A Review of Rhinoceros Horn

Word Count: 3073 words

ENGR3810 STRUCTURAL BIOMATERIALS
FRANKLIN W. OLIN COLLEGE OF ENGINEERING
Sam Yang

May 2, 2011

Abstract

The rhinoceros horn is a unique composite that is made up primarily of keratin. α-Keratin molecules form two-strand molecules that are arranged to form intermediate filaments (IFs). These IFs surround a hair-like core that is generated from the nasal bone of the rhinoceros. The filament density of rhinoceros horn is 7 mm^{-2} and the average filament diameter is 100 μm. Melanin and calcium are the two primary non-keratinous components of the rhinoceros horn: melanin makes the horn more resistant to UV radiation while calcium makes it more resistant to physical wear. The concentration of these two substances is higher in the center of the horn and consequently the horn has a pointed structure. Water content also has a large effect on the behavior of the horn: it has a proportional relationship to the elastic and shear moduli of the horn. In their habitats, rhinoceroses use their horns to spar, dig for water, and guide their offspring. Therefore the horn must be tough while resisting fracture. It has a work of fracture of approximately 10 kJm^{-2}. The values for many other mechanical properties of the horn are unknown due to the difficulty of obtaining the necessary samples. Finally, rhinoceros horn research may lead to innovation within the field of composites, including internal-assembly processes and self-healing mechanisms.

0.1 Introduction

The rhinoceros is a large, heavyset herbivorous mammal whose ancestors first appeared 50 million years ago [Marven 2011]. Throughout these years, many diverse species of rhinoceros were found in North America, Europe, Africa, and Asia. Today only five species remain and less than 17,000 individuals make up the entire wild population [Marven 2011]. The black species in Africa is down to a shocking 2,300 from 65,000 in 1970 [Benyus 1997]. The Sumatran population is faring no better in Asia and now totals fewer than 600 [Benyus 1997]. The sad reality is that this tenacious family is facing extinction. What is responsible for the rapid downward trend? The animals are being hunted for their valuable nasal horns.

The rhinoceros horn has been valued for tens of centuries for its supposed healing powers and material significance [Jones 1992]. The 16th century Chinese pharmacist Li Shi Chen used rhinoceros horn to cure snakebites, hallucinations, typhoid, food poisoning, and even the "devil's possession" [Marven 2011]. Today the material continues to be used in traditional Chinese medicine systems and is said to cure many ailments, including fever, rheumatism, and gout [Marven 2011]. Medicine systems in Malaysia, South Korea, and India also make extensive use of the material. Furthermore, rhinoceros horn is carved into ceremonial cups, hair pins, and belt buckles in these Asian countries. The horn also possesses cultural significance in several Middle Eastern counties. Muslim men from the country of Yemen use rhinoceros horn to form the handles of jambiya, daggers that are presented to the Yemeni boys at age 12 to signify their transition into manhood.

The high intrinsic value placed on the horn makes the material a valuable commodity on the black market. Poachers that hunt the species earn upwards of tens of thousands of dollars for each horn they sell. This high market value has made poachers the greatest threat to the rhinoceros species. As the hunting continues, attempts have been made to save the species from extinction. In Namibia, an official dehorning program was implemented. The aim of the program was to make the animals invaluable to poachers by sawing off their horns, but this attempt did not have the intended results. The slaughter continued as the poachers hoped to increase value of the remaining horns by further reducing the rhinoceros populations. An alternative solution was presented to curb rhinoceros poaching in an indirect manner. Scientists hoped to manufacture a facsimile rhinoceros horn that would act as a viable substitute for the original material. Herein began rhinoceros horn research.

The rhinoceros horn was thought to be similar to many other horns. Most horns are tan or brown in color, homogeneous in appearance, impervious to water, and hard; rhinoceros horn was no exception [Shengqing 2011]. Due to these similarities, the rhinoceros horn was expected to have a bony core surrounded by a keratin sheath. Early discoveries disproved these beliefs as X-ray images confirmed the absence of such a core. The next hypothesis was that the horn was composed of thickly matted hairs [Corrington 1955]. However, this theory was disproved when microscopic examination showed a fiber-matrix structure to the horn. The horn is now understood to be an intricate composite of keratinous filaments embedded in a keratin matrix. This discovery—that a horn could be made almost entirely of keratin but have a similar functionality to other bony-core horns—was remarkable. This unique composite structure remains of great interest to material scientists. The present work investigates keratin, the unique structure of the horn, and the role of non-keratinous components in the behavior of the horn. Its biological function and mechanical properties are also discussed. Lastly, current questions and future direction for rhinoceros horn research is introduced.

0.2 An Overview of Keratin

The primary component of rhinoceros horn is keratin, which is a tough, fibrous protein. It is classified as a scleroprotein: it is insoluble, swells only to a limited extent, resists hydrolysis, and has high sulphur content [Corrington 1955]. Keratins exist in large quantities in many biological materials and form the protective covering of all land vertebrates: skin, fur, hair, wool, claws, nails, hooves, horns, scales, beaks, and feathers are all made primarily of keratins [Bender 2003].

The particular type of keratin that is found in rhinoceros horn is α-keratin. Furthermore, it is classed along with claws and hooves as a hard α-keratin. The degree of 'hardness' is determined by high sulfur content, which relates to the amount of cysteine that exists in the polypeptide chain of the molecule. The molecules of α-keratin bond to one another with disulfide (cross-linked) bridges and hydrogen bonds. Oxidation of its side chain—thio—results in the disulfide derivate cystine that is essential in forming the disulfide (cross-linked) bridges between the molecules. Cystine makes up from 8 to 9% of the total amino acids present in keratin [Wainwright 1982]. Equally important is the amino acid glycine; it has a two hydrogen atom side chain and therefore is important in forming the hydrogen bonds between the molecules. Both the disulfide bridging and hydrogen bonding are responsible for another important characteristic of keratins: they are insoluble. The non-polar, hydrophobic amino acid alanine further contributes to the insoluble nature of keratins.

Keratins are unusual in that they are laid down intracellularly within the membrane of a living cell [Wainwright 1982]. In keratinization—specifically in the rhinoceros horn—precursor cells move from the germinal layer to the base of the nasal bone. The fibers of keratin then invade the precursor cells and displace organelles such as the nucleus and mitochondria; these organelles are then resorbed [Sheen 2002]. Keratinous structures are in actuality composed of dead cells that are packed full of the keratin [Wainwright 1982]. Most other rigid biological materials, such as bone, are deposited extracellularly. This property dictates that the horn cannot immediately repair itself once broken, which is especially important since the horn is constantly subject to large forces.

0.3 A Unique Structure

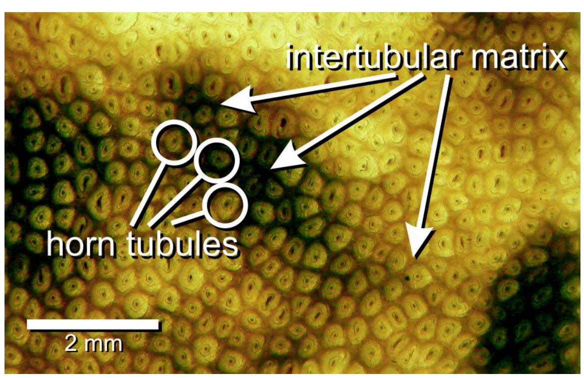

Figure 1: An optical micrograph of the white rhinoceros horn, showing filaments and the interfilament matrix [Hieronymus 2006]. The tubules labeled in the image correspond to the filaments discussed in the present work.

The rhinoceros horn is composed of α-keratin filaments embedded in an amorphous protein (mostly non-crystalline keratin) matrix (Figure 1). Keratin-producing cells surround the epidermal core of the filaments that then produce a multilayered keratin sleeve around the core. These cells lay in a concentric fashion (Figure 2). The result is a keratinous filament that has a diameter ranging from 300-500 μm. This filament is surrounded by approximately 40 lamellae [Hieronymus 2006] and is embedded within the amorphous matrix.

0.3.1 The Filament

The filament itself is an intricate structure: it is composed of an epidermal central structure surrounded by a multilayered keratin sleeve. Each of these components will be detailed further in the following section. First, at the core of each filament is a central structure that resembles the medulla of a hair [Ryder 1962]. This central structure grows from a generative layer of epidermis and below this layer is a dermal papilla [Hieronymus 2006]. The rhinoceros horn is thus attached to the short nasal bone by a mat of connective tissues that generate these hair-like cores [Chernova 1998].The central structure is referred to by Hieronymus as a "medullary cavity;" in actuality, this structure has several gas spaces or one large space occupying the width of the core.

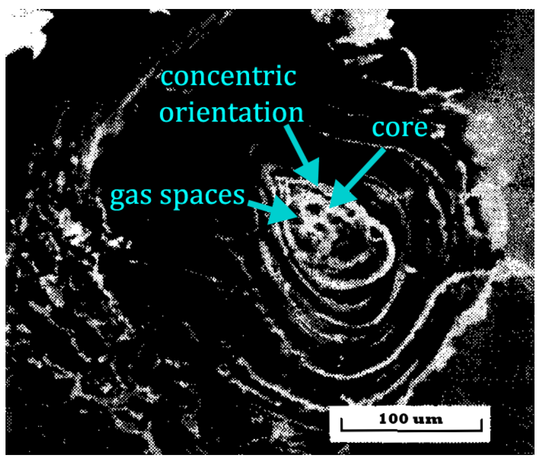

Figure 2: The keratinous filament. The concentric orientation of the keratin sleeves, the gas spaces, and the core structure are labeled [Chernova 1998].

Second, a multilayered keratin sleeve surrounds each of these central structures. The keratin molecules are assembled into two-strain molecules that are approximately 45 nm in length and 1 nm in diameter [Tombolato 2010]. They interact to form helical microfibrils, or intermediate filaments (Ifs), that are 7 nm in diameter [Tombolato 2010]. High resolution micrographs have revealed that the microfibrils are made up of a ring of nine protofibrils with an additional two protofibrils within the circle of nine [Wainwright 1982, quoting Filshie and Rogers 1961]. The filament component, however, accounts for only part of the keratin structure: the other significant component is the cross-linked matrix [Wainwright 1982]. The IFs are embedded within this viscoelastic protein matrix, which provides mechanical continuity between the individual IFs.

0.3.2 The Matrix

The interfilament matrix is composed of non-crystalline keratin; it is a compliant, amorphous matrix that is made up of fusiform interstitial cells [Hieronymus 2006, quoting Lynch 1973]. It is made up of two types of proteins-high sulfur and high glycine-tyrosine proteins. Together they add more cysteinyl and glycycl residues to the filaments. (This product is then organized into circular lamellae that surround the central structure of a filament.)

0.3.3 Interfaces

The filament-matrix interface

The filament and matrix interactions are characterized by strong interfacial bonding. Because both the filament and matrix are made up of keratin, strong bonds are created between the two components. Also noteworthy is that the keratinous filaments are packed closely together in rhinoceros horn, resulting in a polygonal structure. The filament density is small (7 mm^{-2}) compared to that of most other mammalian structural materials, including horse hoof (24 mm^{-2}) and sheep horn (22mm^{-2}) [McKittrick 2010]. These differences in filament density are visible in how differently the materials fracture. Rhinoceros horn frays into filaments whereas horse hoof and sheep horn fray into sheets [Ryder 1962]. The filament diameter is relatively large (100 μm) compared to that of horse hoof (40 μm) but small compared to sheep horn (4,000 μm) [McKittrick 2010]. All three materials have a similar keratinous composite structure, but subtleties such as filament density and size have a large impact on material properties.

The interlaminar interface

In rhinoceros horn, the keratin fibril bundles are not only found aligned in the longitudinal (growth) direction, but are also found attaching adjacent lamellae. The fibril bundles that stretch across the delaminated regions are approximately 40 nm in diameter [McKittrick 2010]. They strengthen the interace between the lamellae and increase the interlaminar shear strength by providing sliding resistance between adjacent lamellae [McKittrick 2010]. Moreover, by increasing the shear strength, the fibril bundles increase the delamination energy, given by the following equation:

$$U_{del} = \frac{2}{9}(\frac{\tau^2}{E})(\frac{wL^3}{t}). \quad (1)$$

where τ is the interlaminar shear strength, w is the width of the plate, L is the length of the plate, and t is the thickness of the lamellae [McKittrick 2010]. In this way, the fibril bundles strengthen the rhinoceros horn not only by bearing load but also by strengthening the interface between adjacent lamellae.

0.4 Calcium and Melanin

Keratin is the primary component of rhinoceros horn; however, calcium and melanin content have significant affects on the behavior and structure of the horn. These two components reinforce the keratin filament-matrix composite; calcium deposits harden and strengthen the keratinous substance while melanin deposits protect the core from ultraviolet rays [Ohio

University 2006]. Together these deposits make the substance more resistant to physical wear and breakdown from UV light exposure [Hieronymus 2006].

In most other horns, the pointed shape of the horn follows the pointed shape of the bony core. Rhinoceros horn is unique in that its shape results from a density gradient of its non-keratinous components. History suggests that the interfilament matrix varies in composition while the composition of the filaments changes very little throughout the horn [Hieronymus 2006]. Calcium and melanin are found in higher concentrations towards the center of the horn. Hieronymus illustrates this gradient (Figure 3) with red marking the densest concentrations of calcium and melanin and blue representing the least dense areas. As a result of the differences in these concentrations, the outer layers of the horn wear more rapidly than the center. The result of continued wear is the pointed structure of the horn. In this way, the rhinoceros horn has a pointed structure despite lacking a bony core.

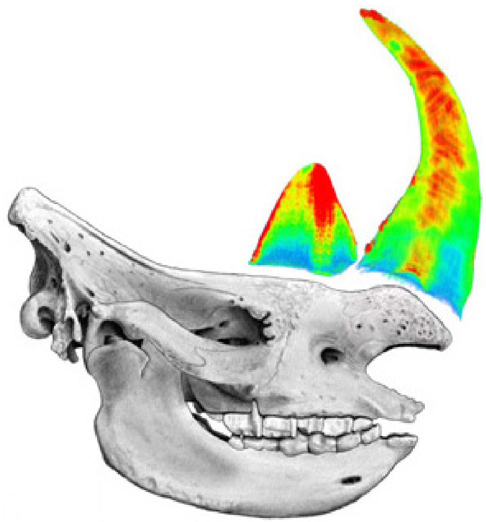

Figure 3: CT scan of rhinoceros horn. The red highlight shows the denser areas of melanin and calcium content while the blue areas represent the least dense areas [Hieronymus 2006].

The varying concentrations of the non-keratinous components suggest that rhinoceros horn exhibits unique growth patterns as well. Most horns are covered with skin which projects from the back of the skull [Tombolato 2010]. The skin is responsible for horn growth as it generates new cells. Rhinoceros horn, on the other hand, is anchored to the dermis at its base. All rhinoceros horn growth occurs at the base in this fashion. As seen in the CT scan (Figure 5), the concentration of the non-keratinous components occurs in visible bands of varying density that alternate approximately every 6 cm. Researchers found from these results that the deposition of calcium and melanin corresponded to annual growth patterns of the horn; the horn grows in pulses throughout the year. The epidermal layer generates new cells periodically rather than a constant supply to the horn.

0.5 Biological Function and Mechanical Properties

Rhinoceroses use their horns to push and spar with other individuals and to puncture adversaries [McKittrick 2010]. They rub their horns on trees and stones to keep them sharp. They dig in waterbeds to find water, uproot shrubbery to eat, and guide their offspring [Marven 2011]. As a result, the horn must provide rigidity and strength while being resistant to fracture. The structure of the rhinoceros horn allows for the desired mechanical properties. Water, however, also has a significant effect on the mechanical properties of the horn. More work must be conducted on the mechanical properties of rhinoceros horn as little research currently exists due to rarity of the samples needed for testing.

0.5.1 Fracture Properties

Keratin is clearly an important component of horn, which needs to be very tough in order to withstand great loads [Currey 2010]. The horn has a work of fracture of about 10 kJm^{-2} [Vincent 1990, quoting Kitchener 1987], which is obviously important for a material that when broken, cannot be instantly replaced. The work of fracture value is similar to that of a high-performance composite [Vincent 1990, quoting Harris 1980]. This similarity between rhinoceros horn and high-performance composites is not surprising; both materials are made up of stiff, inflexible fibers embedded into a flexible resin. The fibers break before they bend while the resin bends before it breaks. The result is a composite that is able to withstand greater loads than either of its parts. When a stress is applied to the material, the matrix inhibits crack propagation and redistributes stress in the direction of the filaments.

0.5.2 Hydration

The horn from a live animal-fresh horn-has a water content of wt.20%; Kitchener and Vincent found that horn can be immersed in water to a saturated value of 40 wt.% water-wet horn [McKittrick 2010]. The insolubility of α-Keratin was mentioned earlier; the filaments did not uptake the water molecules. Instead, the additional water fully hydrated the matrix and likely filled the gas spaces within the cores of the keratin filaments as well [McKittrick 2010]. Fresh and wet horns are both insensitive to notches [Vincent 1990]. This property is obviously of great importance to animals such as the rhinoceros that may face enormous forces from fighting other individuals. The Voigt model was used to experimentally show that calculated horn stiffness becomes progressively smaller as the water content of the matrix increases [Vincent 1990]. Table 4 lists the stiffness values for varying water content. The hydrated horn is less likely to fracture because the wet matrix will yield and flow, dissipating any stress concentrations.

	Dry	Fresh	Wet
Water content (%)	0	20	40
Mean bending stiffness (GPa)	6.1	4.3	1.8
E_f (GPa)	6.1	6.1	6.1
E_m (GPa)	6.1	3.1	0.9
V_f	0.61	0.56	0.53
V_m	0.39	0.44	0.47
G_m (GPa)	2.3	1.1	0.9
estimates of bending stiffness			
Voigt (GPa), infinite fibre length	6.1	4.8	3.6
As above, fibre length of 40 nm	6.1	3.8	1.9

Figure 4: Table of mechanical properties for horn keratin with varying water content (wt.%) [Vincent 1990].

A noteworthy observation is that animals such as the rhinoceros will oftentimes plunge their horns in mud before fighting; thus readying their horns for facing such large forces

[Vincent 1990]. Water also has a plasticizing effect [Maeda 1982]. This can be due to at least two effects: first, the water molecules may interpolate into the hydrogen bonded linkages between the amide and carbonyl groups of the keratin molecules. This interpolation effectively breaks the hydrogen bonds. Moreover, as a result of the broken bonds, extra space is created around the side chains (and the specimen swells). The overall effect is a freedom of rotation about the bonds both in the peptide chain and in the side chains [Vincent 1990] that thus act as a plasticizing agent. The overall effect of hydration is a reduction in the elastic and shear moduli of the composite and the density of the composite.

0.6 Conclusion and Future Direction

The present work investigated the unique structure of the rhinoceros horn, the role of significant non-keratinous components, and its mechanical properties and biological function. Rhinoceros horn has a unique composite structure that makes it able to withstand great loads. Unlike many other horns, the rhinoceros horn lacks a bony core and instead is made primarily of keratin. It is a composite of α-keratin filaments embedded in a compliant matrix with varying concentrations of melanin and calcium. These two components make the horn more resistant to UV radiation and to physical wear and the denser core results in the pointed structure of the horn. Hydration has a directly proportional relationship with the elastic and shear moduli of the horn. Due to its biological functions, the horn must be tough and has a work of fracture of approximately 10 kJm^{-2}. Other mechanical property values for rhinoceros horn do not exist since the necessary samples are difficult to obtain.

Though the exact mechanical property values for rhinoceros horn do not exist in literature, experimental results have shown that rhinoceros horn in similar to many high-performance composites in the market today. The major differences are that rhinoceros horn is made of the naturally pervasive biomaterial keratin and under ambient conditions. Take even the newest generation of manufactured composites, silicon-carbide fibers embedded in aluminum oxide. The composite is made by laying fibers down by hand under a block of ceramic then combining both under pressure and heat so that the ceramic re-solidifies around the fibers. The process is rigorous and if any of the fibers diffuse into another then the resulting composite may be susceptible to breakage [Benyus 1997]. In the rhinoceros horn, on the other hand, the filaments self-assemble from within the composite and do so under ambient conditions. Moreover, keratin is an inexpensive and omnipresent material. If scientists could induce keratin to take this structure, composite technology would be vastly improved.

Rhinoceros horn researches are also investigating the self-healing nature of the horn. The horn is made up of dead tissue; there are no living cells. However, scientists have captured images showing a polymer substitute filling cracks of the horn. One hypothesis is that there are actually living cells in the horn; if true, it may be possible to grow rhinoceros horn in vitro. Another hypothesis is that there may exist an interesting transport mechanism that brings living cells to the horn. This mechanism may make it possible to induce keratin to assemble into rhinoceros horn. The suggested process would involved first depolymerizing the keratin and then reassembling around an epidermal core filament. A similar process is already being utilized in the field of dentistry for new bone growth. Hydroxyapatite is inserted into the jaw of a patient; new bone is made when the bone cells come in contact with and calcify the hydroxyapatite. Researchers are continuing to deepen their understanding of rhinoceros horn and these mysteries may lead to innovative discoveries.

Bibliography

[1] Bender, Hal. (2003). Structural Proteins. *Clackamas Community College*. Retrieved April 2011, from http://dl.clackamas.edu/ch106-08/structur1.htm

[2] Benyus, J.M. (1997). *Biomimicry: Innovation Inspired by Nature*. New York: HarperCollins Publishers Inc, 139-145.

[3] Chernova, O.F. *et al.* (1996). Morphology of Horns in Wholly Rhinoceros. *Russian Journal of Zoology, 2(1)*, 126-138.

[4] Corrington, J. (1955). In Search of Keratin. *Bios, 26(1)*, 23-34.

[5] Currey, J.D. (2010). Mechanical properties and adaptations of some less familiar bony tissues. *Journal of the Mechanical Behavior of Biomedical Materials, 3*, 357-372.

[6] Hieronymus, T. *et al.* (2006). Structure of White Rhinoceros (*Ceratotherium simum*) Horn Investigated by X-ray Computed Tomography and Histology with Implications for Growth and External Form. *Journal of Morphology, 267*, 1172-1176.

[7] Jones, D. (1992). Horn of Plenty. *Nature, 358*, 458.

[8] Marven, N. (2011). Rhino Horn Use. *Nature*. Retrieved April, 2011, from http://www.pbs.org/wnet/nature/episodes/rhinoceros/rhino-horn-use-fact-vs-fiction/1178/

[9] Maeda, Hideatsu. (1989). Water in Keratin. *Biophysical Journal, 56*, 861-868.

[10] McKittrick, J. *et al.* (2010). Energy absorbant natural materials and bioinspired design strategies: a review. *Materials Science and Engineering, 30*, 331-342.

[11] Ohio University. (2006). Scientists Crack Rhino Horn Riddle. *ScienceDaily*. Retrieved April 16, 2011, from http://www.sciencedaily.com /releases/2006/11/061106144951.htm

[12] Ryder, M. L. (1962). Structure of Rhinoceros Horn. *Nature, 193(4821)*, 1199-1201.

[13] Shengqing, L. *et al.* (2011). Identification of Rhinoceros Horn and its Substitutes. *Advanced Materials Research, 177*, 636-639.

[14] Tombolato, L. *et al.*(2010). Microstructure, elastic properties and deformation mechanisms of horn keratin. *Acta Biomaterialia, 6*, 319-330.

[15] Trim, M.W. *et al.* (2011). The effects of water and microstructure on the mechanical properties of bighorn sheep (*Ovis canadensis*) horn keratin. *Acta Biomaterialia, 7*, 1228-1240.

[16] Sheen, J.P. (2002). Keratin. *Macmillan Reference USA*. Retrieved April, 2011, from http://www.novelguide.com/a/discover/ansc_03/ansc_03_00205.html

[17] Vincent, J. (1990). *Structural Biomaterials*. Princeton: Princeton University Press, 138-141.

[18] Wainwright, S.A., *et al.* (1982). *Mechanical Design in Organisms*. Princeton: Princeton University Press, 187-190.