Preparation of speciemens for histological study:

The morphologic study of oral tissues involves the preparation of tissue sections for microscopic exammination, this help to study the structure and functions of oral tissues.

The most common types of microscopes for studying tissues are light microscope and electron microscope.

Light microscope :-

4 techniques for oral tissue preparation are usually used for light microscopic examination :

1- Parraffin embedded section of soft tissue :

This is the most common technique used for soft tissues such as gingiva , cheek , tongue , lips, salivary glands, ect.; that is the tissue which are not calcified ; The steps of tissue preparation in this type are :

A – Fixation of the specimen :

After obtaining the specimen ; it should be immediately placed in fixing solution, the purpose of fixation are to coagulate protein , thus reducing alteration by subsequent treatment , and to make the tissue more permeable to the reagent used. The most common 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 over night in running water.

B- Dehydration , Clearing and infiltration:

Dehydration means water removal from specimen, since water is not miscible with paraffin wax in which the tissue is embedded. Two widely used dehydration agents are alcohol and acetone . The specimen is gradually dehydrated by being passed although 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 $65^{o}C$ until it is completely **infiltrated ;** this process is done in order to distinguish the overlapping cells in a tissue and the extracellular matrix from one another.

C- Embedding: 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 specimen is now ready to be sectioned on a microtome.

D- 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 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 .

C –Mounting the cut section : 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 .

E-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 &E** stain. Hematoxylin is a base , it colors the acidic components of the cells by bluish color. Because the most acidic components of the are cells are DNA and RNA , the nucleus and some regions in the cytoplasm stain dark blue . These components are called **basophilic.**

Eosin is an acidic 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.**

2- 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.

3- 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 undecalcified specimen into a section of about 30-40 microns on a revolving stone or disc and then by grinding on lathe wheel or flat stones.

4- Frozen section for soft tissue: 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) with out being embedded.