***Oral Histology***

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# Slide Preparation

The microscopic examination of tissue sections and their proper preparation is essential in the study of oral tissue morphology. Therefore, a basic knowledge of the various types of microscopes and histological techniques is important to learn. This helps to study the structure and function of oral tissues.

The most common types of microscopes for studying tissues are

light microscope and electron microscope. Under the light microscope, tissues are examined via a light beam that is transmitted through the tissue. Because tissues and organs are usually too thick for light to pass through them, they must be sectioned to obtain thin, translucent sections and then attached to glass slides before they can be examined.

***Preparation of specimen for microscopy:***

Various techniques have been developed to prepare tissues for study so that they closely resemble their natural living state. Four techniques for oral tissue preparation are usually used for light microscopic examination. These are as follows:

**A-Parraffin embedded section of soft tissues**:

This is the most common technique used for soft tissues such as gingiva, cheek, tongue , lip, salivary gland , etc. That is, the tissue, which are not calcified. The steps of tissue preparation in this type are:

**1-Fixation of the specimen:**

Fixation is a complex series of chemical events that differ for the different groups of substance found in tissues.

**The aim of fixation:**

1. To prevent autolysis and bacterial attack.

2- To fix the tissues so they will not change their volume and shape during processing.

3- To prepare tissue and leave it in a condition which allow clear staining of sections.

4- 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 every thing as their in vivo relation to each other.

The most commonly used fixative agents for light microscopical examination are 10% neutral **formalin** and **Bouin’s** fluid . Both of these substances are cross-linked proteins, so maintaining a life like image of tissue after removal from the body. After fixation the tissue is washed overnight in running water.

**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.

**2-Processing of the tissue(Dehydration, clearing and infiltration):**

**Dehydration** :mean remove fixative and water from the tissue and replace them with dehydrating fluid since water is not miscible with paraffin wax in which the tissue is embedded. Two widely used dehydrating agents are ***alcohol and acetone***. The specimen is gradually dehydrated by being passed through a series of increasing percentages of alcohol ( 60% , 70% , 80% and 95% and absolute alcohol).

Then since paraffin and alcohol are not miscible ,the specimen is passed from alcohol through changes of ***xylene*** ,which is miscible with both alcohol and paraffin. This process is called **clearing**, since the tissue becomes transparent in ***xylene***. Then the specimen is placed in a suitable container of melted paraffin wax, which has been in an oven at 65°C until it is completely **infiltrated**. The infiltrated process is done in order to distinguish the overlapping cells in a tissue and the extracellular matrix from one another.

**3-Embedding:**

Is the process by which tissues are surrounded by a medium such as paraffin wax which when solidified will provide sufficient external support during sectioning.

The specimen is embedded in melted paraffin wax after it has been completely infiltrated with paraffin. Once the tissue is impregnated with paraffin, it is placed into a small container, covered with melted paraffin, and then allowed to harden, forming a paraffin containing the tissue. The specimens is now ready to be sectioned on a microtome.

**4-Sectioning of the specimen**

The paraffin blocks are sectioned with **a microtome**, which is a device supplied with a stainless steel blade and an arm that can provide us with equal increments of the tissue thickness (usually from 4 to 10 microns). Then sections are placed on precooled glass slides, permitted to come to room temperature, and stained with specific dyes.

**5-Mounting the cut sections**

The section are placed (mounted) on a glass slides coated with suitable adhesive. The slide is then allowed to dry before staining with water –soluble stains for light microscopical study.

**6-Staining the section**

Paraffin is first removed from the section, then tissue is rehydrated and stain. The most commonly used stains in histology are **hematoxylin and eosin**, commonly referred to as ***H and E stain***. Hematoxylin is **a base**, it colors the acidic components of the cells by **bluish** color. Because the most acidic components of the cells are DNA and RNA, the nucleus and some regions in the cytoplasm stain dark blue. These components are called **basophilic.**

Eosin is **an acid** that dyes the basic components of the cells a **pinkish** color. Because many of the cytoplasmic constitutes have a basic PH, so they are stain pink in color. These elements are said to be **acidophilic.**

**B-Decalcified section for hard tissue:**

The specimens in this section must be decalcified ( the mineral substance removed by acid). This type is used for the tissue containing bone or teeth. Enamel of the tooth contains 96% minerals so it is completely destroyed if decalcified unless it still not fully formed it can be seen.

**C- Ground sections for calcified tissue:-**

Specimens of calcified tissue may be ground into thin section such as bone and undecalcified tooth. This is done by slicing the undecalcifed specimen into a section of about 30-50 microns on a revolving stone or disc and then by grinding on lathe wheel or flat stones.

**D-Frozen section for soft tissues:-**

This type is used to examined the pathological tissue specimens immediately, or when the reagent used for embedding would destroy the tissue characteristics that are to be studied, so specimen of soft tissue may be frozen and sectioned with freezing microtome (cryostat) without being embedded.