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Structural and mechanical properties of alpha-keratin fibers

A dissertation submitted in partial satisfaction of the requirements for the degree

Doctor of Philosophy

in

NanoEngineering

by

Yang Yu

Committee in charge:
Marc A. Meyers, Chair
Shengqiang Cai
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Jan B. Talbot
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Chair

University of California, San Diego

2017
DEDICATION

This dissertation is dedicated to all my family members, especially my parents.

Without your support, I cannot possibly carry on so far and finish this journey.
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ABSTRACT OF THE DISSERTATION

Structural and mechanical properties of alpha-keratin fibers

by

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Doctor of Philosophy in NanoEngineering

University of California, San Diego, 2017

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The understanding on the mechanical behavior of \( \alpha \)-keratin broadens our knowledge in biological materials science. In this study, the hierarchical organization is studied from the intermediate filament to the structural levels. The effects of strain
rate, relative humidity, and temperature are evaluated.

Human hair exhibits a high tensile strength, which is significantly dependent on strain rate and humidity. The strain-rate sensitivity is comparable to that of other keratinous materials. One distinguishing feature, the unwinding of the α-helices and the possible transformation to β-sheet structure of keratin under tension, is analytically evaluated and incorporated into a constitutive equation. The contributions of elastic and plastic strains on reloading are evaluated and correlated to structural changes.

The dynamic mechanical response over a range of frequencies and temperatures is analyzed. The α-keratin fibers behave more elastically at higher frequencies while they become more viscous at higher temperatures. The stress relaxation behavior of α-keratin fibers is established and fit to a constitutive relaxation equation based on the Maxwell-Wiechert model. The two relaxation constants are connected to two hierarchical levels of relaxation: the amorphous matrix-intermediate filaments interfaces, for the short term, and the cellular components for the long term. Results of creep testing provide important knowledge on the uncoiling and phase transformation of the α-helical structure as hair is uniaxially stretched. As the hairs are chemically treated, they exhibit a similar strain-rate sensitivity of ~0.05, which is attributed to the intermediate filaments. As a result, the strain-rate sensitivity of human hair is reduced by half, while that of horse hair remains unchanged. FTIR data confirms that the human hair is more sensitive to the -S-S- cleavage, resulting in an increase of cysteic acid. Therefore, the disulfide bonds in the matrix are
experimentally identified as one source of the strain-rate sensitivity and viscoelasticity in \( \alpha \)-keratin fibers.

In addition to human and horse hair that comprise the primary goal of this investigation, boar, giraffe, elephant, and bear hair were tested in tension, to establish the mechanisms of deformation and failure. In spite of differences in strength attributed to the condition of the hair, no significant changes were observed.
Chapter 1  Introduction of common biological materials

The synergy between biology and materials science has advanced the study of biomaterials and biological materials in the past few decades. The study of biological materials mainly focuses on natural materials (Chen et al., 2012), such as bones, feathers, horns, fish scales and animal hairs. Furthermore, the research on biomaterials aims to apply this knowledge into the biomedical area, for example dental implants and artificial hearts. Researchers are synthesizing novel materials or devices inspired from biological materials, i.e. biomimicking materials, for various applications. Bones, shells, and scales have given us many inspirations for building armor and defensive materials; feathers have helped to advance the industrial design in the areas of aerodynamics; teeth from white shark have given us new ideas about how to design and create the sharpest knife on earth. Numerous structures in nature have broadened our knowledge in designing new biomaterials. On the other hand, by understanding the research path of those biological materials, we can have more ideas on how to advance the research on keratinous materials, such as wool and hair, which have attracted much scientific interest since 1960s (Rouse and Van Dyke, 2010).

The best known bioinspired design from biological materials is Velcro. Georges de Mestral witnessed that burdocks attached onto clothes and animals in order to be carried away. Therefore, after years of mimicking the structure and testing the technique to achieve a similar performance, he patented this brand in the 1950s.
1.1 Bones

One of the best developed areas in biological materials is on bones (Fratzl and Weinkamer, 2007). Bones, as a fundamental structure in our body, have served an important role in constituting the skeleton. Being a composite material, bone is mainly composed of collagen, minerals and water. Volumetrically speaking, bone has 32-44% organic materials, 33-43% minerals and the rest as water (Olszta et al., 2007). Therefore, due to this organic–inorganic hierarchical composite design (shown in Figure 1.1), it is able to yield a high toughness and superior mechanical properties. Mostly, the fracture toughness of bones is of great interest. Since bone is highly hierarchical and the collagens have preferred orientations, its toughness has different values in different directions. For example, in the human cortical bone, the fracture toughness ($K_{IC}$) varies from 2.2 to 5.3 MPa m$^{1/2}$ (Nalla et al., 2003). Another interesting aspect of bones is the self-healing ability, and this has led us to the research on the self-healing composites that can repair their own cracks. During bone regeneration, osteoclasts first dissolve part of the bone and create a cavity, and then osteoblasts deposit new bone to fill the cavity. By adopting this idea, researchers have developed a self-healing material with microvascular networks (Toohey et al., 2007). The design in such material is to first apply a layer of epoxy coating with solid catalyst particles that could interact with the healing agent. The healing agent, on the other hand, constitutes a network underneath the top layer. Therefore, upon external force and in the presence of cracks, the healing agent flows to the surface and starts to polymerize under the influence of the catalyst. Mechanical tests show that this
healing behavior is highly efficient and the performance is good up to seven cycles (as shown in Figure 1.2).

Figure 1.1 Hierarchical structure from a whole bone to nanosized fibril (Weiner and Wagner, 1998).
Figure 1.2 (a) Mechanical behavior and (b) healing efficiency of the self-healing polymer (Toohey et al., 2007).
Another focus of the bone study is on implants and artificial bones (Vagaská et al., 2010). Metallic alloys such as Co, Cr, Ni and Ti have been widely applied; ceramics such as hydroxyapatite have also attracted much attention. Nevertheless, none of these substitutes has the full ability to mimic bone, which has a good elasticity as well as excellent mechanical properties. Therefore, it has occurred to scientists that the most suitable way to achieve such goal is to learn from the bone structure itself, i.e. a natural composite system that has multiple components.

1.2 Nacre

Another example that human beings learn from the nature is nacre (Figure 1.3). Chemically speaking, nacre is mainly made of calcium carbonate (CaCO₃), but it shows a toughness which is orders of magnitude higher than CaCO₃. Although in nacre, aragonite platelets are 95% of the weight, the remaining 5% macromolecules make a huge impact on the nacre’s fracture resistance. Longitudinally, CaCO₃ is comprised of numerous polygonal tablets which have a diameter of 3-5 µm and are separated by an organic gap. Furthermore, these aragonite layers are also separated by nanoscale inter-layers. Due to such organic-inorganic composite system, the tensile strength and Young’s modulus of nacre can reach to 140 MPa and 70 GPa, respectively (Askarinejad and Rahbar, 2015).

One example of bio-inspired design that came from the study of nacre is shown in Figure 1.4 (Bonderer et al., 2008). Researchers first produced alumina platelets and further dispersed them in ethanol. After spin-coating onto a substrate, this film exhibited
a very good extensibility and a high tensile strength. In order to mimic the natural nacreous structure, the alumina platelets were designed to have similar dimension with the aragonite platelets. Furthermore, the aspect ratio was carefully selected so that the load can be effectively transferred onto the platelets; thus, fracture is retarded. Due to this effect, the yield stress increases from 50 MPa to 300 MPa as the volume fraction increases from 0 to 0.15. At low volume fraction (10 % and 15 %), this film exhibits good plastic deformation to ~25 % strain, resulting from the yielding of polymeric matrix. The performances of these films show even more superior results compared to natural bone and nacre. Therefore, by mimicking the structure of nacre, we are able to produce artificial materials with even better performances.

Researchers have also produced another ionically-bonded polyelectrolyte matrix to mimic the nacre structure (Tang et al., 2003). They used the layer-by-layer assembly method to deposit clay platelets and polyelectrolytes and repeated for many times. The mechanical tests show high tensile stress (~120 MPa) and good extensibility. On the other hand, even though researchers have made much progress in mimicking the structures of nacres, they are only able to do so at the micro scale. For the natural nacre, even down to the nanoscale, each platelet is also a composite that is made of organic and inorganic materials. Further effort should be taken in mimicking the structure of those platelets.
1.3 Lotus leaf

The lotus leaf has been studied due to the superior hydrophobicity of its surface. Water droplets are seen rolled off the leaves, which leave little trace on the surface. Therefore, lotus leaf becomes a good example for us to learn in order to design self-cleaning materials. Since the water droplets are able to roll over easily, they can pick up the dust particles as they pass the material surface. This behavior is applicable for modern skyscrapers and light coatings. In fact, a commercial product called Lotusan that mimics
the structure of lotus leaf has now been applied on over half a million buildings all over the world.

The structure of lotus leaf is shown in Figure 1.4. It is composed of small cones that are several micrometers high and spaced 20 µm with each other (Meyers et al., 2011). At a much smaller scale, these cones are further covered with smaller cones/protrusions (Figure 1.5). These protrusions are evenly displaced to create a contact angle higher than 90° for the water spheres. Learning from this phenomenon, researchers synthesized a controlled rough copper surface by electrodeposition (Shirtcliffe et al., 2004). Copper dendrites grow on the pre-existing protuberances, which mimics the arrangement of little cones on lotus leaf. This structure is able to achieve super-hydrophobicity with a contact angle of water droplet from 115° to 165°.

Researchers have also attempted to combine the benefits of nacre and lotus leaf to create a superhydrophobic material (Zhong et al., 2013). Zhong et al. first synthesized graphene oxide (GO) sheets by a modified Hummers method. In the next step, they vacuum filtrated the GO suspension with dopamine hydrochloride. The latter chemical helps to create a graphene-polydopamine composite and this composite turned to be able to change the hydrophobicity of paper. What is also interesting about this treatment of paper is the stable properties towards different corrosive liquids. The nacreous brick-and-mortar structure provides a flexible and good mechanical property for the paper, and the lotus leaf-like hydrophobic surface enables a good self-cleaning effect.
Figure 1.4 Assembly of hybrid platelet reinforced polymer films (Bonderer et al., 2008).

Figure 1.5 Structure and scanning electron microscopy (SEM) images of lotus leaf (Shirtcliffe et al., 2004).
Figure 1.5 Structure and scanning electron microscopy (SEM) images of lotus leaf (Shirtcliffe et al., 2004), Continued.

Figure 1.6 Schematic image of water rolling on two protrusions (Meyers et al., 2011).
Figure 1.7 (a) Mechanical, (b) hydrophobic and (c) self-cleaning properties of the graphene composite paper (Zhong et al., 2013).
1.4 Shark skin

Sharks are able to swim at a very fast speed in the ocean (up to 12 mph) due to their unique skin structure (shown in Figure 1.8). Their skin is covered by numerous dermal denticles of ~ 100 µm long. These tiny scales were proven to be effective in minimizing momentum transfer and drag from the water (Bixler and Bhushan, 2013). These riblets on the dermal denticles help to direct an anisotropic flow, which leads to a much lowered drag. By mimicking the structure of these riblets, applications like swimsuits, airplane and submarine coating have been proposed (Dean and Bhushan, 2010). One of the most popular devices that resemble the shark skin is the swimsuit manufactured by Speedo. It is claimed that such a swimsuit is able to reduce friction by 3%. This will make significant difference in a sport that is measured by hundredth of one second.
1.5 Gecko foot

The above bioinspired designs have demonstrated the possibilities that the naturally refined structures can be mimicked to create new functional materials. Keratins, among the toughest biological materials, show great potential in this aspect (Karthikeyan et al., 2007). Keratin fibers have a highly hierarchical structure that is made with a bottom-up approach. One structure that could give us more ideas about how to achieve in mimicking keratin fibers is gecko feet, which also has a bottom-up hierarchical structure (Figure 1.9). At the nanoscale level, a large number of spatulae that are 200 nm in diameter constitutes one seta. Furthermore, these setae which are 100 µm long will
compose lamellae. These lamellae are the components visible with human eyes that compose the gecko feet pads. Due to the shape and size of the spatula, the gecko is able to produce a high adhesive force (Liu et al., 2012). Starting from understanding the basic structure and mechanics of gecko feet, applications like polyimide films with multiscales that have high adhesive ability (Liu et al., 2012) and elastomer micropillars with micromanipulation ability (Mengüç et al., 2012) have been developed and synthesized.

The Artz group (Arzt et al., 2003; Del Campo et al., 2007; Huber et al., 2005) has done extensive research on the synthetic fibers which mimics the structure of the gecko foot. These structures are hoped to achieve comparable reusable and effective attachment properties. The configuration with the symmetric spatula (Figure 1.10c) has a pull-off force much higher than the spatula with spherical tips (Figure 1.10b) and yields a pull-off stress close to the gecko foot. It was also shown that the pull-off stress is inversely proportional to the size of the pillars according to $R^{-1/2}$ (Figure 1.11).

Therefore, scientists have been demonstrating that by first studying the structure of a biological material, we will be able to mimic it and produce a similar biomaterial with comparable and even superior properties. The examples above illustrate the numerous opportunities that exist to create bioinspired materials and devices.
Figure 1.9 Hierarchical structure of gecko feet (Autumn and Peattie, 2002).

Figure 1.10 (a-d) SEM micrographs of synthetic micro-pillar arrays with different configurations (Del Campo et al., 2007).
1.6 Keratin fibers

Similarly, keratin fibers also show a hierarchical structure, starting from the nanoscaled intermediate filaments to the microscaled cortical cells. These intermediate filaments are embedded in a high-sulfide amorphous matrix; this configuration results in viscoelasticity due to these two major components. Therefore, although seemingly fragile, keratin fibers like wool and hair exhibit even higher toughness and Young’s modulus than wood and skin (Wegst and Ashby, 2004). Mimicking the structure of keratin fibers will be helpful for us to produce composite fibers which are lightweight and exhibit similar or even superior performance. Moreover, if we are able to incorporate two or even
more components when building up from the smallest scale to the entire fiber, we may produce a composite fiber with multiple advantages, such as high tensile strength and toughness, and similar strain-rate, humidity and temperature sensitivities. Nevertheless, we need to first analyze the structure and morphology of these materials, with the help of structural characterization tools at various length scales, to fully understand each component and its interactions and relations; then we also need to know their biomechanical properties and performances under different conditions; furthermore we need to investigate the chemical properties to understand the fundamental mechanisms during the interactions, even though some of these cannot be achieved under the current lab conditions. After achieving all the above steps, we finally may be able to use some available techniques (possibly 3-D printing) to build fibers with comparable performances in order to use them for different applications.

In this dissertation, we first introduce the background of α-keratin fibers with wool and hair as the examples, starting with the structure and morphology; then we further introduce two significant properties of hair, tensile and bending properties and their relevant factors, which include temperature, ethnicity, relative humidity, strain rate, etc.; we then discuss the chemical properties and methods to analyze them; finally we introduce some results on human and horse hair that have been done. We finish by comparing the tensile response and structural changes in the hair of a number of mammal: boar, giraffe, elephant, and bear. It is hoped that our study can provide a deeper understanding of the α-keratin fibers, contributing to the aim of producing bioinspired materials from this seemingly ordinary, yet very unique structure.
Chapter 2  Background studies on α-keratinous materials

2.1  Morphology and structure

The α-keratin fibers of wool and hair have a similar structure (Mercer, 1957), shown in Figure 2.1. The α-keratin fiber consists of an outermost layer, cuticle, which is composed of several sheets. Although this cuticle structure constitutes about 10% weight of the fiber, it was shown not to contribute to the tensile properties. The cortex, covered by the above cuticle, is composed of para cells and ortho cells. The ratio of these two components decides the curvature of the fibers (Fan and Yu, 2011). These cortical cells are usually 100 µm long and are visible under an optical microscope. Furthermore, cortical cells are constituted of macrofibrils, which are several hundred nanometers in diameter. These macrofibrils are further composed of microfibrils and matrix, with the former also called as intermediate filaments. The matrix has high sulfur content, which has a significant effect on the viscoelastic properties of the fibers.

At the nanoscale, two right-handed α-helix chains (Fraser and Macrae, 1973) form a dimer (Pauling et al., 1951). Two such dimers compose a protofilament, and then two such protofilaments associate into a protofibril. Four protofibrils further combine into one intermediate filament, which has a diameter of 7 nm. These helical structures are stabilized and bonded through hydrogen bonds. The intermediate filaments are further embedded in a sulfur-rich matrix which consists of two types of proteins and form one microfibril, as shown in Figure 2.2.
The cuticle of hair fiber is usually 5 µm thick, corresponding to the thickness of 5-10 scales (Figure 2.3). Each sheet is about 60 µm long and 0.5 µm in thickness.
Compared to the cuticle of human hair fiber, wool has a much smaller thickness, only 1-2 scales thick. Moreover, one such scale cell also has a four-layer structure: a thin layer named as epicuticle, a high-cystine exocuticle A, a low-cystine exocuticle B and endocuticle layer at the very bottom. Generally, the endocuticle and exocuticle have differences in the cystine compositions, which greatly influence the chemical linking within the proteins. Due to such reason, the cuticle structure shows a rough surface with intruding cuticle scales while in water and stretched in tension. Overall, the cuticle has a higher ratio of cystine than the rest parts of the fiber.

Figure 2.3 Schematic drawing of the cuticle structure (Swift, 1999).
Since the cuticle is the outermost layer that protects the inner structures of hair fibers, it has the most contact with the outside environment. Therefore, as a fiber is freshly pushed out of the follicle, it has very smooth and unbroken scale edges as shown in Figure 2.4a. This layered structure is packed uniformly. But only a few centimeters away, as the fibers grow, the hair begins to show some broken scale edges, as seen from Figure 2.4b. Such damage is usually caused by weathering and mechanical damage from combing and brushing. And this phenomenon is more obvious seen on long hair fibers.

Figure 2.4 Cuticle structure of (a) fresh and (b) worn hair fiber sections.
The cortex is made of cortical cells, shown in Figures 2.5 and 2.6. A typical cortical cell is usually 1-6 µm in diameter and about 100 µm long. Figure 2.6 shows a broken hair fiber after tension test. These cortical cells were closely packed and separated after breakage. Some studies show that the morphology of hair cortical cells may depend on the sulfur content, as well as on other functional groups (Manogue and Moss, 1953).
Figure 2.5 Split hair cortex with a cortical cell (Robbins, 2002).

Figure 2.6 Cortical cells shown in a hair fiber after tension tests.
The cortical cell, on its turn, is composed of numerous macrofibrils, which have a diameter of 0.1-0.4 µm. As shown in Figure 2.7, the macrofibrils have a splinter shape and combine as bundles. Under the transmission electron microscopy (Figure 2.8), the macrofibril also shows subcomponents which have a diameter of several nanometers.

Figure 2.7 Macrofibrils in a cortical cell (Fortier et al., 2012).

Figure 2.8 Transmission electron microscopy (TEM) image of a macrofibril (Harland et al., 2014).
The subcomponent of the macrofibril is called intermediate filament (or microfibril), as shown in Figure 2.9. The intermediate filaments have an average diameter of ~7.5 nm and are embedded in a matrix with a high concentration of disulfide bonds (Strelkov et al., 2003).

Figure 2.9 (a) TEM image and (b) XRD pattern of intermediate filament (Fraser et al., 1972).
2.2 Mechanical properties

2.2.1 Mechanical models

In order to interpret the mechanical properties of α-keratin fibers in wool and hair, different models were proposed by Feughelman (Feughelman, 1994, 1959), Wortmann and Zahn (Wortmann and Zahn, 1994) and Chapman (Chapman, 1969). Feughelman first proposed a two-phase model, as shown in Figure 2.10a, with microfibrils and matrix as the two distinct components. When stretched, these two exhibit the same strain. According to Feughelman’s model, during the Hookean region, no significant structural change is undergone; only bond angles and distances are changed. In the yield region, the fibers undergo a transition from α-helix to β-sheets (Kreplak et al., 2004).

Based on the two-phase configuration, Wortmann and Zahn differentiated the overall mechanical property with the individual contribution of filament and matrix (Figure 2.10b). In their model, during the Hookean region, the filaments undergo a linear stress-strain relation with bonds stretching, while the matrix yields at around 2% strain and maintains a constant stress at subsequent higher strains. This behavior is thought due to a gel-sol transition. In the yield region, both filaments and matrix exhibit a steady stress, which explains the decreased slope after the Hookean-yield turnover point. Once the transition from α-helix to β-sheets is completed, the filaments begin to show a further increase in stress, due to the stretching of the β-sheets. In the Wortmann/Zahn model, since filaments and matrix are considered separately, the overall property of the fibers comes from the combination of both components. According to their theory, the overall
property of $\alpha$-keratin fiber, $\sigma$, is obtained from two parallel components, $\sigma_f$ for the fiber and $\sigma_m$ for the matrix.

\[
F = F_f + F_m
\]  
(1)

Since,

\[
F = \sigma A, \quad F_f = \sigma_f A_f, \quad F_m = \sigma_m A_m
\]  
(2)

Therefore,

\[
\frac{F}{A} = \frac{F_f}{A} + \frac{F_m}{A}
\]  
(3)

\[
\sigma = \sigma_f V_f + \sigma_m V_m
\]  
(4)

where $V_f$ and $V_m$ are the volume fractions of fiber and matrix, respectively.

However, Chapman and Hearle (Chapman, 1969) gave a different explanation on the properties of filaments and matrix, as shown in Figures 2.10c and d. According to them, the stress-strain curve of intermediate filament shows a critical stress, followed by a constant stress. On the other hand, the matrix exhibits a low slope in the stress-strain curve initially and this slope increased as the extension continues (Figure 2.10d). During the $\alpha$-$\beta$ transition at the yield region, the stress is transferred from the intermediate filament to the matrix. The matrix is thought to be an elastomer and its stress-strain curve fits a large-strain rubber-elasticity stress-strain relationship up to 0.35 strain described by the Treloar equation:
\[ \sigma = \left( \frac{NkT}{3} \right)^{1/2} \left[ L^{-1} \left( \frac{\lambda^2}{n^2} \right) - \lambda^{3/2} L^{-1} \left( \frac{1}{(\lambda n)^{1/2}} \right) \right] \]  

(5)

\( \sigma \) is the stress, \( N \) is the number of chains per unit volume, \( k \) is the Boltzmann’s constant, \( T \) is the temperature, \( n \) is number of random links between the cross-links, \( \lambda \) is the stretch ratio and \( L \) is the Langevin function.

Despite the differences in the above structural models, they are both able to explain the overall properties of the \( \alpha \)-keratin fibers and supported by various experimental results. For example, Feughelman’s model corresponds well to the supercontraction results and the Chapman/Hearle model helps to explain the recovery behavior. Such debate is thought to continue until new evidence on the deformation mechanism is found.

Figure 2.10 (a) Two-phase schematic model of microfibril/matrix system (Feughelman, 1997); (b) series-zone model of the properties of filaments and matrix (Wortmann and Zahn, 1994); (c,d) properties of microfibril and matrix and predicted stress-strain curve (Chapman, 1969).
Figure 2.10 (a) Two-phase schematic model of microfibril/matrix system (Feughelman, 1997); (b) series-zone model of the properties of filaments and matrix (Wortmann and Zahn, 1994); (c,d) properties of microfibril and matrix and predicted stress-strain curve (Chapman, 1969), Continued.

2.2.2 Tensile properties and relevant factors

A typical stress-strain curve of α-keratin fiber is shown in Figure 2.11. It is characterized by three distinct regions. The first part is called as Hookean region, with a linear stress-strain response. During this process, no significant or structural change takes place (Figure 2.12a). Therefore, this deformation is reversible up to ~0.02-0.05 strain. The slope of this region is defined as the Young’s modulus of the keratin fiber. After the turnover point, around 0.05 strain, the curve enters the yield region, from 0.05 to 0.25 strain. During this process, the intermediate filaments undergo a transition from α-helix to β-sheets (Cao, 2002). These α-helical coiled coils (Steinert and Parry, 1985) first unravel, and then refold into the β-sheets. Such process does not require much force; therefore, the stress does not show an increase. The cuticle also shows morphology change as the hair is pulled (Figure 2.12b). The individual scales rotate away from the
tensile axis as they slide on top of each other. Once this transition is mostly complete, the curve goes into the post-yield region. Since the stretching of the β-sheets requires much force, it shows another increase in slope. The curve will keep going up until the hair reaches the final break (Figure 2.12c).

Although tensile tests measure the whole-fiber properties, researchers have reported that tensile properties are mainly provided by the cortex, not the cuticle. Robbins (Robbins and Crawford, 1991) found that even if the cuticle was broken or heavily damaged, there was no obvious difference in the longitudinal properties. But what is also interesting is that cuticle contributes to the bending properties, but this is not fully understood yet.

Figure 2.11 Typical stress-strain curve of hair fiber.
Figure 2.12 SEM images of hair at (a) 0, (b) 0.15 strains and (c) final break.
Researchers have used in-situ Atomic Force Microscopy (AFM) to monitor the surface change during the test. They found out that the cuticle did not lift off from the beginning. Instead of, it is more of a sudden change at around 0.2 strain. As shown in Figure 2.13, the white region indicates about 1 µm increase in height. And this phenomenon happens because the layered structures of cuticle have different extensibilities. Therefore, due to this effect, at 0.2 strain, the inner and outer layers will separate and cause the lift-off.
Figure 2.13 AFM images of hair cuticle during tension test (Seshadri and Bhushan, 2008a).
Starting from the basic tension properties, researchers also studied many related properties. As we know, the viscoelasticity means that the stress is not only dependent on the strain, but also on the time, or strain rate in the experiments. This provides the materials both viscous and elastic properties. Barnes and Roberts did stress relaxation tests on hair (Barnes and Roberts, 2000). They pulled hair fibers up to different strain, 0.5 to 6 percent and then measured the modulus. 20 just-taut hair bundles were stretched by 0.005 in 0.1 s and then monitored from 1 to 1000 s. Figure 2.15 shows that the relaxation modulus depends on the strain as well as time. They also found that stress-strain behavior departs from linearity at about 0.01, as shown in Figure 2.15b. Within a small extension, stress relaxation is the result of deformation and relaxation of peptide bonds and CO-/NH-groups; therefore, the curve shows a linear modulus-strain response.
Figure 2.15 (a) Time and (b) strain dependences of relaxation modulus of hair fibers (Barnes and Roberts, 2000).
It is well known that people of different ethnicities have different hair fibers (Franbourg et al., 2003; Seshadri and Bhushan, 2008b). Color and curvature are two very obvious differences. Franbourg et al. (Figure 2.16) and Seshadri et al. (Figure 2.17) studied the chemical compositions of Asian, Caucasian and African hair. Inherently, there is no apparent difference regarding proteins and amino acids. What is interesting here is that although these two research groups both tested these three ethnicities, the results are slightly different. Both of them found that African hair is very fragile and has low mechanical properties. But the first group reported that Caucasian hair is the strongest while the second group claimed that Asian hair is the strongest. These results indicate that in order to obtain the properties of hair fibers, it is necessary to test a large amount of samples to get the average value among them.

Figure 2.16 Comparison among three ethnic groups by Franbourg et al. (Franbourg et al., 2003).
Robinson et al. (Robinson and Rigby, 1985) studied the stress-relaxation behavior of hair in water. They first pulled the hair up to 0.15 strain and monitored the stress for another 30 minutes. The curve in Figure 2.18 shows a similar result as shown before. It should be noticed that they found that along the hair fiber, the mechanical properties from root to tip also have differences as shown in Figure 2.19. It is thought that these differences are due to the thiol content along the fibers. Therefore, this study shows that even for one fiber, there is a property gradient along the fiber length.

Since the hair exhibits viscoelasticity, we can apply the Maxwell model with a spring and a dashpot in series in order to understand this phenomenon. Since the stress on
both components is the same and the overall strain is the combination of strains of both parts, we have

\[ \sigma = \sigma_{spring} = \sigma_{dashpot} \]  
\[ \varepsilon_{overall} = \varepsilon_{spring} + \varepsilon_{dashpot} \]

After applying a characteristic time \( \tau = \frac{\eta}{k} \), we have

\[ \dot{\varepsilon} = \dot{\varepsilon}_{spring} + \dot{\varepsilon}_{dashpot} = \frac{\dot{\sigma}}{k} + \sigma \]  
\[ k \dot{\varepsilon} = \dot{\sigma} + \frac{1}{\tau} \sigma \]  
\[ 0 = \dot{\sigma} + \frac{1}{\tau} \sigma \]  
\[ \frac{d\sigma}{dt} = -\frac{1}{\tau} \sigma \]  
\[ \sigma(t) = \sigma_0 e^{(-t/\tau)} \]

In the meantime, during the stress relaxation, the \( \dot{\varepsilon} \) is equal to 0, therefore we have

This equation helps to understand the stress relaxation curve exhibited in Figure 2.18 and explain the differences in hair properties from the root to tip ends. The change of thiol content has an influence on the characteristic relaxation time \( \tau \), therefore affects stress during relaxation.
Figure 2.18 Schematic representation of stress-relaxation curve of hair fiber (Robinson and Rigby, 1985).

Figure 2.19 Stress relaxation curve of hair at 0.15 strain in 50 °C water (Robinson and Rigby, 1985).
Among the many factors that influence the mechanical properties of hair, one factor that has been noticed is the humidity (Feughelman and Robinson, 1967). Figure 2.20 shows a typical comparison of hair under 65 % and 100 % relative humidities. Results show that the yield stress under high relative humidity is much lower compared to a smaller relative humidity. On the other hand, the extensibility is much improved at higher humidity, as the breaking strain is greatly increased.

On this subject, wool has been intensely studied compared to hair. Table 2.1 shows here that at 100 % relative humidity, both hair and wool have a lower modulus and wool has a larger breaking strain compared to the dry state. A similar result has also been observed in human hair in our recent experiments.

Figure 2.20 Schematic comparison of hair properties under different relative humidities (Robbins, 2002).
Table 2.1 Elastic modulus vs. relative humidity for human hair and wool (Speakman, 1928a).

<table>
<thead>
<tr>
<th>% RH</th>
<th>65% RH</th>
<th>100% RH&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Human</td>
<td>Merino</td>
</tr>
<tr>
<td></td>
<td>Hair</td>
<td>Wool</td>
</tr>
<tr>
<td>Elastic modulus</td>
<td>5,394</td>
<td>3,040</td>
</tr>
<tr>
<td>% RH</td>
<td>Wool $E_S$ at given RH&lt;sup&gt;@&lt;/sup&gt; / $E_S$ at 100% RH</td>
<td></td>
</tr>
<tr>
<td>0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.76</td>
<td></td>
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<tr>
<td>100</td>
<td>1.00</td>
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</table>

After considering the effect of humidity, some researchers studied the influence of temperature on the tensile properties. Table 2.2 shows that the elastic modulus decreases significantly with increasing temperature, as well as the tensile strength. But, interestingly, the extensibility increases at higher temperatures. Therefore, it seems that high temperature somehow softens the $\alpha$-keratin fibers so that they can extend more before fracture, but they are inevitably more fragile.

Increasing temperature has a similar influence as increasing humidity. Rebenfeld (Rebenfeld et al., 1966) explained that this was due to the disulfide bonds were much relieved under increasing water content and temperature. Therefore, the matrix is thought to be essential to this mechanical behavior of human hair.
Table 2.2 The influence of temperature on elastic modulus, breaking stress and extensibility (Rebenfeld et al., 1966).

<table>
<thead>
<tr>
<th>Temperature °C</th>
<th>pH 7</th>
<th>Elastic modulus MPa</th>
<th>Stress at break MPa</th>
<th>% extension At break</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td></td>
<td>2.080</td>
<td>168</td>
<td>48</td>
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<tr>
<td>35</td>
<td></td>
<td>1.770</td>
<td>129</td>
<td></td>
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<td>50</td>
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<td>1.670</td>
<td>125</td>
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<td>70</td>
<td></td>
<td>1.640</td>
<td>140</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td></td>
<td>1.360</td>
<td>99</td>
<td>72</td>
</tr>
</tbody>
</table>

A further investigation on the thermal effect for the hair properties exhibited that as the temperature increased, it induced glass transition, structural change and denaturation to the keratin materials (Knopp et al., 1997; Mason, 1965; P Mason, 1964; P. Mason, 1964; Milczarek et al., 1992; Phillips, 1985). Figure 2.21 shows the results of temperature dependence of the keratin properties. The glass transition temperature ($T_g$) of hair is highly related to the water content. As the humidity increases, the transition temperature is greatly decreased. It is thought that this phenomenon is helpful in explaining the shape memory effect of hair after immersing in water, since water significantly decreases the $T_g$ so that the matrix returns to the glassy state. Other than this effect at ~35 °C, a further structural change is also observed at 60-70 °C, as shown in Figure 2.21b.
Dankovich et al. (Dankovich et al., 2004) studied the effect of twisting with two sets of experiments. First, they twisted the hair and pulled it without untwisting and they also untwisted the second set after twisting for certain number of turns. They found that after twisting, tensile stress, breaking strain and elastic modulus all decrease, as shown in Table 2.3. Therefore, it is thought that twisting creates damage to the hair fibers and some of these effects are not fully recoverable. Hair is weakened through the combination of tensile and torsional stresses. Even after untwisting the hair, some effects were left in the hair structure so that the mechanical properties are much decreased.
Table 2.3 The influence of twisting on the breaking stress, strain and Young’s modulus, based on (Dankovich et al., 2004).

<table>
<thead>
<tr>
<th>Twists (turns/cm)</th>
<th>Breaking stress (MPa)</th>
<th>Breaking strain (%)</th>
<th>Young's modulus (GPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>196.59±6.58</td>
<td>57.46±4.31</td>
<td>3.02±0.1</td>
</tr>
<tr>
<td>30</td>
<td>195.71±7.43</td>
<td>52.92±1.97</td>
<td>2.57±0.14</td>
</tr>
<tr>
<td>60</td>
<td>178.99±9.56</td>
<td>45.84±2.87</td>
<td>2.39±0.15</td>
</tr>
<tr>
<td>100</td>
<td>113.67±8.34</td>
<td>22.21±3.25</td>
<td>2.24±0.15</td>
</tr>
</tbody>
</table>

Robbins (Robbins, 2002) tested both dry and wet hair after permanent waving and found out there was a decrease of the tensile properties of 5–20% in the dry and wet hair on after permanent waving. The reason for this is that permanent waving breaks about 20% of the disulfide bonds and this will generate tensile damage to the hair fibers. Therefore, research proved that permanent waving significantly decreases the mechanical properties of human hair.

Table 2.4 Effect of permanent waving on the dry and wet hair (Robbins, 2002).

<table>
<thead>
<tr>
<th>Commercial home</th>
<th>Stress to break</th>
<th>Stress to extend 20%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Permanent wave</td>
<td>Dry (65% RH)</td>
<td>Wet</td>
</tr>
<tr>
<td></td>
<td>-7%</td>
<td>-15%</td>
</tr>
<tr>
<td></td>
<td>Dry (65% RH)</td>
<td>Wet</td>
</tr>
<tr>
<td></td>
<td>-11%</td>
<td>-18%</td>
</tr>
</tbody>
</table>
Finally, researchers have studied the effect of pH (Figures 2.22 and 2.23). Although we may not experience such dramatic pH range in our daily life, the results are helpful for our understanding. It turned out that pH does not work alone on the tensile properties. It is a factor because it affects the swelling property of hair. Figure 2.23 shows that the hair swells from pH 2 to 9. And another study by Breuer (Breuer and Prichard, 1967) shows that hair will have irreversible changes in a very low pH environment.

Figure 2.22 20 % index of hair under low pH (20 % index indicates the work to stretch hair to 0.2 strain) (Valko and Barnett, 1952).
2.2.3 Bending property and relevant factors

Since hair fiber is so thin, researchers have been trying many methods to obtain its bending properties. Figure 2.24 shows one of the easiest methods (Scott and Robbins, 1969). It is called balanced fiber method. First, a fiber was attached with two small equal weights on the two ends and hung over a tiny hook. Since the stiffness of fiber will determine the distance between the two legs, through measuring D we can indirectly measure the stiffness of hair fiber. These researchers also provided a stiffness coefficient (G) and bending modulus (E_B) based on the weight and leg distance.

\[
\text{Stiffness coefficient (G): } G = \frac{Td^2}{8}
\]  

(13)
Bending modulus \((E_B)\): \[ E_B = \frac{\pi T d^2}{2A^2} \] (14)

Figure 2.24 Schematic drawing of balanced fiber method (Scott and Robbins, 1969).

In a paper several years later, Scott and Robbins carried out more tests and measurements in order to prove the validity of their method (Scott and Robbins, 1978). Eq. (15) shows if the bending modulus \(E_B\) can be correctly expressed in terms of the small weights and the distance between the two legs, the square of stiffness index \(D\) should be proportional to \(1/T\). Therefore, by changing the loads of the weights, they proved that the equation is valid (Figure 2.25).

Relation between \(d^2\) and \(T^{-1}\): \[ \frac{T}{E_B l} = \frac{8}{d^2} \] (15)
Then these results raised another question: if the stiffness index is related to the modulus, are the bending stiffness and Young’s modulus also related to each other? After testing the properties of many fibers and obtaining the averages of both $E_B$ and $E_S$, they reported that $E_B$ and $E_S$ are almost equal. Table 2.5 shows the averages of bending modulus and Young’s modulus for twelve fiber sections. The ratio between $E_B$ and $E_S$ is approximately to 1. This experiment shows that the bending stiffness and Young’s modulus could be related to each other. At the bending state, part of the fiber is
compressed and the other part is extended. It is considered that the compressed part creates buckling so that it does not contribute to the overall bending properties.

Table 2.5 Comparison between the bending modulus and Young’s modulus for various fibers, adopted from (Scott and Robbins, 1978).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Diameter (µm)</th>
<th>Bending modulus--$E_B$ (GPa)</th>
<th>Young’s modulus--$E_S$ (GPa)</th>
<th>$E_B/E_S$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>98.34</td>
<td>4.23</td>
<td>3.68</td>
<td>1.15</td>
</tr>
<tr>
<td>2</td>
<td>96.04</td>
<td>3.54</td>
<td>3.82</td>
<td>0.93</td>
</tr>
<tr>
<td>3</td>
<td>93.11</td>
<td>4.29</td>
<td>3.43</td>
<td>1.25</td>
</tr>
<tr>
<td>4</td>
<td>83.53</td>
<td>4.25</td>
<td>3.83</td>
<td>1.11</td>
</tr>
<tr>
<td>5</td>
<td>82.01</td>
<td>4.11</td>
<td>3.75</td>
<td>1.1</td>
</tr>
<tr>
<td>6</td>
<td>81.11</td>
<td>3.35</td>
<td>3.96</td>
<td>0.85</td>
</tr>
<tr>
<td>7</td>
<td>72.84</td>
<td>3.6</td>
<td>4.12</td>
<td>0.88</td>
</tr>
<tr>
<td>8</td>
<td>71.70</td>
<td>4.69</td>
<td>3.98</td>
<td>1.18</td>
</tr>
<tr>
<td>9</td>
<td>71.50</td>
<td>3.74</td>
<td>4.03</td>
<td>0.93</td>
</tr>
<tr>
<td>10</td>
<td>64.11</td>
<td>3.23</td>
<td>4.21</td>
<td>0.77</td>
</tr>
<tr>
<td>11</td>
<td>57.82</td>
<td>2.89</td>
<td>4.33</td>
<td>0.67</td>
</tr>
<tr>
<td>12</td>
<td>55.15</td>
<td>3.58</td>
<td>3.59</td>
<td>1</td>
</tr>
<tr>
<td>Average</td>
<td>77.27</td>
<td>3.79</td>
<td>3.89</td>
<td>0.97</td>
</tr>
</tbody>
</table>

Other than the balanced fiber method, some researchers also applied the following bending pendulum method to obtain the bending properties, as shown in Figure 2.26 (Speakman, 1928b). In order to do so, they lined up 39 parallel hair fibers and swung a bar onto the fibers. Eventually the bar was stopped by the hair because the dynamic energy was transferred into the internal energy in the fibers. Therefore, the number of strokes that the bar hit the fibers is related to the stiffness as stiffer fibers result in a lower number of strokes. But since they could not obtain the stiffness from this number, instead they only used it to represent the bending property, as explained in Eq. (16). As shown in Figure 2.27, the method has consistent results for different hair series. In order to prove the validity of this method, Baltenneck et al. related the number of strokes, $N$, to the
number of hair fiber (Figure 2.28). Therefore, this method provides us another easy way to test and obtain the bending properties of fibers.

\[
N = \frac{E_{\text{init}}}{E_{\text{flex}}} = \frac{4}{n \pi k} \frac{E_{\text{init}}}{E} \frac{1}{R^4}
\]  

(16)

where \( n \) is the number of hair fibers, \( k \) is the geometric constant for the bending test (0.2149 mm\(^{-1}\)), \( E_{\text{init}} \) is initial energy of the pendulum, \( E \) is the intrinsic flexural modulus, \( R \) is the mean radius of hair fibers.

Figure 2.26 Schematic drawing of bending pendulum method (Baltenneck et al., 2001) (at each cycle, the pendulum hits the hair and bends it, being slowed down by it).
Figure 2.27 Number of strokes of two measurements for various hair series (Baltenneck et al., 2001).

Figure 2.28 Relation between number of strokes and $1/n$ (Baltenneck et al., 2001).
There are also factors that influence the bending properties. Different from tensile properties, the fiber diameter plays an important role in determining the bending properties. As shown in Figure 2.29 from the balanced fiber method, as the linear density goes down, the stiffness index D also decreases. Similarly, the bending pendulum method exhibited the same trend, as the number of strokes increases with thinner hair fibers. They also found that 94 % of the variation in stiffness is due to the change in fiber diameter. Therefore, the stiffness and bending properties of hair fibers are shown to be related to the fiber diameter.

Moreover, the effect of diameter on the bending stiffness can be directly obtained from the flexure formula:

\[ \delta = \frac{F}{3EI}L^3 \]  

(17)

where \( \delta \) is the displacement, F is the force, L is the length of the hair strand, and I is the moment of inertia as following:

\[ I = \frac{\pi d^4}{64} \]  

(18)

where d is the diameter of hair.
Figure 2.29 Effect of fiber diameter on the stiffness index (D) (Scott and Robbins, 1978).

Figure 2.30 Effect of fiber diameter on the number of strokes (Baltenneck et al., 2001).
Predictably, humidity affects the properties. Experiments using the balanced fiber method (Figure 2.31) show that with increasing humidity, the bending stiffness will drop. The bending pendulum method gives a similar result (Figure 2.32). It shows that as the humidity goes up, the number of strokes increases for different hair fibers.

Figure 2.31 Effect of relative humidity on the stiffness index (D) (Scott and Robbins, 1978).

Figure 2.32 Effect of relative humidity on the number of strokes (Baltenneck et al., 2001).
What should be noticed is that the cuticle contributes to the bending stiffness. Researchers in Japan found out after damaging the scales of wool fibers, the bending stiffness dropped significantly (Kawabata et al., 2004; Yasuda et al., 2002). They first reported the different Young’s moduli for cortex and cuticle and the difference of rigidity for virgin and damaged hair (Figure 2.33). The stripped hair shows a decreased rigidity modulus (0.4 GPa) compared to the intact hair (0.8 GPa). On the other hand, even though cuticle is of several micrometers thick, it contributes ~57 % to the flexural rigidity (Table 2.6). Later they reported the differences in bending stiffness for original wool fiber and scaled-off wool fiber, as shown in Table 2.7. After the removal of the wool fiber cuticle, the tensile modulus did not change much (4.24 and 4.17 GPa), but the bending modulus changed from 0.943 GPa to 0.307 GPa. Considering that cuticle only occupies 14 % in the cross-sectional area, the influence of cuticle on the bending property is shown of significant importance.

![Figure 2.33 Comparison of rigidity modulus for intact hair and stripped hair (Yasuda et al., 2002).](image)
It is possible that the cuticle keeps the fibers of the cortex together and acts as a ‘wrapping’. In its absence, the fibers in compression can buckle during bending.

Table 2.6 Torsional properties of components in human hair (Yasuda et al., 2002).

<table>
<thead>
<tr>
<th></th>
<th>Total hair</th>
<th>Cortex</th>
<th>Cuticle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young’s modulus [GPa]</td>
<td>9.49</td>
<td>5.55</td>
<td>19.85</td>
</tr>
<tr>
<td>Occupied area in cross section [%]</td>
<td>100.0</td>
<td>85.96</td>
<td>14.04</td>
</tr>
<tr>
<td>Contribution to flexural rigidity [%]</td>
<td>100.0</td>
<td>42.36</td>
<td>57.64</td>
</tr>
</tbody>
</table>

Table 2.7 Elastic properties of original and off-scaled wool fiber (Kawabata et al., 2004).

<table>
<thead>
<tr>
<th></th>
<th>Bending rigidity</th>
<th>*Bending Modulus</th>
<th>Tensile</th>
<th>Shear</th>
<th>Transverse</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diameter (μm)</td>
<td>B (mgf·mm²)</td>
<td>E₀ (GPa)</td>
<td>Eₘ (GPa)</td>
<td>Gₘ (GPa)</td>
</tr>
<tr>
<td>Wool (original)</td>
<td>Average</td>
<td>21.6</td>
<td>1.49</td>
<td>0.943</td>
<td>4.24</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>3.23</td>
<td>0.923</td>
<td>0.133</td>
<td>0.753</td>
</tr>
<tr>
<td>Wool (off scale)</td>
<td>Average</td>
<td>18.4</td>
<td>0.211</td>
<td>0.307</td>
<td>4.17</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>3.42</td>
<td>0.0390</td>
<td>0.0780</td>
<td>0.482</td>
</tr>
</tbody>
</table>

2.2.4 Nanomechanical Characterization

Nanomechanical characterization provides us a new tool for understanding the properties of human hair. AFM has been widely used to observe the cross-sectional and lateral surfaces (Chen and Bhushan, 2005; Seshadri and Bhushan, 2008b; Wei and Bhushan, 2006). Figure 2.34 is an AFM image of the hair transverse surface. It shows that the cuticle contains about six layers and it is several micrometers thick.
Nanoindentation is another powerful tool for nanoscale characterization (Bhushan and Chen, 2006; LaTorre and Bhushan, 2005; Wei and Bhushan, 2006). Figure 2.35a shows different hardness and Young’s moduli of human hair, depending on the indentation depth. These researchers also studied the effect of ethnicity, temperature and humidity using the nanoindenter (Figures 2.35 b-d). Mostly these properties are consistent with the macro properties.
Figure 2.35 (a) Nanoindentation results of virgin hair in different depth; comparisons of virgin, damaged and treated hair at different (b) temperatures, (c) relative humidity and (d) ethnicity (Bhushan and Chen, 2006; LaTorre and Bhushan, 2005; Wei and Bhushan, 2006).
2.3 Chemical properties

Since hair fibers are mainly composed of keratins, understanding the chemical properties of human hair requires us to investigate the bonding mechanisms of various chemical groups. Table 2.8 lists the hair amino acid compositions for different ethnicities. The results show much difference in cysteic acid and half cystine, which are thought to be helpful in explaining the mechanical differences of hair among these groups. It should be noticed that there is clearly an overlap in values of most amino acids, except for phenylalanine.
Table 2.8 Amino acid compositions of hair fibers from three ethnicities (µM/g) (Wolfram, 2003).

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>African</th>
<th>Brown/Caucasian</th>
<th>Asian</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>370-509</td>
<td>345-475</td>
<td>370-415</td>
</tr>
<tr>
<td>Arginine</td>
<td>482-540</td>
<td>466-534</td>
<td>492-510</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>436-452</td>
<td>407-455</td>
<td>456-500</td>
</tr>
<tr>
<td>Cysteic acid</td>
<td>10-30</td>
<td>22-58</td>
<td>35-41</td>
</tr>
<tr>
<td>1/2 Cystine</td>
<td>1310-1420</td>
<td>1268-1608</td>
<td>1175-1357</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>915-1017</td>
<td>868-1053</td>
<td>1026-1082</td>
</tr>
<tr>
<td>Glycine</td>
<td>467-542</td>
<td>450-544</td>
<td>454-498</td>
</tr>
<tr>
<td>Histidine</td>
<td>60-85</td>
<td>56-70</td>
<td>57-63</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>224-282</td>
<td>188-255</td>
<td>205-244</td>
</tr>
<tr>
<td>Leucine</td>
<td>484-573</td>
<td>442-558</td>
<td>515-546</td>
</tr>
<tr>
<td>Lysine</td>
<td>198-236</td>
<td>178-220</td>
<td>182-196</td>
</tr>
<tr>
<td>Methionine</td>
<td>6-42</td>
<td>8-54</td>
<td>21-37</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>139-181</td>
<td>124-150</td>
<td>129-143</td>
</tr>
<tr>
<td>Proline</td>
<td>642-697</td>
<td>588-753</td>
<td>615-683</td>
</tr>
<tr>
<td>Serine</td>
<td>672-1130</td>
<td>851-1076</td>
<td>986-1101</td>
</tr>
<tr>
<td>Threonine</td>
<td>580-618</td>
<td>542-654</td>
<td>568-593</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>179-202</td>
<td>126-194</td>
<td>131-170</td>
</tr>
<tr>
<td>Valine</td>
<td>442-573</td>
<td>405-542</td>
<td>421-493</td>
</tr>
</tbody>
</table>

α-keratin fibers such as hair and wool are bonded through several bonding mechanisms (Steinert et al., 1993). Among these mechanisms, weaker bonds such as hydrogen bond, ionic interaction and van der Waals’ interaction are mainly located between the side chain groups and neighboring groups (Akhtar et al., 1997). On the other hand, the disulfide (-S-S-) bonds is considered the strongest and affects the properties of the keratin fibers (Pescatore, 1985; Williams et al., 1994). During an oxidation reaction, two adjacent thiol groups in the polypeptide chains would react with each other and form a molecule of cystine, as shown in the following reaction (Eq. 19).
Figure 2.36 summarizes several important sulfur-containing amino acids existing in the human hair fibers. In order to understand the change in the bond compositions, Fourier transform infrared spectroscopy (FTIR) has been widely applied to monitor certain chemical groups (Table 2.9): cystine dioxide (1121 cm\(^{-1}\)), cystine monoxide (1071 cm\(^{-1}\)), cysteic acid (1040 cm\(^{-1}\)), and cysteine-S-thiosulphate (1022 cm\(^{-1}\)).

Table 2.9 IR absorbance frequencies of several cystine species (Carr and Lewis, 1993).

<table>
<thead>
<tr>
<th>Species</th>
<th>Structure</th>
<th>Wavenumber (cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystine dioxide</td>
<td>(-\overset{\text{O}}{\text{O}}) (-\overset{\text{S}}{\text{S}}) (-\overset{\text{O}}{\text{O}})</td>
<td>1121</td>
</tr>
<tr>
<td>Cystine monoxide</td>
<td>(-\overset{\text{O}}{\text{O}}) (-\overset{\text{S}}{\text{S}}) (-\overset{\text{O}}{\text{O}})</td>
<td>1071</td>
</tr>
<tr>
<td>Cysteic acid</td>
<td>(-\overset{\text{SO}_3^-}{\text{S}}) (-\overset{\text{SO}_3^-}{\text{S}}) (-\overset{\text{SO}_3^-}{\text{S}}) (-\overset{\text{SO}_3^-}{\text{S}}) (-\overset{\text{SO}_3^-}{\text{S}}) (-\overset{\text{SO}_3^-}{\text{S}}) (-\overset{\text{SO}_3^-}{\text{S}})</td>
<td>1040</td>
</tr>
<tr>
<td>S-Sulphonate (Bunte salt)</td>
<td>(-\overset{\text{S}}{\text{S}}) (-\overset{\text{SO}_3^-}{\text{S}}) (-\overset{\text{SO}_3^-}{\text{S}}) (-\overset{\text{SO}_3^-}{\text{S}}) (-\overset{\text{SO}_3^-}{\text{S}}) (-\overset{\text{SO}_3^-}{\text{S}}) (-\overset{\text{SO}_3^-}{\text{S}}) (-\overset{\text{SO}_3^-}{\text{S}}) (-\overset{\text{SO}_3^-}{\text{S}}) (-\overset{\text{SO}_3^-}{\text{S}})</td>
<td>1022</td>
</tr>
</tbody>
</table>
Figure 2.37 shows a typical FTIR spectrum for wool fiber. Among the various peaks, amide III at 1231-1235 cm\(^{-1}\) and CH\(_2\) stretching at 1451 cm\(^{-1}\) are usually used as the internal standards (Joy and Lewis, 1991; Pande, 1994; Strassburger, 1985).

![FTIR spectrum of wool fiber](image)

Figure 2.37 Typical FTIR spectrum of α-keratin fibers (Lipp-sy monowicz et al., 2012).

Researchers have studied the effect of weathering on the human hair (Signori and Lewis, 1997). Figure 2.38 shows the results of fifteen hair fibers. As the fiber is pushed out of the follicle, the weathering effect becomes more prominent; this increases from the root to the tip end. The peak intensities for cysteine-S-thiosulphate do not show much increase regarding the distance from the tip; on the other hand, the peak height of the cysteic acid band increases significantly towards to the tip.
A similar study by Carr and Lewis shows a similar result (Figure 2.39) (Carr and Lewis, 1993). They aged wool to certain amount of time and it introduced a significant increase in the cysteic acid band intensity. Therefore, this confirms that sunlight and weathering induce photochemical changes in the α-keratin fibers.

Figure 2.38 FTIR data of the weathering effect on human hair fibers (Signori and Lewis, 1997).

Figure 2.39 Effect of exposure on the intensity of cysteic acid band (Carr and Lewis, 1993).
Bleaching, on the other hand, has widely studied due to its popularity in the cosmetic industry. The lightening the color of hair is usually accomplished by oxidation (Jachowicz, 1987). During such process, disulfide bonds are oxidized and formed into cysteic acid. FTIR results show that the absorbance band at 1040 cm\(^{-1}\) for the cysteic acid would increase with an increasing bleaching time. Figure 2.40 shows the comparison between hair without bleaching, after 30 minutes and 60 minutes bleaching.

Therefore, FTIR is considered as helpful to investigate the certain effects on the hair fibers. Combining the data from FTIR analysis and mechanical properties can provide new insights into the influences of different chemical groups on the overall fiber properties.

Figure 2.40 FTIR spectra from wavenumber 1000 cm\(^{-1}\) to 1100 cm\(^{-1}\), for chemically-bleached hair (Signori and Lewis, 1997).
2.4 Research objectives

Nature has been perfecting the structure of hair fibers for millions of years. Unlike most techniques available these days under the lab condition, the structure of hair is built in a bottom-up approach. From the molecular level α-helix to the macro level cortical cells, the mechanical properties of hair are strengthened through each component and the interactions between those components. Although seemingly weak, preliminary results show that hair exhibits a very good mechanical properties and different performances under various conditions. This has intrigued much interest in the biological science world since it seems that we still lack a deeper knowledge in understanding even one of the most basic parts of our body.

Besides mere knowledge, understanding the roles of each component will also enable us to find new methods in strengthening our hair, making stronger and more resilient hair. This will greatly benefit the cosmetic industry since “making the hair stronger after using our shampoo” will stop being only a commercial slogan. We will probably be able to target at certain bonds and linkages which we may later find out that are essential for the mechanical properties of human hair. Moreover, we may be able to produce composite fibers with similar structure as the natural hair. Since two or more components are incorporated in such fibers, these materials can exhibit better performances in extensibility and strength, a result that we have known through the study on human hair.

Therefore, as a scientific investigation, we will focus on the following aspects in our experiments:
(i) Structure, morphology and mechanical properties of human hair under various conditions.

We would like to analyze the effects of strain rate, relative humidity and temperature on the mechanical properties of α-keratin fibers. We would like to propose a prediction based on these experimental data, which would also be helpful in analyzing other α-keratin materials.

(ii) Comparisons in structure and property between human and other various animal hairs.

In order to understand the roles of different components in hair fibers, we would like to compare the different animal hairs with the human hair and understand the reasons of different mechanical properties and different strain-rate sensitivities under dry and wet state. Thermal analysis such as TGA and DSC will also be reported on all hair samples. Moreover, experiments will be done on analyzing the performance of fibrils by eliminating the contribution of matrix.
Chapter 3  Morphology of α-keratin fibers

3.1  Structural and morphological characterization

Figure 3.1 shows transmission electron micrographs of transverse cross-section of hair. Within cortical cells, which are separated by a cell membrane complex (the boundaries are marked in micrograph), there are approximately 20,000 intermediate filaments. Two lines are shown between cells, each corresponding to a cell wall (indicated by arrows in Figure 3.1). The darker regions correspond to the remains of organelles and possibly cell nuclei. There is also an intermediate structure, macrofibrils, with a diameter of ~250 nm, formed by the IFs. At the highest magnification, the circles with ~7 nm diameter correspond to the intermediate filaments. They are connected by non-crystalline molecules and there seems to be a profusion of S-bonds that provide strong bonding between them (Hearle, 2007; Marshall et al., 1991).

Figure 3.1 Transmission electron micrographs (TEM) of human hair showing the cell membrane complex and intermediate filaments (detailed on right).
Figures 3.2a and 3.2b show the structure of cuticle on a transverse surface after the hair was fractured in liquid nitrogen; macrofibrils of a hair fiber can be identified after the cross section was exposed (Figures 3.2c and d). The original hair with a diameter of ~90 µm has a layered cuticle structure; sheets overlap and form a lamellar structure surrounding the central cortex (Figure 3.2a). A higher magnification SEM micrograph (Figure 3.2b) shows that the scales of this sample are broken and the edges are damaged. Figure 3.2c shows a fractured surface of the hair. Cortical cells with several micrometers in diameter align parallel to the direction of hair growth. A higher resolution image in Figure 3.2d shows the macrofibrils in the cortical cells. These macrofibrils exhibit a diameter close to ~0.5 µm (indicated by arrows) and are tightly attached to each other even after the cortical cells are torn apart. The structural characterization reveals that the hair has a compact architecture in hierarchy with the scales on the surface.

Figure 3.2 SEM images of (a) and (b) cuticles at different magnifications, (c) cross section, and (d) macrofibrils (indicated by arrows) of a human hair fiber.
Figure 3.2 SEM images of (a) and (b) cuticles at different magnifications, (c) cross section, and (d) macrofibrils (indicated by arrows) of a human hair fiber, Continued.
3.2 Fracture surface and cuticle morphology

The fracture mechanisms at different strain rates were studied by examining the fracture surfaces. Figures 3.3 (a-e) shows the fracture surfaces of the specimens from low strain rate ($10^{-4}$ s$^{-1}$) to high strain rate ($10^{0}$ s$^{-1}$) and Figure 3.3f shows the schematic drawings of three modes of fracture identified. According to Kamath and Hornby (Kamath and Hornby, 1984), there are five fracture modes of human hair: split-ends, fibrillated, angle (Figures 3.3a and 3.3b), step (Figures 3.3c and 3.3d) and smooth (Figure 3.3e). At low strain rates ($10^{-4}$ and $10^{-3}$ s$^{-1}$), hair behaves as a ductile material and shows an angle-end fracture mode (Figures 3.3a and 3.3b). As the strain rate increases, the hair starts to show a step-end mode (Figures 3.3c and 3.3d). The cortical cells break at different time during elongation to produce such fracture mode. However, at the high strain rate of $10^{0}$ s$^{-1}$, the fracture surface exhibits a flat end (Figure 3.3e) indicating a sharp split. This mode resembles the fracture surface of a brittle material. This indicates that the inter-fiber sliding, which takes place at low strain rates, is inhibited at high strain rates. The matrix material, being amorphous, shows significant strain-rate sensitivity, whereas the fibers can be considered as strain-rate insensitive. These results suggest that the fracture of cortical cells spreads gradually between neighboring cells until the whole hair is broken. The changes in the fracture surfaces ranging from low strain rate to high strain rate are illustrated in Figure 3.3f. This embrittlement of the keratin at higher strain rates has been observed earlier by Seki et al. (Seki et al., 2010) for toucan beak keratin and Wang et al. (Wang et al., 2016b) for pangolin scale.
Figure 3.3 Fracture surface of hair specimen tested at (a) $10^{-4}$, (b) $10^{-3}$, (c) $10^{-2}$, (d) $10^{-1}$, and (e) $10^{0}$ s$^{-1}$ strain rates and (f) schematic representations of fracture modes from low strain rate to high strain rate (At low strain rates, fiber pulling and inter-fiber sliding is more prominent. At high strain rates, fracture surface is flat.).
The surface morphology change of hair cuticle at various strains was monitored using an ex-situ method. At a small strain (0.02), the cuticles do not show any lifting in the edges (Figure 3.4a). However, as the strain increases to 0.15 (Figure 3.4b) and 0.35 (Figure 3.4c), the edges start to lift off and the surface becomes rougher; such a change is shown in the schematic drawing in Figure 3.4d. This was also confirmed by an in-situ AFM study (Seshadri and Bhushan, 2008b). The phenomenon is thought to be due to different extensibilities in the layered cuticle structure. During elongation, the separation of these layers in the cuticle causes the roughness in the cuticle surfaces (Seshadri and Bhushan, 2008b).
Figure 3.4 Surface morphology examined ex-situ under SEM at (a) 0.02, (b) 0.15, and (c) 0.35 strains and (d) illustration of scale edge lifting process.
Furthermore, the effect of temperature on the cuticles was studied by observing the surface morphology near the fracture sites. At low temperatures (20 and 40 °C), the hair clearly shows the cuticle edges near the fracture sites (Figures 3.5a and 3.5b). However, as the temperature increases to 60 °C (Figure 3.5c) and 80 °C (Figure 3.5d), the edges of cuticle scales become undiscernible, which suggests that high temperature somehow has a ‘fusing’ effect on them. This further confirms that higher temperature not only changes the mechanical properties in hair, but also affects the surface morphology.
Figure 3.5 Surface morphology near fracture sites at (a) 20 °C, (b) 40 °C, (c) 60 °C, and (d) 80 °C in water.
Figure 3.5 Surface morphology near fracture sites at (a) 20 °C, (b) 40 °C, (c) 60 °C, and (d) 80 °C in water. Continued.
Figure 3.6 shows the surface morphology of a cortical cell in human hair after tension. The surface exhibits a suture-like structure, which greatly increase the surface area and contact area of cortical cells and therefore increase the adhesion between adjacent cells. Moreover, the suture structure with a width of several hundred nanometers creates an interlocking effect, as previously shown in pangolin scales (Wang et al., 2016b). As the hair is stretched under tension, this structure mechanically decreases the sliding behavior between cortical cells.

Figure 3.6 Scanning electron micrographs (SEM) of cortical cell surface in a tensioned untreated human hair fiber (arrows indicate the suture-like morphology).

Figure 3.7 shows the cross-sectional area of a human hair fiber after tension. Four layers of cuticle sheets can be identified in Figure 3.7a. These cuticle sheets overlap onto each other, as indicated by the arrows (Figure 3.7a). An enlarged SEM micrograph
(Figure 3.7b) shows that the cuticle structure exhibits a strong adhesion between the sheets, which leaves no spacing even after the hair is fractured after tension.

Figure 3.7 Scanning electron micrographs (SEM) of (a, b) cortical cell surface in a tensioned untreated human hair fiber (note the layered structure of cuticles) (arrows indicate the edge of one cuticle sheet).
Chapter 3, in part, is a reprint of the materials as it appears in Materials Science and Engineering, C, 2017, Yang Yu, Wen Yang, Bin Wang, and Marc A. Meyers. The dissertation author was the primary author of this paper.
Chapter 4  Tensile properties of α-keratin fibers

4.1  Introduction

Hair, an important part of our body, not only possesses aesthetic significance in our culture, but also offers protection. This fiber-reinforced nanocomposite plays a key role as an outer covering in many vertebrates (McKittrick et al., 2012). Hair fibers have a typical hierarchical structure similar to other α-keratin materials, such as wool, nails, claws, and horns present in mammals. The keratin in reptiles and birds is primarily in the form of β-sheets (Wang et al., 2016a). Keratinous materials are categorized as α-keratin if they exhibit a helical secondary structure or as β-keratin if they are in the shape of sheets. A typical hair fiber has a diameter of 50-100 µm and is covered by an outermost layer, the cuticle. The cuticle consists of thin overlapping scales (Swift, 1999). Each scale has an average length of 60 µm and a thickness of about 0.5 µm. Furthermore, 5-10 such scales overlap to create a total thickness of ~5 µm. The morphology of the cuticle edges is thought to be affected by weathering, combing, and brushing, with more severe damage seen on long hair fibers (Garcia et al., 1978).

Figure 4.1 shows the hierarchical structure of hair. The inner section of hair is called cortex and is composed of cortical cells that are ~100 µm long and 1-6 µm thick. These cortical cells are composed of subcomponents called macrofibrils. The macrofibrils exhibit a diameter of 0.1-0.4 µm (Harland et al., 2014). At the nanometer scale, they are composed of intermediate filaments (IF) embedded in a matrix with high-sulfide content. One IF has a diameter of ~7.5 nm and is formed by eight protofilaments.
Each protofilament is composed on its turn of four right-handed $\alpha$-helix chains; therefore a total of thirty-two chains form an IF (Voet et al., 2008).

![Figure 4.1 Schematic representation of hierarchical structure in human hair starting at $\alpha$-helix chains and progressing to the entire section.](image)

Hair fibers have 65-95 wt% of proteins depending on the humidity and up to 32% of water, with the rest as lipid pigments and other components (Velasco et al., 2009). Therefore, chemically the properties of human hair are dominated by the $\alpha$-keratin (Kreplak et al., 2001). It has been demonstrated that the tensile properties of hair are mostly produced by the cortex, not the cuticle. Robbins et al. (Robbins and Crawford, 1991) damaged the cuticle with chemicals and found no apparent difference in the tensile properties with original hair fibers. Relaxation tests by Barnes et al. (Barnes and Roberts, 2000) and Robinson et al. (Robinson and Rigby, 1985) showed that the moduli are dependent on the time as well as strain and that the thiol content affects the mechanical properties. It was also demonstrated that the tensile properties are highly dependent on
the influences of various factors: a high relative humidity decreases the Young’s modulus and increases the extensibility (Robbins, 2002); an increase in temperature leads to a decrease in Young’s modulus and an increase in extensibility (Rebenfeld et al., 1966); twisting creates damage to the hair fibers (Dankovich et al., 2004) and this effect leads to the decrease in the breaking stress, breaking strain and Young’s modulus. Ethnicities and age will also affect the properties of human hair. It has been shown that hair specimens from different ethnicities exhibit different strains at cuticle lift off (Seshadri and Bhushan, 2008b), topographies (Franbourg et al., 2003; LaTorre and Bhushan, 2005; Wolfram, 2003), surface roughness (LaTorre and Bhushan, 2005), nanomechanical properties (Wei and Bhushan, 2006), and tensile properties (Franbourg et al., 2003; Wolfram, 2003). In the meantime, as hair specimens age, the relaxation time also varies significantly (Benzarti et al., 2011).

Being one of the most important typical $\alpha$-keratin fibers, the mechanical behavior of hair was therefore studied quantitatively with respect to various contributing factors in this study. We report the sensitivities of the hair on the strain rate, relative humidity, and temperature through tensile testing. We also propose a constitutive equation for human hair and compare its predictions with experimental results.

4.2 Materials and Methods

4.2.1 Specimen preparation

Hair specimens were collected from an East-Asian female in her early 30s. All the hair in the experiments was donated from only one individual to avoid the variations in
mechanical properties reported between hairs from different ethnicities and the effect of aging as discussed above. No additional treatment such as straightening or dyeing except daily cleaning was conducted on the hair before collection. The average length of the collected hair was about 30 cm. Before specimen preparation, 2 cm sections were cut off at both ends from the entire hair and discarded. The remainder of the fibers was sectioned into 3 cm long pieces. For each small section, the two ends were glued into sand paper to prevent slipping between the grips during the tensile testing, leaving a 1.0-1.5 cm long hair span between sand papers to be tested. The cross-section area of each fiber was individually gauged using a 0-25 mm range micrometer with 0.001 mm accuracy. At least three measurements were made on one sample to ensure an average value in the diameter. It should also be noted that the pressing on the fiber was carefully avoided during measurements.

Specimens were tested under 20 %, 50 % (ambient) relative humidity (RH), and immersion in water at different strain rates. Specimens tested under water were first prepared by using the same method described above; additionally, the sand papers at the two ends were mounted into epoxy to prevent splitting in water. The hair fibers were then immersed in deionized (DI) water for 24 hours to reach a full saturation before the mechanical tests. A transparent environmental chamber with a dehydrator and hydrometer was built to produce the low-humidity condition. The hair specimens were pre-treated at 20 % RH for 3 days to ensure equilibrium with the environment and tested under such condition.
4.2.2 Mechanical testing

An Instron 3342 system with a 500 N load cell was used for tensile testing. Specimens were tested at strain rates of $10^{-4}$, $10^{-3}$, $10^{-2}$, $10^{-1}$, $10^{0}$ s$^{-1}$ at room temperature and humidity. To determine the effect of humidity on the mechanical properties of hair, the pre-soaked specimens were tested in the DI water at 20 °C at varying strain rates. The effect of temperature on the hair was established at a strain rate of $10^{-2}$ s$^{-1}$ in order to maintain a steady temperature of the hair specimen during one tensile test. Tests at higher temperatures (40, 60 and 80 °C) were conducted in water immersion. Cyclic mechanical tests in air at different temperatures were also conducted by using hair specimens which were heated with a common hair dryer while the temperature was monitored with a thermometer. At least five to eight specimens were tested under one condition (for example, each strain rate at each relative humidity) to ensure an accurate representation of the mechanical properties.

4.2.3 Characterization

For structural characterization, hair specimens were fractured in liquid nitrogen and then fixed using an established method (Yang et al., 2015). The specimens were first immersed in 2.5 % glutaraldehyde solution for 3 hours and further dehydrated with an ascending ethanol series (30, 50, 70, 90, 95 and 100 vol.% twice) for 20 mins in each solution. The surface (cuticle) of the hair before and after testing as well as the fracture surface of the hair specimens was observed in a FEI SFEG ultrahigh-resolution scanning electron microscopy (SEM) (FEI, Hillsboro, OR, USA). The specimens were sputtered with iridium prior to observation.
The hair was also characterized by transmission electron microscopy (TEM) using osmium tetroxide (OsO₄) staining (Filshie and Rogers, 1962) with post-staining of lead. Segments of hair fibers were pre-treated by immersing in 0.5 M thioglycolic acid (pH 5.5) for 24 hours at room temperature to enhance the contrast between the filaments and matrix. They were then washed with double-distilled water for 1 hour and immersed in 1-2 % aqueous OsO₄ for 3 days. Then the segments were washed with distilled water, dehydrated to 100 % ethanol through a series of graded alcohol solutions and then transited to 100 % acetone through graded mixtures of ethanol and acetone. The specimens were subsequently infiltrated using Spurr’s low viscosity epoxy resin through a series of solutions with increasing amounts of resin and decreasing amounts of acetone (25 % resin+75 % acetone, 50 % resin+50 % acetone, 75 % resin+25 % acetone, 90 % resin+10 % acetone, 100 % resin, 100 % resin), each taking one day. Specimens were then placed in fresh resin and polymerized with for 2 days at 65 ºC. The embedded specimens were trimmed and sectioned on a Leica Ultracut UCT ultramicrotome using a diamond blade. Sliced sections were picked up, post-stained with lead for 60 seconds. A FEI Technai 12 (Spirit) (120 kV) transmission electron microscope was used for examination.

### 4.3 Mechanical properties under different conditions

#### 4.3.1 Effect of strain rate

For a thorough understanding of the viscoelasticity of human hair, specimens were tested at different strain rates and temperatures. Typical stress-strain curves are summarized by the band plots in Figures 4.2a and 4.2b, which incorporate the variation
among specimens and indicate the effect of strain rate. As reported previously and also confirmed by our results, a typical tensile stress-strain curve of one hair fiber shows three regions. First it shows a linear section (up to ~0.02-0.05 strain), in which the hair behaves mostly elastically. The chemical bonds are rearranged and no significant structural change is observed. The curve then goes into a transformation region where the α-helix coils uncoil and may transform into β-sheets if conditions are conducive (Kreplak et al., 2004, 2001). This region shows a very slow increase in stress with strain. After a certain strain (~0.25), the curve starts to show an increase in slope and enters the post-transformation region where mostly the remaining α-helices and/or the β-sheets are stretched until the hair reaches the ultimate breaking point. As the strain rate increases, the stress of the “plateau” of the transformation region increases significantly. It is of note that at the highest strain rate (10^9 s⁻¹) there is a peak (circled in Figure 4.2a) at ~0.05 strain followed by a decrease in stress. This behavior becomes more apparent in curves with higher yield stress (the stress at 0.02 strain offset). This peak in stress may correspond to the critical stress of the intermediate filaments proposed in the Chapman/Hearle (C/H) model (Hearle, 2000). According to the C/H model, a critical stress needs to be reached in order to initiate the transformation from α-helix to β-sheet and this is a characteristic of first-order phase changes. Chapman and Hearle (Chapman, 1969) also indicated that a critical force is needed to initiate the formation of β-crystal nucleation. Beyond this critical point, the α-helices will be uncoiled and this may lead to the formation of β-sheets region. This explains the decrease in stress after those peaks since the force drops back to the equilibrium value if transformation occurs. On the other hand, this behavior is only obvious in the tensile tests at 10^9 s⁻¹ with a yield stress higher
than ~150 MPa, suggesting that there could be a critical stress for the sudden nucleation of the β-crystals. For curves with lower yield stress, the transformation region only shows a gradual increase in stress after the elastic region, which may indicate the uncoiling of the α-helices and/or a gradual transformation to the β-sheets. Especially, tensile tests at lower strain rates (10^{-4} and 10^{-3} s^{-1}) do not show a sharp turnover point between the transformation and post-transformation regions, which is thought due to a possibly continuous α-β transformation before the fracture. At a higher temperature of 40 °C (Figure 4.2b), the hair shows a deteriorated performance in both yield stress and ultimate stress at each strain rate compared to that at room temperature. Despite the difference in temperature, there is a similar trend in the strain-rate sensitivity.

Figure 4.2c shows the tensile strength and work-of-fracture (area under the stress-strain curve) as a function of strain rate at room temperature. For the lowest strain rate (10^{-4} s^{-1}), hair exhibits a tensile strength of 152±33 MPa and a work-of-fracture of 30±9 MPa. As the strain rate increases, the tensile strength shows an increase to 267±45 MPa (at 10^{0} s^{-1}). These results clearly confirm the viscoelasticity of hair: tensile strength increases with increasing strain rate. It corresponds to the relaxation tests conducted by Barnes et al. (Barnes and Roberts, 2000), proving that the tensile response of hair is strain-rate sensitive. Work-of-fracture shows a similar trend as the tensile strength. Figure 4.2d shows that as the strain rate increases from 10^{-4} s^{-1} to 10^{0} s^{-1}, the breaking strain first increases from 0.31 to 0.43 and then remains constant at ~0.45. This indicates that although the extensibility of hair is strain-rate sensitive, it can only increase to a certain value (~0.45) under the ambient condition.
Figure 4.2 Band plots of tensile results of human hair at (a) room temperature and (b) 40 °C, and (c) tensile strength, work-of-fracture and (d) breaking strain as a function of strain rate at room temperature (Error bars represent standard deviation.).
Figure 4.2 Band plots of tensile results of human hair at (a) room temperature and (b) 40°C, and (c) tensile strength, work-of-fracture and (d) breaking strain as a function of strain rate at room temperature (Error bars represent standard deviation.)
4.3.2 Effect of humidity

The effect of relative humidity (RH) on the tensile properties was examined by testing hair specimens at 20 % RH and in water at room temperature (20 °C). After the hair is soaked in water, it exhibits a swelling effect as the diameter increases by ~10 %. Figure 4.3a shows three typical stress-strain curves at 20% RH, ambient humidity (50 % RH) and water. At 20 % RH, the hair exhibits a much higher yield stress and tensile stress compared to 50 % and saturated condition. When the hair was tested in water, the Young’s modulus was decreased and the hair fractured at a much lower stress. On the other hand, the hair exhibited a more extended post-plateau region and broke at a much larger strain (~0.75) compared to the ambient humidity (~0.45). Previous studies (Bear and Rugo, 1951; Milczarek et al., 1992) suggest that the IFs are crystalline and not water sensitive, but the matrix proteins are thought to be affected more by water. According to Feughelman et al. (Feughelman, 1959; Feughelman and Robinson, 1967), water works as plasticizer and reduces the interaction between protein chains; at the same time it also works as a swelling agent to increase the dimensions of keratin network. Therefore, water reduces the stiffness and increases the mobility of molecular structure of matrix by increasing the spacing between IFs and by plasticizing the amorphous matrix (Bertram and Gosline, 1987; Feughelman, 1997; Feughelman and Robinson, 1967).
Figure 4.3 (a) Typical stress-strain curves at different relative humidities and (b) yield stress as a function of strain rate (Error bars represent standard deviation.).
4.3.3 Strain-rate sensitivity

Hair specimens were further tested at three humidity levels under different strain rates ($10^{-4}$ to $10^{0}$ s$^{-1}$) and the results are shown in Figure 4.3b. For each strain rate, the yield stress decreases with increasing relative humidity. At the saturated condition, the yield stress increases from 28 MPa at $5\times10^{-4}$ s$^{-1}$ to 54 MPa at $10^{0}$ s$^{-1}$; the increase of stress is much lower compared to 50 % RH (44 MPa at $10^{-4}$ s$^{-1}$ and 149 MPa at $10^{0}$ s$^{-1}$) and 20 % RH (102 MPa at $10^{-4}$ s$^{-1}$ and 192 MPa at $10^{-1}$ s$^{-1}$), which indicates that human hair has different strain-rate sensitivities at different humidities. The corresponding strain-rate sensitivities ($m = \frac{d \log \sigma}{d \log \varepsilon}$) of hair are 0.06, 0.08 and 0.11 at saturation and relative humidities of 50 % and 20 %, respectively.

The strain-rate sensitivities under various conditions are summarized in Figure 4.4. Since hair is a bio-polymer, we compare its strain-rate sensitivity to that of two common polymers reported by Mulliken et al. (Mulliken and Boyce, 2006). The replotted data shown in Figure 4.4 indicate that the strain-rate sensitivity of human hair as a natural polymer is comparable to other synthetic polymers. The pangolin scale, also keratinous (Wang et al., 2016b), shows a strain-rate sensitivity of 0.08, which is in the same range as hair.
Figure 4.4 Strain-rate sensitivities of human hair tested under various conditions, PC, PMMA (adapted from (Mulliken and Boyce, 2006)) and pangolin scales (Wang et al., 2016b) ($\sigma_0 \dot{\varepsilon}_i$ is 1 MPa, $\varepsilon_0 \dot{\varepsilon}_i$ is 1 s$^{-1}$).

4.3.4 Effect of temperature in water

To understand the influence of temperature on human hair, specimens were tested at 20, 40, 60 and 80 °C at the same strain rate of 10$^{-2}$ s$^{-1}$. This strain rate allows a uniform heat distribution in the hair and ensures a steady temperature in the water environment. Figure 4.5 shows the stress-strain curves at various temperatures under water immersion. As the curve shifts downwards with increasing temperature, the yield stress and breaking stress decrease significantly. At 40 °C, the yield stress decreases from 39 MPa at 20 °C to 19 MPa; however, the yield stress only shows a slight decrease (15.5 MPa at 60 °C and
15.4 MPa at 80 °C), as the temperature further increases to 60 °C and beyond. This phenomenon is due to a glass transition reported at 35 °C (Phillips, 1985; Wortmann et al., 2006) and a further structural transition in α-keratin around 60 °C (Knopp et al., 1997; P Mason, 1964; P. Mason, 1964). This explains the decrease in yield stress from 20 to 40 °C as the water works as plasticizer and affects the amorphous matrix in the hair. It was found for wool that, as the temperature is increased to 60 °C, the ductility decreases (P Mason, 1964), opposite to the character of glass transition. It was confirmed by X-ray examination that this is due to a structural change in the crystalline intermediate filaments. A simulation of the α-helical structure in vacuum also confirmed that a high temperature of 67 °C destabilized and changed the helical conformation significantly (Knopp et al., 1997). This transition is thought to cause the decrease of yield stress between 40 and 60 °C. Akkermans and Warren (Akkermans and Warren, 2004) also confirmed that the yield stress of hair decreased with increasing temperatures using a two-state model simulation.

To further study the reversibility of such transition, another group of specimens were first immersed in water at 80 °C for at least five minutes, cooled down in 20 °C water and tested. The inserted diagram in Figure 4.5b shows the comparison between three groups: hair tested at (a) 20 °C, (b) 80 °C and (c) heated-cooled (80-20 °C) hair. The third group showed no difference in yield stress compared to the second group (both ~16 MPa). These last two groups have a much lower yield stress than hair tested at 20 °C (38 MPa). This result indicates that after heating to a high temperature, the human hair undergoes an irreversible structural change, which inevitably lowers the tensile strength and creates permanent damage.
Figure 4.5 (a) Representative stress-strain curves of hair under different temperatures in water and (b) yield stress as a function of temperature (Inserted figure shows the comparison between room temperature, heated and heated-cooled conditions.) (Error bars represent standard deviation.).

4.3.5 Load cyclic effect at different temperatures

In order to investigate the reversibility of the $\alpha$-$\beta$ transformation, specimens were stretched to strains of 0.01 and 0.1 at room temperature, unloaded, and reloaded at strain
rate of $10^3 \text{ s}^{-1}$. These two strains represent the complete elastic and the plateau (transformation) regions, respectively. Figure 4.6a shows the three cycles of loading-unloading at a strain of 0.01. These stress-strain curves all exhibit a linear behavior, with the same Young’s modulus of ~4.2 GPa. At this experimental condition, after each cycle, the specimens return to their original length and the bonds of $\alpha$-helix are only stretched within this region. The process is highly reversible and does not create plastic transformation in the structure.

The loading-unloading stress-strain curves up to 0.1 strain are shown in Figure 4.6b. In the first cycle, the hair exhibits a similar Young’s modulus of 4.1 GPa and a linear elastic region up to ~0.025 strain, followed by the transformation region with a slow increase in stress up to 0.1 strain. Upon unloading, the curve shows a residual strain of ~0.06, indicating the partial reversibility of the deformed structure. In the subsequent two cycles, there is also a hysteresis in the unloading-reloading curve. The linearity of the unloading curve is lost due to the partially reversible changes, which results in a difference from the loading curve. Moreover, there is a significant decrease in yield stress as the specimens are reloaded. Thus, the mechanical strength degrades in this partial phase reversal through the generation of flaws. The recovered strain in each cycle is ~0.03, which is close to the strain of elastic region. These results confirm that the $\alpha$-$\beta$ transformation is only partially reversible upon unloading.

Loading-unloading tests were also conducted at 40 and 60 °C and the results are shown in Figures 4.6c and d, respectively; higher temperature does not contribute to a better reversibility in the $\alpha$-$\beta$ transformation. Moreover, at 60 °C, the mechanical strength
of hair deteriorates significantly compared to that at lower temperatures, as the hair fractured during the 3\textsuperscript{rd} cycle. The hysteresis in unloading-reloading produced by $\beta$ to $\alpha$ transformation is marked by arrows in Figure 4.6. It is assumed that the decrease of yield stress upon reloading comes from damage in previous cycles or during unloading.

\textbf{4.3.6 Weibull analysis among individuals of the same ethnicity}

Although it has been noticed that there are variations in the hair properties among different ethnicities (Franbourg et al., 2003; LaTorre and Bhushan, 2005; Seshadri and Bhushan, 2008b; Wei and Bhushan, 2006; Wolfram, 2003), the variation among the same ethnicity has not been well studied. Figure 4.7 shows the Weibull fits of the yield stress of three females (#1 as the specimen donor above mentioned, #2 as an Eastern Asian female in her late 20s and #3 as an Eastern Asian female in her early 30s). These people were chosen due to their approximately same age group. The same number of specimens (8) from each individual were tested and plotted in the graph. The Weibull moduli, which indicate the variability of the same group of specimens, are 4.50, 4.50, and 5.92 for #1, #2, and #3 individuals, respectively. This shows that the variabilities of the three groups of specimens are within the same range. At the 50 \% probability of failure, the three individuals exhibit yield stresses of 94.8 MPa, 97.5 MPa, 90.5 MPa, respectively. Hence, the mechanical properties of hair from different individuals of the same ethnicity exhibit a very small difference statistically, and the current study is applicable to a broad spectrum of subjects.
Figure 4.6 Cyclic tensile tests up to (a) 0.01 and (b) 0.1 at 20 °C and cyclic tests up to 0.1 strain at (c) 40 °C and (d) 60 °C (Hysteresis marked by arrows.).
Figure 4.6 Cyclic tensile tests up to (a) 0.01 and (b) 0.1 at 20 °C and cyclic tests up to 0.1 strain at (c) 40 °C and (d) 60 °C (Hysteresis marked by arrows.), Continued.
4.4 Analysis

4.4.1 Strain associated with α to β transformation

The parameters of α-helix and β-sheet keratin are shown in Figure 4.8. The right-handed α-helical molecule, which is prevalent in the elastic region, has a diameter of ~1.2 nm and periodicity of 0.52 nm in one turn (Figure 4.8a). Once fully transformed, it turns into a β-sheet structure (Figure 4.8b) and exhibits a periodicity of 0.7 nm with ~0.2 nm in thickness. During the transformation, 3.6 residues of one turn in α-helix (Figure 4.8c) will
experience an elongation in length from 0.52 nm (Figure 4.8c) to 1.2 nm (Figure 4.8d) in β-sheet structure. Therefore, the nominal strain of a theoretical full transformation from α-helix to β-sheet structure is

\[
\frac{1.2 \text{ nm} - 0.52 \text{ nm}}{0.52 \text{ nm}} = 1.31
\]

(1)

Figure 4.8 Schematic representation of (a) α-helix and (b) β-sheet keratin in human hair and transformation of 3.6 residues from (c) α-helical to (d) β-sheet structure.
However, such high strain in the theoretically-complete transformation was not observed in the current experiments (Figure 4.2a). We propose the following reasons for this phenomenon:

i. The tensile stress may induce an unraveling in the α-helices instead of transformation into β-sheets. Kreplak et al. (Kreplak et al., 2001) observed that at a high strain (above 0.2), the α-helical coils experience an unravelling in the structure instead of a phase transition. It is also noted in their work that under conditions with low relative humidity, the former could be the dominant effect in the keratinous fibers.

ii. A complete transformation for all α-helices to β-sheets may not be achievable. In the experiments, this transformation may be only local and not uniform along the entire specimen. During the post-transformation region, the newly-transformed β-
sheets are extended and contribute to the tensile response even though the transformation is not complete.

iii. The experimental condition may not conducive to a full $\alpha$-$\beta$ transformation. Kreplak et al. (Kreplak et al., 2004) showed that under steam condition, horse hair was able to achieve 100% extension. Figure 5a also shows that at a higher humidity, the human hair exhibits a larger extensibility. It is possible that the ambient condition in the current experiments is not suitable for the full $\alpha$-$\beta$ transformation, since the breaking strain is also highly dependent on temperature and strain rate, as shown previously.

iv. The inherent structure, orientation and properties of intermediate filaments (IFs) are not conducive to a full $\alpha$-$\beta$ transformation. Hair is composed of numerous IFs which consist of periodic $\alpha$-helical and non-helical regions (Wang et al., 2016a); only the $\alpha$-helices have the potential to transform to $\beta$-sheets and thus contribute to the tensile strain. In addition, the IFs do not possess exactly the same orientation and mechanical properties; therefore, the IFs that are well aligned with the hair axis will elongate while others may not. Moreover, some IFs would break earlier than others, which creates defects in the hair so that the hair breaks before the full $\alpha$-$\beta$ transformation is completed. The loading-unloading-reloading experiments suggest the existence of permanent damage during tensile tests.

Since the $\alpha$-$\beta$ transformation takes place during the transformation region, which ends at ~0.25 strain, the transformation ratio, $r$, along the fiber direction by the end of this region can be obtained as the following:
This ratio expresses the fraction of α-helices that are fully transformed into β-sheets during the tensile tests.

Wide-angle X-ray scattering (WAXS) patterns for hair before and after stretching are shown in Figure 4.9 to help to understand the α-β transformation during the tensile tests. Prior to tensile testing, α-keratin in human hair exhibits a spot at 0.96 nm indicating the distance between adjacent α-helices and another meridian arc at 0.52 nm embedded into a broader one around 0.5 nm (Kreplak et al., 2004). Figure 4.9a shows the typical α-keratin X-ray diffraction pattern in human hair before stretching. However, after stretching, the 0.52 nm meridian arc becomes less obvious and another equatorial arc at 0.465 nm appears, corresponding to the distance between β-sheets (Figure 4.9b). This indicates that the keratin in hair experiences a transformation from α-helices to β-sheets in the hair during stretching.
Figure 4.9 Wide-angle X-ray scattering (WAXS) patterns of human hair (a) prior to and (b) after stretching to strain of 0.35 (Fiber direction indicated by double-arrowed lines.) (Note that the reflection in meridian arc at 0.96 nm characteristic of α-keratin and the one at 0.465 nm after deformation corresponding to β-keratin.).
4.4.2 Constitutive equation of the tensile response

A constitutive equation based on the experimental results of hair is proposed to obtain a better quantitative description of the tensile properties of similar α-keratin fibers. Firstly, the hair is simplified into only two components: IFs and matrix, as illustrated in Figure 4.10a by Feughelman (Feughelman, 2002, 1959). The IFs and matrix are considered as parallel and aligned with each other and these components can be analyzed individually using the separate stress-strain curves proposed by Wortmann (Wortmann and Zahn, 1994). As shown in Figure 4.10b, the intermediate filaments are characterized by three stages: (a) a near linear region up to 0.02 (elastic region) which only involves the change of bond angles and no significant structural transformation, (b) a flat region with little increase in stress (transformation region) due to the α-β transition, and (c) an increase in stress until the ultimate breaking stress (post-transformation region). On the other hand, the amorphous matrix shows a relatively high modulus at small strain and a gradual rise in stress as the strain further increases.

Figure 4.10 (a) Two-phase composite model for a wool fiber (adapted from (Feughelman, 1959)) and (b) stress-strain curves proposed for IFs and matrix (adapted from (Wortmann and Zahn, 1994)).
As intermediate filaments and matrix are parallel to each other, according to composite theory with the iso-strain approach:

\[ F = F_f + F_m \]  \hspace{1cm} (3)

where \( F_f \) is the force of intermediate filaments, \( F_m \) is the force of matrix.

Therefore,

\[ F = \sigma A, \quad F_f = \sigma_f A_f, \quad F_m = \sigma_m A_m \]  \hspace{1cm} (4)

\[ \frac{F}{A} = \frac{F_f}{A} + \frac{F_m}{A} \]  \hspace{1cm} (5)
We have

\[ \sigma = \sigma_f V_f + \sigma_m V_m \]  

(6)

where \( V_f \) and \( V_m \) are the volume fractions of fiber and matrix, respectively.

According to Feughelman (Feughelman, 1994), in a typical \( \alpha \)-keratin fiber such as Corriedale cool fibers, the volume fraction of fibers, \( V_f \), is 0.56 and the fraction of matrix, \( V_m \), is 0.44. Therefore,

\[ \sigma = 0.56\sigma_f + 0.44\sigma_m \]  

(7)

This relation is further described in Figure 4.10b.

Based on the model developed by Feughelman and Wortmann (Feughelman, 1959; Wortmann and Zahn, 1994), we establish the following equation for a typical stress-strain curve. Therefore, at each strain \( \varepsilon \):

\[ \sigma = C_1\varepsilon - C_2(\varepsilon - \varepsilon_{c1})H(\varepsilon - \varepsilon_{c1}) + C_3(\varepsilon - \varepsilon_{c2})H(\varepsilon - \varepsilon_{c2}) \]  

(8)

where \( \varepsilon_{c1} \) and \( \varepsilon_{c2} \) are the strains at elastic to transformation and transformation to post-transformation transitions (\( \varepsilon_{c1} = 0.02 \), \( \varepsilon_{c2} = \sim 0.25 \) as previously mentioned); \( H \) is a Heaviside function to activate the two terms (Meyers and Chen, 2014) and \( C_1, C_2, C_3 \) jointly define the moduli of the elastic, transformation and post-transformation regions.

To obtain the yield stress and strain, we define the yield point as the intersection between the following two equations:

\[ \begin{align*}
\sigma &= C_1\varepsilon - C_2(\varepsilon - 0.02) \\
\sigma &= C_1(\varepsilon - 0.02)
\end{align*} \]  

(9)

(10)
Therefore, we have

\[
\sigma_y = \frac{0.02 C_1^2}{C_2}, \quad \varepsilon_y = \frac{0.02C_1 + 0.02C_2}{C_2}
\]

As the slope is very small within the transformation region, we have \( C_1 - C_2 \approx 0 \); \( C_2 \approx C_1 \), thus, the yield stress and strain can be further simplified to \( \sigma_y = 0.02C_1 \) and \( \varepsilon_y = 0.04 \).

Hair exhibits strain-rate sensitivity and thermal softening effects, as shown in Figure 4.4; we introduce of a strain-rate sensitivity and a thermal softening (adapted from Chen et al. (Chen et al., 2008)) functions as follows:

\[
\sigma_y = \sigma_{iso} \left( \frac{\dot{\varepsilon}}{\dot{\varepsilon}_0} \right)^m
\]

\[
\sigma_y(T) = \sigma_{iso} \cdot C \cdot \exp \left[ \left( \frac{T - T_0}{T_m - T_0} \right)^\beta \right]
\]

where \( \dot{\varepsilon}_0 = 10^{-2} \text{ s}^{-1} \) is the reference strain rate; \( T_0 = 20 \text{ °C} \) is the room temperature; \( T_m \approx 155 \text{ °C} \) is the melting temperature at 100 % RH (Cao and Leroy, 2005).

Assuming that the strain rate and thermal softening contribute to the overall tensile properties individually, the constitutive equation is:

\[
\sigma = \left\{ C_1 \left( \frac{\dot{\varepsilon}}{\dot{\varepsilon}_0} \right)^m \cdot C \cdot \exp \left[ \left( \frac{T - T_0}{T_m - T_0} \right)^\beta \right] \right\} \varepsilon - C_2 (\varepsilon - 0.02)H(0.02) + C_3 (\varepsilon - 1.31r)H(1.31r)
\]

(13)
The parameters of Eq. (13) are: \( \dot{\varepsilon}_0 = 10^{-2} \text{ s}^{-1}, T_0 = 20 \, ^\circ\text{C}, T_m = 155 \, ^\circ\text{C}, C_1 = 4.7, C_2 = 4.5, C_3 = 0.25, m = 0.06 \, (100 \% \text{ RH}), C = 0.95, \beta = -2.77, r = 0.19. 

Figure 4.11 shows the prediction of Eq. (13), which incorporates the strain-rate, temperature, and transformation functions. The predicted stress-strain curves from this constitutive model agree reasonably with the experimental results at different strain rates.
Therefore, this equation effectively provides help in predicting and analyzing the tensile behavior of hair under various strain rates and temperatures.

Chapter 4, in full, is a reprint of the materials as it appears in Materials Science and Engineering, C, 2017, Yang Yu, Wen Yang, Bin Wang, and Marc A. Meyers. The dissertation author was the primary investigator and author of this paper.
Chapter 5 Strain-rate sensitivity of α-keratin fibers

5.1 Introduction

Keratin hair fibers, such as wool, human hair, and whale baleen have been widely studied due to their superior mechanical properties (McKittrick et al., 2012; Wang et al., 2016a). These fibers usually serve as protective and defensive uses for animal bodies and yet yield some unnoticed properties. For example, wool has a high specific tensile strength of 150-260 kNm/kg, which is comparable to some of the stainless steel (~250 kNm/kg) once the material densities are considered. On the other hand, wool and human hair usually have large extensibility, with a high breaking strain of more than 40 %. Therefore, researchers have been interested in the mechanical properties and morphologies of keratin fibers (Swift, 1999; Velasco et al., 2009) and hoping to be able to mimic these materials in the near future.

Every hair fiber has a hierarchical structure (Rivett et al., 1996), shown in Figure 5.1. A typical human hair fiber has a diameter of 50-100 µm and the outermost layer is called as cuticles. These cuticle scales, which are 0.3-0.5 µm thick and 40-60 µm long, also have a layered structure (Seshadri and Bhushan, 2008a). The inner section, cortex, is composed of cortical cells that are 100 µm long. Moreover, one cortical cell is also built with macrofibrils of ~0.2 µm in diameter and these macrofibrils are further composed of intermediate filaments (IFs) that are 7.5 nm in diameter. These intermediate filaments are made from α-helical polypeptide chains. At the beginning of the tensile tests, these α-helical chains will experience a reversible bond angle rearrangement in the elastic region.
(0~0.02-0.05 strain). Following this region, the structure will have either an uncoiling of the helical chains or a phase transformation from α-helix to parallel β-sheets within the transformation region (~0.02-0.05 to ~0.25 strain). Since there is no or little resistance in these processes, the stress-strain curve of this region will have a very slow increase in stress. Once the strain is larger than ~0.25, the curve goes into the post-transformation region and the unchanged α-keratin chains and possibly-formed β-keratin will be further stretched until breakage.

Apart from the well-studied wool and human hair, horse hair is also a hard α-keratin fiber that has attracted interests during the past decade. Researchers have shown that horse hair has a crystalline structure that is the same with human hair (Kreplak et al., 2004, 2001). Horse tail hair has been widely used in a lot of applications, such as brushes and bows of violins, due to its superior mechanical properties. It is considered very strong but also flexible. Its property is highly influenced by factors such as diet (Kania et al., 2009) and climate such as humidity (Speakman, 1928b) and temperature (Rebenfeld et al., 1966), which is similar to other keratin fibers. Different from human hair that is regularly taken care of, horse hair is usually accustomed to weathering and rough handling. Therefore, it is thought that by studying and comparing the different mechanical properties among these two alike hair samples, we will be able to gain insights in the sources of viscoelasticity and the strain-rate sensitivity in the keratin fibers.
Figure 5.1 Schematic drawing of the hierarchical structure in a human hair fiber.
One of the unique properties of keratin fibers is their viscoelasticity. Stress relaxation studies by Barnes et al. (Barnes and Roberts, 2000) showed that the relaxation moduli change according to different strains and time durations. Robinson et al. (Robinson and Rigby, 1985) showed the differences in the mechanical properties along one hair fiber and the different thiol content along the hair fibers is found to contribute to such phenomenon. The viscoelasticity in the human hair further yields many consequences. Among these consequences, strain-rate sensitivity has been observed in our previous study (Wang et al., 2016a). Generally, the hair fibers become stronger and tougher, as both the yield stress and ultimate tensile stress increase with an increasing strain rate. On the other hand, the Young’s modulus remains similar across all strain rates, indicating that it is an inherent material property. It is suggested that at small extensions, stress relaxation mainly comes from deformation of the peptide bonds and chemical groups; as the strain further increases, the amorphous matrix, which is regarded as an elastomer in the Chapman model to explain the tensile properties of keratin fibers (Chapman, 1969), mainly contributes to the viscoelasticity.

The disulfide bond cross-link is considered as a critical component in holding the α-keratin fibers together (Tombolato et al., 2010). This crosslink has also been shown to mainly contribute to the matrix function inside the fibers (Schweizer et al., 2006). In the meantime, the thiol/disulfide interchange has been shown to largely affect the tensile properties along one hair fiber from root end to tip end (Robinson and Rigby, 1985). Therefore, in this current study, we target on these disulfide bonds and demonstrate that the composition of components in the matrix, particularly disulfide bonds, largely affects
the strain-rate sensitivity and viscoelasticity of the keratin hair fibers. Different strain-rate sensitivities are first observed in two similar keratin hair fibers, human and horse hair. By dissecting these bonds, human hair largely loses the strain-rate sensitivity and the mechanical property under various strain rates becomes uniform; furthermore, human and horse hair exhibit similar sensitivities after the treatment, which largely comes from the tensile property of the intermediate filaments in the hair fibers. The effects of high temperature and saturation are also analyzed and explained from the perspective of these disulfide bonds.

5.2 Experimental methods

5.2.1 Sample preparation and treatment process

Human hair was solely acquired from an East-Asian female in the early 30s. No additional treatments such as permanent waving and dyeing has been previously done on these hair specimens. The hair was directly applied in the experiments to exhibit its normal property without further moistening or shampooing. Horse hair was collected from the tail of a 16-year-old Arabian gelding. The horse hair was briefly rinsed to remove the dirt and further kept under room condition for over 24 hours to remove the moisture. These two groups of hair fibers were further cut into ~3 cm sections and both ends were glued between sand papers. During tensile tests, the two sand papers were pulled to prevent slipping of the hair specimens in the gripping during tensile tests. An
Instron 3342 with a 500 N load cell was applied to test the hair specimens at strain rates of \(10^{-4}, 10^{-3}, 10^{-2}, 10^{-1}, 10^0\) s\(^{-1}\) at room temperature and humidity.

Specimens tested in water were pre-soaked for 24 hours to reach a full saturation. The two ends were mounted into epoxy to prevent splitting during soaking. The human hair tested at 40 and 60 °C are heated within an environmental chamber for at least 20 minutes before testing and maintained at those temperatures during experiments.

5.2.2 Chemical treatment process

The disulfide bonds in both hair samples were cleaved to eliminate the effect of the matrix using an established protocol (Greenberg and Fudge, 2013). ~0.5 g of each hair was first briefly rinsed with acetone to remove surface lipids (Le Blond et al., 2009). The sample was further immersed in 0.1 M 2-mercaptoethanol in 20 % 1-propanol solution for 2 days. This process is necessary to remove the disulfide bonds in the matrix. The sample was then moved to 0.1M methyl iodide in 0.2 M boric acid solution for a day after rinsed with 50 % 1-propanol in between steps. The methyl iodide helps to prevent future cross-linking in the matrix after the reduction (Gillespie, 1973). Both samples were kept under room condition for 24 hours to remove the surface moisture. This process is illustrated in Figure 5.2.
5.2.3 Characterization

Cuticle structures and facture surfaces in both samples before and after treatment were observed in a FEI SFEG ultrahigh-resolution scanning electron microscopy (SEM) (FEI, Hillsboro, OR). The specimens were sputtered with iridium before the observation. Simultaneous thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) analysis was conducted with a TA Instruments Q600 apparatus at a constant heating rate of 20 °C/min up to 500 °C in a nitrogen atmosphere with a purge rate of 10 ml/min. A Nicolet Magna-IR 550 instrument was applied for the Fourier transform infrared spectroscopy (FTIR) analysis on both hair samples.
5.3 Results and discussion

5.3.1 Mechanical properties of original horse and human hairs

The mechanical property of horse hair is compared to the human hair from our previous study (Yu et al., 2017) and the results are shown in Figure 5.3. Unlike human hair, horse hair does not show much increase in stress during the post-transformation region (beyond ~0.25 strain). As the strain rate increases, the Young’s modulus in the Hookean region mainly remains the same, while the yield stress exhibits little increase with an increasing strain rate (Figure 5.3a). The human hair has yield stress (defined as the stress at 2 % offset) changing from 43.45 MPa (at $10^{-4}$ s$^{-1}$) to 126.47 MPa (at $10^{0}$ s$^{-1}$), resulting in a strain-rate sensitivity of 0.11 (Yu et al., 2017). On the other hand, the horse hair has a yield stress of 91.4 MPa and 91.2 MPa at strain rates of $10^{-4}$ s$^{-1}$ and $10^{-3}$ s$^{-1}$, respectively. As the strain rate increases, the yield stress further increases to 102.2 MPa at $10^{-2}$ s$^{-1}$, 114 MPa at $10^{-1}$ s$^{-1}$ and 135 MPa at $10^{0}$ s$^{-1}$. Therefore, horse hair exhibits a strain-rate sensitivity of 0.05, which is much smaller compared to human hair (Figure 5.3c). Since both horse and human hairs are similar $\alpha$-keratin fibers, it is worth of understanding the reasons of such different strain-rate sensitivities.

Figure 5.3d shows the Weibull distributions of the breaking stress from the horse and human hair at the strain rate of $10^{-2}$ s$^{-1}$. At the 50 % probability of failure, human hair shows a breaking stress of 204.76 MPa, which is much higher than the stress of horse hair, 94.62 MPa. This can also be seen from the band plots (Figure 5.3b) that human hair has a higher yield stress from the transformation region. On the other hand, the Weibull modulus (m) of human hair is 3.72, which is smaller than that of horse hair, 6.55. As the
Weibull modulus increases, the data exhibit a much smaller variability and the performance is more stable (Zhan and Wool, 2011). These results show that human hair has a less stable property compared to horse hair, but generally it is much stronger than horse hair. As horse hair has a much larger diameter and cross-sectional area, it requires a larger force to break the fibers. That helps to explain why horse hair is applied in various applications but not human hair.
Figure 5.3 Band plots of tensile results of (a) horse hair, (b) human hair adapted from (Yu et al., 2017). (c) strain rate sensitivities ($\sigma_0$ is 1 MPa, $\varepsilon_0$ is 1 s$^{-1}$) and (d) Weibull analysis of both hair (Note lower strengths of horse hair and strain-rate sensitivity).
Figure 5.3 Band plots of tensile results of (a) horse hair, (b) human hair adapted from (Yu et al., 2017). (c) strain rate sensitivities ($\dot{\sigma}_0$ is 1 MPa, $\dot{\varepsilon}_0$ is 1 s$^{-1}$) and (d) Weibull analysis of both hair (Note lower strengths of horse hair and strain-rate sensitivity). Continued.
5.3.2 Morphology and structure of original horse and human hairs

Since the cuticle morphology is highly related to living environment (Jachowicz, 1987), horse and human exhibit significantly different surface structures. Figure 5.4 compares the cuticle edges in both specimens. Horse hair (Figure 5.4a) shows much more damaged cuticle edges with cracks in some cuticle sheets. This is understandable since horse hair is rarely moistened and taken care of. On the other hand, human hair (Figure 5.4b) shows a very smooth surface with only occasionally lifting in the natural condition. However, since cuticle has been shown not to largely affect the tensile properties even under broken or damaged conditions (Robbins and Crawford, 1991), this difference in surface morphology would not account for the differences in the tensile properties between these two hair fibers.

Figure 5.4 Scanning electron micrographs (SEM) of cuticles in (a) horse hair and (b) human hair.
Figure 5.4 Scanning electron micrographs (SEM) of cuticles in (a) horse hair and (b) human hair.

Wide-angle X-ray diffraction (XRD) patterns of horse and human hair are shown in Figure 5.5. Both hairs exhibit a similar pattern. First, a meridional spacing reflection of 0.52 nm indicates the α-helix pitch projection along the coil axis (Wang et al., 2016a). However, this arc is further superimposed to a broad ring at ~0.5 nm. According to Busson et al. (Busson et al., 1999), this is due to a less ordered structure from the coiled coils. Second, a broad equatorial reflection of ~0.96 nm indicates the distance between various α-helical chains. Since α-keratin exhibits a much different diffraction pattern compared to β-keratin (Kreplak et al., 2004), these results show that horse and human hair are both composed of α-keratin and a similar molecular structure exists in these hair specimens.
5.3.3 Thermal and FTIR analysis of original horse and human hairs

Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) were further conducted to understand the similarities and differences in the two hair fibers. The results of these analysis are shown in Figure 5.6. Figure 5.6a shows the TGA data of horse and human hair. The initial weights of these samples are 10.99 mg and 13.79 mg. Both hairs exhibit a similar trend in weight loss: the initial slower loss from 20 °C to 220 °C and another significant decrease in weight from 220 °C to 500 °C. It has been shown that the first decrease is due to the evaporation and loss of the bounded water in the fibers (Monteiro et al., 2005). Both samples lost ~15 % weight as water evaporated. The further weight decrease in both curves is related to the denaturation of the keratin and degradation of the organic components, which generates a quick material loss from 220 °C to ~320 °C and a slower decrease further up to ~400 °C. After 400 °C, there was some residue from the fibers, resulting 22.7 % and 25.5 % weight left in horse and human.
hair, respectively. These results show that there is little difference in the organic components in two hair fibers.

DSC data examines the stability of specimens within a certain temperature range. Figure 5.6b shows the comparison between the two fibers. The first endotherm in the curve of human hair exhibits a peak from ~50 to ~90 °C, a result from water evaporation (El-Amoudy and Osman, 2012; Humphries et al., 1972). On the other hand, horse hair has a much wider peak at a higher temperature range (~70 - ~120 °C), indicating a firmer bonding of water in the horse hair fibers. The following peaks in both curves show the denaturation of the keratinous structures. Human hair has been reported to exhibit such peak at ~220 °C and horse hair shows a much wider peak ranging from ~220 - ~270 °C. These results indicate that although horse and human hair has similar compositions in chemical components, they exhibit some structural difference within or between these components.

Figure 5.6 (a) TGA and (b) DSC analysis of horse and human hair.
Figure 5.6 (a) TGA and (b) DSC analysis of horse and human hair, Continued.

To understand the reasons for the above differences in the mechanical properties, FTIR analysis was conducted on horse and human hair to investigate a possible variation in the composition of chemical groups. Figure 5.7 shows the FTIR spectra of both hair samples. Along the whole spectrum, several groups are especially analyzed: CH$_2$ stretching at 1451 cm$^{-1}$, amide III at 1231-1235 cm$^{-1}$, cystine dioxide at 1121 cm$^{-1}$, cystine monoxide at 1071 cm$^{-1}$, cysteic acid at 1042 cm$^{-1}$, and cysteine-S-thiosulfate at 1022 cm$^{-1}$ (Carr and Lewis, 1993; Lipp-symonowicz et al., 2012; Signori and Lewis, 1997). These groups attract more interests due to their involvement in the formation of disulfide bonds. Results show that two of the most prominent differences in peak intensity are the cysteic acid and cystine monoxide. The horse hair is thought to have a large concentration of cysteic acid and cystine monoxide, resulting from a higher
oxidation of the cystine disulfide cross-links. Therefore, these data show that in general, horse and human hair are composed of same chemical groups. However, it is possible that the differences in composition and component ratio is a factor in their mechanical properties.

Figure 5.7 FTIR analysis of horse and human hair.
5.3.4 Morphology and mechanical properties of treated hairs

Since the differences in the mechanical property are not explained by the crystal structure and chemical components, both hairs were chemically treated to remove the contribution of matrix in the viscoelasticity. SEM images were taken on horse (Figures 5.8a and 5.8b) and human hair (Figures 5.8c and 5.8d) to understand the effect of this treatment on the inner morphology and investigate if this process changes the physical structure. Before treatment, both specimens show a continuous cortex with hollow cavities indicating the medulla structure (Figures 5.8a and 5.8c). After the treatment, these hair specimens exhibit a similar internal morphology (Figures 5.8b and 5.8d). Therefore, it is confirmed that the treatment does not affect the internal physical structure and the hair fibers still maintain their original morphology; the reduction process can be said only to affect the chemical groups and the effect on the mechanical properties is not achieved by changing the physical structure (such as dissolving the cortex).
Figure 5.8 SEM of cross sectional areas of (a, b) horse and (c, d) human hair before and after the treatment (arrows indicate typical medulla cavities).
Figure 5.8 SEM of cross sectional areas of (a, b) horse and (c, d) human hair before and after the treatment (arrows indicate typical medulla cavities), Continued.
Targeted FTIR analysis on the horse and human hair specimens was conducted at a few related chemical groups. Figure 5.9 shows the spectra with wavenumbers ranging from 2000 cm\(^{-1}\) to 800 cm\(^{-1}\). The peak of amide III at 1231-1235 cm\(^{-1}\) is used as reference since it is not affected during the reduction process. It shows that for horse hair (Figure 5.9a), the peak of cysteic acid at 1040 cm\(^{-1}\) and cystine monoxide at 1071 cm\(^{-1}\) remained unchanged after the treatment, while the human hair (Figure 5.9b) shows much increased peak intensity at these two regions (cysteic acid and cystine monoxide). As the disulfide bonds are dissected, cystine monoxide is created as an intermediate product, while cysteic acid is produced as the final product from the disulfide bonds (Figure 5.10). Compared to horse hair, which is not largely affected with the concentrations of cysteic acid and cystine monoxide before and after the treatment, human hair exhibits a larger content increase in these two groups. Therefore, it is confirmed that among these two hair specimens, the original human hair has a larger concentration of disulfide bonds (-S-S-). Horse hair also shows to have very minimal peak increase from disulfide bonds cleavage, indicating that the horse hair specimen has very few disulfide bonds concentration before the treatment.
Figure 5.9 FTIR analysis at the affected chemical groups of (a) horse and (b) human hair.
The strain-rate sensitivities of horse and human hair are summarized in Figure 5.11. After the treatment, horse hair shows a similar sensitivity, $m$ as 0.06, compared to the original horse hair (0.05). On the other hand, human hair shows a much more affected mechanical property from the treatment: not only the strain-rate sensitivity decreases from 0.11 to 0.05, indicating a smaller response to strain rate, but also the yield stress increases at lower strain rates ($10^{-4}$ and $10^{-3}$ s$^{-1}$) and decreases at higher strain rate ($10^{0}$ s$^{-1}$). It is also worth of noticing that after the treatment, both horse and human hairs show a very similar sensitivity of ~0.05. As both hairs were chemically treated, the disulfide bonds were largely dissected and would no longer affect the viscoelasticity of the $\alpha$-keratin fibers. Therefore, the mechanical property at this stage mainly reflects the property of the crystalline $\alpha$-helix, which is not sensitive to various strain rates. This is thought to be helpful in explaining the similar strain-rate sensitivities of both treated hair fibers and decreased sensitivity of human hair after the treatment.
Figure 5.11 Comparison of strain-rate sensitivities before and after treatment of (a) horse and (b) human hair ($\sigma_0$ is 1 MPa, $\varepsilon_0$ is 1 s$^{-1}$).
5.3.5 Mechanism of strain-rate sensitivity difference

Viscoelasticity has been well observed in wool and human hair fibers (Barnes and Roberts, 2000; Nikiforidis et al., 1992). Barnes and Roberts (Barnes and Roberts, 2000) indicated that this property arises from various bonds, which includes the disulfide type. Therefore, by isolating the influence of the disulfide bonds, it is helpful to analyze the source of viscoelasticity of the α-keratin fibers and the remaining effect from the crystalline chains. The observed different strain-rate sensitivities of both horse and human hairs and the changes of yield stress at various strain rates can be further explained by the different component ratio using the Feughelman’s model (Feughelman, 2002, 1959) and Crewther’s ‘beaded chain’ model (Crewther, 1972) as the structural mechanics of hair fibers (Hearle, 2000).

Based on the findings from FTIR analysis (Figure 5.9), the horse hair is shown to have a lower concentration of disulfide bonds (-S-S-) compared to human hair. This difference can have several sources. First, Greenberg and Fudge (Greenberg and Fudge, 2013) reported a difference in matrix content (percentage of total protein) in both horse and human hairs. On average, human hair has a matrix content of ~32 % of total protein, while horse hair has a matrix content of ~24 %. As the majority of the disulfide bonds exist in the matrix, this result will contribute to a lower concentration of the bonds. Second, environmental conditions, such as photodamage (Pande, 1994) can have an effect of reduction on the disulfide bonds, resulting in a decreased content of these bonds. Weathering (Joy and Lewis, 1991) has also been identified to increase the level of cysteine-S-sulphonate, which is the product of disulfide photooxidation in hair. Since
horse hair is accustomed to the outdoor condition, it is expected that the horse hair specimens would have a further reduced disulfide bond content from an already-lowered matrix concentration compared to human hair. This difference is crucial in analyzing the mechanical property difference observed previously. Figure 5.12 is the schematic drawings which shows the bonds between matrix and intermediate filaments and the disulfide bonds within the matrix. Figures 5.12a and 5.12c represent the horse and human hair, respectively, before the treatment. The intermediate filaments are connected to the matrix through bonds, while the ‘beads’ in the matrix are intermittently connected through disulfide bonds. As shown, the human hair has a higher concentration of the disulfide bonds. When both hairs are under tension, the -S-S- bonds are sensitive to the strain rates. At a low strain rate such as $10^{-4}$ s$^{-1}$, these bonds can have longer time to allow for a slow elongation without contributing to a high yield stress. Since the matrix and intermediate filaments are considered as parallel components in the Feughelman’s model (Feughelman, 2002, 1959), a low stress from matrix yields a low overall yield stress of the hair specimens. However, at a high strain rate (for example, $10^{0}$ s$^{-1}$), the disulfide bonds are not allowed to fully elongate, which leads to a high yield stress in the human hair (Figure 5.11b). On the other hand, as shown in Figure 5.12a, horse hair has a lower concentration of the disulfide bonds. Therefore, as the strain rate increases, it also shows a slightly-increased yield stress (strain-rate sensitivity $m$ as 0.05). However, due to the low concentration, these original horse hair specimens have a much smaller sensitivity compared to human hair ($m$ as 0.11), since only the insensitive IFs contribute to the mechanical property of horse hair specimens.
After the treatment, most disulfide bonds were chemically cleaved (Figures 5.12b and 5.12d). Since the horse hair has a lower disulfide bond content, the strain-rate sensitivity was not much influenced and the property was very close to the data before treatment (Figure 5.11a). This further confirms that the viscoelasticity of horse hair before the treatment mainly results from the intermediate filaments. However, the human hair was largely affected by the chemical treatment, which is illustrated in Figure 5.12d. As the disulfide bonds were cleaved, the intermediate filaments became more dominant in the viscoelasticity. Therefore, the human hair specimens showed a more decreased strain-rate sensitivity from 0.11 to 0.05. Moreover, the treated human hair exhibits a similar sensitivity (0.05) to the horse hair (~0.05), which represents the mechanical property of intermediate filaments. The treated human hair shows a higher yield stress at the strain rate of $10^{-4}$ s$^{-1}$ and lower yield stress at the strain rates of $10^{-1}$ s$^{-1}$ and $10^{0}$ s$^{-1}$. For the original human hair, the yield stress is more dominated by the matrix and disulfide bonds, which leads to a strain-rate sensitive property. After the hair is treated, the disulfide bonds are dissected. The intermediate filaments become more dominant in the yield stress and mechanical property, which is helpful in explaining the different changes in the yield stress of human hair at lower and higher strain rates, as shown in Figure 5.11b. A detailed mechanism of the viscoelasticity in both horse and human hair is summarized in Figures 5.12a to 5.12d.
Figure 5.12 Schematic drawings of the structural changes in (a, b) horse and (c, d) human hair before and after treatment.
Figure 5.12 Schematic drawings of the structural changes in (a, b) horse and (c, d) human hair before and after treatment, Continued.
5.3.6 Thermal and hydration effects on the strain-rate sensitivity

Different strain-rate sensitivities are shown in our previous study by using human hair (Yu et al., 2017). It is also observed that there are thermal and hydration effects on the mechanical property and sensitivity. Figure 5.13 shows the strain-rate sensitivities of human hair tested at 25, 40, and 60 °C in air and 25 °C in water. As the temperature increases from room temperature (25 °C) to 40 °C, the yield stress increases at various strain rates while the sensitivity remains the same as 0.11. However, as the temperature further increases to 60 °C, the strain-rate sensitivity decreases to 0.04, resulting from a higher yield stress at low strain rates and lower yield stress at high strain rates, compared to the 25 and 40 °C conditions. When the human hair specimens are tested in water, both the yield stresses at various strain rates and strain-rate sensitivity decrease compared to the air condition under the same temperature. Therefore, high temperature and humidity are shown to affect the yield stress and largely reduce the strain-rate sensitivity in the human hair. Weigmann et al. (Rebenfeld and Dansizer, 2016) showed that the temperature affects the sulfhydryl concentration, which is also an indication of the disulfide content. Figure 5.13 shows at a medium high temperature (40 °C), the disulfide bonds in the matrix may not be largely affected or dissected. On the other hand, the increased yield stress suggests a stronger cross-link in the hair specimen. As the temperature increases to 60 °C, the strain-rate sensitivity decreases to 0.05. The high temperature is shown to have a similar effect as the chemical treatment in that the yield stresses increase at lower strain rates (10^{-3} and 10^{-2} s^{-1}) and decrease at high strain rate
(10^9 s^{-1}). This indicates that the high temperature affects the disulfide bonds in the matrix similarly to the chemical treatment.

Figure 5.13 Strain-rate sensitivities of human hair under heated and hydrated conditions (Reproduced based on (Yu et al., 2017)) \((\dot{\sigma}_0, \dot{\varepsilon}_0, \dot{\varepsilon}_0 = 1 \text{ MPa, } \dot{\varepsilon}_0 = 1 \text{ s}^{-1})\).

5.3.7 FTIR analysis of saturated and saturated-dried human hair

The difference in the strain-rate sensitivity of the saturated human hair is further analyzed through FTIR analysis. Figure 5.14 shows the FTIR data of some targeted groups for those two samples. As the hair is fully saturated, the peak intensities of cysteic
acid and cystine monoxide increased as compared to original human hair. However, the saturated-dried hair specimen shows similar peak intensities as the original hair, which indicates that the effect of saturation is reversible after the water evaporates from the hair specimens.

Water works at a plasticizer in the hair specimens, which causes a swelling effect in the matrix. Feughelman (Feughelman, 1994) indicated that as water interacts with matrix, it can affect the distance between intermediate filaments themselves and distance between matrix and intermediate filaments. Therefore, as Figure 5.14 shows, it is suggested that as the hair is fully saturated, the swelling causes an increase in the amounts of cysteic acid and cystine monoxide. It is thought to be achievable through the interaction of the disulfide bonds with the water molecules in the matrix. However, as the saturated hair is further dried, the water content in the matrix is decreased and the result shows a decrease of the cysteic acid and cystine monoxide until they reach to the original contents. This is helpful to understand the decreased yield strength and strain-rate sensitivity in Figure 5.13 since water affects the cross-link in the matrix and interaction between matrix and intermediate filaments. As the disulfide bonds are weakened, the saturated hair shows a strain-rate sensitivity of 0.06, which is comparable to those of horse hair and treated human hair. In the meantime, the yield stresses decrease at the same strain rates compared to original human hair, which is also an indication of weakness of the hair specimens.
Figure 5.14 FTIR analysis of original, saturated, and saturated-dried human hair.

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Chapter 6  Viscoelastic properties of α-keratin fibers

6.1  Introduction

Hair, as a common bio-polymer that provides aesthetic and protective significance for many species, exhibits its unique mechanical properties (McKittrick et al., 2012). Among many similar α-keratinous materials, such as wool (Chapman, 1969; Crewther, 1972; Feughelman, 1997), horns (Li et al., 2010; Tombolato et al., 2010), hooves (Bertram and Gosline, 1987; Kasapi and Gosline, 1997), and whale baleen (Szewciw et al., 2010), the mechanical properties of human hair has also been extensively studied (Signori and Lewis, 1997; Swift, 1999; Wortmann and Kure, 1990). A typical human hair fiber is composed of α-helical chains at the nanometer scale (Yu et al., 2017). These right-handed chains will form intermediate filaments (also known as microfibrils), which are further embedded in an amorphous matrix. The intermediate filaments of 7.5 nm in diameter compose macrofibrils, which have a diameter of ~0.2 μm. These macrofibrils form bundles and they are the main components of cortical cells, which have a diameter of 1-6 μm. At the micrometer scale, the cortical cells form cortex, which is covered by cuticles and these two together compose hair fibers. Due to this hierarchical structure and different components of crystalline fibers (intermediate filaments) and amorphous matrix, human hair exhibits strain-rate sensitivity (Yu et al., 2017) and viscoelasticity (Barnes and Roberts, 2000; Nikiforidis et al., 1992). The stress relaxation tests on both human hair (Robinson and Rigby, 1985) and camel hair (Xiao et al., 2016) shows that the stress decreases as a function of time as the hair fiber is strained at certain values. In the
meanwhile, age and ethnicity may also play important roles in the relaxation time (Benzarti et al., 2011).

One important feature in the mechanical properties shown in human hair arises from the crystal structure change during tensile tests. As one hair specimen is stretched, the curve first shows a linear region (up to ~0.02-0.05) where no significant structural deformation happens. Once the strain increases beyond a certain value (~0.05), the curves exhibits a plateau region where possible phase transformation from α-helices to β-sheet will take place. After this transformation region, the curve shows an increase in slope and the residual α-keratin and newly-formed β-keratin are stretched until ultimate rupture of the hair specimen. Research has shown that depending on the experimental conditions, the crystal structure in hair may experience an uncoiling behavior of the α-helical chains (Kreplak et al., 2001) or phase transformation to β-sheet keratin (Chapman, 1969; Mason, 1965). Therefore, the behavior of such phase transformation should also be factored to analyze the viscoelasticity of hair fibers. Moreover, the dynamic mechanical characterizations, which has been applied to analyze various biological materials (Emile et al., 2007; Motokawa and Tsuchi, 2003; Szulgit and Shadwick, 2000), can also provide important information on the viscoelasticity of human hair.

In our current study, the dynamic mechanical property under various frequencies and temperatures are quantitatively studied. A constitutive relaxation modulus equation base on the Maxwell-Wiechert model is developed and compared to the experimental data from the hair specimen stretched within the elastic region. Furthermore, creep test on
single hair fiber also exhibits its viscoelastic property and uncoiling (and possible phase transformation) of the crystalline component in the human hair.

6.2 Materials and methods

6.2.1 Specimen preparation

All hair specimens are donated by only one female individual. This is to guarantee the uniformity of the mechanical properties obtained in our experiments and exclude the influences of age and ethnicity as previously observed (Benzarti et al., 2011; Franbour et al., 2003; Seshadri and Bhushan, 2008b; Wei and Bhushan, 2006). The hair has only experienced regular rinsing and shampooing. No additional treatment such as straightening, dyeing, or permanent waving has been conducted on the hair specimens. The average length of each hair fiber is about 30 cm. To prevent the influences of root and tip ends (Robinson and Rigby, 1985), these fibers are then cut at ~2 cm from both ends. Approximately 3 cm-long sections are made from the long fibers. The two ends of the 3 cm-long sections are further glued between sand papers to prevent slipping during tensile tests, leaving a 1 cm-long hair specimen in between for tensile tests.

6.2.2 Mechanical testing

For tensile tests, an Instron 3342 system with a load cell of 500 N was used. In the stress relaxation tests, two specimens were each stretched to 0.02 and 0.25 strains, respectively at a strain rate of $10^{-2}$ s$^{-1}$. These two parameters were chosen to show
different behaviors at the elastic and transformation regions. As the strains were held, the
stresses were further monitored for another 1000 s. For the creep test, one hair specimen
was stretched within the elastic region (0.02) at a strain rate of $10^{-2}$ s$^{-1}$ until the stress
reached to 100 MPa. The strain was then recorded until the hair specimen broke.

6.2.3 Dynamic Mechanical Analysis (DMA)

The dynamic mechanical behavior of $\alpha$-keratin fibers was analyzed using a DMA
800 Dynamic Mechanical Analyzer (Perkin Elmer). The hair specimens were clamped
tightly in the chamber and the inner temperature was increased at a rate of 5 °C min$^{-1}$
until 30 °C. The specimens underwent an oscillated tensile test within the elastic region as
the total strain did not exceed 0.01 at an increasing frequency from 0.1 to 2.4 Hz. The
storage modulus and tangent delta were recorded at each frequency. Hair specimens were
also tested within the same strain, 0.01, at a set frequency of 0.75 Hz while the
temperature inside the chamber increased from 30 °C to 110 °C at a rate of 5 °C min$^{-1}$.
The dynamic properties of hair under different frequencies and temperatures were thus
obtained to help understand the viscoelasticity of $\alpha$-keratin fibers.

6.3 Results and discussions

6.3.1 Dynamic mechanical properties

Figure 6.1 shows the storage moduli and tangent delta of horse and human hair at
increasing frequencies. The tangent delta, which is the ratio between loss modulus and
storage modulus, represents the viscoelasticity of these α-keratin fibers. As the frequency increases from 0.1 Hz to 0.42 Hz, the storage modulus of horse hair increases from 2.86 GPa to 3.02 GPa while that of human hair increases from 2.28 GPa to 2.51 GPa and the tangent delta decreases in both hair specimens. As the frequency further increases to 2.4 Hz, the modulus only shows a gradual increase to 3.20 GPa (horse hair) and 2.73 GPa (human hair) as the tan delta decreases to 0.07 (horse hair) and 0.12 (human hair).

Figure 6.1 (a) Horse and (b) human hair storage modulus and tangent delta ($\tan \delta = \frac{loss\ modulus}{storage\ modulus}$) as a function of frequency (the storage modulus increases with frequency by virtue of the reduced time for viscous effects).
Both hair specimens were then tested at a certain frequency of 0.75 Hz as the temperature increased from 30 °C to 110 °C and the result is plotted in Figure 6.2. The storage modulus curves exhibit a two-step decrease for both hair: as the temperature of the chamber increases from 30 °C to ~50-60 °C, it shows a decrease from 3.2 GPa to 2.2 GPa for horse hair (Figure 6.2a) and from 2.4 GPa to 1.8 GPa for human hair (Figure 6.2b). As the temperature further increases to 110 °C, the storage modulus eventually reaches to ~1.2 GPa for both hair specimens. However, the tangent delta curve exhibits a
peak at ~55 °C in both curves, which may correlate to the glass transition temperature of \( \alpha \)-keratin fibers under such condition. The tangent delta value further increases as the temperature increases beyond 60 °C.

The above results show that as the frequency increases, the \( \alpha \)-keratin fibers exhibit an increase in the storage modulus and a decrease in the loss storage, which results in an overall decrease of tangent delta. It also indicates that at higher frequencies, the \( \alpha \)-keratin fibers behave more elastically as the degree of viscoelasticity decreases. A similar behavior of human hair from our previous study on the strain-rate sensitivity (Yu et al., 2017) indicates that as the strain rate increases, the work of fracture increases and therefore human hair becomes stiffer. This can be understood in terms of the chemical links within the hair fibers. The amorphous matrix has been thought to be viscoelastic and the crystalline \( \alpha \)-keratin is relatively more strain-rate insensitive. Therefore, the crosslinks in the matrix (probably the disulfide bonds) could possibly contribute to the above change in the storage modulus. Moreover, as the temperature increases from room condition (30 °C), the storage modulus shows a rapid decrease until ~55 °C and reaches to a short plateau until 60 °C. This results in a peak in the tangent delta curve (Figure 6.2), which is related to the structural change of \( \alpha \)-keratin fibers (Mulliken and Boyce, 2006; Phillips, 1985). As the temperature further increases beyond 60 °C, the storage modulus keeps decreasing and the tangent delta increases, which indicates a more viscous and less elastic behavior with an increasing temperature. Therefore, the viscoelasticity of \( \alpha \)-keratin fibers is shown by the dynamic mechanical analysis.
Figure 6.2 (a) Horse and (b) human hair storage moduli and tangent delta as a function of temperature at 0.75 Hz.
6.3.2 Stress relaxation

Figure 6.3 shows the stress relaxation curves of both horse and human hair specimens that were held at 0.02 and 0.25 strains, respectively. These two strains were chosen to exhibit different properties at the elastic and transformation regions. As the specimens were stretched at a strain rate of $10^{-3}$ s$^{-1}$ and held at different strains, the relaxation behaviors show both viscoelasticity and non-linearity. For the specimen that held at 0.02 strain, the stress reached to 50 MPa for horse hair and 92 MPa for human hair at the beginning of the relaxation. As the relaxation starts, the stress in both hair specimens first rapidly decreases and gradually flattens as the time increases over 500 s. However, the specimen held at 0.25 strain shows a different relaxation behavior. During the initial relaxation period (~50 s), the stress shows a similar decreasing rate as the specimens held at 0.02 strain; as time further increases, the stress maintains at a certain value much earlier than the specimens held at 0.02. This can also be seen from the inserted figure which shows the normalized stress as a function of relaxation time. For the specimens held at 0.02 strain, the stresses keep decreasing and slowly reach to 0.55 (horse hair) and 0.57 (human hair) of the stress at the beginning of relaxation. For the specimens held at 0.25 strain, the stresses decrease in the beginning and quickly maintain at 0.70 (horse hair) and 0.75 (human hair) of the initial stress (~200 s compared to ~500 s in the specimens held at 0.02). Therefore, the results show that as the $\alpha$-keratin fibers are stretched to elastic and transformation regions, they exhibit different stress relaxation behaviors. This is thought to be related to keratin phase ($\alpha$ or $\beta$ keratin) within the hair fibers during tensile tests.
The stress relaxation behaviors of α-keratin hair at elastic and transformation regions also reveal significant results of the viscoelasticity. As the hair specimen is stretched to 0.02 in strain, only rearrangements of the bond angles within the hair fibers are thought to take place and contribute to the increase of strain. Our previous study (Yu et al., 2017) also confirmed that as the hair is stretched within the elastic region (0-~0.02-0.05 strain), the elongation is reversible since there is no phase transformation
from α-keratin to β-keratin. The viscoelasticity of α-keratin fiber can be fitted with a simplified version of Maxwell-Wiechert model (Shen et al., 2011). The relationship between relaxation modulus and relaxation time is as follows:

\[
E_{\text{relaxation}}(t) = E_0 + E_1 e^{-\frac{t}{\tau_1}} + E_2 e^{-\frac{t}{\tau_2}}
\]

(1)

where \(E_0\) is an elastic modulus that is not time-dependent, \(E_1\) and \(E_2\) are elastic modulus within two Maxwell elements, \(\tau_1\) and \(\tau_2\) are two relaxation constants, defined by the ratio of viscosity \(\eta\) and elastic modulus \(E\) in each Maxwell element, as shown in Figure 6.4.

Figure 6.4 Schematic drawing of the Maxwell-Wiechert model applied to understand the viscoelasticity of human hair.
As the strain is maintained at 0.02, the stress exhibits a two-stage relaxation: a rapid, almost linear decrease in stress following by a slower decrease and a further gradual decrease (as shown in Figure 6.3). According to Emile et al. (Emile et al., 2007), this behavior can be explained with the hierarchical structure of α-keratin fibers. The cortex of the fibers is mainly composed of uniaxial cortical cells, which are embedded in an amorphous matrix. Those cortical cells are further composed of macrofibrils, which are also made of intermediate filaments and matrix. Therefore, the α-keratin fibers are made from amorphous, viscous high-sulfide matrix and crystalline, strain-rate insensitive fibrils. As previously observed (Yu et al., 2017) in the human hair, intermediate filaments with a diameter of 7 nm and macrofibrils with a diameter of 100-400 nm are the major components at the nanometer and submicrometer scale while at the micrometer scale, cortical cells of 1-6 μm are the major component. As the stress relaxation begins, the lower structure (such as the cortical cells) would contribute to the smaller relaxation constant in Eq. (1), therefore resulting in the rapid decrease observed. The higher structure (such as intermediate filaments and macrofibrils) further contributes to the larger relaxation constant. The parallel configuration between cortical cells and matrix resembles the above Maxwell-Wiechert model and this overall structure contributes to the viscoelasticity of α-keratin fibers.

Based on the experimental data from Figure 6.3, the fitted relaxation modulus curves as a function of time is plotted and shown in Figure 6.5 and the equations for both horse and human hair are as follows:
\[ E_{\text{relaxation}}(t)(\text{GPa}) = 1.23 + 0.46e^{-\frac{t}{14}} + 0.72e^{-\frac{t}{359}} \text{ (Horse hair)} \]  

\[ E_{\text{relaxation}}(t)(\text{GPa}) = 2.65 + 0.71e^{-\frac{t}{11}} + 1.26e^{-\frac{t}{207}} \text{ (Human hair)} \]

where the time-independent elastic modulus \( E_0 \) is 1.23 GPa for horse hair and 2.65 GPa for human hair, two elastic moduli in the Maxwell elements, \( E_1 \) and \( E_2 \) are respectively 0.46 and 0.72 GPa for horse hair and 0.71 and 1.26 GPa for human hair, the two relaxation constants, \( \tau_1 \) and \( \tau_2 \) are 14 and 359 s for horse hair and 11 s and 207 s for human hair, respectively. As time increases, the contribution of viscous components dampens. Therefore, the elastic component dominates after \( \sim 500 \) s in the overall relaxation modulus. This can be explained with the crystalline and elastic components in \( \alpha \)-keratin fibers. The two-stage relaxation of stress observed above is illustrated by the two different relaxation constants (\( \tau_1 \) and \( \tau_2 \)). The smaller constant, \( \tau_1 \), contributes to the rapid decrease while the larger constant, \( \tau_2 \), contributes to the gradual decrease after \( \sim 500 \) s. Meanwhile, similar to the behaviors observed on spider silk (Emile et al., 2007), the larger constant is one order of magnitude higher than the smaller constant (359 s vs. 14 s and 207 s vs. 11 s), which can be explained by the dimensional difference between cortical cells and macrofibrils due to the hierarchical structure of \( \alpha \)-keratin fibers (for example in human hair, \( \sim 1-6 \) μm and 0.1-0.4 μm, respectively). Figure 6.5 shows the fitting curves based on Eqs. (2) and (3), which agree reasonably with experimental results in Figure 6.3.
Figure 6.5 (a) Relaxation modulus curves and (b) their log plots of horse hair and human hair specimens held at 0.02 strain as a function of time (inserted figure shows the applied Maxwell-Wiechert model) (Note that the dots represent experimental data and the solid curve represents the fitting curve).
For the specimens strained to 0.25 and held at such strain (Figure 6.3), uncoiling of the α-helices (Kreplak et al., 2004, 2001) and a possible phase transformation from α-helices to β-sheets are thought to take place during the transformation region. Therefore, it is possible that at the beginning of relaxation, a mix of α-keratin and β-keratin coexist in the hair specimens. As observed in our previous study (Yu et al., 2017), the deformation within this region is partially reversible. Therefore, not only the various components (α-keratin, β-keratin and matrix) would contribute to the stress relaxation, but also the partial reversibility would contribute to the decrease of stress. In the meanwhile, it should be noticed that the stress decreases to ~70 MPa for horse hair and ~110 MPa for human hair after ~250 s, which is close to the yield stress (~70 and ~110 MPa, respectively) (defined as the stress at 0.02 strain offset), indicating a possible residual stress due to phase transformation from α- to β-keratin. This results in a final normalized stress of ~0.7, which is higher of the specimens held at 0.02 strain (as indicated in the inserted figure in Figure 6.3).

6.3.3 Creep

The mechanical behavior of α-keratin fibers under constant stress is analyzed for both hair specimens and the data is plotted in Figure 6.6. The hair specimens were stretched at a strain rate of $10^{-3}$ s$^{-1}$ until the stress reached to 90 MPa (horse hair) and 100 MPa (human hair). These stresses were applied to ensure the strain (~0.02) was within the elastic region as the creep begins.

For the horse hair specimen (Figure 6.6a), as the stress reaches to 90 MPa, the strain first shows a linear increase to ~0.15, followed by a slower increase until rupture at
the strain of 0.47. However, as the strain enters the transformation region, several jumps of strain can be noticed from the curve (marked with dashed ellipses). For the human hair specimen, following the start of creep when the stress reaches to the set value, the curve shows a linear increase of strain up to ~0.17 (stage I as indicated), following by a non-linear increase (stage II). As the strain increases to ~0.24, a different behavior (stage III) can be seen from the curve. During this stage, the strain does not show gradual increase as stage II. Instead, it shows plateaus following by the sudden jumps, as indicated by the dashed ellipses in Figure 6.6b. However, as each jump happens, the strain exhibits a partial decrease from the highest point and further maintained at such strain. The strain increases to ~0.47 after several cycles of such repetitive behavior and the human hair specimen finally broke at ~0.47 after ~7.5 h.

Figure 6.6 Creep curve of human hair under a constant stress of 100 MPa (the hair specimen was first stretched at the strain rate of $10^{-3} \text{s}^{-1}$ until the stress reached to 100 MPa) (Note sudden strain bursts marked by dashed ellipses).
The creep test on α-keratin fibers also helps to understand the viscoelasticity and uncoiling (and possibly phase transformation) of α-helices. As the hair specimens are deformed and the stresses increase to the set value, the α-keratin fibers experience an elastic deformation up to ~0.02 strain. The strains further linearly increase to ~0.17 as the stresses were maintained, which followed by a parabolic increase to ~0.24. It is thought that during these periods, bond angles rearrangement mainly contributes to the
increase of strain. However, for the human hair specimens, the stage III is composed of plateaus followed by sudden increases in strain (marked by dashed ellipses in Figures 6.6a and b). Such sudden increases can be explained by the uncoiling of the α-helices, which opens periodically as illustrated by the model developed by Chapman (Chapman, 1969) and the theoretically full transformation from α-helices to β-sheets (strain as 1.31) (Yu et al., 2017). However, it is also observed that following each jump, there is a partial drop in strain (as shown in circles), which is explained by the structure change from completely straightening to the final configuration. As shown in Figure 6.7, when the α-helical structure is fully extended to the complete straightening state, the nominal strain of one such turn is as follows

\[
\frac{1.39 \text{ nm} - 0.52 \text{ nm}}{0.52 \text{ nm}} = 1.78
\]

(4)

However, a theoretical full transformation from α-helix to β-sheet results in a strain of 1.31 (Yu et al., 2017), which gives a decrease of 0.47 in strain from the complete straightening state to β-sheets structure. Such decrease from the intermediate state is helpful in understanding the partial drop after each jump of strain. During the stage III (Figure 6.6b), the strain increases in such stepped manner until the human hair specimen reaches to the final breakage at 0.47, which agrees with the average breaking strains (~0.45) shown at various strain rates (Yu et al., 2017).
Figure 6.7 Schematic representation of structure transformation from (a) α-keratin to (b) complete straightening state and to (c) β-keratin (reproduced based on (Yu et al., 2017)).

Chapter 6, in full, is submitted for publication in Acta Biomaterialia, Yang Yu, Wen Yang, and Marc A. Meyers. The dissertation author was the primary investigator and author of this material.
Chapter 7  Comparative study of hair from different organisms

Different organisms usually have hair fibers of different diameters. It has been shown in our study that the mechanical properties of horse and human hair differ considerably. Therefore, the mechanical properties of hair specimens from wild boar (Figures 7.1 and 7.2), javelin (Figures 7.3 and 7.4), bear (Figures 7.5 and 7.6), Asian elephant (Figure 7.7), and giraffe (Figure 7.8) are also studied. Scanning electron micrographs (SEM) were taken to observe the surface morphologies of various specimens. It is thought that by analyzing the dimensional effects among various animal hairs, we can gain more insights on the mechanical properties among similar α-keratin fibers.

7.1  Wild boar hair

The boar hair has been widely used in brushes due to its superior stiffness and good mechanical properties. Figure 7.1 shows the scanning electron microscope (SEM) graph on the surface morphology of one boar hair. The cuticle structure, which is similar to both horse and human hair, can be observed on the surface; what also should be noticed is that the cuticles were largely damaged so that the edges are less obvious than human hair. This is understandable since the boars live in the wild and the hair specimens experience rough conditions, such as weathering and dehydrating. Moreover, some cuticle fragments can be identified from Figure 7.1.
The wild boar hair specimens exhibit an average diameter of 220 – 250 µm. These wild boar hair specimens are tested at the strain rates of $10^{-4}$ s$^{-1}$, $10^{-2}$ s$^{-1}$, and $10^{-1}$ s$^{-1}$. The results are shown in Figure 7.2. At the lowest strain rate ($10^{-4}$ s$^{-1}$), the hair shows a yield stress of 77 MPa. As the strain rate increases to $10^{-2}$ s$^{-1}$, the yield stress further increases to 120 MPa. However, the yield stress remains at 130 MPa as the strain rate further increases.

In the meantime, at lower strain rates, the boar hair specimens exhibit a breaking strain of approximately 0.5. At higher strain rate ($10^{-1}$ s$^{-1}$), the breaking strain decreases to 0.4. This confirms that α-keratin fibers tend to break earlier at high strain rates than low strain rates, but the breaking strains exhibit upper limits, which indicates that they are inherent material properties.

Figure 7.1 Scanning electron micrographs (SEM) of a wild boar hair.
It is similar to horse that the wild boar hair does not show much increase in stress after the transformation region. Instead, the stress only shows a gradual increase after the elastic region (0-0.05 strain). It is worthwhile to understand the mechanisms for such behavior.

![Graph](image)

**Figure 7.2** Band plots of tensile results of boar hair at various strain rates.

### 7.2 Javelina hair

Javelinas are mammals with a much smaller size (0.9 – 1.3 m) than wild boars (1 – 2 m). Although similar in appearance and habits, they are considered as distinct species. A typical javelina hair has a diameter of 250 – 350 µm. Figure 7.3 shows a scanning electron micrograph (SEM) of the cuticle morphology. Compared to the previous
organisms, the javelina hair exhibits a much denser packed cuticle structure. The cuticle edges are also damaged and lifted at various locations.

![Figure 7.3 Scanning electron micrographs (SEM) of a javelina hair.](image)

The javelina hair specimens are tested at the same strain rates with a boar hair. At the low strain rate of $10^{-4}$ s$^{-1}$, the hair exhibits a large breaking strain of about 0.75 and a yield stress of 41 MPa. As the strain rate increases, the yield stress also increase to 57 MPa at the strain rate of $10^{-1}$ s$^{-1}$. The yield stress of javelina hair (Figure 7.4) is much lower than that of boar hair. Other than the influences of different living conditions, the size effect of boar hair (220 – 250 µm) and javelina hair (250 – 350 µm) should be considered in understanding this difference.
Figure 7.4 Band plots of tensile results of javelin hair at various strain rates.

7.3 Bear hair

Bear hair has an average diameter of 70 – 90 µm. Figure 7.5 shows the scanning electron microscope graph of the bear hair surface. Lifted cuticle edges can be identified from the surface. Figure 7.6 shows the stress-strain curves of bear hair at three strain rates ($10^{-4}$, $10^{-2}$, and $10^{-1}$ s$^{-1}$). At the lowest strain rate, the bear hair exhibits a yield stress of 70 MPa. As the strain rate increases, the yield stress reaches 120 – 140 MPa. Therefore, it can be seen from the above results, the bear hair has a smaller diameter, but it shows a relatively higher yield stress compared to boar and javelina hair.
Figure 7.5 Scanning electron micrographs (SEM) of a bear hair.

Figure 7.6 Band plots of tensile results of bear hair at various strain rates.
7.4 Elephant and giraffe hair

Elephant and giraffe hair exhibit a very similar appearance and structure. Therefore, they are usually misidentified to each other. We studied the tensile properties of these two different hairs at the same strain rates and the results are shown in Figure 7.7 for elephant hair and Figure 7.8 for giraffe hair. The elephant hair has a diameter ranging from 260 – 430 µm, while the diameter of giraffe hair ranges from 290 – 480 µm. At the same strain rate of $10^{-2}$ s$^{-1}$, the elephant hair exhibits an average yield stress of 67 MPa while the giraffe hair shows an average yield stress of 78 MPa.

Figure 7.7 Representative stress-strain curves of elephant hair at the strain rate of $10^{-2}$ s$^{-1}$. 
7.5 **Size effect on the stress from different organisms**

As we have observed from the above analysis, javelina, elephant, and giraffe hairs exhibit smaller yield stress than horse, boar, and bear hairs. Figure 7.9 summarizes the size effect among these various organisms and Figure 7.10 shows the comparison between predicted breaking stress from Weibull analysis and experimental results. But it should be noticed that the humans have a different living environment compared to other wild animals; moreover, human hair is regularly in touch with hair products such as
shampoo and conditioner. Generally, among the wild organisms, as the average diameter of the hair specimen increases, both yield stress and breaking stress tend to decrease. Therefore, these results indicate that there is a considerable size effect on the mechanical properties among different organisms.

Figure 7.9 Size effect on the yield stress among human, horse, boar, javelina, bear, elephant, and giraffe hairs.
Figure 7.10 (a) Experimental obtained Weibull distribution of breaking stresses for human hair and calculated distribution for different hairs with various diameters; (b) Experimental obtained breaking stress of hair from seven species and Weibull prediction for $F(V)=0.5$. 
Chapters 7 is currently being prepared for submission for publication of the material. Yang Yu, Wen Yang, and Marc A. Meyers. The dissertation author was the primary investigator and author of this material.
Chapter 8  Summary and conclusions

8.1  Summary

8.1.1  Tensile properties

The tensile properties of human hair under various strain rates, relative humidities, and temperatures are investigated. A constitutive equation is developed based on these experimental results. The major contributions are summarized as follows:

(i)  The yield stress of hair decreases with decreasing strain rate, increasing relative humidity, and increasing temperature. The hair exhibits different strain-rate sensitivities at ambient humidity and in water, indicating different responses of the amorphous matrix. Temperature affects the tensile properties at two critical values, a glass transition at 35 °C and a further structural change around 60 °C. Moreover, results show that the structural change is not reversible even after the specimens are cooled back to 20 °C.

(ii) Cyclic mechanical tests show that the hair behaves both elastically and plastically within the elastic and transformation regions. These tests confirm that no substantial structural change is introduced during the elastic region since the deformation is completely reversible; as the α-helical structure uncoils and transforms to β-sheets, it introduces a plastic deformation which is only partially recoverable upon unloading.
(iii) A constitutive equation is developed based on the experimental results and the two-phase model proposed by Feughelman (Feughelman, 1959). Strain-rate sensitivity and thermal softening are included and this model may advance our understanding in the analysis and prediction of the tensile behavior of similar α-keratin fibers.

8.1.2 Strain-rate sensitivity

Different mechanical properties and strain-rate sensitivities of horse and human hair are observed. Both hair specimens are chemically treated to remove the contribution of matrix in the viscoelasticity. The major findings are summarized as follows:

(i) Due to the different natural conditions for horse and human hairs, these two hair specimens exhibit different surface morphologies. The cuticle sheets of horse hair are largely damaged and lifted. On the other hand, the human hair shows a smoother surface. This evidence clearly confirms the two hairs experience different environments.

(ii) XRD patterns, TGA, DSC and FTIR analysis on the original hair specimens show that the horse and human hair are both α-keratin fibers and they are similar in terms of compositions and chemical groups. However, the DSC data shows that there could be possibly different bonding effects within these two hair specimens.

(iii) Horse and human hairs are treated to cleave the disulfide bonds. The strain-rate sensitivities and mechanical properties are both evaluated before and after the treatment. The human hair shows a much-decreased sensitivity after the
treatment, while the horse hair shows little difference. The FTIR data further confirms that it is due to the different disulfide bonds content, which affects the viscoelasticity in these keratin fibers. This difference in the disulfide bonds, resulting from the different contents in the original fibers and weathering conditions, is shown to contribute to the different strain-rate sensitivities in the α-keratin fibers.

(iv) High temperature and water affect the viscoelasticity and strain-rate sensitivity of the α-keratin fibers. Results show that at medium high temperature, the yield stress of hair increases while the hair maintains its sensitivity. However, at high temperature, the strain-rate sensitivity decreases due to the effect caused by the heat on the cross-link in the matrix. At the full saturated state in water, both yield stress and strain-rate sensitivity of human hair decrease due to the interaction between water molecules and the disulfide bonds in the matrix. However, this effect is shown to be reversible after the humidity of hair reaches an equilibrium with the room condition.

8.1.3 Viscoelasticity

In the current study, the viscoelasticity of human hair is analyzed through dynamic mechanical, stress relaxation, and creep tests. The major contributions are summarized as follows:

(i) Human hair behaves more elastically and less viscously when tested high frequencies. Meanwhile, as the temperature increases, the hair exhibits a more viscous property, with a glass transition behavior at \(-55\,^\circ\text{C}\).
(ii) As the hair specimen is stretched up to 0.02 strain and held at such constant strain, it shows a decrease in stress with two relaxation constants, 11 s and 207 s. This behavior can be explained with the hierarchical structure and different levels of the fibrils in the hair specimen. An equation based on the Maxwell-Wiechert model is developed and it agrees reasonably with the experimental data. However, as the hair is stretched and held at 0.25 strain, a possible phase transformation from α- to β-keratin may happen. As the hair is maintained at such strain, it is possible that the responses of various components, including α-, β-keratin, and matrix would together contribute to observed stress relaxation behavior.

(iii) The creep behavior of human hair is composed of three stages: a linear region, a parabolic region, following a repetitive plateau-sudden jump region. It is thought that the uncoiling to the complete straightening state and possible phase transformation of the α-helical structure contributes to the phenomena observed in stage III. The strain increases as various locations in the α-helices uncoil and transform until the hair specimen reaches to breakage at ~0.47 strain.

8.2 Conclusions

(i) This study linked the mechanical properties of α-keratin fibers to the chemical bonds within the hair fibers. Throughout the past decades, researchers have addressed various aspects of the mechanical properties separately. Our study tried to understand the sources of these properties and applied chemical
characterization tools to investigate the role of various chemical bonds and analyze the contributions of them. This idea to understand mechanical properties in terms of chemical bonds is beneficial to research on other biological materials.

(ii) This study unveiled the principal effect of strain rate on deformation and fracture. In our study, we first investigated the effect of strain rate on the mechanical properties and later monitored its effect on the surface morphology and fracture surfaces. This largely contributes to a deeper understanding on the effect of strain rate, not only on mechanics, but also on morphology. In the meantime, we also established a constitutive equation to describe the tensile responses. To our best knowledge, this is the first constitutive equation to describe tensile response of α-keratin fibers at different conditions.

(iii) This study investigated the effect of hair diameter on the yield strength and breaking stress for the first time by selecting hair from seven species. The diameter varied from 80 µm to over 400 µm. It is found that the strength decreases with larger diameter. From a comparative study of hair fibers from various animals, for the first time our study showed the size effects of α-keratin fibers on the yield stress and breaking stress. The important finding is that breaking stress decreases in accordance with a simple Weibull analysis where the strength varies with exp (V/V₀) where V₀ is the reference value and V is the value for the difference species. This is helpful in understanding the influences of size in the mechanical properties of α-keratin fibers.
Chapter 9  Potential future work

We have studied the effect of strain rate, relative humidity and temperature on the mechanical properties of human hair. Results show that the yield stress increases with increasing strain rate, decreasing relative humidity and decreasing temperature. We also compared the results between the constitutive model and experimental data. Furthermore, we exhibited the mechanical properties of horse hair and showed that it has different strain-rate sensitivities compared to human hair under. Following this work, we plan to continue our study on biological materials, especially α-keratin fibers in the following aspects:

1. Shape memory effect of hair fibers:

Hair has been observed to exhibit shape memory effect after immersed in the water. Since hair is also a polymer and shape memory effect in polymers is related to the glass transition temperature (Wu et al., 2013), it is also expected the water may help to lower the glass transition temperature of hair so that the glass transition temperature at that humidity decreased below the room temperature. Therefore, the matrix is able to return to the glassy state in the water and returns to its original shape. We plan to first quantify the mechanical property change after distortion (certain amount of turns/cm) and compare the results after immersing in water. Second, we will put the hair fibers in a mixed salt/alcohol solution and keep above the freezing point (-20 °C). We want to see if the hair still exhibits shape memory effect in the water at a temperature lower than the glass transition temperature. Next, we propose to disrupt bond connections in the matrix
with the above method. Therefore, we hope to fully understand the mechanism of this shape memory effect in the hair fibers.

2. Dynamic behavior of hair fibers:

Dynamic behaviors of human hair fibers also need to be further understood. Therefore, we have proposed to make some modifications on our current Hopkinson bar and test the dynamic property of human under ultra-high strain rates. We are interested in this performance because it will help us to understand the biomechanical properties of similar structures, i.e. one crystalline fibrous component embedded in an amorphous matrix system. Since the ultimate goal of studying α-keratin fibers would be producing fibers with similar configurations, the dynamic behavior would be significant in the real applications for composite fibers.

3. Understanding the ultra-extensibility of nemerteans during tensile tests:

Nemerteans also have a unique structure with multiple layered muscles (Figure 9.1). Besides, this great musculature gives them the ability to stretch up to 10 times of their resting length. One study that tried to calculate the structure change during tensile tests shows that the extensibility is related to the angles of muscle fibers (Figure 9.2) (Clark and Cowey, 1958). It is hoped that by studying this mechanism, we will be able to produce similar biomimetic materials in the near future.
Figure 9.1 Muscle fibers in the nemerteans (Petrov and Zaitseva, 2012).

Figure 9.2 Relations between volume and angles of muscle fibers in nemerteans (Clark and Cowey, 1958).
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