

Al Rasheed College of Dentistry
Oral Histology

Dr. Omar Faridh Fawzi
Lecture 1

An introduction to specimen preparation

Oral histology is the science that study the teeth and it's supporting tissues in the oral cavity. So for studying the tissues there must be preparation of histological section and a microscopic analysis of cells and tissues that requires the preparation of very thin, high quality sections (slices) mounted on glass slides and appropriately stained to demonstrate normal and abnormal structures.

The most common types of microscopes for studying tissues are:

1. **Light microscope:** is a type of microscope which uses visible light and a system of lenses to magnify images of small samples.
2. **Scanning electron microscope:** is a type of electron microscope that produces images of a sample by scanning the surface with a focused beam of electrons. The electrons interact with atoms in the sample, producing various signals that contain information about the sample's surface topography and composition.
3. **Transmission electron microscope:** is a microscopy technique in which a beam of electrons is transmitted through a specimen to form an image. The specimen is most often an ultrathin section less than 100 nm thick or a suspension on a grid. An image is formed from the interaction of the electrons with the sample as the beam is transmitted through the specimen. The image is then magnified and focused onto an imaging device, such as a fluorescent screen, a layer of photographic film, or a sensor such as a charge-coupled device.

Stages in the preparation of histology slides are:

1. Fixing

Samples of biological tissue are "fixed" to preserve the cells/tissue in as natural a state as possible and prevent postmortem decay (autolysis and putrefaction). Chemical fixatives are very carefully selected substances whose properties must meet many criteria. Even the most careful fixation alters the sample to a certain extent and may potentially introduce artifacts that can interfere with interpretation of images of the fine detail of cells, including all their organelles, that can only be

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observed using an electron microscope (such fine detail of cells that can only be seen using electron microscopes is called "cellular ultrastructure"). Part of the skill involved in preparing and interpreting histology slides is in maintaining the structure of the sample in as undisturbed a state and possible, minimizing the introduction of artifacts and recognizing artifacts as such when they are present.

The aim of fixation:

- a) To prevent autolysis and bacterial attack.
- b) To fix the tissues so they will not change their volume and shape
- c) To prepare tissue and leave it in a condition which allow clear staining of sections.
- d) To leave tissue as close as their living state as possible, and no small molecules should be lost.

Fixation is coming by reaction between the fixative and protein which form a gel, so keeping everything as their in-vivo relation to each other.

Factors affect fixation:

- PH.
- Temperature.
- Penetration of fixative.
- Volume of tissue.

According to previous factors we can determine the concentration of fixative and fixation time.

Types of fixative:

Acetic acid, Formaldehyde, Ethanol, Glutaraldehyde, Methanol and Picric acid.

2. Processing

Tissue processing is done to remove water from the biological tissues, replacing such water with a medium that solidifies, setting very hard and so allowing extremely thin sections to be sliced. This is important because biological tissue must be supported in an extremely hard solid matrix to enable sufficiently thin sections to be cut.

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Stages of processing:

- a) Dehydration.
- b) Clearing.
- c) Embedding.

a) Dehydration

To remove fixative and water from the tissue and replace them with dehydrating fluid. There are a variety of compounds many of which are alcohols. several are hydrophilic so attract water from tissue. To minimize tissue distortion from diffusion currents, delicate specimens are dehydrated in a graded ethanol series from water through 10%-20%-50%-95%-100% ethanol.

Types of dehydrating agents: Ethanol, Methanol, Acetone.

b) Clearing

Replacing the dehydrating fluid with a fluid that is totally miscible with both the dehydrating fluid and the embedding medium.

Choice of a clearing agent depends upon the following:

- I. The type of tissues to be processed, and the type of processing to be undertaken.
- II. The processor system to be used.
- III. Intended processing conditions such as temperature, vacuum and pressure
- IV. Safety factors.
- V. Cost and convenience.
- VI. Speedy removal of dehydrating agent.
- VII. Ease of removal by molten paraffin wax
- VIII. Minimal tissue damage.

c) Embedding

After tissues have been dehydrated and before they can be "sectioned" or sliced very thinly they must be secured in a very hard solid block in such a way that the hardened material used to secure all parts of the biological tissues in place is transparent to the optical method used for viewing the finished samples.

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Different types of embedding techniques and materials are used depending on the sample being prepared and the other types of processing involved in preparing that particular sample.

In general, tissue samples are placed in molds together with liquid embedding material which is then hardened. The result of this stage in the preparation of histology slides is hardened blocks containing the original biological samples together with other substances used so far in the preparation process.

The properties of paraffin wax are improved for histological purposes by the inclusion of substances added alone or in combination to the wax:

- improve ribboning.
- increase hardness.
- decrease melting point
- improve adhesion between specimen and wax

3. Sectioning

Sectioning an embedded tissue sample is the step necessary to produce sufficiently thin slices of sample that the detail of the microstructure of the cells/tissue can be clearly observed using microscopy techniques (*either light microscopy or electron microscopy*).

Possible orientations at which tissue samples may be sectioned include:

- a) Vertical sectioning perpendicular (i.e. at right-angles) to the surface of the tissue, and this is the most common method.
- b) Horizontal sectioning is often done for the study of hair follicles and structures that include hairs, hair follicles, erector pili muscles, and sebaceous glands in general. Such structures are sometimes called "pilosebaceous units".

Tangential to horizontal sectioning is done in chemosurgery (also called "Mohs surgery") which is a form of microscopically controlled surgery used to treat certain types of skin cancer.

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the method used to actually cut sections from the hardened block of tissue depends on the type of microscopy that will be used to observe it and hence the thickness of sample required. In the case of samples to be studied using light microscopy, a steel knife mounted in a microtome may be used to cut 10 μ m tissue sections which are then mounted on a glass microscope slide. In the case of samples to be studied using transmission electron microscopy, a diamond knife mounted in an ultra-microtome may be used to cut 50 nm tissue sections which are then mounted on a 3-millimeter-diameter copper grid.

4. Staining

Finally, the mounted sections are treated with an appropriate histology stain.

Biological tissue has very little variation in colors/shades when viewed using either an ordinary light (optical) microscope or an electron microscope. Staining biological tissues is done to both increase the contrast of the tissue and also highlight some specific features of interest (depending on the type of tissue and the stain used).

There are many different histology stains. Histology stains are normally selected according to the type of tissue to be observed. Some stains are more widely used than others while some are only used to study very specific samples.

Hematoxylin and eosin (H&E stain) is the most commonly used light microscopical stain in histology and histopathology. Hematoxylin, a basic dye, stains nuclei blue due to an affinity to nucleic acids in the cell nucleus; eosin, an acidic dye, stains the cytoplasm pink. *Uranyl acetate and lead citrate* are commonly used to impart contrast to tissue in the electron microscope. specific types of biological tissue.

Types of sections for studying tissues are:

1. **Ground section** of non-decalcified tooth and viewing by polarized light has been traditionally used in the analysis of the crystal structure of teeth. However, sections are very thick and preclude the

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use of transillumination to analyze tooth crystal structure such as bone.

2. Decalcified section, decalcify of the tooth and then section and stain as per soft tissue. While this technique is useful in analyzing the soft tissue component of teeth, such as the pulp and periodontal ligament, it loses the bulk of the crystal structure of teeth, making analysis of hard tissue difficult.

3. Frozen Sections

Small pieces of tissue (typically 5mm x 5mm x 3 mm) are placed in a cryo-protective embedding medium then snap frozen in isopentane (an alkane) cooled by liquid nitrogen. Tissue is then sectioned in a freezing microtome or cryostat. Sections are then fixed by immersion in a specific fixative or series of fixatives for carefully controlled period of time.