

## EMBRYOGENESIS

### 1<sup>st</sup> week development

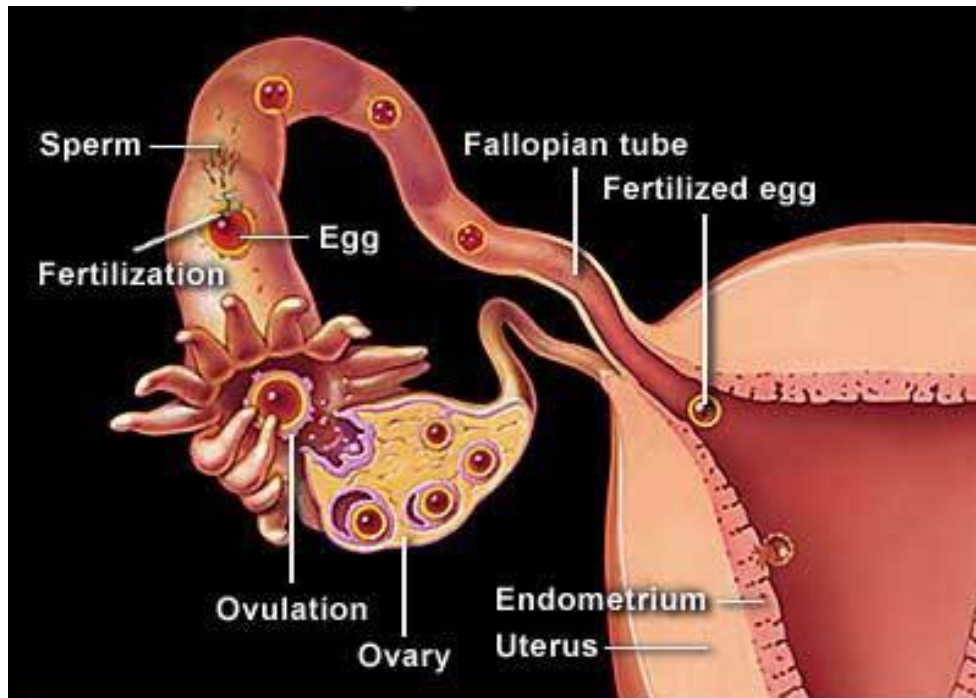
Human embryogenesis is a complicated process by which the fertilized egg develops into an embryo. During the first eight weeks after fertilization, the concepts shift from a single-celled zygote into a multi-leveled, multi-dimensional fetal body plan which utilizes primitively functioning organs.

### The female reproductive system

The female reproductive system is made up of the internal and external sex organs. It performs the following functions:

- a) Formation of eggs
- b) Reception of sperms during copulation
- c) Providing a conducive environment for fertilization
- d) Providing shelter and nourishment to the growing embryo

At puberty, the female begins to undergo regular monthly cycles controlled by the hypothalamus which secretes gonadotropins (follicle-stimulating hormone (FSH) and luteinizing hormone (LH) . At the beginning of each ovarian cycle, 15 to 20, follicles are stimulated to grow but under normal conditions only **one follicle** reaches full maturity.

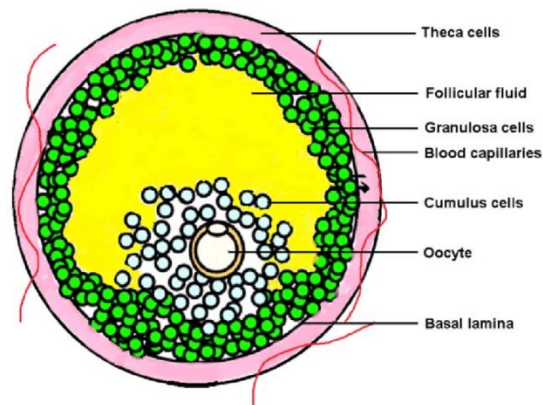


## Ovulation

**In the days immediately preceding ovulation:** under influence of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), the follicle grows rapidly to become a (**graafian follicle**), that cause the surface of the ovary begins to bulge locally. The graafian follicle contain:

1. fully grown **oocyte**
2. **zona pellucida** (*The zona is a glycoprotein shell surrounding the oocyte that facilitates and maintains sperm binding and induces the acrosome reaction*),
3. **cumulus oophorus** (*a cluster of cells that surround the oocyte both in the ovarian follicle and after ovulation*)
4. **Corona radiate** (*is the innermost layer of cumulus oophorus, it is a thick layer that surrounds zona pellucida, formed of proteins, carbohydrates and hyaluronic acid*)

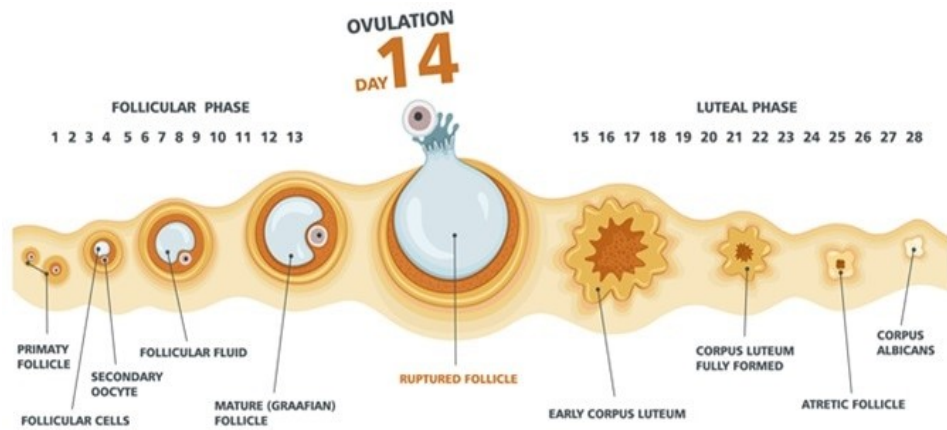
Before ovulation, the oocyte and some cells of the cumulus detach from the inside of the distended follicle



**graafian  
follicle**

**At ovulation day:** the follicle ruptures cause expelling the oocyte. The oocyte remain covered by the zona pellucida and one or more layers of follicular cells. The oocyte that is released is picked up by the fimbriae of the uterine tube (*fingerlike projections at the end of the fallopian tubes, through which eggs move from the ovaries to the uterus*), and the oocyte is transported toward the uterus.

**After ovulation** the follicle turns into the **corpus luteum** which is made up of large conical yellowish cells. Corpus luteum serves as a temporary endocrine gland, by releasing female sex hormones namely **progesterone and estrogen** (*that prepare the endometrium by gets thicker and is well supplied with blood vessels*). The corpus luteum reaches maximum development approximately 9 days after ovulation. It can easily be recognized as a yellowish projection on the surface of the ovary. Subsequently, the corpus luteum shrinks and forms a mass of fibrotic scar tissue, the **Corpus albicans**.



## Fertilization

Fertilization is commonly known as **conception**, is a process by which male and female gametes fuse, occurs in the **ampullary region** of the uterine tube (*the widest part of the tube and is close to the ovary*).

When sperm passing in the uterine tube they undergo a process called **capacitation** (*a process involves epithelial interactions between the sperm and the mucosal surface of the uterine tube*)

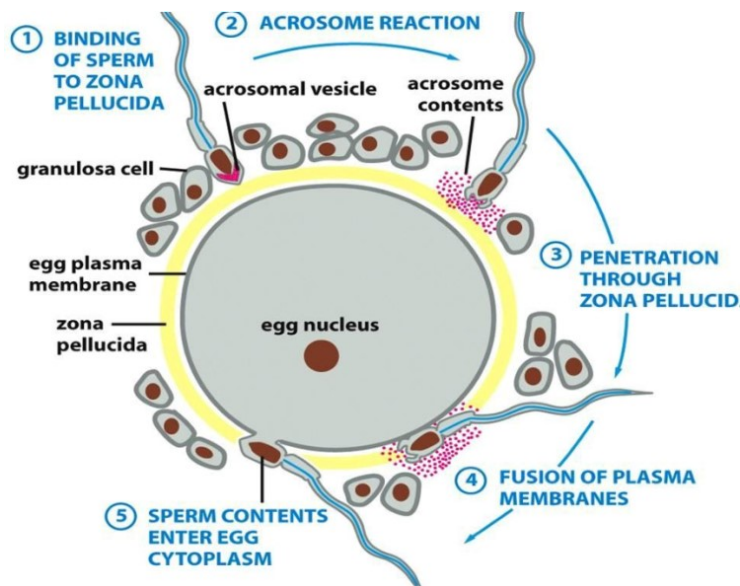
### Phases of fertilization :

Of the 200 to 300 million spermatozoa deposited in the female genital tract, only 300 to 500 reach fertilization site. Only one of these fertilizes the egg. Fertilization passes in three phases:

**Phase I: Penetration of the corona radiate:** Capacitated sperm pass freely through corona radiate

**Phase II : Penetration of the zona pellucida:** Sperms attach to zona pellucida induce **acrosomal reaction** by releasing of acrosomal enzymes (acrosin) that allows sperm to penetrate the zona.

**Phase III :Fusion of oocyte and sperm cell membrane:** because the plasma membrane covering the sperm head cap disappears during the acrosome reaction, actual fusion is accomplished between the oocyte membrane and the membrane that covers the posterior region of the sperm head.



## Results of fertilization :

**A- Restoration of the diploid number of chromosomes**, half from the father and half from the mother. Hence, the zygote contains a new combination of chromosomes different from both parents.

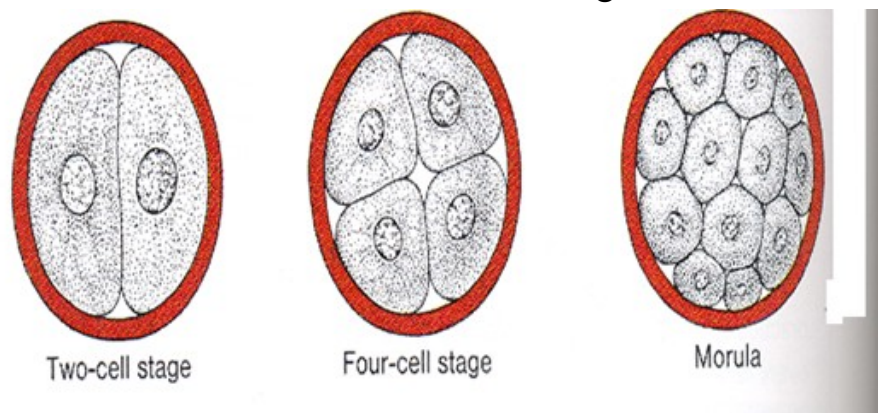
**B -Determination of the sex of the new individual.** An X-carrying sperm produces a female (XX) embryo, and a Y-carrying sperm produces a male (XY) embryo. Hence, the chromosomal sex of the embryo is determined at fertilization.

## C- Initiation of cleavage

### Morula formation:

About a day after fertilization, the resulting one-celled zygote multiple mitotic cleaves as it travels around four days to reach the uterine cavity.

When the cell's number is around sixteen the solid sphere of cells within the zona pellucida is called the **morula**. The different cells derived from cleavage are called **blastomeres**.



### Blastulation (Blastocyst formation)

About the time the morula enters the uterine cavity, fluids begin to penetrate through the zona pellucida into the intercellular spaces of the inner cell mass. Gradually, the intercellular spaces become confluent, the cells realign themselves to form a fluid-filled hollow ball called **blastocyst** with single cavity called **blastocoel**.

**Blastocoel** is the first cavity formed during embryonic development,

**Blastocyst** is a mass of cells that is formed a few days after fertilization.

The blastocyst formed of two cellular populations:

1. **Inner cell mass** that polarized at one end and called **embryoblasts** that subsequently will form the **embryo**.
2. **Outer cell layer** that line the blastocoel called the **trophoblast** that subsequently will develop into **placenta**

The increase in size of the blastocyst causes it to hatch through the zone pellucida , which then disintegrates.



Fertilized egg



2-cell stage



4-cell stage



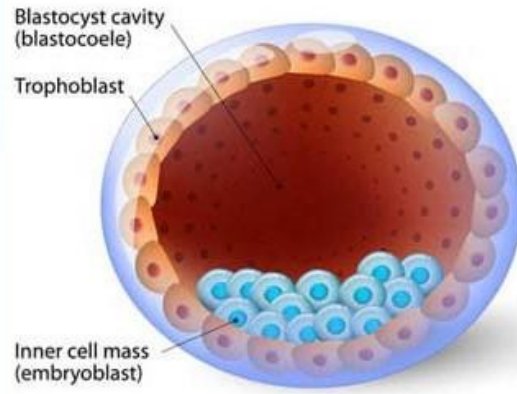
8-cell stage



16-cell stage



Blastocyst



Blastocyst cavity  
(blastocoele)

Trophoblast

Inner cell mass  
(embryoblast)

## EMBRYOGENESIS

### 2<sup>nd</sup> week development

The second week is often referred to as the week of twos. It's the week when the **embryoblast** turns into two layers disk (**epiblast and hypoblast**) .

**Trophoblast** separate into two distinct layers (**cytotrophoblast and syncytiotrophoblast**). Additionally, there are **two cavities** that develop within the embryonic unit at this time as well.

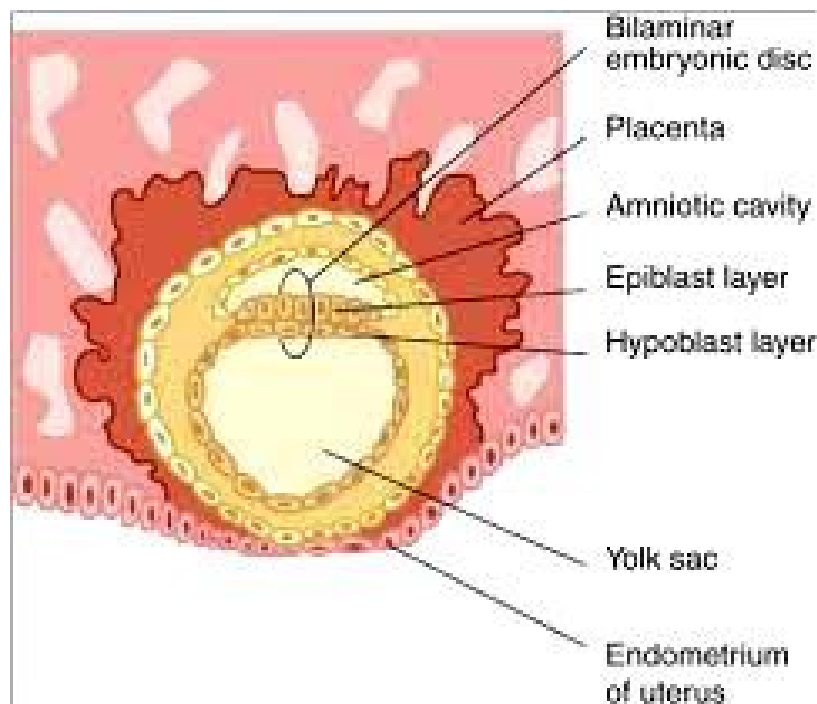
### The Bilaminar Embryonic Disc

The bilaminar embryonic disc, is the distinct two-layered structure of cells formed in an embryo takes place by **day eight** in the development of the human embryo .It is formed when the inner cell mass( embryoblast) forms a bilaminar disc of two layers:

a-an upper layer called the **epiblast**

b-a lower layer called the **hypoblast**

The disc is stretched between tow fluid-filled cavities the **amniotic cavity** and the **yolk sac**.

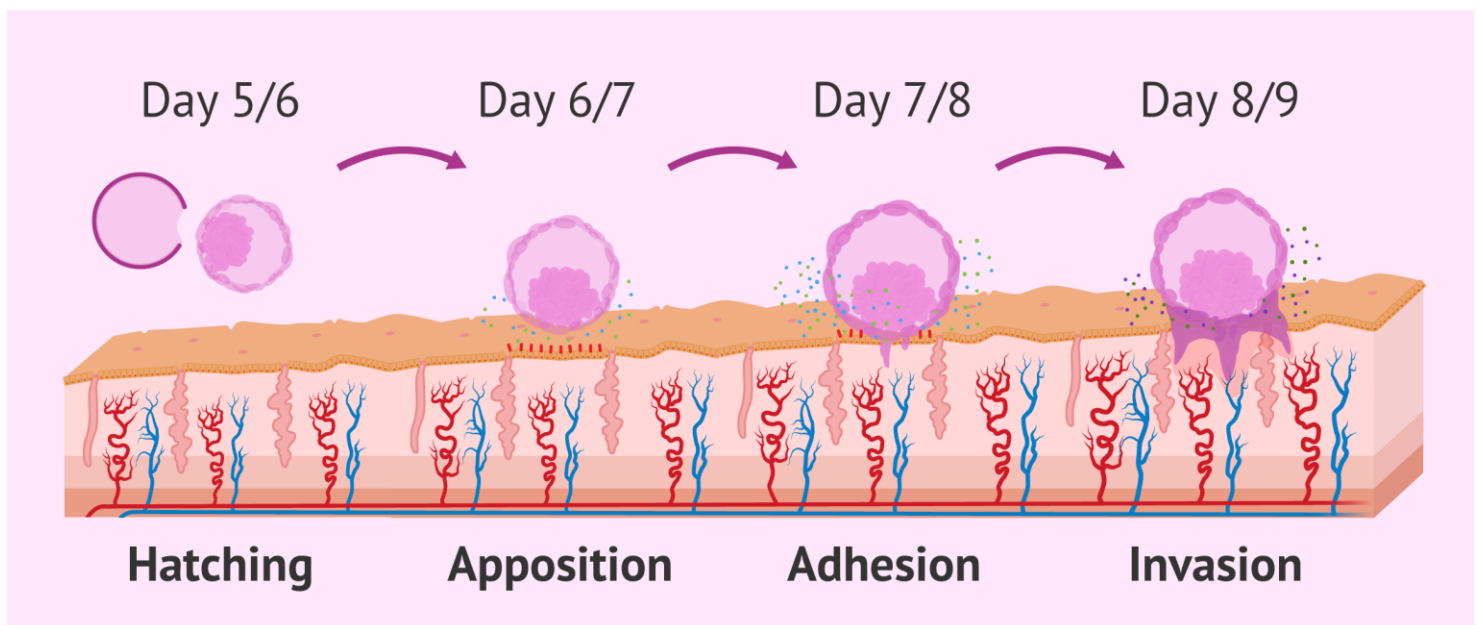


## Implantation

Implantation is a complex biochemical and mechanical process that begins in the first week of gestation and extends into the second week.

**The trophoblast** is a thin layer of cells that helps a developing embryo attach to the wall of the uterus, protects the embryo, and forms a part of the placenta, it will develop two sub-layers: **a-cytotrophoblast** **b-syncytiotrophoblast**

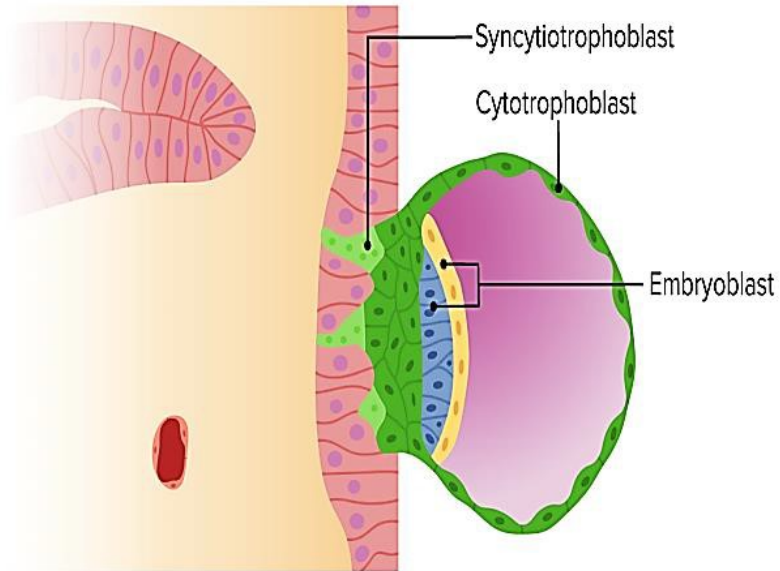
- After ovulation, the endometrial lining becomes **thickened**, with elongated secretory glands and is **increase blood supply** in preparation for accepting the embryo.
- Process of implantation can be subdivided into three phases:
  1. **Apposition phase:** where the blastocyst establishes weak interactions with the uterine wall.
  2. **Attachment phase:** occurs when definitive binding of the blastocyst to the uterine epithelium is more established.
  3. **Invasion phase:** occurs when the blastocyst begins to burrow into the endometrium.



Syncytiotrophoblast expands and erodes maternal capillaries and endometrial glands, the blood will begin to penetrate and flow through the trophoblast to give rise to the uteroplacental circulation.

The syncytiotrophoblast also produces human chorionic gonadotropin, a hormone that stimulates the release of progesterone from the corpus luteum.

The embryo is joined to the trophoblastic shell by a **narrow connecting stalk** that develops into the **umbilical cord** to attach the placenta to the embryo.





## Gastrulation and Neurulation

At the end of second week of development, the cells of the embryoblast differentiate into a two-layered disk, called the **bilaminar germ disk**.

1. The cells situated dorsally, are columnar in shape and called **epiblast**
2. Cells on ventral aspect are cuboidal in shape and called **hypoblast**

### Gastrulation

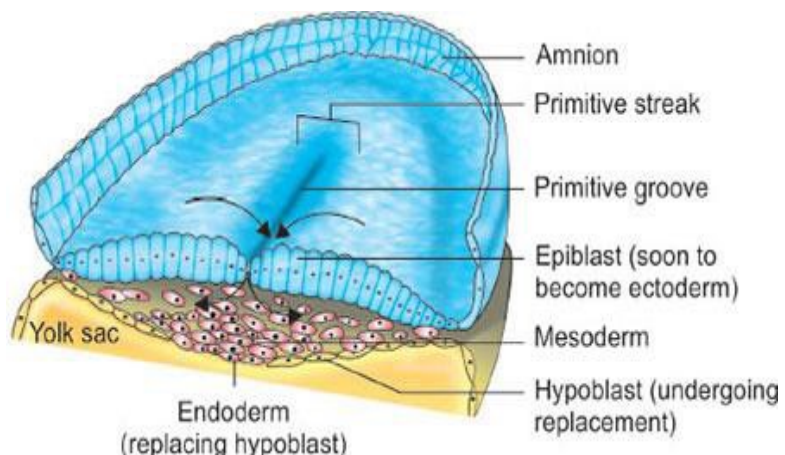
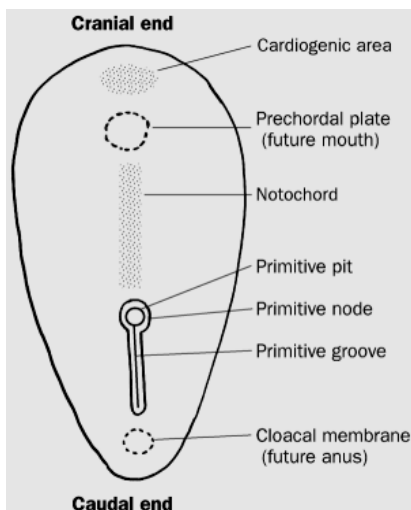
Process by which the bilaminar germ disk differentiate into Trilaminar germ disk involves migration, invagination and differentiation of the **epiblast**. It is largely controlled by the primitive streak.

**Gastrulation occurs in the following sequence:**

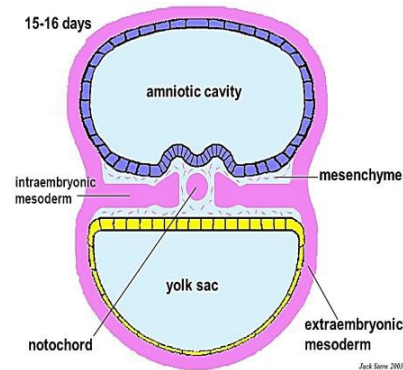
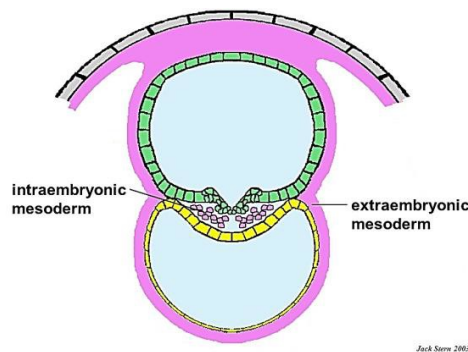
- a. The embryo becomes asymmetric.
- b. The primitive streak forms.

Near the end of 2<sup>nd</sup> week by the 15<sup>th</sup>-16<sup>th</sup> day, a thickened linear band in the caudal end of the dorsal aspect of the embryonic disk called **primitive streak** become prominent. It's appearance enables identification of embryonic axes, cranial and caudal ends, top and bottom surfaces, and sides of the embryo.

- The cephalic end of the streak called **primitive node**, consists of a slightly elevated area surrounding the small **primitive pit**.
- c. At about **day 16** , cells from the epiblast migrate medially toward the primitive streak, detach from the epiblast, and slip beneath it, and organized between the embryonic ectoderm and endoderm to form a layer called **the intraembryonic mesoderm**



- **The Trilaminar germ disk formed of:**
  - **Endoderm** – formed by epiblast cells that migrate through the primitive pit and displace the hypoblast cells.
  - **Mesoderm** – formed by epiblast cells that migrate through the primitive pit and lie between the epiblast layer and the newly created endoderm.
  - **Ectoderm** – formed by the epiblast cells that remain in position.
- By the middle of 3<sup>rd</sup> week, intraembryonic mesoderm separates the ectoderm and endoderm everywhere except at:
  - a-** oropharyngeal membrane cranially
  - b-** cloacal membrane caudally
  - c-** in the midline, cranial to the primitive knot where the notochordal process extends, because embryonic ectoderm and endoderm fuse at these sites and prevent mesenchymal cells from migrating between them.
- Mesoderm also lies outside the embryo *as extra-embryonic mesoderm*
- Invagination of surface cells in the primitive streak and their subsequent migration forward and laterally continues until the end of the **fourth week** when the primitive streak shows **regressive changes**, rapidly shrinks, and soon disappears.
- Gastrulation, continues in caudal segments while cranial structures are differentiating, causing the embryo to develop cephalocaudally.



## The intraembryonic mesoderm

As the notochord and neural tube developed, the intraembryonic mesoderm on each side forms three longitudinal columns which are:

### 1. Paraxial mesoderm

Its gives final count of approximately **42-44 pairs of somites**. The somites are triangular in shape when seen in a transverse section. Somites further differentiate into 3 components:

- a- Sclerotome** (cartilage and bone)
- b- Myotome** (muscles)
- c- Dermatome** (dermis of skin)

### 2. Intermediate mesoderm

Intermediate mesoderm (gives kidneys & genital system)

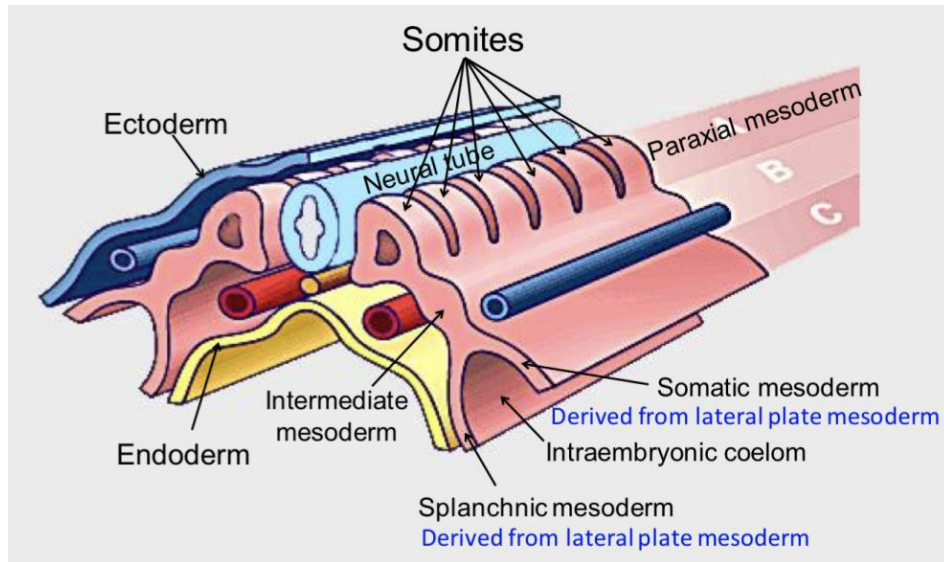
### 3. Lateral plate mesoderm

Its differentiates into two plates:

a-Somatopleure

b- Splanchnopleure

The cavity between these two parts is the **intraembryonic coelom**.

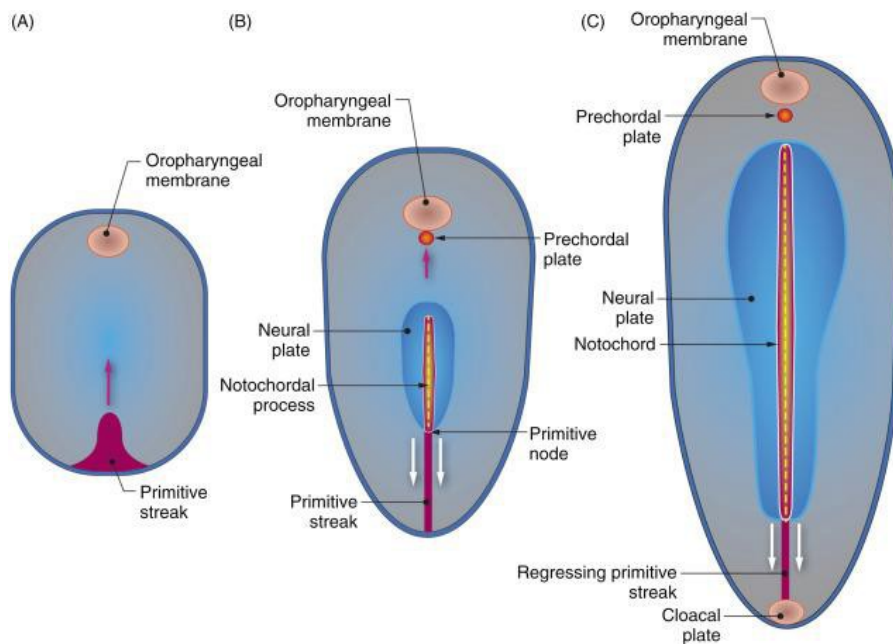


### Notogenesis

It's the process of the **notochord** development in the midline of mesoderm layer. It starts during gastrulation (**3rd week**)

**The notochord** is a cellular rod that forms the first longitudinal midline axis around which the vertebral bodies are organized and is the basis for the axial skeleton.

- It is critical in induction of neurulation.



## Neurulation

It's a process in which the embryo develops structures that will eventually become the nervous system. The embryo at this stage is termed the **neurula**. It appears in 4 stages:

### 1. Formation of neural plate :

The neurulation begins at 3<sup>rd</sup> week when **the notochord induces the formation of the central nervous system (CNS)** by signaling the ectoderm germ layer above it which thickens and forms the **neural plate**. Cells of the neural plate also called the **neuroectoderm**.

### 2. shaping and elongation of the neural plate:

Lengthening of the neural plate and body axis, whereby there is a medial movement of cells in the plane of the ectoderm and mesoderm.

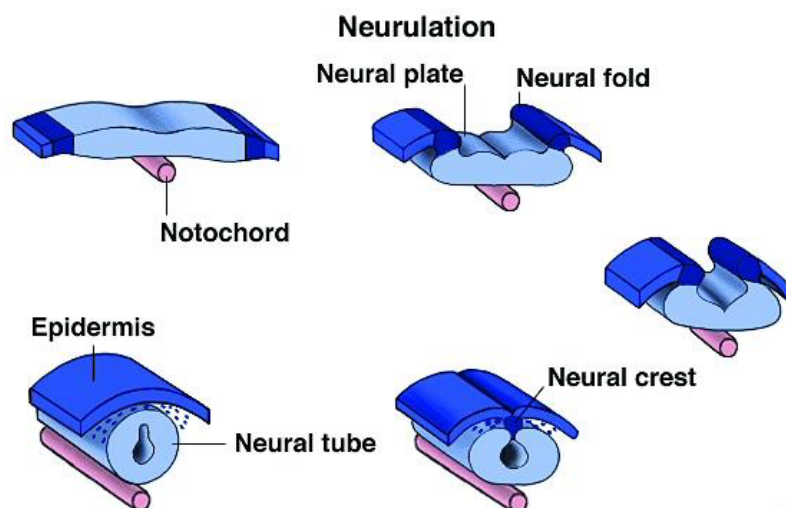
### 3. bending of the neural plate:

As the neural plate lengthens, its lateral edges elevate to form **neural folds**, and the depressed midregion forms the **neural groove**, followed by elevation of the lateral folds

### 4. closure of the neural tube.

Gradually, the neural folds meet each other in the **midline**, where they fuse forming the **neural tube**.

- By the **day 23** the neural tube closed along the embryo except the anterior (cranial ) and posterior (caudal) ends, so the neural tube remained communicate with the amniotic cavity by way of the **cranial** and **caudal neuropores**
- Closure of the cranial neuropores occurs at approximately **day 25**, whereas the posterior neuropore closes at **day 28**.
- Neurulation is then complete, and the central nervous system represented by a closed tubular structure with a narrow caudal portion (the spinal cord ) and a much broader cephalic portion characterized by a number of dilations which are the brain vesicles .

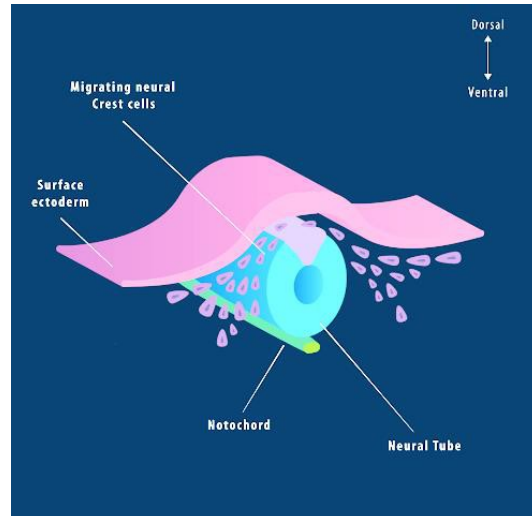


## Neural crest cells

As the neural folds elevate, cells at the crest of the neuroectoderm begin to **dissociate** from their neighbors and undergo an **epithelial-to-mesenchymal transition** as it leaves the neuroectoderm by **active migration** and displacement to enter the underlying mesoderm. Neural crest cells are so important and sometimes called the **fourth germ layer**.

Neural crest cells eventually give rise to :

1. The dorsal root ganglia
2. Schwann cells
3. the autonomic nervous system
4. Meninges
5. Sensory ganglia
6. Bones of the face
7. Teeth
8. Lens of the eyes
9. Melanocytes
10. Adrenal medulla & many glands.



## Germ layers and their derivatives

### ECTODERM

- a. central nervous system (the brain and spinal cord)
- b. peripheral nervous system
- c. epidermis of the skin
- d. cornea and lenses of the eyes

### MESODERM

- connective tissue, cartilage, and bone
- striated and smooth muscles
- the heart walls, blood and lymph vessels and cells (circulatory system)
- kidneys; the gonads (ovaries and testes) and genital ducts

### ENODERM

- epithelial lining of the gastrointestinal and respiratory tracts
- parenchyma of the tonsils, liver, the thymus, the thyroid, the parathyroids, and the pancreas
- liver & pancreas

## PHARYNGEAL ARCHES

Pharyngeal arches are paired structures associated with the pharynx of embryo, which are responsible of head and neck development.

### Development of pharyngeal apparatus

At about the **beginning of the 4th week**, the **1st pharyngeal arch** appears separates the mouth pit or stomodeum from the pericardium, followed by formation of 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> arches more caudally, **the 5th arch fails to form**, so the arches are numbered **1, 2, 3, 4, and 6**.

pharyngeal arch pairs project forward from the back of the embryo toward the front of the face and neck. Each arch develops its own artery, nerve that controls a distinct muscle group, and skeletal tissue. These arches grow and join in the ventral midline.

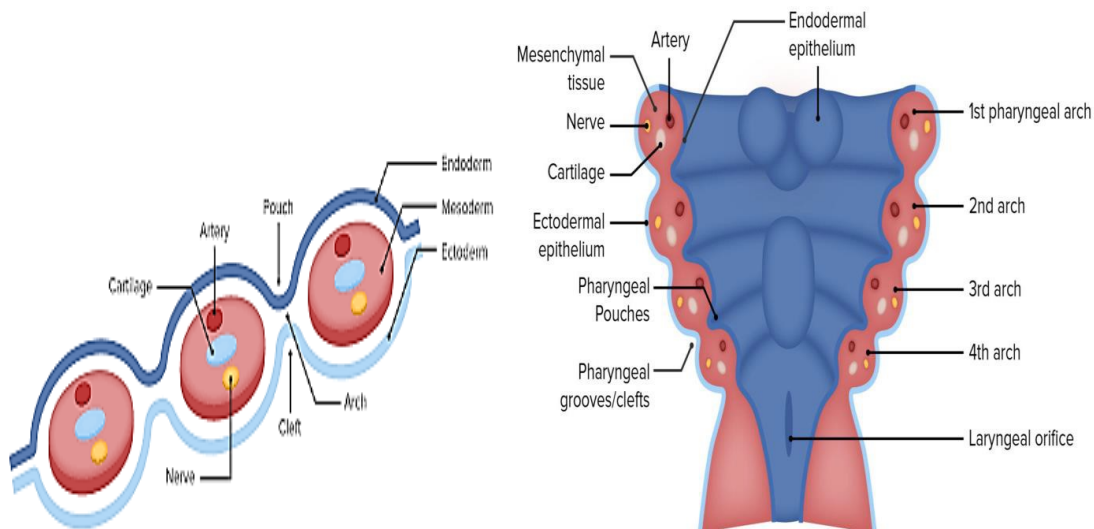
### Structure of pharyngeal arches

Each pharyngeal arch consists of a core of mesoderm tissue covered **laterally by ectoderm** and **medially by endoderm** layer, the mesenchymal core of each arch receives substantial numbers of neural crest cells, also each arch have its own blood vessels and nerve. pharyngeal arches separated from each other by **pharyngeal pouches** from **endoderm side** and **pharyngeal clefts** from **ectoderm side**.

The approximation of the ectoderm of the pharyngeal cleft with the endoderm of the pharyngeal pouch forms the **pharyngeal membrane**

The **5-week embryo** is characterized by the presence of **five arches, four cleft and four pouches**

The 1<sup>st</sup> pharyngeal arch called mandibular arch and the 2<sup>nd</sup> pharyngeal arch called hyoid arch, the remnant arches have no name.



### Derivatives of pharyngeal arches:

arch	Skeletal elements	Muscles	Artery	Nerve
<b>1<sup>st</sup> pharyngeal arch</b> (mandibular arch)	1. maxilla 2. mandible	1. Muscles of mastication (chewing) 2. Mylohyoid muscle 3. anterior belly of digastric muscle	Maxillary artery	Trigeminal nerve
<b>2<sup>nd</sup> pharyngeal arch</b> (hyoid arch)	1. upper part of hyoid bone. 2. Lesser horn of the hyoid bone. 2. Stapes 2. Temporal styloid process	1. Muscles of face 2. Platysma 3. Stylohyoid ligamint 4. Posterior belly of digastric muscle 5. Stapedius muscle	Hyoid artery  Stapedial artery	Facial nerve
<b>3<sup>rd</sup> pharyngeal arch</b>	1. Hyoid (greater horn and lower part of its body)	Stylopharyngeus muscle	1. Common carotid 2. Internal carotid artery	Glossopharyngeal nerve(IX)
<b>4<sup>th</sup> pharyngeal arch</b>	laryngeal cartilages	1. constrictors of the pharynx 2. levator palatini 3. cricothyroid.	1. Right 4th aortic arch: subclavian artery 2. Left 4th aortic arch .	<b>superior laryngeal</b> of the vagus nerve.
<b>6<sup>th</sup> pharyngeal arch</b>	1. Cricoid cartilage 2. Arytenoid cartilage	All intrinsic muscles of larynx except the cricothyroid muscle	1. Right 6th aortic arch: pulmonary artery 2. Left 6th aortic arch: pulmonary artery and ductus arteriosus.	<b>recurrent laryngeal</b> branches of the vagus nerve.

## Pharyngeal pouches

Pharyngeal pouches form on the endodermal side between the branchial arches.

- **First pouch**  
Give endoderm lines the future auditory tube (Pharyngotympanic Eustachian tube), middle ear, mastoid antrum, and inner layer of the tympanic membrane.
- **Second pouch**  
Contributes to the middle ear and palatine tonsils
- **Third pouch**  
Third pouch enlarged and form two wings  
**dorsal wings** form inferior parathyroid glands  
**ventral wings** fuse to form the cytotreticular cells of the thymus
- **Fourth pouch:**  
Fourth pouch enlarged and form two wings  
**Dorsal wing** form superior parathyroid glands  
**Ventral wing** give rise to parafollicular C-Cells of the thyroid gland.

## Pharyngeal clefts

Pharyngeal clefts form on the ectodermal side between the branchial arches.

Developing embryo have four pharyngeal clefts

### 1<sup>st</sup> Pharyngeal cleft

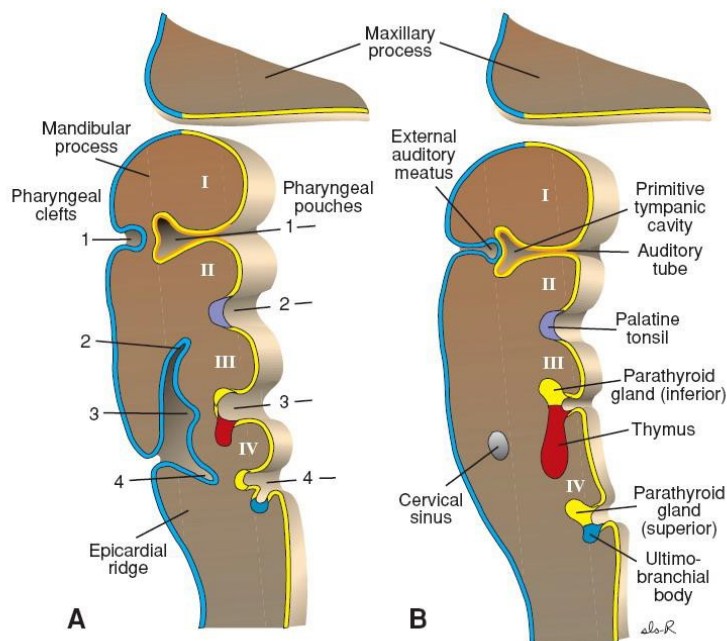
Develops into the external auditory meatus (the corresponding 1st pharyngeal pouch develops into the auditory or eustacian tube, and the intervening membrane develops into the tympanic membrane).

Defects in the development of pharyngeal 1<sup>st</sup> cleft can result in preauricular cysts and/or fistulas (in front of the pinna of the ear).

### Pharyngeal cleft 2,3 and 4

are overgrown by expansion of the 2nd pharyngeal arch and forming the ectodermal depression called cervical sinus.

By the end of 7<sup>th</sup> week the 2<sup>nd</sup> to 4<sup>th</sup> pharyngeal grooves and the cervical sinus have disappeared, giving the neck a smooth contour.



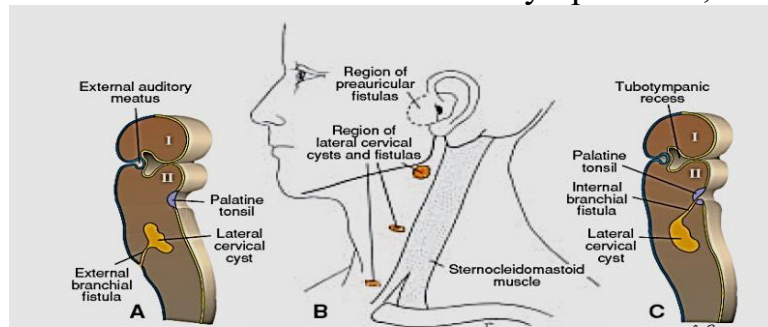


## Branchial cyst

Branchial cyst is a congenital epithelial cyst that arises on the lateral part of the neck usually due to failure from an incompletely closed branchial cleft, usually located between the 2nd and 3rd branchial arches.

Remnants of pharyngeal clefts 2-4 can appear in the form of cervical cysts or fistulas found along the anterior border of the sternocleidomastoid muscle.

Most branchial cleft fistulae are asymptomatic, but they may become infected.



**Figure 15.14** A. Lateral cervical cyst opening at the side of the neck by way of a fistula. B. Lateral cervical cysts and fistulas in front of the sternocleidomastoid muscle. Note also the region of preauricular fistulas. C. A lateral cervical cyst opening into the pharynx at the level of the palatine tonsil.



**Figure 15.15** Patient with a lateral cervical cyst. These cysts are always on the lateral side of the neck in front of the sternocleidomastoid muscle. They commonly lie under the angle of the mandible and do not enlarge until later in life.

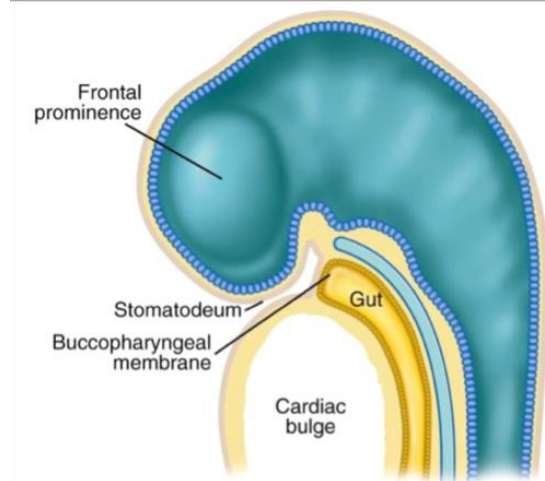


## FACIAL DEVELOPMENT AND ANOMALIES

The human face begins to form during the **4th week** of embryonic development, and continues until the **12th week** with completion of the soft palate.

There are two important tissue structures involved in development of the nose and face { **the pharyngeal arches and neural crest cells** }

The center of the face is formed by the **stomatodeum** that separated from foregut by buccopharyngeal membrane, which is a bilaminar structure consisting from ectoderm outside and endodermal layer from inside, this soon breaks down so that stomatodeum communicates directly with foregut.



### Face initially formed by 5 mesenchymal prominences

1. **Two mandibular prominences** (right and left) derives from 1<sup>st</sup> pharyngeal arch and located inferior to stomodeum.
2. **Two maxillary prominences** (right and left) derives from 1<sup>st</sup> pharyngeal arch located superior/lateral to the stomodeum
3. **Frontonasal prominence** (midline structure) is a single structure that is ventral to the forebrain. It is derived from neural crest cells.

### Nasal development

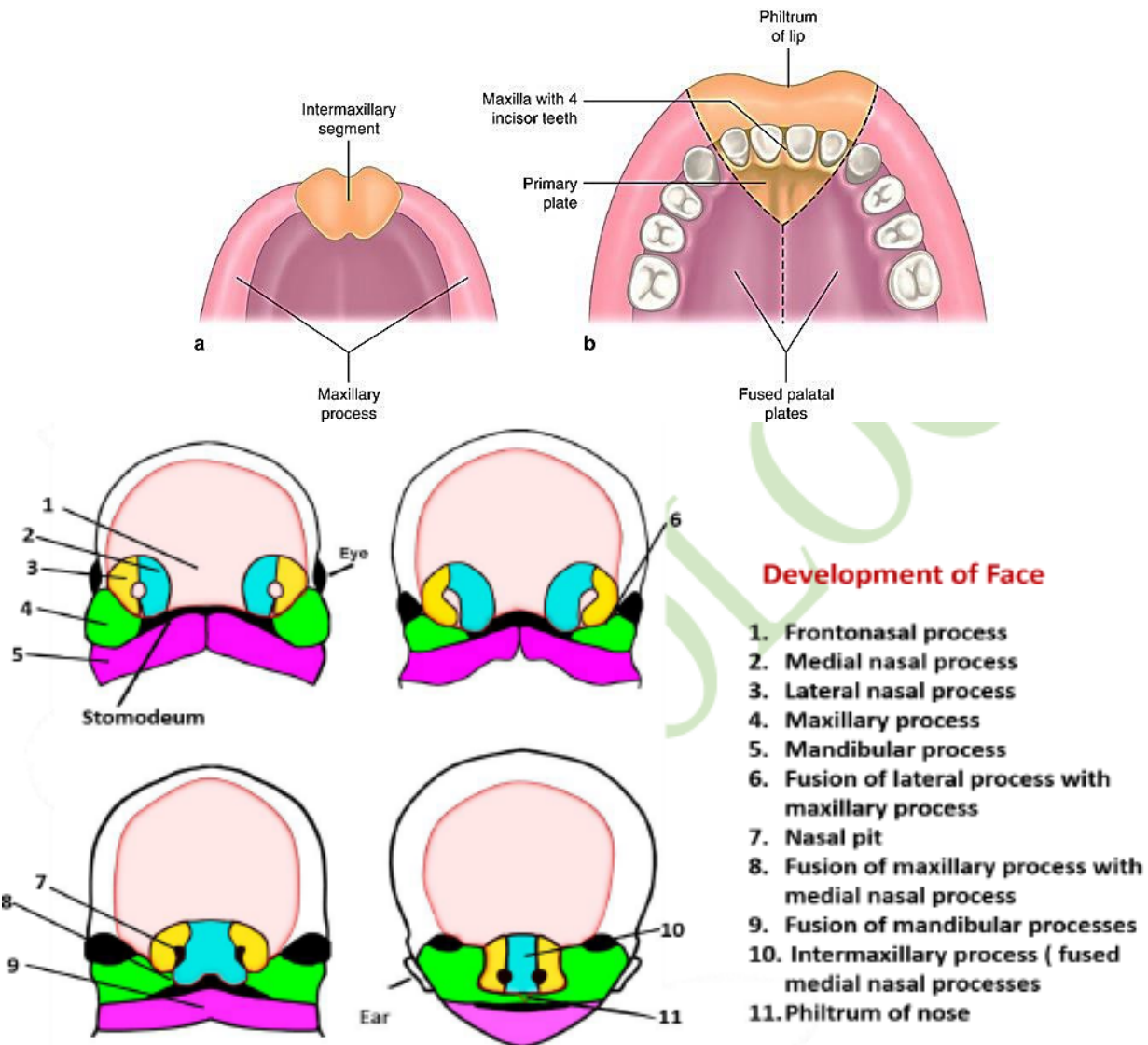
The nose start to develop at end of **4th weeks** of gestation, in the following sequence:

1. on each side of the frontal prominence, and just above the stomatodeum a local thickening of the surface ectoderm developed and called **nasal placodes**.
2. The nasal placodes become depressed due to the rapid proliferation of underlying mesoderm around the placodes, produces a horse shoes shaped ridge convert nasal placodes into **nasal pit**.
3. The nasal pits deepen and develop the **nasal sacs** in the **6th week**.

The horse shoes shaped ridge present as two fast growing ridges, called as **the lateral and medial nasal swellings** surround the nasal sac.

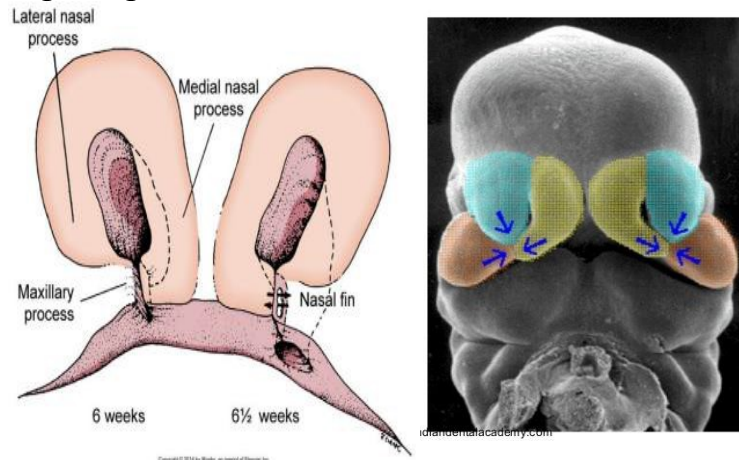
- **Medial nasal swellings** fused at the midline and give rise to **intermaxillary segment** which give rise to:
  - 1-The middle portion of the nose.
  - 2-The middle portion of the upper lip (filtrum).
  - 3-The middle portion of the maxilla which carries the 4 incisors teeth.
  - 4-The primary palate.
- **Lateral nasal swellings** give rise **alae of the nose**, in the same time they fuse with maxillary process laterally, initially they separated by a groove, the epithelium in floor of the groove form a solid core that separate from the surface and eventually canalizes to form **nasolacrimal duct**

➤ The nasal septum grows as a down growth from the merged nasal prominences and fuses with the palatine process.



## The Nasal Fin

The nasal fin is an epithelial seam that develops by fusion between the epithelial linings of the medial and lateral nasal prominences. Shortly after its formation the nasal fin regresses and is replaced by connective tissue growth, which binds together the two maxillary and medial nasal parts of the lip. If this penetration were not to occur, the lip could pull apart.



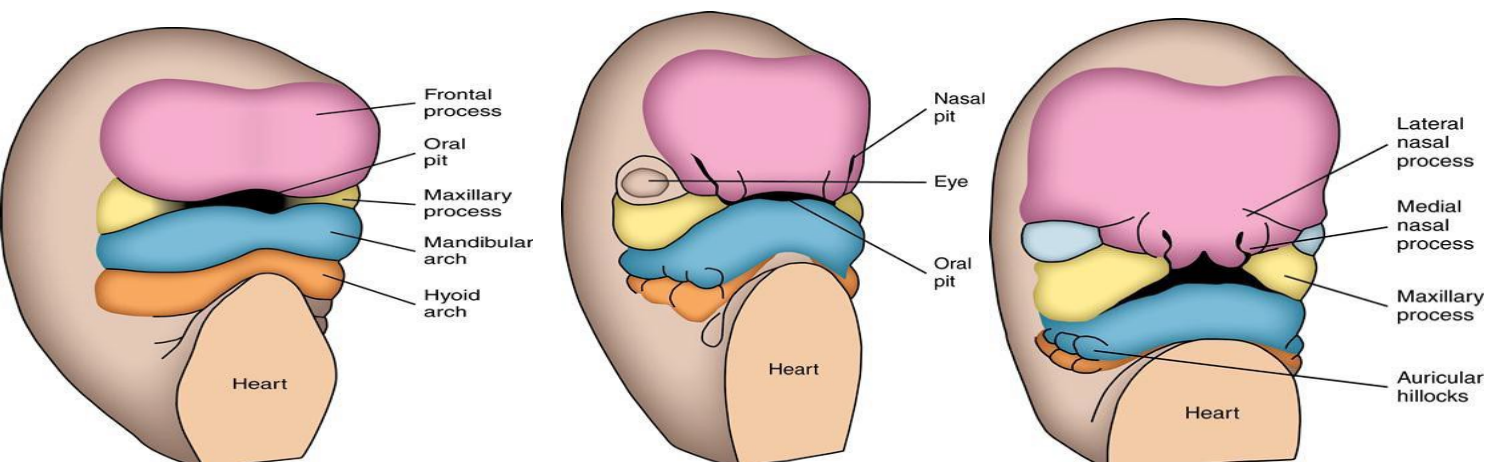
## Cheeks and lips development

As the maxillary prominences continue growing they merge laterally with the mandibular prominences to form the cheeks.

Secondly the cheeks and lips are invaded by mesenchyme of 2nd pharyngeal arch, this mesenchyme will give rise to the muscles of cheek and lips (muscles of facial expression) which innervated by the facial nerve

## Development of lower face:-

It's formed directly by two mandibular swellings, the two mandibular swellings will grow forward, meet and fused at the midline, result in lower lip, chin, lower check region and lower jaw formation.



Human face during the 4<sup>th</sup> prenatal week. Around the centrally located oral pit are grouped the frontal and maxillary processes and the mandibular arch.

Human face during the 5<sup>th</sup> prenatal week. The nasal pits develop and appear on the sides of the face. The frontal process now becomes the frontonasal process

Human face during the 6<sup>th</sup> prenatal week. Nasal pits appear more centrally located in the medial nasal process. This is the result of growth of the lateral face, which also causes the eyes to approach the front of the face. The enlarged maxillary processes are near contact with the medial nasal process.

## **Derivatives Of Facial Components**

### **The frontonasal prominence forms the:**

1. Forehead and the bridge of the nose
2. Frontal and nasal bones

### **The maxillary prominences form the:**

1. Upper cheek regions
2. most of the upper lip
3. Maxilla
4. zygomatic bone
5. secondary palate

### **The mandibular prominences fuse and form :**

1. Chin
2. lower lip
3. lower cheek region
4. mandible

### **The lateral nasal prominences :**

1. alae of the nose

### **The medial nasal prominences:**

1. fuse and form the intermaxillary segment

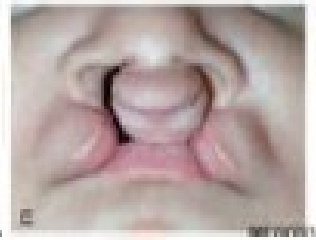
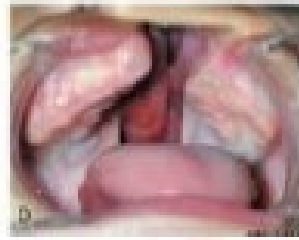
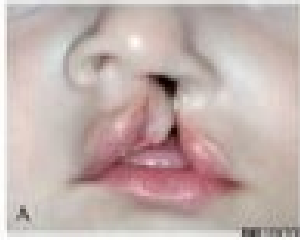
### **Intermaxillary segment**

- 1-The middle portion of the nose.
- 2-The middle portion of the upper lip (filtrum).
- 3-The middle portion of the maxilla which carries the 4 incisors teeth.
- 4-The primary palate.

## **Facial clefts**

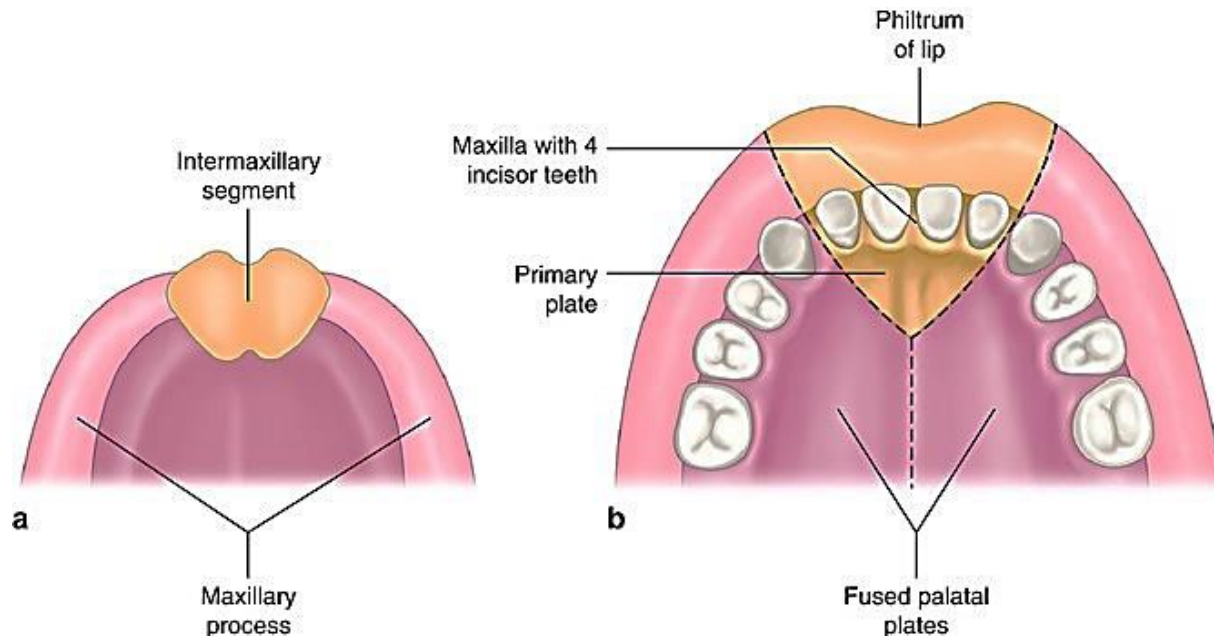
Are malformations that occur very early in pregnancy result from fusion failure among different processes, Types of facial clefts:

- **median cleft lip** (harelip, lack of fusion between two medial nasal process).
- **bilateral cleft lip** (lack of fusion between the maxillary processes and intermaxillary process), its often occurring with cleft palate.
- **macrostomia** (resulting from failure of the maxillary and mandibular processes to fuse).
- **oblique facial cleft** (lack of fusion between the maxillary process and lateral nasal process).
- **mandibular cleft** result from failure of fusion between the two mandibular processes.
- **Frontonasal dysplasia:** hyperplasia of inferior frontonasal prominence ,thus preventing fusion of the medial nasal prominences.



## Palatal Development

The **palate proper** is formed by two parts: the **primary palate** that originated from intermaxillary segment and **secondary palate** which originated from the maxillary prominences.



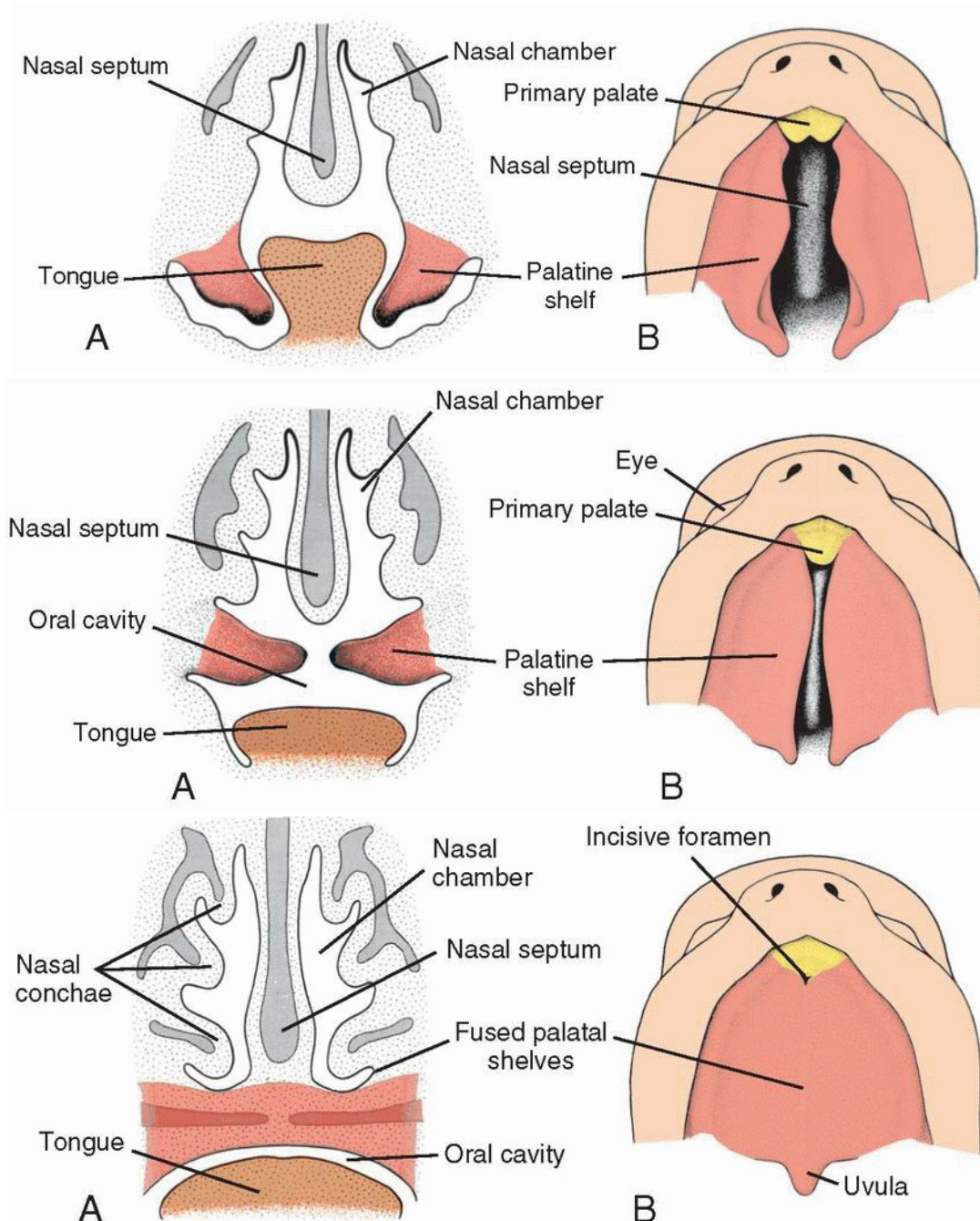
**Palatogenesis** involves the initiation, morphogenesis, and fusion of the primary & secondary palatal shelves to form the intact palate separating the oral cavity from nasal cavity

### Palatal development

- In the beginning, the nasal cavities are separated from the oral cavity by the **oronasal membrane**. This membrane disappears in the **7th week** leaving a connection between the nasal cavities and the oral cavity, called the **oronasal cavity**, which bounded anteriorly by the primary palate and occupied mainly by the developing tongue.

**Development of the primary palate** started at **5<sup>th</sup> week** when the intermaxillary segment formed which gives rise to the primary palate.

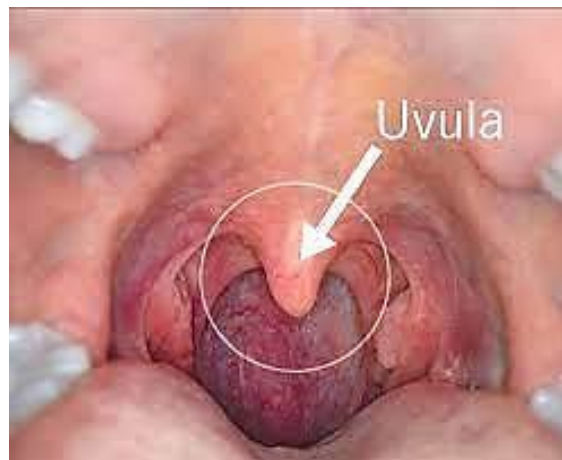
**Development of secondary palate** started at the end of 6<sup>th</sup> week of embryonic development as paired outgrowths called **palatal shelves** (originated from the maxillary process), which initially grow vertically flanking the developing tongue, subsequently with expansion of mandibular process the tongue is withdrawn from between the shelves, and now palatal shelves elevated (this process known as **palatal shelf elevation**) and fuse with each other at the midline above the tongue and with the primary palate anteriorly (the incisive foramen being the landmark between the primary palate and secondary palate). Also palatal shelves fused anteriodorsally with the nasal septum to form an intact roof of the oral cavity





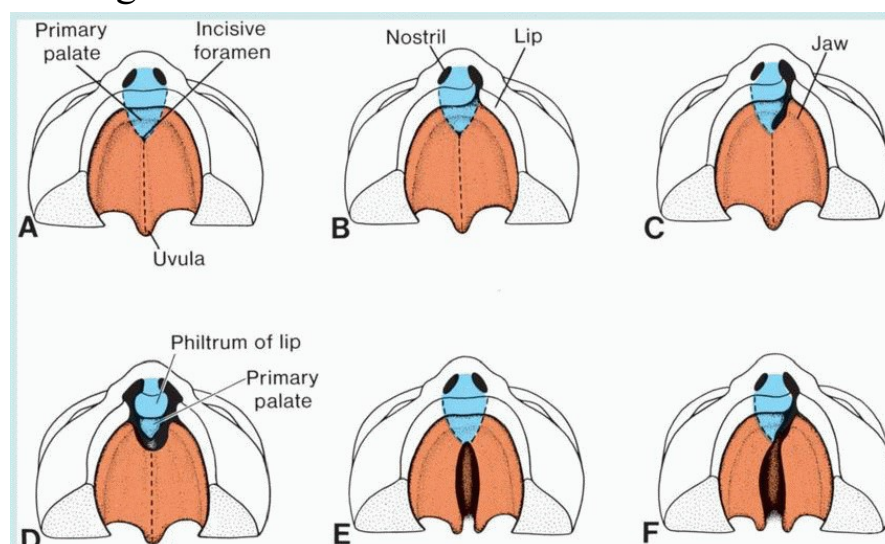
## The Palatine Uvula

It's a little piece of teardrop-shaped tissue that hangs from the back of the roof of the mouth. During swallowing, the soft palate and the uvula move together to close off the nasopharynx, so helps prevent food and liquid from entering the nasal cavity during swallowing. It also secretes saliva to keep mouth hydrated. The uvula is also part of gag reflex. When something touches this area of soft palate, it could induce gagging or vomiting as a safety feature. If uvula senses that something is going down the "wrong way," it course corrects in an effort to avoid choking.



## Palatal clefts:

- **Anterior cleft palate:** located anterior to incisive foramen, results from failure of fusion between the intermaxillary segment and maxillary process.
- **Median palatal cleft:** located posterior to incisive foramen, result from failure of fusion between the two palatal shelves.
- **cleft uvula:** result from failure of fusion between the two palatal shelves in their posterior region.



## Tongue Development

The tongue is muscular structure extend from the hyoid bone to the floor of the mouth. Tongue formed of two parts posterior third and anterior two thirds separated by V-shaped groove (terminal sulcus).

### Tongue development

Tongue development started at the 4<sup>th</sup> week of embryonic development, when the right and left pharyngeal arches meet at the midline they give rise many swellings in the floor of the mouth:

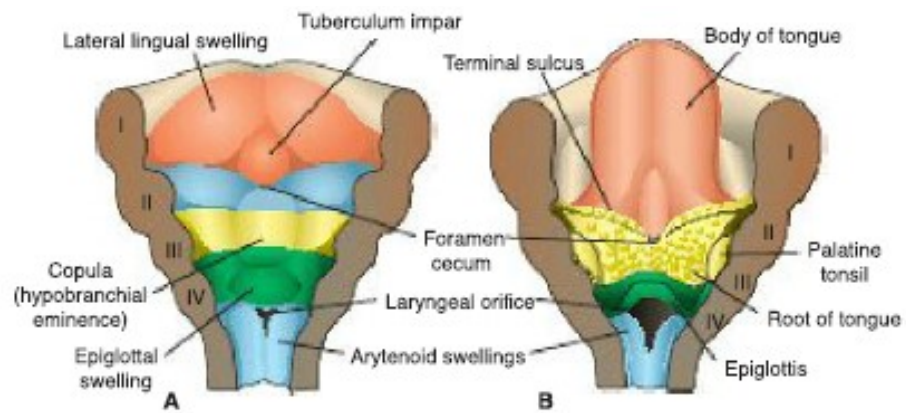
1. **Tuberculum impar** midline swellings arise from mandibular process
2. **two lateral lingual swellings**, that are flanked tuberculum impar arise from mandibular process
3. **copula** midline swelling (associated with the second arch)
4. **large hypobranchial eminence** (from third and part of fourth arches).

Lateral lingual swelling quickly enlarge and merge with each other and with tuberculum impar to form a large mass, which give rise the mucous membrane of the **anterior two thirds of the tongue**.

As the tongue develops, the hypobranchial eminence overgrows the copula and both of them give rise the mucous membrane of the **root of the tongue**.

The anterior two thirds of the tongue separated from posterior one third of the tongue by **terminal sulcus**

So this mucous membrane of anterior two thirds innervated by nerve of 1<sup>st</sup> pharyngeal arch (**trigeminal n.**) The mucosa of posterior part of tongue supplied by the 9<sup>th</sup> cranial nerve (**glossopharyngeal n.**) which related to 3<sup>rd</sup> pharyngeal arch. The epiglottis and the extreme posterior part of the tongue are innervated by the **superior laryngeal nerve**, reflecting their development from the 4<sup>th</sup> arch. Most of tongue muscles are derived from myoblasts originating in occipital somites. Thus, tongue musculature is innervated by the 12<sup>th</sup> cranial n. (**hypoglossal nerve**).



## Tongue anomalies

### Tongue-Tie

Also called ankyloglossia, the tongue is not freed from the floor of the mouth. Normally, extensive cell degeneration occurs, and the frenulum is the only tissue that anchors the tongue to the floor of the mouth. In the most common form of ankyloglossia, the frenulum extends to the tip of the tongue.



### Aglossia:

Failure of the tongue development associated with narrow mandibular arch and crowded teeth.



## **Hypoglossia**

Also called (microglossia), rare anomalies caused by under development of the tongue



## **Bifid tongue:**

Also called (cleft tongue), is a tongue with a groove or split running lengthwise along the tip of the tongue. It is the result of incomplete fusion of the two lateral lingual swellings.



## Dentin Structure and Dentinogenesis

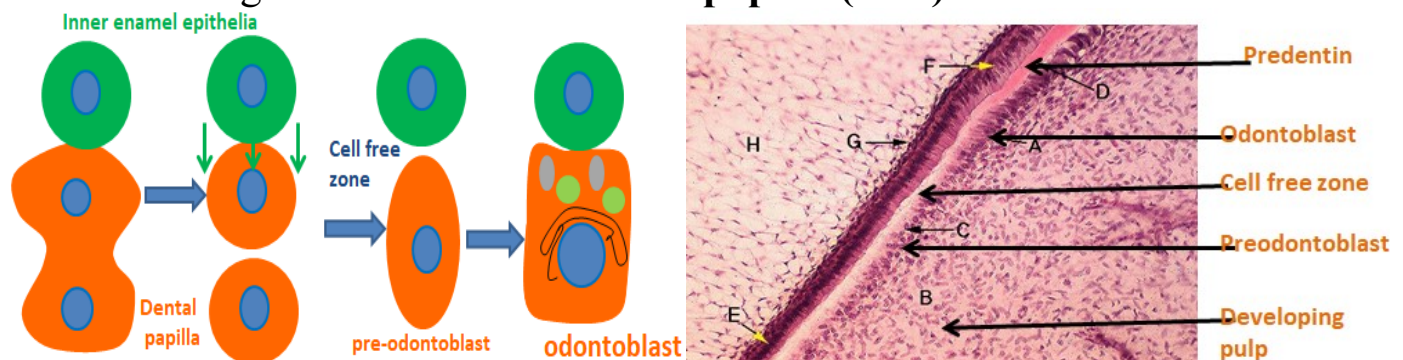
**Dentin** (American English) or **dentine** (UK English) is a calcified tissue that surrounds the entire pulp, secreted by **odontoblasts**, its covered by enamel on the crown and cementum on the root.

- Unlike enamel, dentin formation continues throughout life and can be initiated in response to stimuli, such as tooth decay or attrition.
- Dentin is **yellow color**, it greatly affects tooth color due to enamel translucency
- Dentin is **elastic tissue** which allows the impact of mastication to occurs without fracturing overlying brittle enamel. This resilience is due in part to the presence of tubules throughout the matrix which extend from dentinoenamel junction to the pulp.
- Radiographically: dentin appear more radiolucent than enamel and more radiopaque than pulp.
- By weight, 70% of dentin consists of the inorganic, which represented by hydroxyapatite crystals  $\{Ca_3(po_4)_2.Ca(OH)\}$ , 20% is organic material consists of collagenous protein(fibrils) and ground substance and 10% is water

### Odontoblasts

It's the cell responsible for **dentin formation**, that originated from dental papilla, its first appear at sites of tooth development at 17–18 weeks of development and remain present until death.

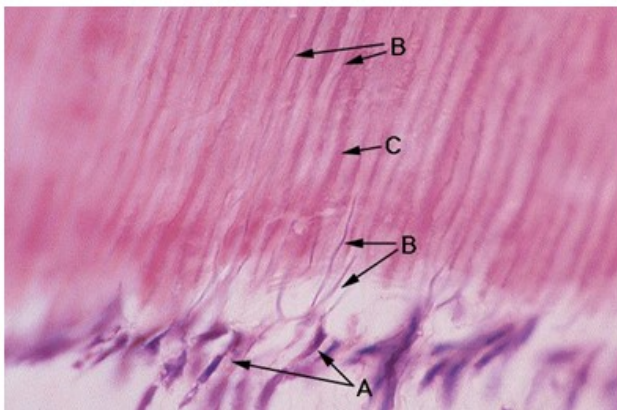
**Odontoblasts differentiation:** In early bell stage the dental papilla formed of **mesenchymal cells** of relatively small uniform size, with large centrally located nucleus, under the influence from inner enamel epithelia (I.E.E.) the peripheral cells of dental papilla will have a short columnar shape and arranged in a single layer, but separated from IEE by **a cell free zone**, as the differentiation progress these cells increase in length to about  $40\mu m$  and  $7\mu m$  in width and become a functioning cells, they will named odontoblasts, at this time the cell free zone will be disappear. Thus the formative organ of dentin is the **dental papilla (D.P.)**.



**Histologically** odontoblasts are large columnar cells in the crown to flatter cells near the apex, whose cell bodies are arranged along the interface between dentin and pulp. Odontoblast passed quiescent and secretory phases:

**Quiescent phase** (resting phase) are flattened with little cytoplasm condensed chromatin and decrease number of endoplasmic reticulum.

**Secretory phase** (active phase): cells show increase in length about  $40\mu\text{m}$  and  $7\mu\text{m}$  in width with increase endoplasmic reticulum, Golgi apparatus and secretory vesicles. It is noted that it is polarized so its nucleus is aligned away from the newly formed dentin, with its Golgi complex and endoplasmic reticulum towards the dentin. As more dentine matrix is deposited, odontoblast cells retreat in the direction of the pulp leaving an elongated process known as the **odontoblastic process** or **Tomes fibers** that occupying the dentinal tubules.



A- odontoblast  
B- odontoblast process  
(tomes process)  
C- predentin

## Dentinogenesis

The process of **dentin formation** by odontoblasts. It starts at the **late bell stage**, it begins at the tip of the crown and it proceeds in a rhythmic pattern to gradually complete cervically. Its occur prenatally as well as postnatally, and to a lesser extent can be seen during the whole life when secondary and tertiary dentin is formed.

**Dentinogenesis occur in two stages:**

### 1-Secretion of dentin matrix (predentin):

After differentiation of odontoblasts, they start in production of the organic matrix (predentin), which consisting of **collagen** fibers which associated with the ground substance which is glycosaminoglycan

The first dentin formed contain bundles of type III collagen fibers with **very distinct large diameter** (0.1-0.2mm) called **vonKorff's fibers** they are perpendicular to the basement membrane and attached to it **supporting the I.E.E.** (in the crown region).. This collagen constitutes the matrix of first formed dentin which is called **mantle dentin**.

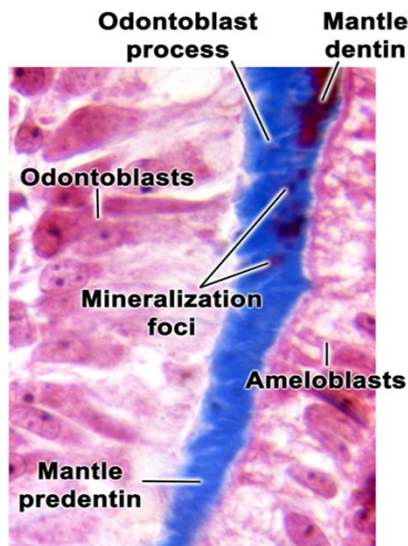
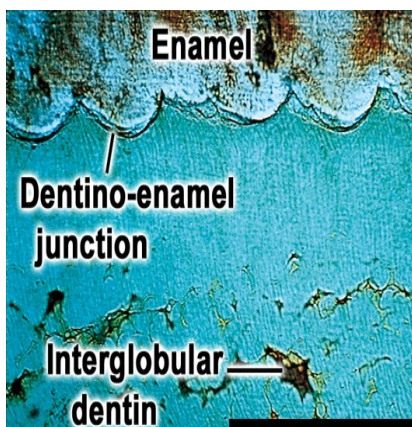
Then korff's fibers fade gradually and smaller fibrils (type I) and arranged parallel to basement membrane , this dentin called **Circumpulpal dentin**.

## 2-Mineralization:-

It occurs parallel to predentin formation

**Mantel dentin Mineralization** appear in in a globular pattern, where small centers of calcification (crystals) which come from matrix vesicle (electron microscopic bud from cell membrane of odontoblast which contain first Hydroxyapatite crystals and alkaline phosphatase enzyme). When the crystals grow the matrix vesicles rupture and their content spread which come to lie between the large diameter collagen fibrils until they fuse together and form globules, these globules fuse with each other to form a uniformly calcified dentin layer. **On occasion** these large globular masses fail to fully fuse leaving small areas of uncalcified matrix known as **interglobular dentin**.

**Circumpulpal dentin mineralization** goes then in linear or occasionally globular pattern begins by crystal deposition in form of fine plates of hydroxyapatite crystals on the surface of the collagen fibrils. The long axes of the crystals are paralleling to the collagen fibrils.



## Histological features of dentin:-

The dentin composed mainly from:-

- 1- Dentinal tubules.
- 2- Peritubular dentin
- 3- Intertubular dentin

### 1- Dentinal tubules (D.T.):-

are fine canals extend through the entire thickness of dentin from dentinoenamel junction (DEJ) in the crown area, or dentinocemental junction (DCJ) in the root area to the pulpal surface.

- In longitudinal ground section, the course of D.T. are **straight** near the incisal edge, cusp tip and in root dentin. While in rest of coronal dentin, the D.T. follow an **S-shaped path** called (**primary curvature**), starting at right angles from

D.E.J, the first convexity of this doubly curved course directed toward the apex of the root ending perpendicular to pulpal surface, this configuration of tubules indicates the course taken by odontoblasts during dentinogenesis.

- **Secondary curvature** also can be distinguished over the entire length of D.T., they probably reflect the minor changes in the direction of movement of odontoblasts.
- D.T. are tapered structures, larger near the pulp about (3-4 $\mu$ m), and thinnest at DEJ. (1 $\mu$ m).
- The terminal part of tubules branches into 2-3 branches (**terminal branches**) near D.E.J. result in increased number of tubules near the D.E.J., some of these branches may pass or cross the D.E.J. and enter the E. which are called (**enamel spindle**).
- **lateral branches** seen between dentinal tubule called **canaliculi** or **microtubules**.

## 2. Peritubular dentin:

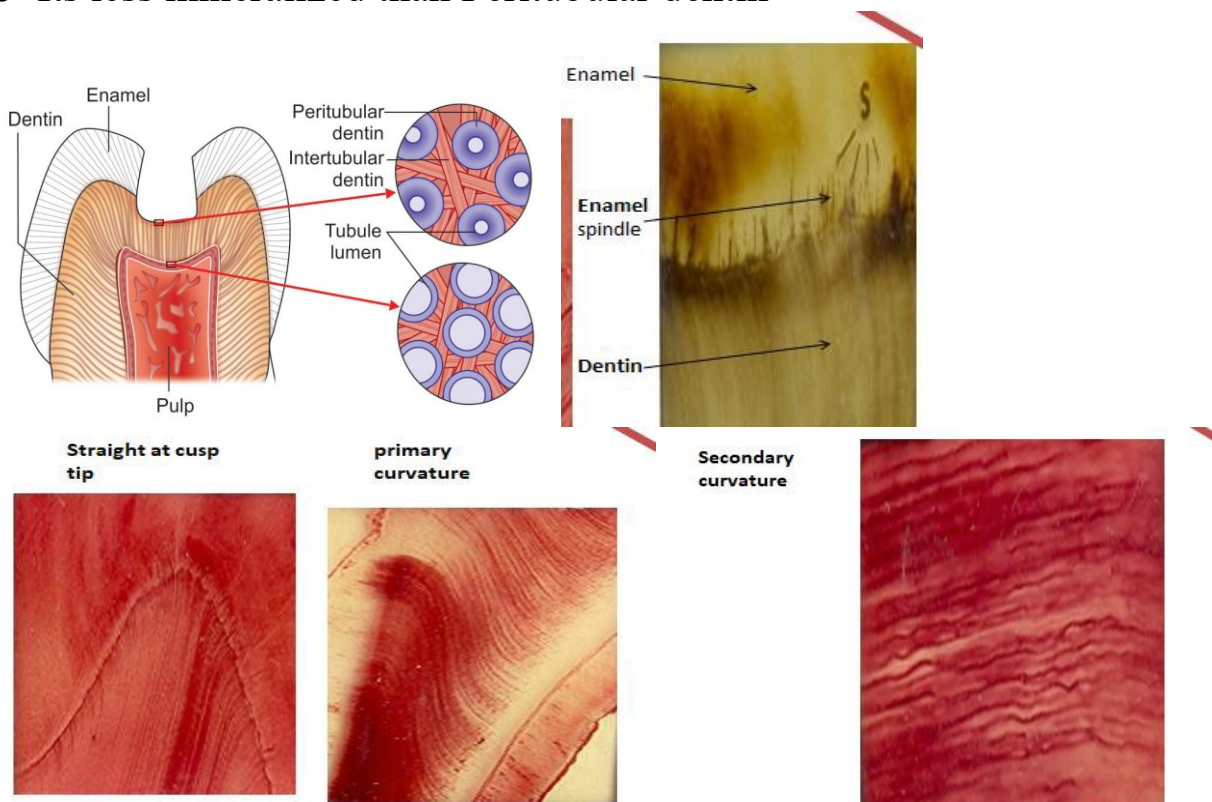
It's the dentin that surrounds the D.T. and form 1 $\mu$ m thick sheath around each tubule, about (0.75  $\mu$ m near DEJ and 0.4  $\mu$ m near the pulp).

- Peritubular dentin is missing from dentinal tubules of interglobular dentin indicating that this a defect of mineralization.

## 3. Intertubular dentin:-

It's the dentin located between the Peritubular dentin it forms the bulk of dentin body.

- It consist of network course of collagen fibers in which apatite crystals deposited on it.
- Its less mineralized than Peritubular dentin





## **Under microscope other structure in dentin can be notes:-**

### **Predentin:-**

It's **unclassified dentin** located adjacent to the pulp tissue lines the innermost region of dentin, it is 10-47 micrometer. It's stained lighter by H.&E. than calcified dentin.

### **Granular layer of tome's:-**

It's **granular zone**, only appear in root dentin adjacent to cementum and only seen in ground section. It appear due to a coalescing and looping of terminal portions of the dentinal tubules. These areas remain **unmineralized**.

### **Interglobular dentin:**

Its **hypomineralized dentin** found in the crown in both sections (decalcified and ground sections) near the D.E.J. and in the root near C.D.J., its formed during globular mineralization, in normal situations small globular areas fused to form a uniformly calcified dentin layer, if fusion does not take place, hypo mineralized regions remain between the globules, which termed **interglobular dentin**. In ground sections is sometimes lost and replaced by air ,so it appear **black**.

## **Incremental lines of dentin:-**

### **1- Incremental lines of von Ebner:-**

All dentin is deposited in daily rhythmic manner, this results in fine lines run at right angles to dentinal tubules seen in cross section. The distance between lines varies from 4-8  $\mu\text{m}$  in crown to much less in root. The course of lines indicates the growth pattern of the dentin.

### **2- Contour lines of Owen:-**

They are **hypocalcified bands**, seen only in longitudinal ground section as accentuated few lines.

### **3- Neonatal lines:-**

It's accentuated contour line separate between prenatal and postnatal dentin seen in deciduous teeth and 1<sup>st</sup> permanent molars. This line is the result of incomplete calcification, due to metabolic disturbances at the time of birth due to the abrupt changes in nutrition of newborn infants. It's zone of **hypocalcification**.

## **Types of Dentin:-**

**1-Primary dentin:** is the outermost layer of dentin, its composed of:

### **a- Mantle dentin:-**

The outer layer dentin closest to enamel formed by new differentiated odontoblasts.

- It's **thin** layer about 150 micrometers wide.
- **Less mineralized** than remainder of primary dentin
- Contain larger collagen fibers, run perpendicular to D.E.J. (Von corff's fiber).

### **b- Circumpulpal dentin:-**

it's secreted after the mantle dentin by the odontoblasts and form the bulk of primary dentin, it's the dentin formed before the complete formation of the root, it's collagen fibrils are small, and densely packed together, it contains more minerals than mantle dentin

### **2- Secondary dentin:-**

It's formed internal to the primary dentin of the crown and root, develops after the crown has come into clinical function and the roots are nearly completed.

- This secondary dentin is deposited more slowly than the primary dentin causing gradual reduction in the size of pulp cavity.
- It has a similar structure to primary dentin the tubules of primary and secondary dentin are generally continuous.

### **3-Tertiary dentin:-**

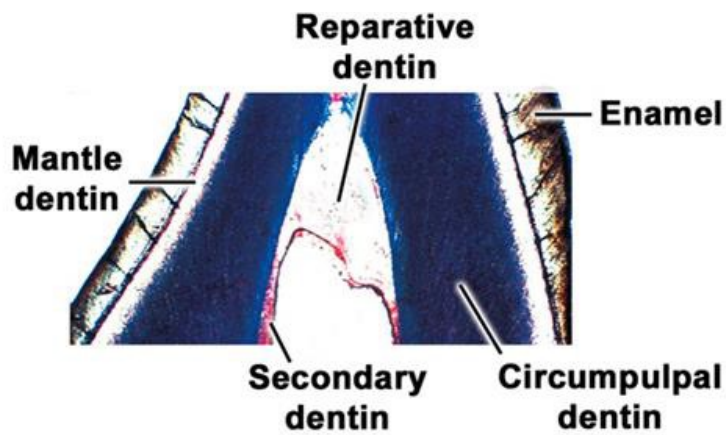
Results from pulpal stimulation, whether due to attrition, abrasion, caries or restoration procedures. Tertiary dentin produced only by cells which directly affected by stimulus. Tertiary dentin is subclassified into **reactionary** and **reparative dentin** depending on intensity of the stimuli.

**Reactionary dentin:** secreted by the odontoblast in respond to chemical attack, either by chemicals diffusing through the dentin or by diffusion of toxic bacterial metabolites down the dentinal tubules in the instance of a carious attack with dental decay. Odontoblast remain survive after injury, and produce **reactionary dentin**

**Reparative dentin:** if odontoblasts killed through injury and it will replaced by undifferentiated mesenchymal cells of pulp (**odontoblast –like cell**), the produced dentin known as **reparative dentin** underneath the site of attack (this action done to seal off the injured site on pulp region and prevents the diffusion of bacteria and their metabolites into the pulp).

Tertiary dentin may be deposited rapidly (in strong or extreme stimuli) resulting in **irregular dentin** formation characterized by sparse and twisted tubules and possible cell inclusions in this case it called **osteodentin** , it lower degree in mineralization than normal, in this case the **tooth has a poor prognosis**.

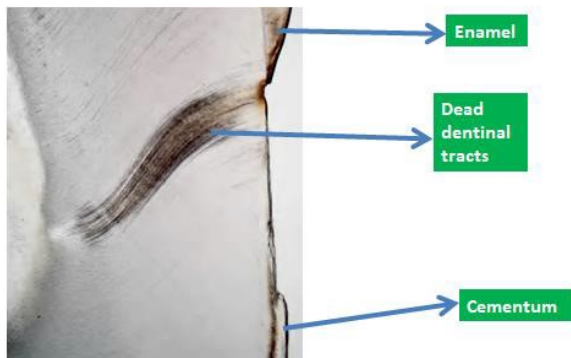
However, if the stimulus is less active, dentin is laid down **less rapidly** with regular dentinal tubules **without any cellular inclusions**, in this case tooth able to be **saved**.



## Dead tracts dentin

The odontoblastic process disintegrated as a result of sever stimuli to the pulp like caries, attrition or abrasion, and the empty tubules are filled with air.

In ground section of dentin, they appear dark in transmitted light and white in reflected light, its area of decreased sensitivity. reparative dentin seals these dead tracts at their pulpal end.



## Enamel Structure and Amelogenesis

Enamel is the hardest calcified tissue in the human body secreted by ameloblast, enamel forms a protective covering over the entire surface of the crown, (E) is ectodermal in origin.

- Unlike other calcified structures in the body enamel is unique as it is totally **acellular, non-vital** and a **vascular** tissue (without blood vessel)
- The Enamel is **white** to **grayish-white** but it appear slightly yellow because it's translucent and the underlying dentin is yellowish, gives the yellow color to enamel.
- The Enamel ranges in thickness from a **knife-edge** at its cervical margin to about **2.5 mm** maximum thickness over the occlusal or incisal surface.
- The Enamel is **very hard** because it composed of 96% mineral (inorganic) in form of hydroxyl apatite (which is crystalline of **calcium phosphate**) **Ca<sub>10</sub>(PO<sub>4</sub>)<sub>6</sub>(OH)<sub>2</sub>**, and 4% organic and water.
- Although Enamel is the hardest tissue in the human body, but it's so **brittle** and may subjected to fracture
- The Enamel is **semi-permeable**, it can permit exchange **ions** and **molecules** specially if the tooth is newly erupted. Fluoride can penetrate enamel easily in newly formed and be less in permeability in adult one.

### Ameloblast

It's the cell responsible for enamel formation

**Ameloblast Differentiation:** ameloblast first seen in the cusp tip region when first layer of dentin had been secreted by odontoblasts, secreted dentin induce adjacent inner enamel epithelium cells (I.E.E.) to differentiate into ameloblasts and amelogenesis process started, so the cells of I.E.E. were needed to induce odontoblasts and now their product (dentin) becomes the **initiator for further differentiation of I.E.E.**

When amelogenesis begins, the tooth germ is described as being at the **crown stage of tooth development.**

## **Life cycle of the ameloblasts:**

### **1- Presecretory stage:**

#### **A- Morphogenic stage**

when first layer of dentin had been secreted by odontoblasts, The delicate basement membrane between I.E.E. and odontoblasts will disintegrate after dentinogenesis and before amelogenesis. The secreted dentin induce adjacent inner enamel epithelium (I.E.E.) to become low columnar cell with large centrally located nuclei and Golgi elements in the proximal portions of the cells adjacent to the stratum intermedium. I.E.E. now called **preameloblast**.

#### **B- Differentiation stage**

The preameloblasts elongate and their nuclei shift proximally toward the stratum intermedium. In each cell the Golgi complex increase its volume and migrates from it's proximal position to occupy major portion of the central core of the cell. The amount of rough endoplasmic reticulum increases significantly, and most of mitochondria cluster in the proximal region. So the majority of cell organelles situated in the cell body distal to the nucleus. Now these cells called **ameloblasts**. Adjacent ameloblasts are closely aligned to each other, due to development of junctional complexes (**proximal terminal bar**) between them.

### **2- Secretory stage (synthesis of enamel)**

Ameloblasts become elongated, the synthesis of enamel protein occurs in the rough endoplasmic reticulum, from where it's passed to the Golgi complex it's condensed and packaged into membrane bound secretory granules migrate to the distal extremity of the cell, and their contents are released against the newly formed mantle dentin.

Secretory ameloblasts secret of first **structureless or prismatic enamel layer**, then ameloblasts will migrate away from the dentin surface to permits the development of a short conical projection which named as **Tome's process**

Tomes' processes interdigitate with the surface of the forming enamel giving it a picket fence appearance. Secretion from proximal part of Tomes process result in formation of inter-rod substance, while secretion from the distal part of Tomes process result in formation of enamel rods.

As the secretory stage is ending Tomes' processes are lost and accordingly the last formed layers of enamel are prismless.

### **3-Maturation stage**

**A- Transitional phase :** After the full thickness of enamel matrix has formed, the ameloblasts enter a brief transitional stage. Their height is decreased and protein synthesizing organelles are drastically reduced. The overall number of ameloblasts is reduced by programmed cell death(apoptosis) and it is estimated that by the end of this stage the ameloblast population is reduced by as much as 25-50%.

## B -Maturation proper:

In this stage ameloblasts modulate between two phenotypes depending on the morphology of their distal ends:

**Ruffled border ameloblasts:** have numerous microvilli in distal end ( 80 % of maturation ameloblasts)

**Smooth-ended ameloblast:** straight distal end ( 20 % of maturation ameloblasts)

Ameloblasts during maturation modulate change their morphology from ruffled to smooth border ameloblasts and back.

In ruffled-ended ameloblasts the massive influx of calcium and phosphates occurs, while in smooth-ended ameloblasts the selective loss of enamel proteins and water occur.

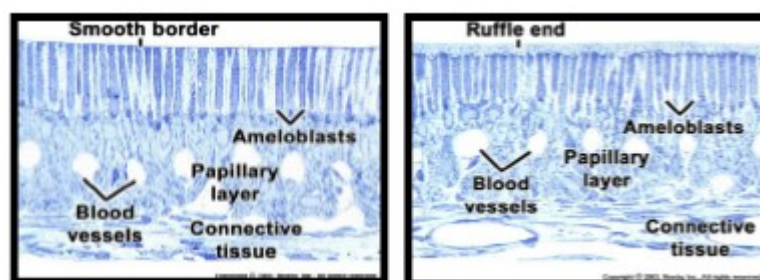
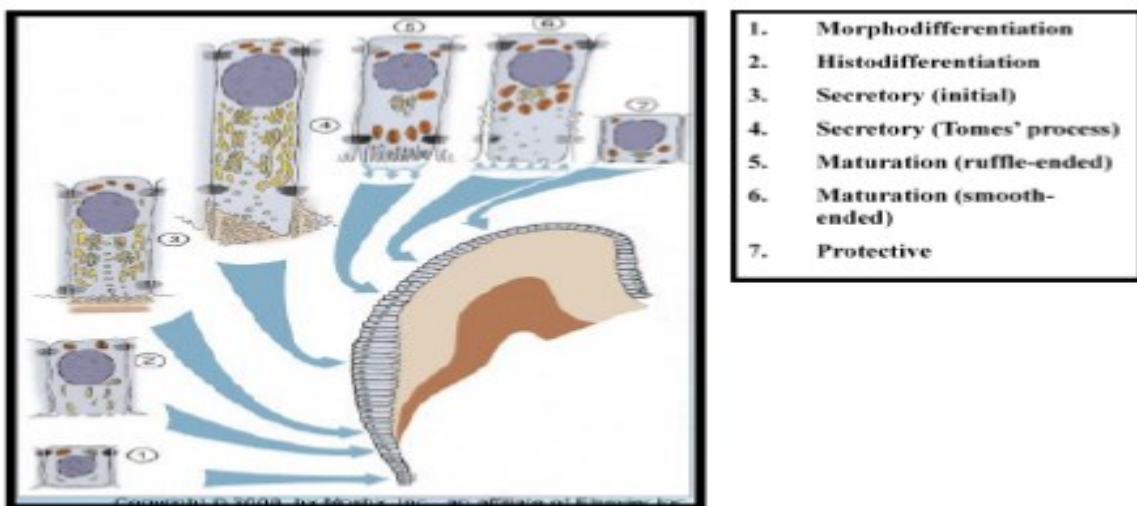
## 4 -Protective stage:

At this stage the ameloblasts become short cuboidal cells which together with the remnants of the other layers of the E. organ form a multilayered structure, namely the **reduced enamel epithelium**.

Reduced enamel epithelium function is protection of formed enamel by separating it from surrounding connective tissue cells in the dental sac until the tooth erupts, otherwise the connective tissues comes in contact with enamel, then it will be resorbed or cementum may be deposited on it.

## 5- Desmolytic stage

The reduced enamel epithelium secrete desmolytic enzyme which induces degeneration of connective tissue which separating it from oral epithelium and enhance the tooth eruption.



# Amelogenesis

It means process of enamel formation by ameloblast cells, it begins shortly after dentinogenesis at the late bell stage.

**Amelogenesis involves 2 stages which are:**

## 1. Enamel matrix deposition:

This stage means production of organic matrix which is composed mainly of **30% minerals** as hydroxy apatite crystals and **70% water and E. proteins** which include 90% amelogenin protein and 10% non-amelogenin protein (enamelin and ameloblastin). The initially and last secreted of E. matrix is described as Rodless or prismless E. (because its secretion is done by ameloblasts without Tomes process). Enamel matrix appears as a deep staining layer in the H. and E. staining sections.

## 2. Mineralization of enamel

When the full thickness of enamel matrix has been deposited, mineralization will be started. This process involves removal of water and protein, followed by the introduction of additional calcium and phosphate, followed by the further removal of water and protein and so on.

Growth of crystals and their fusion occur, these minerals make the initial hydroxy apatite crystals that formed in enamel matrix deposition to grow wider and thicker until full mineralized enamel is formed (until mineralization reaches 96%).

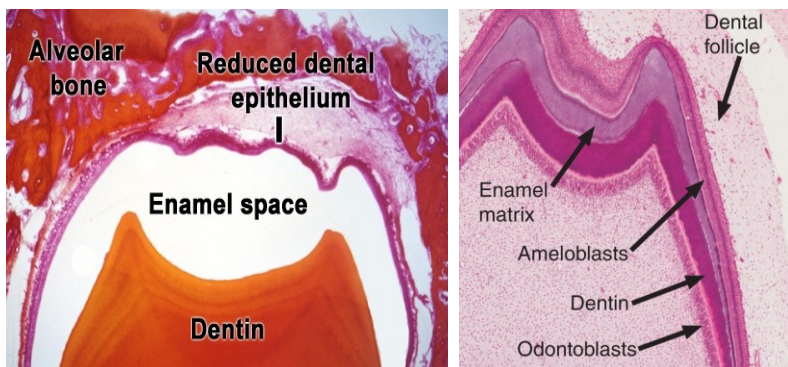
Full mineralized enamel appears as **empty space** in decalcified section.

The source of minerals during maturation are from the:

- 1- Ameloblast itself and other cells of enamel organ like stratum intermedium.
- 2- Capillaries which approach to the outer ameloblasts and are very close to the enamel

## Unique Features of Amelogenesis:

1. Amelogenesis is unique in that the secretory cell is an epithelial cell while all other secretory cells producing hard tissues are ectomesenchymal.
2. In enamel mineralization only noncollagenous proteins are involved; in all other hard tissues collagen plays an important part.
3. After formation of enamel the ameloblasts undergo apoptosis, therefore there is no formation later in life unlike in other hard tissues.



## Histological picture of the Enamel:

Matured enamel only can be studied by **ground sections**, because of the highly mineralized nature of enamel only an empty space can be seen in area of enamel in decalcified section, so it named as **enamel space**, because the mineral ( which represent 96%) has been dissolved and the trace organic material has been washed away during slide processing.

**The Enamel is composed mainly of:-**

- 1- Rods (prisms).
- 2- Rod sheath (prismatic sheath).
- 3- Inter-rod substance (interprismatic subs.).

**1-Enamel Rods (Enamel prisms):-**

It's the basic structural unit of enamel, the number of enamel rods range from 5 millions in lower first incisor to 12 million in upper first molar.

- The rod is shaped somewhat like a cylinder and is made up of millions of crystallites that run with long axes of rod. for the most part.
- Rods are formed perpendicular to DEJ and curve slightly towards the cusp tip. Rods become twisted and intertwine in cusp tip and incisal edges called **gnarled enamel**, it makes the enamel strong and more resistant to fracture.
- The diameter of the rod at the outer surface is double the diameter at DEJ 2:1.

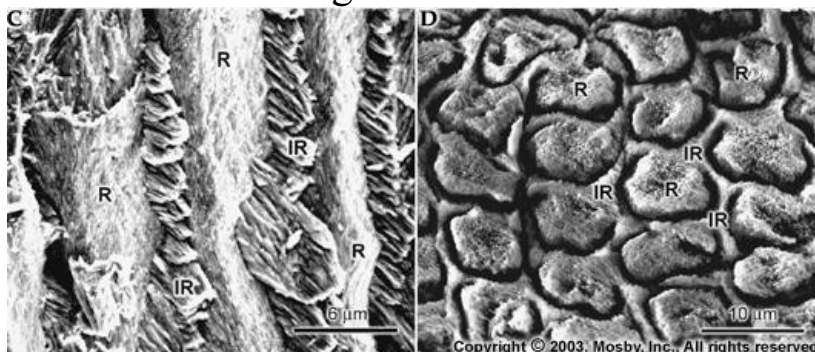
**2- Rod sheath:-**

It's incomplete envelope of organic matter that surround the rods from three quarters, so it separate rods from interrod substance except one quarter

**3-Interrod substance:-**

fill the spaces among enamel rods. In human teeth it may be absent in certain areas.

- Crystals of interrod substances are oriented in a different direction from those making up the rod.
- At the quarter of missing root sheath, the crystals of rods are confluent with crystals of interrod enamel.
- In cross-sectional, rod and inter-rod may appear as hexagonal , round ,oval or fish scale. Sometime has been compared with the shape of a keyhole, the body of it represent the rod which directed occlusally and the tail which directed apically represent the interrod region.





## **Incremental lines of the Enamel:-**

### **1- Cross Striations:-**

are periodic bands of short increments, appear along the full length of enamel rod . Because of this the enamel rod appears **like a ladder**, these segments are about **4 $\mu$ m**, which it represent the **daily rate** of enamel matrix formation by ameloblasts. Its insufficiently calcified.

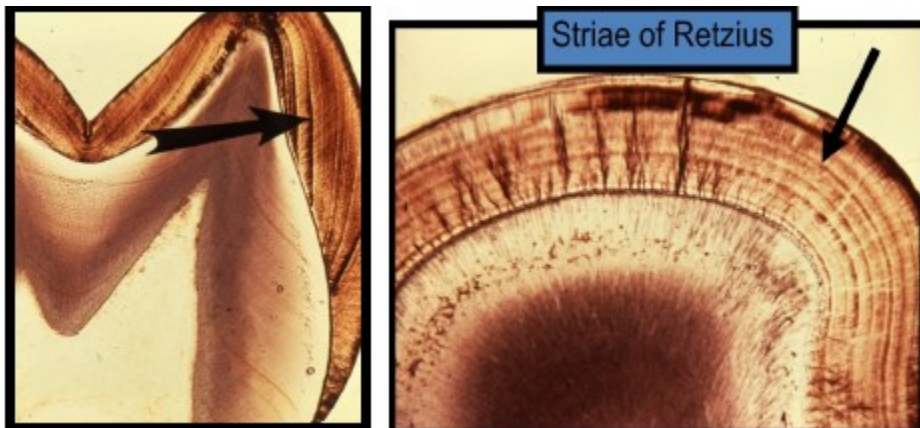
### **2- Incremental lines of Retzius:-**

These are brownish bands in ground section of E. represent incremental weekly growth of E. during formation of crown, reflected variation in structure and mineralization either hypomineralization or hypermineralization.

In ground cross sections they appear like concentric growth rings similar to those found in trees. In ground longitudinal sections they appear to be dark line extending from the DEJ to the tooth surface where they end in shallow furrows know as perikymata (or imbrication lines).

### **3-Neonatal lines:-**

It's incremental lines recorded in E. of deciduous teeth represent part of E.. Is formed before and after birth, appears to be the result of abrupt change in the environment and nutrition of newborn infants.



## **Histological features of enamel:**

### **Hunter-Schreger bands:**

Are light and dark band phenomenon in E., extend about 1/2 the thickness of E. due to change in direction of rods, seen in longitudinal ground section, under reflected light. The dark bands correspond to the cross sectional enamel rods and the light bands represent the longitudinally sectioned interrod enamel.

### **Enamel spindles:**

Before enamel forms, some developing odontoblastic process extend into the ameloblast layer, and when enamel formation begins become trapped to form enamel spindles (which represent the only ectomesenchymal structure present in the E.). It's perpendicular to D.E.J. appear in ground section as dark or black color.

### **Enamel tufts:**

tuft like originate from the DEJ, run 1/5-1/3 E. thickness. They represent protein (enamelin) rich areas in the enamel matrix that fail to mature. They are formed during the formative stages of enamel. They are considered to be 'faults' by some researchers while others consider them to be necessary to anchor dentine to enamel.

### **Surface structures of E.**

#### **1-Perikymata:**

They are transverse, wave like grooves, believed to be the external ending of the striae of Retzius. They are continuous around a tooth & usually lie parallel to each other & to the cemento-enamel junction.

#### **2. E. Lamellae**

Thin leaf like structures that extend from enamel surface toward the dentino-enamel junction and may sometimes extend to dentin. Consist of organic material, with but little mineral content. E. lamellae usually developed in planes of tension

#### **3-E. cuticle**

Primary enamel cuticle covers the entire crown of the newly erupted tooth, secreted by the ameloblasts when enamel formation is completed. probably soon removed by mastication

#### **4-E. Pellicle**

Formed after the tooth is in the oral cavity, acquired from saliva and the oral flora. May contain factors which hinder the attachment of bacteria to tooth surfaces.

### **Hypomineralized structures in E.**

1-E. rod sheath

2-Incremental lines of Retzius

3-E. Lamella

4-E. tuft

5-E. cracks

6-E. spindle

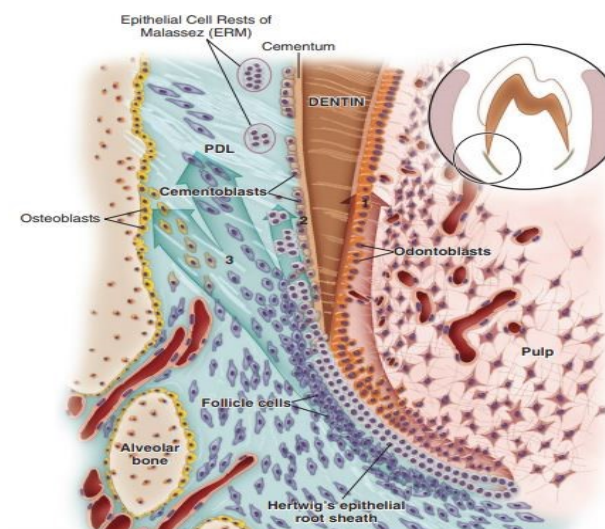
7-Neonatal line

## PERIODONTAL LIGAMENT

The periodontal ligament (PDL) is a fibrous connective tissue ligament located between the alveolar bone and cementum occupies the periodontal space which has thickness of 0.15-0.38 mm, it is thinnest in the mid root zone. PDL composed of cell and extracellular substance.

### Development of PDL

The formation of PDL occurs after the cells of Hertwig's epithelial root sheath have breakdown, permits the cells of the dental follicle to migrate to the external surface of the newly formed root dentin. These migrant follicle cells then differentiate into cementoblasts and deposit cementum on the surface of dentin. Other cells of the dental follicle differentiate into fibroblasts, which synthesize the fibers and ground substance of PDL. Then these fibers become embedded in newly developed cementum and alveolar bone, and as the tooth erupts, they are oriented in characteristic fashion.



### Cells of the PDL.

**1-Formative cells:-** include

#### **a-Fibroblasts:-**

are the **predominant** cell seen in the PDL. These cells are regularly distributed throughout the ligament, and are oriented with their long axis **parallel to the direction of collagen fibrils**. Fibroblasts are **fusiform cells** with many processes, They are large cells with extensive cytoplasm and abundant organelles, associated with protein synthesis and secretion. abundant secretory granules containing type I collagen molecules.

The fibroblasts responsible of collagen and elastin fibers formation, as well as ground substance formation,

#### **b-Osteoblasts**

These are located in PDL along the surface of the alveolar bone, responsible for bone formation on the endosteum surface.

### c-Cementoblasts

They appear near the cementum of variously differentiated stages. These cells are often indistinguishable from periodontal fibroblasts apart from their location adjacent to cementum surface.

## 2-Resorptive cells

### a-Osteoclasts

these are cells that resorb bone. They occupy resorption site in bone surface (Howship's lacunae). The osteoclasts are seen regularly in normal functioning periodontal ligament, but presence of abundance of osteoclasts indicates that resorption was active.

### b- Cementoclasts

They are mononuclear or multinucleated giant cells located in Howship's lacunae on the surface of the cementum, responsible for resorbing cementum under certain circumstances, its resemble osteoclast and found in the normal functioning PDL.

### c- Fibroblasts

The fibroblasts responsible of collagen and elastin fibers formation, as well as ground substance formation, also fibroblasts are characterized by rapid turnover of protein of extracellular matrix in particular collagen.

**So fibroblasts are responsible for the formation and remodeling of the periodontal ligament fibers.**

## 3-Epithelial rests of Malassez

Epithelial rests are normal constituents of the PDL that are seen throughout the life, they are found close to the cementum and are remnants of the epithelial root sheath, they persist as a network, strand, island, or tubule-like structures and parallel to the surface of the root. When certain pathological conditions are present, cells of epithelial rests can undergo proliferation and can produce a variety of cysts and tumor that unique to the jaws.

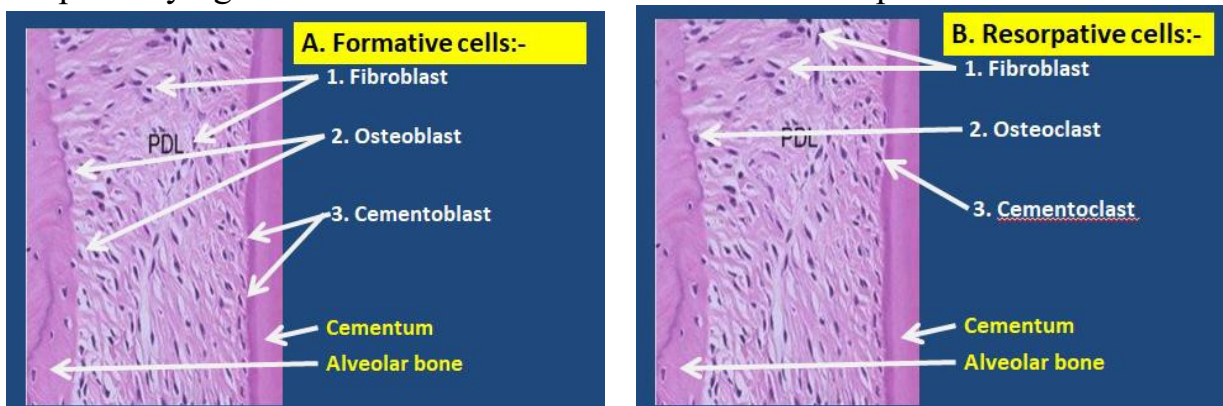
## 4- Defense cells

**Macrophages** are important defense cells because of their phagocytic activity and mobility; they take up bacteria, dead cells and foreign bodies.

**Lymphocytes, mast cells** also appear when PDL is stressed by disease.

## 5- Undifferentiated mesenchymal cells (progenitor cells)

PDL contain progenitor cells that have the capacity to undergo mitotic division, and replace dying differentiated cells at the end of their life span or as a result of trauma.



## **Extracellular substance**

### **1-Fibers:**

The fibers of the PDL are made up mainly of collagen and a small amount of oxytalin fibers and reticulin fibers, and in some species, elastin fibers.

#### **Oxytalan fiber:-**

They are type of immature elastic fibers, small in diameter and run in axial direction one end embedded in cementum or possibly in bone and the other often in the wall of blood vessels, their function may regulate vascular flow in relation to tooth function and support the wall of blood vessels.

#### **Collagen fibers:**

The main types of collagen in the periodontal ligament are *type I* and *type III*. More than 70% of periodontal ligament collagen is *type I*.

The collagen is gathered to form bundles approximately 5  $\mu\text{m}$  in diameter termed *principal fibers*.

### **2. Ground substance**

The ground substance is a gel-like matrix The functions of ground substance are ion and water binding and exchange, control of collagen fibrillogenesis and fiber orientation and binding of growth factors.

The ground substance consists mainly of glycosaminoglycans, proteoglycans and glycoproteins. All components of the ground substance are secreted by **fibroblast**. Ground substance has been estimated to contain 70% water and is thought to have a significant effect on the tooth's ability to withstand stress loads.

### **3. Interstitial tissue**

The blood vessels, lymphatic and nerves of PDL are surrounded by loose C.T., they occupy spaces between bundles of principal fibers called **Interstitial space**, these spaces are designed to withstand the impact of masticatory force.

## **Other structures present in PDL**

### **Blood vessels**

The blood supply is derived from the inferior and the superior alveolar arteries to the mandible and maxilla respectively and reach the PDL from three sources:

- 1-Branches from apical vessels that supply the dental pulp.
- 2-Branches from intra-alveolar vessels, these branches run horizontally, from the alveolar bone to enter the PDL.
- 3-Branches from gingival vessels enter PDL from the coronal direction.

There is rich vascular plexus at the apex and in the cervical part of the ligament. The venous vessels tend to run axially to drain to the apex.

### **Lymphatic vessels**

A network of lymphatic vessels following the path of the blood vessels. It drains lymph from PDL into the adjacent alveolar bone.

### **Nerves**

Nerve supply come from inferior and superior dental nerves.

There are two types of nerve supply :-

- 1- Small fibers that as free nerve endings for pain sensation.
- 2- Large fibers that end as (Knob like or spindle like) for localization of touch or pressure on teeth and known as **mechanoreceptors**.

### **Cementicles**

These are calcified bodies found in PDL seen in older individual, they may remain free in PDL or they may fuse as large calcified masses joined with cementum. The origin of these calcified bodies is not established. It is possible that degenerated epithelial cells form the nidus for their calcification.

## **Functions of the periodontal ligament**

### **1. supportive**

- Attach the teeth to the bone
- Transmit occlusal force to the bone
- Acting as shock absorbent by resisting occlusal impact

### **2.Sensory**

- Tactile sensation
- Pressure sensation
- Pain sensation by the trigeminal pathway

### **3. Nutritive**

The blood vessels of the ligament provide the essential nutrient for the ligaments' vitality and the hard tissue of the cementum and bone. All cells require **nutrition** which is carried by the blood vessels to the ligament and also the blood vessels are concerned with the **removal of catabolite**.

### **4. Homeostatic**

It is evident that the cells of the PDL have the capacity to resorb and synthesize the extracellular substance of the C.T. of the ligament, alveolar bone and cementum.

## **Age changes in periodontal ligament**

The PDL ages as in all other tissues of the body.

- The cell number and the cell activity decreases with aging.
- With aging the activity of the PDL tissue decreases because restricted diets and therefore normal functional stimulation of the tissue is diminished.
- Any loss of gingival height related to gingival and periodontal disease promotes destructive changes in the PDL. Some teeth becomes nonfunctional
- Due to continuous deposition of cementum and alveolar bone, the PDL width also starts to diminished.

## Principle fibers of PDL

**Principal Fibers** are bundles of collagen fibers type I and III approximately 5  $\mu\text{m}$  in diameter, they are embedded in cementum from one side and in alveolar bone on the other side. Principle fibers help the tooth to withstand compression force during mastication and remain attach to the bone, they divided into 5 subgroups according to its region:

**1. Alveolar crest group:** extend obliquely from the cementum just beneath the junctional epithelium to the alveolar crest.

- Functions: (i) Prevent extrusion  
(ii) Prevent lateral tooth movements

**2. Horizontal group** These fibers run at right angles to the long axis of the tooth from cementum to alveolar bone, and are roughly parallel to the occlusal plane of the arch.

**3. Oblique group** Oblique fibers are the most numerous and occupy nearly 2/3rd of the ligament. These fibers are inserted into the alveolar bone at a position coronal to their attachment to cementum.

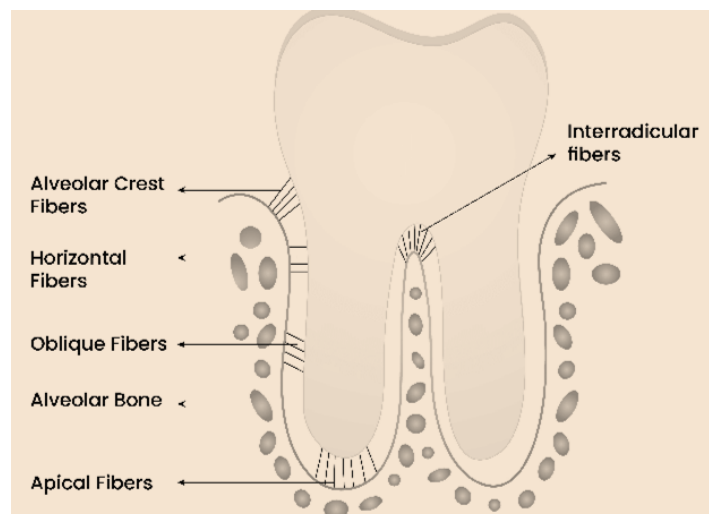
- Functions: (i) Bear vertical masticatory stresses  
(ii) Transform vertical stress into tension on the alveolar bone.

**4. Apical group** run from the cementum at the root tip and anchored into the fundus of the bony socket.

- Functions: (i) Prevents tooth tipping  
(ii) Resists luxation  
(iii) Protects neurovascular supply to the tooth.

**5. Interradicular group** The principal fibers of this group are inserted into the cementum from the crest of interradicular bony septum in multirooted teeth.

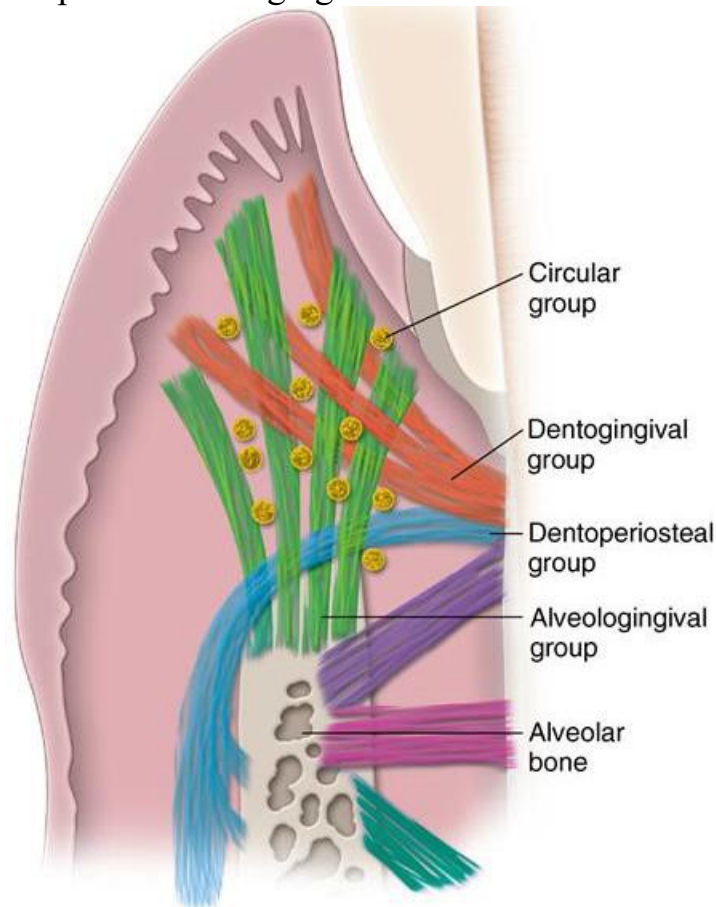
- Functions: (i) Prevents luxation  
(ii) Prevents tooth tipping and torquing.



## Gingival ligament fibers

Lamina propria of the gingiva consist of a dense c.t. that contain dense collagen called **gingiva ligament fibers** which are divided into the following fiber groups:-

1. **Dentoginival fibers**: which extend from cervical cementum to the Lamina propria of the gingiva.
2. **Alveolo-gingival fibers**:- the fibers arise from the alveolar crest extended to the lamina propria of gingiva.
3. **Dentoperiosteal fibers**:- arising from the root cementum and passing over the alveolar crest to be inserted into the periosteum.
4. **Transseptal fibers**:- they running interdentially, passing horizontally from the cementum of one tooth above the alveolar crest to be inserted into the cementum of the adjacent tooth.
5. **Circular fiber**: a small group of fiber that circle the neck of tooth and interlace with other fibers in free gingiva and help to bind free gingiva to the tooth.



## Sharpey's fibers

Principle fibers are embedded into cementum on one side of the periodontal space and into alveolar bone on the other side.



# The BONE

Bone is a living tissue, which makes up the body skeleton and is one of the hardest structures of the animal body. Bone possesses a certain degree of toughness and elasticity.

## Functions of bone:

1. It provides shape and support for the body.
2. It provides site of attachment for tendons and muscles, which are essential for locomotion.
3. It protects the vital organs of the body.
4. Bone serves as a storage site for minerals

## Classification of bones

Bones may be classified according to their shape, mode of development or by histologic appearance.

**A-Classification Based on Shape:** Bones are classified according to their shape as:

- **Long:** ex: Femur bone
- **Short:** ex: Talus bone
- **Flat:** ex: occipital bone
- **Irregular bones:** ex: vertebrae bones

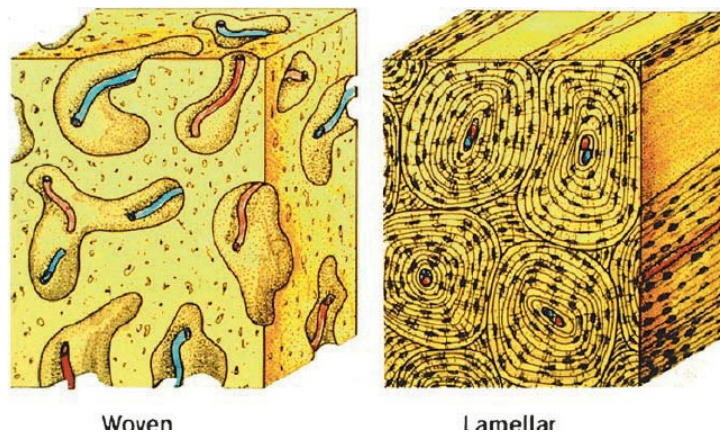
**B-Classification Based on Development :** Developmentally, bones are classified as:

- endochondral bones
- intramembranous bones.

**C-Classification Based on Microscopic Structure:** Histologically, bones are classified as:

- **Woven or immature bone** is the first formed bone with irregularly oriented collagen fibers of varying diameters. This type of bone is **not seen after birth**. However it is seen in the **alveolar bone** and during **healing of fractures**. Since this bone forms rapidly, it incorporates many osteocytes.
- **Mature bone** is further classified as compact bone and cancellous bone.
  - **Compact bone (cortical bone)** consists of tightly, packed osteons or haversian systems, forming a solid mass. Since the bone mass is arranged in layers, it is also called **lamellar bone**.
  - **Cancellous bone (spongy bone)** has a honeycomb appearance, with large marrow cavities and sheets of trabeculae of bone in the form of bars and plates.

Woven Bone	Lamellar Bone
Immature	Mature
Collagen fibers intertwined	Collagen fibers orderly
Mineralization faster	Mineralization slower
Mineral density lower, water content higher	Mineral density higher, water content lower
Osteoclast can remove woven bone totally	Osteoclast can remove portions of lamellar bone at a time



Woven

Lamellar

## Chemical composition

Bone is a specialized mineralized connective tissue. consisting of **33% organic matrix** (fibers (28% of its type I collagen and 5% non-collagenous protein which including, osteonectin, osteocalcin, osteopontin, bone morphogenetic protein, bone proteoglycan, bone sialoproteins and growth factors serum protein).

While the remaining **67% of bone inorganic materials** consist of hydroxyapatite which are deposited in between the molecules of collagen as well as in the ground substance of bone.

## Bone cells:

### 1- Osteoprogenitor cells :-

These cells are undifferentiated mesenchymal cells, located in the deepest layer of the periosteum and lining the vascular canals of the compact bone. They have the capacity to divide and give rise to any of the other three bone cells.

**Morphologically:-** they resemble mesenchymal cells (pale staining large nucleus).

### 2- Osteoblasts :-

Plump, cuboidal, mononuclear cell that responsible for bone formation, found in relation to the bone surface when bone matrix is being deposited. Histologically: Cytoplasm is intensely basophilic, abundant and well developed protein synthetic organelles such as rough endoplasmic reticulum.

### 3. Osteocytes :-

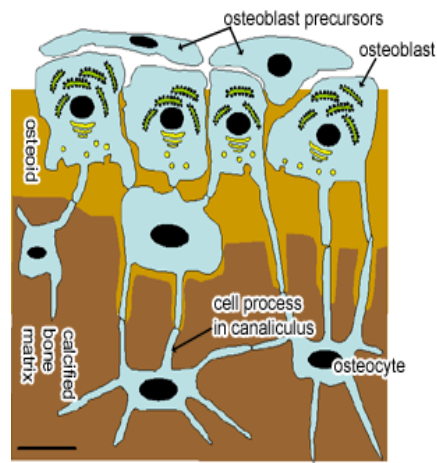
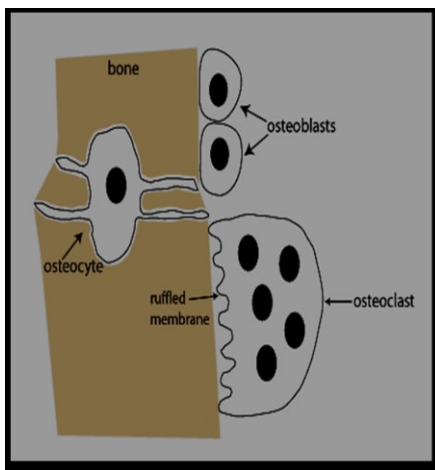
They are the principle cells of bone. It's an osteoblast trapped in bone matrix. Osteocytes possess an ellipsoid cell body with long axis parallel to surrounding bone lamellae. Oval nucleus with narrow rim of faintly basophilic cytoplasm and few organelles but sufficient RER and Golgi apparatus. Cell bodies of osteocytes are located in lacunae and their processes in canaliculi. Osteocyte processes in the canaliculi help maintain contact with adjacent osteocyte and with osteoblast.

Osteocyte function to:

- a. minerals storage
- b. Sense the change in the environment and send signals that affect the other cells involved in remodeling of bone

### 4.Osteoclasts :-

They are located on the surface of bone tissue where resorption is taking place. The cells are large about ( 40-100 um) in diameter, multi- nucleated (15 - 20 closely packed nuclei), more the nuclei the more resorbing capacity. Found in a shallow excavations known as **how ships lacunae**. The portion of the plasma membrane adjacent to the bone surface is wavy known as **ruffled borders**. Osteoclasts are derived from osteoprogenitor cells or probably from circulating blood cells (monocytes) as hematopoietic cells.



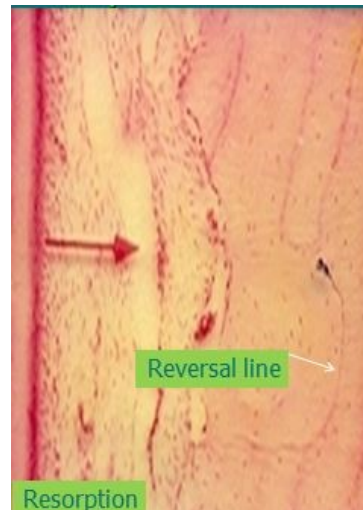
## Incremental lines of bone :-

### 1- Resting lines :-

are smooth straight lines consist of extracellular substance with less collagen and relatively more ground substance, and because of this they stained dark blue with H&E stain, they represent the resting period of the osteoblast during bone formation.

### 2- Reversal lines :-

They are lines that stain dark like the resting lines, reversal lines are scalloped, not smooth, They represent the outline of how ship's lacunae with their convexity toward the old bone. following bone resorption, new osteoblast differentiation and deposited bone tissue leaving these lines separating between old and new bone.



## Bone formation and Growth

Human bones grow until about age 25. The process of bone formation called **osteogenesis** or **ossification**. There are 2 main forms of ossification :

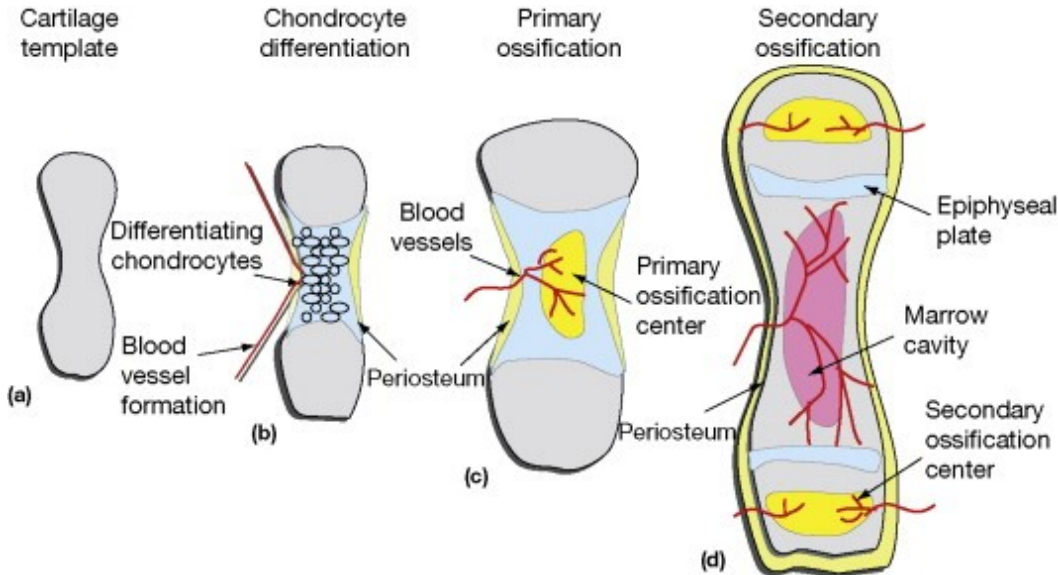
### 1. endochondral ossification

This type of ossification seen in long bones and in fracture healing, bone formation occur within cartilage template in the following steps:

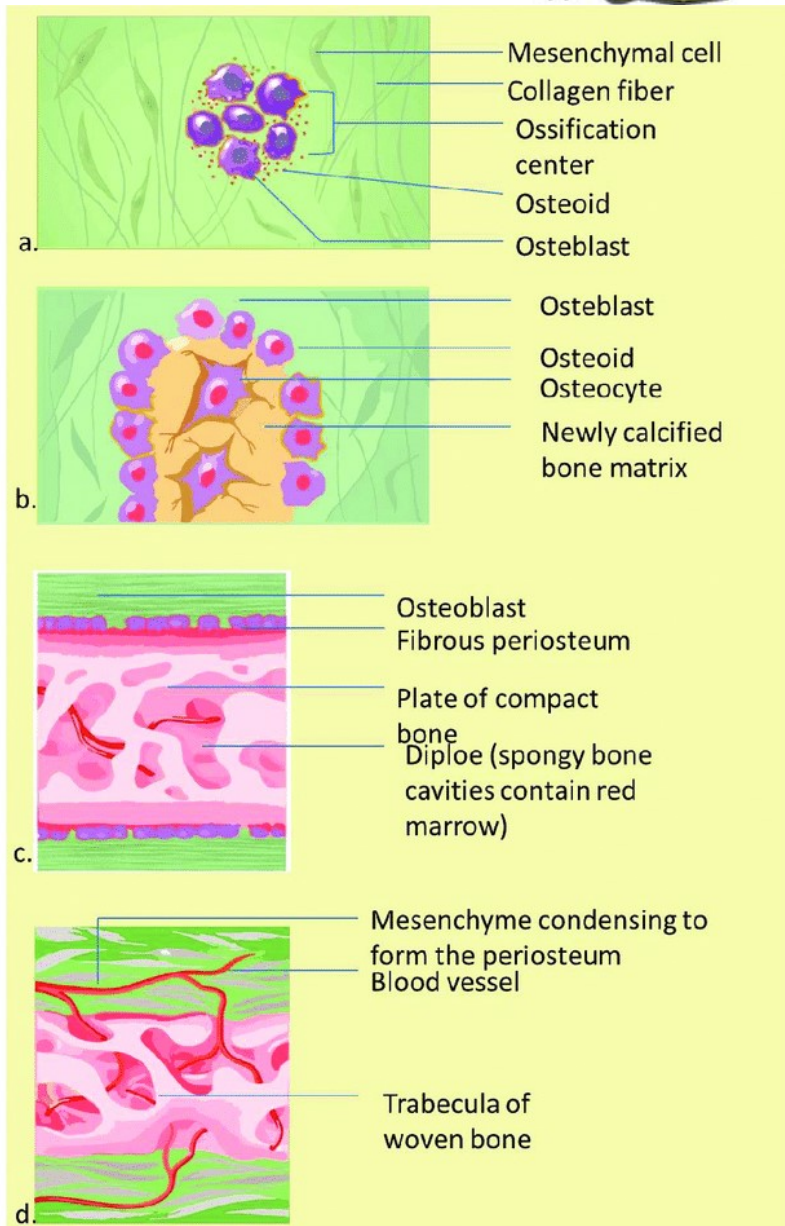
1. Formation of cartilage template by chondroblasts
2. Chondrocyte with in the cartilage undergo hypertrophy (area surrounded by a bone collar) then apoptosis
3. Vascularization, invasion of osteoblast precursors
4. Osteoblasts differentiate, replace cartilage with bone (ossification, bone collar gets longer)

## 2. Intramembranous ossification

occur in flat bones mainly in the skull including maxillary and mandibular bones, in which mesenchymal cells form clusters of Osteoprogenitor that differentiate in place into osteoblasts which form bone.



**endochondral  
ossification**



**Intramembranous  
ossification**

## Bone resorption:

This process done by osteoclasts, its accurate in sequence of event :

1-**Attachment of osteoclast** to mineralized surface through ruffled border.

2. **Decalcification of inorganic material:**

Osteoclast secrete organic acids mainly citric and lactic acid that chelate bone and increase solubility of hydroxyapatite.

3-**Degradation of organic matrix:**

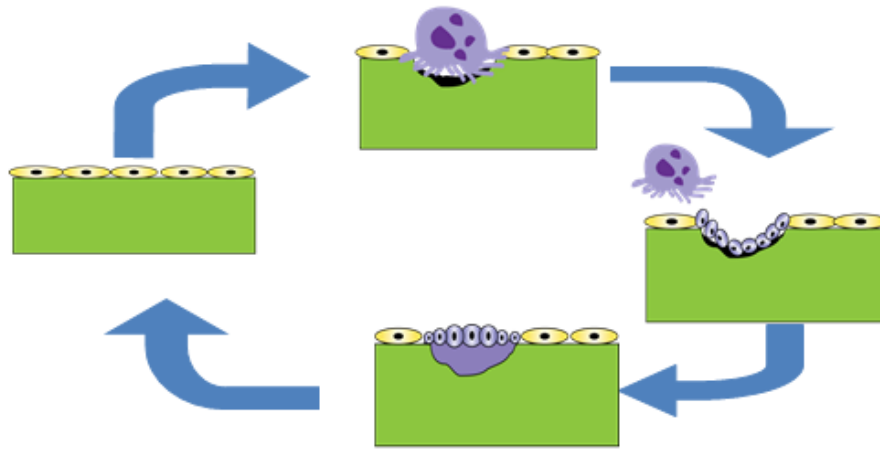
Osteoclast secrete protease and collagenase enzymes which cause break down the collagen extracellularly.

4-**Transport of soluble products:**

The soluble products will be transported to the extracellular fluid or to blood vascular system.

## Bone remodeling:

This means bone deposition by osteoblasts after bone resorption by the action of osteoclasts. This bone remodeling is important in orthodontic treatment because this enables the orthodontist to move the teeth without disrupting their relations to the alveolar bone. Bone resorption occurs on the pressure side and bone deposition occurs on the tension side.



## Bone healing (embryonic bone formation):

During healing of fractures or wound of extracted tooth, an embryonic type bone is formed which is characterized by:

1- Greater number, great size, and irregular arrangement of osteocytes.

2- Coarse irregular fibers.

3- Absence of lamellae and resting lines.

4. Reduced volume of inorganic substance and great amount of organic substances. That's why the socket of the extracted tooth appears empty (dark, radiolucent) in the X-ray film at the time when it's almost filled with immature bone. Then the embryonic or woven bone will be resorbed and replaced by lamellated bone.

## ALVEOLAR BONE

The **Alveolar process** is the specialized part of the maxilla and mandible that **form** and **support** the tooth sockets

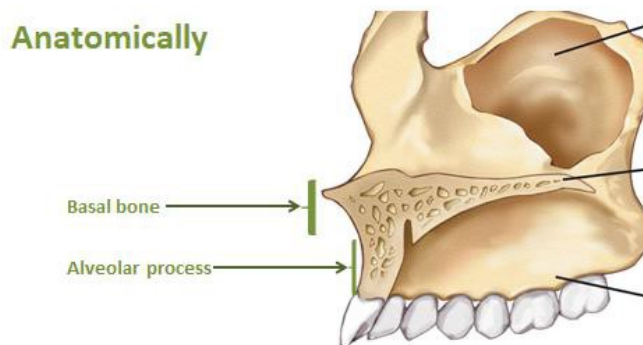
### Functions of alveolar bone:

1. Protect the developing tooth bud
2. Houses the roots of teeth.
3. Anchors the teeth with the help of Sharpy's fibers.
4. Supply blood to the PDL.
5. Organize and controls the eruption of teeth.
6. Absorb and distribute the occlusal forces.

**Anatomically**:- the jaw (maxilla and mandible) composed from 2parts :-

- 1- The alveolar process.
- 2- The basal or body bone.

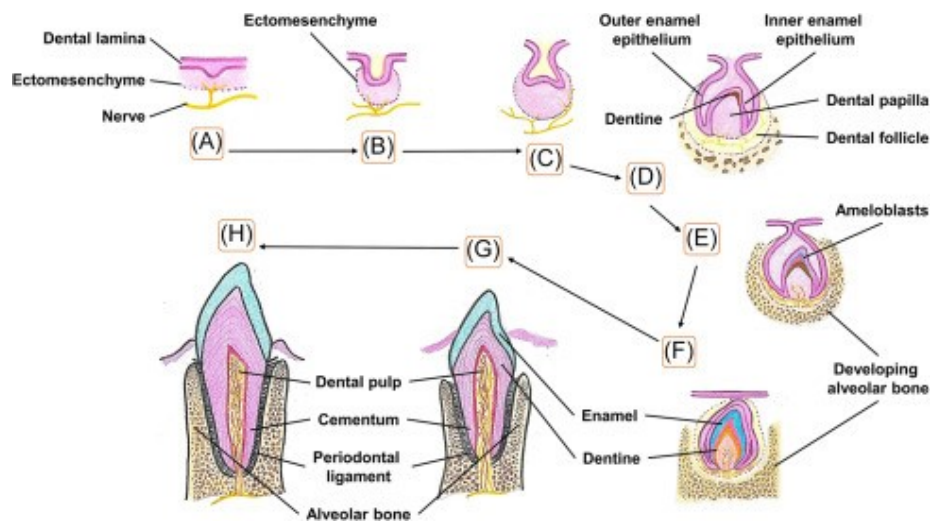
**-Not:-** There is no distinct boundary exists between the above 2parts.



### Development of alveolar bone

Near the end of the **second month of fetal life**, the maxilla and the mandible form a continuous groove at its free surface that it open toward the surface of the oral cavity. The tooth germs are contained in this groove, which also includes the alveolar nerves and vessels.

Alveolar bone start to develop during root formation and continue during tooth eruption, the cells in the dental follicle start to differentiate into fibroblasts and cementoblasts to form periodontal ligament and cementum, at the same time, some cells in the dental follicle differentiate into osteoblasts and form alveolar bone proper



## Structure of the alveolar process:

alveolar process consist of two parts:

### 1-The alveolar bone proper

It surrounded the root of tooth. It consist of two types of bone:-

#### a- Bundle bone

Is the inner wall of the sockets , it provides attachment for the fibers of P.D.L.(Sharpey's fibers). Bundle bone also known as **cribriform plate** (as anatomical term) because its perforated by many foramina transmitting nerves and B.V. to P.D.L.

Radiographically is more radiopaque due to presence of thick bone without trabeculations and is called **lamina dura** (as radiographic term).

Histologically the bundle bone consists of

- a. Bundles of Sharpey's fibers are embedded at right angle to the socket wall.
- b. Other fibrils are less and are arranged in lamella parallel to the socket wall and perpendicular to Sharpey's fibers

#### b. Lamellated bone:

Located between the bundle bone and spongy bone. It mostly consist of haversian systems (**osteons**) each one contain a blood vessel in a haversian canal, blood vessel is surrounded by **concentric lamellae** to form osteon, in between there are **interstitial lamellae**, while **circumferential lamellae** arrange parallel to the surface of adjacent bone marrow.

## 2. Supporting alveolar bone:

Its consist of two parts:

#### a-Cortical bone:

Also called cortical plate It form the outer (labial or buccal) and inner (lingual) plates of the alveolar process. Cortical bone varies in thickness in different areas, its much thinner in the maxilla than in the mandible. The plate adjacent to posterior premolars and molars thicker than anterior region especially on the buccal side. In the maxilla the outer cortical plate is perforated by many small openings through which blood and lymph vessels pass.

**Histologically:** the cortical plates consist of longitudinal lamellae and haversian systems.

## b-Spongy or cancellous bone

It fills the space between the alveolar bone proper and cortical plates. In the region of the anterior teeth and the area close to the alveolar crest of both jaws the spongy bone is very thin or completely absent. The spongy bone consist of :-

### 1- Bone trabeculae (the spongiosa)

which can be classified according to its appearance in the X-ray film into:-

#### Type I:-

In which the interdental and inter-radicular trabeculae are regular and horizontal and arranged in a **Ladder like arrangement**. It's common in the mandible, it's more resistant to the forces of mastication.

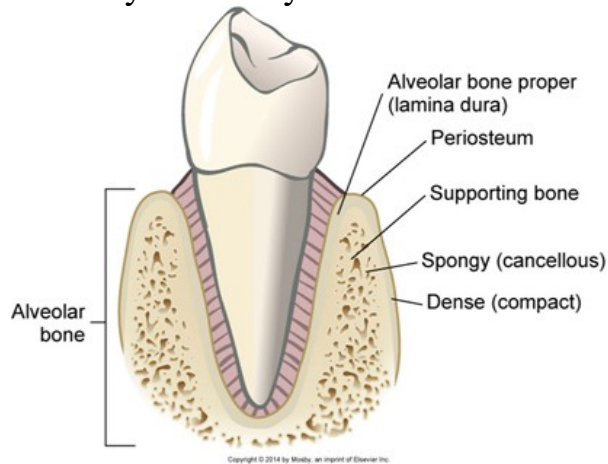
#### Type II:-

The interdental and inter-radicular trabeculae are irregularly arranged, numerous and delicate. It's more common in maxilla.

### 2-Bone marrow :-

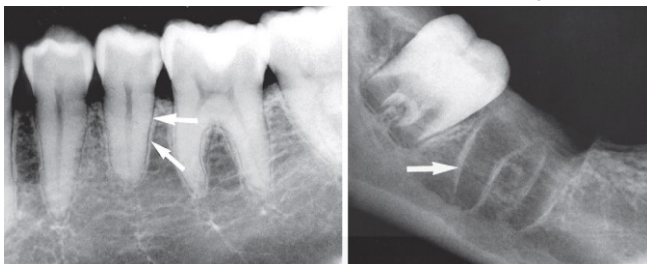
**Its fill the** irregular medullary cavities among bone trabeculae

Histologically the spongy bone consists of **bone trabeculae** of various sizes and shaping enclosing irregular medullary cavities containing **bone marrow**. The marrow spaces contain usually red blood cells forming **hematopoietic marrow**, this red marrow will be transformed into **fatty marrow** by age, but it persist only in the angle of the mandible ,in the head of condylar process and in the maxillary tuberosity.



## Alveolar crest:

Alveolar bone proper meets cortical plates at the alveolar crest. In healthy mouths the alveolar crest is around 1.5 to 2 mm below the **cementoenamel junction**.



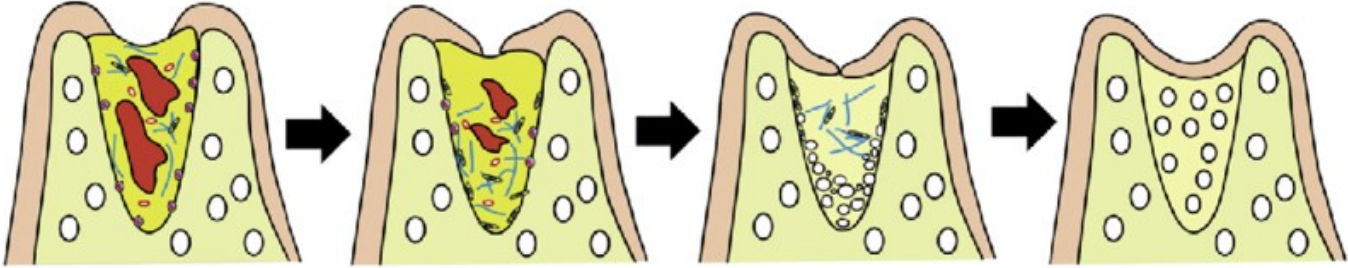
Lamina Dura



## Alveolar bone healing:

The alveolar bone healing process occurs in a dynamic manner and involves many cellular steps:

1. after extraction the tooth socket filled with blood clot that turn into granulated tissue
2. fibroblastic proliferation to form a soft connective tissue
3. connective tissue form a foundation for calcium precipitation, leading to bone trabecular formation (woven bone) in the fundus (apical side) and lateral walls, until the dental socket filled with woven bone.
4. Then the embryonic or woven bone will be resorbed and replaced by lamellated bone.



## Age changes in alveolar bone:

- Brittleness due to decreased water content.
- Sponge bone become thin trabeculae , wide marrow spaces
- Red bone marrow become fatty.
- Osteoporosis.
- Alveolar crest slope distally due to mesial tilting of teeth.
- With loss of teeth and resorption of alveolar ridge mental foramen become very close to the ridge in mandible also maxillary sinus in upper jaw.

## Clinical considerations

1. Lamina dura is an important diagnostic landmark in determining health of the periapical tissues. Loss of density usually means infections, inflammation and resorption of bone socket.
2. The pattern of resorption is different in the maxilla and mandible. The residual alveolar ridge resorbs downward and outward in the mandible, whereas, in the maxilla the resorption is upwards and inwards.
3. The cause for resorption of alveolar bone after tooth loss has been assumed to be due to atrophy, decreased blood supply, localized inflammation or unfavorable prosthesis pressure.
4. The biologic plasticity of alveolar bone that enables the orthodontist to move teeth without disrupting their relations to the alveolar bone. Bone is resorbed on the side of pressure and apposed on the side of tension; thus the entire alveolus is allowed to shift with the tooth
5. During healing of fractures or extraction wounds, an embryonic type of bone is formed, which only later is replaced by mature bone.
6. Increase in functional forces leads to formation of new bone, decreased function leads to a decrease in the volume of bone. This can be observed in the supporting bone of teeth that have lost their antagonists.

# ORAL HISTOLOGY & Embryology

Lec 8

أ.د. انتصار جاسم محمد

## Preparation of specimen for histological examination

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

### Preparation of specimen for microscopical examination:-

Four techniques for oral tissue preparation are usually used for light microscopic examination. These are as follows:

#### A-Paraffin 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:

In light microscopical examination, the most commonly used fixative agents are 10% neutral formalin and Bouin's fluid .

#### 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 everything as their in vivo relation to each other. 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 to remove the fixative agent from the tissue.

#### Factors affect fixation:

Temperature, PH., 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, Infiltration):**

### **Dehydration :**

mean remove water from the tissue and replace it 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).

### **Clearing:**

The 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 called **clearing**, since the tissue becomes transparent in **xylene**.

### **Infiltration:**

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 block 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).

### **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 & 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 constituents have a basic PH, so they are stain pink in color. These elements are said to be **acidophilic**.

### **Other types of tissue techniques are:-**

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

The specimens in this technique must be decalcified ( the mineral substance removed by acid). This type is used for the tissue as 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.

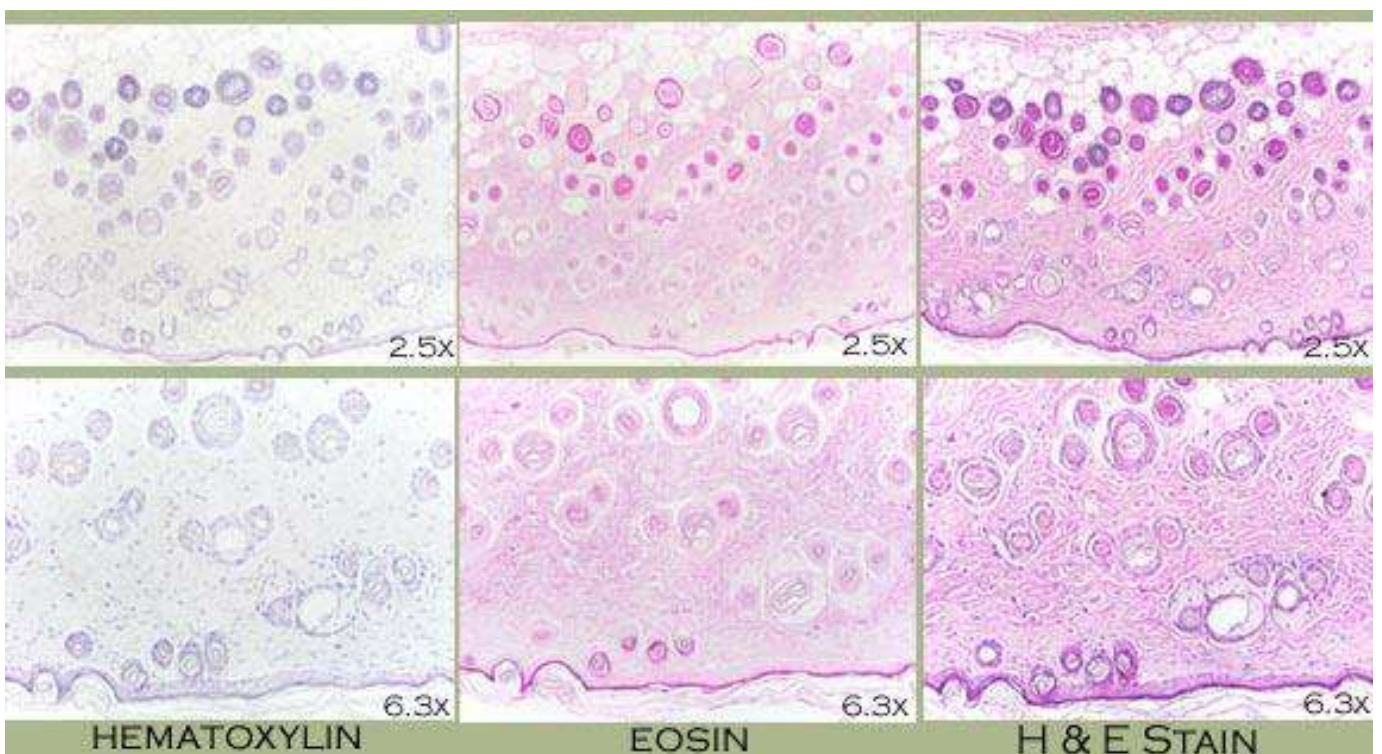
Decalcification accomplished by using 5% nitric acid, the acid changed daily for (8-10) days and then the specimen is tested by pin for complete decalcification.

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

Specimens of calcified tissue may be ground into thin section such as bone and tooth. This is done by slicing the 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.





**Paraffin Blocks**



**Microtome**

# ORAL Histology & Embryology

Lec :9

أ.د.انتصار جاسم محمد

## Development and Growth of Teeth

### **Tooth development (Odontogenesis):-**

Is a complex process by which teeth form from embryonic cells, grow, and erupt into the mouth.

The primitive oral cavity, or **stomodaeum**, is lined by stratified squamous epithelium called the oral ectoderm or primitive oral epithelium. The oral ectoderm contacts the endoderm of the foregut to form the **buccopharngeal membrane**. At about the 27 days ( 4 weeks) of gestation this membrane ruptures and the primitive oral cavity establishes a connection with the foregut.

Most of the connective tissue cells underlying the oral ectoderm are of neural crest or ectomesenchymal in origin. These cells will be induce the overlying ectoderm to start tooth development (epithelial-mesenchymal interaction).

The fundamental developmental process is similar for all teeth(deciduous & permanent teeth).

### **Timing:**

The first sign of tooth development is seen when the embryo is about 6 weeks of intrauterine life ( I.U.L).

### **Primary Epithelial band:-**

Certain areas of basal cells of the oral ectoderm proliferate more rapidly than do the cells of the adjacent areas. This leads to the formation of the primary epithelial band which is a band of epithelium that has invaded the underlying ectomesenchym along each of the horseshoe-shaped future dental arches .

At about 7th week divided and gives rise to:-

1-vestibular lamina :an outer or buccal band of epithelial thickening. Also termed the lip furrow band. it subsequently hollows and forms the oral vestibule between the lips and cheeks from one side and the dental arch from other side.

2-Dental lamina (D.L.): it's the inner band of epithelium it's the primordium for the ectodermal portion of the teeth.

The D.L. is formed by induction of neural crest cells to oral ectoderm, leading to proliferation of the basal layer of the oral ectoderm end with the thickening of the ectoderm tissue, at the region of the future dental arches, this is the first sign of tooth development. This lamina then invaginates as a

sheet of epith. cells into the underlying mesenchym.

Then along the D.L., at a labial side, a small rounded swellings will developed by a localized mitotic activity at a higher rate than the adjacent areas. These will represent the enamel organ (E.O.) of the developing deciduous teeth.

The successors of the deciduous teeth (permanent teeth) develop from a lingual extension of the dental lamina opposite to the enamel organ of each deciduous tooth, which is named the successional lamina and develops from the fifth month of I.U. (permanent central incisor) to the tenth month of age (second premolar).

While the (E.O.) of permanent molars arises directly from a distal extension of the dental lamina.

The functions of dental Lamina:-

- 1- Initiation of all deciduous teeth(2nd month of embryo).
- 2- Initiation of the permanent successor of deciduous teeth (5th month of embryo).
- 3- Formation of the permanent molar tooth germ (4th month of fetal life).

(M1-4th month of I.U. ,M2-12th month, M3-5years).

Fate of the dental lamina:-

- The dental lamina degenerates by mesenchymal invasions in late bell stage. - As the teeth continue in development they lose their connection with the D.L., sometime the remnant stayed in the jaw and gingiva called Serres' pearl's, also named as epithelial rests of Serres.

in the adult life it could proliferate for any reason and developed as pathological cyst.



### Physiological phases in tooth development:-

**1-Initiation** : During this phase, the **sites of the future teeth** are **established** with the appearance of tooth germs along an invagination of the oral epithelium called dental lamina.

**2-Prolifertion**: During this phase, proliferative growth causes regular **changes in the size**

and **proportions** of the **growing tooth germ**.

**3-Histodifferentiation:** During this phase, **differentiation of cell** (begun during morphogenesis) proceed to give rise to **the fully formed dental tissues**, both **mineralized** ( such as enamel, dentin and cementum) and **unmineralized** (such as pulp and periodontal ligament).

4- **Morphodifferentiation** : During this phase, the **shape of the teeth are determined** by a combination of **cell proliferation** and **cell movement**.

**5-Apposition:** During this phase, the **deposition of dental hard tissue** occur in the tooth like **dentin** and **enamel**.

### **The stages of tooth development:-**

The stages of tooth development may described according to the changes of **morphology of developing enamel organ** (morphological stages)

#### **Morphological stages:-**

Although tooth development is a continuous process, it's divided for descriptive purpose into three stages:-

**1-Bud stage.**

**2-Cap stage**

**3-Bell stage**

So the stages named according to the shape of the **enamel organ** of the tooth germ.

#### **1-Bud stage :-**

The epithelium of the dental lamina is separated from the underlining ectomesenchym by a basement membrane. After the differentiation of D.L., round swellings arise from basement membrane at ten different points, corresponding to the future positions of the deciduous teeth so the development of tooth germ is initiated, and the cells continue to proliferate faster than adjacent cells, sinks deeper into the ectomesenchym, to form a bud shape of epithelial structure called the **enamel organ**.

Enamel organ histology in this stage consist of peripherally located low columnar cells and centrally located polygonal cells. The enamel organ is separated from the adjacent ectomesenchym by **basement membrane**.

The enamel organs of the all teeth **are not developed at the same time**.

The buds of the anterior teeth develop before the buds of the posterior teeth, and the buds of lower teeth develop before the upper teeth, so the mandibular anterior teeth firstly formed.

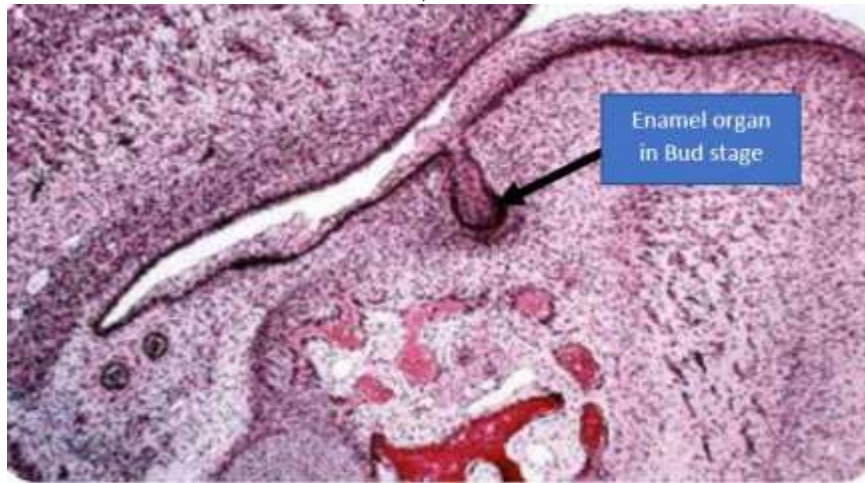
Usually the formation of the enamel organ associated with increasing in mitotic activities of the adjacent ectomesenchymal tissue, which leading to formation of a condensed tissue immediately below the enamel organ which called **dental papilla**.

Also there is a marginal condensation of the ectomesenchymal tissue which surrounding the enamel organ and dental papilla, this condensed tissue named as **dental sac** or **dental follicle**, so these structures which are **enamel organ, dental papilla** and **dental sac** are the main parts of the



## tooth germ.

The enamel organ responsible for the enamel formation, the dental papilla responsible for the dentin and pulp formation, while the dental sac responsible for the formation of the surrounding tissue (cementum, periodontal tissues and alveolar bone).



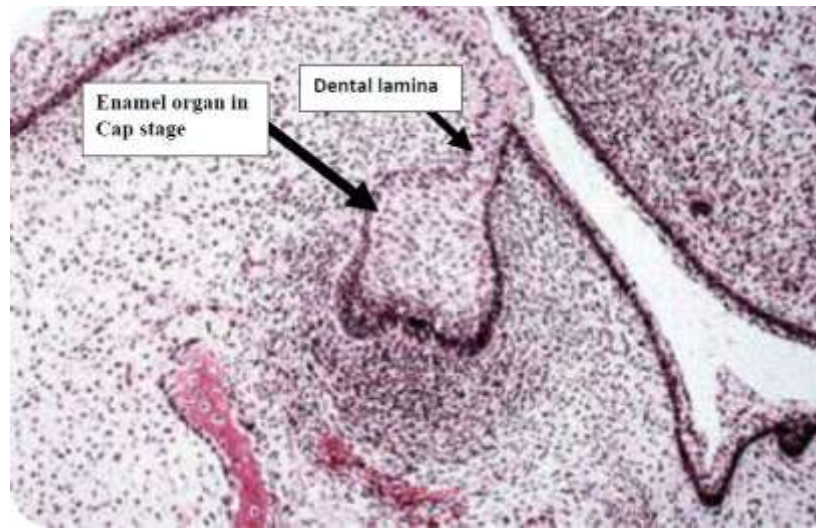
### 1- Cap stage:-

The tooth bud continues to proliferate, it doesn't expand uniformly, so this unequal growth will lead to the cap shape قبة of enamel organ, which is characterized by a shallow invagination on the deeper surface of the enamel organ.

**Histodifferentiation** is started at this stage of development;-

a- The enamel organ shows three types of cells:-

- 1- **Outer enamel epithelium;** the peripheral cells, as a single layer of cubical cells cover the convexity of the cap, forms the outer convex surface of the enamel organ.
- 2- **Inner enamel epithelium:** These cells located in the concavity of the cap, it lines the inner surface of the enamel organ, they are tall columnar cells.
- 3- **Stellate reticulum:** their cells are polygonal in shape, located in the center of the epithelial enamel organ, located between the outer and inner enamel epithelium, the cells assume a branched reticulum form the space in this reticular network (intercellular space) are contain significant quantities of glycosaminoglycan. which gives the stellate reticulum a cushion-like consistency that may support and protect the delicate enamel forming cells (inner enamel epithelium).



### **Transient (temporary) structures:-**

- 1- **Enamel Knot**:- They are condensed cell mass in the center of enamel Organ.
  - 2- **Enamel Cord**: It's strands of cells, which vertically extended from enamel knot to the OEE.
- Both are temporary structures that disappear before enamel formation.

### **B- Dental papilla(D.P.):**

The neural crest cells that is partially enclosed by inner enamel Epithelium proliferate and condenses to form the dental papilla, the changes in the dental papilla occur with the development of the epithelial enamel organ. Dental papilla shows active budding of capillaries and mitotic activity, basement membrane is still present.

### **C-Dental Sac (D.S.)**

At this stage it will be more fibrous.



## Development and Growth of Teeth

### Bell Stage

#### A- Early bell stage

As the invagination of the epithelium deepens and its margins continue to grow the enamel organ assumes a **bell shape**.

a-Enamel organ

It shows four different types of epithelial cells

- 1- Inner enamel epithelium.(I.E.E.).
- 2- Stratum intermedium. (Str. Inter.)**
- 3- Stellate reticulum. (Stel. Ret.)
- 4- Outer enamel epithelium. (O.E.E.)

#### 1- Inner enamel epithelium:-

It consist of a single layer cells that differentiate prior to amelogenesis into tall columnar cells called **preameloblasts**. These cells are 4.5 micrometer in diameter and about 40 micrometer in the height.

These elongated cells are attached together by junctional complex laterally and by desmosomes to stratum intermedium. The IEE. is separated from the peripheral cells of dental papilla by a basement membrane and cell free zone about 1-2  $\mu\text{m}$  wide.

The functions of I.E.E. cells are:-

- 1- Induction: The cells of I.E.E. having an organizing influence on the underlying peripheral mesenchymal cells in the dental papilla, which later may differentiate into **odontoblasts**.
- 2- then it differentiate into **ameloblasts** which form enamel.
- 3- Responsible for crown pattern formation.
- 4- Responsible for formation of the roots of developed tooth: when enamel formation of the crown completed, the IEE with OEE formed the structure from cervical loop which called Hertwig's epithelial root sheath which formed root.

#### 2-Stratum intermedium:-

A few layers of squamous cells form the Stra. Inter. between I.E.E. and Stel. Ret., closely attached by desmosomes and gap junction. this layer seems to be essential **for the mineralization of enamel** during amelogenesis, because it is characterized by high degree of **alkaline phosphates** enzymes which is **essential for the mineralization of enamel**.

Also these cells concern in transport of materials to and from the IEE which later differentiate to ameloblasts.

### **3- Stellate Reticulum:-**

These cell layer become expanded because the intercellular spaces become fluid filled, related to osmotic effects arising from the high concentration of glycos-aminoglycans.

The cells are star in shape with bodies containing prominent nuclei and many branching processes. These cells have numerous tonofilaments and few endoplasmic reticulum and mitochondria which present within the cytoplasm, the long processes of the cell joined with those of adjacent cells by desmosome junction and gap junction.

The main function of stellate reticulum is **a mechanical one**. This relates to the protection of the underlying IEE against physical disturbance and to the maintenance of tooth shape. The hydrostatic pressure generated within the stellate reticulum is in equilibrium with that of the dental papilla, allowing the proliferative pattern of the IEE cells to determine crown morphogenesis, however, a change in either of these pressures might lead to change in the outline of the IEE and this could be important for crown morphogenesis.

### **4-Outer enamel epithelium:-**

These cells are cuboidal form, contain large, centrally placed nuclei. Under electron microscopy, they appear to contain small amounts of the intracellular organelles associated with protein synthesis (e.g. endoplasmic reticulum, Golgi complex, mitochondria) and they contact each other via desmosomes and gap junctions.

It is separated from the surrounding ectomesenchymal tissue of dental sac by a basement membrane 1-2 $\mu$ m thick, which corresponding to basal lamina and hemidesmosome.

The function of OEE involved in the maintenance of the shape of the enamel organ and in the exchange of substances between the enamel organ and the outer environment.

At the late bell stage, when dentin lay down this layer splits in folds, between folds the adjacent mesenchyme of the dental sac forms papillae that contain capillary loops and thus provide a rich nutritional supply for a

vascular enamel organ during enamel formation.

### **b- Dental papilla:-**

It's enclosed in the invaginated portion of the enamel organ, before the I.E.E. begins to differentiate into ameloblasts. the peripheral cells of dental papilla differentiate into odontoblast under the organizing influence of the I.E.E.

The dental papilla ultimately gives rise to **dental pulp**, once the dentin formation begins at the cuspal tip of the bell stage tooth germ.

The basement membrane that separates the enamel organ from the dental papilla, just prior to dentin formation is called the membrana preformativa.

### **c- Dental sac:-**

Before the formation of hard dental tissue begins, the dental sac shows a circular arrangement of its fibers and it resemble a capsular structure. With the development of the root, the fibers of dental sac differentiate into periodontal ligament fibers that become embedded in the cementum and alveolar bone.

### **d- Cervical loop:-**

At the free border of enamel organ, the outer and inner enamel epithelial layers are continuous and reflected into one another as the cervical loop.

### **e- Dental Lamina**

the dental lamina is seen to extend lingually and is termed successional dental lamina as it gives rise to enamel organs of permanent successors of deciduous teeth (permanent incisors, canines and premolars).

The enamel organs of deciduous teeth in the bell stage, and their permanent successor teeth in the bud stage

### **B- Advanced bell stage:-**

During advance bell stage, hard tissues, including enamel and dentin will develop. This stage is also called the crown, or apposition stage, by some researchers. Important cellular changes occur at this time.

- In prior stages, all of the IEE cells were dividing to increase the overall size of the tooth bud, by rapid dividing, called mitosis, **stops** during the crown stage at the location **where the cusps of the teeth form**, by this way the form of crown of developed tooth will established.

- During this stage the boundary between I.E.E. and odontoblasts outlines the future dentinoenamel junction (D.E.J.).

- The first mineralized hard tissues form at this location is dentin.

- At the same time, the IEE cells change in shape from tall cuboidal to columnar and become pre-ameloblasts.
- In addition, the basal margin of the E. organ (cervical loop) gives rise to the Herwig's epithelial root sheath.

## **Nutrition and tooth development**

Nutrition has an effect on the developing tooth. Essential nutrients for a healthy tooth include calcium, phosphorus, and vitamins A, C, and D. Calcium and phosphorus are needed to properly form the hydroxyapatite crystals (minerals), and their levels in the blood are maintained by Vitamin D. Vitamin A is necessary for the formation of keratin, and Vitamin C is for collagen.

Fluoride is incorporated into the hydroxyapatite crystal of a developing tooth and makes it more resistant to demineralization and subsequent decay. Deficiencies of these nutrients can have a wide range of effects on tooth development:

- In situations where calcium, phosphorus, and vitamin D are deficient, the hard structures of a tooth may be less mineralized.
- A lack of vitamin A can cause a reduction in the amount of enamel formation.
- Fluoride deficiency causes increased demineralization when the tooth is exposed to an acidic environment, and also delays remineralization.
- Furthermore, an excess of fluoride while a tooth is in development can lead to a condition known as fluorosis.

## **Developmental disturbances of teeth:**

**1-Anodontia:** is a complete lack of tooth development. Anodontia is very rare, most often occurring in a hereditary condition called ectodermal dysplasia.

**2-Hypodontia** (congenital missing tooth or teeth): It is one of the *most common developmental abnormalities*. The absence of third molars is very common, followed in prevalence by the second premolar and lateral incisor.

**3-Hyperdontia** (supernumerary teeth): It is believed to be associated with the remnant of dental lamina or epithelial rest of Serres.

**4-Dilaceration** is an abnormal bend found on a tooth, and is nearly always associated with trauma that moves the developing tooth bud.

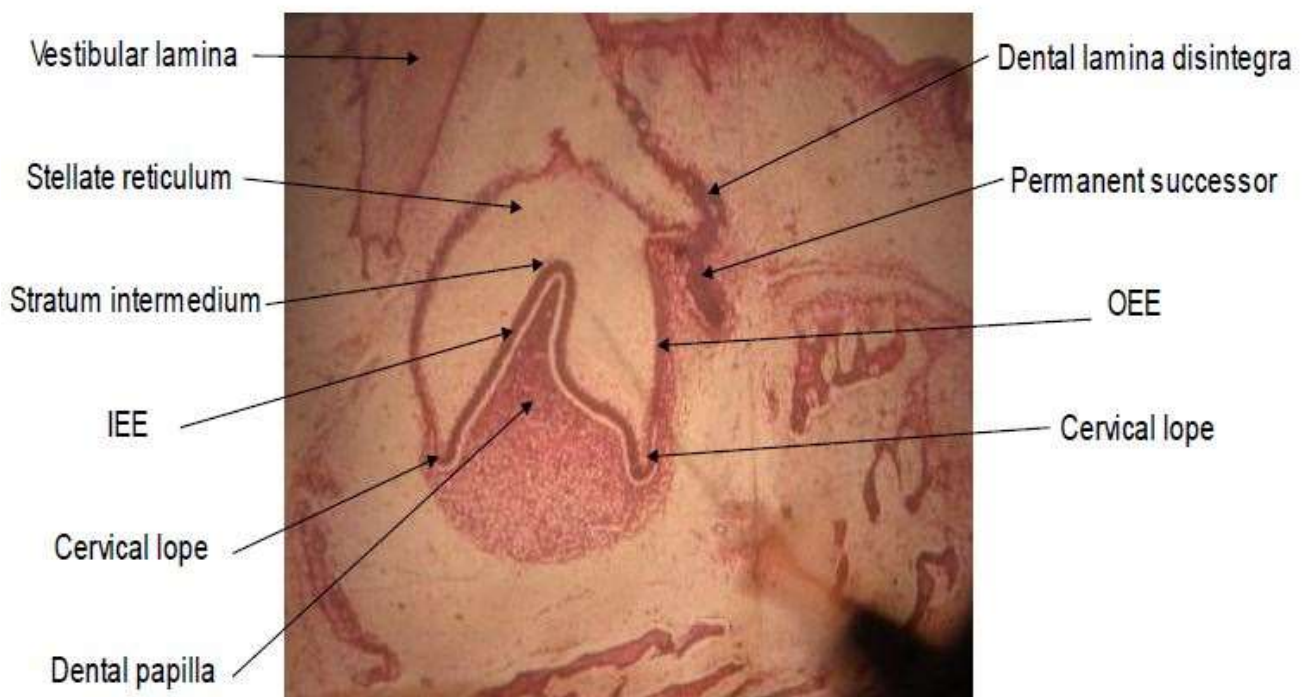
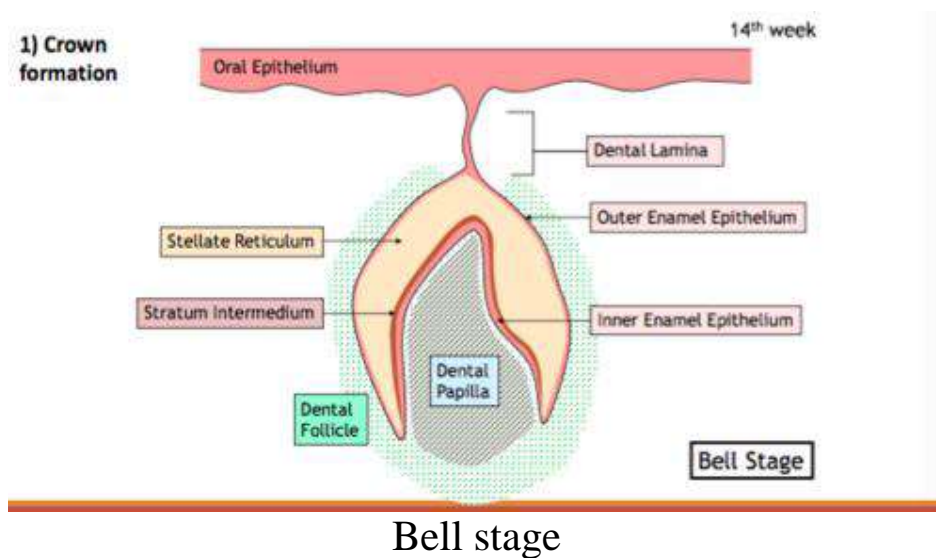
**5-Regional odontodysplasia** is rare, but is most likely to occur in the maxillary anterior teeth. The enamel, dentin, and pulp of teeth are affected, so the teeth are very brittle. On radiographs the teeth appear more radiolucent than normal, so they are often described as "*ghost teeth*".

**6-Amelogenesis imperfect:** is hereditary condition characterized by a defect in enamel formation. Teeth are often free of enamel, small, misshapen, and tinted brown.

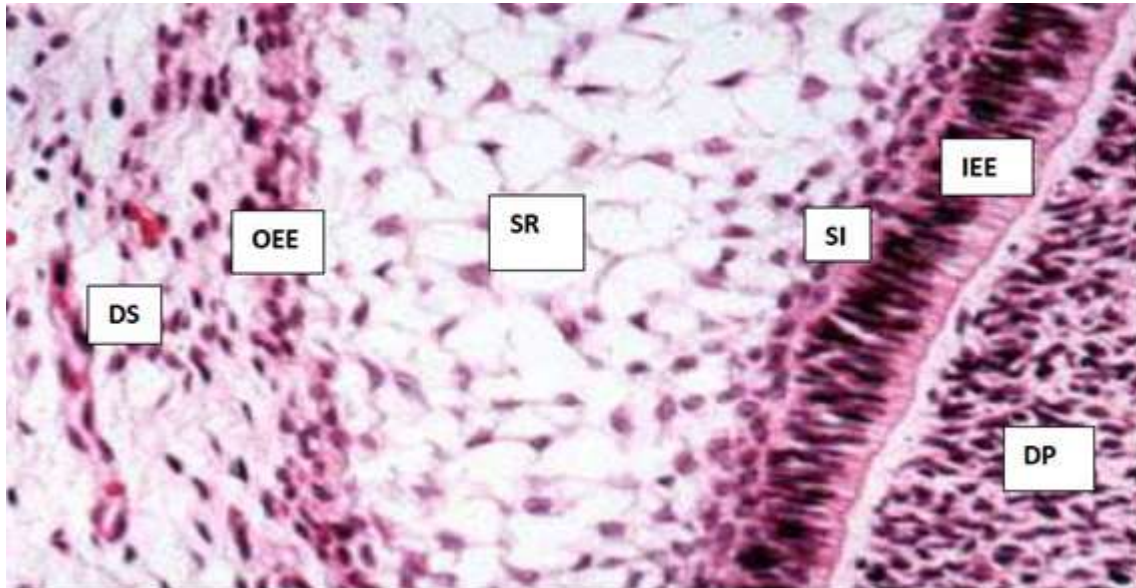
**7- Dentinogenesis imperfecta:** is hereditary condition characterized by a defect in either dentin matrix formation or mineralization of dentin.

**8-Natal and neonatal teeth :** *Natal teeth* are present at the time of birth. *Neonatal teeth* will erupt during the first 30 days after birth. Natal teeth are three times more common than neonatal teeth.

**9- Gemination:** arises when 2 teeth developed from one tooth bud, as a result patient have extra tooth.

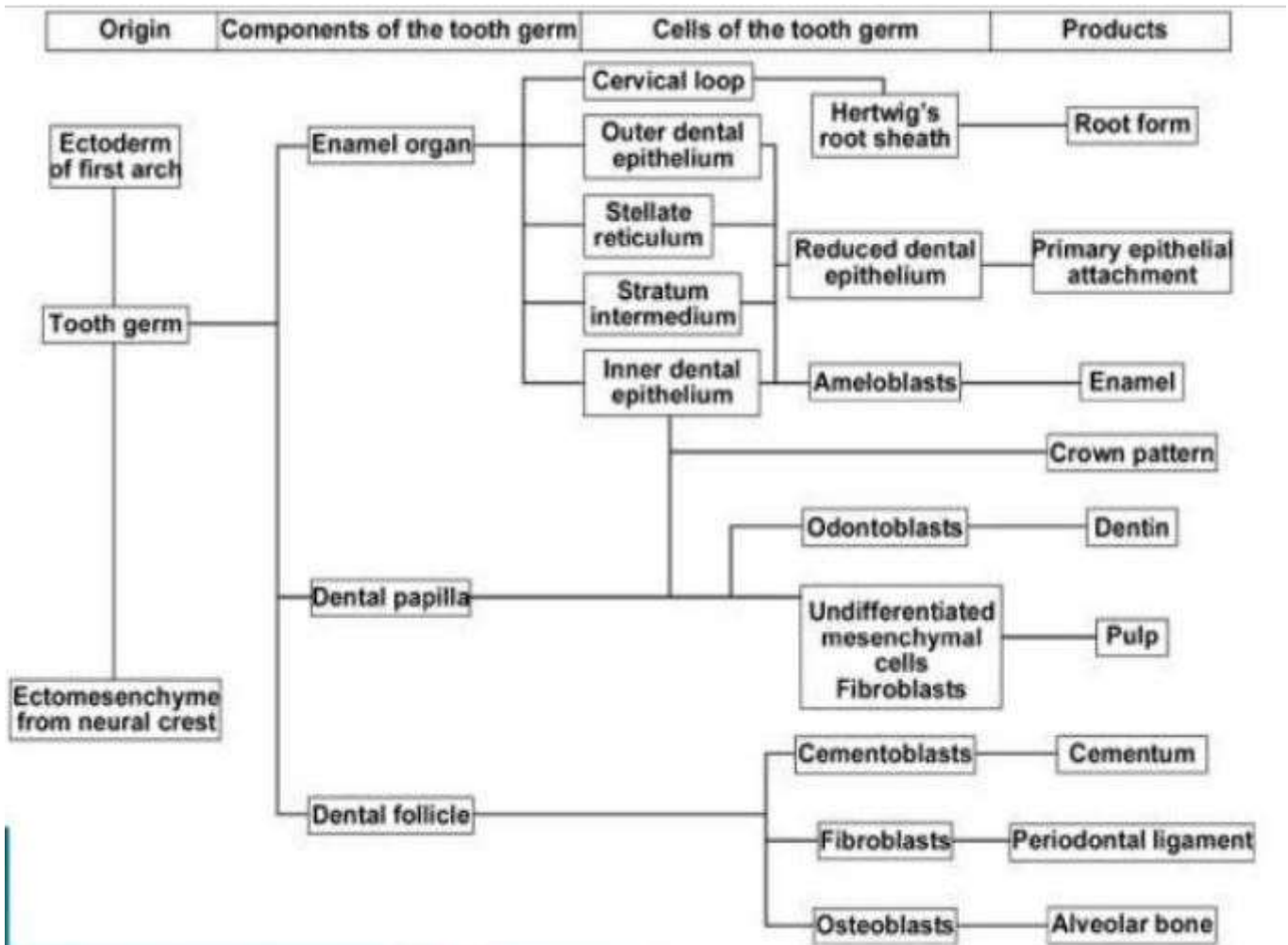


Tooth development at early bell stage



Longitudinal section through tooth germ at early bell stage. DS = dental sac; OEE = outer enamel epith.; SR = stellate reticulum; SI = stratum intermedium; IEE = inner enamel epith.; DP = dental papilla. H&E stain





All processes take place at tooth development

# ORAL Histology & embryology

Lec :13

أ.د. انتصار جاسم محمد

## The dental pulp

Dental pulp consists of soft connective, vascular, lymphatic and nervous tissue, that occupies the center of each tooth, resides in a chamber surround by dentin containing the peripheral extensions of formative cells (odontoblasts) which present at periphery of pulp.

Pulp has a soft, gelatinous consistency, indicates that the majority of pulp (75-80%) is water. The pulp cavity extends down through the root of the tooth as the root canal which opens into the periodontium via the apical foramen, it form the site of communication between the pulp and surrounding tissue, clinically important in the spread of inflammation from the pulp out into the surrounding periodontium.

Pulp and dentin are closely related, both are products of the neural crest-derived connective tissue that formed the dental papilla.

### Anatomy of dental pulp

#### Coronal pulp:-

Occupies the crown, resembles the crown shape, has six surfaces (occlusal, mesial, distal, buccal, lingual and floor). There are also pulp horns which are protrusion of the coronal pulp that extend into cusps of teeth, therefore, the number of pulp horns depends on the number of cusps. At cervical region the coronal pulp joins the radicular pulp present in root.

#### Radicular pulp:-

It's pulpal root canals extend from the cervical region to the apex of the root. The radicular pulp of anterior teeth, is single pulp canal, while the posterior teeth have multiple pulp canals.

The dentinal walls taper, and the shape of the radicular pulp is tubular.

With age coronal and radicular pulp become smaller due to continued dentin formation.

#### Apical foramina:-

An apical foramina is the pulpal opening to the periodontium, this opening varies in size 0.3-0.6 mm, being larger in the maxillary than in mandibular teeth. The apical foramina generally located centrally but become more eccentrically located with age.

The location and shape of the apical foramina may undergo changes as a result of functional influences on the teeth. At sometime change of original foramen may result due to apposition of cementum on opposite side of apical root foramina.

## **Accessory canals:-**

Are canals extend laterally from radicular pulp through root dentin to the periodontal tissue, may seen anywhere along the root but are particularly numerous in apical third of root.

## **Accessory canals may occur in cases:-**

- 1- Where the developing root encounters a blood vessels. If the vessel is located in the area where the dentin is forming, the hard tissue may develop around it, making a lateral canal from the radicular pulp.
- 2- Where there is premature loss of root sheath cells before these cells induce the formation of odontoblasts and dentin formation.

## **Histology of the pulp:-**

The central region of both coronal and radicular pulp contains large nerve trunks and blood vessels, peripherally, the pulp is circumscribed by:-

Odontogenic zone, which composed of:-

- 1-Odontoblast (dentin forming cells).
- 2- Cell free zone (zone of Weil). This zone is a space in which the odontoblast may move pulp word during tooth development.
- 3- Cell rich zone (high cell density). composed principally of fibroblasts and undifferentiated mesenchymal cells, is restricted to the coronal regions, as it is formed during the pre-eruptive phase of the tooth. During early dentinogenesis there are also many young collagen fibers in this zone.
- (4) pulp core which is characterized by the major vessels and nerves of the pulp.

## **Structural Elements of dental pulp:**

### **The cells of the pulp:-**

#### **1- Synthetic cells**

##### **A-Fibroblasts:-**

Are the most numerous cells seen throughout the pulp. These cells producing collagen fibers and ground substance of the pulp, In young pulp, the fibroblasts appear large with multiple processes, centrally placed nucleus, with all protein synthesis organelles as Colge apparatus, rough endoplasmic reticulum and mitochondria ( protein synthetic cell).

By age, the cell become smaller spindle shaped with few organelles.

The fibroblasts in pulp, in addition to forming the pulp matrix they also have the capability of ingesting and degrading the same matrix. These cells have both synthesis and degradation in the same cell.

##### **B- Odontoblasts:-**

The second most prominent cell in pulp, reside adjacent to the predentin with cell bodies in the pulp and cell processes in the dentinal tubules. They are columnar as they become functional. Odontoblasts are larger as in the coronal pulp than in root (which be more cuboidal) and in apical region appear flat.

The odontoblasts when it's active (during dentinogenesis) it has oval nucleus,

abundant rough endoplasmic reticulum, Golgi apparatus, numerous mitochondria.

There are three types of junctional complexes found between adjacent odontoblasts tight, gap and intermediate junctions. Focal junctional complexes are present where the odontoblast cell body gives rise to the process.

## **2- Undifferentiated ectomesenchymal cells:-**

They named also the progenitor cells represent the source from where the connective tissue cells of the pulp are derived. They may give rise to odontoblast, fibroblast when they needed.

They found along pulp vessels, in cell rich zone and scattered throughout the central region of pulp.

Under light microscope they appear as large polyhedral in shape with peripheral processes and large centrally located nucleus.

In old pulp these cells will be decreased in number.

## **3- Defense cells:-**

Macrophages (histocyte), lymphocyte, mast cells, plasma cells, eosinophil's, basophils, in case of pulp inflammation these cells increase in number.

## **Extracellular matrix**

It form the body and the most of its volume and integrity of the dental pulp.

### **Fibers and ground substance:-**

#### **The fibers**

Collagen fibers are present as Type I and type III, found in extracellular matrix surround the cell. Their function is to support other structural elements of the pulp, noticed as a loose reticular network.

The fibers appear sparse delicate in young pulp, after root completion the pulp mature and bundles of collagen fibers increase in number, and they scattered throughout the coronal and radicular pulp.

Fiber bundles are most present in root canal especially near apical region.

#### **The ground substance**

It composed of glycosaminoglycan and proteoglycan and water, its' gel-like in consistency and form the bulk of dental pulp.

## **Supportive Elements**

### **Vascularity:-**

#### **1- Blood vessel:-**

Pulp organ is extensively vascular, with vessels arising from the external carotids to the superior or inferior alveolar arteries. Small venules drain the capillary bed and eventually leave as veins via the apical foramen. Arteriovenous anastomoses of arteriolar size are frequent in the pulp.

As the vessels enter the tooth, their walls become thinner than those surrounding

the tooth, because pulp is protected within hard container of dentin.

These thin walled arterioles enter apical canal and directed toward root pulp and then to coronal area. They give off branches that pass peripherally to a plexus that lies adjacent to the odontogenic zone of the root and crown.

Blood flow is more rapid in the pulp than in most area of the body, and the blood pressure is quite high.

## **2-Lymphatics:-**

Numerous lymphatic vessels are also present in the pulp. These vessels are thin walled, irregularly shaped, larger than capillaries and have an incomplete basal lamina facilitating the resorption of tissue fluid and large macromolecules of the pulp matrix.

Communication of the vessels of pulp with periodontium, take place through apical foramine and accessory canals.

## **Nerves:-**

### **3-Nerve of the Pulp:**

Single large nerve entering the anterior teeth, while several large nerves enter the apical foramen of each molar and premolar, the young premolar may have as many as 700 myelinated and 2,000 unmyelinated axons entering the apex. These nerves have two primary modalities:

#### **a. Autonomic Nerve Fibers:**

Only sympathetic autonomic fibers are found in the pulp. These fibers extend from the neurons whose cell bodies are found in the superior cervical ganglion at the base of the skull. They are **unmyelinated** fibers and travel with the blood vessels. They innervate the smooth muscle cells of the arterioles and therefore function in **regulation of blood flow in the capillary network**.

#### **b. Afferent (Sensory) Fibers:**

They are predominantly **myelinated** fibers, arise from the maxillary and mandibular branches of the fifth cranial nerve (trigeminal).

They may terminate in the central pulp, from this region some will send out small fibers that form the **subodontoblastic plexus** (Raschkow plexus) just under the odontoblast layer. From the plexus the fibers extend in an unmyelinated form toward the odontoblasts where they then lose their Schwann cell sheath.

The fibers terminate as "free nerve endings" near the odontoblasts, extend up between them or may even extend further up for short distances into the dentinal tubule. Their function is transmitting **pain stimuli** from heat, cold or pressure. The subodontoblastic plexus is primarily located in the roof and lateral walls of the coronal pulp. It is less developed in the root canals. Few nerve endings are found among the odontoblasts of the root.

## **Functions of the pulp:-**

**1- Sensory:-** The sensory nerves in tooth respond with pain to all stimuli such as heat, cold, pressure, operative cutting procedures, and chemical agents.

The nerves also initiate reflexes that control circulation in the pulp. This sympathetic function is a reflex, providing stimulation to visceral motor fibers terminating on the muscles of the blood vessels.

**2- Inductive:-** as in early development the odontoblasts of pulp, interacts and initiates tooth formation.

**3- Formative:-**

The pulp organ cells produce the dentin that surrounds and protects the pulp. The pulpal odontoblasts develop the organic matrix and function in its calcification. Through the development of the odontoblast processes, dentin is formed along the tubule wall as well as at the pulp–predentin front.

**4-Nutritive**

high blood supply of the dental pulp transfer the nutrients to the tooth.

**5- protective:-** pulp responds to irritation, whether mechanical, thermal, chemical, or bacterial. A direct response to cutting procedures, caries, extreme pressure, etc., involves the formation of reactive (secondary) dentin by the odontoblast layer of the pulp. Formation of sclerotic dentin, in the process of obliterating the dentinal tubules, is also protective to the pulp, helping to maintain the vitality of the tooth.

**Age- Changes in the pulp:**

**1- The size of pulp**

The most conspicuous changes is decreasing volume of the pulp chamber and root canal due to continuous dentin deposition.

**2-Cell changes**

In addition to the appearance of fewer cells in the aging pulp, the cells are characterized by a decrease in size and number of cytoplasmic organelles. Decrease especially in progenitor cells, leading to reduction in regenerative potential of the pulp.

**3-Fibrosis**

In the aging pulp accumulations of both diffuse fibrillar components as well as bundles of collagen fibers usually appear.

Fiber bundles may appear arranged longitudinally in bundles in the radicular pulp, and in a random more diffuse arrangement in the coronal area. Any external trauma such as dental caries or deep restorations usually causes a localized fibrosis or scarring effect.

**4-Innervation change:**

With age, there is both loss and degeneration of myelinated and unmyelinated nerve fibers that correlated with age- related reduction in sensitivity.

**5- Calcification (pulp stones)**

Is a common age changes of the pulp that may be occurs as localized or diffuse pulp calcification. Pulp stones or denticals are nodular, calcified masses appearing in either both the coronal and root portions of the pulp. They are usually symptomless unless they impinge on nerves or blood vessels.

**The denticals classified according to their structure into:-**

**1-True dental :** They have radiating striations reminiscent of dentinal tubules. Usually those bodies formed by an epithelio- mesenchymal interaction.

**2- False denticals:** These are irregularly calcified tissue generally does not bear much resemblance to any known tissue they are either a hyaline-like homogeneous morphology or appear to be composed of concentric lamellae.

Also denticals may classified according to it's location with dentin:

- 1- **Free denticals** as stone free in pulp.
- 2- **Attached denticals**, attached to the dentin.
- 3- **Embedded denticals** stone embedded within dentin.

They believe that **free denticals** may become **attached** and later embedded as dentin is deposited around the denticals, **the predominance** of denticals are **false free denticals**.

### Clinical Considerations

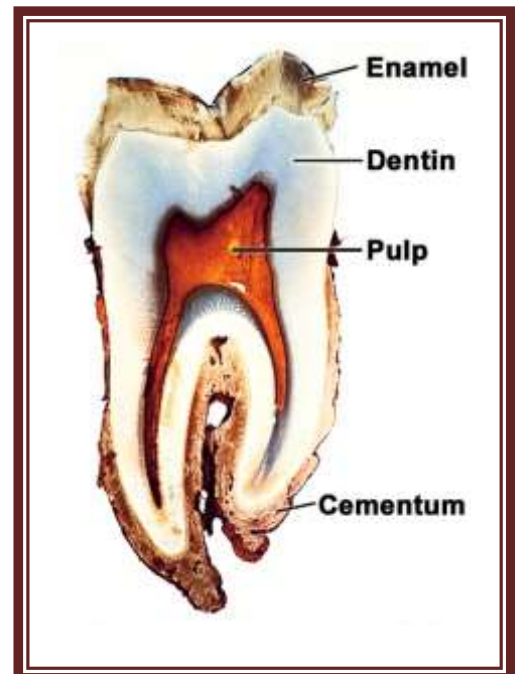
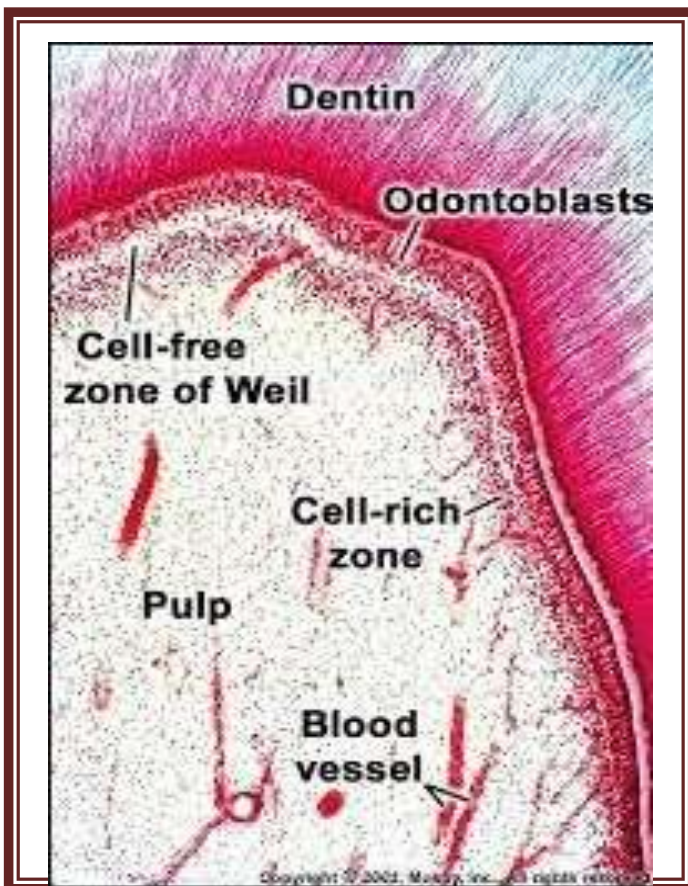
1-With advancing age, there is a difficulty in the endodontic treatment due to:

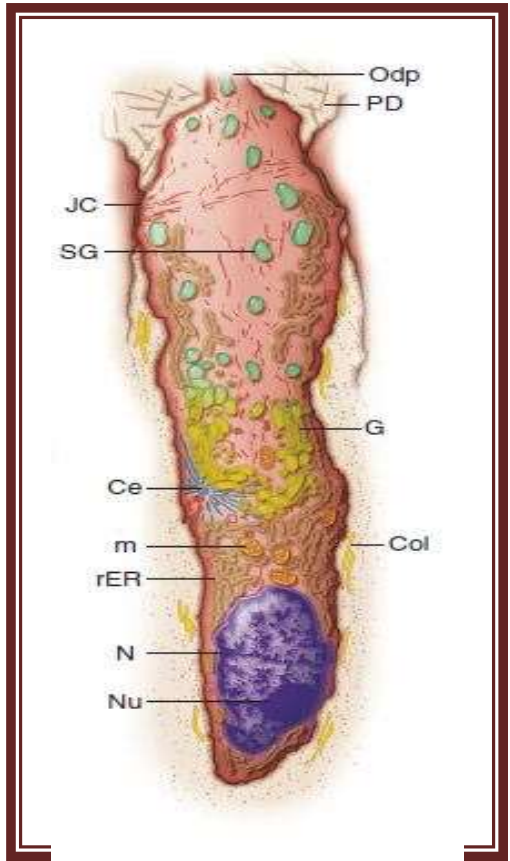
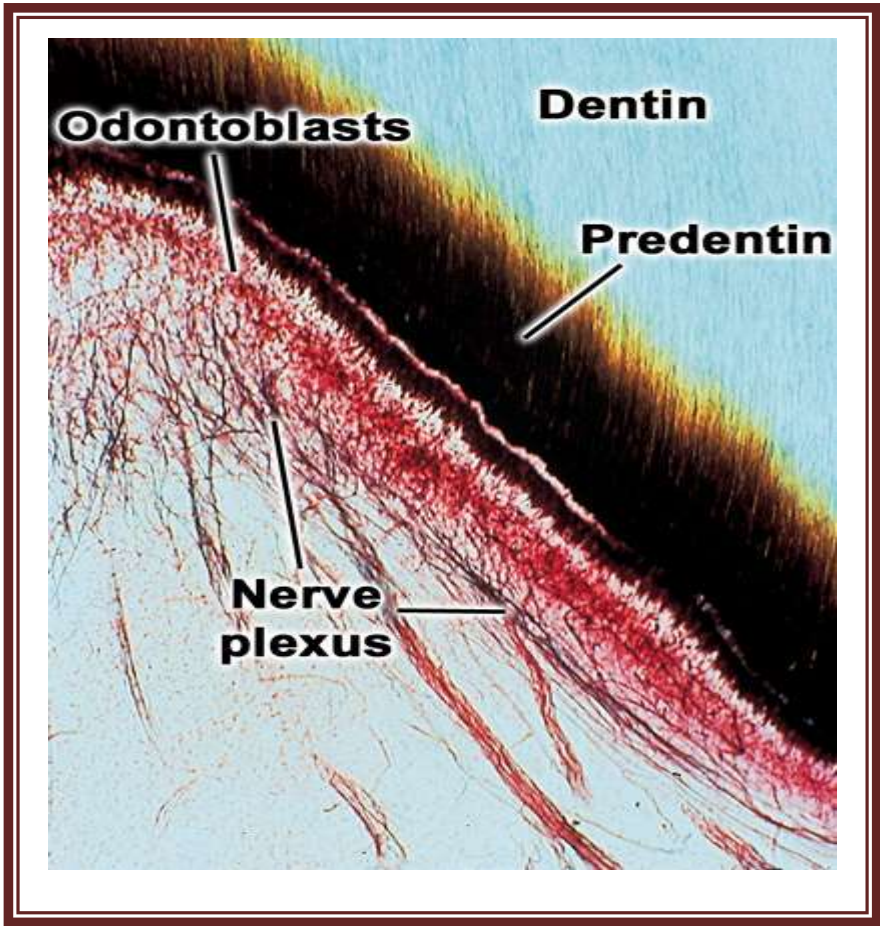
- Excessive dentin formation at the roof and floor of the pulp chamber.
- Presence of pulp stones at the opening of the root canal.
- The apical foramen is narrowed by cementum.

2- when accessory canals are located near the coronal part of the root or in the bifurcation area, a deep periodontal pocket may cause pulp inflammation. Conversely a necrotic pulp can cause periodontal disease.

3- A non-vital tooth become brittle and is subjected to fracture. Therefore, every precaution should be taken to preserve the vitality of the pulp.

4- some materials such as calcium hydroxide seem to facilitate dentin bridge formation. So, they applied in deep cavity preparation when the dentin layer is very thin to protect the healthy pulp.







## Cementum

Cementum is a thin layer of calcified connective tissue covering the external surface of the roots of the teeth, extending from C.E.J. at the cervical portion and continues to the apex.

It's like bone except that cementum is **a vascular** (without b.v.), it's thickness varies according to **site** and **age**.

It's usually much **thicker** in the apical part of the root and in the interradicular area of multirooted teeth than in the cervical region.

Cementum deposition continue throughout the life of the tooth, so it's thickness increase with the **age**.

### Chemical properties:-

The cementum composed of:-

- 1-The inorganic substances is about (45%-50%) which consist mainly of Ca and phosphate in form of hydroxyapatite, with numerous trace element, and the cementum has the highest **fluoride** content of all the mineralized body tissues.
- 2-The organic substance and water is about (50%-55%), the organic material are mainly the collagen fiber type I in addition to the protein polysaccharides ( proteoglycan).
- 3- Also have non-collagenous proteins like, bone sialoproteins, dentine sialoproteins, fibronectin.
- 4- Contain the CAP ( cementum derived attachment protein) promote attachment of mesenchymal cells to extracellular matrix, and may be a marker to differentiate between cementum and bone.
- 5- Cementum is rich in Glucose aminoglucon especially chondroitin sulphate which located around cementum lacunae.

### Physical properties:-

- 1- It's light yellow in color, lighter than dentin color.
- 2- The fully mineralized cementum is softer than dentin.
- 3- Lacks the luster of enamel.

### Types of cementum:-

Types based on the presence or absence of cells and on the nature and origin of the organic matrix

### 1. **Acellular extrinsic fiber cementum (AEFC):**

- Corresponds with acellular cementum (primary cementum), found extend from cervical margin to the apical 1/3<sup>rd</sup> in multirooted teeth. It's the only type of cementum seen in single rooted teeth.
- Fibers derived from Sharpey's fibers (extrinsic fibers) seen perpendicular to root surface, formed by **fibroblast of PDL**.
- Formed in slow rate, regular, thin and root surface is smooth.
- It appears as a homogenous layer in ground section because the cementoblast found on outer surface of cementum.
- Their function is to **provide a medium for attachment** of collagen fibers of PDL, that bind the tooth to alveolar bone.

### 2- **Cellular intrinsic fiber cementum (CIFC):**

- Corresponds to cellular cementum (secondary cementum).
- Having only intrinsic fibers running parallel to the root surface, it formed by **cementoblasts**.
- has **adaptive role** in response to tooth wear in addition to **repair function**, and has no role in tooth attachment.
- Seen in the apical third of the root and in bifurcation area in multirooted teeth, and it's thick.
- Under light microscope we found cementocyte incorporated in the matrix of this type of cementum, similar to osteocyte in bone, lies in a spaces or lacunae the cementocyte has numerous processes pass through the canaliculi, which anastomose with those of a neighboring cells, these canaliculi are directed toward the periodontal ligament, from which the cells obtain their nutrition.
- Less cellular than bone.
- Rapid formation.

Both cellular and a cellular cementum are separated by **incremental lines** which indicated the periodic formation of it. They called the **lines of Salter**.

These lines are far in cellular and closely placed in a cellular cementum.

### 3- **mixed fiber cementum:**

- Collagen fibers are both extrinsic and intrinsic fiber .
- Intrinsic fibers are fewer in number and run between the larger ovoid or round extrinsic fibers.
- If the formation rate is slow cementum may be termed acellular mixed fiber cementum.
- If the formation rate is fast, cementum may be termed cellular mixed fiber cementum.

### 4- **A cellular afibrillar cementum:-**

- Contains no collagen fibers.
- Sparsely distributed and consists of a well mineralized matrix.
- It's thin, acellular layer covers cervical enamel or in between fibrillar cementum and dentine.

- Formed following the loss of reduced enamel epithelium at cervical termination, permitting c.t. to come in direct contact with enamel.

### **The types of cementoenamel junction :-**

1-cementum overlap the enamel for a short distance this occur in 60% of teeth. this occurs when the reduced enamel epithelium degenerates at the cervical termination of unerupted tooth, permitting C.T. of dental sac to come in contact with E. surface, which lead to deposition of cementum named as **a cellular a fibrillar cementum.**

2-Cementum meet the enamel, edge to edge which occur in 30%.

3-cementum fail to meet E., it occur in 10% this occurs when epithelial root sheath in the cervical portion of the root is delayed in its separation from root dentin so cementum not formed in this region.

### **Cementodentinal junction :-**

This junction is relatively smooth in permanent teeth and scalloped in deciduous teeth, the attachment of cementum to dentin in either case is quite firm, because the collagen fibrils of cementum and dentin intertwine at their interface in a very complex fashion, so it's very difficult to identified which fibrils are of cementum or dentin in origin.

sometimes the Dentin separated from cementum by a layer known as **intermediate cementum**, this layer don't exhibit characteristic feature of either dentin. or cementum.

### **Clinical consideration**

1- Cementum is less readily resorbed than bone. so the success of physiological and orthodontic tooth movement relies upon resorption of bone without resorption of the tooth.

#### **2- cemental callus**

- sometimes form around root fractures
- Does not usually remodel to the original dimensions of the tooth.

#### **3- Cementicles**

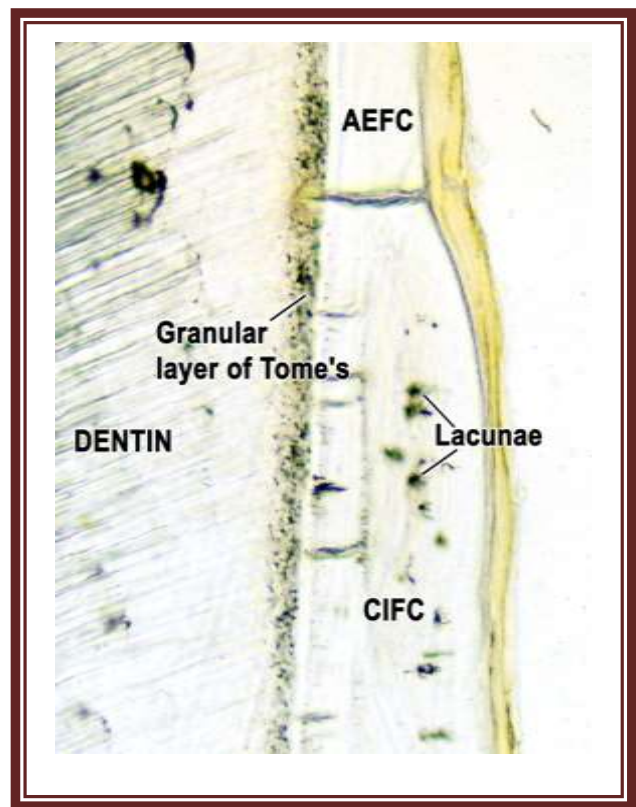
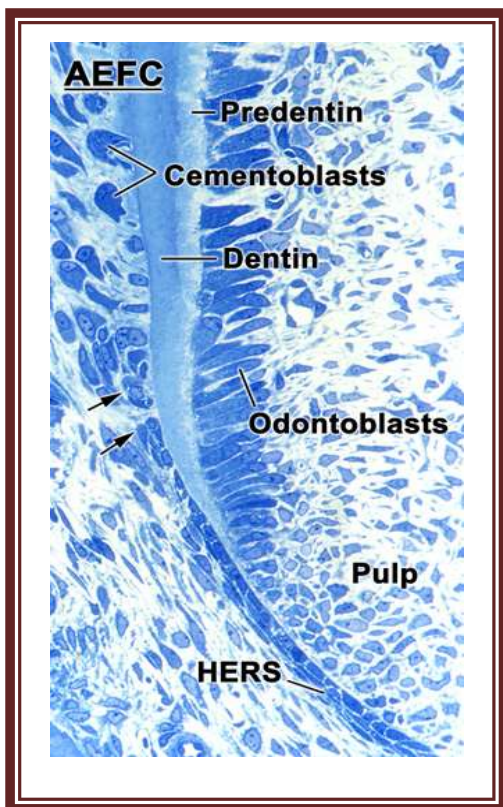
- Are small globular masses of cementum attached to the root or free in the PDL
- As a result of micro trauma and more common in apical and middle thirds of root and in bifurcation areas.

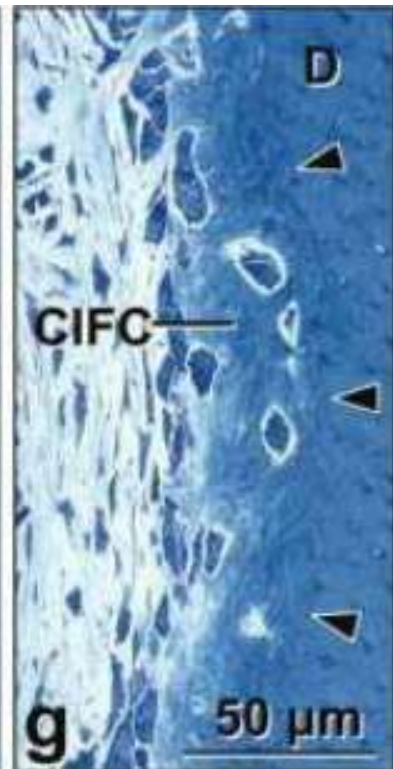
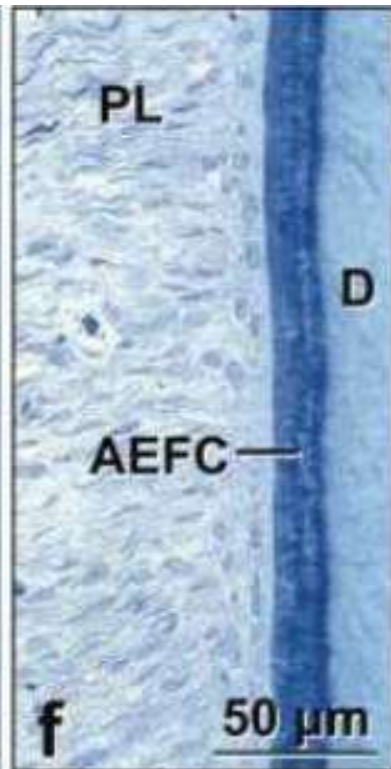
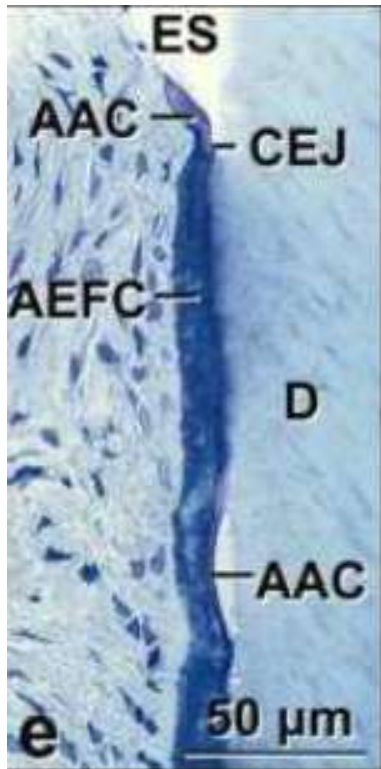
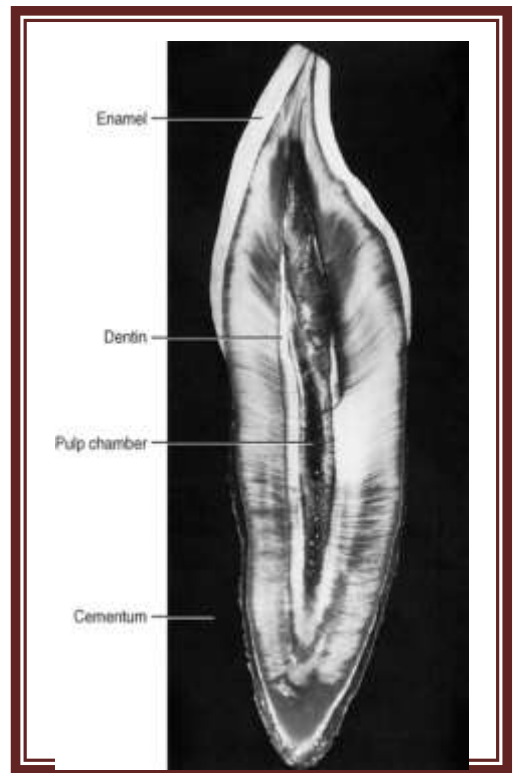
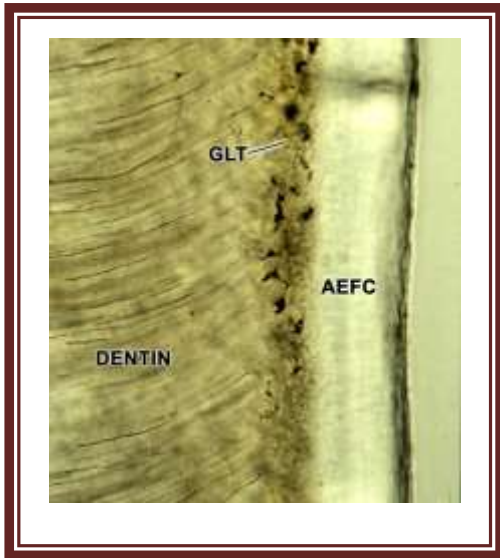
#### **4- Local Hypercementosis**

- In cases of chronic periapical infections.
- Fusion of adjacent teeth called **concrecence.**
- Hypercementosis affecting all teeth is associated with Paget's disease .

#### **5- Hypophosphatasia**

result in reduction of tissue non- specific alkaline phosphatase which causes significant reduction in amount of cementum formed, as a result the attachment of the PDL Fibers is compromised which causes premature loss of teeth ( both primary and permanent dentition).





# ORAL Histology & Embryology

Lec :15

أ.د.انتصار جاسم محمد

## Root formation

The development of the roots begins after enamel & dentin formation has reached the future cementoenamel junction.

The enamel organ plays an important part in root development by forming Hertwig's epithelial root sheath, Hertwig's epithelial root sheath consists of the outer & inner enamel epithelium only.

### Functions of Hertwig's epithelial root sheath :-

- 1-These cells will induce the differentiation of odontoblasts to form root dentin (radicular dentin).
- 2- The root sheath starts from future cementoenamel junction (C.E.J.) and continue to lengthen as the shape, the length, curvature, thickness and number of roots are all dependent on these cells it act as amold.

The IEE cells in the inner root sheath will stimulate the cells of dental papilla to be differentiate into odontoblasts, while the OEE (outer root sheath cells) deposit a cuticle membrane (**enameloid**) composed from enamelin protein, when the 1<sup>st</sup> layer of dentin is produced, the sheath will breakdown and loses its close relation to the dentin while it's remnant will persist either network or isolated masses or columnar of the root, these epithelial remnants are found in periodontal ligament of erupted teeth and they terms as **epithelial cell rest of Malassez**.

Mesenchymal cells from the dental sac move between the epithelial cell rest to contact the root dentin surface, which will differentiate into cementoblast and begin secretion of cementoid (which is uncalcified cementum ) then soon mineralizes into mature cementum.

### Development of the root:

At the beginning of root formation, the root sheath form the **epithelial diaphragm**, this diaphragm bend at the future cementoenamel junction into horizontal plane, **narrowing the wide cervical opening of the tooth germ** so it will form **the primary closure of apical foramine**. In case of the tooth with single root, the sheath grows as **simple tubular structure**.

But in multi-rooted teeth, along tongue like extensions of the horizontal diaphragm will develop, at the region of the furcation where the separate roots are give off these extensions corresponding in number to number of roots.

The cells of the epithelial diaphragm extension grow excessively until they contact the apposing extensions, these extensions then fused and the original single opening is divided into two or three openings each one grows as tubular structure.

In recently erupted teeth the roots are incomplete and only about 2/3 of roots are formed, after eruption the epithelial sheath will continue to grow downward until the entire root is complete.

In the last stages of root development, the proliferation of the epithelium in the diaphragm lags behind that of the pulpal connective tissue. The wide apical foramen is further reduced and narrowed by apposition of dentin and cementum to the apex of the root acting as **secondary closure of apical foramine**.

### **Clinical Consideration**

1- In case that epithelial cells of sheath remain adherent to surface of new root dentin, they may differentiate into ameloblasts and produce enamel such enamel is ectopic enamel called **enamel pearl**, are sometimes found in the area of furcation of roots of permanent molars.

2- Some times, continuity of Hertwig's epithelial root sheath is broken or is not established prior to dentin formation, a defect in the dentinal wall of the pulpal occurs lead to formation of accessory canals.

3- If the epithelial root sheath does not degenerate at the proper time and remain stuck to the surface of the root dentine, then that area becomes devoid of cementum

Areas of root without cementum can be a cause of sensitivity if there is gingival recession.

### **Cementogenesis:-** it means

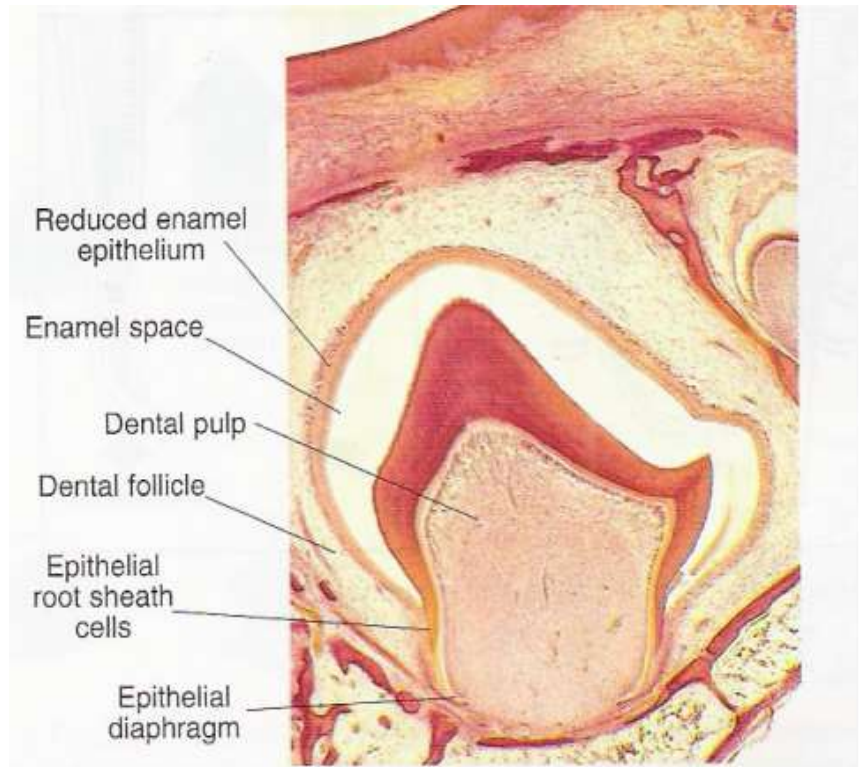
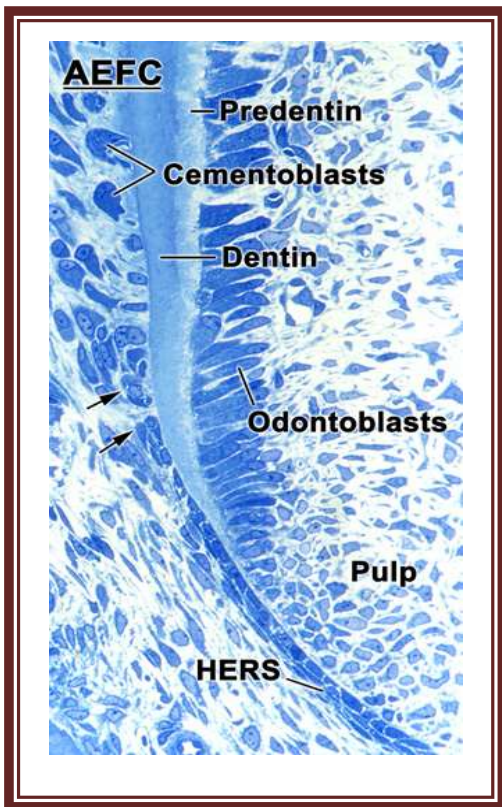
1- Matrix formation.

2- Mineralization.

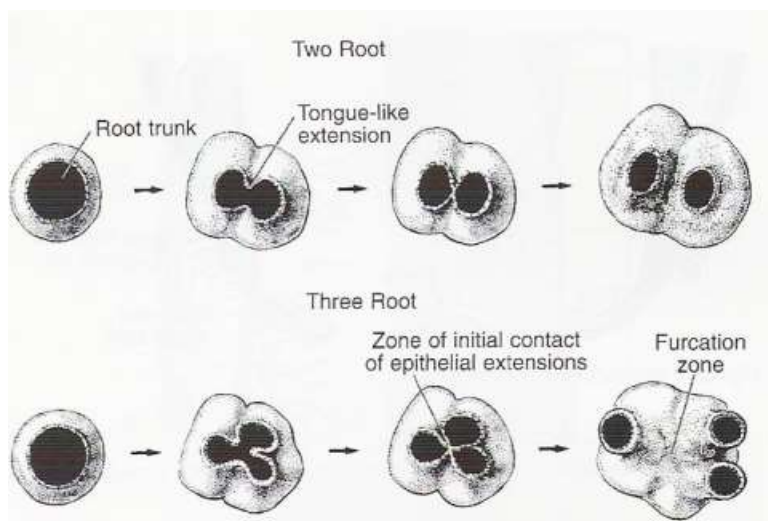
The matrix formation of cementum following the deposition of dentin along the inner aspect of the hertwigs epithelial root sheath. Once the dentin formation is started, the epithelial root sheath will lose its continuity, then the undifferentiated mesenchymal cells from the adjacent connective tissue of the **dental follicle** will be in indirect contact with the newly formed dentin, then these cells started to differentiate into cementoblasts (cementum forming cells).

The **cementoblasts** are protein synthetic cells, so these cells characterized by having abundant cytoplasmic organelles, the main product of these cells are the **collagen fibers** which form the main part of the organic matrix in addition to the **ground substance**.

After some cementum matrix (**cementoid** which is uncalcified matrix) has been laid down which usually lined by cementoblasts, the mineralization of it will begin. that Ca and phosphate ions present in tissue fluid are deposited into matrix, these crystals are arranged parallel to the long axis of the collagen fibers, and the mineralization will take place.

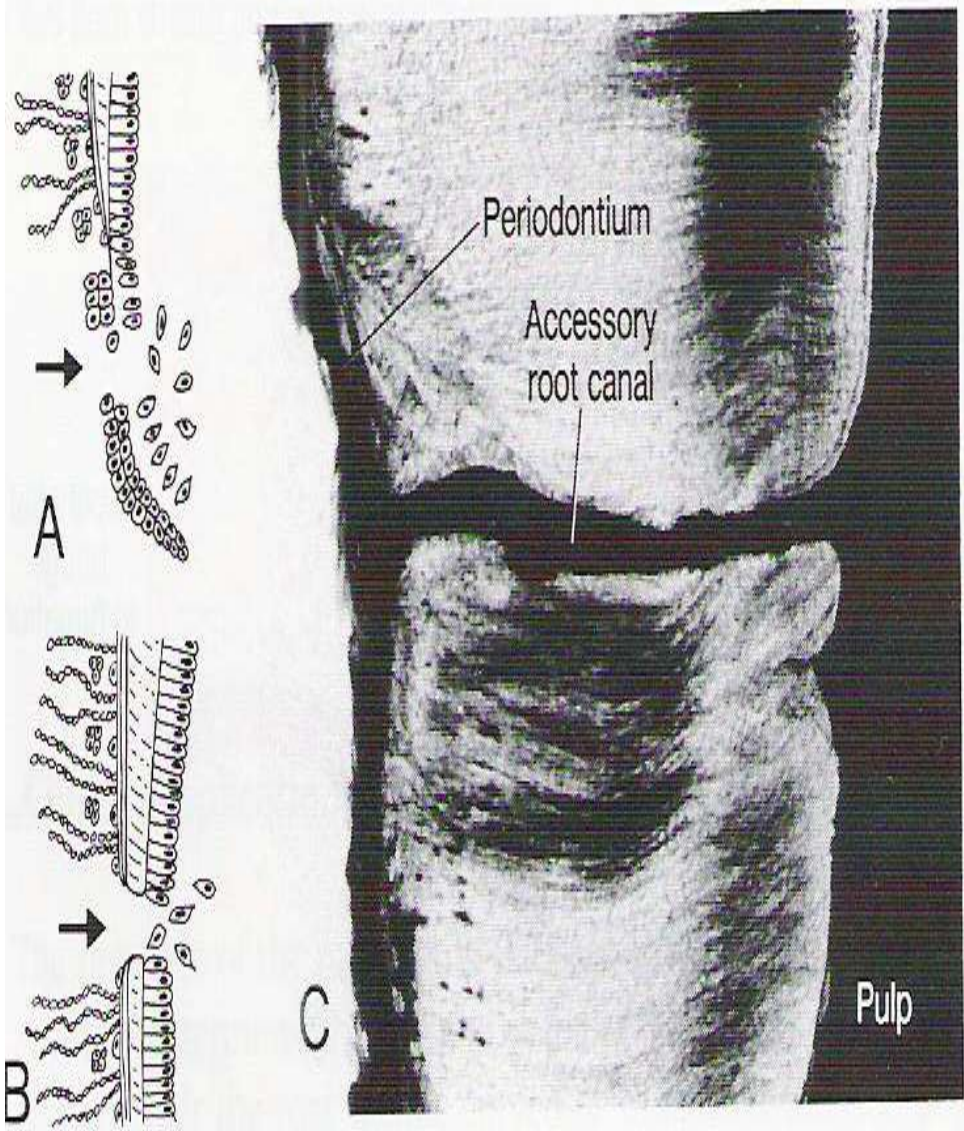


### Root formation



### Formation of multirooted roots





Formation of accessory root canal

# ORAL Histology & Embryology

Lec : 20

أ.د. انتصار جاسم محمد

## Oral mucous membrane (o.m.m)

The mucous membrane, it is a moist lining of any body cavities that communicate with the exterior (such as gastrointestinal tract, respiratory passage, oral cavity ...etc.).

The oral mucosa is a barrier that separates the underlying tissues from their environment.

### Functions of oral mucosa:-

- 1- Protection of the deeper tissues and organs from the environment of oral cavity (ex. mechanical forces during chewing).
- 2- sensory function : important because it provides considerable information about events within oral cavity as heat and cold, touch, pain, thirst; The sensitivity of these sensations vary in different regions. swallowing, retching, gagging and salivating reflexes. These reflexes are initiated by receptors in the oral mucosa, as the touch sensations in the soft palate result in gag reflex.
- 3- Lubrication: the salivary secretion, to maintain the moist surface. A moist oral cavity helps in speech, mastication, swallowing and in the perception of taste.
- 4- Defense: the oral epithelium act as an effective barrier for the entry of the microorganisms. The oral mucosa is impermeable to bacterial toxins. It also secretes antibodies and has an efficient humeral and cell mediated immunity.

### Types of oral mucosa:-

According to their **primary function** it divide into:-

#### (1)- **Lining mucosa:-**

- It represent **60%** of the O.M.
- It lines the cheeks, lips, and cover alveolar mucosa, vestibular mucosa, ventral surface of tongue, floor of mouth and soft palate.
- Its loosely attached to underlying tissue.
- It attach to muscle.

#### (2)-**Masticatory mucosa:-**

- Form only **25%** of O.M.
- Cover the hard palate and gingiva.
- It termed so because it comes in primary contact with food during mastication.
- Its firmly attached to underlying bone.

#### (3)- **Specialized mucosa:-**

- Occupying smaller area about **15%** of O.M.
- Covers the dorsum surface of the tongue.

- It's quite different in structure & appearance from the previous types, composed of cornified epithelial papillae.
- It called so due to contain taste buds.

### **Histological appearance of O.M.M.**

It composed from the following component

- 1- Epithelial component.
- 2- Connective tissue component ( Lamina propria).
- 3- Basal lamina (electron microscope) or basement membrane (light microscope).

Submucosa:- it loose c.t., attaches the mucosa to the underlying structures, it could be **absent** as in masticatory mucosa.

### **Characteristic features of cells of oral epithelium:-**

- The epithelial cell of the O.M. tightly link to each other by desmosomes which are anchored extracellularly by tonofibrils, and arranged in layers.
- called **keratinocytes**, they represent about 90% of epithelial cells.
- The name of these cells not due to its ability to produce keratin but to its content of tonofilaments (fibrous protein) and these keratinocytes are present in all types of oral epithelium.
- The other 10% of cells present in oral epithelium are **non-keratinocyte** cells.
- The interface between epithelium and connective tissue is comprised of a structureless layer called basement membrane.
- The epith. surface area increased through an epithelial projection which extend through underlying c.t. which termed as **epithelial ridges**.

### **Types of oral epithelium:-**

Its stratified squamous variety, it may be:-

- a. Keratinized as in masticatory mucosa (hard palate).
- b. Parakeratinized ( as in gingiva ).
- c. Non-keratinized.( as in lining mucosa).

### **a- Keratinized oral epithelium (orthokeratinized) :-**

It composed from 4 cell layers which are:-

#### **1- Stratum basale.**

- Layer of cuboidal or short columnar cells attached to each other by *desmosomes* (a type of junction between the cells of the same embryonic origin).
- Adjacent to the basement membrane attached to c.t. by *hemidesmosome* ( a junction resemble desmosomes but between the cells of different embryonic origin).
- Basal cell layer consist of two population of cells:-
  - i- Serrated- heavily packed with tonofilaments which are adaptations for attachment.
  - ii- Non-serrated – stem cells – slowly dividing cells which serve to protect genetic information of the tissue.

#### **2- Stratum spinosum (prickle cell layer):-**

- Composed of several rows about 20-25 layers of elliptical or spherical cells ( termed as prickle cell ) because they having large intercellular spaces which contain glycoprotein and fibronectin and these spaces are large in keratinized epith..
- They remain in contact through intercellular bridges or desmosomes.
- They are active in protein synthesis.
- Some mitotic figures may be seen in spinous cell just beyond the basal layer about (2-3 cell layers) .
- So the **basal** cells layer & **parabasal spinous** cells are known as **agerminative layer**.

## 2- Stratum granulosum (Granular cell layer):-

- Composed of 2-3 layers of cell, cells are flatter & wider and larger than spinous cells.
- They contain basophilic keratohyalin granules (blue staining with H&E)..
- The nuclei show signs of degeneration.
- This layer still synthesis protein, but the rat is diminish as the cell approaches the stratum corneum.
- Between stratum granulosum and stratum cornium and between stratum spinosum and stratum granulosum, there are a small organelles called keratinosome or odland bodies.
- they are membrane coating granules has internal lamellated granules, Its function is produce a defense permeability barrier, and it present in all types of oral epith.

## 3- Stratum cornium (keratinized layer):-

- The cells are larger and flatter than granular layer.
- The nuclei and other cytoplasmic organelles such as ribosomes & mitochondria have disappeared.
- The layer is acidophilic ( red staining with H & E).
- Histologically amorphous layer that the cell composed of densely packed filaments (tonofilaments).

### b- Parakeratinized epithelium:-

- The cell layer similar to keratinized epithelium.
- except that the granular layer may be absent or not evident.
- It also differ from that, by the retention of the pyknotic nuclei (condensed) at the cells of stratum cornium.

### c- Non-Keratinized epithelium:-

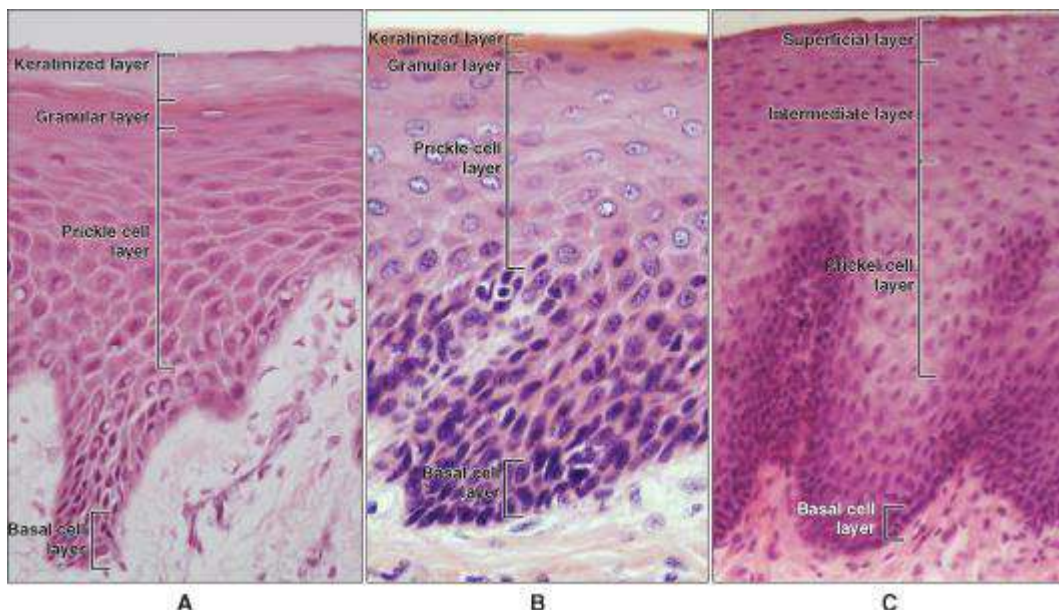
- Primarily there is no stratum granulosum, nor is there a stratum cornium.
- It don't produce keratin surface layer.
- Composed from four layers:-
  - a) **Stratum basale:-** which is similar to that of the keratinized epithelium.
  - b) **Prickle cell layer:** similar to that of keratinized except that the intercellular bridges or prickles are not obvious. For this reasons, some prefer to ovoid the term prickle cell layer for nonkeratinized epith.

**c) Stratum intermedium :-**

- The cells larger than spinosum cells, intercellular space is not obvious, they are more closely applied than spinosum cells, so not have a prickly appearance.
- The cells attached by desmosomes & other junctions.

**d) Superficial layer:-**

- The cells flat with nuclei, ultimately they desquamate.
- So non keratinizing oral mucosa have higher rates of mitosis than do the keratinizing oral mucosa. **why?**



Types of oral epithelium(A: Keratinized epith, B: Parakeratinized epith., C:Nonkeratinized epith.)

**Non-keratinocyte cells:-**

They are present as scattered cells and not as sheet, found in both keratinized and non- keratinized epithelium, doesn't contain the same number of desmosomes at tonofilaments such as that present in keratinocytes, they have different embryonic origin than that of keratinocytes, they constitute about 10% of epithelial cell population. which are:-

- Melanocytes:-** dendritic cell, present in basal layer, originates from neural crest cells, these cells store melanin in form of melanosome which elaborate melanin pigment responsible for pigmentation of oral mucosa.
- Merkel cell:-** non-dendritic, present in basal layer, originates from neural crest cells act as tactile sensory cell.
- Langerhans cell:-** dendritic, present in supra-basal layer, its origin from bone marrow, have an immunologic function.
- Lymphocytes:-** present in any layer, its originate from hemopoietic stem cells and associated with inflammation.

## **Lamina propria (L.P.):-**

- It's a c.t. of variable thickness, support the epithelium.
- consists of fibers (collagen and elastic) embedded in an amorphous ground substance
- with cells, blood vessels (b.v.) and neural elements.
- The lamina propria may attach to the periostum of the alveolar bone, or it may overlay the submucosa as in lining mucosa.
- Its divided for descriptive reason into two parts:-
  - a. Papillary portion.
  - b. Reticular portion.

### **a-Papillary part:**

Is superficial zone of loose C.T. adjacent to epith. and surrounding the epith. ridges. Papillary projection have considerable variations in length and width and depth contain predominantly fine collagen fibers and blood vessels and network of sensory nerve ending.

The interlocking arrangement of papillary part of lamina propria and the epithelial ridges help in 3 functions:-

- 1-Increasing surface contact between epith. and c.t. so it will strengthen attachment.
- 2- It facilitate exchange of material between the epithelium & b.v. in the c.t. of l.p.
- 3- Serving for accurate sensation.

### **b-Reticular part:**

Its denser C.T. contain coarse collagen fibers and are closely packed together besides large blood vessels and nerves, this reticular part is always present, while papillary part may be absent or very short as seen in alveolar mucosa.

### **Cells found in lamina propria**

Fibroblast Histocyte Macrophages Mast cell Polymorph nuclear leucocytes Lymphocytes Plasma cells Endothelial cells

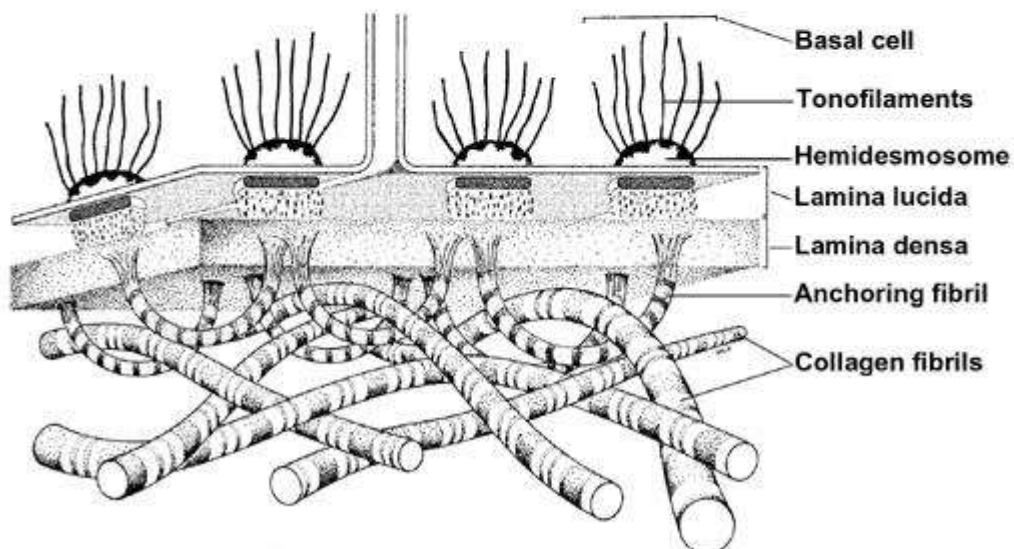
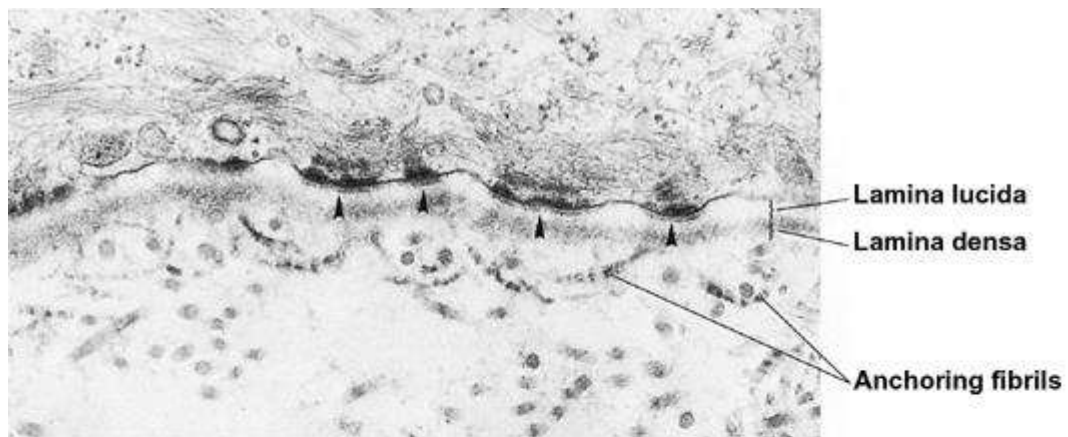
## **Submucosa (S.M.)**

- It consist of loose c.t. of varying thickness and density.
- It contains glands, b.v., nerve & adipose tissue.
- The larger arteries divided into smaller branches, which enter the lamina propria to give an subepithelial branches which remain in the papillary portion of lamina propria.
- It attach the m.m. to the underlying structures.
- The oral mucosa is either loosely attached to the underlying tissue when contain submucosa and called *movable* like in alveolar mucosa, or its firmly attached to underlying tissue(without submucosa) and called *immovable* like in gingiva and hard palate when the oral mucosa attaches directly to the periosteum of underlying bone (*mucoperiosteum*).

## **Basal Lamina ( B.L. ):**

- Under electron microcopy this structure appear as cell free zone about 1-4 micron width, stain positive with periodic acid Schiff method indicate it contain:

- 1- Mucopolysaccharides 2-fibronectin 3-type IV collagen
- 4- proteoglycan 5- glycoprotein 6- special anchoring fibrils.
- composed from two layers which are:-
  - a. Lamina Lucida – present on epithelial side, this is a clear cell free zone, the epithelial cells connected with it by hemidesmosomes cell junction.
  - b. Lamina densa – present on c.t. side, this is dark zone toward c.t. , its filamentous and granular , contain fine collagen fibers called anchoring fibers, connected with c.t. by anchoring fibrils loop.
- B.L. act as anchoring structure between epithelium & c.t. of O.M.M.



Copyright © 2003, Mosby, Inc., All rights reserved.

### Basal lamina under electronic microscopical level

### Effect Of Aging On The Oral Mucosa

- Epithelial thinning
- Decreased keratinization
- Less prominent epithelial ridges.
- Decreased cellular proliferation
- Loss of submucosal elastin and fat
- Increased fibrotic connective tissue with degenerative alteration in the collagen.

# ORAL Histology & Embryology

Lec :21

أ.د. انتصار جاسم محمد

## Types of oral mucus Membrane

### 1-Masticatory mucosa

During mastication, parts of oral mucosa are subjected to forces & pressure like in hard palate and gingiva.

#### a- Hard palate

It's tightly fixed to the underlying bone through mucoperiosteal junction, so its immovable. The epithelium is uniform in form with well-keratinized surfaces, the epithelial ridges are long, numerous, irregular and slender in shape (مخروطية الشكل).

The Lamina propria, presented as layers of dense connective tissue, which has numerous long papillae interdigitate with epithelial ridges. its thicker in the anterior than in posterior parts of the palate.

Various regions in the hard palate can be recognized due to the absence or presence of submucosa, and the varying structures of the submucosa. So the following zones can be distinguished:-

1. Palatine gingiva which is adjacent to the teeth.
2. Median palatine raphe extending from the incisive papilla posteriorly. Radiating from this raphe, transverse folds called palatine rugae which is formed of dense C.T. covered by epithelium.
3. Anterolateral area (fatty zone) between the raphe and gingiva anteriorly.
4. Posterolateral area (glandular zone) between the raphe and gingiva, present posterior to the fatty zone. The submucosa contains minor salivary glands, pure mucous.

The first two zones of the palate, don't have the submucosa, while the submucosal layer occurs in wide regions extending between the palatine gingiva and palatine raphe. The presence of submucosa in the later two zones act as a cushion for the mucosa against mechanical loads and protect the underlying nerves and blood vessels of the palate. The glandular layers of hard palate and soft palate are continues.

#### b- Gingiva:-

Extends from the dentogingival junction to the mucogingival junction, its subjected to the friction and pressure of mastication, the morphology of both epithelium and c.t. indicates it's adaptation to these forces. Epithelium may be keratinized or non- keratinized but most



often it is parakeratinized.

The gingiva can be divided into:-

1. Free gingiva ( marginal gingiva ).
  2. Attached gingiva.
  3. Interdental papilla.
1. **Free gingiva:-** it extends around the cervical level of the teeth its free and movable ends marked by the free gingival groove. The inner side of it forms the gingival sulcus.
  2. **Attached gingiva:-** its immovable firmly attached to underlying cementum or periosteum, extends from free gingival groove to mucogingival junction, which separates it from alveolar mucosa.
  3. **Interdental papilla:-** It's that part of the gingiva that fill the space between two adjacent teeth. It has a pyramidal shape in the anterior teeth, while its triangular shape in posterior teeth. It has a 'facial side' and a 'lingual side'.

Clinically:- the normal healthy gingiva looks pink in color, also the surface of healthy gingiva ( attached gingiva ) shows a characteristic pitting called stippling (orange-peel appearance) which is a characteristic of this type of mucosa. This phenomena disappear where there is gingivitis ( inflamed gingiva ) due to the presence of edema, so usually the inflamed gingiva shows red, shiny, smooth & edematous.

Microscopically:- portions of epithelium appear to be elevated, and between two elevations there are shallow depression. They probably are functional adaptations to mechanical impacts.

## **COL :**

Connecting facial and lingual side of the interdental papilla (on proximal side) is an epithelial structure. It's concave shape means, gingiva is healthy. It becomes dome shaped, in gingival recession and inflammation. It is covered by non- keratinized stratified epithelium.

## **MICROSCOPIC FEATURES OF GINGIVA:-**

Histologically, the gingiva composed from surface epithelium and Lamina propria.

**a- The epithelial part** presented with three types as follows:-

1. Keratinized (orthokeratinized) in 15%
2. Parakeratinized in 75%
3. Nonkeratinized in 10%

**Epith.** faces the oral cavity, which covers the free and attached gingiva, its either keratinized or parakeratinized stratified squamous epith. The epith. ridges are long , slender-like and numerous, lacks separating submucosa layer so its immovably attached to bone and teeth by coarse collagen fibers and has no glands.

## **Sulcular epithelium :**

Faces the tooth without contacting it. Thin non-keratinized stratified squamous epithelium (no granulosum and cornium layers), extends from the coronal end of the junctional epith. to the crest of the gingival margin. Acts as a semi-permeable membrane.

### **Junctional epithelium :**

Provides contact between gingiva & tooth surface. Stratified squamous non-keratinized epithelium, 3-4 cells thick in early life, increasing to 10-20 later. Derived from the reduced enamel epithelium . Attachment to tooth by inner basal lamina . Attachment to gingival connective tissue by outer basal lamina.

### **b- Gingival fibers :-**

About Lamina propria of the gingiva, it consist of a dense c.t. that doesn't contain large vessels, these dense fibers (gingival fibers) are collagen fibers and referred **as gingiva ligament**, arranged as strong bundles that bind attached gingiva to the alveolar bone and tooth. They arise from the cervical area of cementum and from the outer surface of alveolar process (gingival group of p.d.l. fibers which are previously discussed in PDL lecture)which are divided into the following groups:

- 1- Dentogingival:Its fibers run from cervical cementum to lamina propria of gingiva
- 2-Dentoperiosteal :-Its fibers run from cementum to periosteum
- 3-Alveologingival:-Its fibers run from alveolar crest bone to lamina propria of gingiva
- 4-Circular: this small fibers run in circular arrangement around the tooth.
- 5-Transseptal :the fibers run from cervical cementum of one tooth to cementum of adjacent tooth.

## **2-Lining Mucosa:**

Present in areas not subjected to high levels of friction, it is movable and firmly attached to underlying muscles like soft palate , lip , cheek , ventral surface of tongue. Loosely attached to underlying structures as bone, fascia or muscle like floor of mouth , vestibule and alveolar Mucosa.

### **a-Alveolar mucosa:**

Its red, shiny and covering the alveolar bone separating from attached gingiva by mucogingival junction. Its movable (loosely attached to periosteum by a loose well-defined sub mucosa contain mucous minor salivary glands). The epith. is thin, stratified squamous non keratinized and the epith. ridges are low and often entirely missing.

### **b-Lip :**

We can recognize in lip:

- **Oral Mucosa** of the lip lined by non -keratinized stratified squamous epithelium, minor salivary glands are present in the sub mucosa and fibers of orbicularis oris muscle are noted also.
- **Vermillion zone** of the lip is very thin keratinized epithelium can contain sebaceous glands.

### **What gives the vermilion zone the red color?**

- Epithelium is thin
- Epithelium contains eleidin, which is transparent semi-fluid clear substance present in the lamina lucida of the skin epithelium .
- Blood vessels are present near the surface eleidin.

### **c-Soft palate:**

- The mucous membrane on the oral surface of the soft palate is highly vascularized and reddish in color, noticeably differing from the pale color of the hard palate.
- The stratified squamous epithelium is nonkeratinized.
- The lamina propria shows a distinct layer of elastic fibers separating it from the submucosa.
- The latter is relatively loose and contains an almost continuous layer of mucous minor salivary glands.
- The papillae of the lamina propria are few and short.

### **The mucogingival junction:-**

It's the junction between the attached gingiva and the alveolar mucosa. It means keratinized mucosa meets lining mucosa.

It is identified **clinically** by the change from the pale pink color of the gingiva to the bright pink color of the alveolar mucosa.

### **Histologically:-**

The epithelium of attached gingiva is keratinized or parakeratinized, and the lamina propria contains numerous coarse collagen bundles attaching with the underlying tissue by mucoperiosteal junction. While the alveolar mucosa has a thicker, non-keratinized epithelium overlying a loose lamina propria with numerous elastic fibers extending into the thick submucosa.

## **3- Specialized mucosa:-**

The specialized mucosa covering the dorsal surface of the tongue, is unlike that anywhere else in the oral cavity because although covered by functionally masticatory mucosa, it has different types of lingual papillae. Some of them possess a mechanical function, whereas others bear taste buds and therefore have a sensory function.

The tongue is divided into two main parts, anterior  $\frac{2}{3}$  (body of the tongue) and posterior  $\frac{1}{3}$  (base or root of the tongue), separated by a V-shaped line called the terminal sulcus.

The body and the base of the tongue differ widely in the structure of the mucous membrane. The body is termed as papillary and the base as the lymphatic portion.

The lingual papillae are of four types:-

**1-Filiform papillae:** Makes up the majority of the papillae which covers the anterior part of the tongue. They appear as slender, threadlike keratinized epithelial projections covering a core of connective tissue and these papillae do not contain taste buds. These papillae facilitate mastication (by compressing and breaking food when the tongue is opposed to the hard palate) and movement of the food on the surface of the tongue, the papillae is directed towards the throat and assist in movement of food towards that direction.

**2-Fungiform papillae:** scattered between the filiform papillae, are the isolated mushroom-shaped papillae, which are round reddish prominences. Their color is derived from a rich capillary network visible through the relatively thin epithelium. Fungiform papillae contain a few taste buds.

**3-Circumvallate papillae:** (8-10) circumvallate papillae in front of the V-shaped terminal

sulcus between the body and the base of the tongue, each one surrounded by a deep, circular groove into which open the ducts of serous minor salivary glands (the glands of Ebner). These papillae have connective tissue core that is covered on superior surface by a keratinized epith. The epith. covering lateral walls is non-keratinized and contains taste buds.

**4-Foliate Papilla:** Leaf-like, present on the lateral margins of the posterior tongue, a few taste buds are present in the epithelium.

### Taste buds:

It's neuroepithelial structures. avoid-shaped, intraepithelial organs, they extend from the basal lamina to the surface of the epithelium. Unique sense organs that contain the chemical sense for taste. They are small. The outer surface is almost covered by a few flat epithelial cells, which surround a small opening which called as **taste pore**. It leads into a narrow space lined by the supporting cells of taste buds, The outer supporting cells are arranged as a barrel. The inner are shorter and spindle in shape. Between the inner cell arranged **10-12 neuroepithelial cells**, the receptors of taste stimuli. They are with elongated microvilli that project into the taste pore.

A rich plexus of nerves is found below the taste buds. Some fibers enter the epith. and end in contact with sensory cells of taste buds.

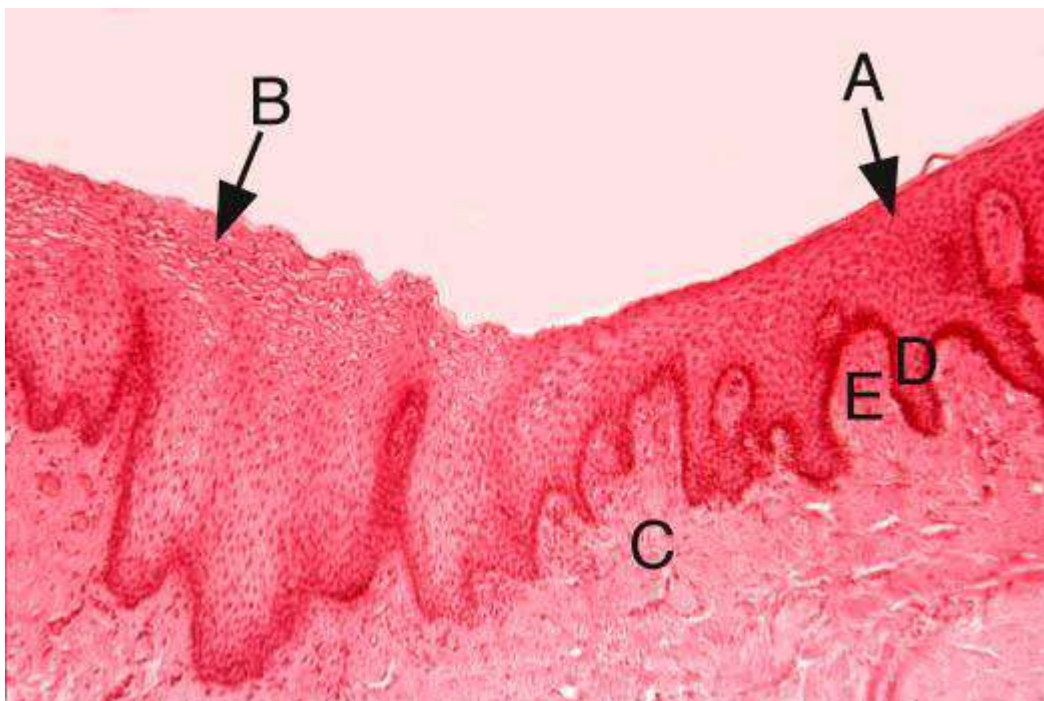
These nerves arise from the chorda tympani in anterior tongue and glossopharyngeal in posterior tongue and come to lie among the taste cells.

4 taste sensations: Sweet, salty, sour and bitter

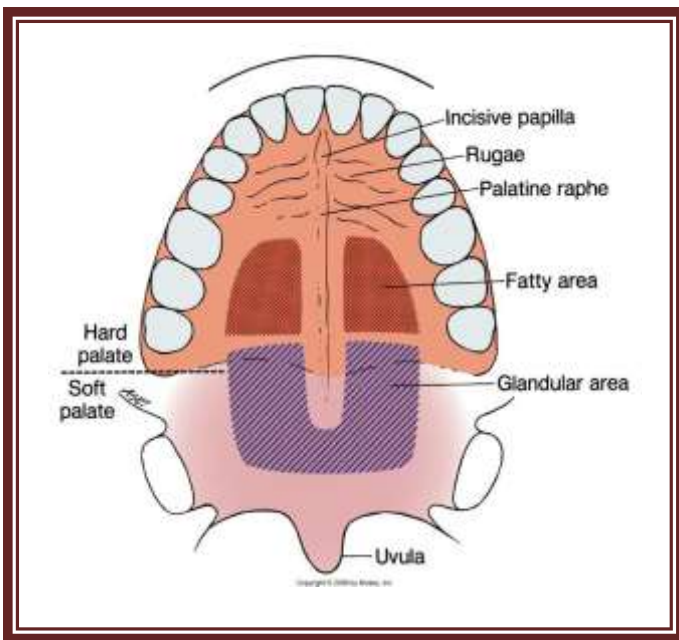
Sweet and salt: anterior tongue

Sour: lateral tongue

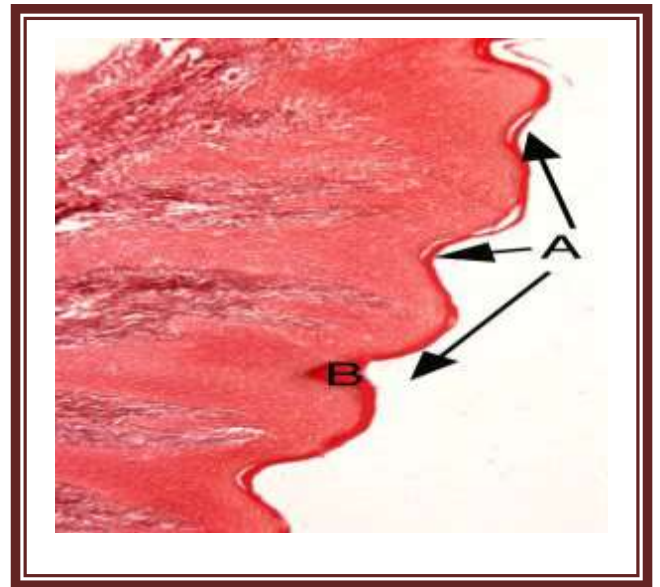
Bitter: region of circumvallate papillae.



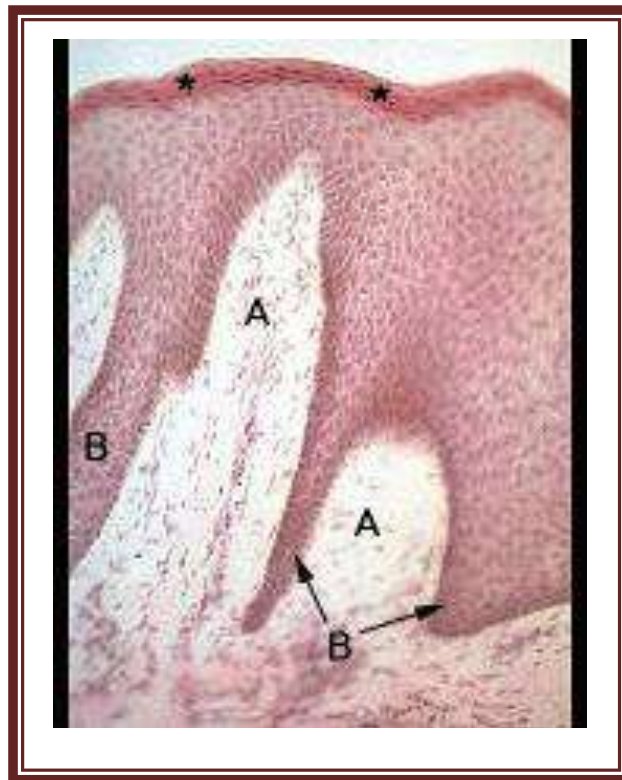
Mucogingival junction between A(attached gingiva) and B(alveolar mucosa).C and E represent reticular and papillary portions of lamina propria respectively and D (epith.ridge).



**Hard palate zone**



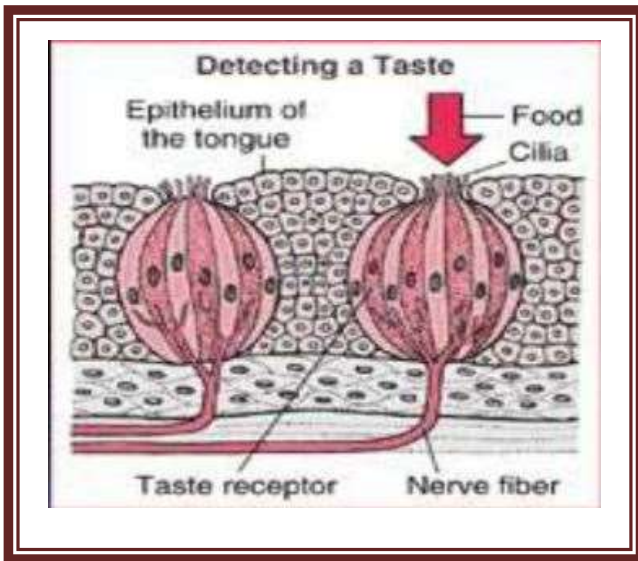
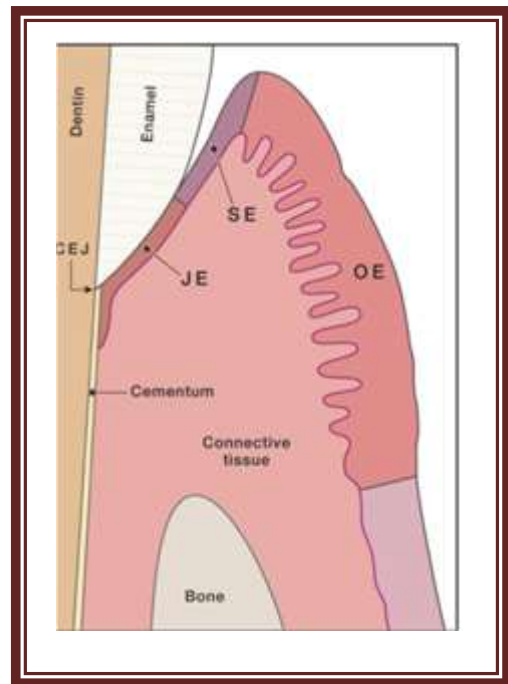
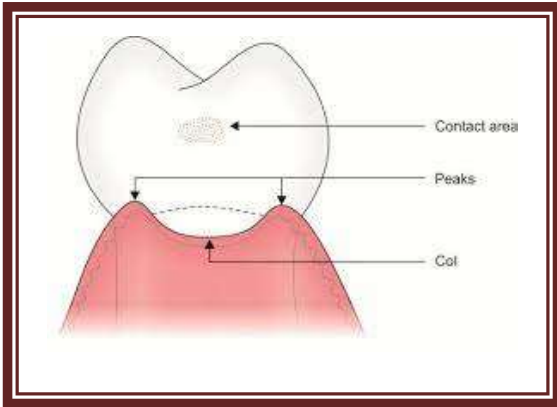
**Part 1**



**Part 2**

**Part-1 show Parakeratinized epithelium of gingiva, A: Connective tissue papilla, B: Rete pegs, and the presence of nuclei (\*) in the stratum corneum.**

**Part-2 show stippling of gingiva which can be observed clinically as little depressions on the surface “orange peel”**



## Junctional epithelium ( Dentogingival junction )

**Junctional epithelium(JE)** is the non-keratinized stratified squamous epithelium which attaches and form a collar around the cervical portion of the tooth.

**Epithelial junction or attachment** is the structural complex (hemidesmosomes & basal lamina) and this combination by which junctional epithelium is attached to the tooth surface

### **Junctional epithelium(JE)**

is a specialized non-keratinized, stratified squamous epithelium. Its the junction of the gingiva with the tooth in the cervical area, and forms the epithelial lined floor of the gingival sulcus.

This is a unique junction of considerable importance because it represent a potential weakness area, the bacteria that are certainly present on the tooth surface continually produce toxins capable of producing inflammation and damage, if they enter the mucosa tissues. so the junction between the epithelium and the enamel or cementum is the principle seal between the oral cavity and the underlying tissue.

### **Development of junctional epithelium:-**

When ameloblasts complete the formation of enamel they secrete a thin membrane on the surface of the enamel called primary enamel cuticle which remain attaching to the reduced enamel epithelium. The primary E. cuticle is soon removed by mastication because it's a very thin membrane, its remnant after eruption of the tooth is called Nasmyth's membrane.

The junctional epithelium derived from the reduced enamel epithelium. How?  
As the tooth erupts and before the crown penetrates the overlying oral epithelium, a fusion occurs between the reduced enamel epithelium and the oral epithelium, and when the penetration takeplace, the epithelial cells above the tip of tooth will be degenerated, and once the tip of crown erupt the reduced enamel epithelium at this time termed as the **primary attachment epithelium**. As the tooth erupts, the REE grows gradually shorter, a shallow groove, which is **gingival sulcus**, may develop between gingiva and tooth surface and extend around its circumference.

### **Histological appearance**

Junctional epithelium is a non-keratinized oral epithelium, consists of cuboidal basal cell layer followed by flattened cell layers aligned parallel to the tooth surface and tapering from 3-4 layers in thickness apically, to 15-30 layers coronally. The epithelium has a

smooth connective tissue interface where a basal lamina has associated hemidesmosomes. Between the plasma membrane of the junctional epithelial cells and the enamel surface a basal lamina structure with similar morphology is present, associated with hemidesmosomes on the membranes of the epithelial cells.

So through an electron microscopical examination, the junctional epithelium showing two basal laminae, an attachment to the enamel surface by **internal basal lamina**, and to connective tissue by **external basal lamina**.

The lamina propria shows an inflammatory infiltration neutrophil leukocytes and mononuclear leukocyte cells, continually migrate into the junctional epithelium, their epith. cells characterized by having large intercellular spaces, so they pass through them to the gingival sulcus and eventually reach the oral fluid.

### **Gingival sulcus (crevice):**

Its invagination made by the gingiva as it joins with the tooth surface. The gingiva does not join the tooth at the gingival margin. It forms a small enfolding known as the sulcus. The sulcus extends from the free gingival margin to the dentogingival junction.

In the average human mouth, the gingival sulcus has a depth of 0.5-3mm, with an average of 1.8mm, any depth greater than 3mm generally can be considered pathologic condition which termed as periodontal pocket.

The sulcular epithelium is non-keratinized in humans, it lacks epithelium ridges and so forms a smooth interface with the lamina propria. It's thinner than the epithelium of the gingiva, the sulcular epithelium is continuous with the gingival epithelium and the attachment epithelium, so these three epithelia have a continuous basal lamina.

Junctional epith. normally it extends from the bottom of gingival sulcus towards the C.E.J. It has very low resistance to the mechanical forces of mastication and to the bacterial toxin produced by dental plaque and calculus.

### **Gingiva can maintain this junction epithelial intact through the followings:-**

1. By the epith. of the gingiva:-when the epith. is injured(due to some reasons), the turnover of the epith. cells and their ability to migrate from the basal layer to higher levels will repair the injured part of the wound.
2. By the lamina propria:- when the C.T. is injured the fibroblasts can form the collagen fibers and ground substance that can repair the wound.
3. By the defense mechanism of the body that can resist the bacterial toxin.

### **Shift of the dentogingival junction:-**

The relation between the gingiva and the surface of the tooth changes with time, when the tip of the enamel first emerges through the mucus membrane of the oral cavity, the epithelium covers almost the whole crown.

Tooth eruption is relatively fast until the tooth reaches the plane of occlusion. This causes the attachment epithelium to separate from the enamel surface, as the crown gradually emerges into the oral cavity, at this stage the primary attachment epith. replaced by secondary attachment epith. which is derived from gingival (oral) epith. When the tooth reaches the plane of occlusion, one third to the one fourth of the enamel is still covered by the gingiva.



The actual movement of the teeth towards the occlusal plane is termed *active eruption* the gradual exposure of the crown by separation of the primary attachment epithelium from the enamel and by the recession of the gingiva is termed *passive eruption*.

### **First stage:-**

this stage persists in primary teeth till about one year before shedding. In permanent teeth it's found nearly till the age of 20 or 30 years. Here we find the coronal end of the primary attachment epithelium (the bottom of the gingival sulcus) is on the enamel, while the apical end of the attachment epithelium remain on the cemento-enamel junction. In this stage the clinical crown is less than the anatomical crown ( the anatomical crown refers to the enamel covered area of the tooth, while the clinical crown is that part of the crown which is exposed in the oral cavity).

### **Second stage:-**

In this stage the coronal end of the attachment epithelium is still on the enamel surface, but the apical end of the attachment epithelium had shifted to the cervical part of the root cementum. This stage may persist to the age of 40 years or even later, the clinical crown is less than the anatomical crown .

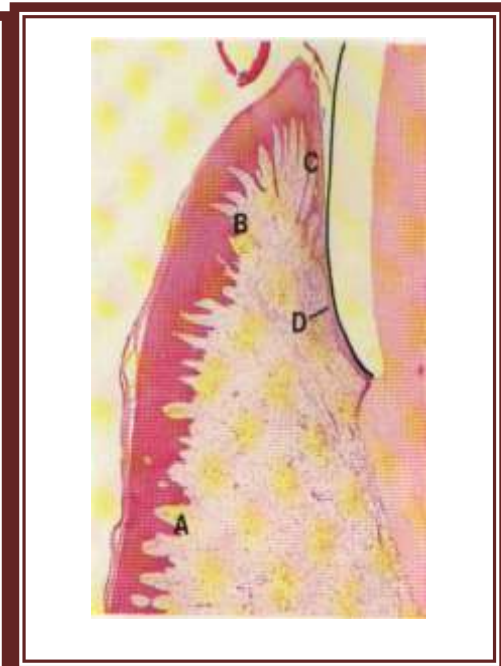
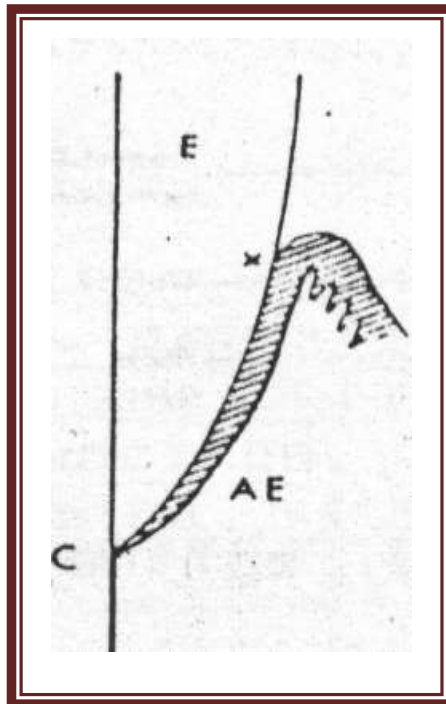
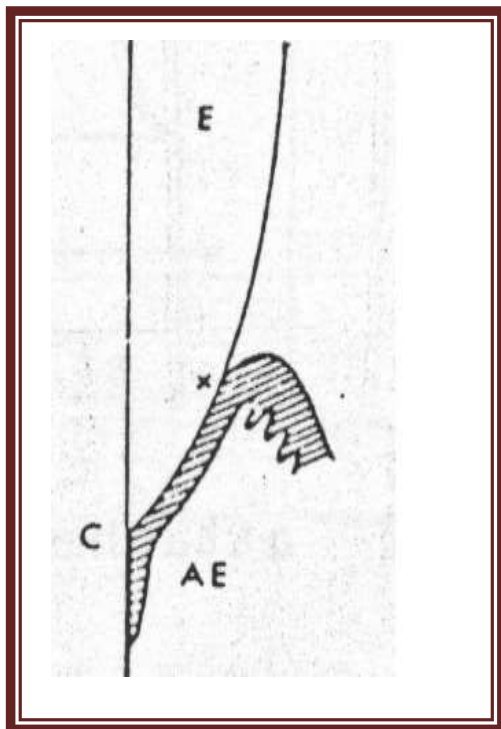
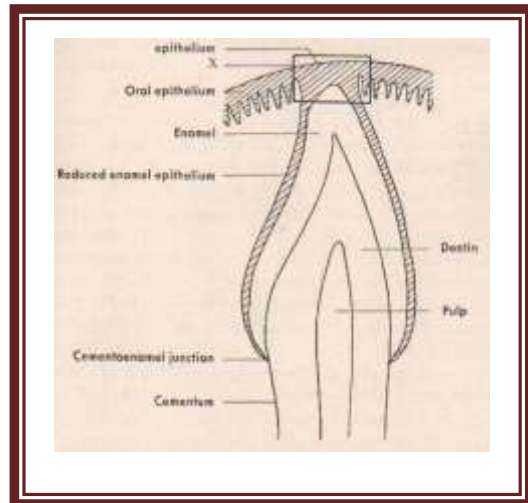
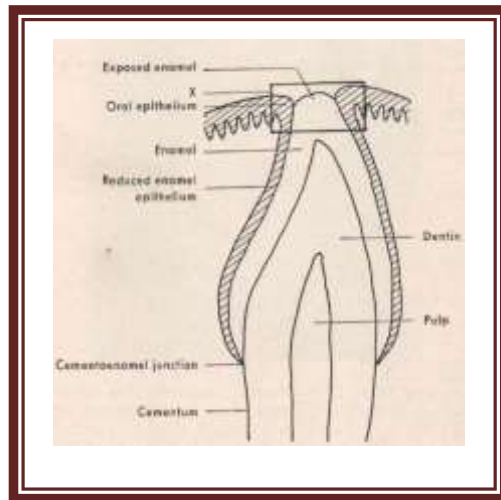
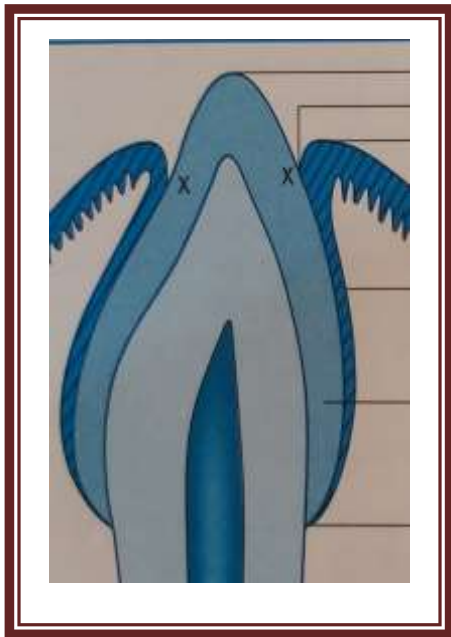
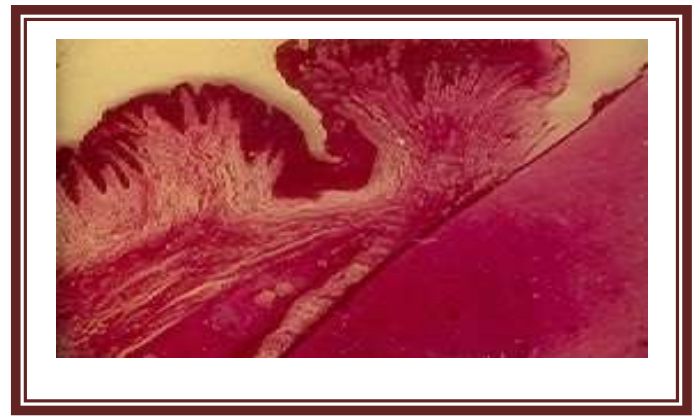
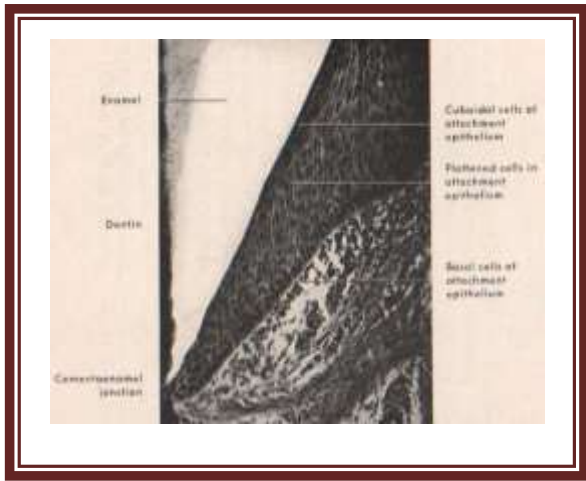
### **Third stage:-**

In this stage the coronal end of the attachment epithelium are on the cemento-enamel junction. The apical end is on the root cementum. This stage is transitory because the epithelium shifts gradually along the tooth surface and the attachment epithelium does not remain at the linear cemento-enamel junction for along time. The clinical crown is equal to the anatomical crown.

### **Fourth stage:-**

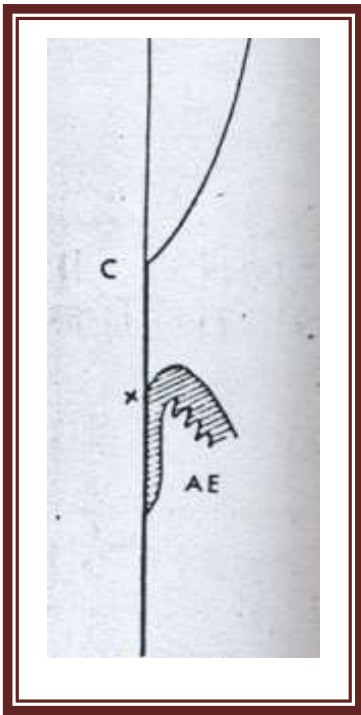
The entire attachment epithelium is present on the root surface i.e. both the coronal and apical ends attached to the cementum of the root. In this stage part of the cementum of the root is uncovered and is exposed to the oral cavity. The clinical crown is greater than the anatomical crown.

The first and second stages may be considered as physiologic, while the third & fourth stages there is a strong possibility that they are pathologic.

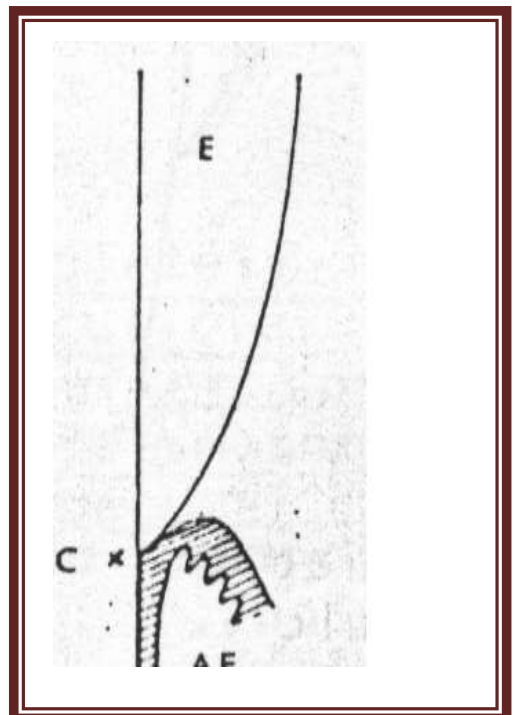


Stage 2

Stage 1



**Stage 4**



**Stage 3**