PREFACE

Keratins represent a group of fibrous proteins produced in some epithelial cells of vertebrates such as reptiles, birds and mammals. These proteins are abundantly present in nature and they constitute the major part of hair, wool, horns, nails, feathers and stratum corneum of the skin. In this book, the authors present current research in the study of the structure, properties and applications of keratin. Topics discussed include simple epithelial keratins K8 and K18; extraction, processing and applications of wool keratin; water sorption of human keratinized fibers and keratin expression in the human pituitary gland and its application to neoplastic pituitary cells.

Chapter 1 - K8 and K18 (K8/18) are the major heteropolymeric intermediate filaments (IFs) present in simple layer epithelia. In hepatocytes, keratin filaments form an extensive cytoplasmic network that is denser at the cell periphery and around bile canaliculi. Keratin filaments are attached to the plasma membrane via desmosomes. K8/18 IFs have long been linked to human chronic liver diseases. In fact, modifications in keratin IFs network organization and the formation, in hepatocytes, of K8/18 containing aggregates, named Mallory-Denk bodies (MDBs), are characteristic of alcoholic and non-alcoholic steatohepatitis, copper metabolism diseases such as Wilson disease and Indian childhood cirrhosis and hepatocellular carcinoma. The formation of MDBs is the consequence of an increase of K8/18 mRNA and proteins, alterations in K8/18 post-translational modifications such as phosphorylation on multiple sites, transglutaminase mediated keratin crosslinking and a defect in K8/18 degradation by ubiquitin-proteasome pathway. The use of transgenic mouse models has allowed unravelling the significance of these changes in K8/18 dynamic in hepatocytes and revealed a function for keratins in protecting hepatocytes against

mechanical and non-mechanical stresses. For instance, mice expressing an ectopic human K14, a mutated human K18 (Arg89→Cys) or K18 (Ser52→Ala) or K18-Gly(-), K8 (Ser73→Ala) or K8 deficient mice are more susceptible to various mechanical and toxic injuries. The recent observation of the existence of K8 and K18 mutations in cases of cryptogenic and non-cryptogenic forms or human liver disease is in total agreement with a role for keratin in maintaining cellular integrity under various threatening conditions. In a pursuit for understanding the molecular mechanism by which keratins could accomplish their protective role in cells, researchers have investigated the relationship between K8/18 and different regulatory pathways. There is now evidence that keratins are involved in signalling pathways regulating apoptosis, cell growth, and motility of various simple epithelial cells. It is noteworthy that expression of keratins is maintained during malignant transformation of simple epithelial cells. The PI3K/Akt pathway plays a pivotal role in apoptosis, cell growth, and motility and over-expression of the active form of Akt1 and Akt2 increase K8/18 protein levels suggesting that IFs are involved in PI3K/Akt pathway. The direct binding of K8 to Akt1 suggests that K8/18 IFs might provide a scaffold for Akt1 in cells. K8/18 interacts directly with other molecules involved in the apoptotic signalling pathway such as tumor necrosis factor receptor (TNFR), TNFR1-associated death domain protein (TRADD), Mrj co-chaperon, with Hsp/c70 and caspase 3. K8/18 also binds to key regulatory proteins of mitosis and cell proliferation such as 14-3-3, Cdc25 and Raf-1 kinase. Therefore keratins which were considered as only structural in the early 90s are now considered as key regulatory elements in modulating multiple signalling pathways.

Chapter 2 - Keratins are proteins characterised by a high sulphur amount and by the presence of strong disulphide bonds which make keratins water insoluble and resistant to different chemical agents.

Keratins with molecular weight ranging from less than 10 to 60 kDa are obtained by cleavage of the cystine bonds with reducing or oxidising agents or by sulphitolysis. Keratin oligopeptides can be obtained by cleavage of peptide bonds using strong acids or strong bases. Recently, green hydrolysis with superheated water and steam-explosion has been proposed with the aim of avoiding the use of harmful agents.

Proteins extracted from wool can be processed alone, or in blend with other polymers, with crosslinking agents, or in the presence of additives (e.g. plasticisers). Keratins are biocompatible, biodegradable, hygroscopic, adsorb heavy metal ions, formaldehyde and other volatile organic compounds. So, keratin can be used in different fields in the form of films, sponges, nanofibres,

microcapsules, hydrogels and powders especially for biomedical applications and filtration.

In this review details on the extraction and processing of keratins from wool are reported, with some example of utilization for different practical applications.

Chapter 3 - Appearance and distribution of different kinds of cytokeratins are closely related to morphogenesis of the various organs. On the morphogenesis of the rat tongue during prenatal and postnatal development, we could recognize the specific appearance and distribution of some kinds of cytokeratins, such as cytokeratins 13 (K13), 14 (K14) and 18 (K18). No immunoreactivity specific for K13 and K14 was detected on the dorsal epithelium of the anterior part of the tongue in fetuses on embryonic day 15 after conception (E15), at which time the lingual epithelium was composed of a few layers of cuboidal cells. No lingual papillae are recognizable in this area. K14-specific immunoreactivity was first detected on the lingual epithelium of fetuses on E17 and K13-specific immunoreactivity on E19. The number of layers of cuboidal cells in the lingual epithelium also increased from E17 to E19. At all postnatal stages, K13-specific immunoreactivity became to be gradually evident in the suprabasal cells of the interpapillary cell columns and K14-specific immunoreactivity did in the basal and suprabasal cells of the papillary and interpapillary cell columns according to the development of filiform papillae.

On the other hand, no immunoreactivity specific for K13 and K14 was detected in the lingual epithelium on E15, at which time the primitive rudiment of the circumvallate papillae was detectable by the thickening of several layers of cuboidal epithelial cells. On E17 and E19, the developing circumvallate papillae were clearly recognizable. No K13 and K14-specific immunoreactivity was evident in the lingual epithelium around these structures. K14-specific immunoreactivity was first detected in the basal layer of the epithelium of the circumvallate papillae on postnatal day 0 (P0) and K13-specific immunoreactivity was detected on P7. Morphogenesis of the circumvallate papillae progressed significantly from P0 to P14, and K13 and K14-specific immunoreactivity was clearly recognizable after P7. K13-specific immunoreactivity was generally evident in cells of the intermediate layer of the epithelium, while K14-specific immunoreactivity was detected in cells of the basal and suprabasal layers.

K18-specific immunoreactivity was detectable in the single layer of periderm cells that covered the dorsal epithelium of the tongue of fetuses on E13. The immunoreactivity was sparsely distributed throughout the cytoplasm

in some periderm cells. On E15, K18-specific immunoreactivity was also detectable in the periderm cells. The distribution of the K18-specific immunoreactivity was sparsely distributed in the cytoplasm in some periderm cells on E13 and E15. On E17, the K18-specific immunoreactivity was very distinct in the periderm cells, which had become swollen and elliptical and covered the dorsal epithelium of the fetal tongues. The pattern of distribution of the immunoreactivity was different from that on E13 and E15, and K18-specific immunoreactivity was compactly distributed over almost the entire cytoplasm in most periderm cells. No K18-specific immunoreactivity was detectable on the dorsal epithelium of the tongue of fetuses on E19. Periderm cells had disappeared completely by E19.

Chapter 4 - Water produces changes in the properties of human keratinized fibers, such as hair and nails, and is therefore of fundamental interest. Water diffusivity in wool, horn, and the corneocyte phase of stratum corneum considerably increases with increased water content in the tissue. However, water sorption of wool is well documented whereas there are few data on human hair and nails. Human hair is a keratinized fiber which is divided into three structural zones: medulla, cortex, and cuticle. Reactive cosmetic treatment of hair often impairs the fiber structure. The resulting damage has an adverse effect on hair water absorption at ambient humidities and leads to an increase in swelling or to liquid retention on wetting. Like hair, the nail plate consists of hard keratin and lipids. The nail plate is an indicator of overall health. The degree of hydration is thought to be the most important factor influencing the physical properties of the nail. The use of nail care products and procedures to beautify and groom the nails is extremely common. Unfortunately, when improperly used, nail cosmetics can lead to nail diseases. Brittleness in the nail may be caused by trauma, such as repeated wetting and drying, repeated exposure to detergents and water, and excessive exposure to harsh solvents, such as those found in nail polish remover.

There is a growing consumer trend toward natural actives that have the potential to maintain hair and nails with a healthy, youthful appearance. Wool proteins are mild, natural, biodegradable and sustainable with multiple functionalities and have potential for use in the personal care and detergent markets. In this work the effect on hair and nails of two keratin proteins isolated from wool has been investigated, an intact keratin intermediate filament protein extract (K-protein) and a low molecular weight keratin peptide from intermediate filament proteins (K-peptide). Both keratins have cystine present in the active S-sulphonated form. This unique chemistry might enable the keratin peptide and protein to reform disulphide bonds in damaged

hair and nails and to replenish the natural disulfides bonds of the hair fibres, directly affecting their properties.

The determination of water sorption isotherm by isothermally applying discrete, cumulative humidity changes involves dynamic and static aspects from which diffusion coefficients and equilibrium water contents have been deduced. Time/absorption isotherms provide a complete description of the absorption phenomenon under particular conditions such as initial regain of the sample, temperature and relative humidity. A number of equations have been proposed for modeling sorption isotherms, being in our case the GAB (Guggenheim-Anderson-deBoer) equation successfully applied.

The main aim of this work is to apply two different S-sulfonated wool keratins, K-protein and K-peptide to untreated hair and nail and to hair and nail subjected to different chemical cosmetic treatments. The moisture absorption/desorption isotherms curves for untreated and treated hair and nail and the kinetics of these processes are studied in this work. The effectiveness of these keratin ingredients at restoring the water sorption characteristics of the keratin tissues is determined.

Chapter 5 - Stratum corneum (SC) is the outermost layer of skin and the skin barrier against chemicals, surfactants, UV irradiation, and environmental stresses. The SC has a heterogeneous structure composed of corneocytes embedded in the intercellar lipid lamellae as illustrated in Figure 1. The morphology of the SC lipids is closely associated with the main epidermal barrier. Knowledge of the lipid structure is important in understanding the mechanism of irritant dermatitis and other SC diseases. The structural information of the SC lipid is obtained by the analysis of aliphatic spin probes incorporated into intercellar lamella lipids using EPR (Electron Paramagnetic Resonance). EPR in conjunction with spin probe method non-destructively measures the ordering of the lipid bilayer of SC.

EPR (or ESR: Electron Spin Resonance) utilizes spectroscopy, which measures the freedom of an unpaired electron in an atom or molecule. The principles behind magnetic resonance are common to both EPR and nuclear magnetic resonance (NMR), but there are differences in the magnitudes and signs of the magnetic interactions involved. EPR probes an unpaired electron spin, while NMR probes a nuclear spin. EPR can measure 10^{-9} M (moles per liter) concentration of the probe and one of the most sensitive spectroscopic tools. Therefore, EPR is able to elucidate skin lipid structures as well as dynamics.

Chapter 6 - The detection of specific keratins may be of diagnostic value in terms of dividing epithelial tissues into various classes depending upon their cellular origin and morphologic and/or growth features. Therefore, this review will focus on the normal patterns of keratin expression in the developing human pituitary gland, in non-neoplastic pituitary cells, and in several pathological conditions such as Rathke's cleft cyst and pituitary adenomas and its associated pathology (i.e. fibrous bodies and Crook's hyaline change).

Chapter 7 - Natural fibers have been involved in an emerging kind of polymer composites taking advantage of the outstanding properties that nature confers them. Thus, several types of biofibers have recently attracted increasing interest of engineers and scientists since mimic their structures or make use of all their potential is a motivating challenge in polymeric materials field. Biofibers can be obtained from different renewable natural sources, those from vegetal origin have been the most exploited, however alternative raw materials, such as keratin biofibers are coming into view. Keratin, as fiber, can be found in hair and feathers. Human hair keratin and wool have been studied since many years ago, due to textile and medical implications, whereas keratin from feathers has not been highlighted enough. Keratin fiber has a hierarchical structure, with a highly ordered conformation, is by itself a biocomposite, product of a large evolution of animal species. Keratin fibers from feathers are non-abrasive, eco-friendly, biodegradable, insoluble in organic solvents, and also have good mechanical properties, low density, hydrophobic behavior and finally low cost. These characteristics make keratin fibers from chicken feather a suitable material to be used as a high structural reinforcement in polymer composites. However the development of keratin fibers as a new reinforcement must be based on a complete characterization in order to know their features, advantages and restrictions. The analysis of keratin fiber from chicken feather include spectroscopic analysis using both Fourier transform infrared and Raman, thermal studies including differential scanning calorimetry and thermogravimetric analysis, also contact angle determinations reflecting the hydrophobic behavior are shown. Morphological details are observed with optical, transmission and scanning electron microscopy. During the last decade these new fibers have been studied by different research groups as reinforcement for several synthetic polymers; their results are reviewed together by first time in concerned literature. Thus, keratin fibers from chicken feathers are shown as a novel eco-friendly material that must be adequately applied in the development of green composites. Finally, the studies reviewed in this chapter can be the scaffolding to increase the use of this protein taking as base the knowledge of its properties and scope,

and therefore the current and future research in keratin could be related to high importance areas as nanotechnology or environmental decontamination, opening interesting gates in multidisciplinary areas that could take advantage of the high performance that nature confers to keratin fibril protein.