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REVIEW

Short histological kaleidoscope – recent findings in histology. Part I

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Abstract

This article is a review of new advances in histology, concerning either classification or structure of different tissular elements (basement membrane, hemidesmosomes, urothelium, glandular epithelia, adipose tissue, astrocytes), and various organs' constituents (blood–brain barrier, human dental cementum, tubarial salivary glands, hepatic stellate cells, pineal gland, fibroblasts of renal interstitium, Leydig testicular cells, ovarian hilar cells), as well as novel biotechnological techniques (tissue engineering in angiogenesis), recently introduced.

***Keywords*:** histological structure, advances, recent findings, angiogenesis, tissue engineering.

Introduction

Since its founding in the 18th century, histology has retained a remarkable place in medicine. Although an ancient science, its dynamic character is preserved through its close connection with multiple important disciplines (molecular biology, genetics, physiology, pathological anatomy) and through diversified and modern study techniques (new microscopic technologies and molecular diagnostic methods), which allow the accumulation of new scientific data and the permanent modification of classical known notions' pattern.

This article is a review of such changes in concept, recently introduced in histology, concerning the structure or the classification of both different tissues' parts and organs constituents or innovatory biotechniques.

New aspects regarding epithelia

The basement membrane and hemidesmosomes – recent findings and pathological implications

Until recently, in traditionally processed tissues for transmission electron microscopy (TEM) (including fixation in glutaraldehyde or formaldehyde) [1] it was considered that the basement membrane (BM) consists of two overlapping well-defined layers: the *lamina lucida*, a homogeneous structure, clear in electrons flow, and the *lamina densa* [2], with anchoring filaments, that are extending from the hemidesmosomes (HDs) through the *lamina lucida* [1]. The preparation techniques by high-pressure freezing (HPF) method and subsequent freeze substitution (FS) can preserve a greater tissue integrity and therefore, it has been found that *lamina lucida* is actually absent and represents only a fixation artifact. Its visibility depends by the organic solvent's extraction of its components in embedding techniques [3].

In the skin, cornea, parts of the respiratory and gastrointestinal (GI) tracts, bladder and amnion, HDs facilitate the anchorage of cytoskeletal intermediate keratin filaments to the extracellular matrix (ECM) components [4]. Hieda *et al.* have described two variants of HDs, according to their ultrastructural appearance and protein components: type I and type II [5]. Type I HDs are found in the stratified and pseudostratified epithelia, such as epidermis, cornea, and oral mucosa; it consists of five major components, belonging to four protein families: integrins ($\alpha6\beta4$), plakins (plectin and the bullous pemphigoid antigen (BPAG) 1 isoform e – BPAG1e, also called BP230), collagens (collagen XVII, also called BPAG2 or BP180) and tetraspanins (cluster of differentiation (CD)151) [5]. Type II HDs are found in simple epithelia, such as intestinal epithelium, endothelium, or mammary gland epithelium, and lack the two BP antigens (BP230 and BP180), thus being formed only by $\alpha6\beta4$ (integrins), plectin, and CD151 (tetraspanins) [5].

HDs integrity is of crucial importance in a series of inherited or acquired diseases; alteration in structure or missing of essential components of HDs result in blistering and skin fragility, collectively known as *epidermolysis bullosa* (EB). Different mutations in 16 specific genes, that encode fundamental elements of HDs, lead to various types of EB. The four major classical types of EB, depending on the level where tissue separation occurs in the skin, are

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EB *simplex* (tissue separation within the basal keratinocytes of epidermis), junctional EB (slit between epidermis and dermis in the *lamina lucida* of the BM), dystrophic EB (underneath the *lamina densa*, involving the anchoring fibrils of the papillary dermis) and Kindler EB (mixed skin cleavage pattern) [6, 7]. Due to new genes and various clinical subtypes of EB reported over the past few years, in the latest Consensus reclassification of inherited EB report from 2020, the notion of "genetic disorders with skin fragility" was introduced. Typical EB disease remains the archetype of this concept, and other conditions with skin fragility (such as erosive diseases, hyperkeratotic diseases, peeling skin syndromes and connective tissue syndromes associating to skin fragility) are now indexed as independent groups [8]. The diagnostic hallmark of this heterogeneous group of diseases is marked by skin fragility, blistering and erosions of the skin. In different forms of EB, in association with skin damage, there can be tooth, hair and nails involvement, ocular abnormalities, and GI, upper respiratory and urogenital tracts fragility of the epithelia [9].

Since the identification of the first constituent of HDs and the first ultrastructural description as small electron-dense domains in the cytoplasmic membrane, much information was over the past 2–3 decades reported, regarding this tightly-ordered and perfectly designed complex of multiproteins. At ultrastructural level, in intact human skin, HDs display a particular appearance having a tripartite structure, with a centrally located less-dense zone, associated with two electron-dense plates: an inner plate, towards the cytoplasm, and an outer plate, slightly longer and parallel to the inner plate, lying on the plasma membrane of the basal keratinocytes. Basal keratinocytes are disposed on the basal lamina. Extracellularly, below the basal side of these keratinocytes a dense plate was observed, having an electron-thin but dense line appearance. The stability of the HDs–ECM complex is maintained by the thin anchoring filaments (that run from the HDs dense plate, pass through the plasma membrane, and insert into the *lamina densa* of the basal lamina) and by the anchoring fibrils (type VII collagen molecules arranged into semi-circular loop structures that encircle dermal collagen fibers and other components, providing sustained connection of basal lamina to underlying structures) [4].

Molecular architecture of HDs organization using super-resolution microscopy in cultured keratinocytes revealed the presence of integrin $\alpha6\beta4$, at the core of each HDs. Integrin $\alpha6\beta4$, a laminin 332 receptor, is a heterodimer with $\alpha6$ and $\beta4$ transmembrane subunits. The $\alpha6$ subunit consists of a short intracellular domain, followed by a transmembrane domain and a long *N*-terminal extracellular domain. This extracellular domain of integrin $\alpha6$ exhibits binding sites for BP180 and CD151 and prefers laminin 332 (formerly known as laminin 5, one of the two principal components of anchoring filaments, next to the type VII collagen). The intracellular interactions of this receptor, with plectin 1a (P1a) and BP230 and with the BP180 transmembrane protein, are mediated by the cytoplasmic domain of $\beta4$. Integrin $\beta4$, rather than running parallel to the plasma membrane, was reported as being disposed along the keratin filaments [10]. The $\beta4$ subunit contains a third and a fourth fibronectin type III domains; they bind to the BP230, but the fibronectin type III third domain also interacts with the cytoplasmic domain of BP180 [11]. BP230 and P1a facilitate the connection of the cytoplasmic intermediate filaments, assembled from K5 and K14 of the basal keratinocytes, to the HDs inner plaque. Also, they link to the transmembrane protein complexes of the HDs outer plaque, namely $\alpha6\beta4$, BP180 and CD151. Spatial distribution of BP230 and BP180 revealed that BP230 mediates the binding process to keratin, through the close association of BP230 C-terminus domain with keratin 14, while the extracellular domain of BP180 was located farther from the keratin filaments [10]. Mutations in either $\alpha6$ or $\beta4$ subunits lead to junctional EB, associated with pyloric or duodenal atresia, and variable involvement of the skin (from mild fragility to death due to severe complications, such as electrolyte imbalance, disseminated infections and urinary tract involvement) [12]. In the epidermis, laminin 332 is secreted by the basal keratinocytes, and deposited into the ECM; it is a heterotrimer assembled from three polypeptide chains ($\alpha3$, $\beta3$, $\gamma2$) into a cross shape. Laminin 332 functions as a catwalk unit linking the basal keratinocytes to the subsidiary reticular dermis. The globular domain of the $\alpha3$ subunit of laminin 332 binds to integrin $\alpha6\beta4$, while $\beta3$ subunit connects to collagen VII within the structure of the anchoring fibrils, thus ensuring a HD–BM stable contact [13]. Integrin $\alpha6\beta4$ binds to laminin 332, making the fundamental initial step in HDs assembly; this idea is supported by the fact that patients having junctional EB with pyloric atresia, or generalized junctional EB, display mutations in the genes for $\alpha6\beta4$ or laminin 332, leading to rudimentary HDs [14]. In the next stage in HDs design construction, after integrin $\alpha6\beta4$ binds to laminin 332, follows the coupling between $\beta4$ subunit and P1a, thus inducing the formation of HDs; the plectin–BP180 interplay, that escalates the bondage between the elements of the complex integrin $\alpha6\beta4$–plectin–BP180 and serves as a scaffold for BP230 embodiment [14].

Tissue morphogenesis, wound healing and cancer metastasis are a variety of physiological and pathological processes in which HDs assembly and disassembly, and they are modulated by structure–function relationship between hemidesmosomal proteins and ECM. In biological settings (such as terminal differentiation), the basal keratinocytes disassemble their HDs, thus inducing their detachment from the BM and displacing themselves into the suprabasal layer of the epidermis. Recently published research on migrating epidermal keratinocytes displayed highly ordered ranges of HDs chevrons, intercalated with focal adhesions (FAs) of actin filaments (FAs represent another type of ECM contact sites beside HDs). The migration follows a regional pattern, that includes assembly activity in the front of the cell, and dissolution in the rear part of the cell, thus supporting controlled translocation of the cell body [15]. For sustaining the directed migration of keratinocytes, nascent FAs appear the first, in pairs, having an oblique angle to the direction of migration, then followed by the accumulation of integrin $\alpha6\beta4$ in the spaces between FAs. Patterned HDs–FAs chevrons remain stationary, without positional changes, with subsequent translocation of keratinocytes towards cell's front, while at cell's rear, old FAs are firstly removed, followed by $\beta4$ accumulation with extracellularly patches formation [16].

Migration, proliferation, and differentiation of keratinocytes are fundamental stages of epidermal component in wound healing process. Losing both cell–cell adhesion and cell–substratum contacts, as well as cytoskeletal remodeling of intact keratinocytes from unwounded epidermis, facilitates their migration to the provisional matrix of the wound. While moving over the surface of the wound they deposit new matrix proteins, including laminin 332, and remodel the provisional matrix that covers the wound bed. After complete epithelization, keratinocytes participate in the reconstruction process of the BM, then finally, they differentiate and stratify to restore skin barrier function [17]. In an *in vitro* wound healing setting, assessing the interplay between adhesion complexes of keratinocytes migration process, the disassembly and reorganization of HDs and FAs generated intrinsic cellular tension and traction forces, with an important role of HDs in counterbalancing the FAs and actomyosin contractility [18].

Surface epithelia: urothelium's membranous plaques

Normal urothelium is characterized by the presence of membranous plaques at the surface of the superficial "umbrella" cells, that marks the terminal differentiation of these cells [19]. From functional point of view, those membranous plaques are considered either a protection mechanism of the epithelium against the urine components, or a necessary reserve. From structural point of view, they are found in two aspects: (*i*) rigid and dense areas (12 nm) of the plasma membrane, made up of specific proteins, the uroplakins, and (*ii*) subsurface fusiform vesicles [20], transporting and delivering the urothelial plaques to the apical plasma membrane, in case of urothelium distension and increasing size [21].

The urothelial plaques are glycosylated inside Golgi apparatus, and then post-Golgi progressively formed, passing through three maturation steps of vesicles (uroplakin-positive, immature fusiform and, finally, mature fusiform vesicles) [22]. Several proteins are playing a role in the vesicular transport of the uroplakins to the lysosomal compartment (*e.g.*, Vps33a) [23], or the insertion of the uroplakins into the apical surface (*e.g.*, myelin-and-lymphocyte protein – MAL) [24]. The uroplakin plaques are associated with the multivesicular bodies (MVBs) and their endocytic degradation in the lysosomes is controlled by the sorting nexin (SNX)31. SNX31 is highly specific for mature umbrella cells of the urothelium and enables the membranes that contain uroplakin to form the intraluminal vesicles [25]. The MVBs are suppressing the excessive/wasteful endocytic degradation of uroplakins [26].

Glandular epithelia: new classification

Regarding the morphological division of exocrine glandular epithelium, it is considered that the compound exocrine glands can also be classified according to the number of the lobules they form; thus, there are: (*i*) unilobulated glands, which contain a single lobule and their intralobular excretory ducts converge into a single terminal duct, opening at the surface (*e.g.*, minor salivary glands) [27]; (*ii*) multilobulated glands, which contain several lobules and intralobular excretory ducts; the lobules are separated by conjunctive septa, containing interlobular excretory ducts, converging into a single terminal duct, opening at the surface (*e.g.*, major salivary glands) [28]; (*iii*) "battalion" glands, multilobulated glands, in which each lobule has a terminal and independent excretory duct (*e.g.*, lacrimal [29], mammary [30] and prostate [31] glands). The epithelia of salivary, mammary, and prostate glands share therefore many similarities, as they are composed of epithelial acinar and ductal cells, myoepithelial cells [32] and neuroendocrine cells [31, 33, 34]. Also, in these epithelial tissues, the myoepithelial cells are the key cellular participants in morphogenesis, maintenance, and repair [35], with similar mechanisms of self-renewing and differentiation (*e.g.*, overexpression of stromal-derived factor-1alpha – SDF-1α or CXCL12α) [32].

New aspects regarding the connective tissues: the adipose tissue

The novel classification of the adipose tissue comprises two new types: the beige and the pink variants. Each type of adipose tissues (white, brown, beige, and pink) presents specific morphological and functional characteristics (inflammatory modulation and endocrine function) of their adipocytes and immune cells [36].

The beige adipocytes are named also bright or brown-in-white adipocytes. They are located inside white adipose tissue, and their cellular structure is similar to that of the brown adipocytes [37]. During basal conditions, beige adipocytes function like white adipocytes. Still, a specific stimulus (*e.g.*, prolonged exposure to cold or caloric restriction) could turn the beige adipocytes into brown-like ones, a process named "browning" [38–40]. In stimulation conditions, a subset of white adipose cells can acquire a thermogenic phenotype without sharing its genetic markers [41]. The genetic lineage, several molecular markers (*e.g.*, uncoupling protein-1 (UCP1), early B-cell factor-2 (EBF2), transcription factor PR-domain containing 16 (PRDM16), transmembrane protein 26 (TMEM26), CD137, and T-box 1 (TBX1)), the maintenance and the thermogenic mechanisms can identify beige adipocytes among white adipocytes [42–47].

The beige adipocytes have a different pattern of gene expression than white and brown adipocytes [43] and a different cellular origin from the classical brown adipocytes [48]. A new subtype of beige adipocytes also emerged, the *g*-beige adipocytes, with a distinct development and thermogenic program from that of conventional beige adipocytes [47]. Recent studies have shown that brown adipose tissue can evolve from the beige adipose tissue, that has acquired a brown-like phenotype [49, 50]; this conversion has beneficial consequences, regarding metabolic changes (treating metabolic syndrome [46], obesity [51–53], type 2 diabetes [38, 50, 54], atherosclerosis, arterial hypertension) [48, 55], ageing [56] and cancer micro-environment [57, 58]. Both brown and beige adipose tissue regulate the systemic metabolism and there is a continuous physiological crosstalk between the beige adipose tissue – the brown adipose tissue – and the muscle [50].

The pink adipocytes were more recently described during pregnancy and lactation, due to the pink color of breast adipose tissue [59, 60]. The pink adipocytes are characterized by microvilli at their apical surface, round and large

nucleus, abundant cytoplasmic lipid droplets and milk-containing granules; like all fat cells, they have a well-developed Golgi apparatus and endoplasmic reticulum (ER), but in a more significant percentage than in other adipocyte types [53].

During pregnancy and lactation, adipocytes have an indispensable role of in mammary gland's epithelium expansion, and they are strongly remodeling by dedifferentiation to preadipocytes [61, 62]; in this process, the hormones released during late pregnancy and lactation are considered to be the key regulators [58]. Also, there is a homeostasis transfer of lipids between the adipocytes and the epithelial alveolar cells [63]. Therefore, some authors sustain that pink adipocyte result from the transdifferentiation of subcutaneous white adipocytes, in a reversible process, called "pinking", to produce and secrete milk [36, 59, 64]. Arguments for this idea would be the pink adipocytes' secretion of milk, leptin [64–66], the 3-phosphoinositide-dependent protein kinase-1 (PDPK1) peroxisome proliferator-activated receptor gamma 1 (PPARγ1) expression [67], or the E74-like erythroblast transformation specific (ETS) transcription factor 5 (ELF5) expression, a transcription factor regulator of alveologenesis [68].

There is also some evidence supporting the hypothesis of pink-brown transdifferentiation and reversible conversion of brown adipose cells to myoepithelial cells [36, 59, 69]. The differences between mice genetic models used in earlier and more recent studies, for tracing the adipocyte lineage, can be the cause of the still present disagreements on the possibility of adipocyte–epithelial cell transdifferentiation [62, 69].

New classification of astrocytes

Macroglial cells, also called astrocytes, are specialized for the structural and functional support of neurons, being present in all regions of the central nervous system (CNS); inhere they perform two, essential, but different, functions: defense, by forming the blood–brain barrier (BBB), and maintenance of the homeostasis, by regulating cerebral blood flow, neuronal metabolism, and neurotransmitter' secretion [70].

Depending on the morphological pattern, at the beginning of the 19th century, two major types of astrocytes were described by Golgi staining: protoplasmic astrocytes (located in the gray matter) and fibrillar astrocytes (located in the white matter) [71]. This classification is currently considered to be outdated, as new particular types of astrocytes or astrocyte subtypes have been identified: *e.g.*, Bergmann and Fañanas cells in the cerebellum, Müller glial cells in the retina, pituitary cells in the neurohypophysis, interstitial cells (ICs) in the epiphysis, tanycytes in the subependymal glia, cribrocytes in the optic disc. In addition, two new subtypes have been described for primates and humans: interlaminar astrocytes and astrocytes with varicose extensions [72, 73]. Given that protoplasmic and fibrillar astrocytes have been extensively researched and documented, we will further describe only the newest identified types of astrocytes.

Veiled astrocytes

Veiled astrocytes are a particular type of densely arranged protoplasmic astrocytes (*e.g.*, in the olfactory bulb or the granular layer of the cerebellar cortex). These astrocytes consist of a small cell body and short, leaf-like extensions, with a substantial surface/volume ratio (20–30 $\mu m^2/L$). At the level of the cerebellum, the extensions of the veiled astrocytes surround several granular neurons, in the form of a velum (hence the name of veiled astrocytes). At the same time, the extensions of these astrocytes surround the glomeruli formed by the rosettes of the mossy fibers, the terminal buds of the axons of the Golgi neurons and the dendrites of the granular cells. The particular arrangement of veiled astrocyte extensions allows them to isolate synaptic structures and separate groups of mossy fibers that conduct different types of information at the cerebellar level [74–76].

Gömöri-positive astrocytes

Gömöri-positive astrocytes are also a specific subtype of protoplasmic astrocytes. Their location is in the arched nucleus of the hypothalamus and in the hippocampus. The cytoplasm has numerous inclusions or granules, which due to the high content in iron (Fe) are intensely highlighted in the Gömöri Chrome Alum Hematoxylin staining. The granules, formed by remnants of degenerated mitochondria and ingested by lysosomes, occur because of oxidative stress processes. These astrocytes play an important role in providing specific metabolic needs for hypothalamic neurons [77, 78].

Bergmann astrocytes

Bergmann astrocytes are located in the middle layer of the cerebellar cortex, also called the Purkinje cell layer. These astrocytes have 3–6 extensions, which extend and cross the molecular layer to the pial membrane. These extensions are strongly branched, with a large area relative to volume ($\approx$20 μm^{-1}) and cover up to 6000–8000 synapses formed by the terminations of granular neurons [79]. There are two subtypes: bifurcated cells and "broom" cells. Having a very small cell body ($\approx$15 μm in diameter), Bergmann astrocytes are difficult to observe in standard sections stained with Hematoxylin–Eosin (HE). Multiple cell extensions serve as a guide in the migration process of granular neurons, from the outer layer of the cerebellar cortex to the inner layer, being a concrete proof of the neuro-astrocyte evolutionary link; the small neurons in the outer granular layer descend along the Bergmann processes to reach their final destination, the inner granular layer. They are hypoxia-resistant cells and can change their mitotic activity (they can double or even triple their number, in cerebellar ischemic disorders, that destroy Purkinje cells located in the same layer). Secondary, reactive gliosis in an area of total infarction allows a retrospective assessment of the degree of hypoxic injury [80].

Fañanas astrocytes

Fañanas astrocytes are located in the molecular layer of the cerebellum. They are small cells with numerous, short, varicose extensions, with a defining "wedge" appearance; the expansions have a parallel path with the extensions of Bergmann cells, without contributing to the formation of the *glia limitans* [81].

Two particular types of astrocytes, characteristic of the human species, were recently described. Studies on nerve tissue, taken from the human adult temporal lobe,

identified four morphological subclasses glial fibrillary acidic protein-positive (GFAP+): densely packed interlaminar astrocytes (in layer 1), protoplasmic astrocytes (in layers 2–4), astrocytes with varicose projections (in layers 5–6), all located in the cerebral cortex, and fibrillar astrocytes located in the cerebral white matter. Similar examinations on nerve tissue, taken from the brain of chimpanzees, identified the same four types of astrocytes, but with a lower cell complexity than in the human species [82, 83].

Astrocytes with varicose projections

Astrocytes with varicose projections exist only in the human and chimpanzees' brain. They are characterized by one to five very long (1 mm) and unbranched primary cytoplasmic extensions, which extend in all directions in the deep cerebral cortical layers (cortical layers 5 and 6). The primary cytoplasmic extensions, intensely GFAP+, are straighter and longer, compared to those of protoplasmic astrocytes that are more wavy and strongly branched. Astrocyte extensions with varicose projections end in the surrounding neuropil or on the surface of the capillaries. These extensions may enter the range of extensions of neighboring astrocytes, but their function is unknown. On the surface of the extensions, these astrocytes have numerous varicose veins arranged evenly and spaced at a distance of 10 μm, an aspect that also gives the name of these cells [84].

Interlaminar astrocytes

Interlaminar astrocytes were first described by Carlo Martinotti & William Andriezen as "caudate caudal fibrous cells" [85], and by Gustav Retzius, as small cells (located in the superficial cortical layers), but with long extensions (reaching to the deep layers) [86]. Many decades later, it was found that these astroglial cells exist only in the brains of higher primates and in humans [82].

Interlaminar astrocytes appear postnatal and are thought to come from some astroglial precursors and not directly from the radial glia [87]. Human interlaminar astrocytes are spherical cells, with a small cell body (≈10 μm), located in the supragranular layer (or layer 1) of the cortex. They have several short extensions, arranged parallel to each other "in palisade". One or two extensions are very long (up to 1 mm), penetrate the cortex, and end in layers 2–4, crossing the domains of protoplasmic astrocytes. Interlaminar astrocyte' extensions often have a spiral appearance, and they terminate in the neuropil and occasionally on the blood capillaries. The terminal portions of cell extensions (similar to axonal terminal buttons) are specific structures, known as "terminal masses" or "terminal bulbs", that contain mitochondria [84]. Interlaminar astrocytes are specifically stained with anti-CD44 antibodies. The specific function of these cells remains unknown, although they may play a key role in connecting distant cells and integrating cell groups into larger structures [88].

Still, a better understanding of normal brain function is needed, to reveal the astrocytes' heterogeneity and their response to injury and disease [89].

Blood–brain barrier: factors secreted by astrocytes and ways of molecular transport

BBB is a complex vascular structure, which regulates the transport of molecules to and from the CNS, thus strictly controlling the chemical composition of the neuronal microenvironment [90]. At the CNS level, the BBB forms neurovascular units, schematically represented by three components: capillary, astrocyte, and neuron [91].

Astrocytes are an important element in the structure of the BBB, forming a bridge that connects over 80% of the blood vessels of the CNS with neurons. Astrocytes are cells with polarized extensions that, on the one hand surround the capillaries, and on the other hand the neurons; consecutively, a double signaling pathway is created between vessels and neurons, with the role of adapting the blood flow to the neural activity. Through signals received from neurons or endothelial cells (ECs) of blood vessels, astrocytes secrete numerous factors that influence the functionality of the BBB [91–93].

Within the neurovascular unit, the factors secreted by astrocytes, through paracrine interactions with pericytes and ECs, maintain the BBB and contribute to the regulation of blood flow.

Recent studies mention the sonic hedgehog (SHh) factor, a component of the hedgehog (Hh) signaling cascade, as having a role in modulating EC function, both during barrier development, and in adulthood. ECs have the Hh patched-1 receptor on the surface, so that, by activating the Hh signaling cascade, the expression of junctional proteins is induced; this determines the characteristic phenotype of the endothelium in the structure of the BBB [94].

Other factors secreted by astrocytes are vascular endothelial growth factor (VEGF), angiopoietins (Ang1 and Ang2) and transforming growth factor-beta (TGF-β), factors involved in vascular growth.

During development, VEGF is necessary for the formation, remodeling, and maintenance of embryological blood vessels; in the early stages of development, the main source of VEGF is radial glia. In adulthood, VEGF reduces the stability of the BBB in inflammatory processes [92].

Ang1 is involved in the process of differentiating the BBB, by promoting angiogenesis and reducing endothelial permeability. Ang2 is involved in the destruction of the BBB in trauma and inflammatory processes [94].

TGF-β secreted by astrocytes and ECs in the CNS has the role of inhibiting leukocyte transmigration through the endothelium; yet recent research concludes that it is difficult to establish the exact role of this factor in the physiology of the BBB [94].

Another factor secreted by astrocytes is the angiotensin-converting enzyme-1 (ACE-1). The role of ACE-1 is to convert angiotensin I into angiotensin II, that acts on angiotensin receptors of ECs in the structure of the BBB. The effect of this angiotensin II–receptor complex is vasoconstriction, reducing barrier permeability and stabilizing the function of junctional proteins [94].

A recent study admits that astrocytes play an essential role in regulating the BBB, but it is difficult to define the quantum of their involvement in specific physiological and pathological processes [93].

BBB is difficult to be penetrated. This phenomenon has huge consequences on therapy. Recent data points to some ways, by which molecules can attend the brain surface. Nonetheless, the effectiveness of transport policy is insufficient. The question of how to overcome transport constraints and develop a fresh mode of transport is a new one.

The bulk-phase/fluid-phase transcytosis techniques (FMT) can transport across the BBB a few soluble plasma molecules at random (*e.g.*, albumin or immunoglobulin G's transferrin). This is due to the presence of high-density clathrin-coated vesicles in ECs, which negatively inhibit charged ligands from passing through FMT. Sinking into the latherin-coated vesicles of the cellular membrane allows the transport of parts of molecules [95]. Based on the transport mechanism, the process is categorized as receptor-mediated transcytosis (RMT) or adsorptive-mediated transcytosis (AMT).

Nanoparticles (NPs) can also be carried across the BBB through carrier-mediated transcytosis (CMT), often used to transfer large molecules, like glucose and acids.

Additional mechanisms include the paracellular aqueous pathway, cell-mediated transcytosis, transcellular lipophilic pathway, and efflux pumps.

Receptor-mediated transcytosis

Specific BBB receptors that transport endogenous polymers based on adenosine triphosphate (ATP) hydrolysis are used in this process. RMT is characterized by a high level of specificity, affinity, and energy dependence [95]. According to the researchers, among the few peptides and proteins that may pass the BBB, can be counted insulin-like growth factors (GFs), Fe–transferrin, insulin, low density lipoproteins and amyloid proteins. Large chemicals can also pass the BBB through the RMT, according to a growing number of studies in recent years. The transferrin-coated polyester NPs, (poly(D,L-lactide-*co*-glycolide), can improve cell surface adhesion and endocytosis, according to research into the transferrin receptor [96]. Carbon nanospheres, large particles with 100 to 500 nm diameter, have been demonstrated to penetrate the BBB *via* clathrin-mediated endocytosis [97].

Adsorptive-mediated transcytosis

Transport across BBB is facilitated by the attraction between the NPs' positive charge and the EC membranes' negative charge. However, AMT has some disadvantages, as poorer affinity, specificity, and targeting capabilities. These limitations restraint AMT's practical applicability. Few cell-penetrating peptides and cationic proteins are transported by AMT [98]. Those materials, however, can mix with a wide range of cargos, and NPs might help AMT deliver medications more efficiently. The bovine serum albumin (BSA) NPs coupled with poly(ethylene glycol)–poly(lactide) can traverse the BBB *via* AMT with 7.76-fold increased permeability following cationization (cationic BSA (CBSA) NPs) [99]. The surface density of CBSA NPs may impact the bridging of the BBB by CBSA NPs in AMT. It is thought that CBSA NPs cross the BBB *via* AMT, by first connecting with the negative charge of BBB's ECs. These findings improved AMT's potential for translocation over the BBB.

Carrier-mediated transcytosis

Multiple carrier proteins have been discovered in ECs that can carry amino acids, nucleic acids, glucose, and other essential nutrients across blood capillaries to brain tissue. The substrate specificity of CMT carriers is really significant, meaning they exclusively connect with specific endogenous molecules. NPs should resemble the chemical that will be carried over the BBB *via* CMT. Glucose transporter proteins (GLUTs) are the most well-known carriers. The membranes of mammalian neurocytes and brain capillary endothelium contain a lot of GLUT1 and GLUT3 [100]. In the mammalian brain, GLUT1 is the primary glucose transporter. Researchers devised a method that takes advantage of GLUT1 transport, to loading glucose onto the liposome surface [101]. Newly modified glucosylated (2-deoxy-D-glucose (D-Glu)) NPs loaded with Paclitaxel (PTX) have a better ability to penetrate the BBB and are less toxic to the cells than non-glucosylated NPs loaded with PTX. In chemotherapy of multiform glioblastoma, D-Glu NPs has emerged as a viable targeted delivery system due to its ability to improve BBB penetration (GLUT-mediated transcytosis) and drug accumulation (GLUT-mediated endocytosis) [102].

Current state of knowledge regarding the origin and classification of human dental cementum

Root cementum has given rise to a number of controversies related to origin, classification and structure. In fact, it is the most publicized mineralized tissue in our body. Still, a lot of data on its regard was obtained from the laboratory animals, information that is not actually in accordance with the human structural reality [103, 104]. This brief presentation refers to the current state of knowledge regarding part of the histology of human dental cementum only.

Root cementum was highlighted by optical microscopy at the beginning of the 19th century (1835) by Frankel & Ratskov, its presence being important to be distinguished from the coronal cementum, that occurs in some animals, *e.g.*, horse, sheep, rabbit, and guinea pig [103, 105].

It is a hard, mineralized tissue, a thin strip, that covers the dentin of the root. The variance in chemical composition during the mineralization process is due to diet and other environmental influences [106]. Human cementum has a calcium (Ca)/phosphorus (P) ratio of 1.51, a Ca/zinc (Zn) ratio of 595–990 and a mineral density of 1240–1340 mg/cm^3 [107]. Immunohistochemistry (IHC) has proven a small number of ECM glycoproteins: dentin matrix protein 1 (DMP1), bone sialoprotein (BSP), osteopontin (OPN), all in connection with tissues that possess a mineralization capacity [108]. Potential biomarkers for human cementum were identified in cementocytes: superoxide dismutase 3 (SOD3) and serpin family A member 1 (SERFINF1), considered to be indigenous products [109].

Functionally, cementum is part of the periodontium, having a role of protection and regeneration of periodontal tissues [103, 104].

The origin of human cementum is controversial [103]. There were two hypotheses regarding the nature of root cementum, the mesenchymal one, in which cementoblasts derive from both the fibroblasts of the follicular sac or of the desmodont, and the epithelial one, in which cementoblasts arise from the epithelial cells of the Hertwig sheath [103].

It is worth emphasizing that the epithelial theory, which has gained a lot of momentum in the last two decades, is supported today by a very small number of researchers and practically it is disproved [103]. Yet, HERS-C2, a cell line obtained from Hertwig's epithelial root sheath

(HERS), can differentiate into cementoblasts *via* the process of epithelial–mesenchymal transition and generate cementum-like tissue *in vivo* (immunohistochemically positive for the three ECM glycoproteins discussed above) [110].

The classification of cementum is also highly controversial [103]. The most complete is the Schroeder classification, which uses three criteria: (*i*) the submission time (primary/secondary cementum), (*ii*) the presence of cells (acellular/cellular cementum) and (*iii*) the source of its collagen fibers (intrinsic/extrinsic fibers cementum).

Historically, the cementum has been classified into two major types: acellular variant, in the superficial third, and cellular variant, in the deep third [103, 104]. Over time, these two types were reconfigured and subdivided according to the structural identification of new elements, resulting in the description of 11 classes of cementum [103].

The first three types of cementum are the most important and correspond to the old historical classes of acellular and cellular cementum. These are: acellular extrinsic fiber cementum (AEFC), cellular intrinsic fiber cementum (CIFC) and cellular mixed stratified cementum (CMSC). In addition to these and their subtypes, there are other varieties of cementum, more or less controversial [103].

• AEFC contains dense extrinsic fibers, without cementocytes, and corresponds to the classical acellular cementum [103]. According to IHC, the ECM glycoproteins, BSP and OPN, revealed a very strong pattern in the thin AEFC layer. Functionally, the acellular cementum binds the periodontal ligament and thus, it participates to the stabilization of the tooth in the dental alveolus [108]. Its subtype, the acellular intrinsic fiber cementum (AIFC), is challenged by certain researchers! [103].

• CIFC contains intrinsic fibers and cementocytes [103]. BSP revealed a diffuse localization; OPN is present in CIFC too, providing a good contrast with the underlying dentin [108]. Its subtype, the cellular extrinsic fiber cementum (CEFC), has not been distinctively classified, but more, after the amount of the extrinsic fibers: CIFC rich in extrinsic fibers and CIFC poor in extrinsic fibers [103].

• CMSC corresponds to classical cellular cementum; it contains layered CIFC (Salter incremental lines) and AEFC [103]. It is present on the apical roots and the furcation area [111]. The localization of the three ECM glycoproteins is different: DMP1 is found only in the matrix at the cementum–dentin junction (CDJ), and BSP and OPN through the whole cementum stratum [108]. The role of DMP1 here is not completely understood: it is possible that it plays a local function at CDJ, or in the process of cementum development [108]. There is a CMSC' subtype, the CMFC; it is fast formed, with less mineralized fibers; it contains extrinsic and intrinsic fibers and cementocytes [103].

• Acellular afibrillar cementum (AAC): it does not contain fibers or cells. It is a notion under debate! [103, 111].

• Intermediate cementum: it also represents a notion that should be reconsidered, being more probably part of the dentin! [103].

• Aberrant cementum: coronary cementum with cementicles [111].

Acellular extrinsic fiber cementum

AEFC covers 1/3 of the cervical surface; it is made up of collagen fibers and contains completely mineralized non-collagenous proteins [103]. According to IHC staining pattern, both BSP and OPN are locally highly concentrated, possibly playing a role in cementum development [108].

The extrinsic fibers come from those of the periodontal ligament; they are thick, branched, and have a perpendicular disposition to the dentin. It features incremental Salter lines, due to the rhythmic deposition process, intensely stained with HE [103, 104].

In its structure, the controversy implies the CDJ. 80% of the authors consider this junction made from the inter-digitations between the cementum extrinsic fibers and those of the dentin matrix; 20% of the authors discuss the presence of an amorphous material, formed from non-collagenous proteins with rare fibrils, that serve as a binder between dentin and cementum [103].

Acellular intrinsic fiber cementum

It represents a contested variant of acellular cementum; it occurs at the end of cementogenesis, in the apical zone and in the interradicular region. It does not show cementocytes; the cementoblasts are withdrawing among the desmodontal cells. Collagen fibers are intrinsic. It probably plays an adaptive role [103].

Acellular afibrillar cementum

It represents an isolated area, just below the enamel–cementum junction; also, it can partially cover the cervical enamel. It is made up of non-collagenous proteins similar to those from AEFC. It is a controversial type in origin and function. The origin resides in either: cementoblasts, or Hertwig epithelial sheath cells, or it is just a precipitate from tissue fluid (serum). Its function is generally unknown. In case that it is originating from cementoblasts, its role must be of protection, the deposition of cementum by the cementoblasts adding strength to the reduced layer of enamel [103].

Cellular mixed stratified cementum

It forms 2/3 of the root, being located in the apical region and the furcation zone of the tooth; it contains CIFC, layered by incremental lines and occasionally some AEFC. CIFC contains both intrinsic and extrinsic fibers. The content in extrinsic fibers varies from individual to individual, depending on their density; thus, the following varieties can be distinguished [103]:

▪ CIFC rich in extrinsic fibers is an inconsistent and controversial structure! The extrinsic fibers are surrounded by intrinsic fibers and are thicker than those in AEFC; they present a central unmineralized core, surrounded by a highly mineralized area.

▪ CIFC low in extrinsic fibers.

▪ CIFC without extrinsic fibers (pure CIFC) is characterized by the presence of lamellae, obtained by the longitudinal and transverse arrangement of collagen fibrils: alternation of clear and dark lines (2.5 μm diameter), more numerous towards the dentin.

Cellular mixed cementum

It is a variant of the CMSC, but faster formed, with less

mineralized fibers [103]. It covers the root surface and anchors the periodontal ligament, and it is very sensitive to disturbances in the phosphate/pyrophosphate (Pi/Ppi) ratio [112].

The origin of collagen fibers is twofold: extrinsic (desmodontal) and intrinsic (cementoblasts). There are differences between the fibers: the extrinsic ones are ovoid/round in shape and thicker (5–7 μm in diameter); the intrinsic fibers are more delicate, and they are smaller (1–2 μm in diameter) and numerically reduced. Intrinsic and extrinsic fibers form a complex model at almost right angles with different orientations. Regarding the cells from its structure, the cementocytes are phosphate-responsive cells, able to regulate cementum homeostasis. They express sclerostin, SERFINF1, DMP1, SOD3 and E11/gp38/podoplanin, but no markers for odontoblasts [109, 113, 114].

Intermediate cementum

It is the most controversial type of cementum, being regarded as an overlapping of notions. The denomination should be reconsidered to prevent further confusion! Currently, it consists of the Hopewell–Smith hyaline layer in the AEFC region and Bencze intermediate cementum in the CMSC region. It is formed by a thin cementum strip between the dentin and the AEFC/CMSC, respectively. It occurs at the beginning of cementogenesis. It covers the dentin mantle of the molars and premolars. Also, its origin is controversial. In 90% it is considered as part of the dentin (the observed lacunae are dilated dentinal tubules or dilated odontoblastic processes, in continuity with typical dentinal tubules); still, there are authors that take into consideration an enamel origin: an enamel like tissue secreted by the cells of the Hertwig sheath; this theory is currently disproved, data being obtained from rodents! [103].

Possibly, the role of the intermediate cementum is to seal the sensitive root dentin.

Aberrant cementum

It refers to coronal cementum or cementicles [111].

The coronal cementum characterizes teeth with imperfect amelogenesis or early degeneration of the adamantine organ (it causes the differentiation of follicular sac cells into cementoblasts with coronary cementogenesis).

The cementicles are free or attached to root cementum. They are obtained either by the mineralization of Mallasez's epithelial remains or by the mineralization of degenerate desmodontal vessels.

Despite all the progress in understanding the cementum and its developmental biology, additional fundamental questions remain, including: the factors directing cementum formation, the origin of the cementoblasts, potential functions for cementocytes, and prospects for directing cementum regeneration [115].

We can conclude that cementum is a highly unique mineralized tissue, whose controversial structure may possibly benefit from dental tissue engineering and the development of new biotechnologies.

The tubarial salivary glands – a new type of salivary gland?

The tubarial salivary glands appear to be the fourth pair of macroscopic salivary glands; they are located between the nasal cavity and the neck, in the posterolateral walls of the nasopharynx on the medial side of the *torus tubarius*. Their name came from their location [116].

These glands were described using positron emission tomography (PET) and computed tomography (CT), with radiolabeled ligands to the prostate-specific membrane antigen (PSMA PET/CT) [116], as structures with a cranio-caudal length, with a median value of 3.3 cm (2.2–4.6 cm) in females (only six patients studied) and of 3.9 cm (1.0–5.7 cm) in males (99 cases studied) [117]. Several ducts open on the medial side of the *torus tubarius*, towards the nasopharyngeal wall [116]. Yet, the recognition of the tubarial salivary glands as individual anatomical organs is still under debate due to three elements: (*i*) the previous anatomical description of clusters of glands around the auditory tube's mucosa and in the regions adjacent to the *torus tubarius* [118, 119]; (*ii*) the previous description of salivary glands cancers at the nasopharynx level [120]; and (*iii*) the issue of gender representation (sexual dimorphism) [121].

It has been hypothesized that they may contain many mucous PSMA-positive acini, with a physiological role in the lubrication of the nasopharynx and oropharynx, as well as in swallowing. Consistently with a reduced number of serous acini, there was no amylase expression in the gland cells [116].

As clinical relevance, during an intensity-modulated radiotherapy for head and neck cancer, a radiotherapy mean dose can have significant local toxicity, expressed by dysphagia and xerostomia [122].

Activation and cross-talking of hepatic stellate cells, key players, and prognostic markers in hepatic diseases

The hepatic stellate cells (HSCs), initially named the Ito cells, are representing approximately 15% of the total resident cells and 1/3 of the nonparenchymal cells, in the normal human liver [123].

The HSCs are located inside the Disse space, a virtual, permeable, subendothelial connective tissue space delimitated by the basolateral surface of hepatocytes and the anti-luminal side of the sinusoidal ECs layer [123, 124]. They are the main perisinusoidal cellular type [125]. In the Disse space occurs the exchange of biomolecules between the hepatocytes, the portal blood flow from the GI tract, and many other cells located at this level [126].

The HSC origin is both mesodermic and endodermic, with characteristics of resident fibroblasts in the stromal matrix and pericytes attached to capillaries [123, 127]. They are mainly considered as pericytes, located in the perisinusoidal Disse space [128].

Morphogenetically, the HSCs are involved in growth and differentiation of all the hepatic parenchymal cells and in the liver regeneration (they are members of the hepatic progenitor cell' niche and they are recruited in the regeneration process) [129].

In healthy liver, the HSCs have an important involvement in homeostasis and lipidic metabolism, being a lipid-storing cell [125], that store and control the release of the retinol and vitamin A from their cytoplasmic lipid droplets [128]

and synthesize some lipoproteins. They also contribute to liver immune system, to xenobiotic metabolism, to pH regulation and to oxidant stress response [127].

All types of liver pathology activate the regenerative role of HSCs. It is well documented the HSCs involvement in liver fibrosis [130], viral hepatitis [131], liver tumor microenvironment and cancer generation and arrest [132]. In the space of Disse of the fibrogenic liver, quiescent HSCs are activated and transdifferentiated into myofibroblasts, whose secretion accumulates ECM components that form scar tissue [126]. The liver fibrosis and disease progression processes depend on the dynamic cell-to-cell communication among the hepatic cellular types, through activation of novel pathways and their messengers, and through receptors that translate information into cellular pathophysiology and fate [133]. These very intricate and dynamic cell-to-cell communication mechanisms include autophagy [134], ER stress [131], oxidative stress, retinol and cholesterol metabolism, epigenetics, and receptor-mediated signals [123, 135].

Activated HSCs are interacting with other elements, such as: injured epithelial cells (hepatocytes and cholangiocytes) [136], altered components of ECM (ECM proteins progressively stored in Disse space) [137], immune regulatory cells (through paracrine regulation of Kupffer macrophages [138], T-helper 17 (Th17) cells [139], $\gamma\delta$ T-cells [140], B-cells [141], natural killer (NK) cells [142], and with the other surrounding hepatic cell types (such as sinusoidal ECs [143] or hepatic oval progenitor cells) [129]. Other factors are mitigating the HSCs activation: the enteric microbiome [144], the chronic infection by hepatotropic viruses [145] and/or the co-infection with human immunodeficiency virus (HIV) [146].

The activation and crosstalk of the HSCs induce changes in other communicating/interacting cells, and involve the production of GFs, and cytokines with autocrine and paracrine functions – substances which promote lipophagy, cell proliferation and fibrogenesis [134]. In activated HSCs, their homeostatic function is dysregulated, and they obtain energy by enhanced lipophagy of vitamin A-storing lipid droplets and depletion of the vitamin A stores [134].

Activated HSCs contribute to hepatocellular carcinoma (HCC) development by promoting fibrogenesis and inflammation and by modulating tumor microenvironment [147]. Therefore, HSCs and their cellular crosstalk mediators, such as fibroblast growth factor 9 (FGF9) [147, 148], SDF-1 protein [149], TGF-β [125], PDGF-α [150], microribonucleic acids (microRNAs)' expression [151] and the extracellular vesicles [152], can be used as prognostic biomarkers in chronic fibrotic processes and in HCC.

With the aid of the newly experimental methods (*e.g.*, multi-cell type and/or three-dimensional (3D) cell culture, or animal-based models [153, 154]), the complex HSC's signaling is still under study. Deciphering the regulation of HSC activation promises remarkable possibilities for novel human clinical therapies, with successful long-term results.

Kidney interstitium – a new insight of resident fibroblasts

The renal interstitium is situated between the BM of the renal tubules and vessels, having several functions: to support the elements of the renal parenchyma, to secrete hormones or other substances (erythropoietin, renin, adenosine), to mediate the exchange processes between the tubules and vessels, maintaining homeostasis, to protect against anemia and to regulate the inflammatory responses [155–158].

The renal interstitium is divided into different compartments, corresponding to the cortex and medulla. The cortical interstitium represents about 10% of the human cortex volume [156], while in the deeper part of the medulla, surrounding the renal papilla, the interstitium volume can increase up to 30–40% of the renal medullary volume [155, 156]. The cortical interstitium contains different regions: between the renal tubules (peritubular interstitium), between the arteries (periarterial interstitium) and the mesangium [159].

The renal interstitium contains a loose ECM, interstitial fluid, and different cell types, such as fibroblasts, mononuclear immune cells (macrophages, antigen-presenting dendritic cells) and perivascular cells or pericytes (mostly in the medulla) [155–158, 160].

A lot of differences were described between the cortical and medullary interstitium, regarding the interstitial volume, the histological constituents, and the endocrine synthesis function [156].

Resident fibroblasts in the renal interstitium, also called type 1 ICs of the kidney, are stellate cells, with long cytoplasmic processes. The cytoplasm contains an important protein synthesis apparatus: rough ER, free ribosomes, as well as Golgi complexes, mitochondria, lysosomes and a lot of microfilament bundles and microtubules. Fibroblasts are involved in the synthesis of ECM components (type I, III, VI collagen fibers in the matrix, collagen IV and V in the BM, ground substance constituents) and several hormones [157, 161].

Cortex

In the cortex, the peritubular interstitium is a narrow space found between the renal corpuscles or glomeruli, tubules, and capillaries, whereas the periarterial interstitium is wider, located between the renal arteries and containing the lymphatic vessels [156].

The cortical peritubular fibroblasts (cortical type 1 ICs) represent the major population of ICs, having similar characteristics as fibroblasts in other organs [157, 161]. Their long cytoplasmic processes contain microfilaments and dense plaques [161], which are in contact to the BM of the renal tubules, capillaries, and Bowman capsule. Moreover, there are junctional complexes that connect the fibroblast extensions, thus, forming a continuous network that support the renal parenchyma and maintain the 3D architecture of the renal tissue [157, 158, 161]. All these connections may suggest that the intercellular communication between interstitial fibroblasts, epithelial tubular cells and ECs are mediated not only by paracrine factors or exosomes, but also by mechanical forces [157, 160]. As a result, fibroblasts integrate all these cellular responses, being key participants in different physiological and pathological processes [157].

In healthy kidney, occasionally, cortical fibroblasts accumulate a few lipid droplets.

Different subpopulations of cortical fibroblasts were described, according to their origin, morphological characteristics, and functions [157].

The ecto-5'-nucleotidase (ecto-5'-NT) enzyme or CD73, that converts extracellular nucleotides to adenosine, is expressed in the plasma membrane of cortical peritubular fibroblasts, but not in fibroblasts of other compartments of the renal interstitium (periarterial connective tissue, medullary interstitium) [157]. The renal adenosine production plays an important role in controlling the glomerular filtration (due to the constriction of afferent arteriole), as well as, in the modulation of the inflammatory responses [156, 157, 162, 163].

A subpopulation of cortical peritubular fibroblasts, which are ecto-5'-NT-positive peritubular fibroblasts, is responsible for erythropoietin (EPO) production in the kidney, a glycoprotein hormone that regulates hematopoiesis, inducing proliferation and differentiation of erythroid progenitors [156, 157, 161, 163, 164]. The kidney is the main site of EPO synthesis [156]. In healthy kidney, EPO is produced in response to hypoxic insults, to maintain homeostasis [156, 158]. An interesting fact is that Ito cells in the liver, which also produce EPO, are positive for ecto-5'-NT, as well [157].

Renin, a hormone secreted by juxtaglomerular cells, may be produced by some cortical fibroblasts, which are located nearby juxtaglomerular cells, but also by perivascular cells (pericytes) in kidney diseases [157].

The phenotype of cortical fibroblasts may change in different pathological conditions.

Medullary interstitium

Medullary fibroblasts (medullary type I ICs or lipid-laden type I ICs), resemble the cortical fibroblasts and they have structural relations to medullary renal tubules and vessels, forming a continuous network and providing a local structural support. The connection between medullary fibroblasts and the BM of the thin loops of Henle and *vasa recta* are very well observed at the electron microscopy, but the contact with the collecting ducts is rarely noticed. They increase in number in the deeper part of the medulla and are often named renomedullary ICs.

Unlike the cortical fibroblasts, the lipid-laden type I ICs do not secrete EPO and adenosine, but they have a high content of lipid droplets, that vary in size and number, and almost fill the whole cell [156, 157].

Renomedullary ICs have a characteristic arrangement, in rows, perpendicular to the tubules and vessels, connecting all these structures like the rungs of a ladder.

Besides their role in ECM production, renomedullary ICs are an important site for prostaglandin E2 (PGE2) synthesis, being a mediator of inflammatory responses [158]. The lipid droplets within the cytoplasm represent the precursors for PGs secretion. Additionally, these medullary interstitial type I cells have endocrine antihypertensive functions, secreting medullipin I, which is converted to medullipin II in the liver.

Recent studies revealed that fibroblast subpopulations may have different origin. Even though usually fibroblasts are considered to be derived from mesenchymal cells in classical studies, new data suggest that EPO-secreting fibroblasts may have a neural crest origin, from myelin protein zero (P0)-Cre lineage-labeled cells, whereas the renin-produced fibroblasts derive from forkhead box D1 (FoxD1) mesenchymal cell progenitor cells [156–158, 165]. In addition, some authors have shown that almost 98% of all cortical fibroblasts originate from neural crest [158, 165, 166].

The literature related to type I ICs in healthy kidney is less consistent, but there are a lot of studies that have examined their involvement in kidney diseases, especially their contribution to renal interstitial fibrosis development [156, 160].

During chronic kidney disease (CKD), resident fibroblasts may contribute to development of the fibrotic process, being also involved in regeneration of the damaged renal tubular epithelium and in inflammatory responses [158, 166]. Besides, dysfunctional fibroblasts lose the role in maintenance the organ architecture and detach from the BM of the capillaries. Therefore, peritubular capillary is no more structurally stable, they become fragile and rare, leading to hypoxia, which amplify the pathological condition [166].

In a severe renal fibrosis, the interstitium may occupy up to 60% of the total kidney volume [3, 9], with increasing numbers of ECM-producing fibroblasts (and also renin-producing cells) and accumulation of excessive ECM [155–158]. Increased number of ecto-5'-NT-positive peritubular fibroblasts lead to high concentrations of adenosine in chronically injured kidney, thus, contributing to the vasoconstriction of the afferent arteriole and a lower glomerular filtration rate (GFR) [156, 164, 167]. Meanwhile, EPO-secreting cells are lost, leading to renal anemia, even though the hypoxic conditions represent a key pathological condition in renal fibrosis [156, 158].

In physiological conditions, adult fibroblasts are inactive but, following tubular lesions or inflammatory conditions, these cells may acquire an activated phenotype and convert into myofibroblasts, which synthesize components of the ECM and express alpha-smooth muscle actin (α-SMA) [156–158, 166]. Recent studies revealed that EPO-secreting cells also may transdifferentiate into myofibroblasts in renal interstitial diseases, so, a deficiency in EPO production and anemia occurs [166]. Besides, in chronic renal disease, renal fibroblasts can acquire a pro-inflammatory phenotype, producing cytokines (interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-α), etc.) [158, 166]. Some authors reported that anti-inflammatory agents provide the phenotypic reversion, thus, offering a promising therapeutic approach for severe renal fibrosis [158].

Several pathways that regulate the phenotype transition were described, especially PDGF receptor (PDGFR) signaling pathway [158, 168]. Resident fibroblasts in the kidney are positive for CD73 and PDGFR-β. Activation of PDGF-BB/PDGFR-β axis is responsible for the regenerative function of the renal tubular epithelium [158, 160]. However, in chronic kidney diseases, the long-term activation of PDGF-BB/PDGFR-β axis accelerate the renal fibrosis, therefore, PDGF-BB/PDGFR-β signaling pathway may be advantageous or troublesome [158, 160, 166].

In conclusion, resident fibroblasts in healthy kidney represent a heterogeneous cell population, with distinct molecular characteristics and various functions in the cortex and the medulla. In CKD, fibroblasts are versatile cells and display a functional heterogeneity and plasticity, playing a pivotal role in fibrinogenesis and in mediating inflammation.

New insights about the pineal gland calcification

The pineal gland is a vital homeostatic photo-neuro-endocrine organ, that synthesizes melatonin, a substance with a myriad function: a signaling molecule in regulating the circadian rhythm (by acting on the way of the super-chiasmatic nuclei) [169], a strong antioxidant for neural protection [170] and a powerful anti-inflammation, anti-tumor, and anti-aging agent [171, 172].

The pineal gland has the highest rate of calcification of all organs and tissues of the human body [173, 174] and is the most common site of physiological calcifications (71.6%) [175]. The pineal calcification limits the synthetic capacity of melatonin and earlier studies sustained an association with a variety of neuronal diseases, as schizophrenia [176, 177], dyskinesia [178, 179], Alzheimer's [180] and Parkinson's disease [181]. Recently, several studies have shown that the increasing of pineal calcification decreases the melatonin production in humans, and that the melatonin and its metabolite levels are positively correlated to the uncalcified gland volume and negatively linked to the size of the pineal calcification [182–184]. The decreased endogenous melatonin level lowers the neuronal resistance to oxidative stress and the inhibition of amyloid secretion and deposition; consequently, the hyperphosphorylation of *tau* protein is increasing [185–187]. Therefore, the pineal calcification seems to have a direct influence on neurodegenerative diseases and aging, even on cancer immunosuppression [174, 188]; the melatonin treatment is relieving the clinical symptoms, even if it does not cure the diseases: a meta-analysis showed that the melatonin treatment leads to a modest improvement of tardive dyskinesia symptoms in schizophrenia patients [189].

Pineal calcification was identified in the human species even since 1653. Several studies indicate that the pineal calcification is a natural aging-related process, though with considerable interindividual variability, linked with sex, altitude, and duration of sunlight exposure [190, 191]. Males are more likely to present pineal calcification then females [192, 193]. Higher altitude and duration of sunlight exposure are also correlated with an increased pineal calcification [190]. These changes can be caused by an aging-related deterioration of the suprachiasmatic nucleus (the neuronal circadian pacemaker's transmission to the pineal gland) [194], similar to neurodegenerative disorders [191, 195].

The pineal calcifications are strongly associated with aging even if they have been detected in newborns or children. Yet, in children they are rare [196, 197] (under the age of six years old being present in 1% of children) [196].

However, their number and proportion (total volume calcification toward total volume of the gland) increase with age [174, 198]; in humans, the incidence of the visible calcification is at 5% of the 0–9 years old people [197], 32% of the 10–19 years old people, 53% of the 20–29 years old people and 83% of the over 30 years old people [174].

The formation of pineal calcified concretions ("*corpora arenacea*" or "brain sand") is incompletely elucidated, it can have a physiological, maturational, degenerative, or combined cause [174, 197]. The concentric laminated pineal calcification is not a random process, but a complex, well-structured and programmed mechanism: the number of lamellae is directly proportional to human age [174, 199] and the laminated pineal concretions are structurally similar to the osteons of the compact bone [174]. Two main types of pineal intraparenchymal calcifications are observed, in young and old people, with different shapes and size of calcifications; in young people, the calcifications are globular, lobulated, and localized in the proximity of the pinealocytes, while in elderly patients, calcifications are larger, lamellar, concentric, associated with glial cells [197, 200–203]. Kim *et al.*, studying the pineal gland of older adults (62–80 years), found conglomerate areas, calcified in a concentrical manner, distributed over the entire parenchyma, but preponderantly with a central concentration [204] (they may be related to the circannual changes in Ca level) [199].

In the pineal gland are significantly present two different crystalline compounds; this fact suggests two different biologically mechanisms of formation and biological functions [201]. There are small, well-defined crystals, that have less than 20 μm in length (microcrystals) [205] and large polycrystalline complexes, of hundreds of micrometers in length (often called "mulberry-like" structures) [201]. Electron microscopy studies detailed three types of the small crystals: cubical, cylindrical (95%) and hexagonal [201, 202].

The chemical components of pineal crystals are mainly represented by salts of Ca and magnesium (Mg) [199, 206]. Ca^{2+} tend to concentrate along the cellular plasmalemma [197]. New analyzing methods of spectroscopy (energy dispersive spectroscopy and infrared Raman spectroscopy) and selected area electron diffraction have shown that the tiny crystals are usually made of calcite (calcium carbonate, mainly containing Ca, carbon, and oxygen), comparable to the otoconia from internal ear. The presence of calcite inside epiphysis represents its only known nonpathological occurrence in the human body (excepting the otoconia of the inner ear) [201, 207]. The large, laminated concretions were found to be nanocrystalline hydroxyapatite (containing Ca, Mg, and ammonium phosphates, with a mean Ca/P molar ratio between 1.65 to 1.68) [207, 208], but traces of sulfur (S), Mg, and sodium (Na) were also detected [206].

Numerous theories are trying to explain the mechanisms for pineal calcification formation. Mast cells, which secrete tryptase, that participate in calcification, have been found in perivascular areas, where Ca deposits originate [197, 199, 209]. Another possible mechanism of pineal calcification is represented by the extrusion of polypeptides in the extracellular space, with an active transfer of Ca. In the center of the concentrical "*corpora arenacea*", exophytic membrane debris was found; this could represent a by-product of pineal neuronal and glial polypeptide exocytosis [197]. There is also the possibility of osteoblast-like and osteocytes-like transformation of local, or vascular migrated mesenchymal stem cells (MSCs), under pathological conditions [174, 203, 210]; thus, the high melatonin levels promote calcification by stimulating mesenchymal cell differentiation in bone cells [174, 211, 212].

The mechanism of pineal calcification is therefore considered to be multifactorial; with the advance of age, the calcification is intensifying and its pattern changes, the number of pinealocytes decreases and the secretory function of the pineal gland is diminishing. In the light of recent research, inevitable as it seems, the pineal calcification should not be considered a common physiological process.

New findings in Leydig cells

Leydig cells (LCs) are polygonal, with a centrally located, large, and round nucleus presenting a marked nucleolus and an eosinophilic cytoplasm. These cells produce testosterone and have the morphological characteristics of steroid-secreting cells, with a prominent smooth ER, sizable and multiple lipid vacuoles, and numerous mitochondria. The lipofuscin inclusion represents a usual presence in LCs, appearing as rounded irregular bodies composed of accumulated lipid droplets in the lysosomes. They also contain specific cytoplasmic inclusions, the Reinke crystalloids, arranged in a linear pattern. These crystalloids are by-products of steroid metabolism and testosterone production in testes [213–215].

LCs present a unique cytoarchitecture in cord-like structures surrounding the seminiferous tubules. Only very few LCs are present among the seminiferous tubules [216]. They share the same thin basal lamina and present small canaliculi, rudimentary desmosomes, and microvilli processes.

From the interstitial LCs, the testosterone diffuses into seminiferous tubules and influences the seminiferous epithelia and the Sertoli cells, generating and sustaining sperm production.

In mammals, two distinct LC populations appear during pre- and post-natal testis development: the fetal LCs (FLCs) and the adult LCs (ALCs). FLCs and ALCs may share the same progenitor pool in the fetal testis [217].

Activation of the sex determining region Y (*SRY*)–SRY-box transcription factor 9 (*SOX9*) genetic cascade induces Sertoli cell differentiation. Sertoli cells produce desert hedgehog (DHh) and PDGF to induce FLC differentiation in the interstitial zone [218, 219].

NR5A1 (or the steroidogenic factor 1 – SF1) is a nuclear receptor expressed in various tissues, such as the ventro-medial hypothalamus, pituitary gonadotropic area, adrenal cortex, spleen, as well as testis, and ovary. Its activation is essential for steroidogenic cell differentiation, and this factor is expressed in the adreno-gonadal primordium [220, 221]. From stem cells of non-steroidogenic tissues transfected with SF1 were obtained *in vitro*, differentiated steroidogenic cells. Without the SF1, female internal genitalia were developed by male mice, who died early after birth due to a lack of testosterone and adrenocortical-producing cells [222]. Was proves, thereby, that FLC's androgen production during the fetal period is essential for the masculinization of the brain and male genital tract [223].

The stem LCs (SLCs) generate during puberty adult LCs (ALCs), which will produce androgens in the adult, under the control of the hypothalamic–pituitary–gonadal (HPG) axis.

A new treatment for androgen deficiency in hypogonadism, which affects males of all ages, could be offered by stem cell therapy: from SLCs were generated *in vitro* ALCs, and Leydig-like cells, which were transplanted into ALC-null animals, restoring the serum testosterone levels successfully under HPG control.

The multipotent SLCs persist in the adult testis and have the capacity to form ALCs, and also all the three major lineages from MSCs (adipocytes, chondrocytes, and osteoblasts) [224].

In fetal and neonatal mouse testes, ALCs can have three possible sources: peritubular progenitors (Hes1+/Arx+) originating from coelomic epithelium [225], perivascular progenitors (nestin+/Notch+) originating from the gonadal–mesonephros border [226], and dedifferentiated FLCs, appearing by the end of the fetal stage [227].

Because ALCs are considered postmitotic cells that do not divide [228], the SLCs have an essential role in maintaining adult ALCs' homeostasis [229, 230]. It is not clarified if the SLCs represent a unique subset of stem cells or a mixed origin population from mesenchymal progenitors or pericytes [231].

Ovarian hilar cells

In 1922, Berger described the morphology of the ovarian hilar cells for the first time and referred to them as "sympathicotropic cells" [232].

Ovarian hilar cells are located in the hilum of the ovary, adjacent to the mesovarium; cells are organized in unencapsulated aggregates of different size and shape, more numerous in the medial and lateral poles of the hilum, near the insertion of the ovarian ligament [233]. At the interface between hilum and medullary stroma, these cell aggregates are closely related to large veins and lymphatic vessels and may protrude into their lumen. Characteristically, the hilar cells unsheathe unmyelinated nerve fibers and occasionally extend into the medulla, surrounding the *rete ovarii*. The hilar cells are surrounded by thin collagen fibrils and are associated with fibroblasts and intermediate cells that share common features of fibroblasts and hilar cells. Both hilar cells and intermediate cells are in close relationship with the hilum nerves, and even establish synapses with the nerves; this particular arrangement suggests that hilar cells may originate from hilum fibroblasts and the differentiation of fibroblasts into hilar cells could be induced by the nerve impulses [233–235].

Morphologically, cells are round or oval shaped, ranging between 15 and 25 μm in diameter, and contain an abundant acidophilic cytoplasm and a round, euchromatic vesicular nucleus with one or two nucleoli. In postmenopausal women, the nuclei may be heterochromatic. The cytoplasm contains perinuclear acidophilic granules, lipid vacuoles located peripherally, and inclusions of lipochrome pigment, golden-brown in color.

Ovarian hilar cells are similar to LCs in the testis and contain specific Reinke crystals unevenly distributed in the cytoplasm, arranged either in parallel arrays, or staked. The crystals are homogenous, acidophilic structures, shaped as rods with rounded or tapered extremities and their length often equals the largest diameter of the cells. The crystals are present only in a few cells and difficult to identify. Reinke crystals may be revealed by Fe Hematoxylin or Masson's trichrome staining, and appear black and magenta, respectively [234]; in sections stained with HE and viewed in ultraviolet light, crystals fluorescence yellow. Additionally to Reinke crystals, hyaline structures, spherical or ellipsoidal in shape, are present in a large number within the hilar cells; these are regarded as precursors of the crystals [234].

Ultrastructurally, ovarian hilar cells exhibit the features characteristic to cells that secrete mitochondria with tubular cristae, lysosomes, and lipid inclusions. Reinke crystals have a crystalline aspect, and consist of hexagonal microtubules, densely packed and parallel, separated by clear spaces [235].

Immunohistochemically, the hilar cells are intensely positive for inhibin, calretinin and Melan-A [236].

Ovarian hilar cells should be differentiated from the ectopic adrenal cortex tissue and the theca lutein cells [237]. The adrenal cortical rests are rare in the ovary but can be present in the mesovarium or in the ovarian hilum; these cells resemble to normal adrenal cortex and contain abundant lipid vacuoles [234].

Ovarian hilar cells are present in various number and have different distribution depending on the age: these cells can be observed in the fetal ovary, but are absent in children, reappear at puberty and also persist after menopause [234]. Mild hyperplasia of the hilar cells is common in postmenopausal women and can be associated with the proliferation and luteinization of the ovarian stroma [234].

The morphology and the enzymatic equipment of the ovarian hilar cells are consistent with the steroid hormone-secretion profile, but their contribution to the steroid hormone pool has not been established in normal females [233]. *In vitro* studies demonstrated that ovarian hilar cells secreted high amounts of androstenedione and low amounts of estradiol E2 and progesterone [234]. *In vivo*, hilar cells are stimulated by exogenous and endogenous human chorionic gonadotropin (hCG), which induces cell proliferation and growth [234]. Moreover, their secretory activity is influenced by the hormonal changes that occur at puberty, during pregnancy and after menopause [238]. Since ovarian hilar cells secrete androgens, their hyperplasia or the tumors arising from these cells lead to masculinization [238].

According to the *World Health Organization* (WHO) Classification of tumors of the female reproductive organs, hilar cell tumors are classified as sex cord-stromal tumors [239]. These tumors usually occur in women after menopause, and are unilateral, small, with a benign evolution and good prognosis [232, 239]. The differentiation between extensive hyperplasia and hilar cell tumors is based on the size of the lesion. Hilar cells tumor may be associated with other benign or malignant tumors of the female genital tract, including polycystic ovaries, uterine myoma, granulosa cell ovarian tumor and endometrial adenocarcinoma [232]. Tumor hilar cells are polygonal or oval-shaped, rarely elongated, contain abundant granular acidophilic cytoplasm and oval nuclei with coarse chromatin. For the diagnosis of such tumors, the presence of the Reinke crystals is pathognomonic (even though the crystals are found only in half of the cases), especially if tumor cells display vacuolated or acidophilic granular cytoplasm [232, 240]. Moreover, tumor cells are commonly IHC positive for inhibin and melanoma antigen recognized by T cells-1 (MART-1), and sometimes can express vimentin, keratin, or actin [241, 242]. It has been hypothesized that the hilum of the ovary is the niche for putative tumor-initiating stem cells, with high potential for cancer transformation by the inactivation specific tumor suppressor genes; these cancer-prone stem cells seem to be responsible for tumor growth and chemoresistance [243–245].

Angiogenesis – the contribution of tissue engineering

Angiogenesis is the development of blood vessels. The formation of new vessels can take place under normal and pathological conditions. It is very rare in the adult human subject. Two types of angiogenesis are described: angiogenesis that occurs especially in the areas of scarring and recovery of lesions and that which occurs in the process of tumor growth.

Although the effects of the action of different types of angiogenesis are not identical, they are driven by similar signals. Through angiogenesis from normal tissue repair processes, interconnected and functionally intact vessels are generated. In contrast, in angiogenesis from tumor growth processes unformed vessels are numerous, but destructured and immature [246, 247].

Knowledge of the mechanisms involved in the regulation of normal and pathological angiogenesis will open important ways for the realization through tissue engineering of new reconstructive models that integrate a strong and fully structured vascularization. However, the results of tissue engineering research have only partially materialized, so that clinical applications are still very low [248].

Spatial control of the scaffold architecture

In vivo, angiogenesis is controlled by spatial landmarks that direct the growth and maturation of vessels. It has been shown that the intervention of factors, such as inflammation or ischemia, results in a stimulation of the local release of cytokines, GFs, and chemokines, causing the formation of a molecular gradient in the extracellular space [249].

The difference in concentration of the molecules determines the formation at the cellular level of a frontal edge, with spatially controlled arrangement. The difference in molecular concentration causes locally angiogenesis and increased perfusion.

Currently, tissue engineering has attempted to replicate this process of angiogenesis. To reproduce the spatial control of cell disposition, the two methods used consisted in the direct modeling of the cells by the bioprinting process and by the distribution technique of the molecules involved in the proangiogenic stimulus.

The most advanced techniques in the field based on recent advances are 3D bioprinting and electrospinning. These two methods represent the most advanced tissue engineering constructions which simulate natural vascular development [250].

3D bioprinting

In regenerative medicine, the technique of 3D printing is widely used. Through this procedure, the process of angiogenesis in the modified tissues can be performed. There are two methods of bioprinting: the direct method (direct bioprinting) and the indirect method (indirect bioprinting) These printing methods can combine cells, biomaterials, and GFs. Thus, complex constructions are obtained that have pores and channels the size of microns, capable of guiding the process of angiogenesis.

The direct 3D method (direct bioprinting) uses bio-ink that contains cellular and extracellular components. The bio-ink drops are printed in defined shapes. This technique consists in the rapid crosslinking or gelation of hydrogels to obtain a structure with high stability.

The indirect bioprinting method consists in printing “sacrifice” channels that will later be encapsulated by the biomaterial loaded with cells. Then the “sacrifice” channels

will be removed. This process is performed either by using a solvent or by thermal action. The remaining capillary network will be seeded with dormant ECs. This will play a role in guiding angiogenesis [251]. The direct 3D method is a 3D printing method that uses inkjet. The principle of the method consists in using an instrument called thermal or piezoelectric actuator by which the layer-by-layer (LBL) technique dispersion of the bio-ink droplets on a substrate is achieved. Hydrogels with fast gelling properties combined with crosslinking agents are used to print very well-organized networks by the direct 3D bioprinting method. Thus, by this method it was possible to create vessels with a diameter of 200 μm [252].

Another method used in tissue engineering is pressure-assisted bioprinting, to initiate the process of vascularization and angiogenesis [253]. In this technique, the bio-ink is pressurized and stored instead of a thermal or piezoelectric actuator, as is done in the direct bioprinting technique. Some researchers obtained infusible vascular structures loaded with very well-organized cells. They used an extrusion system and a mixture of bio-ink [254, 255]. Other researchers obtained infusible vascular structures loaded with very well-organized cells. They used an extrusion system and a mixture of bio-ink. A system of nozzles that are arranged coaxially was used to produce tubes with diameters between 500–1500 μm and vessel wall thicknesses of 60–280 μm. It can be considered that, by the method of bioprinting by pressure, together with the one of direct bioprinting made with bio-ink containing encapsulated cells, it will be possible to induce the formation of functional vessels [255].

Laser bioprinting is a less widely used printing method. This technique uses direct laser-induced transfer or the light curing process [256]. The method has the advantage that the cells can be printed with a very high resolution, without subjecting them to the so-called shear phenomenon [257]. In humans, through this method of biological printing with the help of laser, smooth muscle cells were obtained, interconnected ECs from the umbilical vein, and through the photopolymerization technique, tissues with a complex prevascularized structure were obtained [258]. Two weeks after the application of the biological impression, the obtained tissue was analyzed and a development of an anastomosis between it and the vessels of the host tissue was found [255]. Compared to the control tissue, a significant increase in the number of vessels as well as their density was observed in the tissue that underwent prevascularization.

3D printing has a great advantage because this technique can control angiogenesis and the appearance of new vessels in the tissues that have undergone changes. In the future, by developing 3D bioprinting techniques and increasing the accuracy of these methods, the prevascularization of many tissues can be achieved on a large scale.

Electrospinning

The electrospinning technique has been used by researchers in recent years. This manufacturing method uses a nanofiber-based electrospinning technique to obtain new vascular networks.

The electrospinning technique allows precise control over the diameter (50 to 500 nm, similar to the ECM's fibers), porosity and degradation rate of the newly formed fibers [259].

Kenar *et al.* used a poly(L-lactide-*co*-ε-caprolactone) (PCL) mixture (with collagen and hyaluronic acid) and significantly improved the vessels' length in modified tissues. This mixture was used to form a fibrous matrix, which implanted in the host tissue, promoted integration with the vascularization of this tissue [260].

Other studies have used electrospinning, implemented at the nanometer scale to produce matrices capable of being identical to the extracellular bone matrix and thus managed to significantly improve the process of angiogenesis by spatial organization of fibers [261, 262].

Biomaterials and their role in intracellular angiogenesis

To increase the performance of tissue regeneration through cell transplantation, release of GFs and gene therapy, the effective way to achieve these targets is represented by angiogenesis. Cell dynamics and implicitly angiogenesis is stimulated or inhibited by several factors, such as 3D cellular arrangement, the chemical composition of the ECM, cellular ultrastructure, as well as the physicochemical and morphological properties of the cells. Proteoglycans with hydrophilic groups and structural or adhesive glycoproteins, such as fibronectin, laminin, tenascin, vitronectin influence the mutual interaction between cells and surrounding patterns, making possible external transduction, *i.e.*, the initiation of specific signaling.

Integrins expressed on the cell surface represent the superfamilies of immunoglobulins representing adhesive molecules, such as cadherins, selectins, etc., and through them the cell can attach to surfaces. The juxtacrine cell–matrix interaction between cellular receptors and corresponding elements in the ECM induces a series of biochemical reactions at the cellular level, resulting in the maintenance of cell–matrix interaction, cell motility and migration, their growth, gene expression, improving the intercellular connection, as well as modulating the cellular phenotype. A reciprocal connection is made between the cells determined by the activation of the surface mechanoreceptors, which determine the juxtaposition of each cell with chemical groups from matrix. As a result of this connection between cells and matrix and by the involvement of adhesive proteins, cytoskeletal contractile agents will be released. The use of appropriate models, which have adequate physicochemical stability and combined with factors and components of the ECM, could reorganize the arrangement of cells to promote the process of angiogenesis.

Conclusions

Histology is the key element for the microscopic study of cells, tissues, and organs. It remains one of the fundamental elements of science, being essential in understanding and interpreting new scientific discoveries. If today, in the field of research, this discipline may seem outdated and surpassed by the *in vitro* cell and molecular biology', genetic' and proteomic' studies, it definitely remains the cornerstone (along with the *in vivo* study of tissues and organs) for the effective diagnosis in clinical practice. Although most new advances in science are submicroscopic, the final expectations of these discoveries will eventually be assessed on their microscopic effects on the cells, tissues, and organs of an individuum. HE staining is still

the "gold standard" for histological diagnosis, while IHC and molecular biology are ancillary tools that can provide additional information in confirming histological diagnosis.

Conflict of interests

The authors declare that they have no conflict of interests.

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