Introduction to Histology

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2.1 Introduction to Histology

"Histology" is the examination of normal cells and tissue and is performed with the aid of a microscope. In contrast, "histopathology," a subdiscipline within pathology, refers to the study of diseased tissues, and will be discussed separately in Chap. 3. Histologists have the specialized skills necessary to process and stain various tissue samples, while histopathologists are physicians with the skills necessary to interpret the histological slides. The routine specimen types received in a histology laboratory, and their preparation using routine histological techniques, are first introduced below, followed by an introduction to the important components of a normal human cell and the histology of various normal tissue types.

2.2 Specimen Types

Specimens received for histological examination include both cytology specimens and histopathology specimens, examples of which are listed in Table 2.1. Cytology specimens are taken with the aim of examining tissue at a cellular level [1]. These specimens, therefore, include samples of free cells or tissue fragments. The most common sample type is a fine needle aspiration (FNA), where a very thin needle and a syringe are used to acquire a small amount of cells or fluids from a lesion (e.g. thyroid cyst [2]). Bodily fluids, such as urine [3, 4], or cerebrospinal fluid [5], etc., can also be processed. Another special sampling technique is when cells are gently scraped or brushed from an organ (e.g. cervical smear [6]). In contrast, histopathology specimens include whole organs or small samples of larger tissues. A needle core biopsy is the most common type of sample where, in comparison to a FNA, a large needle is used to remove a greater quantity of tissue. Other sampling techniques available to clinicians include excisional biopsies where an entire lesion is surgically excised, or incisional biopsies where part of a larger lesion is removed.

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Table 2.1 Examples of cytopathological and histopathological specimens

Cytopathology	Histopathology
Fine needle aspiration (e.g. thyroid cyst)	Biopsies (e.g. biopsy of a breast mass)
Smears (e.g. cervical)	Surgical specimens (e.g. prostatectomy)
Bodily fluids (e.g. urine)	Autopsy specimens (e.g. kidney)

2.3 Specimen Examination and Sampling

Grossing of histopathology specimens [7] involves careful examination by the pathologist including a specimen description, weight, and measurement of dimensions [8]. Photographs can be taken and relevant surgical margins inked. Thorough dissection is performed in order to locate representative areas suitable for sampling. Biobanking [9] can also be completed at this stage, which involves taking small samples of fresh tissue and is used, for instance, to create cell lines, isolate stem cells, generate organoids or ex vivo organotypic cultures [10, 11] for storage in a tissue biobank. Touch preparations (or "touch preps") can also be made using fresh tissue [12, 13], where the specimen is gently touched against a clean glass slide, an imprint made, and later examined.

2.4 Preparation of Histological Slides

The sampled tissue is placed into a plastic cassette and undergoes a series of steps in order to prepare the tissue for histological examination. Table 2.2 highlights the various stages of tissue preparation and summarizes the purpose of each stage mentioned below.

2.4.1 Fixation of Tissue

Fixation involves submerging the sampled tissue in chemical substances (i.e. fixatives) in order to prevent tissue digestion by enzymes or bacteria and to preserve as much as possible of its morphologic and chemical characteristics. Fixatives promote cross-links between proteins and form a gel that maintains the in vivo relations of tissue components to each other [14]. There are a number of reagents that can be used for fixation, each of which has differing penetration rates. Formaldehyde [15] is the most commonly used agent for histopathology and when dissolved in water, it is referred to as "formalin." One part formalin is typically diluted with nine parts water to produce a 10 % formalin solution, a concentration that is optimal for tissue fixation [16]. This solution penetrates tissue at about 1 mm an hour [17], therefore, biopsies are generally submitted for processing the same day as received, while larger specimens (e.g. a mastectomy) are not processed the same day as received as they require a longer fixation period.

Table 2.2 The stages of tissue preparation and the purpose of each stage

Stage	Purpose
Sampling	To choose the most representative areas of the specimen
Fixation	To preserve tissue morphology and chemical composition
Dehydration	To remove fixative and cell water and replace with dehydrating fluid
Clearing	To remove dehydrating fluid and replace with clearing fluid
Embedding	To impregnate with liquid paraffin and make the tissue resistant to sectioning
Sectioning	To make tissue sections available for histological analysis

2.4.2 Processing of Tissue

The processing of tissue includes dehydration, clearing, and embedding steps. Dehydration involves the removal of fixative and water from the tissue and their replacement with dehydrating agents by placement in increasing concentrations of ethanol. Next, the clearing step involves replacing the dehydrating fluid with a lipid solvent (e.g. xylene). The tissue is subsequently removed from the cassette and placed in a molten wax-filled mold for embedding. At this point, orientation of the tissue within the mold is critical, as it will determine the plane through which the section will be cut. Incorrect placement of tissues may result in diagnostically important areas being missed or damaged later [18]. Elongate tissues should be placed diagonally across the block (e.g. core biopsy), while tubular structures (e.g. vas deferens) are embedded so as to provide transverse sections showing all tissue layers. Specimens that have an epithelial surface (e.g. skin surface) are embedded in such a way as to provide sections in a plane at right angles to the surface.

2.4.3 Sectioning of Tissue

The waxed cassette is next placed in an instrument with fine blades called a microtome. Rotation of the drive wheel moves the block holder a controlled distance forwards, the blade's edge strikes the tissue block, and thin sections are cut and affixed to a glass slide. Sections are usually four microns thick so that a single layer of cells can later be seen under the microscope. Following thorough drying of the tissue sections are ready for staining.

2.5 Staining of Tissue

As most tissues are colorless, methods of staining tissues have been developed to make them visible, while also allowing distinctions to be made between tissue components. This is done by using mixtures of acidic or basic dyes that selectively stain various tissue elements. The constituents that react with basic dyes do so because of acid in their composition (e.g. nucleoproteins), while acidic dyes stain basic tissue components (e.g. cytoplasmic proteins). Of all routine stains, the combination of hematoxylin and eosin, or the "H&E stain" [19], is the most commonly used and dates as far back as the 1870s. This stain is considered the gold standard in histology and in a typical tissue section, nuclei are stained blue/purple, whereas the cytoplasm and surrounding matrix have varying degrees of pink staining [20]. Therefore, the H&E stain has the ability to reveal structural information with specific functional implications. Special stains [21] use a slightly different technique to stain particular structures (e.g. Masson's trichrome for muscle and collagen fibers [22]) or pathogens (e.g. Ziehl–Neelsen for acid-fast bacteria [23]). When routine or special staining cannot provide all the diagnostic answers required, histopathologists can use advanced staining techniques including immunohistochemistry (IHC) or in situ hybridization (ISH).

2.5.1 Immunohistochemistry

IHC is a multistep technique involving the interaction of a target antigen (i.e. the protein of interest) with a specific antibody tagged with a visible label [24]. The aim is to detect the presence of elevated levels or the absence of a particular target antigen. Antibodies can be coupled with an enzyme, such as horseradish peroxidase (HRP), requiring the use of a light microscope for visualization, or with fluorescent chemical compounds requiring the use of a fluorescent microscope. Different labeling techniques are available including the direct or indirect labeling method. In a direct assay, a fluorophore-labeled antibody, such as fluorescein isothiocyanate, can react directly with the target antigen. This tag allows immediate visualization of the antigen. In contrast, in an indirect assay, an unlabeled primary antibody is used. This binds to the target antigen and an enzyme-labeled secondary antibody binds to the primary antibody. In general,

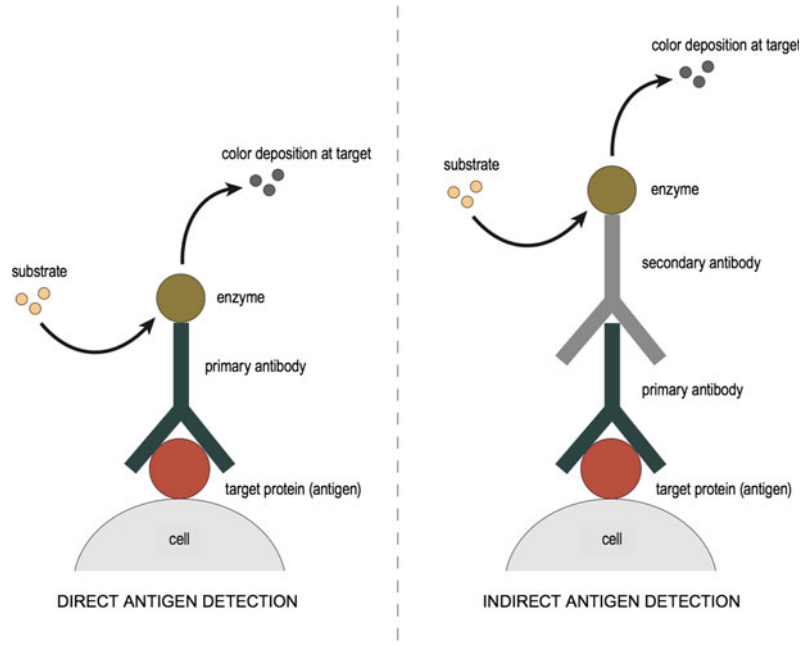

Fig. 2.1 Illustration of immunohistochemistry

primary antibodies are raised against the antigen of interest and are unlabeled, while secondary antibodies are raised against IgG of the primary antibody. Finally, a chromogenic substrate must be added for visualization (e.g. diaminobenzidine), which reacts to produce a brown precipitate in the presence of the HRP enzyme. A simplistic illustration of these two assays is shown in Fig. 2.1. Recent advances in this area include multiplexing of antigens [25, 26], whereby multiple stains can be performed on the same tissue section, followed by analysis using digital imaging software.

2.5.2 In Situ Hybridization

While IHC involves the detection of marker proteins in tissue, ISH can detect target ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) sequences [27]. Different detection systems are then used to visualize the presence of the target sequence. Fluorescence ISH (FISH) uses fluorescent dyes and fluorescent microscopy while chromogenic ISH (CISH) uses chromogenic dyes and brightfield microscopy [28]. In this process, a complementary DNA strand (or probe), or RNA strand (or riboprobe) is used to localize a specific DNA or RNA sequence. The probe hybridizes to the target sequence with elevated temperature (i.e. denaturation) and excess probe is washed away. If the probe is already fluorescent, it will detect the site of hybridization directly. If the probe is chromogenic, an additional step is needed to visualize the probe [29]. A simplistic illustration of ISH is shown in Fig. 2.2. Multiplexing using ISH can also be performed [30], followed by spectral imaging for the detection and subsequent deconvolution of multiple signals.

2.6 The Frozen Section

The "frozen section" is an alternative tissue preparation technique where, in contrast to routine processing, is a rapid histological examination done on fresh tissue. The sample is quickly placed into cryoprotective embedding medium and cut in a refrigerated microtome (i.e. cryostat). The sections are then stained with H&E. This technique is used, for example, where the surgeon needs a tumor margin to be examined to ensure that it has been adequately removed, or to

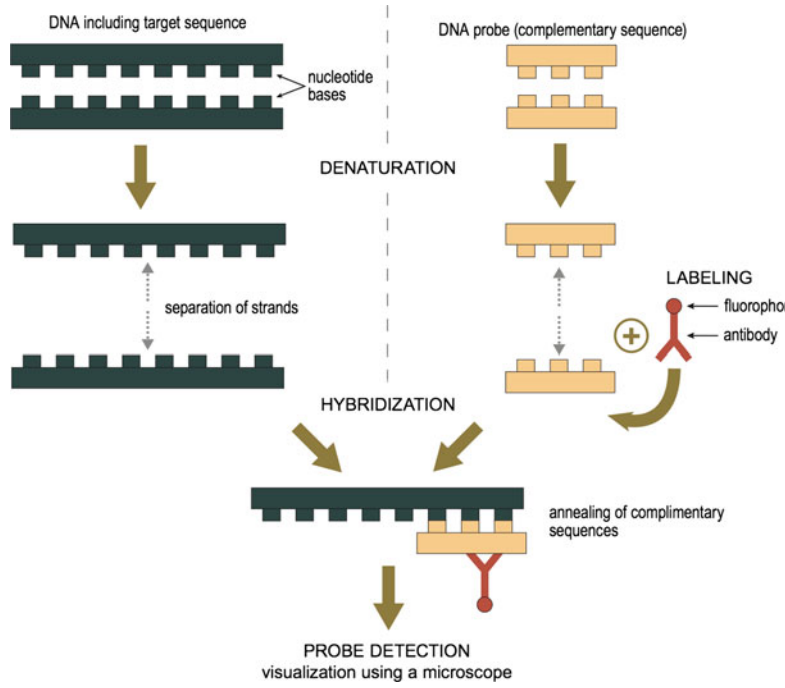

Fig. 2.2 Illustration of in situ hybridization

confirm a diagnosis of cancer intraoperatively [31, 32]. The diagnosis should ideally be conveyed to the clinician within 20 min after receipt of the tissue within the pathology laboratory, termed "the turnaround time" [33]. Despite the speed of the procedure, one major disadvantage of the frozen section technique is that "freezing artifacts" are frequently seen which can obscure tissue morphology and cellular detail. Examples of freezing artifacts seen, include nuclear ice crystals, bubbles, vacuolated cytoplasm, nuclear chromatin changes, tissue cracking, etc. Therefore, this method is only used when an urgent intraoperative diagnosis is needed.

2.7 Tissue Microarray Construction and Evaluation

The tissue microarray (TMA) construction process [34] involves a region of interest first being identified and marked on a histology slide. This same area is then marked on the corresponding paraffin tissue block (i.e. the donor block) and core biopsies are taken [35]. The cores are then inserted into a separate paraffin block (i.e. the recipient block) [36] in a precisely spaced, array pattern. This process is repeated multiple times where finally, one paraffin block is made up of hundreds of tissue core biopsies from many donor blocks (Fig. 2.3). Simultaneous analysis of molecular targets at the DNA, mRNA, and protein levels can then be performed under identical conditions. Epidemiologists, histopathologists, and researchers can subsequently analyze data following quantitative digital image analysis [37]. Therefore, the development of TMA technology has allowed the efficient study hundreds of different tissue samples concurrently [38] and is an invaluable research tool.

2.8 Microscopes, Automated Imaging, and Digital Software

Many different microscopes are available today, each of which have varied applications and modifications that contribute to their usefulness

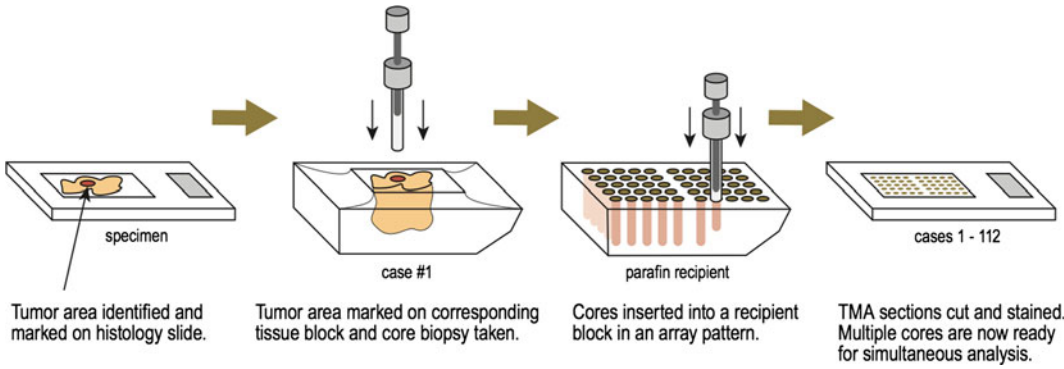

Fig. 2.3 Tissue microarray construction

Table 2.3 Classification, type, light source, and function of various microscopes

Classification	Microscope type	Light source	Description
Optical	Light microscope (or "compound")	Visible light	This is the most commonly used microscope with strong magnifying power, used for the study of cells, chromosomes, and DNA
	Dissecting microscope (or "stereoscope")	Visible light	This contains lenses in different angles that provides 3D viewing, used for forensics, fine repair, microsurgery
	Fluorescence microscope	UV light	This is a special type of light microscope where, instead of light reflection and absorption it uses UV light to view cells
	Digital microscope	Visible light	This makes use of the optical lens and charge-coupled device (CCD) sensors to magnify objects and includes a camera for high quality recording
Electron	Transmission electron microscope (TEM)	Electron beam	This microscope is used for studying cells and microorganisms and can produce images as small as 1 nm in size
	Scanning electron microscope (SEM)	Electron beam	This is less powerful than the TEM but can provide 3D viewing of objects and is used for studying cells and small particles of matter

(Table 2.3). The upright microscope is the most common configuration and has binocular eyepieces, high power compound objective lenses, and a precision sample stage [39]. In contrast, an inverted microscope is essentially an upside-down, upright microscope. As brightfield and fluorescence microscopy are the most frequent types performed in histology labs, only these will be introduced in this chapter.

Brightfield microscopy is performed using a light microscope with a light source commonly projecting from the back of the microscope. This light travels upwards from beneath, projects through the field diaphragm and condenser lens beneath the stage, up through the histology slide containing tissue, into the objective lens, and finally up to the camera and/or eyepiece. A simplistic illustration of this light path is shown in Fig. 2.4. The fluorescence microscope is similar to the conventional brightfield microscope with added features to enhance its capabilities [39]. While the conventional microscope uses visible light ($\sim$400–700 nm), the fluorescence microscope uses much higher intensity light source that causes excitation of fluorophores (i.e. the excitation light). The light is absorbed by the

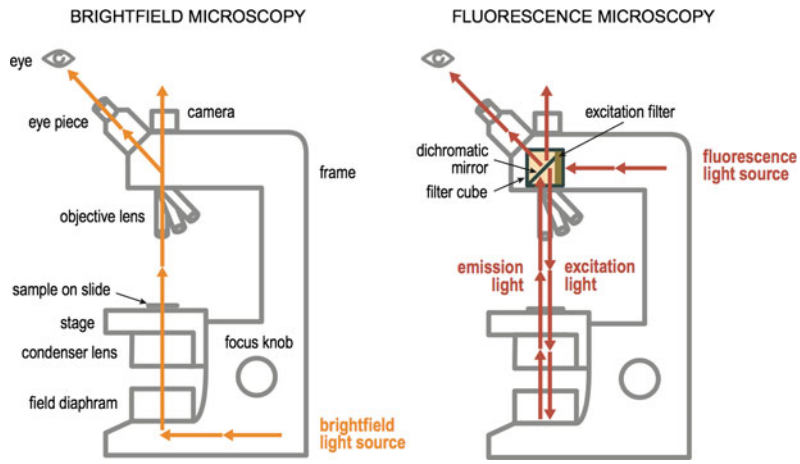

Fig. 2.4 Light paths of brightfield and fluorescence microscopes

fluorophores, which causes them to emit a longer, lower energy wavelength light. This fluorescent light (i.e. the emission light) can be separated with filters designed for that specific wavelength. In the fluorescence microscope (Fig. 2.4), the light initially travels to a filter cube. Inside the filter cube it passes through an excitation filter to a dichroic mirror (or beam-splitter), which sends light down through the objective lens and onto the histology slide containing tissue. The emitted fluorescence from the specimen then travels back up through the objective lens, into the filter cube, through the dichroic mirror and through an emission filter. From here it continues traveling upwards toward the camera and/or eyepiece and can be recorded or viewed [39].

Modern digital pathology combines the power of the microscope with electronic detection and advanced computerized analysis and is now progressively replacing previously subjective, semiquantitative manual scoring with precise quantification of protein expression [40]. A sensor is used to obtain an image, which is then displayed on a computer monitor using charge-coupled device technology. Automated scanning can also be performed using a robotic loader. Associated imaging software packages for both brightfield and fluorescence purposes provide complex algorithms for quantitation of immunostaining. Tissue can be automatically segmented into gland or stromal targets and cells can also be segmented into nuclei and cytoplasm using various algorithms. This allows the translation of extent and intensity of immunostaining into a continuous variable, more amenable to large-scale bioinformatics analyses.

2.9 The Normal Human Cell and its Components

The human cell is the basic structural and functional unit of the body and its main compartments are the nucleus and the cytoplasm, which are completely separate entities that work together to keep the cell functioning [41]. Histologically, the nucleus, nucleolus, and cytoplasm can easily be distinguished on routine H&E. Antibodies can also be used to selectively stain the nucleus, cytoplasm, or cytoplasmic membrane also, depending on the target of interest. While the structures within the nucleus and cytoplasm cannot be seen using routine microscopy, their functions are introduced briefly, as knowledge of this basic information is imperative for an understanding of the topics discussed in forthcoming chapters.

2.9.1 The Cytoplasm and its Organelles

The cytoplasm surrounds the cell nucleus and is surrounded by a plasma membrane, which separates the interior of the cell from the outside environment. This membrane is selectively permeable and controls the movement of substances in and out of the cell. The cytoplasm is made largely of cytosol fluid containing multiple organelles ("little organs") suspended within it that carry out specific directions of the nucleus. These organelles include mitochondria, endoplasmic reticula, the Golgi apparatus, vacuoles, and lysosomes (Fig. 2.5) among others. Mitochondria function to produce most of the cell's energy in the form of adenosine triphosphate (ATP) [42] and are also involved in processes such as cell signaling [43], cellular differentiation [44], cell growth [45], cell cycle control [46], and cell death [47]. There are two types of endoplasmic reticula, the rough endoplasmic reticulum (RER) and the smooth endoplasmic reticulum (SER) [48]. The SER is involved in lipid metabolism [49], carbohydrate metabolism, and detoxification, while the RER contains ribosomes on the surface where active protein synthesis occurs [50, 51]. The Golgi apparatus concentrates and packages proteins from the RER inside the cytoplasm prior to being transferred to their appropriate destinations [52]. Vacuoles are involved in the storage and intracellular digestion of molecules and lysosomes contain enzymes responsible for the breakdown of proteins, nucleic acid, carbohydrates, lipids, cellular debris, and foreign organisms [53].

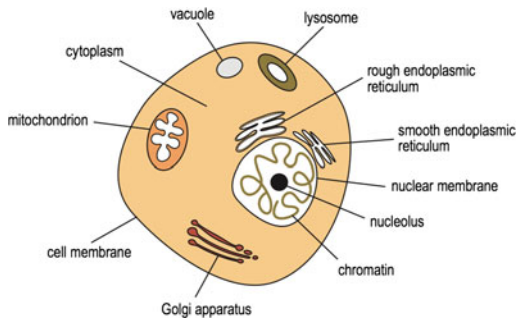

Fig. 2.5 The human cell and its components

2.9.2 The Nucleus and Gene Expression

The nucleus is the largest organelle found in human cells and is surrounded by a nuclear envelope with nuclear pore complexes that allow material to move in and out [54]. It contains genetic information in the form of DNA. DNA is a complex molecule consisting of two antiparallel strands of nucleotide bases, each with a backbone of sugar (deoxyribose) molecules linked together by phosphate groups [55]. Each sugar molecule is linked to a base, which is attached by hydrogen bonds to a base on the other strand in a complementary fashion so that adenine (A) bonds with thymine (T) and guanine (G) bonds with cytosine (C) [56]. DNA is organized into highly compact, regular units called chromosomes. Within the nucleus is a structure called the nucleolus, which contains RNA, ribosomal proteins, and functions as the site of ribosome synthesis. RNA differs from DNA in that RNA molecules are single-stranded, the backbone sugar is ribose, and it contains uracil (U) in place of thymine [57].

Human genetic testing has made significant advances in the past decade [58]. The identification of certain sequences in order to diagnose genetic diseases can be done by performing sequencing on a blood sample, fresh tissue, or paraffin embedded tissue. DNA sequencing [59] is the process of determining the nucleotide order of DNA fragment. As mRNA is generated by transcription from DNA, reverse transcription must be performed (using a reverse transcriptase enzyme) for RNA sequencing [60]. Following this, complementary DNA (cDNA) fragments are generated, PCR amplification executed, a library created, and sequencing performed comparing results to a reference genome.

2.10 Basic Histology of Normal Tissues

"Tissues" refer to groups of similar cells performing similar functions (e.g. cardiac myocytes). "Organs" refer to groups of tissues (e.g. the heart)

and "organ systems" include groups of organs that function together (e.g. the cardiovascular system). Tissue is composed of various cells together with a surrounding extracellular matrix (ECM). There are four fundamental tissue types including epithelia, connective tissue, muscle and nervous tissue, each of which will be introduced separately below.

2.10.1 Epithelia

Epithelial cells are generally classified as "covering and lining epithelia" which are found lining the cavities of the body and surfaces of structures, or as "glandular (or secretory) epithelia" [41]. Glands refer to single cells or groups of cells that secrete protein, mucus, or lipid. This includes "endocrine glands" which secrete into extracellular spaces (i.e. secrete internally) and "exocrine glands" which secrete into ducts (i.e. secrete into the external environment). Epithelial cells can also be classified based on the number of cell layers present and the shape of the cells in the top layer (Fig. 2.6). Epithelial tissue can, therefore, be one cell thick (i.e. simple epithelium), or two or more cells thick (i.e. stratified epithelium) [61]. There are three basic cell shapes based on microscopic appearance, including squamous (flat and wide), columnar (tall), and cuboidal (cube shaped) cells. Consequently, by describing the number of cell layers and the surface cell shape the different forms of epithelia can easily be classified. In some cases a third feature, namely specialization of the cell surface, e.g., keratinization [62] or the presence of cilia [63], is included. Stratified squamous epithelium that is exposed directly to the environment (e.g. the skin), can show keratinization (i.e. a layer of dead cells is present on the surface), while those that are not directly exposed (e.g. the oral cavity) are only partially keratinized or nonkeratinized. The presence of cilia (i.e. hair-like motile processes) is another specialization seen in simple columnar epithelium present on the surface of these cells. This surface adaptation helps propel substances along, e.g., the airways contain cilia to propel mucus.

Cells of epithelial tissues are usually tightly packed together and form a continuous sheet or a solid aggregation of cells. They lack intracellular spaces and are united by several types of junctional specializations [64] (i.e. tight junctions [65], desmosomes [66], hemidesmosomes [67] etc.). Therefore, epithelia have only one free surface (i.e. apical surface), which is exposed at the body surface or at the lumen of a duct, tube, or vessel. The lower surface of an epithelium (i.e. basal surface) [68] rests on an underlying basement membrane, which is a thin sheet of collagen and glycoproteins, which acts as both a scaffold and a selectively permeable membrane allowing water and small molecules through [69].

Two special categories of epithelium are pseudostratified and transitional epithelium [41]. Pseudostratified columnar epithelium is so called, because of an apparent stratification, however, all of the cells are attached to the basement membrane (Fig. 2.6). Therefore, it is really simple epithelium, despite giving the impression of stratification. Transitional epithelium (or urothelium) is stratified epithelium lining the walls of the urinary tract. The term refers to the fact that it may appear as stratified cuboidal to squamous in appearance depending on the extent of bladder distention. Specific names are also given to epithelium in certain locations. Endothelium is a term given to simple epithelium lining blood vessels (vascular endothelial cells) [70] or lymphatics (lymphatic endothelial cells) [71]. Mesothelium is a name given simple squamous epithelium lining the major body cavities [72], for example, the peritoneal epithelium lining the abdominal organs [73].

2.10.2 Connective Tissues

Connective tissues form a scaffold that epithelial tissues lie on, and nerve and muscle tissues are embedded. They are classified as connective tissue proper and specialized connective tissues, including adipose tissue, cartilage, bone, and blood (Fig. 2.7). All connective tissues are characterized by various individual cells scattered within an extracellular space filled with an ECM

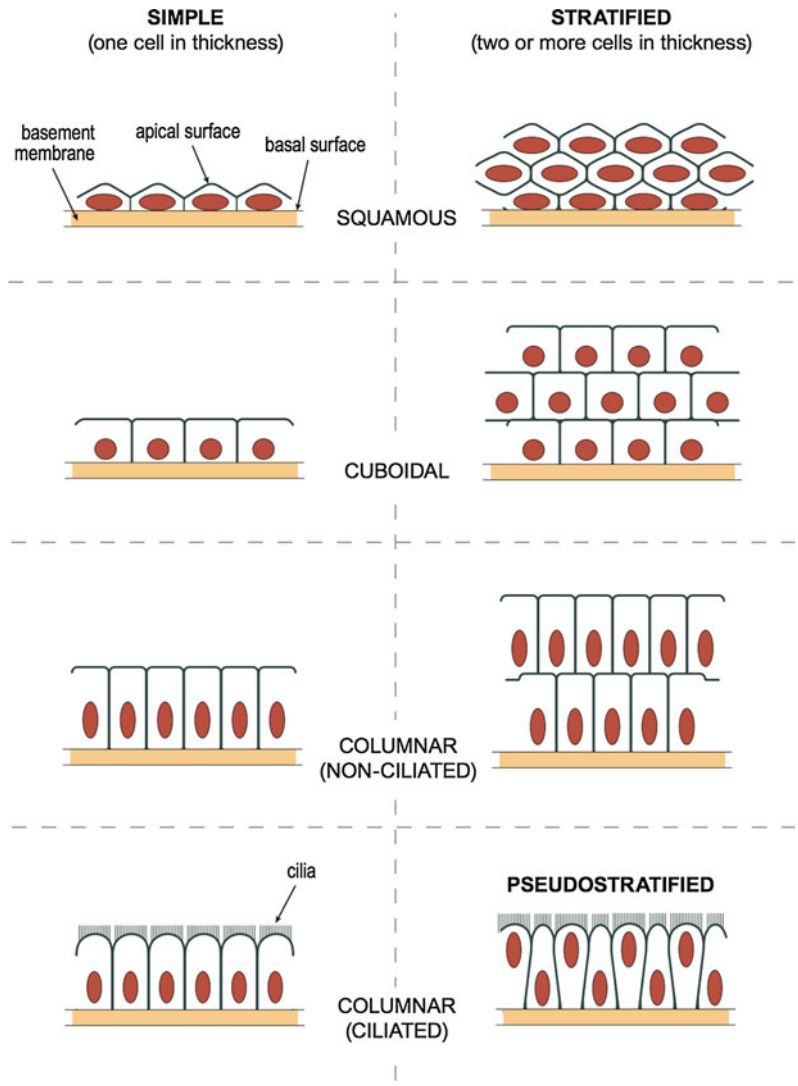

Fig. 2.6 Illustration of epithelial cell types

[41]. Variations in the composition of the ECM determine the properties of the connective tissue. In general, the ECM comprises ground substance and various fibers (i.e. collagen, reticular or elastic fibers) woven into a network. Ground substance supports the connective tissue cells, binds them together, and permits the diffusion of nutrients and other dissolved substances between capillaries and cells. There are many known types of collagens, with type I being the most abundant. Histologically, collagen appears as irregular, wavy fibers arranged singly or in small groups. Reticular fibers are very fine fibrils consisting of another type of collagen (type III). They are usually not visible using routine H&E, but can be demonstrated using special stains (e.g. reticulin) [22]. Like reticular fibers, elastic fibers [74] require special stains to be visualized also (e.g. elastin). Once stained, elastic fibers appear as fine, dark, undulating fibers within the tissue.

The principle cells in connective tissue proper include fibroblasts that secrete collagen fibers and ground substance [75], together with macrophages, mast cells, and adipocytes among others. Fibroblasts have elongated nuclei with a moderate amount of cytoplasm that tapers at the

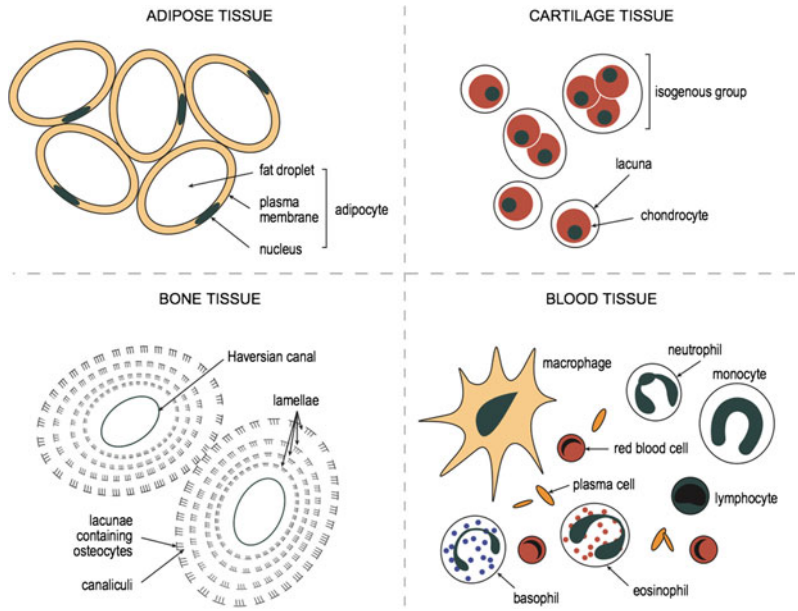

Fig. 2.7 Illustration of connective tissue cell types

ends under the microscope. They are usually found alone and contain an elliptical nucleus showing finely stippled (i.e. "dot-like") chromatin and one or two nucleoli. Macrophages are large, round cells, with vesicular nuclei [76]. In some cases, a brown pigment is seen within them, which is the result of lysosomal action on ingested red blood cells. Mast cells are small, ovoid cells with spherical, eccentric nuclei, and basophilic granules [77]. In adipocyte cells, the nucleus appears flattened with the cytoplasm forming a very narrow rim around a large central lipid droplet. During routine preparation of histological slides fat is dissolved, therefore, the adipocytes actually appear empty. When adipocytes are seen in large numbers, the tissue is referred to as adipose tissue.

There are three types of cartilage tissues that differ in the type of fibers they contain within the ECM. These include hyaline, fibrocartilage, and elastic cartilage, with hyaline being the most abundant type. Histologically, hyaline cartilage has a basophilic appearance on H&E. Chondrocytes (cartilage cells) produce the matrix of cartilage and are seen within lacunae (i.e. matrix cavities), singly or in clusters of 2–8 cells. These groups are called isogenous groups and are derived by mitosis from a single chondrocyte. In contrast, the principle cells in bone tissue are osteoblasts, osteocytes, and osteoclasts. The osteoblast is involved in bone deposition at the bone surface and secretes the matrix of bone (i.e. osteoid), which becomes calcified following deposition [78]. As they are trapped within the ECM, they become osteocytes [79]. The osteocytes are located in lacunae and fine channels (canaliculi) containing osteocyte cell processes, connecting lacunae to each other. The third cell type, the osteoclast, is associated with bone resorption [80] and does this by secreting enzymes that acidify the matrix. These are large, multinucleated cells with a ruffled border histologically. In order to be able to visualize bone tissue, the specimen is usually placed in a decalcifying solution (e.g. formic acid) [81], which removes calcified material so that good quality paraffin sections can be prepared that will preserve the microscopic elements.

Blood is traditionally classified as a specialized form of connective tissue, even though it has a different function in comparison to other connective tissue types. It has a highly fluid ground substance (i.e. plasma), which comprises mainly water together with salts, proteins, nutrients, hormones, and waste material. The cellular component of blood is produced by the bone

Table 2.4 Histologic description and function of hematopoietic cells

Cell type	Histologic description	Function of hematopoietic cell
Erythrocyte	Flat or oval-shaped cell with no nucleus	Responsible for the transportation of oxygen in the body
Neutrophil	Multilobated nucleus, often with 3–5 lobes	Involved in the acute inflammatory response
Eosinophil	Eosinophilic granular cytoplasm and a bilobed nucleus	Associated with the allergic response and parasitic infections
Basophil	Basophilic granular cytoplasm and a bilobed nucleus	Responsible for allergic response by releasing histamine
Monocyte	Large cell, with a large, indented ("kidney-bean" shape) nucleus and abundant cytoplasm	Precursors for tissue macrophages, which engulf and digest foreign microorganisms, dead cells, or debris (i.e. phagocytosis)
Lymphocyte	Small, round cell with a deeply staining spherical nucleus surrounded by a thin rim of basophilic cytoplasm	Precursors of natural killer cells, B and T lymphocytes involved in the acute inflammatory response. B cells further differentiate into plasma cells
Megakaryocyte	Large cell, with a large pale multilobated nucleus and abundant cytoplasm	Precursors of thrombocytes (or platelets) formed by budding from megakaryocytes and contribute to hemostasis

marrow in a process termed hematopoiesis [82]. These hematopoietic cells are derived from multipotent hematopoietic stem cells. Following division, the resulting daughter cells (myeloid or lymphoid progenitor cells) can commit to alternative differentiation pathways depending on growth factors involved. Finally, blood cells are divided into three lineages including the erythroid lineage (reticulocytes and erythrocytes), the lymphoid lineage (T cells, B cells [83] and natural killer cells [84]) and the myeloid lineage (granulocytes [85, 86], megakaryocytes [87], and macrophages [88]). Table 2.4 lists the histologic description and function of various hematopoietic cells, and their basic structures are also illustrated in Fig. 2.7.

2.10.3 Muscles

Muscles are responsible for maintaining posture, locomotion, and movement of the internal organs (e.g. contraction of the heart). Three kinds of muscle tissues are found in different organs of the body, including skeletal muscle, cardiac muscle, and smooth muscle (Fig. 2.8). Skeletal muscle generally forms the muscles attached to bones and by contracting, these muscles move joints. Cardiac muscle (myocardium) forms the mass of the heart. Smooth muscle is a component of the walls of many hollow organs within the body, such as the digestive tract. By contracting, smooth muscle propels the contents along the tube it surrounds (e.g. the intestine), or regulates

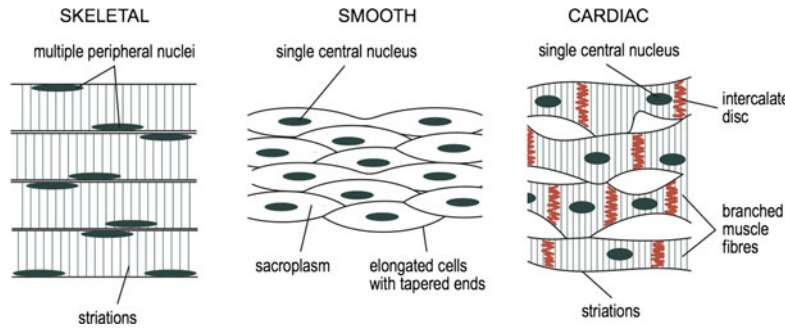

Fig. 2.8 Illustration of three kinds of muscle tissue cell types

the amount of fluid flowing through it (e.g. the blood vessels). Skeletal and cardiac muscle is referred to as "striated" muscle as they show light and dark bands when viewed under the microscope. Smooth muscle cells do not have visible striations, however, they do contain the same contractile proteins arranged in a different pattern. While muscles are conventionally classified based on morphology (i.e. striated or smooth muscle), they can also be classified based on function (i.e. voluntary or involuntary muscle). Histologically, muscle tissue cells (myocytes) are elongated and spindle-shaped with little intervening extracellular material. Smooth muscle and cardiac muscle myocytes contain one nucleus, while skeletal muscle is multinucleate. The unusual microstructure of myocytes has lead to the use of specialized terminology, including the sarcolemma (plasma membrane), the sarcoplasm (cytoplasm), the sarcoplasmic reticulum (endoplasmic reticulum), and sarcosomes (mitochondria).

2.10.4 Neural Tissues

The nervous system is divided anatomically into the central nervous system (CNS) and the peripheral nervous system (PNS). The CNS consists structurally of the brain (within the skull) and the spinal cord (within the vertebral canal), while the PNS is composed of nerves (i.e. cranial nerves from the brain and spinal nerves from the spinal cord) and ganglia (i.e. nerve cell clusters). Neurons (nerve cells) are specialized cells that respond to stimuli and conduct electrical impulses to and from all body organs. All neurons have the same basic structure consisting of the cell body (soma) containing the nucleus, cytoplasm and organelles, and nerve processes that conduct the signals [89]. Nerve processes include the axon, which carries signals away from the cell body, and dendrites, which carry signals toward the cell body. There are three basic shapes to neurons, bipolar (i.e. consisting of a single axon and single dendrite), pseudounipolar (i.e. consisting of a single axon with a central and a peripheral branch), and multipolar (i.e. consisting of a single axon and numerous dendrites), illustrated in Fig. 2.9. There are four types of supporting cells (or central neuroglia) in the CNS, including oligodendrocytes [90], microglia [91], astrocytes [92], and ependymal cells [93]. In contrast, the Schwann cell is the principle supporting cell in the PNS (or peripheral neuroglia) [94]. The oligodendrocyte or Schwann cell wraps around axons of neurons to form the myelin sheath that ensures rapid conduction of nerve impulses [95]. This sheath is not continuous and gaps between neighboring cells

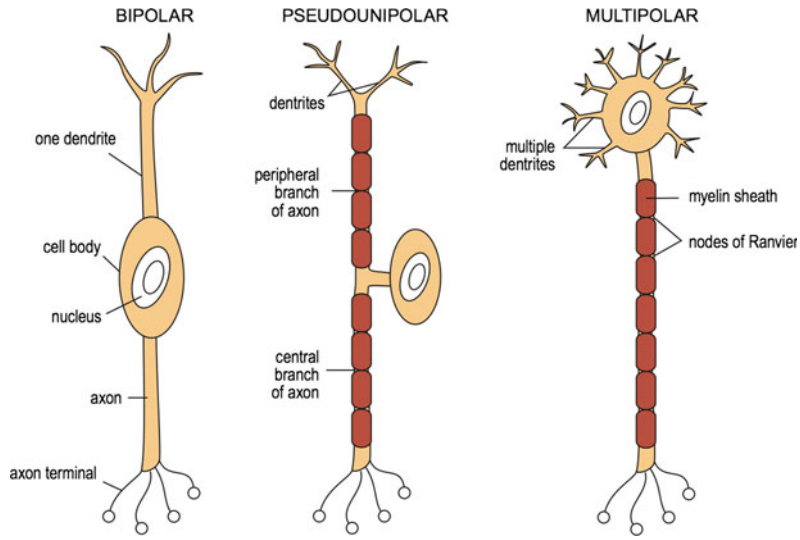

Fig. 2.9 Illustration of basic neuron types

are called nodes of Ranvier [96]. Neurons are seen histologically as irregular or stellate in shape and are multipolar. They have a large cell body with a large, round, and pale (euchromatic) nucleus and a single prominent nucleolus. The supporting cells are quite difficult to distinguish using routine H&E, however, and immunocytochemical methods are therefore necessary to demonstrate them adequately, e.g., glial fibrillary acidic protein (GFAP) highlights astrocytes [97].

2.11 Summary

In summary, this chapter has introduced basic terminology and important techniques and instruments used in histology. This information should be of practical use to the reader and will help to develop and refine the body of knowledge necessary for understanding the forthcoming chapters.

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