Tissue Engineering

Essentials for Daily Laboratory Work

W. W. Minuth, R. Strehl, K. Schumacher

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Important Note:
As research and clinical work are constantly expanding our knowledge, we would like to emphasize that when this book was written, all dosage and application specifications reflected the state of the art. However, users are strongly advised to check the instructions that come with the preparations and medical products used and use their own judgement on dosage according to specific recommendations in their own countries.

Finding Literature Using Search Criteria
Due to the rapid growth of information, constantly transforming our knowledge in the area of cell biology and tissue engineering , it made sense to collate a number of search criteria rather than a bibliography. Put in a medical or biological database such as PubMed or Biological Abstracts, these search criteria will lead you to the most up-to-date literature on the subject.

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Preface

Why this book at this time? A number of things have come together. In restructuring our lab, we needed to clear out, organize and archive. A lot of interesting material from the past was lying around that, for various reasons, was not being further investigated and thus had never been published. On inspection of the data and images, we realized that we had actually learned much more from unsuccessful experiments than from the successful ones that had seamlessly fit into the experimental design. When we came upon difficulties, we did not give up. We continually asked new questions and carried out further experiments until we came to logical explanations.

In addition, we have offered many courses in cell and tissue culture as well as tissue engineering over the years, for participants from both Germany and abroad. The participants often asked interesting and fundamental questions which were insufficiently or completely unanswered by previous books. To solve this problem, it was necessary to do a great deal of research in the various databanks. We have sketched, structured and worked the answers to those questions into the text as fundamental information.

Although we train students daily in microscopic anatomy, it has become increasingly evident to us how little is known about the development of functional tissues. However, it is exactly this aspect that is of particular importance for the future production of tissue constructs, from adult cells or stem cells, for use in patients. Socially interactive cell networks must be produced out of individual cells and implanted into the patient as functional tissue, and no health risks should be added in the process.

This book introduces theoretically fundamental and experimental concepts, which should open the door into the field of tissue engineering. Additionally, it should give students, technicians and young scientists a look into the fascinating world of differentiable cells and tissues. We must make clear that we stand at the beginning of a very exciting and future-oriented scientific development. For this reason, we must adjust ourselves to learning about the development of tissues. After sufficient experimentation, and in the course of this decade, tissue engineering will change from a purely empirical to an analytically reproducible science. We will get an overview of each step in tissue development and learn to simulate it experimentally. Apart from molecular biological processes, epigenetic factors and microenvironments will also play a major

roll. In addition, we must adjust to the fact that it will not be possible to generate functional tissues with cell culture methods.

Will W. Minuth, R. Strehl, K. Schumacher Regensburg, February 2003

Contents

1
Developmental processes

Cell, tissue and organ cultures today are no longer to be ignored, for a variety of reasons. For one, in recent years enormous progress has been made in the clarification of molecular and cell biological processes with the help of cultivated cells. Another reason is that without various cell cultures, the industrialized production of many medications and antibodies would be unimaginable. Finally, cultivated cells are repeatedly brought up in discussion as an alternative to animal experimentation.

All the cells of an organism can be isolated from tissue using the modern methods at our disposal today. In addition, nearly all cells can be cultivated without major difficulties for various purposes, both in analytically small as well as technically large scales. The scale can vary from single cells in a droplet to bioreactors with thousands of liters of culture medium. Through these techniques one can build on about 50 years of experimental experience in cell culture. Key phrases for the industrial use and the work associated with it are “cell culture engineering”, “metabolic engineering”, “bioprocessing”, “genomics”, “viral vaccines”, “industrial cell culture”, “medium design”, “viral vector production”, “cell line development”, “process control” and “industrial cell processing”. However, almost all of these terms involve a particular type of culture. The cells in question should divide as fast as possible in order to more efficiently synthesize a bioproduct, medication or vaccine. A wide variety of innovative instruments have been developed in recent years for all these techniques. In addition, these methods have been so well optimized that little increase in efficiency can be expected in the next few years. A great deal of information on this topic is available in previously published books.

Tissue culture, and therefore tissue engineering, must been seen very differently. The purpose here is to achieve, or produce, functional tissue and sections of organs through cultured cells. These constructs should support regeneration as implants or be used as bioartificial modules at the patient. Tissue engineering is a relatively young technique, building on 10–15 years of understanding in the field. For this purpose, whole branches of science in the areas of biomaterial research, engineering science, cell biology, biomedicine and individual disciplines in surgery must work closely together.

Considerable progress has been made in the production of artificial tissue with the presently available methods. Nevertheless, it is a fact that the constructs currently produced still do not have sufficient tissue specificity. Liver parenchyma in bioartifi-

cial modules shows only a fraction of the original detoxification capacity, implanted pancreas cells lose their ability to synthesize insulin over time, kidney epithelia tend not to maintain the necessary barrier and transport functions, and cartilage constructs build an extracellular matrix (ECM) with too little resistance to mechanical load. In addition, proteins that are not typical to the specific tissue are often synthesized by the constructs and can cause inflammation or even a rejection reaction.

In the media, one has the impression that many currently incurable diseases will very soon be treatable with cell therapy, tissue engineering or the manufacturing of organs. It is envisioned is that stem cells will primarily be used. In the spotlight, in particular, are embryonic stem cells whose future significance in this area is still undetermined and whose cell biological capacity seems to inspire enthusiasm without critique. On closer consideration, however, it becomes clear that most current knowledge has been obtained from pluripotent stem cells of the hematopoetic system. Far less experience has been gained in embryonic stem cells from experimental animals and there is very little truly validated experimental data for embryonic stem cells in humans. The existing results in this area often do not seem to be thrilling and highlight many unsolved problems.

There is, also, comparatively little knowledge about the development of totipotent human stem cells. In this case, international research will only show in the coming decade if the promises of many biotechnology firms hold up to critical analysis. The regeneration of functional tissue cannot be solved with isolated stem cells alone. Stem cells, as with all other cells, must first divide in sufficient quantities, form social networks and then develop into specialized tissues, through mechanisms still unknown at this time. These processes are carried out automatically in a developing organism. When trying to simulate these processes *in vitro*, however, one realizes that the characteristics developed in the constructs using the currently available strategies are insufficient.

From our perspective, future key issues to be clarified in tissue engineering are how functional tissue can be generated in culture and how the development of tissue properties can be individually controlled. Artificial tissues will only then be considered a meaningful form of therapy, when a disease can be overcome without harm to the patient. In order to do this, a tissue must exhibit the necessary functional characteristics as a regenerational tissue, an implant or biomodule.

Every day we are faced with all types of functional tissues in the adult organism, in terms of both macroscopic and microscopic anatomy. The adult organism and, therefore, the endpoint of development are fairly well known to us. There are, also, numerous verified discoveries in the early development of humans, as much research has been done in the field of embryonic development and germ tissues. The point and location that a tissue or organ originates from has been specifically studied. Surprisingly little is known, in contrast, about the mechanisms in the development of functional tissues. Understanding this development, however, is key to the production of optimal artificial tissue.

The only available databanks are not very productive if data regarding functional tissue development is requested. It may be surprising, but we could also not find any book about the processes of tissue development. Recently, however, increased

activity in this area can be observed. There are various attempts to explain the development of ground tissue with its functional facets through molecular biology. The driving force for this is certainly stem cells. It has been shown that individual functional tissues cannot even be developed out of this type of cell. Only precise understanding of the specific developmental physiology can lead to the generation of tissue.

In the area of regenerative medicine, there are many fascinating and unanswered questions, such as why certain cells in an organism cease to divide after months, years or life-long, whereas other cells are renewed after days. Often these processes even happen side by side in an individual tissue. This alone cannot be explained by the effects of growth factors or morphogenic substances. The microenvironment and cell interactions must have much more effect on the individual regenerational behavior. This means that future perspectives into the developmental needs of tissues must be sharpened and expanded accordingly.

[Search criteria: cell culture organ culture tissue culture tissue engineering]

2
Cells and Tissue

2.1
The Cell

Natural tissue as well as artificial tissue is composed of many different cellular elements and their associated ECM. The cells build multicellular networks and interact with the ECM. Before one can consider the production of artificial tissue, it is necessary to have a fundamental comprehension of cells and natural tissue. The following, however, can understandably convey only certain important aspects of microscopic anatomy.

2.1.1
The Cell as a Functional Unit

Human cells should first be schematically introduced as the smallest functional unit of life. It is generally accepted that a typical characteristic of a living cell is its adequate response to stimuli, such as hormones. Another typical property of cells is that they double their number at regular intervals. This is true for all embryonic cells, as well as all cells of the maturing organism. For cells in a tissue of the adult organism, on the other hand, there are specific differences. Cells in the intestinal epithelium are renewed within a few days, whereas parenchymal cells of the liver or kidney divide only after years. Heart muscle cells and neuronal cells will not normally divide again, even after a lifetime.

The human body possesses around 1×10^{13} tissue cells, living in close contact. In addition, 3×10^{13} blood cells, for the most part in isolated form, can be found in the bloodstream. At the same time, cell size varies widely. The diameter of glia cells (neuronal tissue) is 5 μm, that of sperm cells 3 – 5 μm, that of liver cells 30 – 50 μm and that of a human oocyte 100 – 120 μm.

As with the size, the shape of cells is quite variable. Between the round or spindle shaped and the strict geometric shape of cells in epithelia, all transition shapes can be found. The cell surface can be smooth or uneven. Furthermore, individual surface enlargements from single microvilli to specialized brush borders can be developed. Animal and human cells are surrounded by a selectively permeable membrane (Fig. 2.1), inside which the cytoplasm with the nucleus and other essential organelles

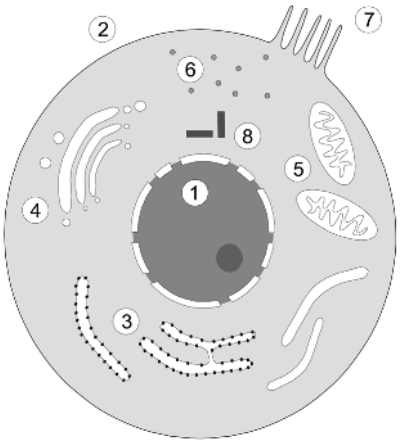

Fig. 2.1: Illustration of a cell with its organelles: nucleus (1), plasma membrane (2), ER (3), Golgi apparatus (4), mitochondria (5), secretory granules (6), microvilli (7) and centrioles (8).

are located. Under light microscopy, the predominantly basophilic cells can easily be differentiated from the mostly acidic cytoplasm.

2.1.2 Plasma Membrane

The plasma membrane is a biological membrane that divides physical/chemical compartments from each other. It is composed of a phospholipid bilayer, through which unipolar molecules such as O_2 and CO_2 can freely diffuse. It serves as a barrier to electrolytes, amino acids and sugars. Under light microscopy, it appears as a trilaminar structure: light–dark–light. Built into this lipid bilayer are numerous proteins that, among other tasks, through targeted transport or as hormone receptors have a mediator function for the information exchange between the cytoplasm and the extracellular environment. A cell membrane, however, is not a mechanically fixed and therefore rigid structure, but rather a fluid, viscous and, accordingly, fragile mantle. The individual phospholipids and the membrane proteins, both, are more or less mobile within this layer. Apart from phospholipids, other lipid molecules, such as cholesterol, are present which provide a certain amount of stability in the bilayer. The outer lipid layer of the plasma membrane contains many glycolipids and glycoproteins, whose sugar residues, oriented outwardly, form their own layer, referred to as the glycocalyx. The proteins that are built into the plasma membrane are made of integral and associated membrane proteins, each with hydrophobic and hydrophilic sections. The hydrophobic sections provide anchoring in the lipid layer, whereas the hydrophilic sections reach out to the extracellular space or into the cytoplasm. Many of these proteins are actually glycoproteins. Functionally, they are transport proteins for electrolytes and amino acids, receptor proteins for hormones or anchoring proteins.

One of the main functions of the plasma membrane is as a diffusion barrier. It can control which molecules pass into or out of the cell by means of various active or passive transport processes. A further function of the plasma membrane specific

to tissue cells is its communication ability. Cells are able to communicate with each other over the plasma membrane and build mechanical cell contacts through tight junctions or communication channels through gap junctions. This serves to control cellular exchange, as well as cell recognition or signal processing. These functions are particularly important when social networks develop from isolated cells and, from there, form functional tissue.

2.1.3 Nucleus

With the exception of red blood cells or erythrocytes, all human cells contain a nucleus. The most important component of the nucleus is the chromosomes. They contain the complete set of genetic information. In addition, the nucleus is the control organ for many cell functions. The nucleus, with individual chromosomes, can only clearly be seen under light microscopy during interphase, i.e. between mitotic cycles. In a similar manner, the nucleolus is only observed during this phase. A cell has, as a rule, only one nucleus. However, some cells of particular tissues may have two or even more nuclei. These can be found in the parenchymal cells of the liver, in osteoclasts and in striated musculature.

2.1.4 Mitochondria

The mitochondrion represents the power station of the cell and is a carrier of enzymes, which enable it to produce energy, in the form of adenosine triphosphate (ATP). The characteristic reaction processes in the mitochondria are the energy-producing citric acid cycle and the β-oxidation of the fatty acids. In places where many mitochondria are found within a cell, it can be assumed that synthesis or working processes with increased energy requirements are also taking place there. This process can be identified by, among other things, the fact that the plasma membrane is strongly folded (Fig. 2.2). Within the folds are many mitochondria. Physiological transport investigations in such cells have shown that here increased energy-consuming transport pumps are also inserted, which manage the increased cellular exchange. Such processes can be clearly observed as a morphological correlate on the cells in the salivary glands.

2.1.5 Endoplasmic Reticulum (ER)

The ER plays the decisive role in protein synthesis. Cytoplasmic proteins are built on free ribosomes (polyribosomes), whereas proteins of the plasma membrane as well as secretory proteins are built in the ER. In the cytoplasm, ribosomes can exist individually or in chains, referred to as polysomes. Polyribosomes are connected by a single-strand messenger RNA (mRNA). The oxygen-binding protein, hemoglobin, for

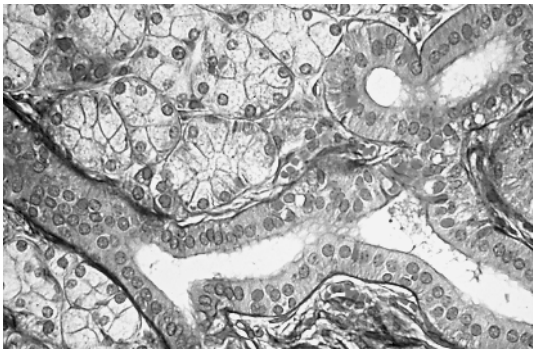

Fig. 2.2: Histological representation of an exocrine gland with part of the duct. The basolateral plasma membrane is largely unfolded. Mitochondria that supply the necessary energy for the pumps in that area are inserted in the folds of the plasma membrane. Due to the folding, the nuclei are crowded into the luminal cell side.

example, is formed on such polyribosomes. Ribosomes involved in the formation of glycoproteins and lipoproteins, on the other hand, do not simply release their protein product into the cytoplasm, but pass it on into the lumen of the ER. The ER is a net-like membrane system of tubules and cisterns, found throughout the cell. It is partly covered with numerous ribosomes and designated as the rough ER (rER). Ribosomes are macromolecules composed of proteins and ribonucleic acids, and not contained within a membrane.

2.1.6 Golgi Apparatus

The Golgi apparatus is found in direct vicinity to the ER. Depending on the cell type, it consists of a varying numbers of dictyosomes and Golgi vesicles. The dictyosomes, or Golgi fields, appear in electron micrographs as stacks of membranous sacs, surrounded by numerous vesicles. In the Golgi apparatus, transport vesicles coming from the ER and containing newly synthesized proteins are processed. As an example, proteins delivered to the Golgi are modified with particular sugar molecules (glycosylation). The end result is glycoproteins or proteoglycans. Frequently, proteins are only biologically active after this step.

2.1.7 Endosomes, Lysosomes and Peroxisomes

Endosomes and lysosomes are a heterogeneous group of organelles, which serve very diverse metabolic processes. Lysosomes are membrane vesicles with particular enzymatic equipment for intracellular metabolic processing, separation and digestion. The metabolic products produced in the lysosomes can be passed on into the surrounding cytoplasm or reused if necessary. On the other hand, lysosomes also serve as a storage place for metabolites that cannot be further broken down. They are then referred to as residual bodies and can be seen as pigment or lipofuscin granula for diagnostic purposes. If the contents of the lysosome enter the cytoplasm uncontrolled, the entire cell, as well as the adjoining cells, can be destroyed by autolysis.

Peroxisomes do not occur in all cells. On the other hand, some cells, e.g. liver cells or tubule cells of the kidney, are particularly rich in peroxisomes. The most important function of these organelles is to house hydrogen peroxide-producing oxidases and catalases, which play an important roll in gluconeogenesis, fat metabolism and various detoxification reactions.

2.1.8 Cytoskeleton

The cytoskeleton (Fig. 2.3) forms the scaffold for other important components of the cell. It consists of microtubules, microfilaments and intermediate filaments. These form a micro-network and function as the skeleton of the cell. Important proteins of this network are tubulin, actin filaments, myosin filaments, the many different keratins, nexins, vimentin, desmin and neurofilaments. Microtubules serve the directed transport of molecules within the cell. Neurons, for example, can possess axons which are 1 m long. Even the synapse, as the end of the neuron, must be controlled by the neural cell body. With the microtubule system, a transport speed of up to 400 mm/day is ensured so that even the most distant end of the cell is provided for.

Microfilaments such as actin filaments and myosin filaments are found in cells in differing quantities. Cells that form extensions, and change form and location exhibit a particularly large amount of microfilaments. Intermediate filaments, such as cytokeratins, build the skeletal system in epithelial cells, and give them their specific shape and stability.

2.1.9 ECM

Most cells produce not only their own organelles, but also proteins of the surrounding ECM. This is an interactive scaffold that provides mechanical stability and cell anchorage, and is also able to control cell functions. In building the ECM, cells synthesize mainly high-molecular-weight fibrous proteins, which are secreted out of the cell and

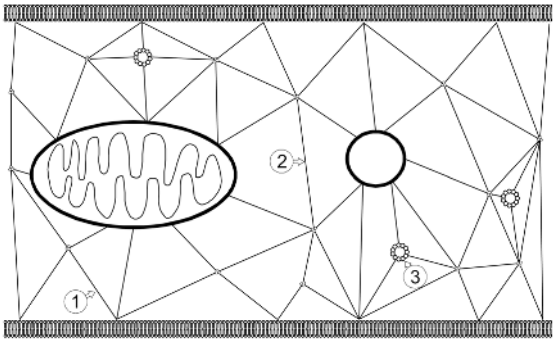

Fig. 2.3: The cytoskeleton of a cell consists of microfilaments (1), intermediate filaments (2) and microtubules (3). From the three-dimensional linkage of these structures, a meshwork results into which the individual cell organelles, such as the mitochondria, are built. Thus, in homogenous tissue cells, the organelles are always found in the same position.

built up in the surrounding environment to form an insoluble network. In epithelia or muscle cells this is a leaf-like basement membrane, whereas connective tissue cells form a three-dimensional network, called the pericellular or ECM. The basement membrane and the pericellular matrix consist mostly of the same protein families; however, due to the varied amino acid sequences, the individual components are differently interconnected. Components of the ECM include the various collagens, laminin, fibronectin and individual proteoglycans. In many tissues the ECM is soft and elastic, whereas mechanically strong structures are formed in tendon, cartilage and bone.

2.1.10 Cell Cycle

Cells must proliferate in order for tissues to develop, as well as for the replacement of dead cells through regeneration in the adult organism,. This is carried out within the framework of the cell division cycle (Fig. 2.4). First, cells double their contents and replicate their DNA in interphase. Next, the cells divide in mitosis. A cell in interphase can usually be recognized by the clearly defined nucleolus. If the decision is reached for a cell to divide, the cell continues into the G_1 phase, where the formation of important molecules, such as RNA, proteins and lipids, take place within about 24 h. In addition, the volume of the cell increases. In the subsequent S phase, the DNA in the cell is replicated. If this important phase is complete, the cell continues into the G_2 phase. Replication of the DNA is completed and everything is prepared for the actual division of the cell.

Mitosis itself takes about 4 h. In prophase, DNA/histone complexes condense into 46 chromosomes. The mitosis spindle is formed on the developing centrioles. The nuclear envelope and the nucleolus dissolve. Phosphorylation of the lamina in the nuclear membrane follows and, eventually, reusable vesicles are formed again. In metaphase, chromosomes arrange themselves in the equatorial plane or at the site of future division, each chromosome consisting of two sister chromatids. At this stage, long and short sections of the individual chromosomes are clearly visible under light microscopy. As the process continues, the chromosomes divide into the sister chromatids and, with the assistance of motor proteins, are transported along the mi-

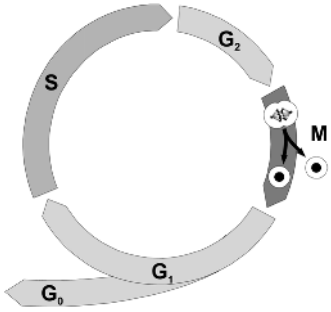

Fig. 2.4: Schematic of the cell cycle, which is divided into the G_0, G_1, S and G_2 phases. The actual division of the cell takes place in the M phase.

crotubules to the centrioles during anaphase. In the following telophase, a new nuclear envelope is synthesized. Cell division is terminated by the production of a ring of actin and myosin filaments, which cleaves the cell in two. In this phase of cytokinesis, each daughter cell receives one of the newly synthesized nuclei and half of the cytoplasm, along with necessary organelles.

Depending on the tissue type, cells can divide within days or only after months or years. In addition, some cells will not divide again during the life of the organism. Non-proliferating cells are said to be in G_0 phase.

[Search criteria: cell cycle mitosis division interphase]

2.2 Tissue Types

The development of cellular networks in complex organs is reflected in the structural and functional characteristics of tissues. Tissue is not only an accumulation of individual cells, but consists of defined cellular and specific extracellular structures. Both parts are functionally irreplaceable.

Surprisingly, humans only possess four different kinds of basic tissue – epithelia, connective tissue, muscle tissue and nervous tissue. From these come four completely different functions, such as the division of the organism from other compartments, the connection of structures, movement and control.

No organ of the body consists of only one basic tissue. Nearly all need each of the four tissues in a particular arrangement in order for each special function to become effective. The vascular system is one example. It consists of epithelial tissue, which lines the vessel lumen, smooth muscle tissue, in order to change the blood flow, nervous tissue, for controlling the rate of blood flow, and connective tissue, which connects the individual structures to each other and the surrounding environment. Tissues can consist of either homogenous or quite different cell types. Particularly characteristic is that clearly defined social contact (sometimes close, sometimes loose) is cultivated for the maintenance of specific functions in individual tissues.

Typically, one finds many mobile cells in tissue, such as leukocytes, plasma cells and macrophages, which react to cell metabolites, antigens or bacterial infection, and thus serve in immunological defense. Accordingly, few of these cells are to be observed in healthy tissues, whereas the cell number drastically increases during illness.

[Search criteria: tissue muscle epithelium connective neural]

2.2.1 Epithelia

The epithelia consist of geometric, spatially closely connected cells, which are anchored to a basement membrane (Fig. 2.5). Virtually no intercellular substance is to be found between epithelial cells.

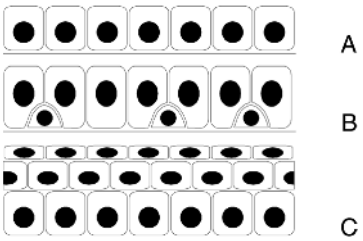

Fig. 2.5: Structural drawing of single squamous and pseudostratified epithelia. It is typical of all epithelia that the cells have a particularly close relationship to neighboring cells and are anchored to a basement membrane. (A) Schematic illustration of cuboidal epithelia. The basal side of each cell is anchored to the basement membrane and the apical plasma membrane borders the lumen. The lateral cell borders are in contact with the adjoining cells. (B) With the pseudostratified epithelia, several cell types are present. All cells are anchored to the basement membrane, but not all reach the lumen, giving the illusion of multiple layers. (C) With the stratified epithelia, only the basal cell layer has contact to the basement membrane.

Epithelial tissue forms a multitude of biological barriers – the central function of epithelia in an organism. It covers surfaces in a layer of closely connected cells, thereby forming a barrier between air- or liquid-filled compartments of the body. For this reason, it is the epithelia alone that determines what is taken up by or excreted by the body at the cellular level. It regulates the uptake of gas and fluid, and output by means of active or passive transport mechanisms. Epithelial cell layers have, for the most part, no gaps between cells and, with the exception of the stria vascularis in the inner ear, have no blood vessels.

Epithelial cells sit with their basolateral side on a basement membrane. The basement membrane is the structural element which separates epithelia from the connective tissue beneath it. If this barrier is no longer functionally intact, carcinoma cells can leave the epithelial compartment and infiltrate the connective tissue.

At their surface, epithelia exhibit various cell differentiations. On the one hand, they may have a more or less smooth surface. On the other hand, there may be a dense brush border for surface area enlargement or kinocilia for increased transport function. Characteristic of all epithelia is their polarization. This means that each cell has one side oriented toward the lumen and one toward the basement membrane, through which uptake and output of molecules take place.

2.2.1.1 **Building Plans of Epithelia**

Epithelia lining the body surfaces may consist of one layer (simple), multiple layers (stratified) or one layer appearing to be multiple layers (pseudostratified). Epithelia can take on completely different forms. They may be flat (squamous), cuboidal or cylindrical (columnar). Simple epithelia are characterized by the contact of all cells with the basement membrane. Flat epithelia form the typical squamous-shaped epithelia which occur in the lining of blood vessels as endothelial cells. Vascular endothelial cells are continually exposed to the bloodstream and therefore need to be anchored particularly firmly to the basement membrane (Fig. 2.6). Adhesion molecules for leukocytes, in the form of selectins, are located on the endothelial surface are. These are able to bind sugar molecules on leukocytes and thus facilitate their exit from the bloodstream. Endothelial cells further possess contractile filaments, with which they can regulate the width of their intercellular gap, to a certain extent. Endothelial cells additionally

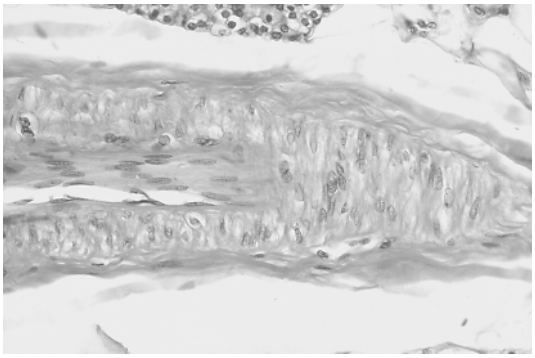

Fig. 2.6: Microscopic view of an arteriole – diagonal cut. The lumen is left in the center, lined by endothelium. Numerous smooth muscle cells are found in the media of the vessel wall.

produce nitrogen oxide (NO), which leads to a reduction in the smooth muscle tone surrounding blood vessels and thus to a increase in flow.

Single squamous epithelia, however, occur outside of blood vessels as well. They line the alveolar space in the lung, and provide a short diffusion gap for carbon dioxide and oxygen, due to their flat shape. Beyond that, this type of epithelia is found both in the thin part of the loop of Henle (kidney) as well as in the epithelial lining of the serous pleura and the peritoneum.

Cuboidal epithelia can be recognized under light microscopy by the fact that their cell width and height are about the same (Fig. 2.5A). Cuboidal cells are found, among other places, in renal tubule structures, where they serve in transport processes, and in urine production or in salivary glands, where they are active in saliva production. Likewise, cuboidal cells in the follicles of the thyroid are shown to work as storers and donors of hormones. Columnar epithelia are higher than they are wide, and line the lumen of the whole small and large intestine in the form of enterocytes, for example, where they serve the uptake of nutrients.

In common with simple squamous epithelia, pseudostratified epithelia cells are also in contact with the basement membrane (Fig. 2.5B). However, they differ from simple squamous epithelia in that not all cells reach the upper surface of the epithelia and the cells, as well as their nuclei, are at different levels. The pseudostratified epithelia of the respiratory tract are specialized with moveable kinocilia on their surface and are therefore referred to as ciliated epithelia (Fig. 2.7).

Cells are found in three different layers in pseudostratified epithelia, one above the other (Fig. 2.5C). Basal cells are anchored to the basement membrane and are not in contact with the epithelium surface. From this basal cell layer, epithelial cells are regenerated continually and life-long from stem cells. In the intermediate zone, immediately above the basal cell layer and in the luminally situated stratum superficiale, the cells are no longer in contact with the basement membrane. The cells of the outer epithelium surface are periodically sloughed off. In stratified squamous epithelia, the surface cells are flattened and, in contrast to the basement cells, oriented parallel to the epithelium surface. The basal cells are usually cuboidal to columnar. Cells of the intermediate zone lose this orientation. They become polygonal and their nuclei become more parallel to the epithelium surface. The mucous membranes from the oral

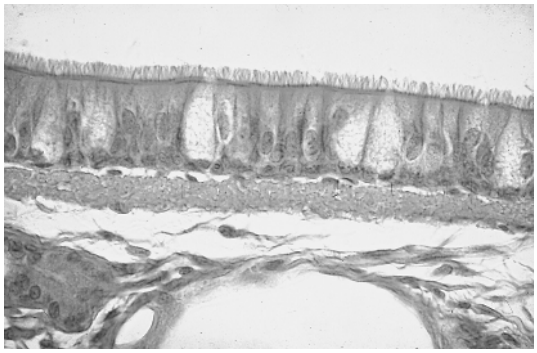

Fig. 2.7: Histological illustration of pseudostratified ciliated epithelium in the respiratory tract. The epithelia borders the airway. The luminal epithelial side with kinocilia serves the function of cleaning and moves dirt particles toward the oral cavity.

cavity to the lower third of the esophagus (Fig. 2.8), of the vagina, as well as transition areas of the urogenital and digestive tract to the outer skin exhibit this stratified squamous epithelia.

In contrast to the stratified squamous epithelia in the mucous membrane of the mouth, the stratified squamous epithelia of the outer skin is keratinized. The stratified squamous epithelium of the skin shows characteristics of the dynamic keratinization process, which begins in the stratum granulosum. The regenerative basal cells lie in contact with the basement membrane. However, they do not occur here alone, e.g. neighbor melanocytes, which through their pigment are responsible for the brown coloring of the skin. In the intermediate zone is first the stratum spinosum. The spiky appearance of this cell layer is due to the numerous occurrences of desmosomes, into which bundles of condensed cytoskeletal components lead. These cellular characteristics serve as protection against shearing stress which can affect the outer skin. In the next layer, the stratum granulosum, are the cytoplasmic keratohyaline granula, which contain the protein filagerin, visible under light microscopy, to a high degree. Apart from the transverse cross-linking of proteins, the superficial cells experience organelle degradation, including that of the nucleus. In the end, the cells of the stratum corneum consist only of a closely packed keratinized substance, surrounded by a modified cell membrane. The Langerhans cells, which carry out immunological tasks, are also next to the melanocytes.

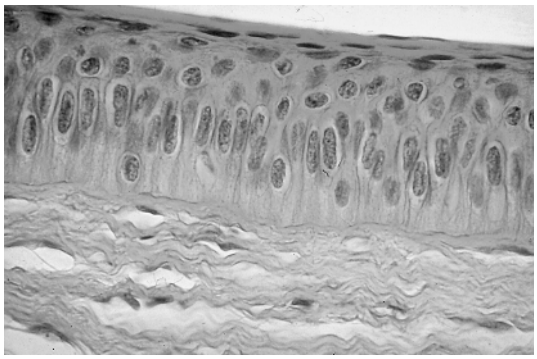

Fig. 2.8: Histological illustration of the non-keratinized stratified epithelium of the oral cavity. The epithelial cells form close cellular networks. Thus, a biological barrier between the luminal and basal sides of the epithelium develops.

A further stratified epithelium is the transition epithelium, also known as the urothelium, which is exposed to urine on its luminal side. Biologically aggressive substances, such as urea and the changing pH of the urine, have led to its particularly pronounced tight junctions and the condensed cytoskeletal elements found on the luminal side of its cells. These consist of actin filaments and intermediate filaments, as well as uroplakin. The superficially located cover cells have a polygonal form and branch-like cell extensions, which should reach the basement membrane. In this sense they differ substantially from the other stratified squamous epithelia. The name transition epithelia suggests that the epithelium can be stretched to accommodate different volumes and because of this can present a large range of cell heights.

[Search criteria: tissue epithelial morphology histology]

2.2.1.2 Glands

Glands result from cells of the surface epithelia budding into the connective tissue beneath. If the gland forms a duct, one speaks of an exocrine gland (Fig. 2.9A and B). If, however, this developed epithelium loses its contact with the original surface epithelium, the island remaining in the connective tissue can secrete only into the

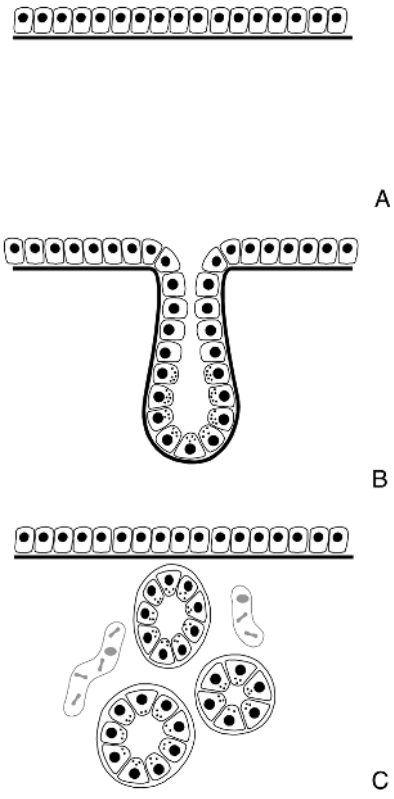

Fig. 2.9: Schematic illustration of gland formation. (A) Exocrine and endocrine glands are formed from the simple squamous embryonic epithelium. (B) A glandular duct results from the invagination of the epithelium into the connective tissue beneath it. If the lumen maintains contact to the surface, then an exocrine gland is formed. (C) If the encompassed epithelial cells lose contact to the lumen, an endocrine gland develops. At the same time, capillaries are increasingly developed in this area.

interstitium and into capillaries. One speaks of the development of an endocrine gland and of internal secretion, whereby the secretion contains hormones (Fig. 2.9C).

Glandular tissue consists of epithelial cells, which form a substance and then secrete this from the cell. The basolateral cell side remains in constant close contact with blood vessels, since it needs to take up numerous nutrients from the blood for synthesis. Secretion takes place on the luminal side of the acinar epithelia in the salivary glands, whereas the hormone is always delivered toward the capillary with endocrine glands.

If a connection between the epithelia grown into the connective tissue and the surface epithelium remains, the secretion formed in the gland will exit through a duct. The secretion can then be modified by special cells of the duct in terms of its water and electrolyte composition, similar to in the kidney. This process is possible, for example, in duct epithelia of the parotis (Fig. 2.10). The cells of exocrine glands are polarized, since they take up material from the interstitium over the basolateral side and secrete from it, which is then transferred to the luminal side into the duct. Whole organs, like the salivary glands, can have a purely exocrine function.

Apart from purely exocrine functions, endocrine output can be found in a gland. The classical example is the pancreas with the endocrine islets of Langerhans, which deliver insulin and glucagon into the blood stream in order to regulate sugar metabolism. The exocrine portion of the pancreas produces digestive enzymes, such as amylase and lipase, which are then delivered into the ductus pancreaticus and further into the duodenum.

The end sections of exocrine glands are distinguished by their type of secretion – serous, mucous or mixed seromucous end sections. The epithelia of the gland show corresponding histological characteristics (Fig. 2.10). The cells of the serous end sections possess a round nucleus, which lies in the center of the cell. The cytoplasm presents itself as homogenously reddish in routine staining. The serous secretion is non-viscous and enzyme-rich. In the cells of mucous glands, on the other hand, the nucleus is markedly flattened and lies near the basolateral side of the cell. The cytoplasm appears foamy and whitish. The secretion is more viscous and contains fewer enzymes than the serous secretion. A mucous gland cap sits at the end of some serous end sections and these areas are then designated as Ebner half moons.

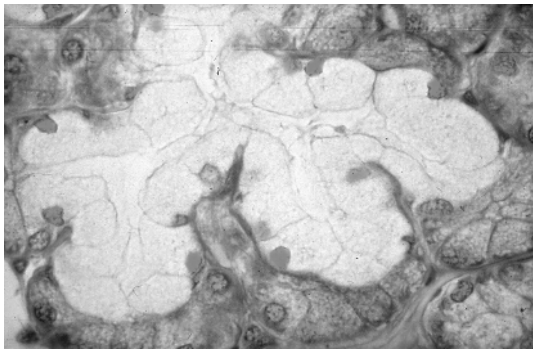

Fig. 2.10: Light microscopy view of a salivary gland, which is composed of mucous and serous acini.

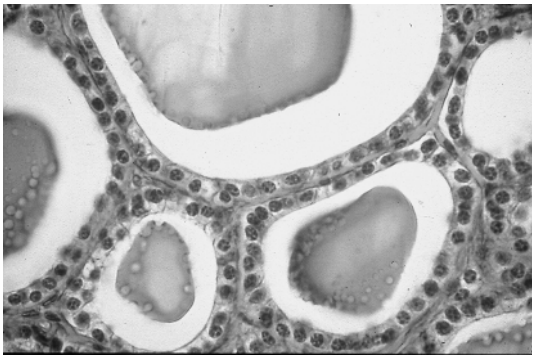

Fig. 2.11: Histological representation of a thyroid. The epithelium forms balloon-shaped follicles, which are filled with colloid in their lumen.

Secretions are delivered by glandular cells in completely different ways. The merocrine form of secretion is based on exocytosis. The intracellular secretory vesicles fuse with the luminal plasma membrane, whereby the secretion is delivered outwardly, without any loss of the cell membrane. In apocrine secretion, the apical portion of the cell, containing secretory products, is pinched off. This process then includes a structural loss to the cell. With holocrine secretion, the secretory product in the cell is released as the cells apoptose and are sloughed off.

The glandular end sections can also take on different forms. In principle, they can be tubular or coiled and tubular. Acinar end sections have a sac-like form. It may be that both forms are found in a gland, with large common end sections. This is then referred to as a compound gland.

Usually the hormone-producing, i.e. endocrine, cells are not polarized. An exception here is in the thyroid, where polarized epithelium is exhibited in an endocrine gland (Fig. 2.11). Here, polarization serves the storage of hormones, which can be mobilized as necessary for delivery into the interstitium.

[Search criteria: glands morphology histology mucous serous seromucous]

2.2.1.3 Epithelia in Sensory Perception

Sensory epithelia are groupings of cells tat can receive and transmit stimuli. In the retina they serve vision; in the inner ear, hearing. They are taste cells (Fig. 2.12), and mechanoreceptors in the outer layer of the skin and on the roof of the nasal cavity are the olfactory epithelium.

In principle, the receptors of sensory epithelia can be divided into primary and secondary groups. Primary sensory cells receive a stimulus on one side of a cell and pass on the excitation over its own axon. This is the case in olfactory cells (Fig. 2.13). They can also be described as nerve cells, which express receptors for certain olfactory molecules at one end. Thus, the olfactory epithelium is the only place in the body in which a nerve cell has direct contact with an exposed surface. Secondary sensory cells, on the other hand, have one sensory end with the appropriate receptors, but are connected synaptically to nerve cells on the other end, such as in the taste epithelium.

Sensory cells never comprise the epithelium alone, but always occur in combination with basal cells and support cells. Basal cells are thought to serve as stem cells for both