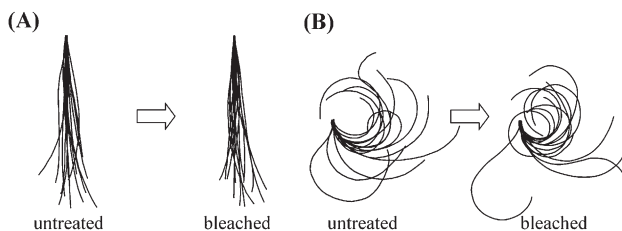


Figure 1. Changes of appearance in untreated and bleached hairs. (A) Shapes of straight hairs and (B) shapes of curly hairs; each sample was $N = 20$. A picture was taken of each hair sample, and the outline of the hair was traced in black to clarify the observation of the change in hair shape. Results were compiled from 20 independent hair samples.

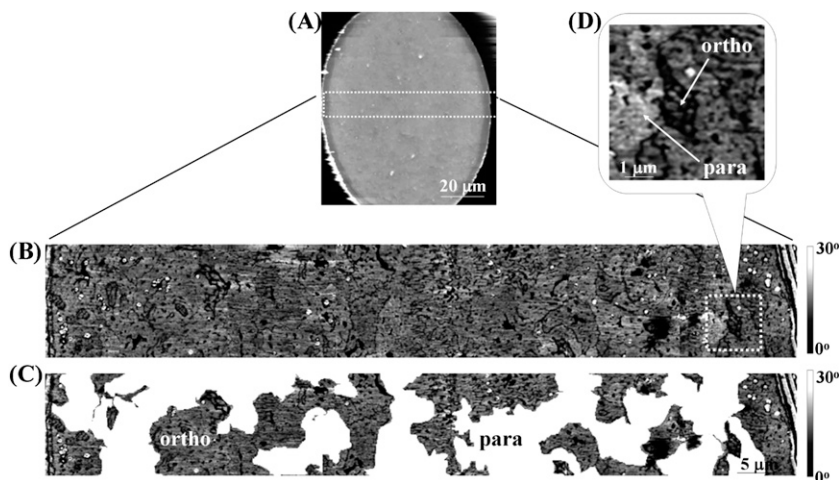


Figure 2. AFM images of a cross section of virgin straight hair in water. (A) Height image. (B) Phase image. (C) Phase image, with the paracortex-like structure painted in white. (D) Phase image containing a magnified $5 \times 5 \mu\text{m}$ section of the image in Figure 2B.

could be distinguished from those on the convex side in the curly hair (Figures 3C and D). On the other hand, in the case of straight hairs (Figures 3A and B), it was not possible to define concave and convex sides; for convenience, straight hairs were divided into left and right sides instead of concave and convex sides. To date, it has been commonly believed that the distribution of the paracortex-like structure on the concave side was related to curliness. If this biased distribution were solely responsible for curliness, however, it would be difficult to explain why straight hairs (Figures 3A and B) also exhibited biased distribution of the two types of cortical cells. Conversely, not all curly hairs had biased distribution (Figure 3D). Therefore, it was not possible to definitively state that the shapes of hairs were solely influenced by the distributions of the two types of cortical cells.

COMPARISON WITH THE YOUNG'S MODULUS IN STRAIGHT AND CURLY HAIRS

The differences in the Young's modulus of the inner components of straight and curly hairs were investigated, again using AFM force curve measurement. The Young's modulus in cross sections of virgin straight, bleached straight, virgin curly, and bleached curly hairs were measured. Representative data from bleached curly hairs is shown in Figure 4 (for other hair types, see the Supplementary Figures 4–6). It was noteworthy that a difference existed between the moduli of the concave and convex sides of curly hairs (Figure 4C); this difference could hardly be observed by conventional AFM phase imaging (Figure 4B).

Young's modulus of macrofibrils was analyzed because of previous reports that the macrofibrils are significantly influenced by bleach treatment (15). Young's modulus of macrofibrils in the four types of hair was measured at 20 random points; the average Young's modulus is shown in Figure 5. Macrofibrils on the concave and convex side of curly hairs, or on the left and right sides for straight hairs, could be distinguished from each other in the same manner as in Figure 3. In addition to those sections, macrofibrils on the orthocortex-like structure could be distinguished from those on the paracortex-like

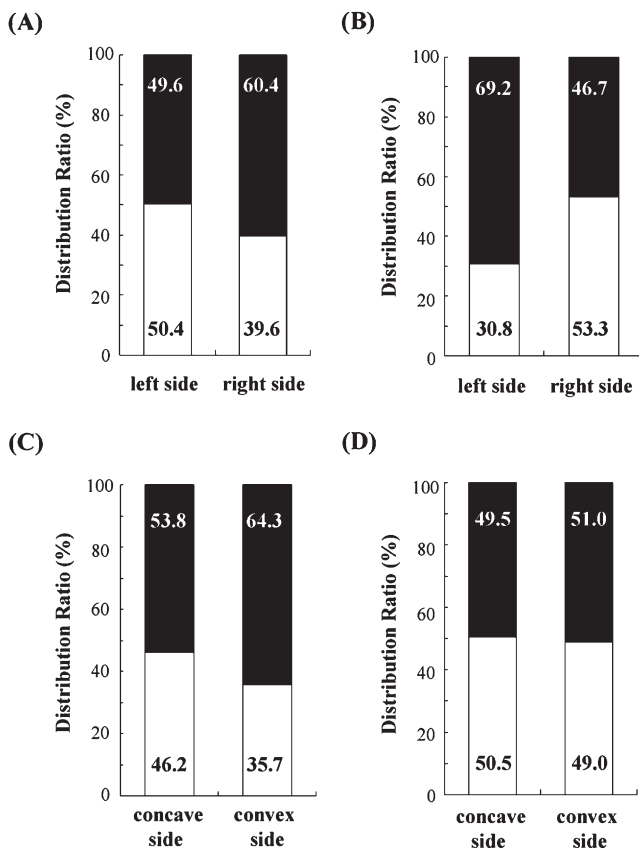


Figure 3. Comparison of spatial distribution of the two types of cortical cells among four types of hairs. Spatial distribution of the two types of cortical cells (black: orthocortex-like structure; white: paracortex-like structure) on the concave side (left) and convex side (right): (A) in virgin straight hair, (B) in bleached straight hair, (C) in virgin curly hair, and (D) in bleached curly hair.

structure. Figure 5 depicts the ratio of the Young’s modulus of each macrofibril to the average Young’s modulus of the macrofibril on the paracortex-like structure on the concave side (for curly hairs; for straight hairs, the left side was arbitrarily chosen).

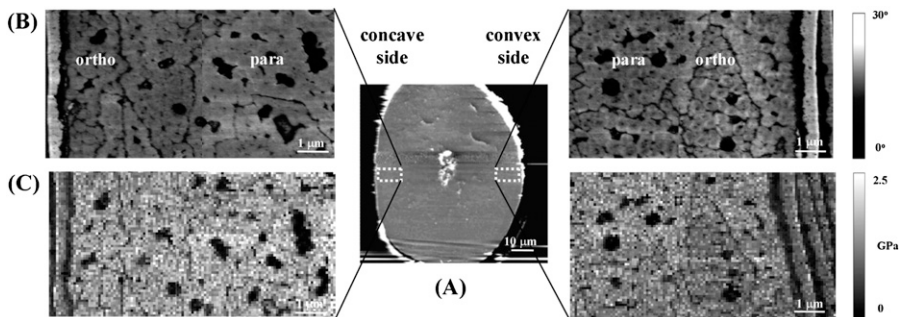


Figure 4. AFM images on cross section of bleached curly hair in water. (A) Height image. (B) Phase images (left: concave side; right: convex side). (C) Young’s modulus images corresponding to the phase images above.

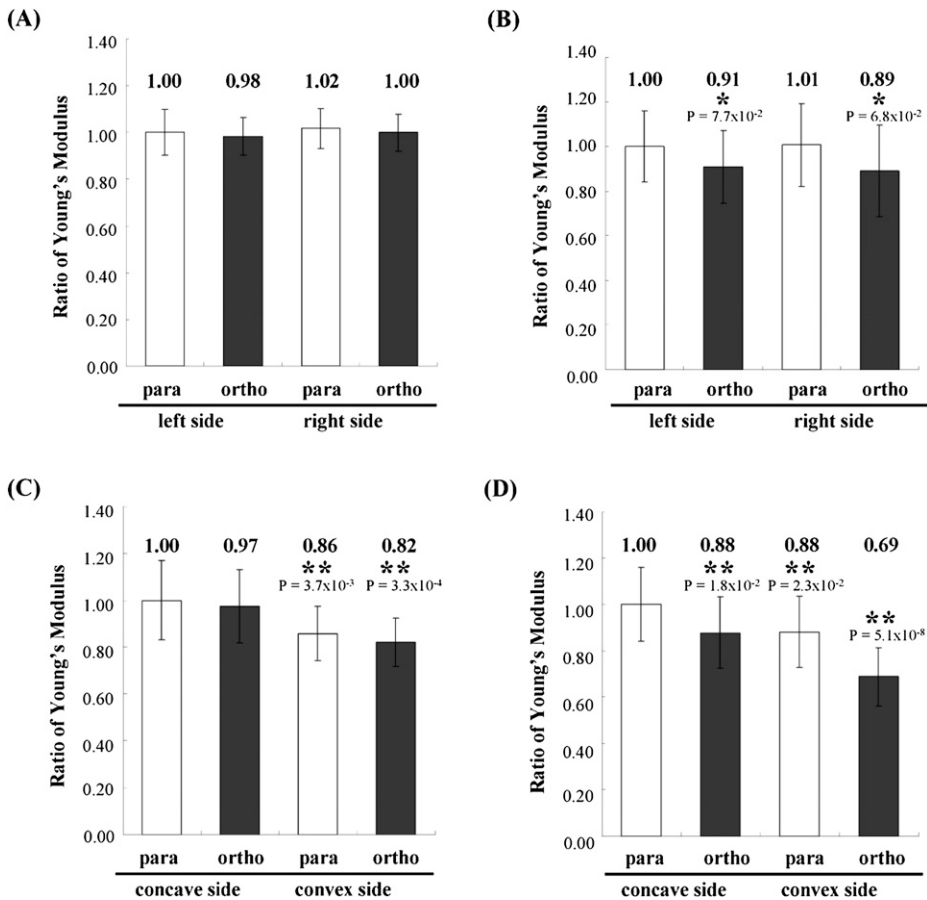


Figure 5. Comparison of Young's modulus of four types of macrofibrils among four types of hairs. Ratio of average Young's modulus of macrofibrils on the paracortex-like structure on the concave side to that of Young's modulus of macrofibrils on the concave side (left) and the convex side (right): (A) in virgin straight hair, (B) in bleached straight hair, (C) in virgin curly hair, and (D) in bleached curly hair. Statistically significant difference compared to the average of Young's modulus of the macrofibril on the paracortex-like structure on the concave (left) side by using two-tailed T test ($n = 20$, $\alpha = 0.05$).

Figure 5A reveals that Young's modulus of macrofibril in virgin straight hairs had almost the same value throughout, without regard to the type of cortical cell or whether the scan area was on the left or right side.

Comparison of Figures 5A and B reveals that Young's modulus of macrofibrils was lower on the orthocortex-like structure than on the paracortex-like structure. Macrofibrils on the paracortex-like structure were damaged by bleach treatments; furthermore, macrofibrils on the orthocortex-like structure were severely damaged, as reported by Kitano *et al.* in 2008. Figure 5B also confirmed that the intentional division of the straight hair into left and right sides was not meaningful, as expected.

Importantly, comparison of Figures 5A and C revealed that in many curly hairs, Young's modulus of macrofibrils of cortical cells on the convex side was lower than on the concave

side, even between the same type of cortical cells. This observation clarifies the fact that four types of macrofibrils (on the orthocortex- or paracortex-like structure, and on the concave or convex side) are present.

Another interesting finding is shown in Figure 5D. In comparison with Figures 5C and D, Young's modulus of macrofibrils on the orthocortex-like structure decreased upon bleach treatments, as seen in straight hairs. Young's modulus of macrofibrils on the orthocortex-like structure was decreased by a factor of 0.91 ($= 0.88/0.97$) on the concave side, whereas it was decreased by a factor of 0.84 ($= 0.69/0.82$) on the convex side. Comparison of Figures 5B and D reveals that the influence of bleach treatment on macrofibrils on the orthocortex-like structure on the concave side of curly hairs was similar to its influence in straight hairs. However, it is worth noting that macrofibrils on the orthocortex-like structure on the convex side of curly hairs was more significantly influenced by bleach treatment than corresponding macrofibrils in straight hairs.

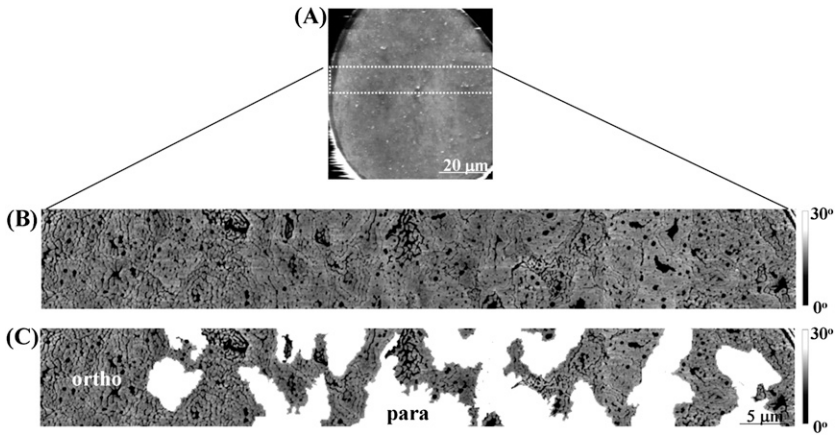
CONCLUSION

In summary, in the case of curly hairs, bleach treatments decrease the ROC. Furthermore, force curve measurements of the inner components revealed a difference in Young's modulus between macrofibrils on the concave and convex sides. Previously, it was considered that the curliness of hair is primarily influenced by the distributions of the two types of cortical cell. However, this study demonstrates that there are four types of macrofibrils (on the orthocortex- or paracortex-like structure, and on the concave or convex side) whose mechanical properties are different. Among these, macrofibrils on the orthocortex-like structure on the convex side of curly hairs are especially affected by bleach treatment. This could play a role in shape changes that result in unintentional unruly hair acquired by chemical bleaching with alkaline peroxide.

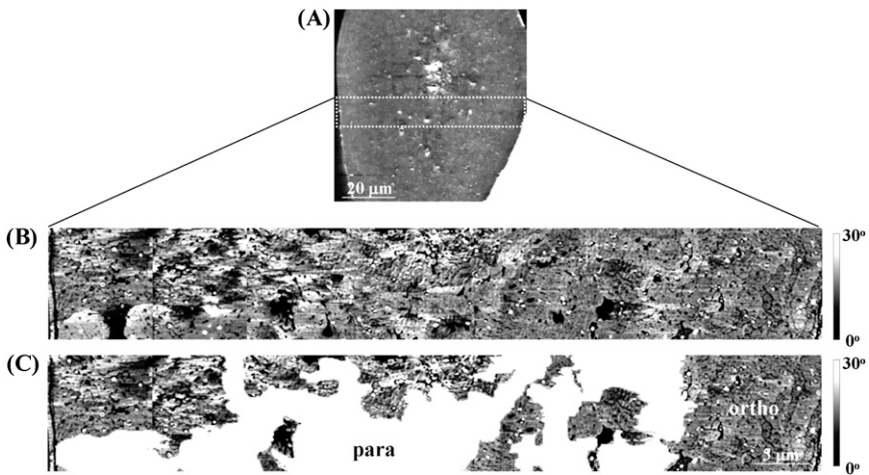
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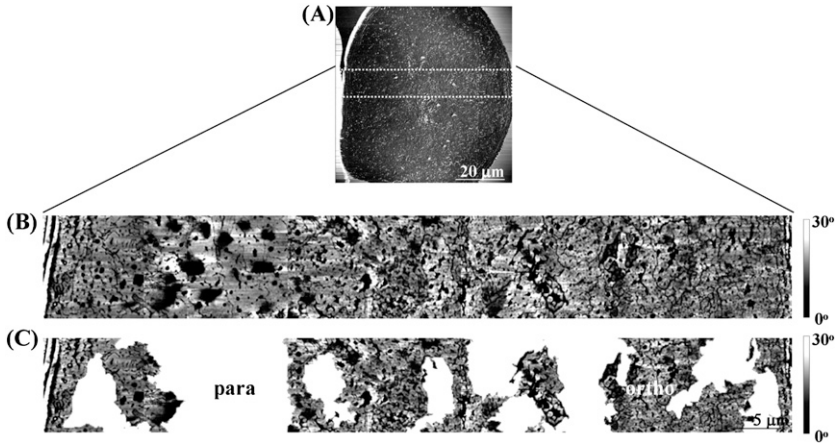
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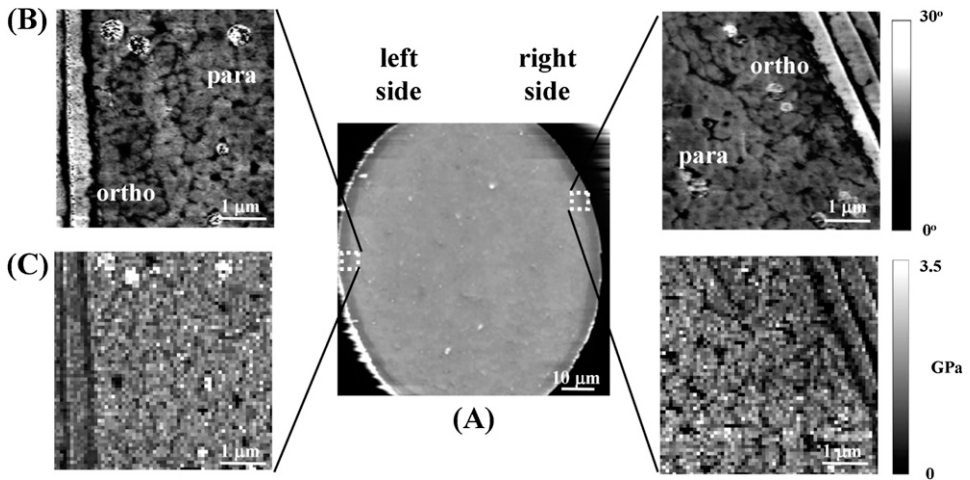
Supplementary Figure 1



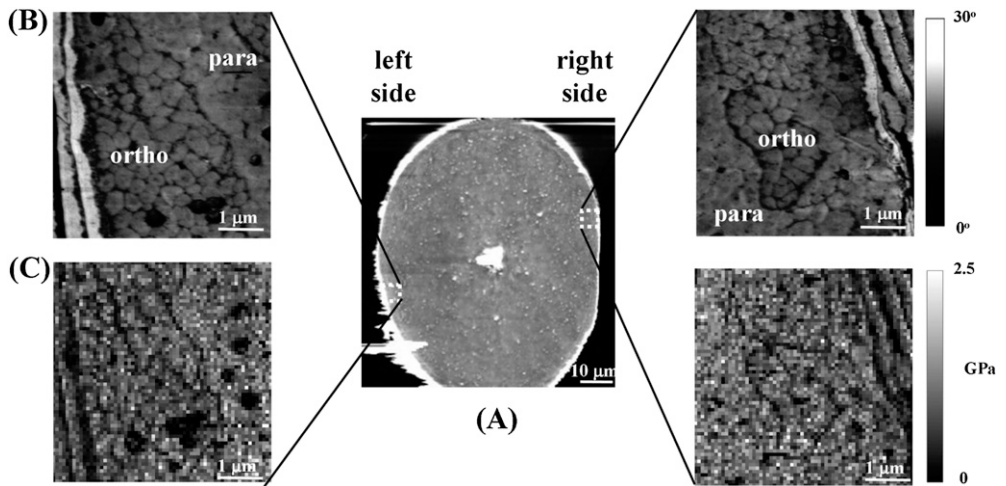
Supplementary Figure 2



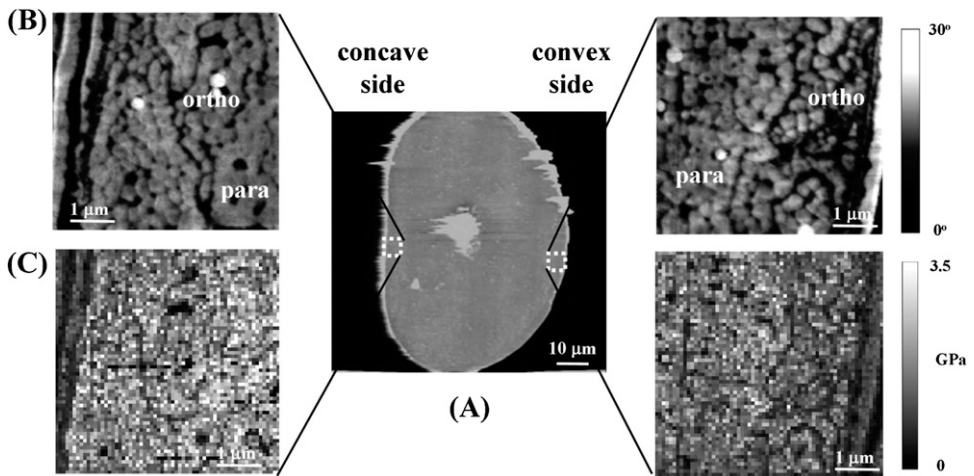
Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6

