History of Keratin Research

The earliest documented use of keratin in medicine comes from a Chinese herbalist named Li Shi-Zhen. [2] Over a 38-year period, he wrote a collection of 800 books known as the Ben Cao Gang Mu. These books were published in 1596, three years after his death. Among the more than 11,000 prescriptions described in these volumes is a substance known as *Xue Yu Tan*, also known as *Crinis Carbonisatus*, that is made of ground ash from pyrolized human hair. The stated indications for *Xue Yu Tan* were accelerated wound healing and blood clotting. Not much is known about how the discovery of human hair's biological activity came about, but it took modern scientists several more centuries to find out what the Chinese had revealed.

In the early 1800's, when proteins were still being called albuminoids, many different kinds of proteins were being discovered. Around 1849, the word "keratin" appears in the literature to describe the material that made up hard tissues such as animal horns and hooves (keratin comes from the Greek "*kera"* meaning horn). This new protein intrigued scientists because it did not behave like other proteins. For example, the normal methods used for dissolving proteins were ineffective with keratin. Although methods such as burning and grinding had been known for some time, many scientists and inventors were more interested in dissolving hair and horns in order to make better products.

The resolution to this insolubility problem came from a trade more than 700 years old - the tanning industry. In the years preceding World War I, lime was applied to the manufacture of keratin gels. In a United States (US) patent issued in 1905, John Hoffmeier described a process for extracting keratins from animal horns using lime. [3] He then used the extracted keratins to make gels that could be strengthened by adding formaldehyde (formaldehyde "crosslinking" is a popular method of strengthening such gels and is still used today to "fix" tissues containing structural proteins like keratin and collagen).

During the years from 1905 to 1935, many methods were developed to extract keratins using oxidative and reductive chemistries.[4-9] Not only was this technology applied to animal horns and hooves, but many scientists and inventors extracted keratins from human hair as well. The driving force behind these developments was the medical and biological properties of these extracts. Among the first inventions were keratin powders for cosmetics, composites, and coatings for drugs, respectively.[10-12] In 1925, one of the first companies to make commercial use of keratins was founded in the United Kingdom (UK). Croda International produced lanolin from wool but soon discovered the value of wool keratins as products for many cosmetic and personal care markets. Today, Croda is one of the top producers of specialty keratin-based products.

By the late 1920's many techniques had been developed for breaking down the structures of hair, horns, and hooves, but scientists were confused by the behavior of some of the extracted proteins. Based on what was known about other proteins, there were several things that were very different about the wool keratins that remained unexplained. In 1927, scientists began to openly discuss their theories and publish papers that speculated on the structure of keratins and hair fibers.[13] During this decade, keratin research changed its focus from what could be made from keratins to the structure and function of keratins. Several key papers were published by scientists in Russia and the US that analyzed oxidatively and reductively extracted keratins.[7,8]

These scientists soon concluded that many different forms of keratin were present in these extracts, and that the hair fiber must be a complex structure, not simply a strand of protein. In 1934, a key research paper was published that described different types of keratins, distinguished primarily by having different molecular weights.[8] This seminal paper demonstrated that there were many different keratin homologs, and that each played a different role in the structure and function of the hair fiber.

It was during the years of World War II and immediately after that one of the most comprehensive research projects on the structure and chemistry of hair fibers was undertaken. Driven by the commercialization of synthetic fibers such as Nylon and polyester, Australian scientists were charged with protecting the country's huge wool industry. Synthetic fibers were seen as a threat to Australia's dominance in wool production, and the Council for Scientific and Industrial Research (later the Commonwealth Scientific and Industrial Research Organisation or CSIRO) established the Division of Protein Chemistry in 1940. The goal of this fundamental research was to better understand the structure and chemistry of fibers so that the potential applications of wool and keratins could be expanded.

Earlier work at the University of Leeds and the Wool Industries Research Association in the United Kingdom (UK) had shown that wool and other fibers were made up of an outer cuticle and a central cortex. Building on this information, scientists at CSIRO conducted many of the most fundamental studies on the structure and composition of wool. Using X-ray diffraction and electron microscopy, combined with oxidative and reductive chemical methods, CSIRO produced the first complete diagram of a hair fiber.[14] This schematic is shown in **Figure 1**.

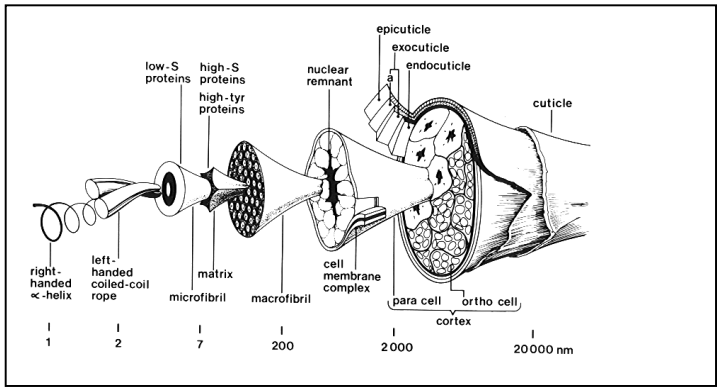

Figure 1. Schematic of a wool fiber drawn by Bruce Fraser and Tom MacRae of CSIRO and taken from Rivett DE, Ward SW, Belkin LM, Ramshaw JAM, and Wilshire JFK; "Keratin and Wool Research"; The Lennox Legacy, CSIRO Publishing; Collingwood, VIC, Australia; 1996. Used with permission.

CSIRO scientists also conducted extensive studies on the wool proteins themselves. Many methods for the extraction, separation, and identification of these keratins were developed. Other fundamental studies included wool surface chemistry, processing and products, fellmongering (harvesting of wool from sheep), felting, carbonising, surface treatments, flammability, denaturation, chemical modification, dyeing, photochemical degradation, and application of polymers to wool. This monumental effort was conducted over a period of more than 30 years and resulted in over 660 publications, 20 patents, and 3 books.

In the meantime, the use of oxidative and reductive chemistry to extract keratins from hair fibers was being applied by other scientists across the world. In the Netherlands, researchers patented a method for making films and textile fibers from reductively extracted keratins from

ground up hooves.[15] Probably nowhere in the world was keratin research more active than in Japan. Between the years of 1940 and 1970, applications for keratin-based inventions submitted to the Japanese patent office numbered more than 700. This was a renaissance in keratin research that was trending toward the fundamentals of materials science and biomaterials. Driven by the development of reliable methods to solubilize keratins, researchers were beginning to understand the many sub-classes of keratins and their different properties.[16-20] In 1965, CSIRO scientist W. Gordon Crewther and his colleagues published the definitive text on the chemistry of keratins.[21] This chapter in *Advances in Protein Chemistry* contained references to more than 640 published studies on keratins.

Once scientist knew how to extract keratins from hair fibers, purify and characterize them, the number of derivative materials that could be produced with keratins grew exponentially. In the decade beginning in 1970, methods to form extracted keratins into powders, films, gels, coatings, fibers, and foams were being developed and published by several research groups throughout the world.[22-24] All of these methods made use of the oxidative and reductive chemistries developed decades earlier.

New companies also formed and offered some of these keratin products to growing industries. In addition to Croda in the UK, Rita Corporation was founded in the US and began offering naturally derived products to the food, drug, cosmetic, and personal care markets. Around 1980, Rita Corp. partnered with a Japanese company, Seiwa Kasei, Ltd., to bring a host of keratin-based products to these same markets. Another Japanese organization, the Kao Soap Company, holds more than 72 keratin-based patents.

The prospect of using keratin as a biomaterial in biomedical applications was an obvious one. During the 1980's, collagen became a commonly used biomolecule in many medical applications. Other naturally derived molecules soon followed such as alginates from seaweed, chitosan from shrimp shells, and hyaluronic acid from animal tissues. The potential uses of keratins in similar applications began to be explored by a number of scientists. In 1982, Japanese scientist published the first study describing the use of a keratin coating on vascular grafts as a way to eliminate blood clotting,[25] as well as experiments on the biocompatibility of keratins.[26] Soon thereafter in 1985, two researchers from the UK published a review article speculating on the prospect of using keratin as the building block for new biomaterials development.[27] In 1992, the development and testing of a host of keratin-based biomaterials was the subject of a doctoral thesis by a French graduate student.[28] Soon thereafter, Japanese scientists published a commentary in 1993 on the prominent position keratins could take at the forefront of biomaterials development.[29]

The value of hair proteins was not limited to pure keratin materials. While earlier research demonstrated that keratin powders could be mechanically combined with other materials, the possibility of chemically combining keratin with other compounds began to emerge as a research area as early as 1967.[30] By the 1980's Japanese companies began to patent methods of chemically reacting keratins with other materials to form products used in hair treatments (shampoos, conditioners, etc.). By 1989, the concept of producing keratin that was chemically combined with silicone (so called keratin-silicone copolymers) was patented in Japan.[31] Other keratin-silicone copolymers were also patented by Croda International around this same time period.[32]

Due to the fundamental research of scientists all over the world, and more importantly the publication of their experimental results, the field of keratin research has continued to grow.

Wound healing, drug delivery, tissue engineering, cosmetics, and medical devices continued to be popular subjects for keratin-based research in the past decade. Wound healing in particular was the subject of patents granted to an international collection of inventors in 1986, 1991, 1992, and 1998.[33-37] Japanese companies, government, and academic laboratories continue to look to keratins as a source of economic development in the field of medicine.[38-41] Other countries including France, Russia, the United States, Great Britain, Australia, and New Zealand continue to make significant contributions to the growing base of keratin knowledge.

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