Course: Histology

Study: Cosmetology

Form of classes and number of hours: lectures 15 h, laboratory classes 15 h

Number of ECTS credits: 4

Educational outcomes:

knowledge of the histological structure of the subcutaneous tissue, dermis, epidermis and its appendages

The method of verification of the learning outcomes:
examination

Subject matter of the classes:

1. Histological structure of the subcutaneous tissue.
2. Histological structure of the dermis.
3. Histological structure of the epidermis.
4. Histological structure of skin appendages.

Bibliography

Books

Baumann L., *Cosmetic Dermatology*. MacGraw Medical, New York 2002.

Błaszczyk M., *Histologia dla kosmetologów*. Oficyna Wydawnicza PWSZ, Nysa 2013.

Pawlina W., *Histology a Text And Atlas: With Correlated Cell and Molecular Biology.* Wolters Kluwer Health, Warszawa 2020.

The histology of the skin includes the structure of the subcutaneous fatty tissue, dermis and epidermis. In order to understand these topics comprehensively, it is strongly advised to refresh the knowledge about all the cellular and intercellular structures, and about all the tissues. The following text is just a general view of the main subject.

Histological structure of the skin

The skin and epithelium covering organs in contact with the external environment constitute a common integument of the body. Its role is to provide an optimal environment for deeper tissues, by separating them from the external environment, and at the same time, ensuring contact with it by exchanging substances and receiving stimuli.

The skin is divided into three layers: the subcutaneous tissue and the dermis (made of connective tissues) and the epidermis (made of epithelial tissue).

Several classes of cells can be found in the connective tissue: fat cells, histiocytes, mast cells, plasma cells, blood-derived leukocytes. However, the basic cells of connective tissue are fibroblasts and fibrocytes. Their shape is oval, they have protrusions located in one plane and they have single-cell nuclei. They are indirectly responsible for the mechanical properties of connective tissues, thanks to the synthesis of most intercellular substances. Fibroblasts are the term for the more metabolically active juvenile form, although in practice there are no anatomical differences between them and the fibrocytes, and some of them can only pass into the other by changing the level of metabolism.

1. Intercellular substance of connective tissue

Connective tissue cells are loosely dispersed, there are large areas of intercellular substance between them. It is synthesized mainly by fibroblasts and fibrocytes and consists of fibers and unformed intercellular matter.

1.1. Connective tissues fibers

Collagen fibers, as the name suggests, are made of collagen. This protein is synthesized in the rough endoplasmic reticulum (mainly fibroblasts) as a preprocollagen – a protein α chain ending at both ends with globular fragments. The characteristic amino acids of preprocollagen are glycine, which is every third amino acid, proline, which usually takes the place behind glycine, and lysine. The terminal segments of the chain are recording peptides that will be then cleaved off. Translation is followed by a multi-stage maturation of collagen:

- cleavage of the signal sequence from the amino terminus,
- hydroxylation of proline and lysine in the presence of vitamin C, resulting in the amino acids hydroxyproline and hydroxylysine,
- at the carboxyterminal end, globular structures are formed that allow for the appropriate spatial arrangement of the three collagen molecules during the building of disulfide (-S-S-) bridges and hydrogen bonds between them. If the three molecules forming a triple molecule are the same, it is homotrimeric, on the other hand, if the molecules are different, the triple molecule is heterotrimeric,
- the procollagen triple helix is bound to the hsp47 protein which prevents premature aggregation into fibers,
- Procollagen is packed into secretory vesicles and exocytosis in the Golgi apparatus. Outside the cell, the recording peptides at the ends of the molecule are cut off by the protocolagen protease.

The mature form of the collagen molecule, formerly known as tropocollagen, is 300 nm long and 1.5 nm in diameter.

The described model of synthesis applies to type I collagen, but it is similar for all fibrillar collagens (I, II, III, V and XI).

The collagen fiber in the connective tissue is composed of such connected triple molecules, between which lysine oxidase cross-links. Collagen molecules aggregation into fibers takes place in the extracellular space, under the control of fibroblasts that release subsequent secretory vesicles. Fibroblasts form coves in the cell membrane, in the appropriate places in which collagen molecules are arranged during the formation of cross-bonds between lysine and hydroxylysine, with the participation of lysine oxidase (the activity of this enzyme depends on the presence of copper cations).

In young tissues, the fibers are 15-20 nm in diameter, in mature tissues they are up to 300 nm. Due to the regular arrangement of collagen molecules, transverse striation is visible on the fibers every 68 nm. The molecules are arranged in parallel, in the same direction, overlapping each other in 1/4 of their length. Between successive molecules, along the fiber there are gaps of 60% of the collagen molecule length.

Excess collagen fibers are removed by specific enzymes – *matrix metalloproteinases* (MMPs), e.g. collagenases (breaking down collagen I, II, III, X), gelatinases (degrading denatured collagen, laminin, fibronectin, elastin), matrilysin (degrades collagen IV and proteoglycans), stromelysin (digests, among others, proteoglycans, fibronectin), membrane MMPs, macrophage metalloelastases. Moreover, the fibers can be removed by macrophages in the process of phagocytosis.

So far, 29 types of collagen differing in α chains and 42 types of α chains which build fibers (encoded by 42 different genes, although it has been written about 44 types too) have been described. The number of amino acids in the molecule of different types of collagen ranges from 600 to 3000.

The most interesting types of collagen for cosmetology are:
Type I constitutes 90% of the total collagen in the human body, it largely builds the skin, bones, dentin, ligaments and tendons. It makes up 85% of collagen in the skin. It has been shown that its amount decreases in skin exposed to intense photoaging.

Type III is found mainly in the reticular tissue, blood vessels, spleen, kidneys, lungs, and also predominates in the skin during fetal life. In adults, it accounts for up to 15% of skin collagen. Its fibers are thinner than collagen I.

Type IV builds the basal membranes of the epithelium, it occurs in the lens of the eye.

Type VII builds basement membrane anchoring fibers.

Collagen in connective tissue, together with glycosaminoglycans, determines the level of its hydration, because it forms a colloidal system with water. The skin owes its mechanical strength to collagen fibers. Collagen fibers easily undergo reversible deformation – bending, however they are not stretchy. The direction of the arrangement of fibers in the tissues depends on the direction of physical stresses. Hence the differences in the elasticity and extensibility of the skin in different locations and in different directions. The arrangement of the fibers is described by the so-called Langer's lines, being of great importance in plastic surgery. If the skin is cut along these lines, the damage heals quickly and without scarring. If the injury is perpendicular, then the fibers are cut and regenerated fibers are overgrown, then thick scars called keloids are formed. The arrangement of fibers is also included in **Kraissl lines** marked with lines of wrinkles, and in **Borges lines** dependent on skin tension (RSTL – *relaxed skin tension lines*).

Reticulin fibers: (reticulin) resemble like type I collagen fibers, but type III is predominant. They are only 20 nm thick. In their chemical structure, they are distinguished by the presence of numerous

sugar groups. These fibers form spatial networks or, if they are arranged in a plane – a regular grid. As in collagen I, they show striations every 68 nm. They build basal membranes, they are located around the endocrine organs and form the framework of the organs of the lymphatic system.

Elastic fibers are also thinner than collagen fibers and they are also branched. The core of the elastic fiber is made of elastin – a protein weighing 72 kDa. The core is surrounded by a network of 350 kDa microfibrillin (**fibrillin I**).

Compared to collagen, the elastin molecule does not contain hydroxylysine and it includes only a small amount of hydroxyproline. The irregular arrangement of amino acids makes the molecule hydrophobic in proline, valine, and glycine-rich domains, and hydrophilic in lysine and alanine-rich regions, resulting in irregular twists rather than parallel patterns. A consequence of this is the ability of the elastic fibers to stretch. There are atypical amino acids in elastin: desmosine and isodesmosine, formed from 4 lysine molecules. In the intercellular space, individual elastin molecules form cross-links between these amino acids. It is so thanks to lysine oxidase.

Elastic fibers undergo stretching, and after the stretching force ceases, they return to their previous position. Thanks to this, they determine the skin's ability to reversible deformations. They are associated with collagen fibers and in the event of a collagen fibers change, they are the elastic fibers that bring them back to their resting location.

Elastin is not synthesized *de novo* in adult humans.

Oxytalan and elaunin fibers are also sometimes distinguished, however, they are considered to be the immature form of elastic fibers; similarly to them they have a fibrillin scaffold.

1.2. The basic nature of connective tissues

Between the cells and fibers of connective tissues there is an aqueous solution of inorganic and organic chemical compounds. Among the organic polysaccharides, glycosaminoglycans and proteins play a special role. The basic substance of connective tissue is abbreviated as **ECM** (*etracellular matrix*).

Glycosaminoglycans (GAGs) are composed of repeating disaccharide molecules, containing an amino sugar (acetylgalactosamine or acetylgluco-samine) and a sugar derivative – glucuronic or iduronic acid.

In an aqueous environment, glycosaminoglycans, as polyanions, bind, for example, sodium cations and water (1000 times bigger than their own mass). It is primarily the GAGs that are responsible for the degree of hydration of the dermis.

There are seven glycosaminoglycans in the human body: chondroitin sulfate (two different types, with a sulfur valence of 4 and 6), keratan sulfate, dermatan sulfate, heparan sulfate and heparin. One the other hand, **hyaluronic acid** deserves a special attention. It is composed of several thousand sugar subunits, other GAGs of several hundred. Glycosaminoglycans combine with proteins to form proteoglycans, while hyaluronic acid does not do so. However, it is a core to which numerous proteoglycan molecules can attach to form conglomerates: aggrecan, betaglycan, perlekan, agrin, syndecan, decorin, serglycine, and versican. These are structures with a molecular weight of up to 250 kDa (aggrecan) or 260 kDa (verse).

The spatial arrangement and the chemical composition of the sugar and protein parts determine the properties of individual glycosaminoglycans and proteoglycans.

Hyaluronic acid can selectively bind specific molecules, thanks to receptors for e.g. growth factors, which influences the metabolism and distribution of other substances as well as the migration of substances and cells.

Basic **non-collagen proteins** include:
- **Fibronectin** found, inter alia, in plasma (but also in other connective tissues). Thanks to the collagen IV binding sites, integrins, heparin enables the adhesion of cells and other proteins.
- **Laminin** is a component of the basal membrane, it enables cells to bind to the basal membrane, it also has binding sites for collagen IV, GAG, laminin, and fibronectin.
- **Tenascin** has binding sites for fibronectin, heparin, growth factors, and integrins. It is a protein characteristic of growing tissues.
- **Entactin**, or nidogen, is a glycoprotein specific for the basal membrane, where it connects laminin with type IV collagen. It has binding sites for fibronectin and perlecan.

2. Subcutaneous tissue

The subcutaneous tissue (Latin *hypodermis, tela subcutanea*) is composed of lobules of adipose tissue surrounded by connective tissue proper.

There are two types of adipose tissue: white and brown adipose tissue. **Brown adipose tissue** is characteristic of fetal development. Over time, after infancy, it gradually gives way to the developing yellow adipose tissue. The cells contain a lot of small drops of fat. It enables faster and more efficient access to backup materials. Moreover, the cells of this tissue contain an exceptionally large number of mitochondria, the cytochrome oxidase of which gives the cells a brownish colour. This tissue is highly vascularized and innervated. Its function is to generate the heat quickly.

White adipose tissue contains adipocytes with a thin layer of cytoplasm with organelles and a flattened cell nucleus surrounding a single large drop of triglycerides. This tissue is abundantly supplied with blood, and the cells are surrounded by a network of type III collagen fibers, secreted by adipocytes.

3. Dermis

The dermis (Latin *cutis vera*) is made of the connective tissue. There are two layers: papillary and reticular, however, there is no clear border between them. The basic elements that give the skin mechanical properties are GAGs, the collagen and elastic fibers.

The **papillary layer of the dermis** lies between the epidermis and the subpapillary vascular plexus. It is made of loose connective tissue, containing a lot of basic substance, relatively fewer collagen fibers (type I and III) and elastic fibers. Among the elastic fibers, oxytalan fibers, running perpendicular to the skin surface, prevail here. The outer border (from the side of the epidermis) of the papillary layer is wavy, the papillae are bent towards the epidermis. Apart from the connective tissue components, there are also blood vessels, lymph vessels, nerve fibers, receptors, and smooth arrector pili muscles.

The **reticular layer** is located between the superficial vascular plexus and the subcutaneous tissue. It is made of dense, irregular connective tissue containing a lot of collagen fibers (type I) intertwined with elastic ones. The elastic fibers in this layer are mainly elaunin fibers running parallel to the surface of the skin, deeper, thicker, mature elastic fibers prevail. These layers do not have clear boundaries, the transition between the layers is smooth.

The blood is supplied to the skin through the subcutaneous arteries, which form the arterial network of the dermis on the border of the subcutaneous tissue and the reticular layer. The second arterial network is located more outside, it is the sub-papillary arterial network. The artery goes to each dermal papillae. There are three venous networks in the skin (two analogous to arterial net-

works, the third in between them). The arterial networks intertwine with venous networks, forming plexuses: superficial (sub-papillary) and deep (in the dermis, on the border of subcutaneous tissue). The arteries arranged perpendicularly to the skin surface are accompanied by veins whose course is parallel over large sections, which is very important for thermoregulation.

Blood vessels only reach the skin – the epidermis is not vascularized. Its nutrition takes place only thanks to the diffusion from the dermis.

There are also numerous lymphatic vessels in the skin that drain lymph from the papillary layer to the lymph nodes. Due to the presence of numerous lymphocytes, skin is an important element of the immune system (**SALT**, *skin-associated lymphoid tissue*).

4. Epidermis

The epidermis (Latin *epidermis*) is the outermost layer of the body. It is formed by a stratified keratinizing squamous epithelium. The cells of this epithelium are constantly renewed: in the innermost layer, new keratinocytes are synthesized, and then, they are gradually pushed out by the newly formed cells. They undergo the process of keratinization, they die off (it is a kind of apoptosis), and they peel off the outer surface. The passage of cells through successive layers until exfoliation is called a ***turn-over***, it usually takes from 20 to 30 days depending on the thickness of the epidermis, and it gets longer with age.

4.1. Protein and structure characteristic of the epidermis

Keratins are proteins that are characteristic components of the epithelium, including the epidermis and its products (skin appendages). These are fibrillar proteins that build intermediate filaments. 54 genes encoding keratin have been described in the human genome, including 28 type I (acidic) and 26 type II (basic/neutral) genes located in sets of 27 on two chromosomes. Type II epidermal keratins are K1-K8 and K71-K80, and type II hair keratins: K81-K86.

Keratin filaments are made of polymerized systems of paired keratins type I and II.

Human keratins belong to the α keratins. B keratins are much harder, for example they build beaks and claws of birds, claws, reptile scales.

Desmosomes (*macula adherens*) are structures that bind adjacent epithelial cells. Adhesion occurs thanks to cadherins – desmoglein and desmokoline. On the inner surface of the cell membranes there are desmosomic attachment plaques 400 x 250 x 10 nm made of desmoplakin and placoglobin, with cytokeratin filaments attached to them. In the area covered by the plaque, the cell membranes of neighbouring cells are at a distance of 30 nm from each other farther apart. Transmembrane glycoproteins, desmokoline and desmoglein – Ca^{2+} – dependent cadherins protrude from the plate through the membrane. They come together to bind the cells.

The **basal membrane** (Latin *basalis membrane*) is produced by the epithelial tissue at the border with the connective tissue. It consists of collagen, proteoglycans, and glycoproteins such as laminins and entactin/nidogen. Laminins are glycoproteins with a mass of 140 kDa to 400 kDa, they are composed of subunits including 3 polypeptide chains with binding sites for integrin receptors located in the cell membranes of epithelial cells. By polymerising, laminins create large surfaces. Entactin (nidogen) is a glycoprotein with a mass smaller than laminins (approx. 150 kDa). It combines laminins with type IV collagen and requires Ca^{2+} cations to function. Proteoglycans, thanks to a significant negative charge, control the flow of ions through the basal membrane and bind the water. Moreover, they generate additional cross-links with laminins, collagen IV and entactin.

In the **basement membrane**, three layers can be distinguished (according to the classic description):

- ***lamina lucida*** made of laminins, nidogen, fibulin and BM40 protein – the glycoproteins allowing adhesion to epithelial cells, and of proteoglycans: agrin and perlate.
- ***lamina densa*** is a network of collagen IV (constituting 50% of the basement membrane proteins), building the scaffolding of the basement membrane with which the loops of type VII collagen anchor fibers are intertwined, which "attach" the dense plaque to the connective tissue, most often in the vicinity of hemidesmosomes .
- Collagen III ***lamina reticularis***, which is already part of the connective tissue, not the epithelium.

The basement membrane is firmly attached to the epidermis and connective tissue (the dermis). It is bound to the epidermis by hemidesmosomes with a structure similar to that of the desmosome, but the attachment plates are located only on one side – in the cells, they are not in the basement membrane. The second structure that connects cells with the basement membrane are focal attachments of actin filaments to the basement membrane, built, among others, by from integrins (transmembrane proteins), α-actinin, and vinulin. From the outside, integrins bind to glycoproteins – laminin and fibronectin.

The basal membrane is anchored to the connective tissue by three structures:

- type VII collagen fibers form loops "sewing" a dense plate to the collagen fibers of a mesh plate,
- fibrillin microfibrils with a diameter of 10-12 nm, connects a dense plate with elastic fibers,
- slight folding of the dense lamina creates a kind of protrusions, into the connective tissue, enabling direct connection with type III collagen.

4.2. The structure of the epidermis

Due to the orientation of metabolism to the synthesis of, inter alia, keratin, epidermal cells are referred to as **keratinocytes**, and after being saturated with keratin proteins, they are called corneocytes (Latin *squamulae corneae*). In addition to keratinocytes, the epidermis also includes melanocytes, Langerhans cells and Merkel cells.

On the cross-section of the epidermis, five layers of cells are distinguished, clearly differing in morphological features and the nature of metabolism (synthesized substances, the content of synthesis products).

4.2.1. Basal layer

The **basal layer** (Latin *stratum basale*) is the innermost layer of the epidermis, tied with the basal membrane. The cells are connected to each other, as well as to the cells of the spinous layer by desmosomes, and to the basement membrane by hemidesmosomes and focal attachments. This layer is made up of one row of cylindrical keratinocytes. These cells have one cell nucleus, very large and oval, arranged perpendicular to the basement membrane. Most of the cells in this layer are able to divide themselves but only 10% of them are stem cells. The divisions occur in such a way that one of the daughter cells still adheres to the basal membrane and remains in the basal layer, and the other is separated from the basal membrane and belongs to the second layer – the spinous layer. In addition, the described process explains a very important phenomenon for the epidermis: new cells arising in the divisions of the basal layer constantly push older cells outwards (further away from the basement membrane).

4.2.2. Spinous layer

The **spinous layer** (Latin *stratum spinosum*) is several cell layers thick, ranging in shape from polygons of all dimensions to equal to more and more flattened. The cells of this layer are tightly bound to each other by the desmosomes. The connection of desmosomes persists even after cell death. The synthesis of keratins, initiated in the basal layer, is still taking place, and they gradually merge into intermediate – keratin filaments, referred to as tonofilaments. The synthesis of horny plate proteins, mainly involucrin, also begins.

4.2.3. Granular layer and water barrier of the epidermis

The **granular layer** (Latin *stratum granulosum*) consists of several layers of cells with a spindle-shaped (cross-sectional) and flattened cell nuclei. The cells are live and they are metabolically active, but some cell organelles, including the nucleus, begin to decline. In the cytoplasm there are keratohialin grains containing a mixture of keratin proteins rich in cystine and histidine – the precursors of filaggrin (profilaggrin weighing over 400 kDa) and trichohialin. Filaggrin and trichohialine initiate the aggregation of keratin filaments into bunches – tonofibrils, parallel to the cell axis. The binding of individual components of the resulting protein envelope takes place through cross-links, formed with the participation of epidermal transglutaminase. This process which takes 2-6 hours, is called **keratinization**. The cell then loses the nucleus and other organelles, it interrupts metabolism and passes to the stratum corneum. The second characteristic structure are lamellar bodies composed of glycosphin-golipids, sterols, ceramides and phospholipids. These substances are secreted into the intercellular space in the last layers of the granular layer and the first stratum corneum in the form of lipid plaques (*intercellular lamellae*). Moreover, they prevent water from penetrating the epidermis.

The water barrier of the epidermis consists of a cell envelope and a lipid envelope.

The cellular envelope, approx. 15 nm thick, consists of insoluble proteins of the inner membrane surface: SPR (small proline rich) proteins, cystatin, desmoplakins, elafin, envoplakin, filaggrin, involucrin, 5 different keratins, and lorikrin which constitutes 80% of the cell envelope.

The lipid envelope is located extracellularly, it is 5 nm thick. It is made of sphingolipids, cholesterol, free fatty acids and acylglucosylceramide.

4.2.4. Translucent layer

The translucent layer (Latin *stratum lucidum*) is visible only in the thick epidermis, on the soles of the feet and on the inner surfaces of the hands. It consists of several layers of cells in which organelles and the cell nucleus continue to disappear, although desmosomes are still preserved. In this layer, the cell boundaries observed in the optical microscope meet.

4.2.5. The stratum corneum

The stratum corneum (Latin *stratum corneum*) consists of flat, tightly arranged cells devoid of organelles, filled with keratin filaments. They are defined as corneocytes (Latin *Squamulae corneae*) with a core of keratin filaments and a keratinized sheath with, among others, involucrin, loricrin, SPR proteins. The cells lying in the more inner layers retain desmosomes, while the outer part peels off gradually.

The thickness of the epidermis varies from 0.1 to 2 mm depending on the location. The thickest skin is on the soles of the feet (heel) and on the palms. In these areas, there is a distinct light layer, and the callous layer is definitely the thickest layer of cells, it contains several dozen of cell layers.

In total, in the five mentioned layers there are from several to several dozen layers of cells (usually up to 25), which within 20-30 days (most often 26-28) shift from the basal layer to the keratinized layer.

4.2.6. Natural moisturizing factor of the stratum corneum

Filaggrin that binds keratin fibers after about 2-3 days from synthesis is gradually degraded to single amino acids, e.g. histidine, arginine, glutamate. Then, histidine is metabolized to urocaine, arginine to citrulline, and pyroglutamic acid is formed from glutamic acid. In addition, the breakdown products are lactic acid and urea. This mixture is joined by other amino acids, lactic acid, Na^+, K^+, Cl^- ions, e.g. from sweat. This mixture has the properties of a humectant, it binds water in the outer layers of the epidermis. In cosmetology, the described mixture of filaggrin decay products is treated as a separate substance and it is called **NMF** – ***natural moisturizing factor***.

4.2.7. Characteristic cells of the epidermis

In addition to keratinocytes, there are three types of cells in the epidermis:

- **Melanocytes**, pigment cells from the nervous system. They make up about 10% of the cells of the basal layer of the epidermis. Their function is the synthesis of melanin – the pigment of the skin, hair and iris. The cells themselves are located in the basal layer, but the synthesized dye reaches the granular layer through the branched outgrowths of melanocytes.
- **Merkel cells** are the modified epithelial cells and they are located in the basal layer, connected to other cells by desmosomes. In conjunction with the sensory ending of the neuron, they constitute sensory receptors – Merkel sensory bodies. Most of them can be found in the places which are particularly sensitive to touch, e.g. on the tips of the fingers. Their less important function is the synthesis of local active substances (vasoactive intestinal peptide VIP, enkephalin, pancreostatin).
- **Langerhans cells** make up 3-8% of the spinous layer cells. They are stellate (dendritic) cells that do not form desmosomal connections with other cells. The characteristic organelles of these cells are the so-called Birbeck granules, small vesicles stalk-tapered on one side. Histocompatibility antigens and receptors for Fc fragments of immunoglobulins and the complement component C3 are located on the surface of the membrane. Their presence is related to the basic function of Langerhans cells, the presentation of antigens. This is part of a delayed-type hypersensitivity reaction.

4.5. Skin pigment

The color of the human body results from the color of the blood that shines through the epithelium covering the body. So it is red – dark pink where the epithelium is thin and transparent (lips, oral cavity, parts of the reproductive organs), and light pink on the rest of the surface, where red light reflected from hemoglobin is partially absorbed by keratin and mixed with white diffused light on the cells containing e.g. numerous keratohyalin grains and lamellar bodies. However, thanks to the skin's pigment – melanin – the skin can be darker, or it can darken under the influence of UV rays.

Melanin is a derivative of the amino acid tyrosine. The enzyme tyrosinase plays a special role in the synthesis. The synthesized melanin goes from the Golgi apparatus to the melanosomes. They go through four phases of formation and differentiation, from round to oval premelanosomes with much more concentrated contents. They are transported to the ends of the melanocyte processes, reaching the granular layer, and secreted into the intercellular space, they proceed to the target cells through endocytosis. Melanin is then placed over the cell nuclei to shield them from UV radiation. One melanocyte thus supplies about 36 keratinocytes.

There are two forms of melanin – black or brown **eumelanin** (there are two types: black and dark brown) and orange-pink **phaeomelanin**. Depending on the genome, the ability to synthesize these melanins varies from person to person and it determines the color of the body and hair. The amount of the dye also depends on hormones, nutrition, and metabolic characteristics. Depending on the amount of pigment in the skin, the resistance to UV radiation varies. Negroid people have the same number of melanocytes as Caucasians (2000/mm^2 on the face and forearms, twice less than on the rest of the body). However, their melanosomes are larger and more evenly distributed throughout the epidermis. Only on the surface of the hands and feet they are grouped, as in Caucasians, over the entire surface of the body.

Based on the differences in response to exposure to UV radiation, skin phototypes are distinguished (proposed by Thomas B. Fitzpatrick).

4.6. Skin appendages

Skin appendages are additional structures that have supporting functions. They are produced by the epidermis and are continuous with it. The tissue that builds the sebaceous glands, hair, nails – is an anatomical extension of the epidermis, it is its deep entanglement. The hair root matrix, the nail matrix, is a layer containing reproductive cells which is an extension of the reproductive (basal) layer of the epidermis. Skin appendages are hair, nails, sebaceous, sweat and milk glands, as well as horns, hooves, scales, claws and feathers. Only selected structures will be discussed here.

4.6.1. Hair

Hair (Latin *pilus*) is composed of keratinized cells similar to the stratum corneum, although there is hard keratin, with non-soft keratin proteins, and significant amounts of **trichohialin**. Among the hair there are long (covering the head, including the chin and mustache, armpits, genital area), short, thick (eyebrows, eyelashes), vellus hair covering most of the body surface, and fetal hair – lanugo.

The nature of the hair depends on the structure of the root matrix, but also on the sex hormones for which the receptors are located in the hair follicles. Therefore, e.g. a hair follicle can transform into terminal hair depending on the level of androgens. Humans have got a similar amount of hair as other mammals. Seemingly, its amount decreases because the vellus hair is almost imperceptible.

The root of the hair lies within the hair follicle. It is a deep, tubular indentation of the epidermis that reaches the subcutaneous tissue and it expands to form the hair bulb. The hair papilla with blood vessels and nerves penetrates the hair bulb from the side of the connective tissue. The hair follicle contains cells of the hair matrix, which divide to form the hair proper and the inner hair sheath.

The proper hair consists of: the core, the cortex and the unicellular thickness of the hair coat, made of cells arranged in tiles. The hair is outside surrounded by a three-layer inner sheath. Its successive layers are: cuticle of inner root sheath, Huxley's layer and Henle's layer. The sheath only reaches the sebaceous glands. The outer sheath which is a direct extension of the convex epidermis is placed on the outside. It is built at the root of the basal and spinous layers, and from the side of the skin surface it is formed by the entire convex epidermis.

From the outside, the hair follicle with the sebaceous gland is surrounded by a connective-tissue hair capsule, separated from the epithelial cells of the sheath by a hyaline membrane. The hair is accompanied by the arrector pili muscle, anchored between the hair follicle and the papillary layer of the dermis.

A **hair bulb** is a thickening of the hair follicle with stem cells. It lies between the mouth of the sebaceous gland and the bulb. It enables hair bulb reconstruction after its damage or hair loss.

Hair follows a cycle in which it grows continuously at a rate of 1/3 mm a day – this is the anagen phase. After a few months, growth is stunted, then it begins to start again or the hair falls out, and a new hair growing from the same bulb pushes out the old one. About 1% of hair is in this stage – a catagen phase. Telogen phase is the stage of hair loss and matrix rest. The hair bulge remains intact, so the root can then regrow and start new hair growth.

4.6.2. The nails

The nail (Latin *unguis*), like hair, is formed by divisions of epithelial cells that make up the nail matrix. The matrix is continuous with the epidermis, although there is no granular layer. As in the case of hair, the cells become keratinized. However, they do not exfoliate, so the nail plate continues to grow.

A skin fold covers the proximal ends of the nail plate – the nail skin. Its part covering the matrix is called eponychium. It protects the nail matrix against infections. The lighter part of the nail – on the matrix side – is the lunula. In the transitional phase of keratinization, the gradually deposited substances are not transparent, hence the whitish shade, only after the structure of secreted proteins and lipids has been fully built up, the cell layers gain transparency. Thanks to that the old nail plate is transparent and becomes pink (the color of the deeper vessels blood).

The plate lies on the placenta, made of fibrous connective tissue, covered with epidermis. The function of the placenta is to nourish the nail, of course only right next to the matrix, where the plaque cells are still alive. The distal edge of the placenta ends with a layer of keratinized epidermis – a hyponychium.

The nail grows at a rate of 3 mm per month.

4.6.3. Sweat glands

Sweat glands (Latin *glandulae sudoriferae*) are divided into two types.

Eccrine sweat glands are straight tubular glands, they form a ball on the border of the dermis and subcutaneous tissue. The secretory part consists of cells of a single layer cuboidal or columnar epithelium. The excretory duct forms a two layer cuboidal epithelium that ends when it reaches the epidermis. The rest of the sweat way is the opening between the horny cells in the stratum corneum. **Clear cells** of the secretory part secrete especially water and electrolytes (they secrete Na^+ in an active way). **Dark cells** synthesize and secrete proteoglycans. Between the basement membrane and the secretory cells there are myoepithelial cells, responsible for contracting to facilitate the secretion mechanism.

Sweat is made of water, sodium chloride, urea, uric acid, ammonia, vitamin C and a small amount of proteoglycans. Secretion is of **merocrine** nature in these glands (secretion leaves the cells by exocytosis without disturbing the cell).

Eccrine glands are present on the entire surface of the skin, except for the lips, penile glans, labia minora and clitoris, but the most of them are on the surface of hands and feet.

Man is one of the very few species of mammals that have developed eccrine sweat glands and whose thermoregulation is based on such glands.

The apocrine sweat glands are larger than the eccrine ones, they are branched and follicular. The excretory duct is made of a single layer cuboidal epithelium and opens into the hair follicle. The name of the gland is misleading: it was once assumed that there was a type of apocrine secretion

(i.e., synthesized substances accumulate at the apical pole of the cell, which bulges out and is cut off from it). However, it turned out that a merocrine release, such as in the eccrine glands, is taking place. The secreted substance is thick, it contains a large amount of lipids and proteins, some carbohydrates, ammonia. It is odorless, but when broken down by skin bacteria, it produces a smell that has very important social functions: it is characteristic of the breed and individual. These glands are found mainly around secondary sexual characteristics (perineum, genitals, anus, armpits, external auditory canal). Secretion is not related to thermoregulation; it is regulated based on emotional stimuli.

4.6.4. Sebaceous glands

The **sebaceous gland** (Latin *glandula sebacea*) is formed from a deep indentation of the outer sheath of hair. Considering that the outer sheath itself is an inversion of the epidermis, the sebaceous gland also maintains its continuity with it. The sebaceous glands always go to the hair follicle except around the anus, foreskin and nipple.

The sebaceous gland forms branched vesicles. The cells synthesize the components of sebum, fill them up, increase their volume by 150 times. This is accompanied by the disappearance of organelles – apoptosis, the entire cell turns into a secretion (example of holocrine secretion). This process takes about a week. The dead secretory cells are replaced with new ones, resulting from dividing stem cells.

The excretory duct of the sebaceous gland is made of a stratified squamous epithelium (an extension of the outer hair sheath). The release of larger amounts of sebum supports the contraction of the arrector pili muscles. The arrector pili muscle simultaneously moves the hair away from the opening of excretory duct, opening it.

Depending on their location, the sebaceous glands range from 100 per 1 cm^2 on the most of the body surface to as much as 900 per 1 cm^2 on the face. The soles of the feet and the inner surface of the hands contain the least amount of them.

Sebum is composed of lipids (triglycerides, diglycerides, free fatty acids) – 57% in total, 26% of waxes (also classified as lipids), 12% of squalene (an unsaturated hydrocarbon also classified as lipids) and 2% of cholesterol. The sebum and sweat form a thin acidic film on the skin surface (pH 5.5).

There are specialized sebaceous glands in the given locations:
- Meibomian glands (tarsal glands) on the eyelids, their secretion seals the closure of the eyelids and prevents tears from flowing,
- Montgomery glands on the nipples. There are between 4 and 28 tubercles per nipple. Their secretion protects the nipples, they have a structure resembling mammary and sweat glands,
- Tyson glands on the genitals,
- Fordyce spots in the vicinity of the mouth.

Histology

Sample control questions:

1. Keratin proteins – structure and functions.
2. Hair structure and functions.
3. Glycosaminoglycans – structure and properties.
4. Structure and functions of a nail.
5. The keratinization processes.
6. Skin pigment.
7. Skin receptors.
8. Dermal-epidermal junctions.
9. Structure and functions of subcutaneous tissue.
10. The concept of NMF.
11. Structure and functions of sweat glands.
12. Structure of connective tissue proper.
13. Structure and functions of the granular layer of stratified squamous epithelium.
14. UV influence on the epidermis.
15. Structure and functions of the basal layer of stratified squamous epithelium.
16. Collagen synthesis.
17. The structure of the dermis.
18. Basal membrane – structure and functions.
19. Structure and functions of sebaceous glands.
20. Epidermal barrier.
21. Vascularization of the skin.
22. Elastic fibers.
23. Structure and functions of basal layer cells of the epidermis other than keratinocytes.

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