Tissue Processing for Microscopic Analysis with Applications in Oral Histology

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Keywords

Histopathology, Fixation, Ground Section, Decalcified sections, Hematoxylin and Eosin staining.

Abstract

Tissue processing is a critical step in microscopic studies, enabling detailed visualization of cellular and structural components in both soft and hard tissues. In oral histology, proper processing preserves tissue morphology, enhances staining, and facilitates accurate analysis. This review summarizes the major tissue processing techniques, including formalin-fixed paraffin-embedded (FFPE) sections, decalcified sections, ground sections, and frozen sections, with emphasis on their applications in oral and systemic tissues. While FFPE sections are widely used for soft tissue examination, decalcified and ground sections allow for a detailed study of hard tissues such as bone and teeth, and frozen sections provide rapid intraoperative evaluation. Key staining methods, including hematoxylin and eosin (H&E) and special stains, are highlighted for their role in differentiation of tissue components. This review further discusses applications in immunohistochemistry, molecular pathology, forensic studies, and pharmacological research, providing a comprehensive guide for researchers and dental professionals aiming to optimize tissue processing and microscopic analysis.

Introduction:

Tissue processing is essential in biomedical research and diagnostic pathology, as it enables the preparation of tissue samples for microscopic examination and provides insights into tissue structure, function, and abnormalities.[1]It is widely used in histopathology for disease diagnosis and plays a key role in immunohistochemistry, where processed tissue sections are incubated with antibodies to detect specific proteins or antigens, revealing their localization and distribution.[2]Tissue processing is also critical for transmission electron microscopy, allowing for high-resolution visualization of cellular ultrastructure and subcellular components. In molecular pathology, it facilitates extraction of nucleic acids, such as DNA and RNA, for analysis using techniques

like PCR and blot hybridization.[3] Additionally, it is employed in forensic science to determine cause and manner of death,[4]and in pharmaceutical research to evaluate drug effects on tissues, aiding the development of new therapies.[5] The process begins with collecting tissue specimens from biopsies, autopsies, or experimental models.

1.Formalin-Fixed Paraffin-Embedded (FFPE) Sections

FFPE sections are widely used for routine histopathology to diagnose and characterize diseases, including cancer. This technique preserves tissue architecture and cellular morphology, assisting in the visualization of pathological changes. Biopsies can be obtained from multiple tissues, including:

- **Organ tissues:** Liver, lung, kidney, heart, spleen, pancreas, and gastrointestinal tract.
- **Skin and soft tissues:** Skin, subcutaneous tissue, muscle, adipose tissue.
- **CNS tissues:** Brain, spinal cord, and peripheral nerves for neurological studies.
- **Bone and joints:** Bone biopsies and synovial tissues.
- **Reproductive system:** Uterus, cervix, ovary, testes, prostate, breast.
- **Lymphoid tissues:** Lymph nodes and spleen.
- **Endocrine glands:** Thyroid, parathyroid, adrenal, pituitary.
- **Oral cavity:** Oral mucosa, tongue, salivary glands, palate, tonsils, tooth etc.

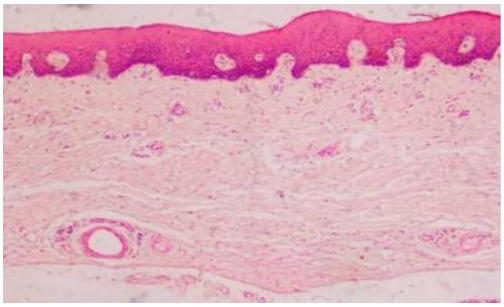

Figure 1: Histology of keratinized stratified squamous epithelium stained with hematoxylin and eosin (H&E).

Sequential Steps in FFPE Processing:

1.1 Fixation: Tissue is immersed in a fixative to preserve structure and prevent autolysis. Dietrich's fixative (30% formalin, 10% formaldehyde, 2% acetic acid, 10% buffered formalin, and water) is commonly used. Fixation time varies (48–72 h) depending on tissue size, followed by washing in running water.[6]

Aims: Terminate metabolism, preserve tissue morphology, and prevent shape changes during staining.

1.2 Dehydration: Water is removed to allow wax penetration using ascending alcohol concentrations (70–100%) for ~45 min per step.

1.3 Clearing: Tissue is treated with a clearing agent (commonly xylene) to make it transparent and replace alcohol for wax infiltration.

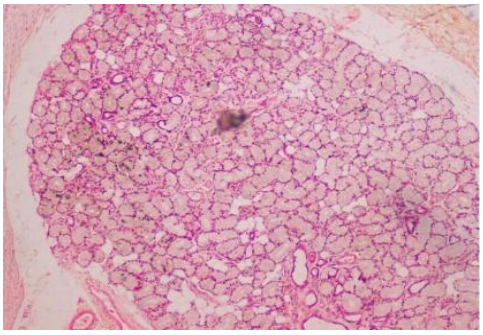

Figure 2: H&E stain of a mucous salivary gland showing mucous acini.

1.4 Impregnation: Tissue is immersed in molten paraffin wax, allowing xylene to be gradually replaced, ensuring hardness for sectioning.

1.5 Embedding: Tissue is oriented in paraffin blocks and cooled to 4 °C, ensuring proper alignment of epithelium and connective tissue.

1.6 Sectioning: Microtome sections (3–5 µm) are placed on albumin-coated slides, dried, and prepared for staining.

1.7 Staining: H&E staining differentiates tissue structures. Hematoxylin (with mordant aluminum) stains nuclei blue/purple, while eosin stains cytoplasm pink/red. Slides undergo dewaxing, rehydration, staining, and dehydration before mounting with DPX. Special stains (PAS, PTAH, Von Gieson, reticulin) are used depending on the tissue.[7]

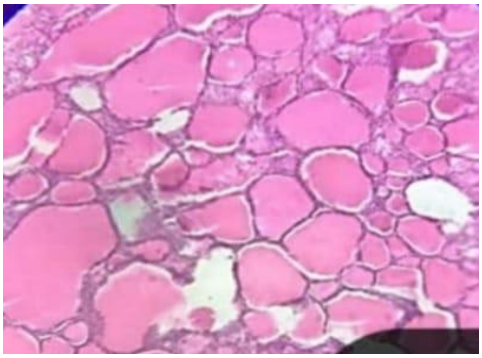

Figure 3: H&E stain of thyroid gland showing round follicles filled with colloid.

2) Decalcified Sections

Hard tissues like bone and teeth contain calcium salts (e.g., hydroxyapatite) that make them brittle, preventing thin sectioning for microscopy. Decalcification removes these minerals, allowing study of dental pulp and bone. In teeth, enamel (96% inorganic) is removed to visualize the pulp, which contains blood vessels, connective tissue, and cells. Bone, composed of 67% inorganic and 33% organic matter, retains its organic matrix after decalcification. This allows for observation of lacunae, osteocytes, and capillaries, though microscopic clarity is lower than in ground sections.

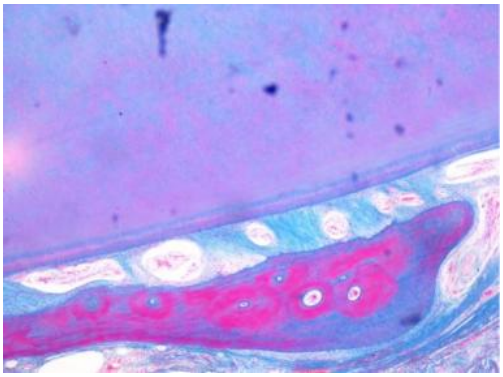

Figure 4: Decalcified section of alveolar bone showing cementum and periodontal ligament.

After extraction, a hole is drilled in the tooth crown or apical root to allow fixative penetration. Tissues are fixed in 10% neutral buffered formalin for 24 hours and decalcified in 5% nitric acid, with daily solution changes. Complete decalcification is confirmed when a pin passes easily to the center. Tissues are then washed, embedded in paraffin, sectioned, stained, and examined under a microscope.[8]

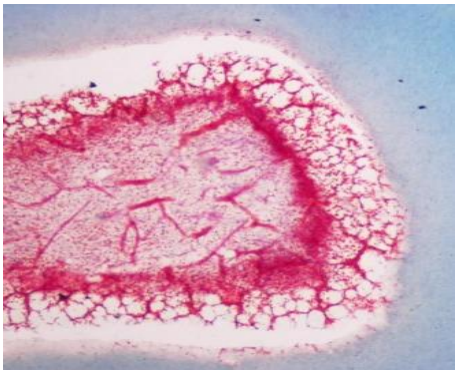

Figure 5: Decalcified section of Dental Pulp.

Histological examination of decalcified pulp identifies cell types, blood vessel integrity, pulp stones, and pathological changes.[9] Pulpitis can be reversible—characterized by localized inflammation, collagen deposition, minimal necrosis, and tertiary dentin formation with mild stimulus pain—or irreversible, showing coronal pulp necrosis, PMN infiltration, biofilm colonization, and spontaneous pain.[10]

Decalcified bone sections allow for the evaluation of bone cells (osteoblasts, osteocytes, osteoclasts) and detection of abnormalities like osteoporosis, osteomalacia, fractures, tumors, metabolic bone diseases, and metastasis, which aids in diagnosis, staging, and treatment planning. The decalcification process is time-consuming, and tissue preservation may be affected by fixation, processing, and choice of decalcifying agent.[11]

3) Ground Section

Ground sections are used to study hard tissues like bone and teeth, focusing on inorganic components, while organic substances are removed. Unlike decalcified sections, enamel is preserved. Thus, this method is ideal for analyzing enamel, dentin, cementum, and bone microstructures. Bone features such as osteons, Haversian canals, lacunae with osteocytes, Volkmann's canals, and interstitial lamellae are well visualized. Although preparation is labor-intensive, it is simple, cost-effective, and highly suitable for research and forensic investigations.[12]

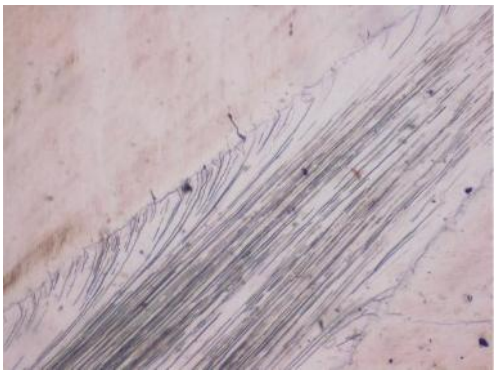

Figure 6: Ground section of tooth showing the dentin-enamel junction (DEJ), the wavy interface that strengthens the bond between enamel and dentin.

In forensic odontology, ground sections assist in age and sex estimation, pathological assessment, and bite mark analysis by examining enamel, dentin, and pulp microscopically. Extracted teeth are preserved in formalin, saline, distilled water,

or dilute hydrogen peroxide prior to sectioning.

Teeth are first cut longitudinally with a micromotor and diamond disc, then thinned using a laboratory lathe, Arkansas stone, or graded emery papers (200–600) to approximately 50 µm. Thin sections are lifted with a camel hairbrush, floated on water, and mounted on glass slides with a mountant (DPX or Canada balsam) and coverslip for microscopic examination.[13]

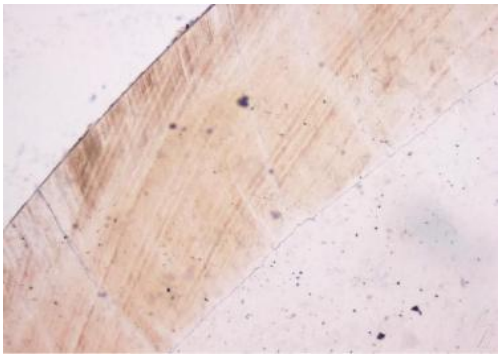

Figure 7: Ground section of enamel showing enamel rods and incremental lines of Retzius, indicating periods of enamel formation.

4) Frozen Sections

Frozen sections are prepared for rapid evaluation of tissue during surgery, particularly when no preoperative diagnosis exists, or intraoperative findings require immediate assessment. They assist surgeons in decision-making, ensure sample adequacy, and serve as quality control. Combined with MALDI (matrix-assisted laser desorption ionization) imaging, frozen sections support cancer research, drug development, and personalized medicine through analysis of the molecular composition and spatial distribution of proteins, lipids, and metabolites.[14]

Tissues are frozen in a cryostat at –40 °C, where water in the tissue acts as the embedding medium. Sections of 10–15 µm are cut and stained with hematoxylin and eosin, methylene blue, or PAS. The process takes approximately 10 minutes and provides immediate microscopic evaluation, which can be crucial in surgeries such as cancer resections, organ transplants, or procedures involving critical structures.

Acknowledgment

We sincerely express our heartfelt gratitude to Dr. Thirumal Mohan for his invaluable guidance throughout this research. We are also privileged to have worked under the mentorship of Dr. Rajagopal Appavu, whose unwavering support, guidance, and commitment to academic excellence were instrumental in the completion of this paper.

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Peer Review Status

Reviewer 1: Accepted

Reviewer 2: Accepted

Reviewer 3: Accepted