ISSN: 2181-4341, IMPACT FACTOR (RESEARCH BIB) – 7,245, SJIF – 5,431

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PEDAGOGICAL METHODS IN TEACHING HISTOLOGY TO STUDENTS

Annotation: Histology (a combination of the Greek words тканьстóς - "tissue" and-λογία-logia) is the study of the microscopic anatomy of plant and animal cells and tissues. It is performed by examining a thin section of tissue under a light or electron microscope. The ability to visualize or differentiate microscopic structures is often enhanced by the use of histological dyes. Histology is the most important tool in biology and medicine.
Key words: histology, anatomy, plant, microscopic structure.

Histopathology, the microscopic examination of affected tissues, is an important tool in anatomical pathology, since accurate diagnosis of cancer and other diseases usually requires histopathological examination of samples. Trained physicians, often certified as pathologists, are personnel who conduct histopathological examinations and provide diagnostic information based on their observations.
Trained scientists who prepare histological sections are histotechnicians, histology technicians, histology technologists, medical scientists, medical laboratory technicians, or biomedical scientists. Their specialty is called histotechnology.

Chemical fixation with formaldehyde or other chemicals.

Chemical fixatives are used to preserve tissues from destruction, as well as to preserve the cell structure and subcellular components, such as cellular organelles (for example, the nucleus, endoplasmic reticulum, mitochondria). The most common fixative for light microscopy is 10% neutral buffered formalin (4% formaldehyde in phosphate buffered saline). For electron microscopy, the most commonly used fixative is glutaraldehyde, usually in the form of a 2.5% solution in phosphate-buffered saline solution. These fixatives preserve tissues or cells mainly by irreversible crosslinking of proteins. The main action of these aldehyde fixatives is to cross-link amino groups in proteins by forming a CH2 (methylene) bond, in the case of formaldehyde, or C5H10 cross-links in the case of glutaraldehyde. This process, while preserving the structural integrity of cells and tissues, can disrupt the biological functionality of proteins, especially enzymes, and also denature them to a certain extent. This can be disastrous for some histological methods. For electron microscopy, additional fixatives such as osmium tetroxide or uranyl acetate are often used.

Fixation with formalin leads to degradation of mRNA, miRNA and DNA in tissues. However, isolation, amplification, and analysis of these nucleic acids from formalin-fixed and waxed tissues is possible using appropriate protocols.

Fixing a frozen slice

Frozen section is a quick way to fix and mount histological sections. It is used for surgical removal of tumors and allows you to quickly determine the edge (that the tumor has been completely removed). For this purpose, a refrigerating device called a cryostat is used. Frozen tissue is cut using a microtome, frozen sections are mounted on a slide and painted in the same way as with other methods. This is a necessary way to fix tissue for certain staining applications,

such as antibody-associated immunofluorescence staining. It can also be used to determine the malignancy of a tumor if it was discovered accidentally during surgery in a patient.

The purpose of fabric processing is to remove water from fabrics and replace it with a medium that solidifies, allowing you to cut thin sections. The biological tissue must be fixed in a solid matrix so that sufficiently thin sections can be cut, usually 5 microns (micrometers; 1000 micrometers = 1 mm) thick for light microscopy and 80-100 nm (nanometers; 1,000,000 nanometers = 1 mm) thick for electron microscopy. For light microscopy, paraffin is most often used. Since it does not mix with water, the main component of biological tissue, the water must first be removed during the dehydration process. To remove water, the samples are passed through baths with a gradually increasing concentration of ethanol. This is followed by a hydrophobic cleaning agent (such as xylene) to remove the alcohol, and finally molten paraffin, an infiltration agent that replaces xylene. Paraffin does not provide a solid enough matrix to cut out very thin sections for electron microscopy. Instead, resins are used. Epoxy resins are the most commonly used embedding medium, but acrylic resins are also used, especially in cases where immunohistochemistry is required. Thicker sections (from 0.35 microns to 5 microns) of resin-impregnated tissue can also be cut out for light microscopy. Again, the immiscibility of most epoxy and acrylic resins with water leads to the need for dehydration, usually with ethanol.

Embedding

After the fabrics are dehydrated, cleaned and soaked with the embedding material, they are ready for external embedding. During this process, tissue samples are placed in molds along with a liquid embedding material (such as agar, gelatin, or wax), which then solidifies. This is achieved by cooling in the case of paraffin and heating (curing) in the case of epoxy resins. Acrylic resins are polymerized by heat, ultraviolet light, or chemical catalysts. The hardened blocks containing tissue samples are then ready for sectioning.

Since tissues fixed in formalin and paraffin (FFPE) can be stored indefinitely at room temperature, and nucleic acids (DNA and RNA) can be extracted from them decades after fixation, FFPE tissues are an important resource for historical research in medicine.

Embedding can also be performed using frozen, unfixed fabrics in a water-based environment. Pre-frozen fabrics are placed in molds with liquid embedding material, usually glycol, OCT, TBS, cryogel, or water-based resin, which are then frozen to form solidified blocks.

Partitioning

Partitioning can be performed in a limited number of ways. Vertical cross-section perpendicular to the fabric surface is the usual method. Horizontal sectioning is often performed to evaluate hair follicles and hair follicles. Tangential to horizontal dissection is performed in Mohs surgery and in CCPDMA techniques.

For light microscopy, a steel knife installed in the microtome is used to cut off sections of tissue with a thickness of 10 micrometers, which are mounted on the slide of the microscope. For transmission electron microscopy, a diamond knife installed in an ultramicrotome is used to cut sections of tissue 50 nanometers thick, which are mounted on a copper grid with a diameter of 3 millimeters. The mounted sections are then treated with appropriate dyes.

Frozen tissues placed in a freezing environment are cut on a microtome in a refrigerated machine called a cryostat.

Staining

Biological tissue has a low contrast characteristic of a light or electron microscope. Staining is used to give a contrast to the fabric, as well as to highlight certain features of interest. In cases where the basic mechanistic chemistry of staining is understood, the term histochemistry is used. Hematoxylin and eosin (H&E stain) is the most commonly used light microscopic stain in histology and histopathology. Hematoxylin, the main dye, turns the nuclei blue due to its affinity for nucleic acids in the cell nucleus; eosin, an acidic dye, turns the cytoplasm pink. Uranyl acetate and lead citrate are commonly used to contrast tissue in an electron microscope.

Special Staining: There are hundreds of different other methods that are used to selectively stain cells and cellular components. Other compounds used to dye sections of fabrics include safranin, oil red o, Congo red, fast green FCF, silver salts, as well as numerous natural and artificial dyes that have generally emerged from the development of dyes for the textile industry.
Histochemistry refers to the science of using chemical reactions between laboratory chemicals and components within a tissue. A commonly used histochemical technique is the Perls reaction with Prussian blue, used to demonstrate iron deposits in diseases such as hemochromatosis.
Histological samples are often examined using radioactive methods. In autoradiography, the slide (sometimes histochemically colored) is X-rayed. More commonly, autoradiography is used to visualize locations where a radioactive substance has been transferred in the body, such as cells in the S phase (undergoing DNA replication) that include tritiated thymidine, or sites where radiolabeled nucleic acid probes bind during in situ hybridization. For autoradiography at the microscopic level, the slide is usually immersed in a liquid emulsion of the nuclear path, which dries to form a film for exposure. Individual silver grains in the film are visualized by dark-field microscopy.
Recently, antibodies have been used to specifically visualize proteins, carbohydrates, and lipids. This process is called immunohistochemistry or, if the stain is a fluorescent molecule, immunofluorescence. This technique has significantly expanded the ability to identify categories of cells under the microscope. Other advanced techniques, such as non-radioactive in situ hybridization, can be combined with immunochemistry to identify specific DNA or RNA molecules with fluorescent probes or tags, which can be used for immunofluorescence and fluorescence amplification by enzymes (especially alkaline phosphatase and tyramide signal amplification). Fluorescence microscopy and confocal microscopy are used to detect fluorescent signals with good intracellular detail. Digital cameras are increasingly being used to capture histological and histopathological images.

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