

HISTOLOGY AND EMBRYOLOGY FOR DENTAL HYGIENE



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Histology and Embryology for Dental Hygiene (Sheldahl)

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Preface



Figure 0.1: This record player stand is used as a metaphor

Why study histology and embryology?

This home-built stand for a record player is going to serve as a metaphor. Measurements were made to ensure there would be enough space for records. It is built out of wooden dowels, and used some floor tiles to create a smooth surface. Why those two? Actually, those building materials were re-used, they were left over from previous projects. Sure, a trip to the hardware store could have meant more appropriate materials, but would the hassle have been worth it? Currently, there is a Covid-19 pandemic, the answer is no. This is why you should study embryology, many instructions and materials are re-used during human development. Some things should make sense in this book, such as the instructions which guide even-spacing between teeth. But some things won't make sense initially, such as why is enamel made by epithelial cells, when it *looks* like bone tissue? Why can a piece of pig heart be used to surgically repair gingival tissue? What is the philtrum for? These are questions that can be better answered by studying where they came from (embryology) rather than studying their appearance in adults (anatomy). Imagine not knowing that flies develop from maggots, you might believe in [spontaneous generation](#) after observing the appearance of maggots on rotting food. Then why study histology? The answer to that is simple: embryos are tiny, you need a microscope to see what is going on. But more importantly, things happen in the oral cavity you can't see, but you should understand conceptually. For instance, with a basic understanding of histology you will understand why a pocket depth over 3mm is considered unhealthy. You can conceptualize what makes the linea alba appear white in some patients. You can explain what causes perikymata. You are on your own, however, on how to pronounce perikymata.

Author bios:

Laird C. Sheldahl, Ph.D. is the lead author and illustrator of this textbook. He has a Ph.D. in physiology and pharmacology. He currently teaches anatomy and physiology, as well as histology and embryology for dental hygiene, at Mt. Hood Community College in Gresham, OR. His Ph.D. thesis studied a morphogen involved in the formation of the head, including neural crest cell migration. This morphogen, a Wnt, is re-used during development of the teeth, which in turn involves neural crest cells. To study this process in frogs (their embryos are not inside uteruses, which makes them much easier to study) he did a lot of microscopy, which has helped considerably in the writing of this textbook. He does not have a background in dental hygiene, and is therefore very happy to have expert collaborators.

Student editors

Amen Mohammed

Conventions used in this eBook

There are two types of links found in this eBook. Internal links will bring you to the section of the book where that word is defined. These links should be in blue, regular text, such as *junctional epithelium* or *calcium hydroxyapatite*. Use the back button to return to your original spot. Other links in the book will take you to an external website, such as Wikipedia. These links should be a purple monospace font, such as this: [external link](#). We don't have control over how they appear on your eReader, however. External links are for further reading if you are interested, but are not required reading to understand the material we present. The basic format of each chapter is as follows:

chapter outline	
Philosophy	Where we try to provide a road-map for what I will be covering, and why we are covering it.
Physiology	Where we cover histology and embryology.
Clinical significance	Where we cover why the histology or embryology is relevant to your practice.

Table 0.1: Basic format of this textbook

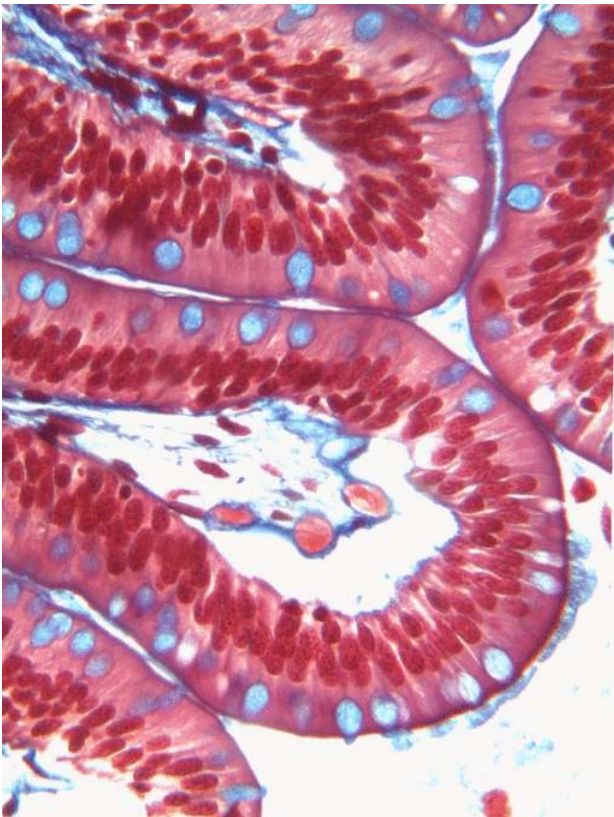


Figure 0.2: Example of an animated image

Animated images

The image in Fig. 0.2 is an animated .gif file, it should be cycling through a series of changes. The authors wish to take advantage of the things that can be done in an eBook, but not a print book.

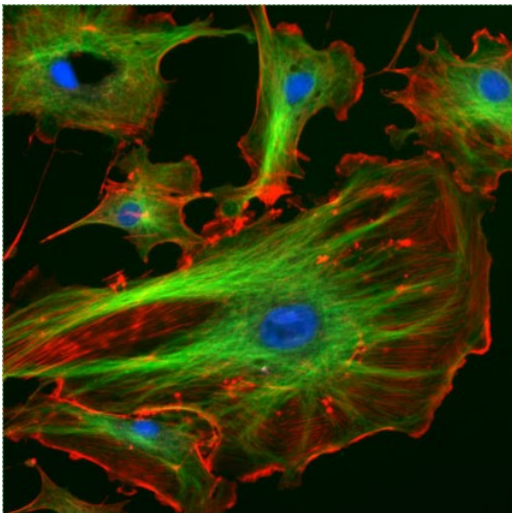


Figure 0.3 : Example of an image created by someone else. Image credit: [Fluorescent cells](#) by [NIH image](#) is in the Public Domain CC0

Other images

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Course objectives

Course objective	Textbook chapter(s)
1. Identify core concepts of cell biology and general histology related to the face and oral cavity	1,2
2. Demonstrate an understanding of the role of epithelial tissues, underlying connective tissues and neural crest cells in the face and oral cavity	3,4,5
3. Identify the basic patterns of early human developmental biology related to formation of the face and oral cavity	6,7
4. Describe the major steps of amelogenesis and enamel structure	8
5. Describe the major steps of odontogenesis and formation of the dentin-pulp complex	9
6. Describe the major steps of development of tooth roots and the periodontium	10
7. Identify mesenchymal-epithelial relationships in tooth development and tooth eruption.	8, 9, 11

Table 0.2: Course objective (for instructor use)

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CHAPTER OVERVIEW

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1.1: Cell Biology Review

1. Overview of cell biology
2. Inside the cell:
 - Cell membrane, cytoplasm
 - Organelles
3. Outside the cell:
 - Extra-Cellular Matrix (ECM)
 - Extra-Cellular Fluid (ECF)
4. Neither in nor out:
 - Cell division
 - Cell death
 - Cell junctions

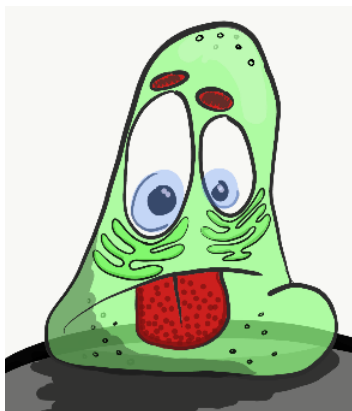


Figure 1.1: Cartoon of a cell.

Overview of cell biology

The cell is the basic unit of life. It is the smallest thing that we call living (without arguing about viruses), and the human body is made of 10 trillion of them. We started off as a single cell, and the purpose of this class to learn a little bit about how that one cell developed into the trillion-celled organism you are today. Nearly all of the instructions for making trillions of cells— how to make them, when to make them, where to make them— are found within that single cell. Different cells have different functions, even though almost every cell in one person has the exact same DNA. To become different from one another, cells express different DNA. This is an important process called **differentiation**, which is really no more complicated in concept than cells going from looking like boring generic cells to looking and behaving differently from other cells. We give different names to cells as they make these changes. For instance, a stem cell that gives rise to, oh let's say, a smurf, would be named a Smurfal Stem Cell. As the Smurfal Stem Cells divide and differentiate into cells that make a smurf, the cells actually making the smurf would be named Smurfoblasts, and when the Smurf was finished, the cells inside that Smurf would be named Smurfocytes. The stem cells found in a tissue called mesenchyme are named mesenchymal stem cells, the cells that make dentin are named odontoblasts, the cells within mature cementum are named cementocytes. Note that differentiation is not a single step, but a series of steps along a spectrum, from the least differentiated, to more differentiated, until we reach a terminally differentiated cell. The aim of this chapter is to review aspects of a cell biology class that come up in this textbook, and not much more. We assume you have covered cell biology in a per-requisite to this class. We need to review the parts of a cell that help us to explain difficult concepts like differentiation and development. If you find you need more than a quick refresher, here are some link from the NIH, who have a number of very useful publications and videos (for free), such as:

- [3D animations of the human cell](#)
- [Learn genetics \(interactive website\)](#)
- [Inside the Cell \(pdf and epub downloads\)](#)

Two useful (and free) eBooks you may wish to download are from OpenStax:

- [Biology 2ED](#)

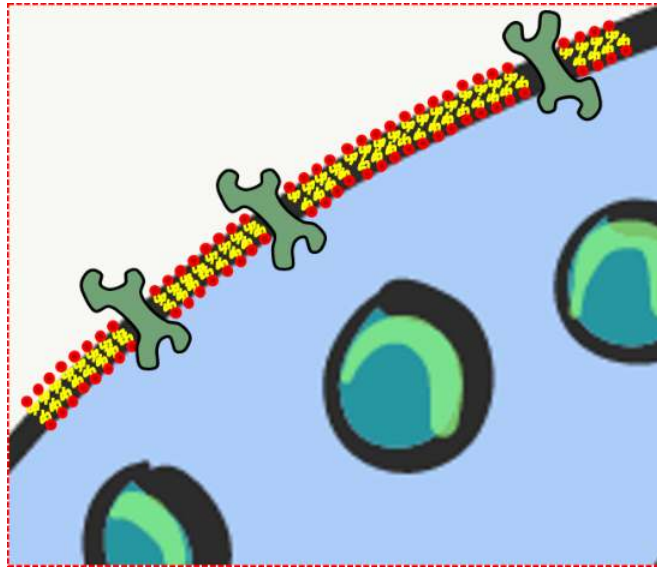


Figure 1.2: The plasma membrane

Inside the cell

Cell membrane and cytoplasm

Every human cell is surrounded by the **plasma membrane**. The plasma membrane separates the cell from its environment, and allows certain materials to enter and leave the cell. Phospholipids and cholesterol form a barrier that separates the cell from the external environment, which in turn allows us to point at that cell and call it a thing. **Trans-membrane proteins** span the plasma membrane, and regulate what goes in or out. Other trans-membrane proteins receive signals from other cells or the environment, and relay that information to the inside of the cell. Still others mediate attachment to structures outside of the cell. Other integral membrane proteins are located on the outer or inner surface of the plasma membrane, but do not span the entire width of the phospholipid bilayer. Many biology classes focus heavily on the nucleus, a trend that was admonished by one of the first African Americans to earn a Ph.D., Dr. Ernest Everett Just, in his book [Biology of the Cell Surface](#). Dr. Just was an embryologist, and in embryology the plasma membrane proteins—especially receptors and attachment proteins—are very important. The **cytoplasm** is the gelatinous filling of a cell, and is sometimes referred to as Intra-Cellular Fluid (ICF). Cytoplasm includes nutrients and electrolytes absorbed from the fluid surrounding all cells. In addition, the cytoplasm includes a number of proteins and glycoproteins synthesized by the cell. These molecules may have other important functions, but they attract water from the ECF. The end result is that cells have a gelatinous filling, rather than a watery one. This gelatinous filling is filled with a number of organelles, similar to the way my grandmother's jello salad contained grapes, raisins and other food or food-like substances that did not in any way turn that jello into an actual salad.

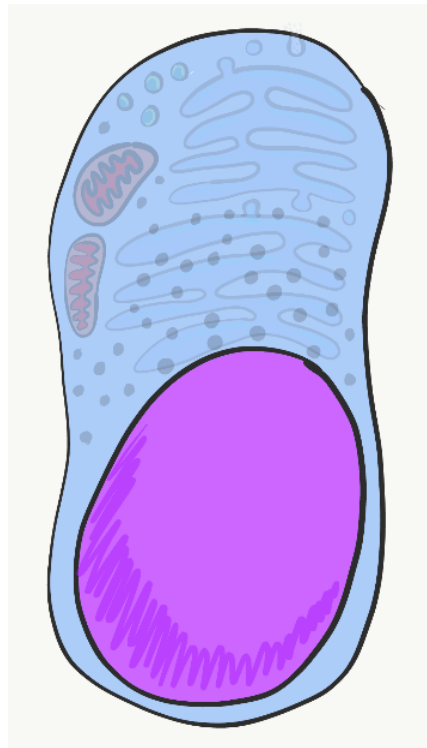


Figure 1.3: The nucleus.

The nucleus

The nucleus contains practically all of cell's **Deoxyribonucleic acid** (DNA). DNA is the instructions for making a **Ribonucleic Acid** (RNA) copy, and most RNA is translated in the cytoplasm into proteins found inside of and outside of a cell. It is also the instructions for when and where to make these proteins. For instance, epithelial cells of the oral mucosa do not make enzymes that secrete calcium and phosphate into the extra-cellular matrix. On the other hand, epithelial cells that differentiate into ameloblasts make these proteins, by **expressing** the DNA for these enzymes, after being told to do so by other cells called neural crest cells. All cells in the human body have the same DNA (with a few exceptions). However, different cells express different DNA at different times. DNA can be divided into 2 basic types. There are **genes**, each gene is more-or-less the instructions for a single protein. The rest of DNA folds up into unique shapes that provides instructions for when and where to express these genes. The latter is referred to as **non-coding DNA**. For DNA expression to occur, proteins called **transcription factors** (with help from special small molecules) bind to regulatory regions of DNA, open up the appropriate region, and recruit enzymes that copy one strand of DNA into **messenger RNA** (mRNA), which leaves the nucleus to be translated into protein. Other transcription factors may temporarily inhibit the expression of genes.

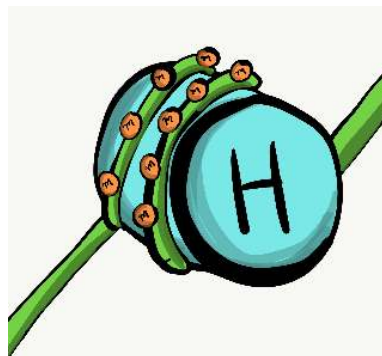


Figure 1.4: Methylated DNA wrapped around a histone.

Transcription factors turn on and turn off genes quickly, in response to changes in a cell's environment. But when cells differentiate, they shut down genes they don't need more permanently. Rather than relying on the on and off switches of transcription factors binding to regulatory DNA regions, these inactivated genes are **methylated** (a -CH_3 group is attached), packed

up around **histone** proteins, and put into long-term storage. The pattern of DNA methylation and histone packaging is copied during mitosis. This means the pattern of genes that are available or packaged away is inherited by both daughter cells, but because this inheritance is not a difference in the DNA sequence, it is known as **epigenetic** inheritance. **Epigenetic** traits are more capable of modification in response to changes in the environment than genes (DNA), and play an important role in cell differentiation and cell fate which is covered in more detail in chapters 8-11. We have 46 molecules of DNA in the nucleus– they are freakishly long molecules, but only 46 in number– 23 maternal, and 23 paternal. During mitosis, these 46 molecules are packaged up tightly into 46 **chromosomes** (times 2). This packaging involves using histones along nearly the entire length of a DNA strand, and allows chromosomes to be seen under the light microscope. The rest of the time, DNA is mostly unwound (un-needed instructions are wound around histones, the rest are free to be transcribed), and fills up the nucleus in a way that doesn't look very exciting. We call that DNA **chromatin**, and functionally it is by far the more exciting of the two forms.

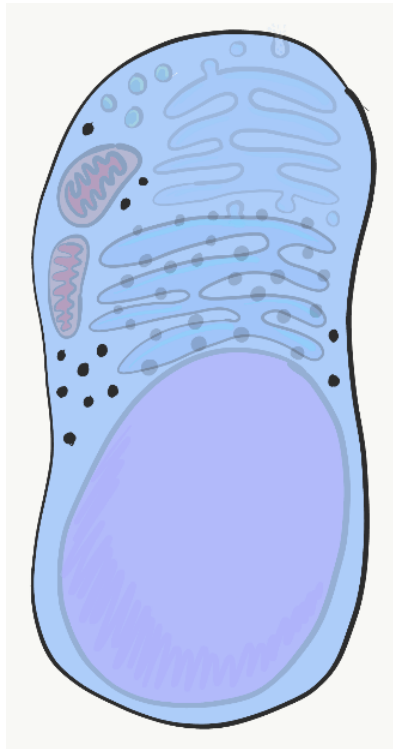


Figure 1.5: Free ribosomes in the cytoplasm.

Ribosomes

Visible throughout the cytoplasm are small specks made of protein and RNA called **ribosomes**. These structures **translate** mRNA instructions that came from the nucleus, which means the mRNA instructions are used to guide the linkage of a series of amino acids into a long protein. Groups of three mRNA nucleotides, called codons, instruct the ribosome which amino acid to add to a protein. Like regulatory regions of DNA, RNA found in the ribosome itself– not the mRNA instructions but ribosomal rRNA, is a type of RNA that folds up into specific shapes that, along with the ribosomal proteins, perform the chemical reaction of translation. Free-floating ribosomes in the cytoplasm synthesize proteins that remain in the cytoplasm, such as keratin or enzymes that mediate apoptosis.

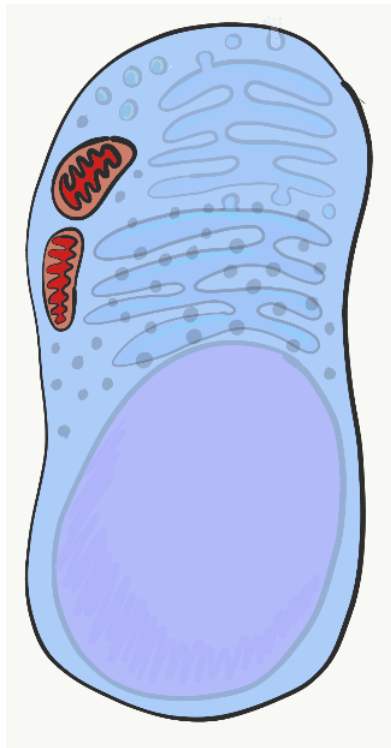


Figure 1.6: Mitochondria have two phospholipid bilayers.

Mitochondria

The mitochondria are where the majority of **Adenosine Tri-Phosphate (ATP)** is produced. ATP is made of Adenosine, plus three phosphate (PO_4^{3-}) groups— pay attention to the phosphate part, it is also a major component of bone, enamel, dentin and cementum. ATP powers almost all cellular processes, including the transcription and translation of mucous proteins within a salivary gland, the electrical signals sent by neurons in the tongue when food enters the oral cavity, and the contraction of myo-epithelial cells to cause salivation. Mitochondria burn glucose, using oxygen, and harness some of the energy released in the form of ATP. Mitochondria are different from other organelles in that they contain a little bit of their own DNA, which is inherited just from mother. Mitochondria also contain two phospholipid bilayer membranes, not one like other organelles. They use this extra membrane to generate ATP. Perhaps you covered glycolysis and the citric acid cycle before. The part to remember is that mitochondria use a proton (or H^+) gradient, which makes the inside of mitochondria acidic, and therefore potentially toxic to the rest of the cell.

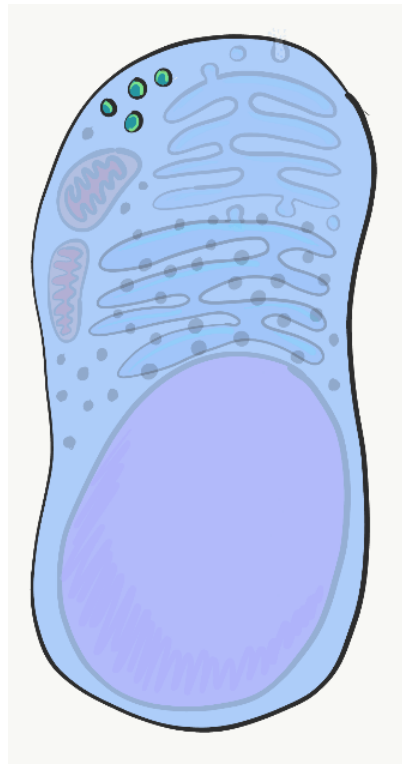


Figure 1.7: lysosomes

Lysosomes

Lysosomes are small compartments surrounded by the same phospholipids as in the plasma membrane. Inside lysosomes are acids and digestive enzymes that can be used to destroy stuff inside the cell when it wears out, or materials that the cell has gobbled up from outside (e.g. debris, bacteria). When a cell dies and begins to break apart, neighboring cells are in danger of being damaged from the acids and enzymes found within lysosomes. In the oral cavity, epithelial cells only have a life-span of days before they wear out, so it is very important for these cells to neutralize acids and enzymes in their lysosomes first, in a process called apoptosis.

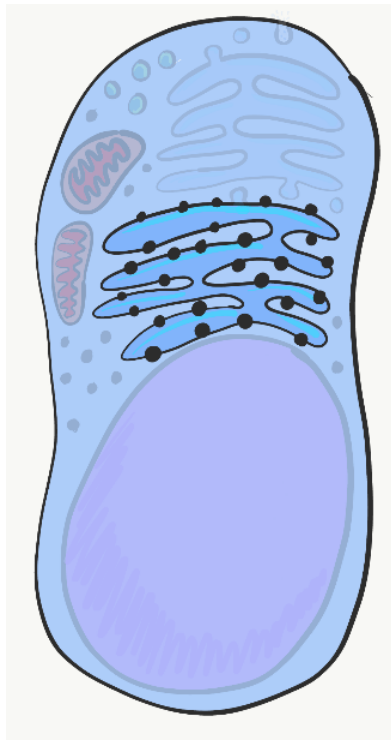


Figure 1.8: The rough Endoplasmic Reticulum (rER), with bound ribosomes.

Endoplasmic Reticulum

The **Endoplasmic Reticulum** (ER) is a series of interconnected tubes surrounded by a phospholipid bilayer– similar to lysosomes, only bigger, more tubular, and not full of acid. The **smooth Endoplasmic Reticulum** (sER) is where cells produce lipids and store calcium. The **rough Endoplasmic Reticulum** (rER) is covered in ribosomes. Proteins made by these ribosomes wind up inside the rER, then travel to the Golgi apparatus, and either wind up being secreted (such as mucous proteins) or stay within the plasma membrane (such as cell-junction proteins, or receptors for morphogen molecules).

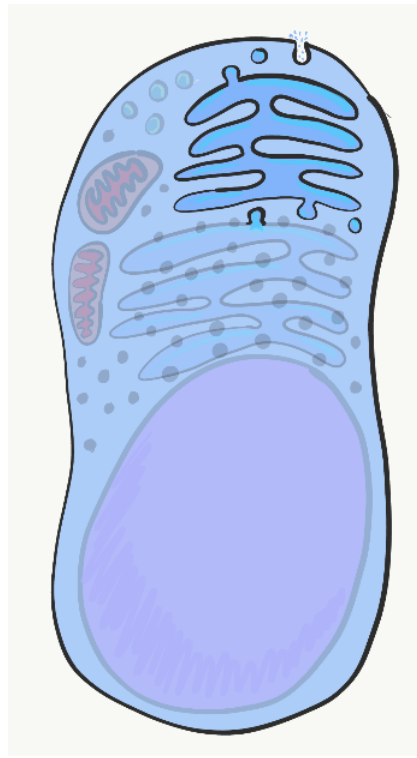


Figure 1.9: The Golgi apparatus is where proteins synthesized in or on the rER are taken next, via vesicles, before being transported to the plasma membrane.

Golgi apparatus

The **Golgi apparatus** is another set of tubes, similar to the rER. Small membrane-enclosed spheres called **vesicles** shuttle proteins made in the rER to the Golgi apparatus, where the proteins are modified. Often, these proteins have sugars attached to them, making them **glycoproteins**. Vesicles take these proteins to the plasma membrane, where they are either secreted or become a part of the plasma membrane. We cover the role the secreted protein collagen plays in enamel and the periodontal ligament. We also cover the shared role of the secreted glycoprotein fibronectin and the membrane-bound protein integrin have in healing damaged gingival tissue.

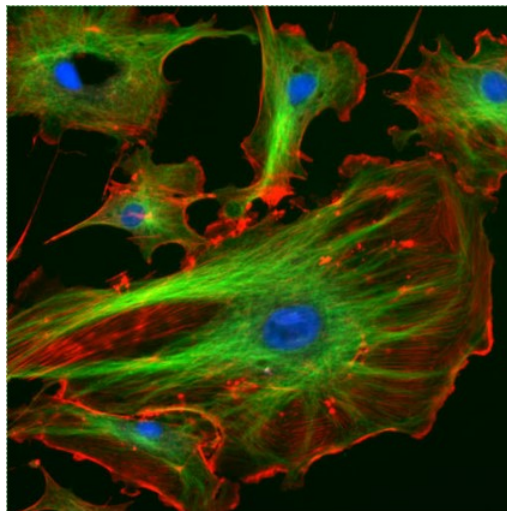


Figure 1.10: The cytoskeleton, viewed by confocal microscopy. Image credit: [Fluorescent cells](#) by [NIH image](#) is in the Public Domain CC0

Cytoskeleton

The **cytoskeleton** is a network of structural proteins within the cytoplasm. This network gives the cell its shape, the ability to change its shape, or to migrate. Shown in Fig 1.10, cells have microtubules and actin filaments stained red and green, respectively. These proteins are not generally visible on more old-fashioned histology images– both would be pink on a H&E stain, as would soluble proteins in the cytoplasm.

Outside the cell

Extra-cellular matrix:
Ground substance
Fibers

Table 1.1: Two major components of ECM. **Extra-Cellular Matrix** (ECM) includes all the material found outside of cells. It is usually broken down into the two components listed in Table 1.1.

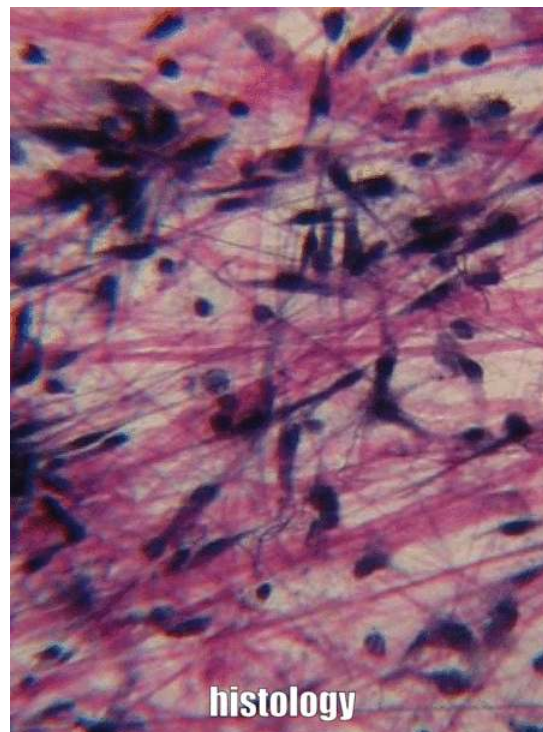


Figure 1.11: Illustration of areolar CT, highlighting the major components.

Ground substance

Ground substance is the name for all of the material outside of the cell that has no particular shape when viewed under traditional microscopy. One part of ground substance is **Extra-Cellular Fluid (ECF)**, which is the water and nutrients surrounding cells that was called plasma when it was inside of a blood vessel. Once fluid exits the blood and surrounds cells, it is called ECF. Unlike plasma, this fluid is held in place by the other major part of ground substance, which includes proteins, glycoproteins, and polysaccharides. These molecules are solutes, and their size makes them immovable, therefore they are good at holding the fluid in place, forming a gel. It doesn't look like much under a microscope, no more than if you looked really closely at some jello. These proteins, glycoproteins and polysaccharides are made and secreted by cells (these cells probably have a lot of rER and Golgi apparatus). One of the glycoproteins found in ground substance is **fibronectin**, which is a really long protein. Cell may recognize, bind to and move along fibronectin if they have the correct **integrin** protein spanning their plasma membrane. Therefore, fibronectin acts not only as a road along which cells travel, it is also a road map. Getting the right cells to the right place at the right time is very important both in healing and in development. In fact, what you learn about development is re-used (recapitulated) in healing. Another important molecule found in ground substance is a large polysaccharide called **Hyaluronic Acid (HA)**. Like fibronectin, cells can bind to and travel over HA (using a different type of plasma membrane protein), which has [applications in](#)

dentistry, such as helping cells of the gingiva stick to a dental implant and form a bacteria-resistant seal. We can't see fibronectin or HA without using some modern imaging tricks, which is why they are listed in as ground substance, and not in the next section, fibers. Because cells migrate over ground substance proteins, we say that ECM proteins function as a **scaffold**. Without scaffolds, tissues grow only from their edges. This is fine if speed is not important, such as when enamel and dentin are forming. However, in wound repair, it is optimal for a wound to heal everywhere at once, rather than from the edges. The body, therefore, often puts down some form of scaffolding first, such as a scab. In dentistry, artificial scaffolds can be created to help the body heal, based on our knowledge of the functions of ground substance. Examples of artificial scaffolds include some bone grafting materials, periodontal membranes and materials. In addition to their structural role as a scaffold, guiding cells to new locations, ground substance molecules provide cells with information. This information tells cells where they are located, and what they should be doing. For instance, when a stem cell binds to fibronectin, fibronectin can instruct the stem cell to express different genes and differentiate into a new type of cell, such as an odontoblast, and begin secreting dentin. Getting cells to the correct location is nice, but they need to know what to do when they get there. This happens during tooth formation and in response to tooth injury. As we learn more about how ground substance instructs stem cells, we get better at helping teeth repair themselves. It is unfortunate that many textbooks gloss over ground substance as just the gelatinous material outside of a cell, which is why we have gone into it in more detail, and instead glossed over mitochondria.

Fibers

Three extracellular proteins were visible under a light microscope a century ago, and they were grouped together as **fibers** of the extra-cellular matrix. Like fibronectin and other ground-substance proteins, fibers are secreted by cells called fibroblasts.

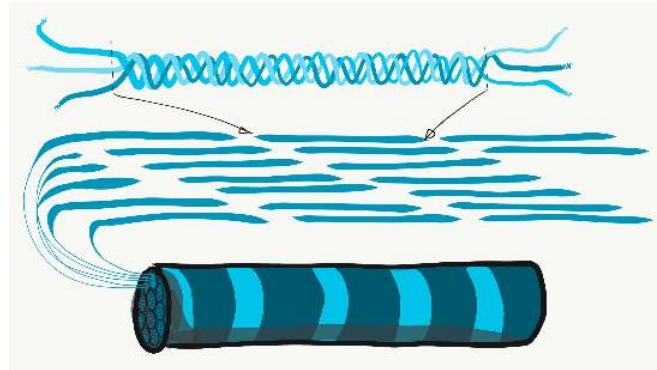


Figure 1.12: Collagen fibers are made of numerous individual proteins woven together, which are in turn woven together.

Collagen is the strongest of the three fibers, and is therefore often referred to as a structural protein—it gives many organs their shape. This fiber is made of 3 coiled α -helices, that are in turn coiled and cross-linked together, making a very strong macromolecule with the same basic shape as a rope. It is very strong if you pull on it from the ends, but bends if you apply force from the side. For instance, an area of advanced caries on a tooth can be as soft as wet driftwood, as it contains its collagen fibers without the plentiful minerals that used to surround them. Collagen fibers are found in regions of the oral cavity where the ability to resist force is important, such as in enamel or the periodontal ligament. In fact, it's found throughout the human body, accounting for 25% of our protein content. In addition to having a structural role, collagen also acts as a scaffold, similar to fibronectin and HA, which allows cells to migrate across the long fibers. Getting collagen from the rER (where it is translated) to the Golgi body for modification, and finally secreted from the cell requires **extra-large vesicles** for transport. **Reticular fibers**, which are not shown in Figure 1.11, look like a fine, spider web-like mesh under the microscope. Later it was discovered that reticular fibers are different form of collagen, but they are still called by their own name, and often receive equal footing with collagen on the list of ECM fibers. This web-like network of proteins isn't as strong, but provides enough of a frame for blood cells to rest throughout organs like the spleen and lymph nodes, as opposed to sinking to the bottom. **Elastic fibers** are thinner than collagen fibers, and often look like fine hairs in the ECM under a microscope. They are made from a different type of protein (elastin). As their name suggests, these fibers can be stretched and then spring back to their original length. This is not something collagen can do (if you can't put your palms on the floor while keeping your legs straight, the collagen fibers of your hamstring and gastrocnemius muscles and their tendons limit your range of motion. Regular stretching can activate fibroblast activity and lengthen them). Elastic fibers are found in higher quantity in regions of the oral cavity that change shape during speech or swallowing, such as the soft palate.

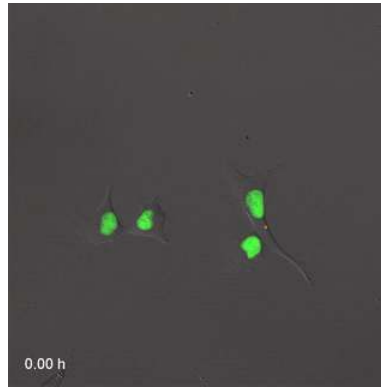


Figure 1.13: Mitosis. Image credit: Far-Red & Near-infrared Fluorescent Ubiquitination-based Cell Cycle Indicator (FUCCI) by Erin Rod is licensed under CC BY-SA 4.0

Mitosis

Cell division, or **mitosis**, the process by which one cell makes a copy of itself, producing two identical daughter cells. Early in development, as we are growing from a single cell to a trillion cells, lots of mitosis occurs. When a cell is not undergoing mitosis, it is said to be in interphase. This is the time where a cell might be doing its job, such as producing fibers for new ECM, or a cell might be preparing for mitosis. Before cell division can occur, a cell must have roughly double of everything. During mitosis, everything is divided in half between two new daughter cells. Not all cells are capable of mitosis– in fact, most cells in an adult have differentiated and are performing tasks, they are too busy to reproduce. We say these terminally differentiated cells have exited the cell cycle. To repair damage, tissues have **stem cells**. Stem cells are undifferentiated cells that are capable of dividing and differentiating into one or more different types of cells. A stem cell divides into two daughter cells, one daughter typically remains a stem cell, and the other differentiates into whatever cell type it is instructed to. A tissue can therefore have a constant supply of stem cells, as long as the stem cells don't die before they undergo mitosis. As we get older, our tissues don't heal as well because we have lost stem cells. Generally speaking, when a stem cell dies it is gone, another stem cell does not undergo mitosis to produce two stem cells and replace it. Stem cells are named based on how many different types of cells they can potentially become. The uni-potent stem cells of the oral epithelium become keratinocytes, and only keratinocytes. The multi-potent neuro-mesenchymal stem cells turn into dentin, pulp, cementum and periodontal ligament. The omni-potent fertilized egg becomes every cell in a human, plus more.

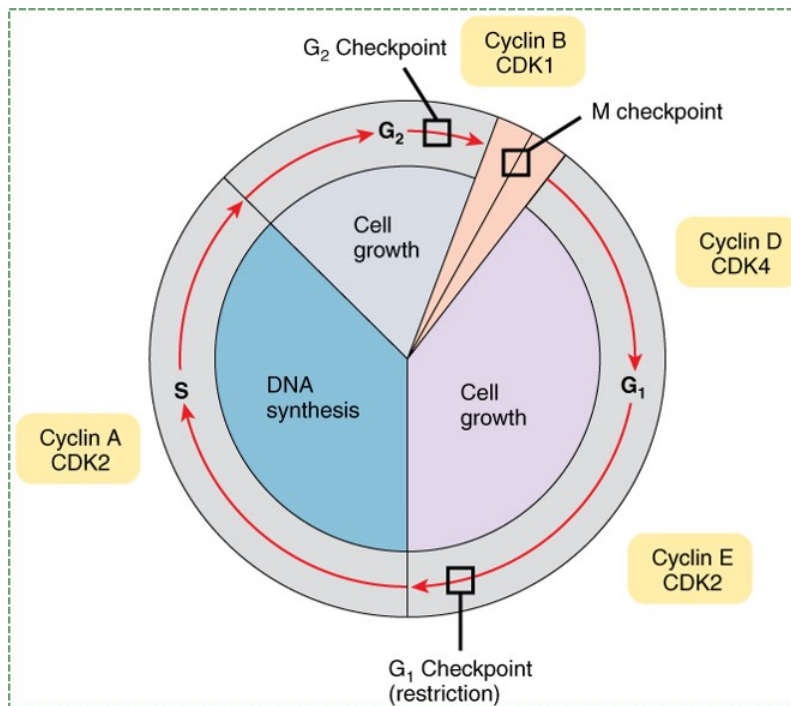


Figure 1.14: The cell cycle. Image credit: [Anatomy and Physiology Fig 18](#) by OpenStax is licensed under CC BY SA 4.0

To go through interphase and prepare for another round of mitosis, cells go through a series of **cell cycle checkpoints**. These are enzyme-catalyzed reactions which prevent progression to the next phase until a certain amount of a product build. This sets up the base rate for cell division, which helps ensure that the correct amount of tissue growth occurs. The passage through cell cycle checkpoints is regulated by the phosphorylation of proteins called cyclins. Cyclins are transcription factors that activate genes that allow progression through the checkpoint. The speed of this process can be sped up or slowed down by external signals, such as **growth factors**. Growth factors are hormones that are secreted into the ground substance of a tissue. The density and stickiness of the ground substance influences how far the growth factor diffuses. If diffusion is limited, the growth factor only speeds up growth in a localized area. This is important in the formation of new organs, such as teeth. Other growth factors might spread over a wide area, especially if they are secreted into the bloodstream. Secretion of Growth Hormone into the blood, for instance, allows different organs to grow at roughly the same speed. Mutation in a gene for a growth factor, or the receptor for a growth factor, can lead to a **gain-of-function**. If cells receive a constant on-signal for passage through the cell cycle as result, we call these genes oncogenes. It takes just one mutated copy of a receptor gene to gain a function. The cyclins and other genes that regulate the cell cycle, however, are called tumor suppressor genes. For a cell to lose its ability to regulate cell cycle checkpoints, both copies of a **tumor suppressor gene must be mutated**. In your car, it would take one foot to step on the gas too hard, but you'd have to be missing both feet to not be able to hit the brakes. Usually, cancers form when a single cell acquires mutations to both oncogenes and pairs of tumor suppressor gene **alleles**. An allele is one of the two copies of a gene found in the same position on two homologous chromosomes. Even if this occurs, there is another layer of protection covered in the next section.

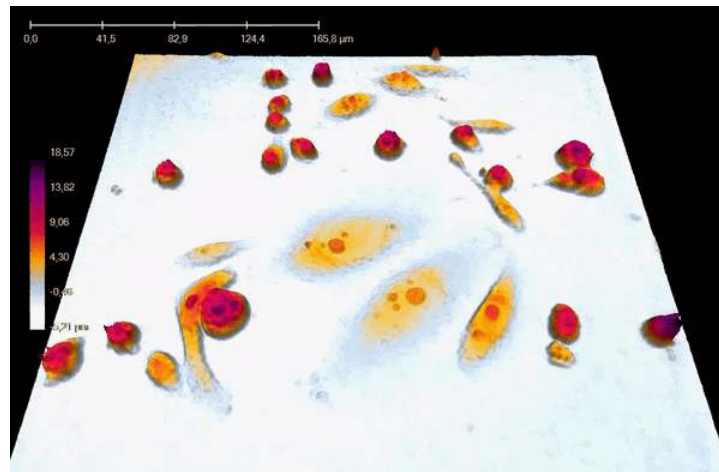


Figure 1.15: Cells undergoing apoptosis. Image credit: 43705 by Birgite Janicke is in the Public Domain CC0 / converted to gif

Apoptosis

All cells contain a group of cell-surface receptor proteins and intra-cellular enzymes that allow them to undergo programmed cell death, or **apoptosis**, when instructed. Programmed cell death is critical to multi-cellular life, which is an odd thing to say. Without it, our bodies would be full of very old, non-functioning cells. Or worse, as cells reached the end of their lifespan– which for epithelial and blood cells is really short– they release the contents of their lysosomes and mitochondria. As you may recall, the contents of these organelles are highly acidic, which damage or kill neighboring cells. If those neighboring cells died as a result, they too would release their lysosomal and mitochondrial contents, causing even more damage. When this happens in the human body it is called tissue **necrosis**. That's not all, a dead cell spews out DNA, and DNA is really long and stringy and tends to be very sticky. This can trap other cells and prevent them from migrating properly. This is great if a cell is trapping and killing bacteria and doesn't mind dying in the process, but not something good to do to neighboring human cells. Therefore, cells are instructed to undergo apoptosis as they are reaching the end of their lifespan, if the immune system has determined them to be infected or cancerous, or just aren't needed anymore. Apoptosis ensures that before a cell dies, it neutralizes the pH of its lysosomes and mitochondria, and chops up its DNA into safe, small bits. During development, more cells are produced than needed, and the extra cells are removed later in an organized fashion– similar to the way construction of a large building involves building scaffolding first, and the scaffolding is removed towards the end of the project. The process of wound repair also involves an over-production of cells followed by their organized removal. Hopefully, this will seem logical by the time you finish this course. During wound repair, DNA instructions are turned on that were used by cells when they were developing embryonically. If you want to sound fancy, and don't we all, you can say wound healing recapitulates embryonic development. **Recapitulate** means to state again (repeat). The process of apoptosis begins either with an internal or an external signal. For instance, when a cell's DNA becomes too mutated, or if there are an odd number of chromosomes during mitosis, this triggers apoptosis. Alternatively, a cell can be instructed to undergo apoptosis from an extracellular signal called a Tumor Necrosis Factor (TNF). A series of enzymes called caspases are activated, which ultimately leads to the neutralization of acids, destruction of DNA, and cause the cell to explode into numerous small bits, which can be cleaned up by macrophages.

Cell-to-cell junctions

Types of cell junctions	Examples
Cell-to-cell	Desmosomes, tight junctions, gap junctions
Cell-to-ECM	Hemi-desmosomes

Table 1.2: Major types of cell junctions. Junctions are specialized groups of proteins on or near the cell surface that make connections to some other structure. These connections can be to other cells, or to the ECM, as listed in Table 1.2.

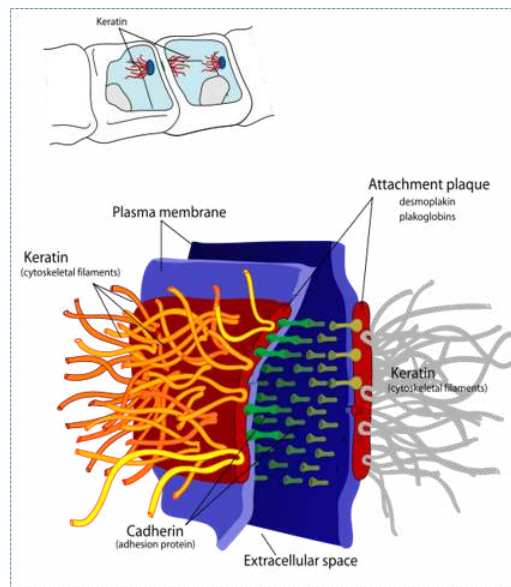


Figure 1.16: Desmosome (anchoring junction). Image credit: A desmosome, also known as macula adherens by Mariana Ruiz LadyofHats is in the Public Domain CC0

Desmosomes

Anchoring junctions (**desmosomes**) are strong connections between two cells. The anchoring junctions pair up and anchor the cytoskeleton of one cell to its neighbor. A large group of cells anchored together by these junctions are much stronger as a group. “A single twig breaks, but the bundle of twigs is strong” —Tecumseh. **Hemi-desmosomes** are half of a desmosome anchored to the ECM, such as the seal between the gingival epithelium and the non-cellular surface of a tooth. One of the many proteins in a desmosome is an integrin. This protein recognizes and binds to proteins in the ECM such as fibronectin. When the integrin protein of a cell connects to fibronectin, this not only anchors the cell’s cytoskeleton to the ECM and anchor the cell in place, the integrin also signals to the inside of the cell, allowing the nucleus to know what type of tissue the cell is located in. Before a cell can migrate to a new location, it must first remove its anchoring junctions. During development, cells migrate to new locations and form new structures. During wound healing, stem cells detach from their neighbors, migrate into the injured area, and begin cell division to create enough cells to fix the injury.

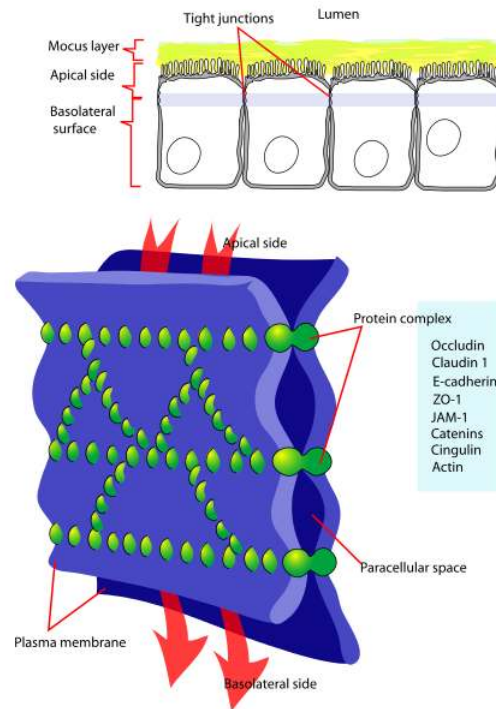


Figure 1.17: Tight junctions. Image credit: Diagram showing a tight junction by Mariana Ruiz LadyofHats is in the Public Domain CC0

Tight junctions

Tight junctions are smaller junctions between cells. Tight junctions completely encircle a cell, and create a water-tight seal between that cell and another cell. This serves to create barriers between one part of the body and another, allowing the cells to regulate what goes across and what does not. This also gives cells **apical-to-basolateral polarity** (or a difference between top and bottom), which is especially important to an epithelium. The apical side of an epithelial cell faces the **lumen** (hollow center) of an organ, while the basolateral side is closest to underlying connective tissue. Proteins synthesized on the rER can be sent to either the apical or basolateral portion of the plasma membrane. Once there, trans-membrane proteins of the apical side of the cell cannot diffuse to the plasma membrane on the basolateral side, because the ring of tight junctions blocks their movement.

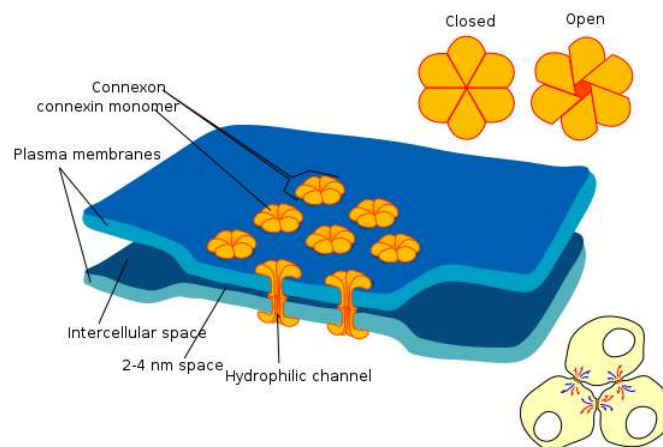


Figure 1.18: Gap junction. Image credit: the diagram shows a cell union called gap junction By Mariana Ruiz LadyofHats is in the Public Domain CC0

Gap junctions

Gap junctions (or connexons) are a group of proteins that form a small passage between cells, and can be opened or closed. This gap allows cells to communicate directly with one another. Because of the way epithelial cells are connected to each other– in a sheet– this communication occurs across a plane. This is one way that cells know their position relative to one of the body’s axes, and is a process called **Planar Cell Polarity** (PCP) (more reading [can be found here](#)). PCP is side-to-side polarity, while apical-to-basolateral polarity is top-to-bottom. PCP allows cells to know what direction they are facing in the body, ensuring that the structures they are forming are not only in the correct location, but in the correct orientation. For instance, this type of signaling allows teeth to form so that a tooth’s lingual side is facing the lingual side, not facing the buccal, mesial or distal sides. Planar cell polarity also helps teeth to erupt straight into the oral cavity, rather than at an angle.

Chapter 0 * Chapter 2

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1.2: Histology Review

- 4 main tissue types (by adult appearance)
 1. epithelia
 2. connective tissue
 3. muscle
 4. nervous
- 3 main tissue types (by embryonic lineage)
 1. ectoderm
 2. mesoderm
 3. endoderm

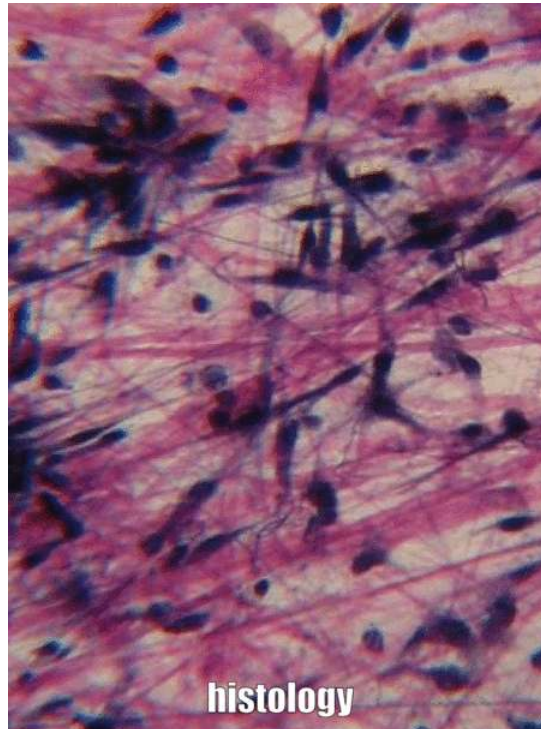


Figure 2.1: Histology of areolar CT, highlighting the cells, collagen fibers, elastic fibers and ground substance.

A short history of histology

The aim of this chapter is to review histology covered in your Anatomy & Physiology classes– but only the tissues that appear in the head and neck, and only the parts that are relevant to this course. This is by no means a comprehensive review. If you need an extra review on basic histology, try these links:

- [An interactive histology flowchart](#)
 - by Laird C. Sheldahl, Ph.D.
- [The Internet Pathology Laboratory for Medical Education](#)
 - at The University of Utah, Eccles Health Sciences Library.
- [Histologyguide.com](#)
 - by T. Clark Brelje, Ph.D. and Robert L. Sorenson, University of Minnesota.

Histology is the study of what tissues look like under a microscope. A **tissue** is a group of cells, all of the same major type, working together to perform a function. Because most cells are transparent, microscopists use of a number of different stains to highlight different parts of cells. Fig 2.1 is an image of a very common staining technique using the dyes **Hematoxylin and Eosin** (H&E). These two stains turn molecules with negative charges blue, and positive charges pink. DNA and RNA take up both colors, which makes nucleuses purple, while most proteins turn pink. Unfortunately, most cells are full of proteins and their cytoplasm

turns pink, but the extra-cellular matrix is also full of protein, which turns pink as well. We are therefore often trying to make sense of a sea of pinks with purple spots. On the plus side, if you purchase a histology coloring book, you only need two crayons.

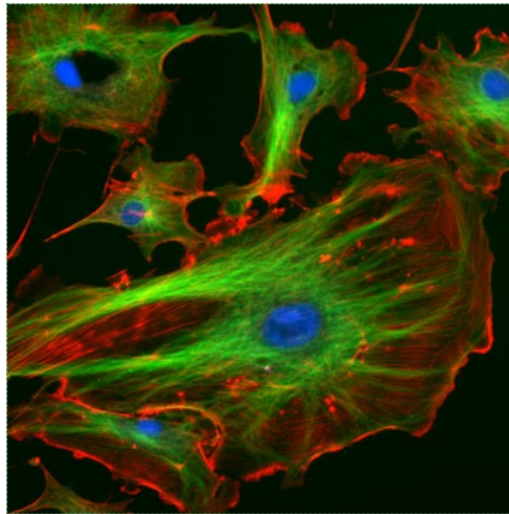


Figure 2.2: Example of a confocal microscope image. Image credit : [Fluorescent cells](#) by [NIH](#) image is in the Public Domain CC0

Imaging

Most of the images you see in histology textbooks use stains such as H&E stains— this type of technology dates back to the 1800s. Microscope technology has improved since then, such as the image in Fig. 2.2, which comes from a confocal microscope. The confocal microscope uses expensive lasers to generate [very beautiful images](#), but relies on stains which are theoretically similar to H&E. Sadly, most textbooks still use the old-fashioned (uglier) images. Since you should be using neither the newer nor the older microscopes in your line of work, we have illustrated most of the histology images in this book. This allows us to focus on the concepts, and spend less time developing the skills necessary to interpret pictures of pink blobs with purple spots. We presume that in the Anatomy and Physiology classes you took to get into this program, you learned to spot the differences between different types of tissues. We review those differences in this chapter. We ask you to keep an eye out for similarities between different tissues (aside from they all look like pinkish blobs with purple spots). If you jump ahead and read the embryology chapters, you'll have a better idea of what to look for, but for now just keep that thought in the back of your head as we cover the major tissues of the human body.

Enough embryology to start learning about histology

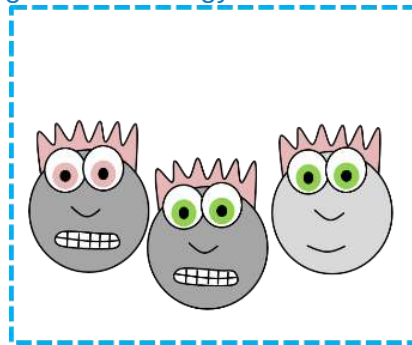


Figure 2.3: One possible way to group cartoon faces, based on a few physical characteristics.

Classification: the old way, and a better way.

Histology categorizes tissues based on what the cells look like in adulthood. If we determined people's families based off what they look like as adults, we might do an OK job of it, or we might make mistakes. For instance, we might lump the cartoon faces in Fig. 2.3 into two families based primarily on hair and eye color. That is how the 4 major tissues types we are going to review are categorized. The major problem with this sort of classification system is it is hard to tell what physical traits are due to nature (genetics, such as eye color) versus nurture (environmental factors, such as hair dye).

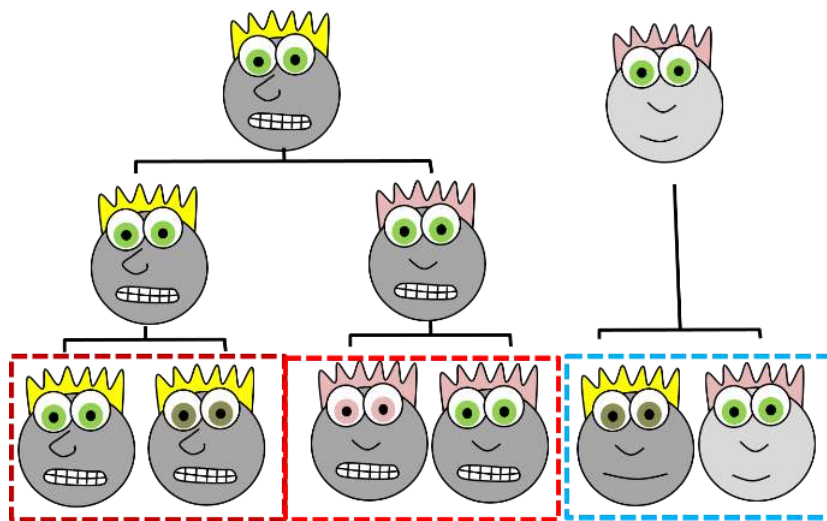


Figure 2.4: Another way to categorize cartoon

faces, based off of lineage data.

When we learn about embryology, however, we see we can do better. We can categorize tissues using cell **lineage** instead, tracing who the adult cells are related to by following which cell they came from during embryogenesis. Early embryologists stained individual cells and followed them through cell division after cell division to see what they became. Others, such as Hans Spemann, moved cells from one place to another and asked whether they still turned into what was expected, based on their new location. When transplanting just a few cells lead to embryos with two complete heads, Spemann concluded that was unexpected. To get a better idea how that happens, science had to wait for the discovery of DNA-storing genetic information for an organism. Then, developmental biologists like Christiane Nüsslein-Volhard were able to determine the mechanisms by which a single embryonic cell became different types of cells in an adult organism—her organism of choice was flies, and for that work she won the Nobel Prize. Unlike earlier histologists and embryologists who named everything after themselves, we get fun names like Bicoid, Dickkopf, Frizzled and [Sonic Hedgehog](#). These scientists followed the lineages of cells, watched what they become, and determined how the cells make decisions as to when and what to become. Rather than use the *appearance* of our cartoon people, if we knew their **lineage** (you might also see the fancier term **ontogeny**), we can classify them into more accurate families, with one sub-family (Fig. 2.4). When you see the word lineage, think family line, where differentiated cells are the youngest generation, and stem cells are their parents and grandparents. Sadly, this is not how histology is taught, so we're going to look at what adult cells look like and classify them based on physical characteristics. When we do, look to see whether two tissues have distinct borders (likely different lineages) or blended borders (often the same lineage).

The 4 main tissue types
Epithelial
Connective Tissue
Muscle
Nervous

Table 2.1: the 4 main tissue types

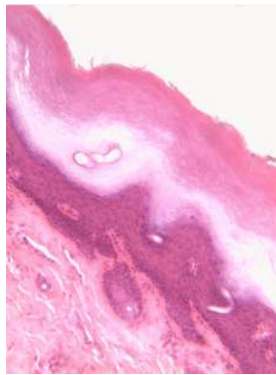


Figure 2.5: Example of an epithelium (with connective tissue below).

Epithelia

Location of epithelia

An **epithelium** is composed of epithelial cells, usually connected to one another in a sheet. There is very little ECM in an epithelium, mostly cells. The cells are anchored together by desmosomes, which holds them in a sheet. Tight junctions ensure that the things that travel from one side of the epithelium to the other pass through the epithelial cells, and therefore must either be lipid-soluble, or be recognized by transport proteins on the apical and basolateral plasma membranes. An epithelium therefore has **apical-to-basolateral polarity**. The apical surface faces the outside of the body, and the basolateral inward, and polarity means the two sides are different. The *outside* can mean the outer surface of the skin, or an inner surfaces, such as lining the oral cavity, stomach, and bladder. Every surface of the human body is an epithelium. Therefore, under the microscope, if you see an empty space, an epithelium borders that space. The inner lining of a sweat duct is an epithelium. The inner lining of a blood vessel is an epithelium. All other tissues are between that epithelium and the epithelium on the outer surface of our body. Even enamel is made by an epithelium, so if anyone tells you enamel is a connective tissue because it is *looks* similar to bone tissue, they haven't studied embryology, and need to be educated. Use words like *ontogeny*.

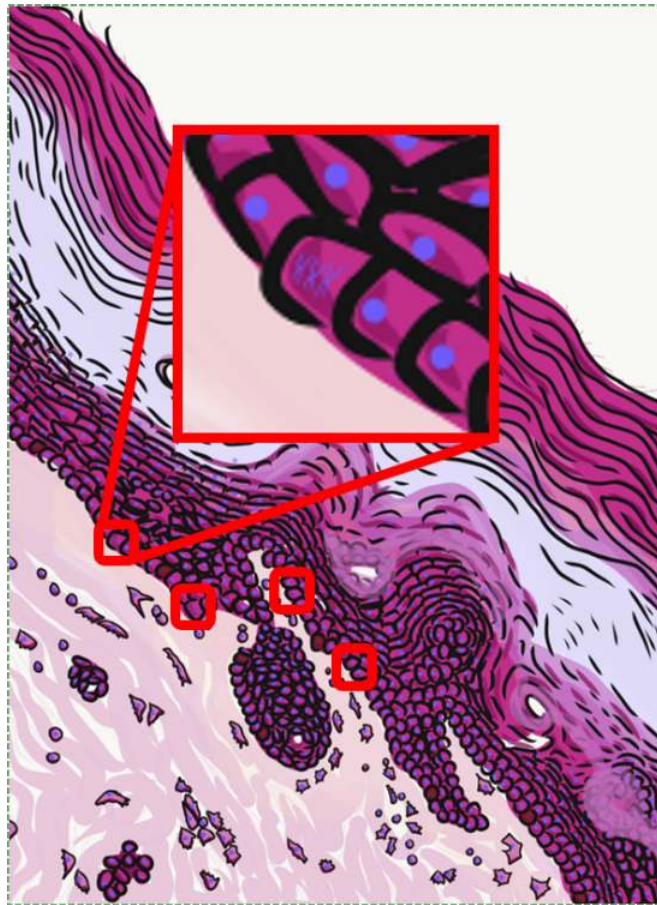


Figure 2.6: Stem cells in the basal layer of a stratified epithelium.

Epithelia are thin

Epithelial tissue is good at healing because it contains numerous stem cells capable of undergoing mitosis (you can tell by the presence of visible chromosomes, rather than chromatin). For thicker epithelia, stem cells reside in the deepest layer. Epithelia cannot be terribly thick, however, because they are **avascular** (without their own blood vessels). Nutrients for an epithelium must diffuse from underlying connective tissue, which is why in Fig 2.6, the epithelial cells on the apical side lack nuclei. They are dead, but still connected to the rest of the epithelium. The down-side to being good at mitosis is it means those cells are a few steps closer to becoming cancerous. Many people regularly expose themselves to high doses of carcinogens found in alcoholic beverages and tobacco (chewing tobacco in particular), which leads to oral cancers. In addition to membranes, glands are made of epithelial cells. Exocrine glands, such as the salivary glands, contain a single layer of epithelial cells, rolled up into tubes called ducts. These ducts bring exocrine secretions to a surface of the body. Endocrine glands, however, are different. They are not arranged in a sheet, but are amorphous clusters of epithelial cells. Endocrine glands aren't avascular, either. Blood vessels grow right into the epithelia of an endocrine gland. This is important because endocrine glands secrete hormones directly into the blood.

Number of layers	Shape of cells
Simple	Squamous
Stratified	Cuboidal
	Columnar

Table 2.2: The basic categorization of most epithelia.

Epithelia are mostly classified based on two criteria. With a few exceptions, epithelia have a name from the first column and a name from the second column of Table 2.2. **Simple epithelia** have just one layer of cells, while **stratified epithelia** have more than

one. **Squamous** means the cells are flat, like fried eggs. **Cuboidal** means square-ish in appearance (cells are 3D, but look 2D under the microscope). **Columnar** means the cells are tall (like columns).

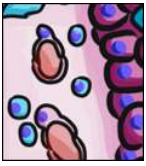


Figure 2.7: Simple squamous epithelia (endothelia) forming the walls of capillaries.

Simple squamous epithelia

If you look at the lining of a capillary, you *might* be able to see the **simple squamous epithelium** that lines the inner surface. These cells, called endothelial cells, are functionally really cool cells, even if they are hard to see. Normally, endothelial cells keep blood inside of a capillary, but they can become damaged, such as during probing, flossing or coughing.

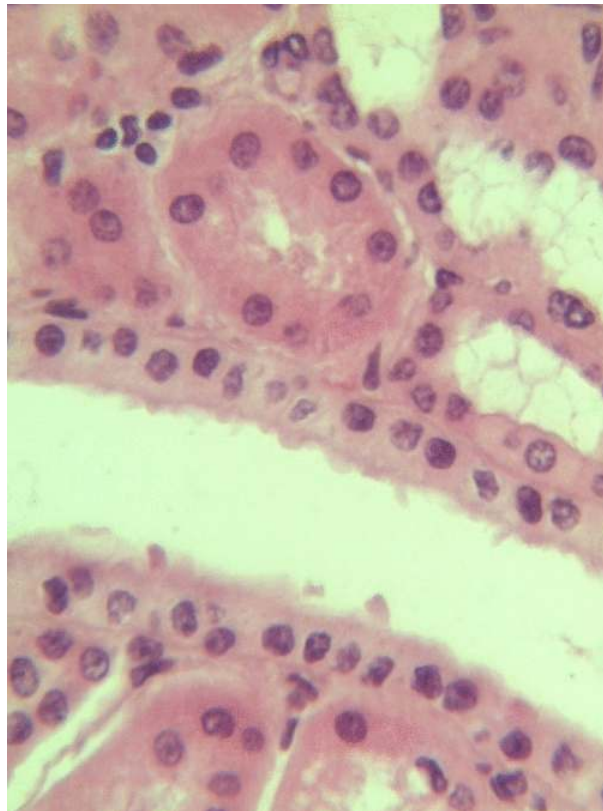


Figure 2.8: Simple cuboidal epithelia, histology and illustration. Legend: cuboidal epithelial cells (pink). underlying connective tissue (light blue) and lumen of tubes (white).

Simple cuboidal epithelia

Simple cuboidal epithelia line most exocrine gland ducts, such as sweat and salivary glands. In Fig 2.8, you should see two ducts coming out towards the camera, and one duct running side-to-side. The lumen of each duct is lined by a simple cuboidal epithelium. The cuboidal cells in the epithelium are connected to their neighbors on the sides by anchoring junctions. Where two ducts get close together, it is two simple cuboidal epithelia, not one stratified one, there are two separate layers of epithelia, with a little ground substance between them. Stratified cuboidal epithelia exist, such as in parts of the parotid salivary gland, but they are rare enough that we can skip them. The capillaries in Fig. 2.7 are lined by flat cells to allow for diffusion of nutrients out of the capillary and waste products in, while salivary ducts in Fig. 2.8 have thicker cells to keep saliva from leaking out and dissolving connective tissues of the face.

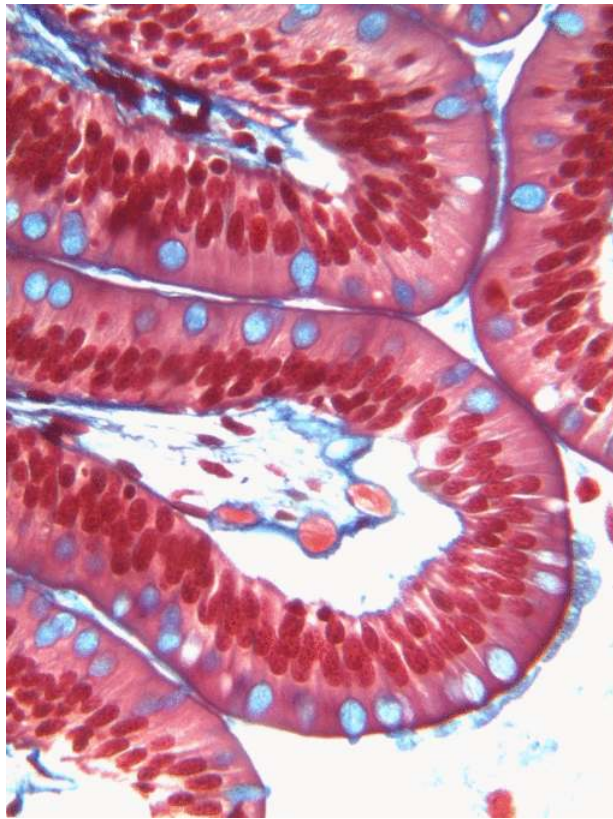


Figure 2.9: Histology of a simple columnar epithelium (highly folded), with illustrations highlighting the columnar cells (pink), goblet cells (blue) and the underlying connective tissue (lighter blue).

Simple columnar epithelia

Simple columnar epithelia are not found in the adult oral cavity, but in an embryo the columnar Inner Enamel Epithelium differentiates into enamel-producing ameloblasts during embryogenesis. The cells' shape isn't important other than it helps histologists to identify these cells in a developing tooth.



Figure 2.10: Lateral

view of bed, showing a cat between the basolateral surface of the comforter and the apical surface of the thin bed sheet. Image credit: “photo” by unknown is in the [Public Domain, CC0](#)

If you are looking at the illustration in Fig 2.9, you may wonder why there are fewer cartoon nucleuses than visible in the histology picture. Did the illustrator mistakenly give you a picture of a stratified columnar epithelium and try to pass it off as simple? No. When we say an epithelium is simple, it means one cell thick from apical-to-basolateral. But when we cut a tissue sample to view under the microscope, the slice may be several cells thick from side-to-side. And when we lay the tissue sample on its side, the cells that were running side-to-side are now sitting on top of one another. We have illustrated what a simple epithelium *should* look like if we had the ideal one-cell-thick slice of tissue. This might be a good time to bring up perspective. Epithelia form flat sheets, and in Fig. 2.9 we are looking at a (folded) sheet in cross section. Imagine slicing through your bed and looking at it from the side view. You could make out the individual, single layers of your bed sheet and comforter. Furthermore, you might note that while each is a single layer, the bed sheet is thin and the comforter is thicker. You could identify the basolateral surface of your bed sheet (the side that touches you when you sleep) and the apical surface (the side close to the comforter). In Fig. 2.10, if there was a second cat on top of the first cat, we could say the layers of cats is stratified. But if the bed sheet was covered in a single layer of cats, side-to-side and front-to-back, the layer of cats would be simple. Under the microscope, we’re doing the same thing, but unfortunately cells are semi-transparent, so it gets messy. When we call this a simple epithelium in Fig. 2.9, that means there is only one pink cell between the white space (here, the lumen of the intestines) and the very light-blue space (the connective tissue, where nutrients can be absorbed into blood vessels). Those cells are thicker than cells we looked at in Fig. 2.8, but there are no columns on top of other columns.

Figure 2.11: Histology and cartoon of a stratified squamous epithelium (and a little connective tissue below).

Stratified squamous epithelia

Stratified squamous epithelia can be found in the outer lining of the skin, and the inner lining of the oral mucosa. While we have different names for the skin and the oral mucosa, if we were to name them based off of embryology, rather than superficial appearance, they would both contain a stratified squamous epithelium. Chapter 3 covers in more detail the epidermis of the skin, which is a keratinized stratified squamous epithelium. Keratin is a tough, water-resistant protein made by the main type of epithelial cell in this tissue, the keratinocyte. The stratified squamous epithelium changes at the vermilion border to less-keratinized, and the stratified squamous epithelium of the oral mucosa is either less or not keratinized. Based on that information, should we classify the vermilion zone as an extension of the labial mucosa, or as part of the skin and lips? Or is there a line in the vermilion zone that divides into a skin half and an oral mucosa half? It’s times like this we could use the advice of an embryologist

to inform us we shouldn't always look for lines in adult tissues, nor define borders between the skin, the vermilion and the oral mucosa based on color. If you change careers and go into proctology, use this same information down there.

Stratified cuboidal, stratified columnar and transitional epithelium

These tissues are not appearing in this textbook.

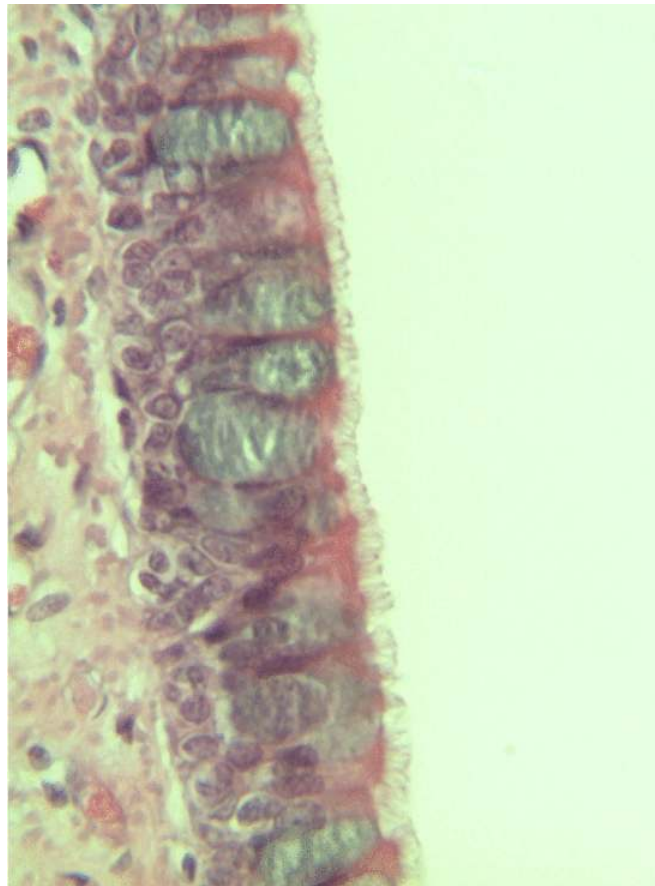


Figure 2.12: Pseudostratified epithelium, histology and cartoon. Order of appearance: epithelial cells, goblet cells, underlying connective tissue.

Pseudo-stratified epithelia

Ciliated pseudo-stratified columnar epithelium, or as many people say, **pseudostratified epithelium**, can be found in upper portions of the respiratory tract, such as lining the nasal cavity and the para-nasal sinuses. This tissue definitely deserves a different name from the stratified squamous epithelia of the skin, labia and oral mucosa. However, it probably didn't need to break the naming rules outlined in Table 2.2. A pseudostratified epithelium has more than one layer, but it is hard to count how many. As a result, someone named this tissue *pseudo*-stratified. The big fat blue cells in Fig. 2.12, which don't have cilia, are **goblet cells**. These cells produce **mucus**. Goblet cells synthesize mucous proteins within their rER and secrete them, and when they attract water, they become mucus. This is very similar to ground substance, however mucus is secreted out of the body, ground substance is a part of the body. The blue coloring of the goblet cells tells us that mucous proteins do not carry the same ionic charges as most proteins found within the epithelial cells of the pseudo-stratified epithelium. Due to this difference in color, they are classified as a distinct cell— a unicellular gland found within the pseudo-stratified epithelium. But what does our friend the embryologist say? Well, the columnar cells can't become goblet cells, and the goblet cells can't become columnar cells, but they do share a common ancestor (or stem cell), so we probably should consider the goblet cells a part of the pseudo-stratified epithelium. Does it matter? No, but questions like this matter later in the book, it's good to start practicing now.

Connective tissue (CT)

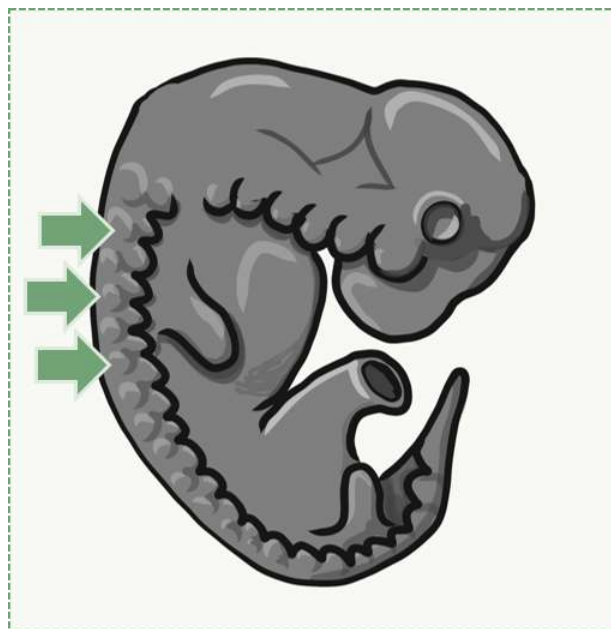


Figure 2.13: Somites of an early stage embryo, from which connective (and muscle) tissue develop

General characteristics of connective tissue

Many textbooks tell you **connective tissue** connects 2 other tissues. That's like saying your smart-phone connects you to other people. It is technically true, but glosses over the fact these could be people you know, people you don't know, or people you don't want to know. It ignores the fact you could be communicating synchronously with people on the other side of the world, communicating asynchronously over email, accessing information from the near sum-of-all human knowledge (the internet), or reading the sophomoric rant of some disgruntled knuckle-dragger who, years ago, posted a rude comment on a YouTube video recording of a histology class lecture (also the internet). So far, we've tried to keep you, the reader, safe from extraneous information about histology that is not be relevant to your career. Here we go into *more* detail than your average undergraduate histology class. Connective tissues come from segments of tissue in an embryo, found between two layers of epithelium, called somites (Fig. 2.13). Because of their shared lineage, connective tissues share a number of common features. The average connective tissue has few cells, and is mostly composed of Extra-Cellular Matrix. That matrix includes glycoproteins and polysaccharides made by the cells, which attract water to form the gel matrix of ground substance. It also includes more visible fibers. Some important ground substance molecules covered earlier are Fibronectin and Hyaluronic Acid. The three main types of visible fibers are collagen, reticular and elastic fibers. Connective tissues are typically highly **vascular**, meaning they contain blood vessels. There are a number of different cell types found within a connective tissue. The stem cells are **mesenchymal stem cells** (MSCs). Mesenchymal stem cells are extremely important cells (here is further reading), and since you can find them in adult tissues they are called a type of **adult stem cell**. These cells are capable of undergoing mitosis to produce more adult stem cells, which can differentiate into fibroblasts, lipoblasts, chondroblasts, osteoblasts, hemocytoblasts, myoblasts and neuroblasts. Thus, they form most connective tissues, including bone, cartilage, blood (both red and white blood cells), plus muscle tissue. That's pretty much everything except epithelia, which mesenchymal stem cells can form as well after going through a transition.

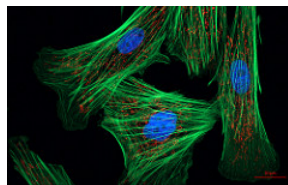


Figure 2.14: Fibroblasts, as seen under a confocal microscope. Image credit: Indian Muntjac fibroblast cells By Michael W. Davidson is licensed CC BY 2.0

In a basic connective tissue, mesenchymal stem cells divide and differentiate into **fibroblasts**, the cells that blast out the fibers and ground substance found in ECM. There may be other cells found in a connective tissue, including adipocytes, red and white blood cells, or other cells that have emigrated from their tissue of origin. In a mature connective tissue, the fibroblasts are sometimes called fibrocytes, which fits with our regular nomenclature for a cell going through stages of differentiation. More frequently,

however, they are called fibroblasts and we don't worry about whether they are actively blasting out fibers at the time or taking a rest.

Figure 2.15: Mesenchyme, the generic embryonic connective tissue, doesn't have any specialization. Image credit: Human Mesenchyme By Jpogi at Wikipedia is in the Public Domain CC0

Mesenchyme

Mesenchyme is the first type of connective tissue an embryo makes. It differentiates into other connective tissues. It is an embryonic tissue that has yet to decide what it will become when it grows up. It is composed mainly of mesenchymal stem cells and **mucous** ground substance (*mucous* is an adjective to describe loose ECM, while *mucus* is a substance excreted from the body). There is a special type of mesenchyme important to face and tooth development, called **neuro-mesenchyme**. The lineage of neuro-mesenchyme is not from mesoderm like most connective tissues, but from a special type of neuronal cell called a Neural Crest cell. Neuro-mesenchyme forms special connective tissues we have yet to mention: dentin, cementum and the periodontal ligament.

Figure 2.16: Areolar connective tissue, histology and cartoon.

Areolar connective tissue

Areolar connective tissue is the quintessential connective tissue, or the most boring depending on how you look at it. It contains a little of everything other connective tissues might have: cells, ground substance, and all 3 fibers. Because it has a good amount of ground substance, it is an ideal tissue to occupy places where blood vessels might need space to grow in the future. Hence areolar CT is found in regions that are highly vascular. This is why you can find a small layer of areolar connective tissue directly underneath nearly every epithelium, including the stratified squamous epithelium of the various types of oral mucosa. Ground substance also absorbs water and swells in size, which is why the areolar connective tissue of the nasal cavity is also referred to as erectile tissue.

Figure 2.17: Dense irregular connective tissue, histology and cartoon

Dense irregular connective tissue

Underneath the areolar CT of the oral mucosa is **dense irregular connective tissue**. Fibroblasts make the bulk of the tissue: collagen fibers, the strongest of the 3 fiber types. In a dense irregular CT, the collagen fibers point in all directions. This makes this tissue particularly strong in all directions. In Fig. 2.17, most of the pink is extracellular collagen fibers, and the white space ground substance. You can find dense irregular CT (along with its buddy, areolar CT) in the dermis of the skin. Why in both places, you ask? Lineage.

Figure 2.18: Histology of dense regular connective tissue.

Dense regular connective tissue

Like dense irregular CT, **dense regular connective tissue** is mostly collagen fibers, only the fibers run parallel to one another. That makes this tissue strong in one direction. You mostly find dense regular CT between muscle and bone, or between bone and bone. The only place in this book where we find dense regular CT is the periodontal ligament, between bone and cementum. You might be thinking the collagen fibers look wavy, not parallel. When someone sliced this section of a tendon, they sliced through the tissue using a thin blade. As a blade cuts the tissue, the tissue moves, and so the blade pushes fibers in one direction, then pulls them back, and pushes again as the person slices as thin of a slice as they can. For better results, use an expensive device that freezes or embeds soft tissues in wax, such as a microtome, cryotome, or vibratome. We bring this up because many textbooks suggest histology is a nearly-perfect representation of what is found in the human body, when in reality there are **artifacts**, or errors, that histologists have learned to ignore when we see them. We have chosen to illustrate many of the images in this book to avoid discussion of artifacts where possible, and focus on concepts relevant to dental hygiene.

Figure 2.19: Adipose connective tissue, histology and cartoon

Adipose connective tissue

Adipose tissue is a type of connective tissue. It is special in that it has very little ECM. It contains mostly **adipocytes**, the mature cells that store triglycerides. It also contains mesenchymal stem cells, which if a person needs more adipose tissue, divide and differentiate into more adipocytes. Technically, Mesenchymal Stem Cells differentiate into a lipoblast first, but this is only important for the sake of staying consistent with the naming system. Maybe if they had named the cells adipoblast and adipocyte,

we'd use the -blast name more frequently, but someone messed that up. Many textbooks place adipose tissue next to areolar connective tissue and categorize them both "loose", on account of their low number of visible fibers. That's fine if you like categories based on appearances, but you don't need to call either of them "loose". It is descriptive and does not correspond to anything embryological. We find adipose tissue in many places throughout the body, either between two other tissues, or embedded within another tissue (such as the adipose you find in bacon). On the other hand, areolar connective tissue is found next to dense irregular connective tissue, which is why we've deviated from the grouping found in most textbooks.

Figure 2.20: Hyaline cartilage, histology and illustration. Order of appearance: lacunae, chondrocytes, ground substance, perichondrium, surrounding dense connective tissue.

Cartilage connective tissue

Cartilage connective tissue comes in three basic flavors: hyaline cartilage, fibrocartilage and elastic cartilage. The hallmark feature of cartilage connective tissue is its ample amount of ground substance. **Chondroblasts** secrete glycosaminoglycans and glycoproteins, such as Hyaluronic Acid, which attract water, forming a very dense but slippery gel. The ECM of cartilage contains collagen— there is more collagen in the fibrocartilage of the temporo-mandibular joint, whereas the elastic cartilage of the epiglottis has more elastic fibers. After secreting ECM, the chondroblasts differentiate into **chondrocytes**, and reside within the tissue in spaces called **lacunae** (lakes). The outer edges of cartilage are a thin layer of parallel collagen fibers known as the perichondrium. If you look at Fig 2.20, the perichondrium appears like a layer of dense regular connective tissue (it is, it just has a second name), and the edge of the perichondrium blends into dense irregular connective tissue. The reason the cartilage blends into dense irregular connective tissue is they share the same lineage: chondroblasts and fibroblasts both differentiate from mesenchymal stem cells.

Figure 2.21: Bone connective tissue, histology and cartoon highlighting osteocytes and lamellae of ECM.

Bone connective tissue

Bone tissue (or osseous tissue) is the last type of connective tissue we cover in detail. Because of its similarity to dentin, cementum and enamel, we cover bone tissue in a fair amount of detail. Like any connective tissue, bone tissue starts as mesenchymal stem cells, which differentiate into an intermediate stem cell called an osteo-chondro-progenitor cell, which can in turn decide to adopt an osteoblast or a chondroblast fate, depending on what signals it receives. A stem cell that has adopted the osteoblast fate (and denied the chondroblast fate) would be an **osteo-progenitor cell**. These osteo-progenitor cells are stem cells within the connective tissues surrounding bones, the **periosteum** (superficial) and **endosteum** (deep). When stimulated, they undergo mitosis, and one daughter differentiates into an osteoblast. An **osteoblast** is the cell that secretes the ECM that makes up the bulk of bone tissue. Osteoblasts secrete collagen fibers and ground substance, which later mineralizes, trapping the osteoblasts inside of a **lacuna** (a small space within dense ECM). At this point, they differentiate into **osteocytes** and maintain the bone tissue. Next, a new set of osteoblasts lay down another layer of bone tissue around the previous one. This creates either the concentric layers of bone tissue found in the osteons of **compact bone**, or the thinner spiral layers of bone tissue found in the trabeculae of **spongy bone**.

Figure 2.22: Three (of four) major cell types found in bone tissue and their lineage. MSC = mesenchymal stem cell, OCP = osteo-chondro progenitor cell.

Figure 2.23: Calcium hydroxyapatite crystal structure of bone ECM. Legend: Red/orange = phosphate, white = calcium ions, yellow = hydroxide ions. Image credit: Fluorapatite by Von Benjah-bmm27 in the Public Domain CC0

The extra-cellular matrix of bone tissue is roughly 1/3 collagen fibers (organic protein) and 2/3 mineral (inorganic). The mineral component is a mixture of positive-charged Ca^{2+} ions, which react with negative-charged phosphate (PO_4^{3-}) to form a hard,

crystalline matrix. Because most cells are full of phosphate, Ca^{2+} levels in the cytoplasm must always be kept very low, so if a cell like an osteoblast wishes to store Ca^{2+} , it does so within its smooth Endoplasmic Reticulum. The $\text{Ca}^{2+}\text{PO}_4^{3-}$ further react with water (or more specifically, hydroxide ions OH^-) and small amounts of fluoride (F^-) to form a crystal named **calcium hydroxyapatite**. Dentin, enamel and cementum have ECM very similar to bone tissue, only with varying amounts of collagen versus mineral components. Collagen fibers run parallel within one layer of bone tissue, and 90° in the next layer. While the mineral component of bone and tooth tissues provides strength, the collagen provides the ability to bend or flex, reducing the chances the tissue will shear off under stress. Collagen fibers, therefore, have a function similar to the rebar in reinforced concrete. The lower percentages of collagen found in enamel makes it harder and more resistant to caries than dentin or cementum, but still susceptible to fracture if enough force is applied.

Figure 2.24: Collagen fibers of a tendon are continuous with the periosteum and Sharpey's fibers of bone tissue.

Bones are surrounded by a layer of dense regular connective tissue called the periosteum. This tissue is a continuation (not a separate structure) of the same tissue of tendons and ligaments. The periosteum also contains osteo-progenitor cells, the semi-differentiated cells that came from Mesenchymal Stem Cells, as well as osteoblasts and osteoclasts (coming up). Collagen fiber bundles from this layer named **Sharpey's fibers** penetrate the superficial layers of compact bone. This makes a very strong connection between bone tissue and tendons or ligaments.

Figure 2.25: Osteoclasts demineralize bone tissue.

Another important cell found in the periosteum and endosteum is the **osteoclast**. This cell is not derived from the osteo-chondro-progenitor cell that gives rise to osteoblasts and osteocytes. The lineage of an osteoclast traces to the bone marrow, from a stem cell that also gives rise to red and white blood cells. An osteoclast is a close relative of a white blood cell called a macrophage, more than it is to bone cells. Osteoclasts demineralize bone tissue, releasing Ca^{2+} into the bloodstream. This is important because muscle and nervous tissue cannot function without Ca^{2+} , which our diet cannot provide continuously. Hence, bone tissue can be thought of as a calcium storage organ.

Figure 2.26: The bone remodeling unit: osteoblasts plus osteoclasts. Image credit: Own work By Shandristhe Azylean is licensed under CC BY-SA 3.0

Osteoclast activity is important during the exfoliation of teeth, as bone tissue is removed to loosen the connection between bone and tooth. Osteoclasts activity is also key to the mechanism by which orthodontia works. Lastly, osteoclasts play an important role in maintaining bone health— this may seem counter-intuitive, because osteoclasts destroy bone tissue. Bone tissue is constantly being repaired by a group of osteoblasts and osteoclasts working together, known as a **remodeling unit**. Because compact bone tissue is dense, there is little room for cells to work. Osteocytes can repair small amounts of damage, but larger amounts of damage would build up over time. To prevent this, remodeling units constantly work to remove bone tissue and replace it with fresh bone tissue. The remodeling units cannot find damaged bone tissue, they simply keep removing and replacing bone tissue. Physical stress on bone tissue causes osteoblasts to work a bit harder, which leads to increased bone density. Loss of osteoblast-stimulating hormones (such as estrogen) can cause osteoblasts to work more slowly than osteoclasts, which can lower bone density and potentially lead to [osteoporosis](#).

Figure 2.27: The basic steps of endochondral ossification.

Bones form either by **endochondral ossification**, or by **intra-membranous ossification**. The two processes are similar, the major difference is that endochondral ossification begins with a hyaline cartilage model, whereas intra-membranous ossification begins with a dense connective tissue model. Most of the skull develops by intra-membranous ossification, except the mandible and maxilla, which use both. Because of their similarity, we cover only endochondral ossification.

Figure 2.28: Blood vessels and nerves develop first, and are later surrounded by bone tissue. Image credit: "Dissected skull, Maison Tramond model, Paris, nineteenth century" by Centre for Research Collections University of Edinburgh is licensed under CC BY-SA 2.0 Most of the skeleton starts off not as bones, but as cartilage. Cartilage is typically avascular, but when chondrocytes receive the correct signals, blood vessels grow into the ground substance. The growth of new blood vessels into a tissue is called **angiogenesis**. Angiogenesis provides the pathway that mesenchymal stem cells use to migrate into the central area, where they differentiate into osteoblasts. After that, osteoblasts replace cartilage tissue with spongy bone tissue. This first site of ossification is named the *primary ossification center*. As remodeling occurs at the primary ossification center, blood vessels grow into the epiphyses and begin the same process at what are named the *secondary ossification centers*. Ultimately, most of the cartilage is replaced, except in two important places. First, cartilage may remain between the primary and secondary ossification centers, leaving growth plates (such as in bones of the arms and legs). Cartilage also remains at the ends, providing articular cartilage found in synovial joints, such as the temporo-mandibular joint. Intra-membranous ossification, because it begins with a dense connective tissue, leaves fontanelles (soft-spots) between bones until ossification is complete, rather than cartilaginous connections.

Figure 2.29: The basic steps of bone repair. Note the similarity to endochondral ossification.

When bone tissue is injured, it goes through a healing process similar to endochondral ossification. Perhaps you remember mention of wound healing recapitulating development? Here is an example of an adult tissue re-using the same mechanism to heal that it used during embryogenesis to grow in the first place. Similar to an injury to the skin, bone tissue first goes through an inflammatory process, and damaged blood vessels form clots. External blood clots are known as scabs, but inside the body they are named a **hematoma**. A blood clot contains an ECM fiber named **fibrin** that acts as a scaffold. This scaffold allows blood vessels to grow into the area, by angiogenesis. This in turn allows mesenchymal stem cells to migrate into the injured area, leave the blood and migrate along the scaffold, and differentiate into chondrocytes. The chondrocytes replace the fibrous hematoma with fibrocartilage—this cartilage step is really the only step that does not have a **homologous** step (a shared step) in wound repair in the skin. Unlike repair of the skin, bone tissue growth needs a cartilage model first. With this new cartilage scaffold, more mesenchymal stem cells migrate and differentiate into osteoblasts, and replace the cartilage with bone tissue. Later, spongy bone tissue is remodeled so that it roughly matches the original compact and spongy layers. One difference can be seen afterwards, the former site of injury is a little bit thicker after it is repaired—similar to the way skin tissue can be made stronger after an injury because the collagen fibers in scar tissue have more covalent cross-links than in dense irregular CT.

Figure 2.30: Artificial scaffolds help mesenchymal stem cells migrate into an injured area, differentiate into osteoblasts, and produce bone tissue. Image credit: Alloplastic particulate graft by Coronation Dental Specialty Group is licensed CC BY SA 3.0

Knowing the steps of endochondral ossification and bone fracture repair are important in instances where stimulating or mimicking the natural healing process can reduce the need for surgeries and implants. For instance, patients may lose a significant amount of bone tissue with prolonged periodontitis. If enough bone tissue is lost, a surgeon may use a bone tissue graft to replace it. Bone tissue from a cadaver can be ground up, destroying the cells but leaving the collagen and minerals. Other sources may include a patient's own bone tissue (a full set of ribs or fibulas is less important than an intact set of jaw bones and teeth). The harvested ECM is spread into the injured site, where it acts as a scaffold, not as a replacement. The patient's mesenchymal stem cells migrate through the scaffold and begin the healing process, which can proceed quickly because all the raw materials are in close proximity. The grafted scaffolding does not have to come from actual bone tissue. It can be [ground-up dentin](#), fibrin, or synthetic polymers printed using a 3-D printer. The scaffolds, synthetic or biological, are ultimately replaced, which is why you may see this referred to as Guided or Resorbable Tissue Regeneration. Later we learn a scaffold is just one part of the healing process, there are important signals that can be used to guide mesenchymal stem cell migration, division and differentiation. If you wish to know more, We have a short [YouTube video](#) that covers this new technology in more detail, aimed at 200-level Anatomy and Physiology students.

Figure 2.31: Mineralized tissues first evolved in the ectoderm (similar to enamel and were later produced by mesoderm (such as bone). Image credit: "The origin of bone." by Darja Obradovic Wagner and Per Aspenberg is licensed under [CC BY-NC-SA 4.0](#)

Lastly, it is interesting to consider, from an evolutionary viewpoint, [the lineage of the four mineralized tissues](#) (bone, enamel, dentin and cementum) in humans and other vertebrates. Did one evolve first, and the others modified from the parent tissue? Fig. 2.31 provides a hint. We consider this question of lineage from a developmental viewpoint, in chapters 8-11.

Figure 2.32: Hypothetical comparison between blood connective tissue and reticular connective tissue.

Blood, Reticular, and Lymph Connective Tissue

There are other connective tissues covered in histology classes, here we cover them briefly. **Blood** is a type of connective tissue, composed of cells (red blood cells, mostly), liquid ground substance (blood plasma) and fibers (fibrin). **Lymph** is primarily white blood cells, plus liquid ground substance. **Reticular connective tissue** contains blood cells, liquid ground substance and reticular fibers. It can be found in lymphatic organs where blood cells tend to rest rather than flow, such as lymph nodes.

Figure 2.33: Scar tissue in the heart after a myocardial

infarct. Image credit: “Histopathology of dense fibrous scar replacing myocyte loss in myocardial infarction” by [Katarzyna Michaud et al](#) is licensed under [CC BY-SA 4.0](#)

There are other forms of connective tissue that aren’t typically covered in 200-level histology classes, we cover one of them: **scar tissue**. Scar tissue is made by fibroblasts following an injury. This occurs after an injury to any tissue that is large enough that it cannot be replaced quickly by stem cells. One example is sclerotic dentin. Scar tissue is very similar to dense regular connective tissue, except the collagen fibers in scar tissue are highly cross-linked. This makes scar tissue very strong, but also reduces its mobility and capacity to be remodeled. Scar tissue can be formed wherever there are large injuries to an organ– fibroblasts can often fill up the space more quickly than waiting for regeneration to occur. Once formed, scar tissue is difficult to remodel, so it is more-or-less permanent.

Muscle tissue

Figure 2.34: Somites, from which connective tissue and muscle tissue develop.

General characteristics of muscle tissue

Muscle tissue, like connective tissue, is derived from somites. The stem cell that differentiates into one of the several types of muscle cells is called a **myo-satellite cell**. Given the correct set of extra-cellular signals, myo-satellite cells differentiate into skeletal, smooth or cardiac muscle cells. Skeletal muscle cells are long multi-nucleate cells that were once hundreds of **myoblasts** that fuse together. Like a fibroblast, a myoblast is stem cell that has undergone some differentiation, enough to limit its fate, and is responsible for building the adult tissue. In muscle tissue, myoblasts create protein fibers that are mostly intra-cellular (such as actin thin-filaments and myosin thick filaments). After fusing with other myoblasts, the adult muscle cells are often confusingly referred to as muscle fibers, but they are definitely cells, not molecules like the protein and glycoprotein fibers found within the ECM of connective tissue. Thus, they can also be called **myocytes**. In fact, they aren’t the only multi-nucleate cell you read about in this chapter, is perplexing as to why this cell often receives a special suffix, while osteoclast cells do not. In adult muscle tissue, myo-satellite cells remain, ready to undergo mitosis and fuse to form larger muscle cells in response to exercise or injury. We see skeletal muscle in the tongue and underneath regions of oral mucosa, but we do not cover smooth or cardiac muscle tissue.

Figure 2.35: Muscles (organs) contain muscle tissue and dense regular connective tissue.

Muscle tissue is composed primarily of muscle cells, connected by collagen fibers. Unlike connective tissue cells, which may bind to collagen or other ECM proteins for migration using trans-membrane linkers such as integrins, muscle cells anchor in place to the ECM. Muscle cells express different linker proteins to do this, such as the protein Dystrophin. The layers of collagen are thin between cells, and thicker on the outer surface of a muscle. These layers of collagen fibers ultimately become the dense regular connective tissue of a tendon. It is often difficult to determine where muscle tissue ends and connective tissue begins, because of the collagen fibers in both tissues. By extension, it is also difficult to tell where the tendon ends and the periosteum begins. Some histologists draw lines between these tissues, but the junction is better described as a blending. This is because both muscle tissue and connective tissue have the same lineage, from the mesenchyme of somites. For people studying musculo-skeletal diseases like

Muscular Dystrophy, there is interest in identifying the instructions that guide mesenchymal stem cells to differentiate into myo-satellite cells— especially considering the larger number of mesenchymal stem cells in the bone marrow compared to myo-satellite cells in the muscle of a child or adult. If novel treatments result from this research, it could make **dental hygiene procedures for patients with Muscular Dystrophy** much simpler.

Nervous tissue

Figure 2.36: Confocal image of neural tissue, including neurons and glial cells. Image credit: Desert rose by Tarzerind is licensed under CC By SA 4.0

General characteristics of neural tissue

Shown to the left is a confocal image of **neural tissue**. What I like about this image, and is often absent from images of neural tissue, is that this one shows all of the cells. The typical image of neural tissue uses stains to highlight **neurons**—the cells specialized at conducting electricity— leaving out the **glia**—the majority of cells in the nervous system that do everything else, including guide neurons connections and change the strength of those connections when we learn. What you should see at this time is neural tissue is composed primarily of cells, and has very little ECM. While the brain especially has a huge number of blood vessels inside of it, technically they do not run within the neural tissue. Instead, there is a thin blood-brain-barrier separating the brain from the blood vessels. Normally, we wouldn't call neural tissue avascular, we do so now only to make a point: neural tissue shares a lot in common with epithelial tissue. By now, we hope you have guessed why: neural and epithelial tissues share the same lineage.

3 major embryonic tissues

Figure 2.37: Animated illustration of gastrulation.

Gastrulation

When an embryo implants in the uterus, it is a hollow ball of identical, omnipotent stem cells that resemble a simple cuboidal epithelium. But soon, some of these epithelial cells migrate inwards, in a very important process called gastrulation. At this stage, we have two distinct layers of cells. The layer on the outside becomes the epidermis, oral mucosa and the central nervous system. The layer one on the inside becomes the lining the gut and other hollow organs. Next, some cells from the outer layer change. They lose contacts with neighboring cells, and migrate into the middle. As this happens, they stop looking like epithelial cells and begin to look like mesenchymal cells. This produces a third layer of cells in an embryo. The three layers are listed below, along with what they form in the adult:

3 embryonic layers	Cell fate
Ectoderm	Epithelium of skin and oral mucosa, neural tissue
Mesoderm	Connective and muscle tissue
Endoderm	Epithelial lining of hollow organs

Table 2.3: the three embryonic tissues We have now sneakily introduced you to two important terms used in development. The first is cell lineage, which means where a cell came from in the past, and now **cell fate**, which means what that cell *can* become in the future. If we didn't think your license exam would include questions on the four major tissue types, we would stick with the three embryonic tissues, with neural being a sub-family of epithelia (you'll see why later). We'd have put connective and muscle tissue within the same lineage, rather than separate them. But we can see why academia hasn't changed the way it teaches histology, the confusion that comes with change would not be worth the benefits we'd gain by grouping cells by lineage rather than appearance. But this, we think, is more than enough basic histology for a dental hygienist, it is time to move on to the histology of the oral cavity.

Chapter 1 * Chapter 3

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1.3: Histology of the Oral Mucosa

- Overview
- Skin histology
- Oral mucosa histology:
 - Lining mucosa
 - Masticatory mucosa
 - Dento-gingival mucosa
 - Specialized mucosa
- Clinical applications of:
 - Lining & masticatory histology
 - Dento-gingival histology
 - Specialized histology

Chapter overview

We start this chapter reviewing the histology of the skin. The different regions of oral mucosa are similar to the skin because they share the same lineage: ectoderm and mesoderm. There are a few small differences. One unfortunate difference is the names we use for the skin are not the same names we use for oral mucosa. Otherwise, it is faster to focus primarily on the small differences, there is no need to cover the histology of the epidermis or dermis each time. In subsequent chapters on the teeth, pay attention to how the lineage of tooth tissues is different from that of oral mucosa, as well as to how their basic pattern is similar.

For more practice with histology of the oral mucosa (and more), websites worth visiting are:

- [The oral histology lab](#)
 - by Brian R. MacPherson, Ph.D. and James G. Tieman, Ed.D. at the University of Kentucky College of Medicine.
- [The virtual oral histology lab](#)
 - By Roger Shore at the School of Dentistry, University of Leeds



Figure 3.1: The three major layers of the skin, and the major tissue types in each.

Histology of the skin – for comparison

The skin and the oral mucosa share a lot in common because of their shared lineage from ectoderm and mesoderm. Both are composed of a stratified squamous epithelium, just deep to that areolar CT, followed by dense irregular CT. Unfortunately, parts of

the skin and oral mucosa receive different names and are classified differently based on their location. That means you have more names to memorize... Boo!

The **dermis** is the connective tissue layer of the skin. It is composed in part of a layer of dense irregular CT, the old-fashioned name for which is the reticular layer of the dermis. The other part of the dermis is a layer of areolar CT, which has an old-fashioned name, too the papillary layer of the dermis. It received that name for having finger-like **dermal papillae** on the apical surface, compared to the smoother transition between the papillary and the reticular layer. In Fig 58, the upward-pointing dermal papillae meet downward-pointing **rete pegs** of the epidermis. The **epidermis** is composed of stratified squamous epithelial tissue. The dermal papillae of the dermis meet the rete pegs of the epidermis like inter-meshed fingers from two hands, which makes for a strong connection between epidermis and dermis. Some regions of the oral cavity won't require such a strong connection, and the rete pegs and dermal papillae are smaller or absent. Note that the border between epidermis and dermis is distinct, whereas the border between the reticular and papillary layers of the dermis is blended. That is because the epidermis is derived from the ectoderm, while the two layers of the dermis are derived from the mesoderm.

The epidermis of the skin is highly **keratinized**. The epithelial cells make a large protein called **keratin** (or more accurately, **keratins**, as there are over 50 genes for slightly different keratin molecules), which is similar to collagen, except keratin is not secreted. These long fibrous proteins accumulate within **keratinocytes**, the principle cell of a stratified squamous epithelium. Epithelial stem cells in the basal layer of the epidermis give rise to new keratinocytes, and as keratinocytes mature, they are pushed towards the apical surface. As the keratinocytes move superficially, they fill up with keratin, and receive fewer nutrients (an epithelium is avascular), until ultimately the keratinocytes at the surface are dead and completely full of keratin. The keratin fibers are cross-linked to each other and to desmosomes, which anchor the dead cells together, making for a very tough and water-resistant barrier. Keratinized regions of oral epithelium are located where there is a lot of abrasion. In the rest of the oral cavity, we want moisture, so there is less or no keratinization.

Skin color

Pigment	Color	Source	Location
Melanin	red or brown/black	melanocytes	epidermis/oral mucosa
Carotene	orange-yellow	diet (plants)	dermis/sub-mucosa
Hemoglobin	Red-maroon	blood	Dermis/sub-mucosa

Table 3.1: The three major skin pigments.

There are 3 major pigments (a molecule that absorbs certain frequencies of visible light) that contribute to skin color, listed in Table 3.1. Keratin has no color, but it can obscure the visibility of the deeper pigment hemoglobin. **Melanin** levels vary naturally in the skin. Everyone is born with roughly the same number of **melanocytes**, the cells that synthesize the melanin pigment. Melanocytes are derived from neural crest cells that migrate from the CNS to the deepest layer of the epidermis. They are not anchored to keratinocytes, and their shape more closely resembles neurons or glia than they do epithelial cells. Melanocytes can produce a lighter form of melanin (pheomelanin) or a darker form (eumelanin). After synthesizing melanin, melanocytes transfer it to keratinocytes inside of vesicles (melanosomes). The amount and type of melanin produced has a baseline rate set at birth—this is not regulated by genes we inherit from our parents, but by more complex epigenetic factors.

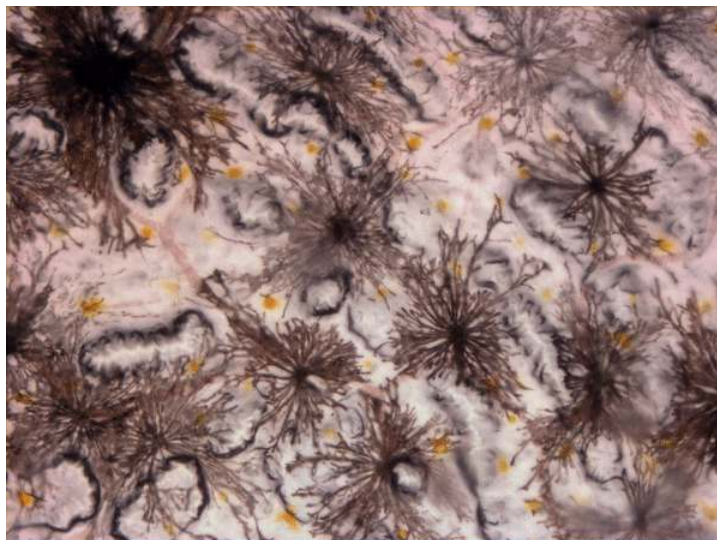


Figure 3.2: Melanocytes in some animals, such as these from a fish, move melanosomes to quickly change skin color (camouflage). Human melanocytes work more slowly, but similarly. Image credit: “7x speed timelapse video of fish melanophores responding to 200uM adrenaline” by Zephyris is licensed under CC BY-SA 3.0 / converted to gif

Environmental changes trigger melanocytes to change the levels of melanin they produce, and these changes illustrate several functions of melanin. The most obvious function of melanin is to absorb UV light. When UV light causes DNA damage to keratinocytes, this induces them to secrete a hormone (Melanocyte Stimulating Factor) which activates nearby melanocytes to produce more melanin. Extra melanin, when deposited into new keratinocytes, reduces damage to DNA and helps prevent skin cancer. Melanin (in the epidermis) also protects folic acid levels in the blood (the dermis), which can be destroyed by UV light. Folic acid is required for cell division, low levels during pregnancy can lead to congenital malformations of the neural tube (spina bifida).

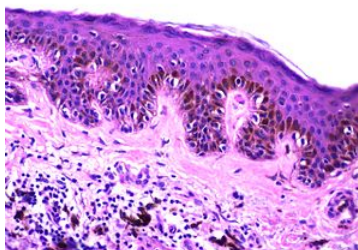


Figure 3.3: Histology of focal melanin in a melanocytic nevus (mole). Note the brown color of melanin is inherent, not added to the tissue like the pink and purple H&E stains. Image credit: “Lentigo simplex or simple lentigo or lentiginous melanocytic naevus” by Leszek Woźniak & Krzysztof W. Zieliński is licensed under CC BY-SA 3.0

Melanin may also be present within the oral cavity, despite much lower levels of exposure to UV light. This can be explained because of two more functions of the molecule melanin. Melanin protects tissues against damage from abrasion. Pregnancy leads to an increase in the amount of melanin in the areolas and labia minora, areas more likely to suffer abrasion during or after childbirth than they are to suffer UV damage. A third function of melanin is its ability to bind free radicals. After being transferred to keratinocytes, melanin binds to toxic free radicals, and as keratinocytes are exfoliated removes free radicals from the body.

Two other major skin pigments are hemoglobin and carotene. These are found below the epidermis, higher levels of the darker eumelanin may obscure them. **Hemoglobin** allows red blood cells to pick up and dump off oxygen molecules. It undergoes a color change when it picks up oxygen, shifting from maroon to red. Healthy people of any skin color do not exhibit variation in hemoglobin. **Carotene** is an orange pigment molecule made by plants which accumulates in the epidermis. Carotene is converted into Vitamin A, and without Vitamin A human cells cannot make the important morphogen **Retinoic Acid**. The amount of carotene that accumulates in the skin, along with melanin, contributes to diversity in skin tone, although carotene levels usually do not change in response to environmental factors.

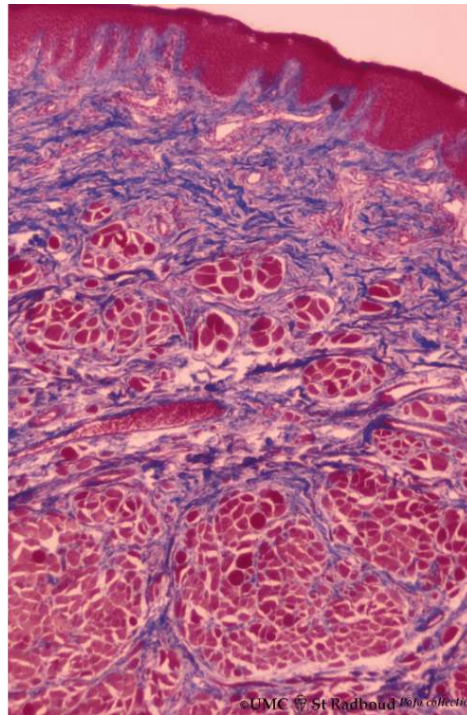


Figure 3.4: Histology of the vermilion border using Azan stain, at the border between the oral mucosa (left side) and the red zone of the lip (right side). Image credit: "Lip (human), region between red zone (vermilion border) and mucosa inner surface" by Poels, Lambert G. is licensed under [CC BY-NC-ND 3.0](#)

Vermilion border

The **vermilion border** (or margin, or zone) is usually defined as part of the skin. It contains less keratin, no hair follicles and few or no sebaceous glands. Furthermore, the keratinocytes in the vermilion border synthesize a clear protein (Eleidin), which allows the reddish color of hemoglobin in the capillaries of the dermis and muscle tissue to be more visible in the vermilion zone than in the more highly keratinized neighboring regions of skin.

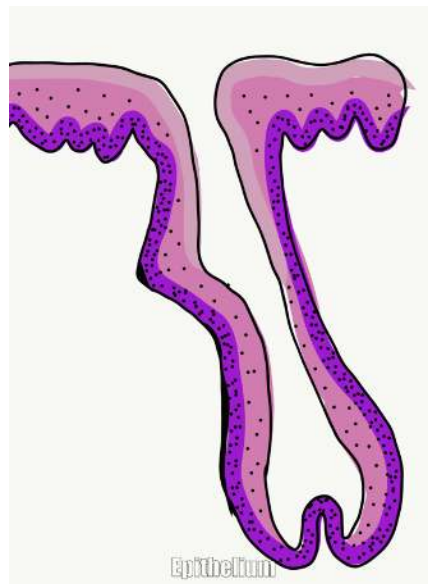


Figure 3.5: Basic components of a hair follicle.

Hair follicles

Hair follicles share developmental processes with teeth. That gives these appendages of the skin and oral mucosa a similar pattern, only one makes keratin and the other calcium hydroxyapatite. A hair follicle is an invagination (an inward-folding) of the

epidermis. The stem cells in the basal layer of the hair bulb divide, differentiate into keratinocytes, and die to form the hair itself. A hair, therefore, is an epithelial structure. Surrounding– or deep to– the hair follicle are the connective tissue layers of the dermis.

Where and when hair follicles invaginate from the surface epidermis is regulated by Planar Cell Polarity signals, which ensure more-or-less even spacing between follicles. Similar signals govern the spacing of the teeth. Also similar to teeth, new hair follicles grow beneath old ones, pushing the old ones out in a process called **exfoliation**. Of course, hair follicles grow and exfoliate more times than a tooth. Nevertheless, it might be a good idea to keep scientific developments in the hair-loss treatment industry somewhere on your radar, advances there may have applications in some future [tooth-growth industry](#).

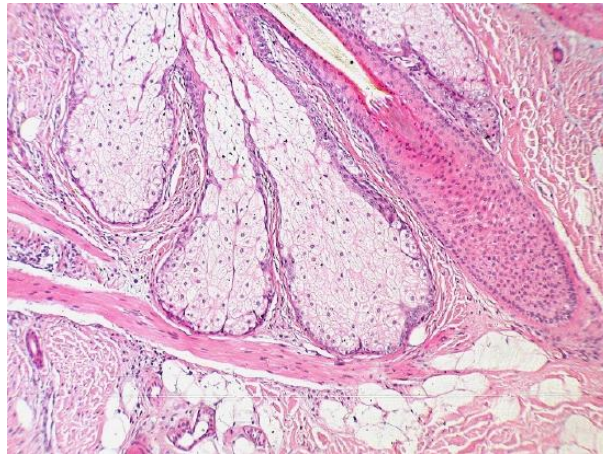


Figure 3.6: Sebaceous gland (H&E stain), associated with a hair follicle. Image credit: “Base of pilosebaceous unit” by Kilbad is in the [Public Domain](#), [CC0](#)

Sebaceous glands

Sebaceous glands are oil-producing glands within the skin. They may discharge directly to the surface of the skin, but are mostly associated with hair follicles. The material they secrete, called sebum, is mostly lipids (triglyceride and others). Unlike proteins, these molecules are not synthesized on the rER, modified in the Golgi apparatus nor secreted from vesicles. Instead, sebum accumulates in the cytoplasm and is secreted by destruction of the cell. New glandular cells are produced from stem cells in the basal portion of the gland. The vermilion zone and oral mucosa contain few or no sebaceous glands, but when sebaceous glands are present in these regions, they form benign whitish spots known as **Fordyce Spots**.



Figure 3.7: Tissues found in the oral mucosa and sub-mucosa layers.

General histology of the oral mucosa

The **oral mucosa** is the **mucous membrane** that lines the oral cavity. It shares the same lineage as the skin, and we therefore see the same tissue types in the same order. However, its layers get different names and are classified differently. Based on the 3 major embryonic tissues, the layers of the skin are divided appropriately, but the oral mucosa is divided incorrectly.

First, stratified squamous epithelium and underlying areolar connective tissue are lumped together and called the oral mucosa. The stratified squamous epithelium may be referred to individually as the **oral epithelium**, and like the epidermis, is derived from the ectoderm. The layer of areolar connective tissue, which is homologous to the papillary layer of the dermis in the skin, is called the **lamina propria**. It is produced by cells derived from mesoderm. Deep to the oral mucosa, the layer of dense irregular connective tissue is called the **sub-mucosa**, which is the homologue of the reticular layer of the dermis in the skin. The sub-mucosa is also produced by cells derived from mesoderm.

Embryonic tissue	Skin	Oral mucosa		
Ectoderm	Epidermis	Stratified squamous epithelium	Stratified squamous epithelium	Oral mucosa
Mesoderm	Dermis	Areolar CT		
Dense irregular CT	Dense irregular CT	Sub-mucosa		

Table 3.2: Summary of the layers of the skin compared to the layers of the oral mucosa

Figure 3.8: Increased amounts of keratin in the oral mucosa obscure underlying pigments. Image credit: leukoplakia by dozenist is licensed CC BY 3.0

The amount of keratinization of the oral mucosa reflects the amount of stress or abrasion that region experiences. This is similar to the formation of a callus on the hands or feet. Higher-than-normal levels of keratinization are clinically relevant when they indicate bruxism, tobacco use, or other health-related issues. Keratin doesn't have a color, but higher levels of keratin in the epithelium obscures the maroon-ish color of blood found within the sub-mucosa, hence non-keratinized mucosa look more reddish, while keratinized mucosa more whitish. Levels of keratinization are categorized into three or four groups listed in Table 3.3.

Type of stratified squamous epithelium	Level of keratinization	Location
Keratinized	Full	Skin
Ortho-keratinized	Partial	Masticatory mucosa
Para-keratinized		
Non-keratinized	None	Lining mucosa

Table 3.3: Different types of oral mucosa and their level of keratinization

Histology of oral mucosa

Figure 3.9: Lining mucosa (upper half) is non-keratinized, masticatory mucosa (lower half) is partially keratinized.

3 classes oral mucosa

Lining mucosa is found in most regions of the oral cavity, and is not involved significantly with mastication. These are regions more important for speech and swallowing. They are therefore mostly non-keratinized. They may have higher levels of elastic fibers within the lamina propria. Because lining mucosa does not get as much friction and abrasion, it has small or no visible dermal papillae and rete pegs between the epithelium and connective tissue layers. The upper-half of Fig. 3.9 illustrates a lining mucosa.

Masticatory mucosa is found in regions of high abrasion caused by mastication, such as the attached gingiva. The epithelium is either be **ortho-keratinized** or **para-keratinized**, which are both partially keratinized. An ortho-keratinized epithelium contains keratinocytes with keratin and nucleuses, whereas the para-keratinized epithelium lacks nucleuses. Differentiating between ortho- and para-keratinized tissue is based on appearance, and has no clinical significance. For the rest of this book, we refer to them together as “ortho- or para-keratinized epithelium”. Because this mucosa is generally under higher levels of stress, it has more pronounced dermal papillae and rete pegs than lining mucosa. The lower-half of Fig. 3.9 illustrates a masticatory mucosa, whose apical surface contains a degree of keratinization.

Specialized mucosa is found on the dorsal surface of the tongue. It does not display a uniform level of keratinization.

Lining mucosa, by region

Figure 3.10: Buccal mucosa. Image credit: [buccal mucosa](#) by the [NIH](#) is in the Public Domain CC 0

Labial and buccal mucosa

Labial mucosa and **buccal mucosa** both have a non-keratinized stratified squamous epithelial layer. This gives them a more reddish or pinkish baseline appearance (see alveolar mucosa pigmentation below). As with all oral mucosa, there are no hair follicles, but in places sebaceous glands may be present, forming Fordyce spots. As a lining mucosa, the epithelial layer is generally non-keratinized, but there can be regions of keratinization where stress occurs. Most notably is likely the **Linea Alba** (or “white line”), running along the line in the buccal mucosa where the teeth meet.

Figure 3.11: Specialized mucosa of the tongue. Image credit: [Ventral tongue](#) by the [NIH](#) is in the Public Domain CC 0

Ventral surface of the tongue and the floor of the mouth

The **ventral surface of the tongue** and the **floor of the mouth** both contain very thin, non-keratinized stratified squamous epithelium. The thin-ness gives these surfaces a more reddish-appearance than other lining mucosa. The thinness of the epithelium, coupled with the rich blood supply in the deeper lamina propria are also why some medications are given sub-lingually.

Figure 3.12: Illustration of the histology of the soft palate.

Soft palate

The **soft palate** is lined by a non-keratinized stratified squamous epithelium, with a very thin layer of sub-mucosa deep to it. This gives the epithelium a firm attachment to deeper muscle tissue, which is important for speech and swallowing.

Figure 3.13: Alveolar mucosa. Image credit: Gingiva of the human mouth by John Crawford is licensed CC BY 3.0. / Arrows added

Alveolar mucosa

Alveolar mucosa is lined by a non-keratinized stratified squamous epithelium. It has a rich blood supply and numerous elastic fibers within the lamina propria, but few dermal papillae and rete pegs. Because of the large blood supply and low levels of keratin, you may often hear healthy attached gingiva should appear pinkish, but this assumes the absence of melanin.

Figure 3.14: Example of healthy human gingival coloration. Image credit: “Preoperative picture of 25-year-old female complaining of black-colored gums” by Arthiie Thangavelu et al is licensed under [CC BY-NC-SA 4.0](#)

Clinical disorders may alter the coloration of the gingiva, but to assume all healthy gingiva are pinkish ignores healthy variation in skin color. The gingiva of darker-skinned patients may be darker and be completely healthy. Melanin can be deposited in gingival mucosa, either uniformly (evenly) or focally (localized, such as a freckle or macule— a freckle on the labia). Unlike tanning, which represents a change in melanocyte activity in response to UV damage to keratinocytes, melanin in the gingiva rarely represents a homeostatic change (hence, not a pathological change). Instead, melanin levels in the oral mucosa are linked to inborn melanin level in the skin (the patient’s skin color), a concept that should hopefully be more obvious once we consider the shared lineage of these two tissues.

Masticatory mucosa

Figure 3.15: Attached gingiva. Image credit: Gingiva of the human mouth by John Crawford is licensed CC BY 3.0 / *arrows added*

Attached gingiva

Attached gingiva is a type of masticatory mucosa, lined with a para-keratinized stratified squamous epithelium. The increased amount of keratin, compared to alveolar mucosa, obscures the underlying blood supply, creating a lighter appearance (which can be described as whitish in the absence of melanin).

Figure 3.16: Illustration of a partially keratinized epithelium of the attached gingiva.

Large dermal papillae and rete pegs create the stippled (rough surface) appearance of the attached gingiva.

Figure 3.17: Interdental gingiva. Image credit: Gingiva of the human mouth by John Crawford is licensed CC BY 3.0 / *cropped and brackets added*

Interdental gingiva

Interdental gingiva is similar to the attached gingiva.

Figure 3.18: Marginal gingiva. Image credit: Gingiva of the human mouth by John Crawford is licensed CC BY 3.0 / *cropped and brackets added*

Marginal gingiva

Marginal gingiva is similar to the attached gingiva.

Figure 3.19: The hard palate. Image credit: [Pleomorphic adenoma of the left palate](#) by the [NIH](#) is in the Public Domain CC0

Hard palate

The **hard palate** is lined by an ortho-keratinized stratified squamous epithelium and mostly lacks a sub-mucosa, making for a rigid connection to underlying bone tissue.

Mucosa of the Dento-Gingival junction

Figure 3.20: Sulcular epithelium (red bracket).

Sulcular epithelium

Sulcular epithelium (or crevicular epithelium) is lined by either a non-keratinized or para-keratinized stratified squamous epithelium. It creates a space between the gingiva and tooth, named the **gingival sulcus**. Sulcular epithelium is not attached to the surface of the tooth. Under the microscope, the absence of dermal papillae and rete pegs indicates this tissue gets very little abrasion.

Figure 3.21; Junctional epithelium (red bracket).

Junctional epithelium

Junctional epithelium (JE) is a non-keratinized stratified squamous epithelium. It is special in that its apical surface attaches to the tooth by way of hemi-desmosomes. Other epithelia attach to connective tissue only on their basolateral surface, their apical surface faces the external environment. This unique attachment to the tooth surface is referred to as the **epithelial attachment** (EA). This uniqueness is challenging to re-create with dental implants. In a subsequent chapter, we cover the special lineage of the junctional epithelium that gives the adult tissue its uniqueness.

Junctional epithelium is thinner than other gingival mucosa, only five cells thick at the end. It is also more permeable, having fewer desmosomes between cells. This allows white blood cells from the underlying vascular sub-mucosa to migrate through junctional epithelium and enter the gingival sulcus. But this also increases the potential for oral cavity bacteria to do the same in reverse, especially if the epithelial attachment is lost.

Specialized mucosa

Figure 3.22: Illustration of the histology of the tongue.

Tongue histology

The **dorsal surface of the tongue** contains more than one type of mucosa. The epithelial surface is mostly an ortho-keratinized stratified squamous epithelium, and can therefore be thought of as a masticatory mucosa. Scattered across the dorsal and lateral surfaces are four different shapes of bumps called **lingual papillae**. The most numerous are filiform papillae, which contain only keratinocytes, and may be ortho- or para keratinized. The other three lingual papillae also contain taste buds, which are not keratinocytes, and therefore not a masticatory or lining mucosa, but a specialized mucosa. These structures are appendages of the oral mucosa.

Anteriorly, deep to the oral mucosa and sub-mucosa, the tongue contains numerous bundles of skeletal muscle tissue, and some adipose tissue. Posteriorly, the tongue contains more adipose and salivary gland tissue.

Figure 3.23: The Tongue by the OpenStax is licensed CC BY 4.0

Filiform papillae contain an ortho-keratinized or para-keratinized epithelial layer. These papillae function for providing friction only, their mucosa contain no taste buds.

Fungiform papillae contain an ortho-keratinized or para-keratinized epithelial layer over a highly vascular sub-mucosa, giving these structures a more reddish-appearance than neighboring filiform papillae. The epithelial layer contains taste buds, which detect the sense of gustation, which is in turn a part of the perception of taste.

Foliate papillae are found on the lateral edges of the tongue. They contain an ortho-keratinized or para-keratinized epithelial layer with taste buds.

Circumvallate papillae are found at the border between the anterior and posterior portion of the tongue, the sulcus terminalis. They contain an ortho-keratinized or para-keratinized epithelial layer with taste buds and minor salivary glands.

Turnover time of epithelia

Epithelium	Turnover time (days)
Skin	27-38
Hard palate	24
Floor of mouth	20
Buccal and labial mucosa	14
Attached gingiva and taste buds	10
Junctional epithelium	5

Table 3.4: Turnover time of the various regions of oral mucosa and skin

The time it takes to replace all of the cells within the epithelial layers of the skin and oral mucosa is shown in Table 3.4. As you should see, the oral epithelium grows quickly, which means it can regenerate quickly following injury. This is largely due to the presence of growth factors in saliva. This also means the lifespan of these cells is short, which means oral cancers are relatively rare in the absence of large doses of carcinogens (tobacco and alcohol). The epithelial cells of oral mucosa do not live long enough to easily acquire the multiple mutations to oncogenes and tumor-suppressor genes required to cause cancer.

Clinical applications of lining and masticatory mucosa histology

Hyper-keratosis

Hyper-keratosis is a homeostatic response of the oral mucosa to stress, either chemical or physical. In response to stress, epithelial cells express more keratin, causing an increase in the degree of keratinization. Vitamin A deficiency can lead to generalized hyper-keratosis.

Figure 3.24: Leukoplakia, and example of hyper-keratosis. Image credit: leukoplakia by dozenist is licensed CC BY 3.0

Leukoplakia

If the increase in keratinization is localized, it is referred to as **leukoplakia**. Parafunctional habits can cause regions of the buccal mucosa to undergo hyper-keratosis. Bruxism may cause the linea alba to appear more white-ish. Chemical stress caused by use of smokeless tobacco products (snuff) cause leukoplakia at the site of use.

Figure 3.25: Nicotinic stomatitis, an example of hyper-keratosis. Image credit: [Nicotinic stomatitis](#) by DVIDS is in the Public Domain CC0

Nicotinic stomatitis

Hyper-keratosis can be caused by the chemical stress of cigarette smoke. It is not caused by nicotine, which is an addictive substance but mostly non-toxic, despite what many [otherwise reliable resources](#) suggest. We cover adverse effects of nicotine when we discuss PDL loss in chapter 11. For the oral mucosa, the chemical stress from smoking is caused by benzene, formaldehyde, poly-aromatic hydrocarbons, and other toxic chemicals produced by combustion (burning). At the time we are writing this, there is no evidence that nicotine gum causes hyper-keratosis of the oral mucosa, and the link between vaping and hyper-keratosis is not strong, despite both containing nicotine. If your patients smoke, these two nicotine-delivery methods are considerably safer for both the lungs and oral cavity, and can be helpful tools in smoking-cessation (nicotine is the addictive component of tobacco products). This is not to be mistaken for an endorsement of taking up an e-cigarette habit, nor maintaining one, we are discussing *relative* risk here.

Nicotinic stomatitis is a visible change to the hard palate. In response to chronic stress, the ortho-keratinized epithelium produces more keratin, leading to a more white-ish appearance. However, epithelial cells of the minor salivary glands do not respond to stress in this fashion, and remain pinkish (the genes for keratin production are possibly methylated and packed around histones in these cells). This same pattern can also be caused by ingesting hot liquids.

Clinical changes to the gingiva

Figure 3.26: Migration into an injured area by leukocytes (identified as masses of purple nucleusses in the dermis) during an inflammatory response. Image credit: “[Dermal perivascular lymphoeosinophilic infiltrate – very low mag](#)” by Nephron is licensed under [CC BY-SA 3.0](#)

Inflammation of any tissue is referred to as tissue-name-itis, hence **gingivitis** is inflammation of the gingiva, while **periodontitis** is inflammation to the gingiva and periodontium. The redness, swelling, heat and pain symptoms indicate the body has likely suffered trauma, and is undergoing a response to that trauma. Mucosa exude more liquid into an area as a part of the inflammatory process, known as **edema**, making that region of the oral mucosa larger and paler when inflamed. Edema may give gingiva a puffy or rolled appearance.

Ideally, an inflammatory response limits the spread of the initial damage and set the stage for regeneration. When a tissue regenerates, stem cells migrate into the affected area, divide, and differentiate into the cells needed to repair the damage, such as keratinocytes or fibroblasts.

Figure 3.27: The fluids that cause edema in a tissue are easily compressible. Image credit: “Pitting edema” by James Heilman, MD is licensed under [CC BY-SA 3.0](#)

Chronic inflammation, on the other hand, leads to cell death and the loss or recession of a tissue. This is because stem cells generally halt progression through the cell cycle until the inflammatory process removes the source of the stress. Without stem cells generating new cells, chronic stress allows everyday wear on a tissue to accumulate.

Figure 3.28: C Collection of Gingivo-Crevicular Fluid (GCF). Image credit: “[Extracrevicular GCF collection](#)” by Zeyad Nazar Majeed et al is licensed under [CC BY 4.0](#)

During an inflammatory response, the gingival sulcus fills with **gingivo-crevicular fluid** (GCF). GCF contains breakdown products of human cells undergoing necrosis or apoptosis, the breakdown products of bacteria being killed by white blood cells, bacterial toxins, and inflammatory molecules released by human cells. The dead-end of the gingival pocket minimizes mixing with the secretions of salivary glands, therefore taking a sample of gingival fluid is a diagnostic tool for measuring gingival health

Figure 3.29: Really bad gingivitis. Image credit: [Really bad gingivitis](#) by D. Rosenbach, is licensed CC BY 3.0

Gingivitis

Gingivitis is any inflammation of the gums. It includes edema in the ECM of connective tissues in the lamina propria and sub-mucosa, as well as inside the epithelial cells of the oral mucosa. This causes the marginal, attached and inter-dental gingiva to become visibly swollen. When marginal gingiva is swollen, it may produce a crescent-shaped edema known as a **McCall's festoon**. This inflammatory response generally causes no damage, but untreated gingivitis can progress to periodontitis, which in turn can lead to bone and tooth loss. Gingivitis also increases the risk of developing Alzheimer's Disease and [heart disease](#), making it all the more important to intervene as early as possible. Currently, [half of American adults over the age of 30 suffer from periodontal disease](#), and seventy percent of people over the age of 65, there is plenty of room for more work to be done.

Figure 3.30: Gingival hyperplasia. Image credit: Gingival enlargement due to S – amlodipine by the NIH, is in the Public Domain CC0

Gingival hyperplasia

Hyperplasia means the increased growth of a tissue, which could mean an increased number of cells, an increased amount of ECM produced by cells, or both. **Gingival hyperplasia** is an abnormal growth of gingival tissue. It may look similar to edema, but the underlying cause (and therefore treatment) is different. A tissue that has undergone hyperplasia is not as squishy as a tissue with edema. Hyperplasia is a side-effect of certain medications, such as phenytoin and cyclosporine. Other triggers exist, including pregnancy, hormonal disturbances, or it may even be a hereditary condition ([Hereditary Gingival Fibromatosis](#)). Like edema, gingival hyperplasia may be caused by poor oral hygiene. The first response of the immune system to oral microorganisms is inflammation (and therefore edema). Over time, the immune system may respond to continued stress by releasing growth factors that trigger increased cell division of nearby stem cells, and other signals (morphogens) that trigger cell differentiation and cellular activity (such as the production of ECM proteins). This is a perfectly healthy response to stress in the palms of the hands or soles of the feet, generating a callus. However, the body sometimes has trouble distinguishing between physical stress and other types of stress, such as chemical stress caused by toxins produced by microorganisms.

Figure 3.31: Cosmetic surgery for gummy smile. a) before, b) laser incisions, c) immediately after surgery, d) 2-week post-operative. Image credit: “[Esthetic crown lengthening](#)” by Shanmukha Srinivas Manikanta Kumar Tirumalasetty et al is licensed under [CC BY-NC 4.0](#)

The increase in the size of the gingiva, even if not caused by poor oral hygiene, may make maintaining oral hygiene difficult, and should therefore be addressed. In some cases, it may not be possible to remove the underlying cause (such as a hereditary condition, or a life-saving anti-seizure medication), in which case surgical removal of gingival tissue (gingivectomy) or gingival reshaping (gingivoplasty) may be warranted. Excess gingival tissue can be removed using a scalpel or a laser tool. The use of lasers can reduce bleeding and pain by cauterizing damaged blood vessels and ablating nerve endings.

Figure 3.32: Gingival pigmentation, before and during pigment removal surgery. Image credit: “[Pre-operative view and Maxillary pigmentation removal using scalpel surgical technique](#)” by Rehab A.Abdel Moneim et al is licensed under [CC BY-NC-ND 4.0](#)

Pigmentation of the gingiva

Similar to increased levels of keratin in the attached gingiva, increased levels of melanin may obscure underlying healthy level of hemoglobin. The presence of melanin alone should not be mistaken for unhealthy gingiva. Unlike keratin, melanin is a *pigment*, meaning it absorbs only certain wavelengths of light (has a color). Surgeries and treatments to remove gingival pigment exist, often advertising what is found in textbooks: *healthy gingiva should be pinkish or whitish in color* (try a google search for gingival hyperpigmentation if you are skeptical). It is worth noting skin whitening products exist because systemic racism in the United States and other countries has led many people of color to feel displeasure with darker skin pigmentation, illustrated by experiments such as [the Doll test](#) by Drs Kenneth and Mamie Clark. Furthermore, the concept that pink is healthy and brown is unhealthy is not shared across cultures. For instance, dental offices in Ethiopia and surrounding areas may offer ethnobotanical tattooing of the maxillary gingiva to add a blue-ish or grey-ish coloration to the gingiva, masking pinkish regions.

Figure 3.33: Smokers melanosis. Image credit: “[Smoker’s melanosis in oral mucosa with brown black pigmentation](#)” by Skinstudy is licensed under [CC BY SA 3.0](#)

A rapid, focal change in melanin production in the oral cavity may be a response to a medical condition. Most common among dark-skinned women between the ages of 30-50, increased melanin production in response to acute trauma or prolonged irritation (such as smoking) may arise, termed a melanocanthoma (further reading can be found [here](#)). Despite the name this type of skin lesion has, it is not a tumor, but a homeostatic change in cell activity. Tobacco smoke creates numerous free radicals that can be absorbed by epithelial cells, thus increased melanin production helps protect the oral mucosa. Tetracycline antibiotics bind to melanin, which triggers melanocytes to up-regulate melanin synthesis. Tetracycline drugs are very common, found in over-the-counter first-aid creams, added to animal feeds, and were even in beers brewed in Sudan 2,000 years ago.

There are other pigments that cause color changes to the gingiva, and they are classified as either **endogenous** (produced by human cells) or **exogenous** (environmental). Endogenous pigments commonly found in the gingiva, besides melanin, include hemoglobin

and breakdown products of hemoglobin. A reddish lesion caused by hemoglobin or its breakdown products is a sign of damage to capillaries (bruising). An example of an exogenous pigment is jimsonweed (*Datura stramonium*), which can be used in the ethnobotanical gingival tattoos mentioned previously.

Figure 3.34: Gingival recession. Image credit: Class II gingival recession on the left maxillary canine and lateral incisor by Nitin Khuller, is licensed under CC BY 3.0

Gingival recession

Chronic inflammation of the gingiva can lead to **gingival recession**, exposing deeper tissues of the tooth, which in turn may make teeth more susceptible to tooth decay. The most common cause of gingival recession is gingivitis and periodontitis. Gingival recession can also be caused by abrasion (improper tooth brushing), abfraction (bruxism), improper tooth position, and aging.

Figure 3.35: Stillman cleft. Image credit: [V-shaped gingival recession](#) by Ana Suzy Jati et al, is licensed CC BY 3.0

A **Stillman cleft** is a V-shaped region of gingival recession. It is often caused by occlusal trauma.

Figure 3.36: Sub-epithelial graft. Legend: 1) donor site (ipsilateral mucosa), 2) recovered connective tissue, 3) connective tissue added to recipient site, 4) sutured recipient site. Image credit: "Retrieval of the subepithelial connective tissue graft from the palate and placement at the recipient site" by DRosenbach is licensed under [CC BY-SA 4.0](#)

Gingival grafting

A **sub-epithelial connective tissue graft** (SECT graft) may be performed to repair gingival recession. Unlike a skin graft, which grafts epithelial plus some or all of the connective tissue from a donor, a sub-epithelial graft transplants only connective tissue from the lamina propria and/or sub-mucosa. The goal of a sub-epithelial graft is not to replace damaged tissue, but to provide a scaffold that promotes healing of the patient's own tissues. The connective tissue provides a scaffold which epithelial stem cells are attracted to and migrate over. These stem cells undergo mitosis, producing more epithelial cells which differentiate into keratinocytes and regenerate the oral epithelium. Similarly, the patient's mesenchymal stem cells migrate through the scaffold and replace the transplanted collagen, regenerating the lamina propria and sub-mucosa.

with transannular pericardial patch being sutured">

Figure 3.37: Use of synthetic polymer scaffold in heart surgery. Image credit: "[Intraoperative view showing hand sewn polytetrafluoroethylene bicuspid pulmonary valve in situ with transannular pericardial patch being sutured](#)" by Prashant Ramdas Wankhade et al is licensed under [CC BY-NC-SA 4.0](#)

Sub-epithelial grafts splice connective tissue from nearby regions of healthy gingiva (such as from neighboring gingiva or hard palate). This leaves behind small wounds, which heal quickly. Nevertheless, damaging healthy tissue is not optimal. Another option is to use tissue from a human cadaver. Because the connective tissue used in such a procedure is mostly collagen fibers, options include the use of synthetic collagen-like polymers (Fig X) or a **pericardial patch** procedure. Unlike human tissue, there is a large supply of cow and pig connective tissue. First, the connective tissue that surrounds a cow or pig heart (the pericardium) is harvested and stripped of any pig or cow cells. The acellular tissue that remains should not trigger tissue rejection (collagen is collagen across vertebrates), but it acts as a scaffold. Ultimately, the patient's own epithelial stem cells migrate over the scaffold to regenerate the oral mucosa. In addition, the patient's mesenchymal stem cells migrate into the scaffold from nearby healthy sub-mucosa, and replace the cow or pig collagen. This procedure is very similar to different types of bone tissue grafts and is not limited in use to the gingiva—a video of a heart valve replacement using bovine pericardium can be [viewed here](#). We hope you appreciate the amount of histology and cell biology required to understand how pieces of cow hearts can be used to repair damaged gingiva.

Figure 3.38: Free gingival graft. Image credit: [Free gingival graft placed around the implants](#) by Danny Omar Mendoza Marin, is licensed CC BY 3.0

A **free gingival graft** may be done around dental implants, or used to repair gingival recession. Similar to the sub-epithelial graft, the free gingival graft harvests healthy tissue from the donor; only in this case epithelial cells from the oral mucosa are also transplanted. When placed around dental implants, attached gingival tissue can adhere to the implant, as opposed to junctional epithelium which adheres to a tooth. Coating a dental implant with Hyaluronic Acid helps grafted tissue adhere to the implant. Without adhesion to the implant, oral microorganisms can bypass the oral mucosa and enter the sub-mucosa. Similar to the sub-epithelial graft, connective tissue from a pig or cow can be used as a scaffold, removing the need for a second surgery site. Connective tissue grafts are relatively common in dentistry and maxillofacial surgery, but these technologies are starting to generate changes in [tissue grafting below the neck](#), too.

For further reading on dental biomaterials

[FDA information](#) on GINTUIT

[Mucograft](#) – acquired by Geistlich biomaterials

Gengigel Hyaluronic Acid gel

Table 3.5: Further reading about currently available dental biomaterials

Clinical applications of dento-gingival junction histology

Figure 3.39: Illustration of periodontal pocket morphology (note: blood vessels are present deep to both JE and pocket epithelium, they do not suddenly appear).

Periodontal pockets

The depth of a **periodontal pocket** can be measured using a calibrated probe. In a healthy state, the distance from the marginal gingiva to the epithelial attachment should be between 1-3mm. Pockets within this range typically have an intact epithelial attachment, which prevents oral bacteria from entering the sub-mucosa and causing gingivitis or periodontitis. Poor oral hygiene, however, can lead to increased levels of oral bacteria within the periodontal pockets. Because the junctional epithelium is more permeable than other regions of the oral mucosa, white blood cells come into contact with this bacterial population and trigger inflammation. With chronic inflammation comes a loss of junctional epithelium, which can reduce the thickness of the junctional epithelium further, potentially causing the loss of the epithelial attachment. At this point, the pocket is said to be lined with **pocket epithelium**. Since it is no longer attached to the tooth, the probe can likely be inserted over 3mm. The thinness of the pocket epithelium brings the probe closer to blood vessels in the lamina propria, making it more likely to damage these vessels, causing **Bleeding on Probing** (BoP, or Bleeding after Probing, BaP).

It is possible that a periodontal pocket deeper than 3mm can have junctional epithelium with an intact epithelial attachment. These exhibit minimal Bleeding on Probing, and can be considered uncharacteristically deep pockets rather than a clinical manifestation of periodontitis.

Figure 3.40: Bleeding on probing (BoP). Image credit: [Bleeding after probing](#) by Luigi Checchi et al, is licensed CC BY NC 3.0

The major risk with Bleeding on Probing involves the oral microbiome, which is a large collection of microorganisms. These microorganisms come into contact with the bloodstream with disruption to the epithelial barrier. Even so-called “good bacteria” trigger inflammation when they move inside the body. Until damage to the barrier is repaired, inflammation continues. Chronic inflammation within the pockets leads to damage to nearby tissue, such as alveolar bone. This leads to further damage to the oral cavity which we discuss later. A second risk with migration of the epithelial attachment is that acid-producing bacteria now come into contact with softer cementum, leading to root caries.

Pseudopockets, which may also be called false pockets or gingival pockets, are caused by gingival hyperplasia or edema. Enlargement of the gingival margin, whether by mitosis or fluid buildup, deepens the measurement of a pocket, but does not share the same harmful loss of the epithelial attachment. The underlying cause of the pseudopocket may need to be addressed.

Clinical applications of specialized mucosa histology

Figure 3.41: Geographic tongue. Image credit: [geographic tongue](#) by Jbarta, is licensed CC BY SA 3.0

Geographic tongue

Geographic tongue is a condition where filiform papillae on the dorsal surface of the tongue become non-uniformly hyperkeratinized, giving some filiform papillae a more white-ish appearance. Other papillae may be lost due to prolonged inflammation, leaving more reddish spots on the tongue. The pattern of keratinized versus partially-keratinized papillae changes over weeks. The result is almost always a cosmetic concern, although some patients may describe periods of heightened sensitivity to hot, acidic and/or spicy foods. There are currently no treatments for geographic tongue. The underlying causes of geographic tongue are unknown.

Figure 3.42: Black hairy tongue. Image credit: black hairy tongue by Com4, is in the Public Domain CC 0

Black hairy tongue

Black hairy tongue occurs when filiform papillae shed epithelial cells more slowly, thus the papillae become enlarged. This allows the papillae to pick up more stains from tobacco smoke, foods, or oral bacteria, creating thicker, darker bumps on the tongue. It is thought that this condition might be triggered by overgrowth of certain oral fungi, possibly following the loss of competition with the use of certain antibiotics. The reason the filiform papillae appear hair-like is that both filiform papillae and hairs are composed predominantly of dead keratinized epithelial cells. Patients are encouraged to brush their tongue when brushing their teeth.

Chapter 2 * Chapter 4

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1.4: Histology of the Teeth and Periodontal Tissue

- Overview
- Bone tissue review
- Histology of teeth
 - Enamel
 - Dentin
 - Pulp
 - Cementum
- Histology of the periodontium
 - Periodontal Ligament
 - Alveolar bone
- Summary of hard tissues

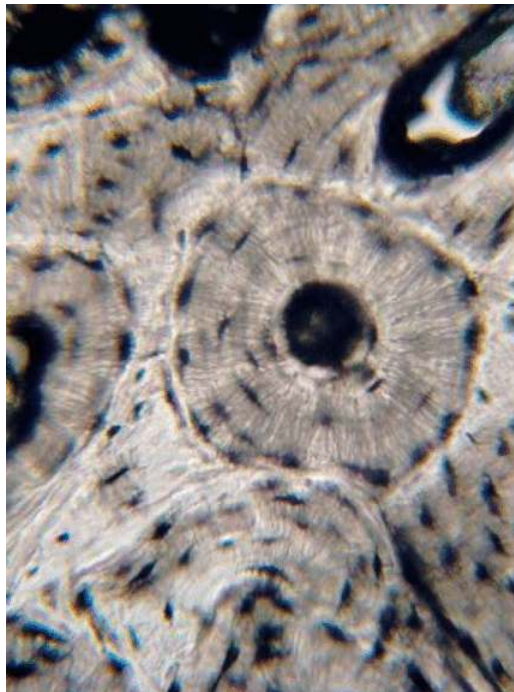


Figure 4.1: Histology and illustration of compact bone tissue, highlighting cells and layers of ECM.

Overview

This chapter briefly covers the histology of the hard tissues enamel, dentin, cementum and bone, as well as the soft tissues of pulp and the periodontal ligament. The definitions and clinical considerations of these tissues are addressed in the chapters on the development of these tissues (Chapters 8-11)

For more practice using histology images, we share these useful links

- The [Oral histology Lab](#)
 - by Brian R. MacPherson, Ph.D. and James G. Tieman, Ed.D., at the University of Kentucky (*we linked to this in the previous chapter as well*)
- The [Microanatomy Web Atlas](#)
 - by Gwen V. Childs, Ph.D., FAAA at the [University of Arkansas for medical sciences](#). Jump to the digestive system/tooth unit.

Histology of bone tissue (brief review)

Bone tissue is deposited in layers by osteoblasts. These cells become trapped within lacunae between layers of bone tissue, and differentiate into osteocytes. Bone tissue can either be compact and made of osteons, or spongy bone made of trabeculae. Bone tissue is almost entirely ECM, summarized in Table 4.1. Bone tissue is highly vascular—compact bone contains central canals and perforating canals, whereas in spongy bone the space between bony trabeculae is filled with red bone marrow or highly vascular adipose connective tissue.

Components of bone tissue	
<1%	Osteocytes
66%	Mineral ECM: Calcium Hydroxyapatite
33%	Protein ECM: Collagen

Table 4.1: Components of bone tissue

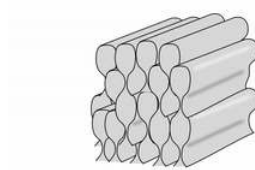


Figure 4.2: The ECM of enamel is laid down in rods next to other rods, each rod is secreted by one cell. In contrast, bone tissue is deposited in a circular layer upon circular layer, each cell working only in one layer.

Histology of teeth

Histology of enamel

Enamel shares some mineral characteristics with bone tissue, but it is acellular and avascular. Enamel matrix is deposited in columns called enamel rods by cells called ameloblasts. Enamel is the strongest substance in the human body, on account of its high mineral content, listed in Table 4.2.

Components of enamel	
0.0%	Cells (<i>there are no cells</i>)
96%	Mineral ECM: Calcium Hydroxyapatite
4%	Protein ECM: Amelogenins and Enamelin (<i>not collagen</i>)

Table 4.2: Components of enamel



Figure 4.3: Cross-section of tooth enamel, highlighting the Striae of Retzius and Hunter-Schreger bands. Unlabeled image credit Tooth of *Paranthropus robustus* SKX 21841 from Swartkrans by Didier Descouens, is licensed CC BY 3.0 / boxes and animation added

During embryonic development, ameloblasts work on a circadian rhythm, depositing ECM at a regular pace for 4 days, then changing speed. The changes in speed leads to changes in enamel density, which can be seen as horizontal Striae of Retzius. Another pattern is visible because ameloblasts do not create an enamel rod in a perfectly straight line, but changes directions slightly over days. This leads to the pattern known as Hunter-Schreger bands. The border between enamel and underlying dentin is a distinct line named the **Dentino-enamel Junction** (DEJ).

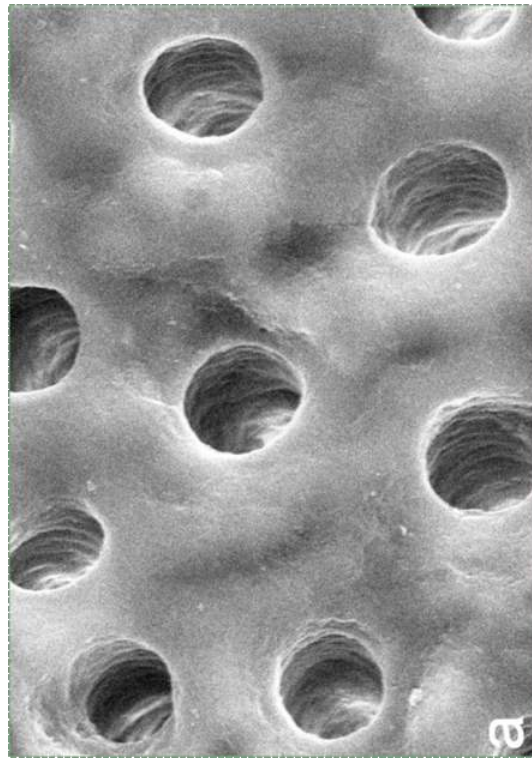


Figure 4.4: Surface view of dentin. Image credit: [Dentinal tubule occlusion of dentine discs after treatment](#) by Peiyan Yuan, is licensed CC BY SA 3.0 / *labels, arrows and animation added*

Histology of dentin

Dentin, like enamel, is avascular, but it should not be called acellular. Dentin matrix is deposited by cells called odontoblasts. These cells—or more accurately, their cell bodies—are found within the pulp cavity, immediately adjacent to the dentin. Each odontoblast has an arm-like protrusion called an odontoblastic process that extends nearly the entire thickness of the dentin ECM that cell secreted. The odontoblastic process secretes more dentin (in a soft form that hardens later), as well as a fluid called **dentinal fluid** into the space known as a dentinal tubule. Dentin is not as strong as enamel, because of its lower mineral content, listed in Table 4.3.

Components of dentin	
—	Cells (odontoblasts cell bodies in pulp, odontoblastic processes in dentin)
70%	Mineral ECM: Calcium Hydroxyapatite
30%	Protein ECM: Collagen , mostly

Table 4.3: Components of dentin.

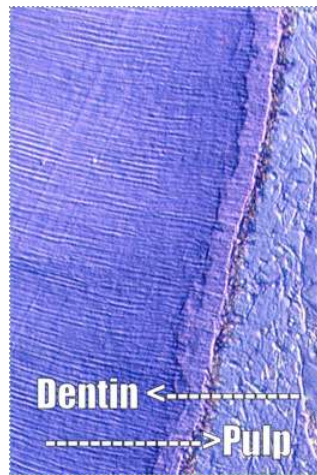


Figure 4.5: Histology of dentin, highlighting the cell bodies of odontoblasts and the imbrication lines of Von Ebner, Image credit: "histological section of tooth" by Doc. RNDr. Josef Reischig, CSc. is licensed under CC BY-SA 3.0 / labels and animations added

Similar to the lines of Retzius, dentin is deposited at different rates over days, which creates bands called the Imbrication lines of von Ebner. These lines run perpendicular to the dentinal tubules. Exceptionally-pronounced imbrication lines are named Contour lines of Owen, and occur with exceptional changes in nutrition (such as childbirth).

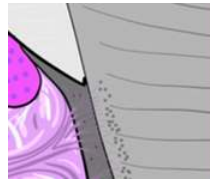


Figure 4.6: Illustration of Tome's granular layer in root dentin (small dark spots).

Root dentin has a layer of speckles near the border with the cementum named Tome's granular layer. It has no known function. It is a good landmark when looking at dentin under the microscope, its presence indicates you are viewing root dentin, not mantle dentin, and a thin layer of cementum should be close by.

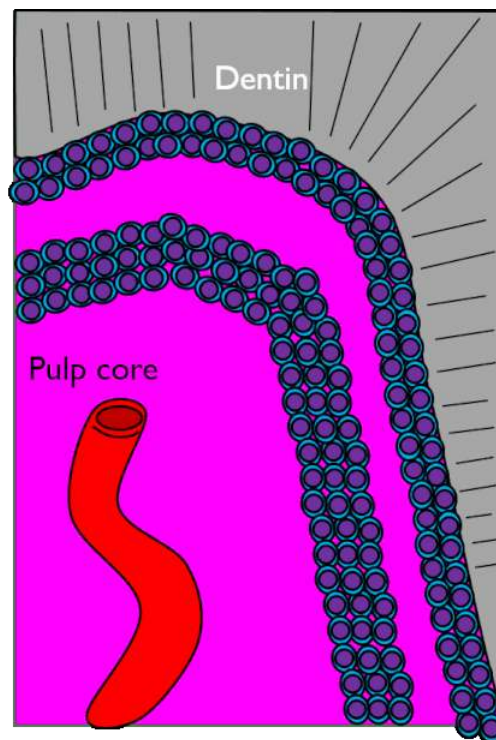


Figure 4.7: Illustration of the histology of the pulp chamber.

Histology of pulp

The pulp chamber is composed of an indeterminate type of connective tissue, although it most resembles areolar connective tissue. The most common cell types found within the pulp core are fibroblasts and mesenchymal stem cells. The pulp houses the blood, nerve and lymphatic supplies for each tooth. The pulp has 4 layers that appear distinct under the microscope listed in Table 4.4.

Layer	Predominant feature
Odontoblast layer	Odontoblast cell bodies
Cell-free zone	Not actually free of cells, cells aren't visible
Cell-rich zone	Cells are visible and densely packed
Pulp core	Location of capillaries, nerve endings and lymphatic vessels

Table 4.4: The four layers of the pulp chamber and their predominant features.

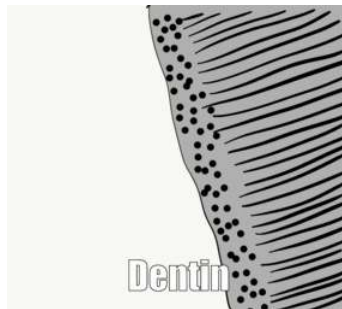


Figure 4.8: Illustration of cementum, and surrounding tissues.

Histology of cementum

Cementum forms a thin layer (roughly 60 μ M, but see below) over the surface of root dentin, which is roughly the width of a hair. The ECM of cementum differs significantly enough from enamel that the two can be differentiated by their color (cementum is yellowish in hue), whereas no significant color difference exists between cementum and dentin. The ECM of cementum is deposited by cementoblasts, which can be found on the surface of cementum throughout life. As a result, the thickness of cementum can triple in thickness between the ages of 10 and 70 years of age. Cementum incorporates more fluoride than the other hard tissues.

Cementum, like dentin and enamel, is an avascular tissue, but it can contain cells. There are two types of cementum based on histological (visual) studies dating back to the 1830s. These two types are cellular and acellular cementum. More [recent advances in molecular biology and genetics](#) are yielding newer concepts, but the old classification system still rules.

In **cellular cementum**, cementoblasts are trapped within the ECM and differentiate into cementocytes. In the mature tissue, Cementocytes are found within lacunae, similar to bone tissue. However, canaliculi only extend in the direction of the periodontal ligament. In the opposite direction is avascular dentin, which cannot provide cementocytes with nutrients. Cellular cementum is found in the apical half of the root.

Acellular cementum does not contain cementocytes, and can be found in the coronal half of the root. The border between cementum and dentin— the **Dentino-cemental junction**— is less distinct than the DEJ. Cementum is slightly less strong than dentin, and is composed of the materials listed in Table 4.5.

The lower mineral content of cementum compared to bone tissue suggests it is also less strong than bone, but as we see in subsequent chapters, the loss of bone, dentin and cementum in response to periodontitis or orthodontia is complex and requires discussion of morphogens and the differentiation of resorptive cells.

Components of cementum	
—	Cellular cementum (apical portion) Acellular cementum (coronal portion)

50%	Mineral: Calcium Hydroxyapatite
50%	Protein: Collagen and proteoglycan mixture

Table 4.5: Components of cementum

Fibers in cementum

Acellular cementum contains **extrinsic collagen fibers** which travel perpendicular to the surface of the root, and are likely secreted by both fibroblasts and cementoblasts. These fibers attach to alveolar bone, and represent the ends of the principal fibers of the periodontal ligament embedded within cementum (also called Sharpey's fibers, just as in bone tissue).

Cellular cementum contains intrinsic fibers and possibly extrinsic fibers. **Intrinsic collagen fibers** are secreted by cementoblasts, and do not extend beyond cementum. They are oriented parallel to the root surface and are mainly involved in the repair of cementum (a scaffold). More recent publications subdivide cellular cementum into Cellular Intrinsic Fiber Cementum and Cellular Mixed Stratified Cementum, based on the amount of extrinsic collagen fibers present. Cementum that does not contain extrinsic collagen fibers (closer to the crown, the cervical region) does not contribute to the attachment to bone tissue.

Histology of periodontal tissues

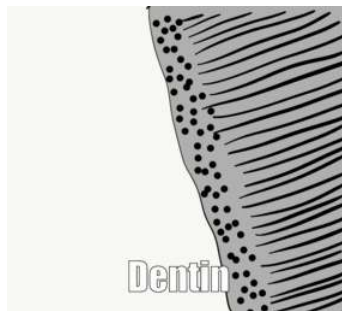


Figure 4.9: Illustration of the periodontal ligament and nearby tissues.

Histology of the Periodontal Ligament

The **Periodontal Ligament** (PDL) is a region of dense regular CT that connects the cementum to alveolar bone. Sharpey's fibers extend from the PDL into cellular cementum. Like the dentin-cemental junction, the border between cementum and PDL is blurred due to their shared lineage. Clusters of rogue epithelial cells can be found within the PDL which are named the epithelial rests of Malassez.

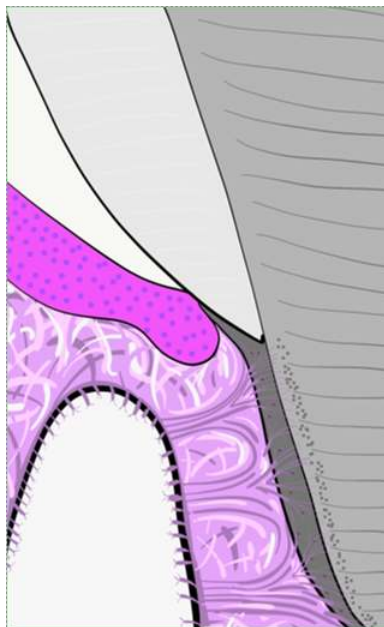


Figure 4.10: Illustration of alveolar bone, and nearby tissues. Look for Tome's granular layer as a landmark, if needed.

Histology of alveolar bone

Alveolar bone is compact bone tissue, with a semi-unique feature of containing dozens of tiny holes through which Sharpey's fibers from the PDL insert, as well as larger holes for the nerves, blood and lymphatic vessels than exit bone tissue and into the apical foramen of the tooth. This makes the surface of the alveolar sockets appear porous, which is almost unique for the surface of bone tissue. The porous surface is called the **cribriform plate**, which is not to be confused with the other region of compact bone riddled with tiny holes, the cribriform plate of the ethmoid bone (whose holes are for smell nerve endings, connecting the nasal cavity to the brain).

Resorptive cells in hard tissues

The periosteum is a layer of dense connective tissue that surrounds bone tissue. In addition to collagen fibers, it contains osteoblasts and osteoclasts involved in bone remodeling. Similar cells exist for cementum and dentin, but not enamel. **Cementoclasts** and **Odontoclasts** are capable of demineralizing cementum and dentin, respectively. Like osteoclasts, these cells express genes involved in acid secretion as well as digestive enzymes, including members of the matrix metalloproteinase family. Unlike osteoclasts, they are not active continuously in a remodeling unit, they have no baseline activity and are typically only activated during tooth exfoliation.

Odontoclasts and cementoclasts likely differentiate from the same bone marrow stem cell that can also become an osteoclast. The exact signals involved in determining whether the multi-potent stem cell differentiates into an osteoclast, odontoclast or cementoclast is not well understood. The body may regulate this process by long or medium-range hormone and growth factor signals. The trigger may also be very short-range: binding of the stem cell to proteins or glycoproteins within the ECM of target tissues, providing the stem cell with positional information. While roughly identical to osteoclasts under the microscope, odontoclasts and cementoclasts have important physiological differences. For instance, the hormone calcitonin inhibits osteoclasts but triggers odontoclast activity during root resorption. The significance of this detail is that root loss does not always match bone loss. For instance, in osteoporosis, bone loss leads to the loss of teeth, not root loss. During tooth exfoliation, however, root dentin is resorbed but alveolar bone undergoes both resorption and deposition, to form a pathway through bone above the permanent tooth and fill in alveolar socket space below.

Some publications suggest cementoclasts are distinct cells found in the periodontal ligament and that they play a role in the maintenance of cementum throughout life. Other publications suggest cementoclasts are the same cell type as odontoclasts and osteoclasts. Without further study, it is hard to tell whether a cementoclast is a distinct cell type, or an osteoclast found near cementum. The mechanism of resorption is likely the same (acids and proteinases), but whether these cell are regulated the same is an important question during root exfoliation or orthodontic movement.

Summary of hard tissues

Table 4.6 compares the components of the ECM of hard tissues.

Hard tissue	Components	
Enamel	96%	Mineral: Calcium Hydroxyapatite
	4%	Protein: Amelogenins and Enamelins (<i>not collagen</i>)
Dentin	70%	Mineral: Calcium Hydroxyapatite
	30%	Protein: Collagen, mostly
Cementum	50%	Mineral: Calcium Hydroxyapatite
	50%	Protein: Collagen and proteoglycan mixture
Bone	66%	Mineral: Calcium Hydroxyapatite
	33%	Protein: Collagen

Chapter 3 * Table of Contents * Chapter 5

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1.5: Histology of Glands, Lymphatics and Sinuses

- Basic anatomy
- Salivary glands
 - Components of saliva
 - Dental pellicle
 - Histology of salivary glands
 - Anatomy of salivary glands
 - Clinical applications of gland histology
- Lymphatic system
 - Lymph nodes
 - Tonsils
 - Clinical application of lymphatic histology
- Sinuses
 - Para-nasal sinus anatomy
 - Clinical application of para-nasal sinus histology



Figure 5.1: Basic anatomy of an exocrine gland.

Basic anatomy of exocrine glands and lymphatic organs

The average exocrine gland is composed of simple cuboidal epithelial cells that form grape-shaped structures called **acini** (although other glands have tube-shaped ends). These grape-shaped structures are where secretions are produced. The liquids produced by these cells enter a duct, which can be made of simple cuboidal or simple columnar epithelial cells. It is possible for larger glands to have regions of stratified cuboidal epithelial cells. The outside of the gland is surrounded by a dense connective tissue **capsule** (not quite thick enough for us to worry about whether the collagen fibers are regular or irregular). The connective tissue extends inwards forming trabeculae (or septa, not to be confused with the trabeculae of spongy bone tissue), which divide the organ into lobes.

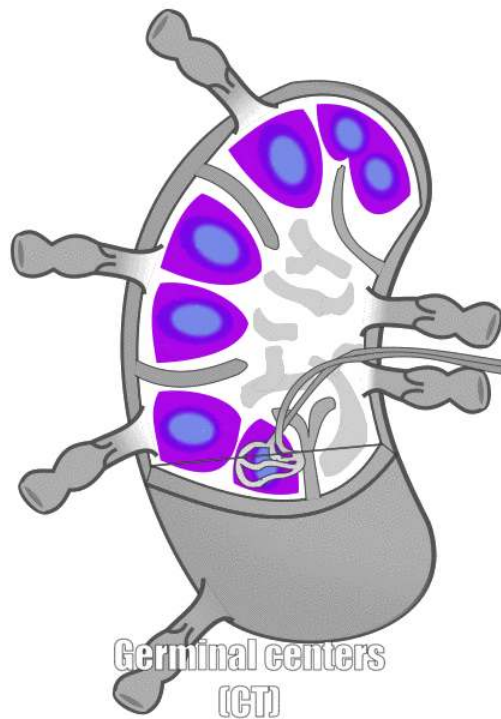


Figure 5.2: Anatomy of a lymph node.

The average lymphatic organ shares patterns with exocrine glands. A lymphatic organ is surrounded by a dense connective tissue capsule, and that connective tissue extends inwards forming trabeculae. Inside a lymphatic organ, clusters of white blood cells suspended within reticular connective tissue form **germinal centers** (or nodules) where white blood cells filter cellular debris and scan body fluids for pathogens. If they detect a pathogen, they multiply, release inflammatory signals, and migrate through the body's connective tissues looking for more pathogens.

Salivary glands



Figure 5.3: Accelerated tooth decay in meth mouth. Image credit: Suspected meth mouth by Dozenist, is licensed CC BY SA 3.0

Components of saliva

The major function of salivary glands is to produce **saliva**, which helps maintain the health of the oral mucosa and teeth, as well as assist in mastication. Acinar epithelial cells transcribe and translate long mucous proteins on the rough endoplasmic reticulum, modify them in the Golgi apparatus to form large glycoproteins, and secrete them. These cells also pump electrolytes from their cytoplasm into the lumen of the acinus, which, along with the glycoproteins, attract water by osmosis. These components make saliva moist and sticky enough to adhere to all surfaces of the oral cavity, rather than sink to the floor of the mouth.

Other molecules are secreted to become a part of saliva. Buffers within saliva help to maintain a stable pH, despite the acidity or alkalinity of different foods, or the acidity of certain oral bacterial secretions. Buffers and electrolytes also help disrupt the formation of bacterial biofilms, which bacteria use to adhere to teeth. The watery nature of saliva helps to moisten food and the oral mucosa, assisting in mastication, speech and swallowing. Watery saliva helps to limit the population of oral bacteria. People swallow saliva even when they are not eating, flushing a percentage of oral microorganisms down to the stomach, where certain death awaits in the form of Hydrochloric Acid and the protein-destroying enzyme pepsin. The enzymes salivary amylase and lysozyme are produced by acinar cells to serve a similar purpose, breaking covalent bonds within the cell walls of viruses and bacteria, or their surface glycoproteins used to adhere to tooth surfaces. Without hours of mechanical digestion by the teeth and stomach, there is very little surface area for salivary amylase to digest carbohydrate molecules for nutritional purposes. Chemical **digestion** of carbohydrates for nutrition is carried out in the small intestines by the much more abundant pancreatic amylase. Bacteria, however, do not form a bolus (a large solid mass), their cell walls and surface glycoproteins are open to attack by enzymes without prior mechanical processing. A second function of salivary amylase is to cleave a small amount of small sugars from starch, allowing taste buds to detect them, so Salivary amylase and other carbohydrases in saliva function in the sense of gustation as well as in limiting bacterial populations.



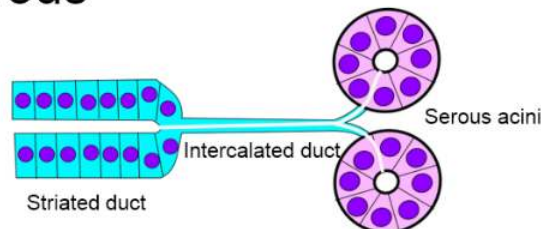
Figure 5.4: Dental pellicle. Image credit: own work by Jost Jhan, is licensed CC BY SA 4.0

Dental pellicle

The **dental pellicle** is a coating of glycoproteins from saliva on the surface of teeth. It normally prevents excess deposits of $\text{Ca}^{2+}\text{PO}_4^{3-}$. It also prevents demineralization of enamel. Both depositing bumps or eroding crevices allows bacteria to adhere to teeth and avoid being washed into the stomach. Saliva also contains the minerals that produce calculus, so its role is complex.

Histology of salivary glands

Serous



Mucous

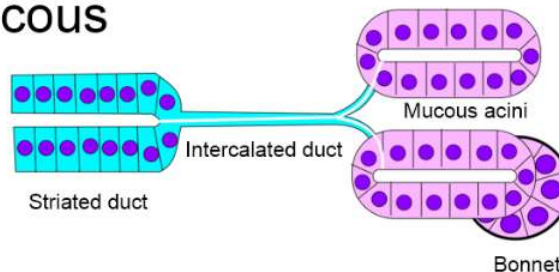


Figure 5.5: Illustration of the basic histology of serous versus mucous salivary glands.

Serous acini produce more watery secretions. These are composed of simple cuboidal epithelium. Serous acini tend to stain more faintly using a traditional H&E stain, because they make fewer proteins.

Mucous acini secrete more glycoproteins, making their secretion thicker and stickier (more mucous). Mucous acini are made of a simple columnar epithelium. Mucous acini tend to stain a little darker using a traditional H&E stain, because they make more proteins.

Muco-serousacini produce secretions halfway in-between the first two in consistency. These look like mucous acini under the microscope, with an additional bonnet (a hat) of **myo-epithelial cells**. These cells contract like smooth muscle cells. For most epithelial cells, the actin and myosin genes are not highly expressed, except by epithelial stem cells, which may need to migrate to a different area to help repair a wound. Myo-epithelial cells, on the other hand, use some of the DNA instructions for making larger amounts of actin and myosin and organizing them into filaments more similar to muscle tissue than generic cytoskeleton. This makes these myo-epithelial cells appear more similar to smooth muscle than an epithelium. However, these cells have an epithelial lineage—they differentiated from an epithelial stem cell, not from a Mesenchymal Stem Cell or myoblast.

All three secretions mix in the oral cavity to produce saliva. These secretions reach the oral cavity by way of different ducts, which can be identified under the microscope by their different types of epithelial cells. We know of no clinical significance to the types of ducts or their cells.

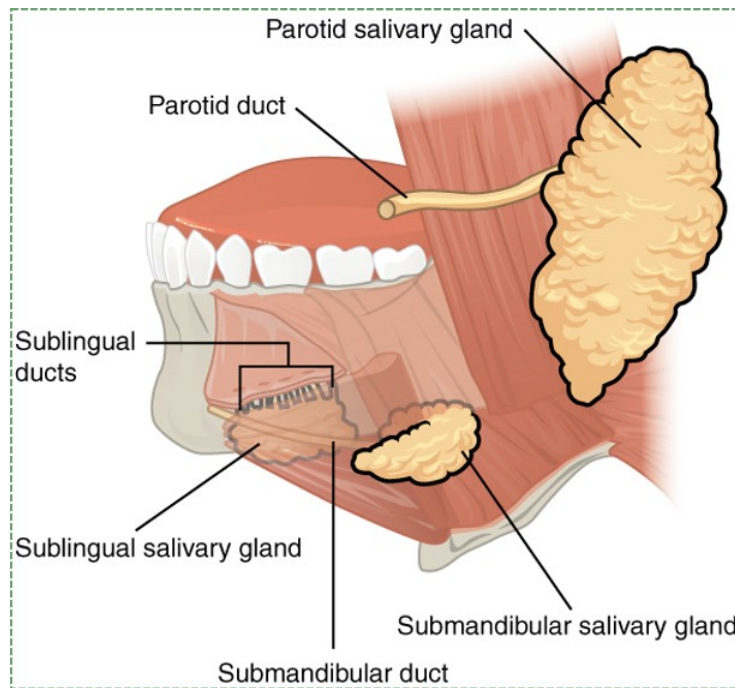


Figure 5.6: Salivary gland anatomy. Image credit: Salivary glands by [OpenStax college](#), is licensed CC BY 3.0

Anatomy of exocrine glands

There are three major and numerous minor salivary glands. The three major salivary glands are listed in Table 5.1.

Major salivary gland	Details
Parotid salivary glands	Largest
	Serous (mostly)
	25% saliva by volume
Sub-mandibular salivary glands	2 nd largest
	Muco-serous
	65% saliva by volume
Sub-lingual salivary gland	Mucous
	10% saliva by volume

Table 5.1: Summary of the three major salivary glands.



Figure 5.7: Parotid duct papillae. Image credit: Parotid duct papillae by D Rosenbach, is licensed CC BY SA 3.0

The **parotid glands** drain into the oral cavity by way of the parotid duct (or Stensen duct). The ducts travel through the masseter muscle and enters the oral cavity at a papilla usually located lateral to the 2nd maxillary molar.

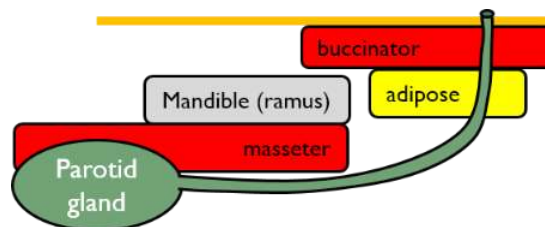


Figure 5.8: Illustrated representation of the path of the parotid duct.

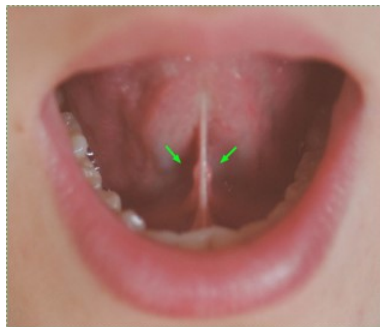


Figure 5.9: The sub-lingual caruncle. Image credit: Carruncula sublingularis by Hellerhoff, is licensed CC BY SA 3.0

The **sub-lingual glands** and **sub-mandibular glands** have ducts that share an entrance on the floor of the mouth at the sub-lingual caruncle. The sub-lingual gland also has numerous smaller ducts that open onto the floor of the mouth.



Figure 5.10: Minor salivary glands visible thanks to Nicotinic Stomatitis. Image credit: [Nicotinic stomatitis](#) by DVIDS is in the Public Domain CC0

Numerous **minor salivary glands** are located throughout the oral cavity. They are normally not visible, unless the oral mucosa is hyper-keratinized. Luckily, no one has bothered to name these glands or their ducts, except for the **von Ebner salivary glands**, which are minor salivary glands associated with the circumvallate lingual papillae. The minor salivary glands are predominantly mucous, with exceptions, which helps their secretions to stick to the roof and sides of the oral cavity.

Clinical application of salivary gland histology



Figure 5.11: A patient with mumps. Image credit: Mumps by Photo CDC/NIP/Barbara Rice, is in the Public Domain CC0

Mumps is a viral infection that causes swelling of the Parotid glands. Vaccination has reduced the number of mumps cases in the US by over 99%. Unfortunately, [mumps outbreaks](#) still occur, often in high-density living areas (such as college dormitories), aided by [fraud, misinformation campaigns, and conspiracy theories](#) targeting vaccines (the MMR vaccine in particular, at least in the U.S.). Unfortunately, it is difficult to counter these or other beliefs in pseudoscience, although [some strategies work better than others](#).



Figure 5.12: Angular chelitis, a sign of hyposalivation. Image credit: Angular chelitis by Matthew Ferguson, is licensed CC BY SA 3.0

Hypo-salivation

Hypo-salivation, or decreased saliva production, can be caused by certain diseases, medications, cancer treatments, and aging. It may result in **xerostomia**, or dry mouth. Reduced saliva can decrease the healing ability of the oral mucosa, leading to sores. Reduced saliva also reduces limits to growth placed on the oral microbiome, leading to infections and caries. Reduced taste can also be a consequence, which patients might compensate for by flavoring their food with excessive levels of salt (NaCl). Using [Monosodium Glutamate](#) (MSG, or $\text{NaC}_5\text{H}_8\text{NO}_4$) to enhance both flavor and saliva production is a significantly safer option, despite fears of the non-existent (not to mention racist) [Chinese Restaurant Syndrome](#).



Figure 5.13: An impressive sialolith and its former location. Image credit: Salivary gland stone and the operation mark by Peter Nickson, is in the Public Domain CC0

Mineralized saliva (sialoliths)

Electrolytes in saliva can precipitate and form salivary gland stones, or **sialoliths**. Large sialoliths may block the duct of one of the salivary glands, which stops the flow– but not the production– of saliva. As a result, saliva builds up in the gland, causing swelling and possibly inflammation. Blockage of a minor salivary gland produces a swelling within the oral mucosa known as a **mucocoele** (Fig. 5.14), while blockage of a major salivary gland produces a **ranula** (Fig. 5.115). These are fairly common disorders. Inflammation of the parotid gland must be clearly distinguished from mumps before any treatment is given.



Figure 5.14: Example of a mucocoele. Image credit: Mucocoele by Dozenist is licensed CC BY SA 3.0



Figure 5.15: Example of a ranula. Image credit: Ranula human by Ph0t0happy is licensed CC BY SA 3.0



Figure 5.16: Sialograph. Image credit Own work by Hellerhoff, is in licensed CC BY SA 3.0

Sialography

Sialoliths may be felt by technicians. Alternately, an image of the blockage known as a **sialograph** can be taken by injecting a radiopaque dye as far into the salivary gland duct as it can go (until further flow is blocked by the sialolith). Usually, sialoliths are removed with minimal discomfort. Sialography should not be done in cases of acute infection, as the dye may push infectious exudate, spreading the infection.

Lymphatic system

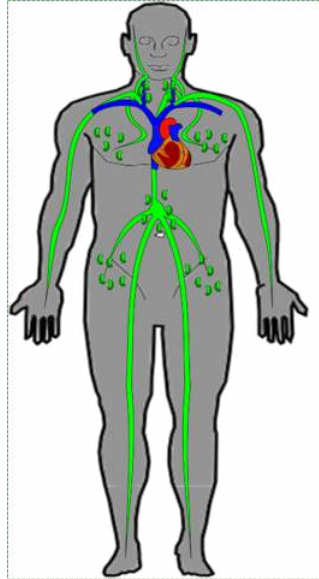


Figure 5.17: Basic anatomy of the lymphatic system.

Very basic overview of the lymphatic system

Lymphatic organ	Major function
Lymphatic vessels	Drain ECF off organs, including teeth
Lymph nodes	Filter debris and identify pathogens
Tonsillar tissue	Identify pathogens
Other lymphatic organs	Located below the neck

Table 5.2: Functions of the major lymphatic organs

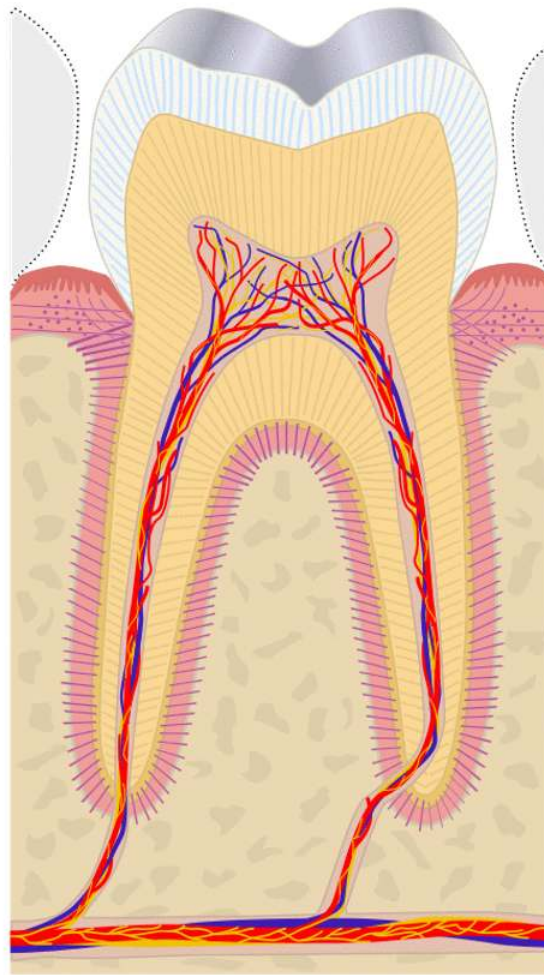


Figure 5.18: ECF production and drainage in a tooth. Legend: Red = blood, Blue = ECF, Green = lymph. Image credit: Own work by Goran tek-en, is licensed CC BY SA 3.0 / Arrows and animation added

Lymphatic drainage of the teeth

Fluid travels to the teeth via capillaries. As plasma exudes from the capillaries, it becomes ECF. Veins collect most of this fluid, the rest is collected by lymphatic vessels. ECF absorbed into a lymphatic vessel is called **lymph**, and it is returned to the circulatory system. Lymphatic vessels are more permeable than capillaries, having **mini-valves** on their outer edges. Mini-valves allow solids to enter the lymphatic vessels, including cellular debris, microorganisms, and cancer cells. The lymphatic vessels are connected to a number of lymph nodes, which clean up debris and mount an immune response against the microorganisms and cancer cells. Knowing the anatomical connections of the lymphatic vessels helps locate periodontal disease by the inflammation triggered in down-stream lymph nodes.

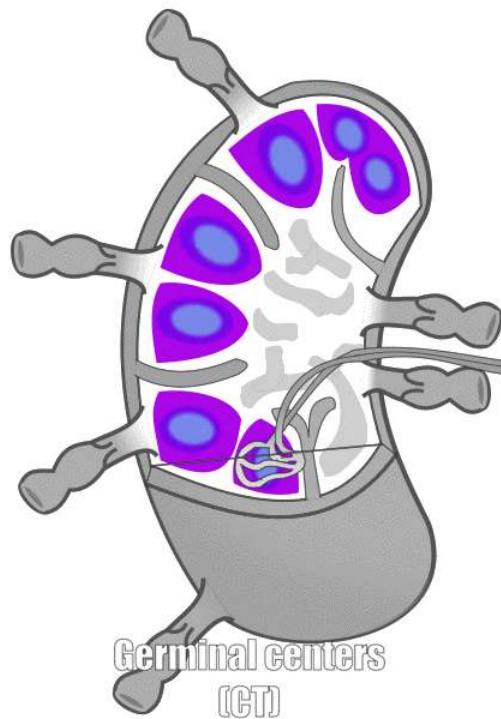


Figure 5.19: Illustration of lymph node histology.

Lymph nodes

Lymph nodes are found throughout the body. A large cluster of lymph nodes can be found in the neck, called the **cervical lymph nodes**, in addition to other locations. Lymph nodes are composed primarily of reticular connective tissue, which provides support for resident clusters of white blood cells. Afferent lymphatic vessels bring lymph to the node, while efferent vessels drain lymph towards the circulatory system.

Lymph nodes serve two major functions. One is to clear cellular debris before lymph reaches the circulatory system, becoming blood plasma again. Two is to identify pathogens and begin an inflammatory response when necessary. If this occurs, white blood cells multiply within the node and secrete inflammatory molecules.

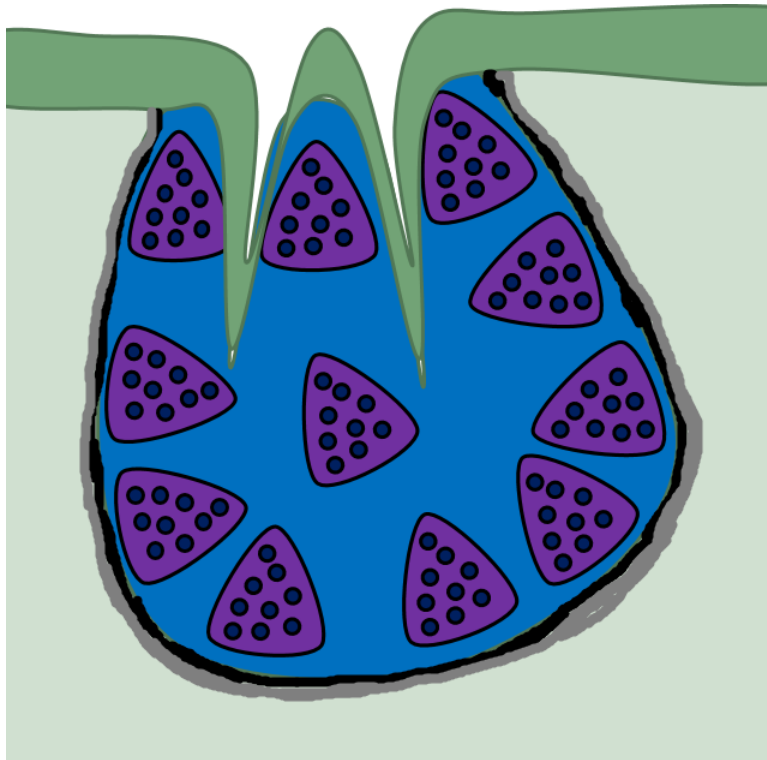


Figure 5.20: Illustration of the basic histology of a tonsil.

Tonsillar tissue

Tonsillar tissue is similar to lymph nodes, except there are no lymphatic vessels, and one end of the tonsil is un-encapsulated (an broad opening). Clusters of white blood cells can be found in germinal centers, suspended within reticular connective tissue.

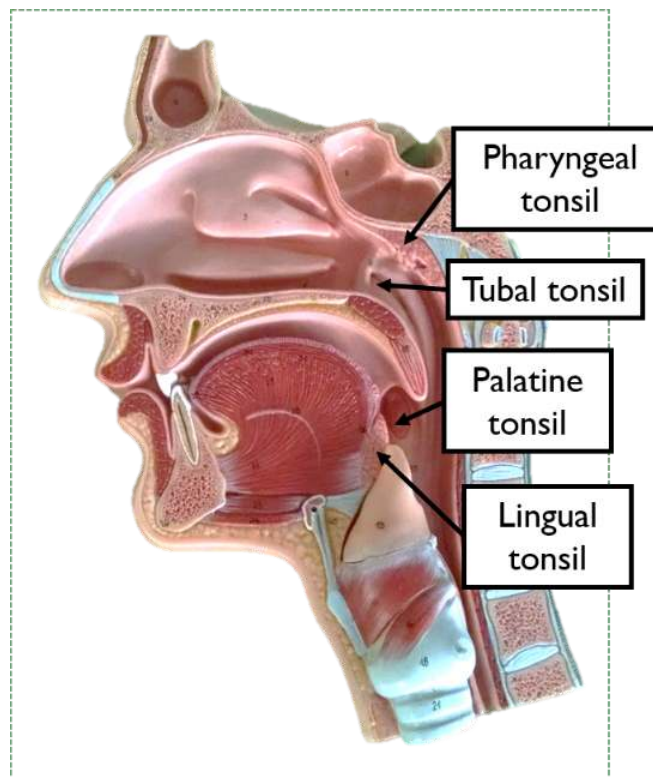


Figure 5.21: Location of tonsils on an anatomical model.

The tonsils

There are 4 major tonsils located the border of the oral cavity and pharynx: lingual, palatine, pharyngeal and tubal. These tonsils form a ring, called **Waldeyer's ring**, which serves to identify pathogens that are either ingested or inhaled, and begin an immune response before they reach deeper locations, such as the lungs or stomach. Because of their anatomy (no vessels), tonsils do not filter debris or microorganisms out of bodily fluids, they only identify pathogens. The un-encapsulated side faces the lumen of the pharynx, coming into contact with many foreign molecules.

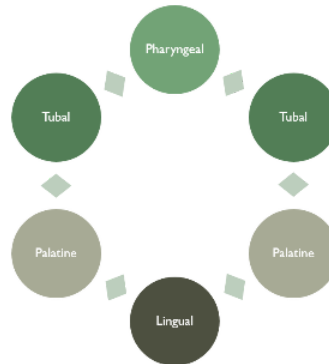


Figure 5.22: Schematic of Waldeyer's ring, as if looking at tonsils from the anterior view of the fauces.

Clinical applications of lymphatic system histology



Figure 5.23: Lymphadenopathy. Image credit: own work by James Heilman MD, is licensed CC BY SA 3.0

Lymphadenopathy of lymph nodes

In the absence of infection, lymph nodes are soft and moveable. However, if white blood cells detect a pathogen, they undergo cell division and release inflammatory molecules. This leads to lymph nodes becoming palpable, possibly visibly swollen, and likely sensitive to touch. The swelling of lymphatic tissue is called **lymphadenopathy**, and is a non-specific sign of immune system activation.



Figure 5.24: Pathway of the drainage of lymph from teeth into lymph node clusters

The mandibular incisors drain into **sub-mental lymph nodes**. The sub-mental lymph nodes and the rest of the teeth (other than maxillary 3rd molars) drain into **sub-mandibular lymph nodes**. The sub-mandibular lymph nodes then drain into **superior deep cervical nodes**. Swelling of some or all of these lymph nodes can indicate active periodontal disease.



Figure 5.25: Lymphadenopathy of tonsillar tissue. Image credit: own work by James Heilman MD, is licensed CC BY SA 3.0

Lymphadenopathy of tonsillar tissue

Tonsillar tissue can become inflamed, and when it does it is usually called **tonsillitis**. When it does, the increased number of white blood cells (living and dead) may be visible as white-ish patches within the inflamed tonsil(s). Tonsillitis is more common in children between pre-school and pre-teens because of the way the immune system develops immunocompetency and tolerance.

Para-nasal sinuses

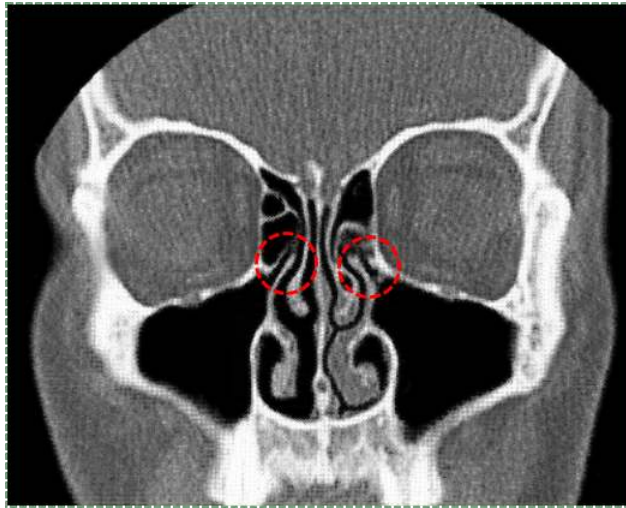


Figure 5.26: Para-nasal sinuses. Image credit: conchae nasalis by Simplicius, is licensed CC BY SA 3.0 / red circles added

Basic anatomy of the para-nasal sinuses

The **para-nasal sinuses** are spaces within the frontal, sphenoid, ethmoid and maxillary bones surrounding the nasal cavity. The maxillary, frontal and sphenoid sinus communicate with (connect to) the nasal cavity via small passageways. The para-nasal sinuses are lined with pseudostratified epithelium. This epithelium produces mucus which can trap pathogens and debris. With the aid of cilia, these harmful agents are removed from the body. Within the nasal cavity are 3 bumps called nasal conchae, which divide the nasal cavity into three winding spaces called the nasal meatuses. (for those who are counting, there is a fourth space above the superior nasal conchae called the spheno-ethmoid recess, it is not a meatus because it does not form a pathway from the nasal vestibule to the nasopharynx).

Clinical applications of para-nasal sinus histology



Figure 5.27: Inflammation of the left para-nasal sinus (black arrowhead indicates swollen mucosa). Image credit: own work by James Heilman MD, is licensed CC BY SA 3.0

Inflammation within the para-nasal sinuses

When the para-nasal sinuses become inflamed (known as **sinusitis**), the small ducts leading to the nasal sinuses may become obstructed. The increase in fluid that accompanies inflammation has nowhere to drain, leading to pressure within the sinuses. Furthermore, one response of goblet cells to inflammatory signals is to produce more mucous proteins, which again has nowhere to drain.

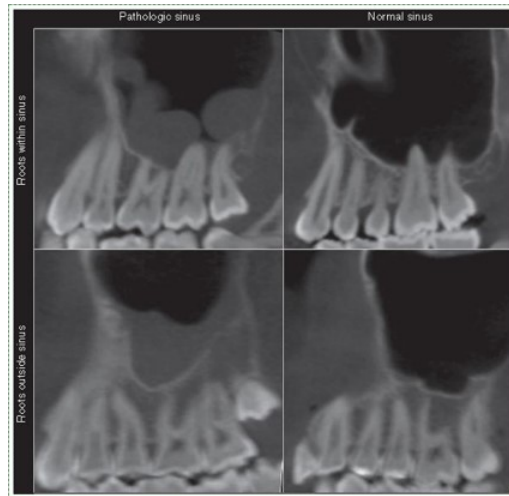


Figure 5.28: Tooth roots can extend into the maxillary sinus. Image credit: Figure by Gina Roque-Torres, is licensed CC BY 4.0

The posterior maxillary teeth lie close to, or partly within the maxillary sinuses. Therefore, if inflammation occurs within these sinuses, it can cause discomfort to the significantly more-sensitive teeth. Furthermore, sinus infections may spread to the posterior maxillary teeth.

Chapter 4 * Chapter 6

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1.6: Early Development

- Overview of early development
- Patterning
- First trimester:
 - Pre-implantation
 - Gastrulation
 - Differentiation of the ectoderm
 - Neurulation
 - Neural crest migration
 - Differentiation of the mesoderm
 - Somite formation
 - Heart formation
 - Differentiation of the endoderm
 - Pharynx formation
 - Development of other oral cavity structures
 - Salivary gland development
 - Lingual papillae development
- Clinical applications of early development

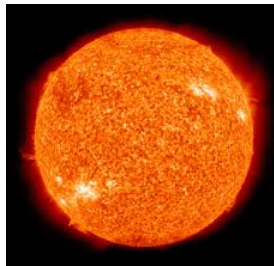


Figure 6.1: From this photo, the polarity of the Sun is probably not recognizable. Image credit: [The Sun photographed at 304 angstroms](#), by NASA/SDO (AIA) – is in the public domain CC0

Overview of early development

Human **development** is the process where a single cell, a visually unremarkable cell except for perhaps its size, slowly changes into trillions (37) of different-looking cells. **Embryology** is the study of the phases of development, especially the early phases where most of the interesting stuff happens. A round cell, just a ball with no sides and no special parts, grows into an organism with different organs in different places. Fig. 6.1 is the Sun. You see it every day, can you tell whether the photo right-side up? Is there even a right-side up? We are not astronomers, but the Sun looks a bit like a fertilized egg, and in Fig. 6.1 there are no obvious landmarks to orient our view. An egg has a similar **amorphous** (without shape) appearance, but it **morphs** (smoothly changes shape) into the embryo shown in Fig. 6.2, which has a head-end and tail-end, and clear beginnings of arms and legs. The first part of this chapter focuses on *concepts* common to many developmental events seen in chapters 7 through 11. The second half of the chapter covers the details of early development.



Figure 6.2: 7-week-old human embryo. [Human embryo 7 weeks](#), by GoldenBear is licensed CC BY SA 3.0

Pregnancy is divided into three equal time periods named **trimesters**. During the first trimester, a single cell multiplies into a multicellular organism with no discernible shape (other than it is a round blob, not an icosohedron). Starting at week 2, the blob of cells develops into an **embryo**, which has different shapes. The embryo keeps developing new shapes until it has all of the basic human shapes, such as a head and arms and heart. At this time (week 8) it is a **fetus**, and the first trimester has ended. The next two trimesters are relatively boring, the fetus grows bigger and the shapes mature, but nothing terribly exciting happens until birth. That's why this is a Histology and *Embryology* book, not Histology and *Fetology*.

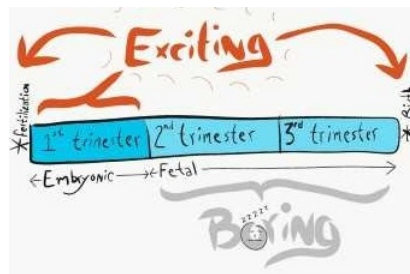


Figure 6.3: The three trimesters, and how much detail each gets in an embryology textbook.



Figure 6.4: Director of the film NoBody's Perfect, a documentary on surviving the teratogenic effects of thalidomide. Image credit: Filmmaker Niko von Glasow, is licensed CC BY SA 2.0

As human cells divide during the first trimester, they make decisions as to their cell fate, which ultimately determines the number and location of our arms and legs, our head and heart. These decisions involve signals being sent between cells. Errors in these signals or decisions lead to **congenital disorders** (or congenital malformation, or birth defects (but see below)). These can happen without known cause. Or, chemicals may interfere with developmental signals, and we call these chemicals **teratogens**. The most famous teratogen in my country is the drug thalidomide, which— if taken by pregnant women at the wrong time— interferes with the decision-making processes an embryo uses to decide where to grow limbs. This causes phocomelia, or malformation of the limbs. Not every country shares this bias, the teratogenic effects of [agent orange](#) (dioxin, which is also found in cigarette smoke) and atomic radiation ([pdf download](#)) are notably absent in most American school classes ([agent orange](#) and [atomic radiation](#) also have

adverse effects on adults, and the rate this information reaches the American public is subject to similar biases). We will cover a small number of signals involved in some important decisions that are made during face and tooth development. 37 trillion decisions are made between the single-cell stage and birth, errors are inevitable. Some errors are minor, changing the appearance but not the function of an organ; we might refer to this as a birth mark or human variation. Other mistakes are more severe, interfering with a person's health, and we refer to those as congenital disorders (or perhaps inborn errors of development, but preferably not birth *defects*, because our choice of words may influence the [amount of health care](#) people seek, it depends on the patient). If you at any time feel like you need more information on embryology, these links are helpful:

- The [Embryology Education](#) page
 - by Dr. Mark Hill at the University of New South Wales, Sydney, Australia
- [Embryo Images Normal and Abnormal Mammalian Development](#)
 - by Dr. Kathleen K. Sulik and Dr. Peter R. Bream Jr. at the University of North Carolina School of Medicine.
- The [3D Atlas of Human Embryology](#)
 - an open-source collection of 3D pdf files (readable using adobe acrobat reader) by over 70 different students at the Academic Medical Center, Netherlands.

Patterns

This section covers the basic processes that occur during development. Often, more than one process is occurring in the same place at the same time. However, it is useful to think about the following processes separately, the way you might focus on just the vocals– or cello, or guitar, or accordion– in a song.



Figure 6.5: Illustration of cell cloning, followed by signaling.

Cloning

Mitosis produces clones, or identical cells with identical DNA. However, cells differentiate and express different genes to become different tissues and organs. In the first 2 weeks of development, cells divide and produce more identical (albeit smaller) cells. Very soon, cells make decisions as to what they are going to become, and these decisions are coordinated with other cells. Without coordination, an embryo could wind up with two butts and no head.



Figure 6.6: Differentiation is caused by changes in gene transcription that leads to cells looking (and behaving) different from other cells. These changes are usually triggered by morphogens.

Differentiation

Differentiation is the process where stem cells begin to express different genes, causing them to appear and function differently from other cells. All cells in the human body have the same DNA, because all cells copied their DNA from the DNA in the zygote (a few exceptional cells change their DNA a bit). The zygote is a toti-potent stem cell, transcription factors could potentially bind to and activate any gene. As cells differentiate, however, they limit their cell fate by putting some genes into deep storage (not actually changing the [genetic code](#), but changing what may or may not be accessible to RNA polymerase).

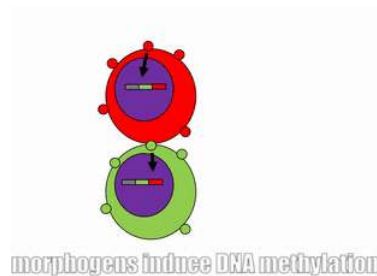


Figure 6.7: Differentiation alters cell fate, which is passed on to daughter cells in the form of conserved DNA methylation and histone packaging.

Differentiation is triggered upon receiving chemical signals. These signals cause target cells to methylate the DNA of certain unnecessary genes, pack those DNA regions around histones, and permanently shut those genes off. For instance, cells fated to become ovaries never synthesize insulin, the gene for insulin is packaged away long before ovaries secrete estrogen or ovulate. DNA methylation and histone packaging limits the fate of these cells. After that, the signals can disappear because the methylation pattern is copied during mitosis and passed to both daughter cells (and their daughter cells). In Fig. 6.7, imagine a signal instructs one cell to methylate green genes, leaving the red genes open to potentially be transcribed. A different signal does the opposite to the other cell. As the red cell duplicates its DNA, it copies the methylation pattern, so that all of its daughter cells cannot express green genes. Meanwhile, the green cell produces a number of daughter cells who have the red gene methylated, none of its daughter cells can express the red gene. We now have 4 red cells, who belong to one lineage, and 4 green cells who belong to a different lineage. Further patterning arises as cells transcribe un-methylated genes. In the cartoon, as red cells transcribe the red gene, this creates a short-range signal which induces nearby green cells to transcribe the dark-green gene. We would say those target cells have differentiated further, and this organism has become even more complicated (3 different cell types). This must continue until we have **200 or so different cell types**. Generally speaking, as a cell becomes more specialized, its fate becomes more limited (more DNA methylation and histone packaging), possibly to the point of being limited to just one cell fate.

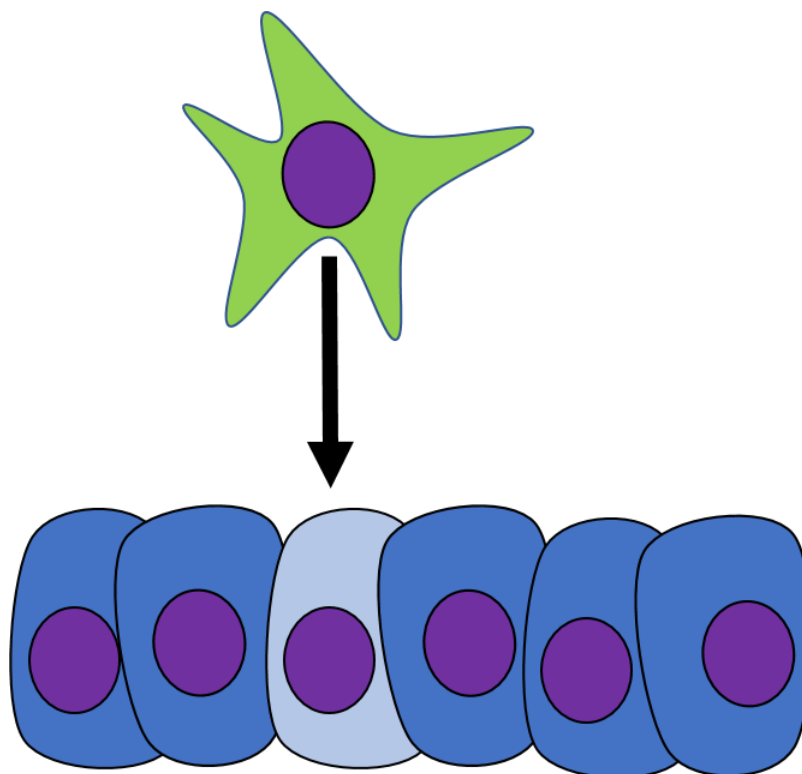


Figure 6.8: Green cell releasing a morphogen to induce the differentiation of a dark blue cell into a lighter blue cell.

Induction

One cell may signal to another cell and **induce** (instructs) that cell to differentiate. Chemicals that can induce another cell to change its shape (to morph) and change cell fate are called **morphogens** (or sometimes called Growth Factors). Morphogens are similar to

neurotransmitters or hormones, they often bind to cell-surface receptor proteins. Activation of morphogen receptors leads to activation of intracellular signals, which in turn can either activate or inhibit specific transcription factors to alter the expression of different genes. Changes in gene expression cause the target cell to look and function differently. Morphogens also change the methylation of genes and their packaging around histones, which is a more permanent change to gene expression. This pattern of gene regulation is passed down to daughter cells, which we learned in Chapter 1 is an example of epigenetic inheritance. By regulating gene expression through both transcription factor activity and methylation, morphogens change not only the morphology the target cell, but their cell fate as well. There are often several closely-related molecules that belong to a *family* of morphogens, each chemical having a name like morphogen-1, morphogen-2a, morphogen-2b, and morphogen-3. There are a very large number of different morphogens, in this textbook we discuss only a few by name, mostly ones directly related to tooth development, including the **Bone Morphogen Protein (BMP)** family, the **Wnt** family, and the **Fibroblast Growth Factor (FGF)** family. Morphogens can act over different distances listed in Table 6.1. The names of specific morphogens are very important on embryology exams, much less so on dental hygiene exams.

Distance	Type	Examples
Direct	Cell-to-Cell	Epithelial-to-neuro-mesenchymal stem cell (Odontoblast differentiation)
	Cell-to-ECM	Integrin-to-collagen (Ameloblast differentiation)
Short-range	Diffusion through ground substance	Bone Morphogen Protein (BMP), Wnt, Fibroblast Growth Factor (FGF). (Tooth bud induction)
Body-wide signals	Hormones	Growth Hormone, Calcitonin.

Table 6.1: Examples of different type of signals seen during embryogenesis.

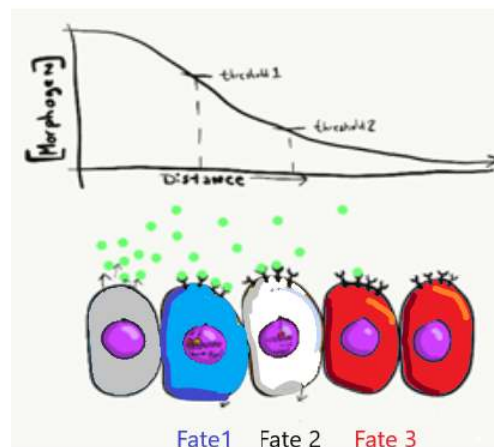


Figure 6.9: Illustration of a morphogen gradient having different effects at different concentrations.

Morphogens are secreted into ground substance, creating a concentration gradient of morphogen levels as they diffuse slowly away from the source. The amount of morphogen molecules produced, the relative stickiness of the ground substance, the distance away from the source and the number of receptor proteins on the target cell determine level of a signal cells receives. Sometimes, a morphogen produces visible effects that fall along a gradient (or spectrum), which conceptually matches the gradients we just listed, such as skin color (lighter to darker) or body size (shorter to taller). However, sometimes the effects of a morphogen are more discrete (tooth bud or no tooth bud, there are no half tooth buds). To help explain these observations requires a little knowledge of how transcription factors work. For example, in the French flag model (Fig 141), imagine receiving high levels of the morphogen signal triggers the transcription of a blue gene and inhibits a red gene, causing those cells to differentiate into blue cells. Moderate amounts of the morphogen signal may not be enough to recruit RNA polymerase to the blue gene, but enough to inhibit the red gene, causing cells in regions of moderate morphogen levels to differentiate into white cells. Lastly, cells that receive little or no morphogen signal do not inhibit the red gene, causing them to differentiate into red cells. There are other models that help explain the link between induction and morphogenesis— between a chemical and an organism—for instance a morphogenetic field

is less Francophile in nature but harder to visualize. These models all share an important concept: chemical signals can communicate **positional information** in a developing embryo.

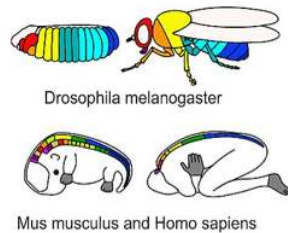


Figure 6.10: Hox (homeobox) gene expression in fruit flies, mice and humans. Image credit: “Hox genes in various species” by Stefanie D. Hueber, Georg F. Weiller, Michael A. Djordjevic, Tancred Frickey is licensed under [CC BY 4.0](https://creativecommons.org/licenses/by/4.0/)

During very early development, regional expression of **homeobox genes** controls cell fate, not morphology. Homeobox genes are transcription factors that specify which part of the body a particular cell is now a part of, such as part of an arm or face, even if that area has no specific shape yet. They do not encode proteins cause cells to become part of a specific organ. Instead, homeobox genes activate or inhibit a collection of other genes (a program), setting the stage for future changes. In contrast, morphogens like FGF and BMP activate genes that alter a cell or tissues shape (morphology). Homeobox gene expression represents a very specific plan in embryology. It is highly conserved across animal species, and the pattern is very predictable in embryos. The pattern is also relatively simple and linear (Fig. 6.10). Think of homeobox genes like surveyors who determine the locations where houses will be built in a subdivision, while morphogens are the contractors who follow the DNA blueprints for each house, which are built by cells. We can extend this metaphor one step further: in a subdivision, houses are similar, derived from one basic blueprint, but given slight modifications with each re-use.

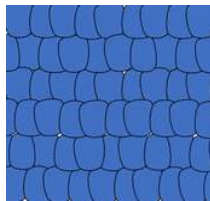


Figure 6.11: Illustration of a stable pattern arising in response to two morphogens.

The ability of a tissue to create its own patterns is called **self-organization** (in contrast, maternal effect genes from mom’s cells guide patterns in the embryo’s cells). This involves multiple morphogens, initially expressed randomly. Interactions between these morphogens leads to a stable pattern of expression, and a complex pattern of differentiation. For instance, some morphogens block other cells from producing the same morphogen. In Fig. 6.11, imagine a blue morphogen induces one cell to differentiate into a blue cell, but blocks neighboring cells from becoming blue cells. These neighbors would be free to differentiate into red cells. At the same time, a distant cell produces red morphogen, which induces it to differentiate into a red cell, but blocks its neighbors from doing the same. Its neighbors would be free to produce blue morphogen and differentiate into blue cells. The initial pattern that arises is alternating rings of red and blue cells. However, rings produce regions of red-next-to-red and blue-next-to-blue, which violates our make-believe rules, and this starts a battle. To minimize the number of red-next-to-red and blue-next-to-blue, cells change their cell fate until a stable pattern arises, in this case stripes. It would be hard to predict from the start which cells would be red versus blue, but you could predict the development of red and blue stripes. If these were pigment-producing cells in a zebra, we’d have a zebra with stripes (the best type of zebra). But this pattern could also be alternating regions of oral mucosa that grow a tooth bud or don’t grow a tooth bud, ensuring each tooth bud has enough space to grow (if you want to go down a rabbit-hole, these interactions can be modeled mathematically). When a morphogen blocks another signal, we call that **antagonism**, and antagonist signals are just as common as induction signals. They must, after all, it is fundamentally just as important to grow one head as it is to not grow two heads.

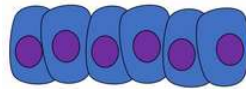


Figure 6.12: Cell proliferation can occur appositionally or interstitially.

Proliferation

Growth of a tissue is called **proliferation**. Proliferation occurs either by mitosis (adding cells) or by adding extra-cellular matrix (synthesizing proteins or other molecules). When new growth happens next to a region of older growth, this is **appositional growth**, whereas if growth happens from within and pushes older tissue outwards, that is **interstitial growth**. Dense tissues, such as bone, enamel and dentin, undergo appositional growth. Softer tissues like mesenchyme grow interstitially.



Figure 6.13: Interstitial growth in an epithelium can cause budding or invagination.

The growth of a tissue is regulated by hormones, such as Growth Hormone, which coordinates the rate of growth of tissues body-wide. On the other hand, local-acting morphogens cause small regions to grow faster than neighboring regions. To make room for new cells while still maintaining a single-layer of cells connected to their neighbors by desmosomes and tight junctions, an epithelium bulges inwards or outwards if it can't grow wider. An outward growth is known as a **bud**, and an inwards growth is an **invagination**. Arms and legs grow from buds, while teeth, hairs and the brain grow from invaginations. These are both forms of interstitial growth.

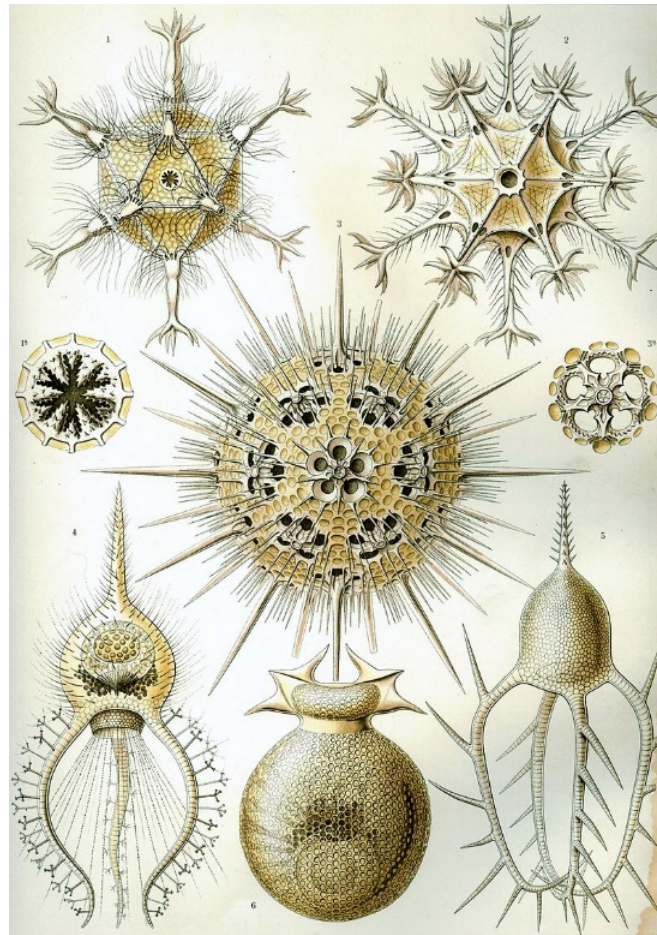


Figure 6.14: An example of different morphs (shapes) of the same basic pattern in dinoflagellates. Images credit: Kunstformen der Natur (1904), plate 1: Phaeodaria, by Ernst Haeckel is in the Public Domain CC0

Morphogenesis

Morphogenesis is the appearance of new shapes in an organism. It means to go from no shape to some shape. The word **morphology** can mean the study of these shapes, or be used as an adjective to mean the shape of something (For example, a cleft palate is a change to the healthy morphology of the palate.) From the zygote to the blastula stage (sometimes referred to as the germinal stages) the ball of cells is amorphous, it has no shape (other than being a sphere). At the start of gastrulation, however, the embryo undergoes numerous morphological changes. New shapes arise in the developing embryo. It develops an inside and an outside, a tail end and a head end, soon it develops a left and right, and a front and back. After that, a nervous system and a circulatory system become visible. How long do you think it takes before teeth develop? The answer may surprise you.

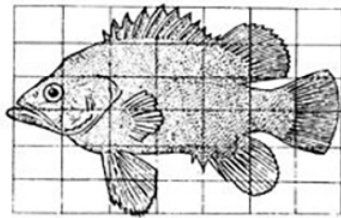


Fig. 150. *Polyprion*.

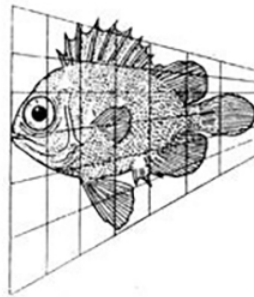


Fig. 151. *Pseudopriacanthus altus*.

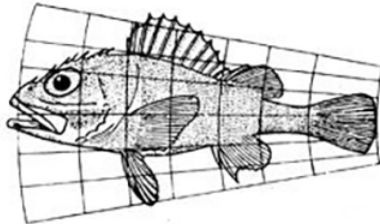


Fig. 152. *Scorpaena* sp.

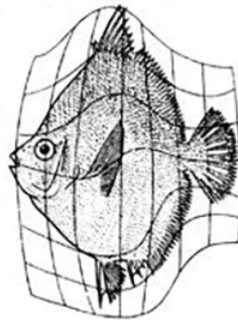


Fig. 153. *Antigonia capros*.

Figure 6.15: D'Arcy Wentworth

Thompson proposed that what may look like different shapes in related species may be *transformations* of one basic pattern. Image credit: "Fig 150-153" by D'Arcy Wentworth Thompson, On Growth and Form is in the [Public Domain, CC0](#)

Fig. 6.14 is a famous drawing of tiny sea creatures with mineralized exo-skeletons. This image was later analyzed by a mathematical biologist named [D'Arcy Wentworth Thompson](#), who noticed different biological shapes can be different transformations of the same shape. Transformations, he proposed, occurred by different localized rates of growth— something our current knowledge of morphogen signaling supports— as he illustrated in Fig 6.15. He is regarded as the first person to describe morphogenesis. When Thompson looked at the tiny sea creatures, he didn't see different shapes, he saw changes to one shape. These changes happened over evolutionary time, and led to different-looking sea creatures (who shared a single lineage). In this book, we look at different body parts that share the same lineage but change over developmental time, such as hair follicles and tooth buds. Thompson's ideas were expanded upon by the mathematician Alan Turing, who coined the term morphogenesis. His other achievements include inventing computer science and breaking the Nazi code during WWII (with the help of [many female mathematicians](#) at Bletchley park). Sadly, he committed suicide after his arrest, conviction and imprisonment for homosexuality by the British government he helped save. Their government's view on homosexuality has developed since then, we can understand the current view better by following its lineage.

Morphogenesis and genetics

To have a well-rounded understanding of how morphogen signaling works, it helps to understand some concepts of genetics. When we say understand, we don't mean memorize the specific details, focus on the concepts. First, pay attention to when we discuss a morphogen, we likely mean the signaling molecule itself. We at times discuss the morphogen *signal*, and that extra word *signal* makes a big difference. Mutations that alter morphogen signals include mutations to the gene for the *extra-cellular* morphogen molecule itself (if it is a protein such as FGF and BMP) or enzymes that synthesize the morphogen (as is the case with Retinoic Acid), *cell-surface* receptors for the morphogens, *cytoplasmic* second messengers and *nuclear* transcription factors. Discussing morphogen *signaling levels* saves us from the complexity of including all possible targets. Next, some mutations might be **gain-of-function**, such as a mutation to a receptor that prevents it from shutting off, and these are often inherited in an autosomal dominant fashion (it only takes 1 faulty [allele](#) to create a signal where it shouldn't be). Alternatively, **loss-of-function** mutations prevent proteins from functioning properly, and are often inherited in an autosomal recessive pattern (it takes both copies to be faulty to lead to a loss of a signal where it should be, one good allele would be enough). Some mutations fully stop, or fully start, morphogen signals. and in such cases we would say these mutation have complete penetrance. Other mutations might partially block or partially start morphogen signals, and have [partial or incomplete penetrance](#), the severity of the symptoms fall on a spectrum.

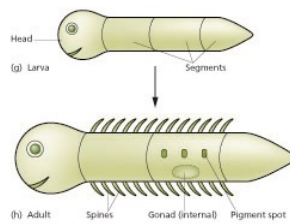


Figure 6.16: Maturation involves the changing of already present patterns. Generalized scheme of embryonic development, by JMW Slack is licensed CC BY SA 4.0

Maturation

Maturation is the process by which new shapes created by morphogenesis continue to develop and take on a more mature form. By the end of week 9, all of the basic organ shapes have formed, and we say the embryonic period is over and the fetal stages begin. The fetus undergoes maturation, and maturation involves bits of the previous processes (another way of saying that is maturation recapitulates other developmental processes).

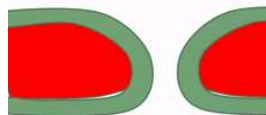


Figure 6.17: Illustration of the fusion of two separate structures and their tissues.

Fusion

Sometimes, two separate bits of the same type of tissue come into contact with one another and **fuse** into a single tissue. For an epithelium, this requires the cells share the same type of trans-membrane protein known as a cell adhesion molecule. For a connective tissue, this requires the correct integrin binding to fibronectin. In Fig 147, the basic pattern of green being on the outside and red in the middle is initially disrupted by the fusion of two structures. There are three ways to remove green cells from the middle area. The first way is for cells to receive **guidance cues** and cause cells to move. Some guidance cues are repulsive, such as the lack of correct cell-to-cell contacts, or high concentrations of a repulsive morphogen. Other guidance cues are attractive, such as the correct cell-to-cell contact or a positive guidance morphogen. Whether a cell-to-cell connection or morphogen is attractive or repulsive depends on how the target cell has been programmed (what receptor genes it is expressing). Cells in the incorrect location can also be induced to undergo apoptosis (see below). Lastly, another way to re-organize cells is to induce them to **de-differentiate** (revert to a stem cell) and differentiate into the correct cell type. This is called a transition; both early development and tooth development involve examples of epithelial-to-mesenchymal transitions and mesenchymal-to-epithelial transitions.

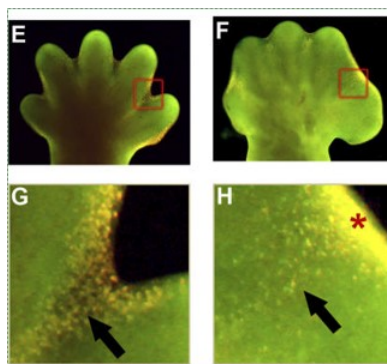


Figure 6.18: Growth of fingers occurs by apoptosis of regions of a hand plate, guided by morphogen gradients. Image credit: [Depletion of BMP Signaling Causes Interdigital Syndactyly](#), by Bandyopadhyay A licensed CC BY SA 4.0

Apoptosis

In the construction of tall buildings or large ships, scaffolding is built first to allow the construction workers to get where they need, then removed after construction is complete. Similarly, during development, apoptosis is an integral part of the construction of body parts. Many tissues grow more cells than are needed in the mature form. To undergo maturation, un-needed cells undergo apoptosis. For instance, during the growth of bone tissue, extra cartilage tissue is made to act as a scaffold for osteoblasts. Shown in

Fig. 6.18, the hands and feet start off as **paddles**, and only with apoptosis in alternating regions does morphogenesis of fingers and toes occur. Without enough apoptosis, people are born with webbed fingers or toes, or suffer ankyloglossia (their tongue is rigidly anchored to the floor of the mouth by a pronounced lingual frenulum).

Early development

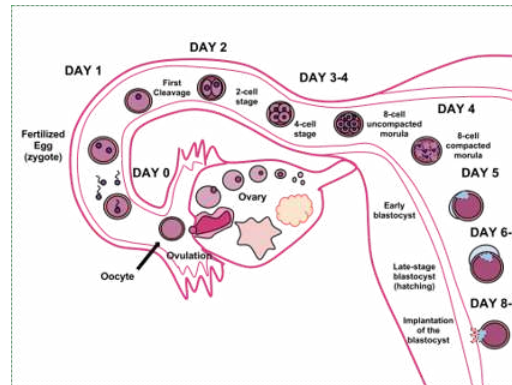


Figure 6.19: The pre-implantation period generally occurs in the fallopian tube. Image credit: Fertilization in humans, by Ttrue12 is licensed CC BY SA 3.0

Pre-implantation period

The **pre-implantation period** begins with fertilization, spans a week or so of development, and ends with an embryo implanting into the uterus. **Fertilization** is the union of one sperm cell and one egg—thousands of sperm are needed, but only one is allowed into the egg. This union joins 23 maternal chromosomes with 23 matching paternal chromosomes, forming a **zygote**. Fertilization most often occurs towards the distal end of a fallopian tube. Over the next week the zygote undergoes mitosis and increases in cell number, but it does not grow in size. All of the amino acids and nucleotides for making new cells during this first week come from the cytoplasm of the zygote, which is why the egg is a very large cell. After implantation, the uterus provides the raw materials for an embryo to grow larger.

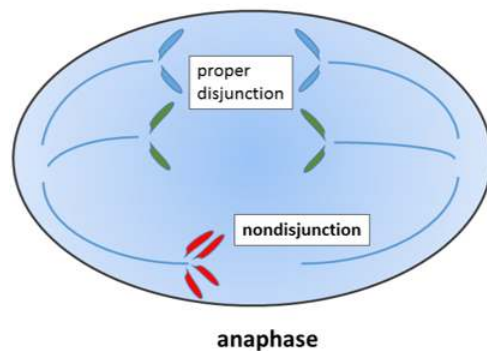


Figure 6.20: Errors separating chromosomes during mitosis generally triggers cell-cycle arrest. Image credit: Non-disjunction of sister chromatids, by Wpeissner is licensed CC BY 3.0

During mitosis, the 23 pairs of chromosomes are duplicated and divided between 2 daughter cells evenly. If mistakes are made and an odd number of chromosomes wind up in a daughter cell, this fails a cell cycle checkpoint and triggers apoptosis. If a chromosome breaks, it can lead to the same result.



Figure 6.21: Cells with an extra copy of chromosome 21 can survive and develop. Image credit: Boy with Down Syndrome using cordless drill to assemble a book case, by Rob Kay is licensed CC BY SA 3.0

There are exceptions, however. A cell with an odd number of chromosome 21 or 23 can survive. If that cell is the egg, then the zygote and all subsequent cells will have an odd number of chromosomes. This leads to congenital disorders such as Trisomy 21. If the chromosomal segregation error happens at the 2-cell stage of development, then only half of the cells in the adult would have an extra chromosome, and the condition would be less severe. Extra chromosomes interfere with decision-making processes in development. People with Trisomy 21 frequently develop microdontia, and may suffer from hypo-salivation and bruxism. They therefore need extra care maintaining healthy oral hygiene.

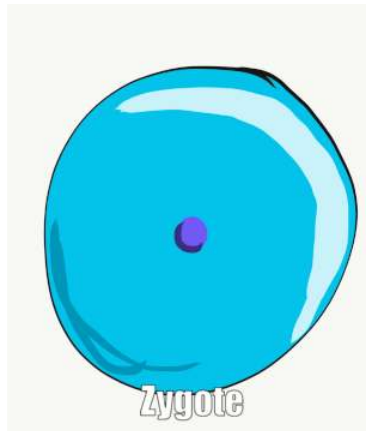


Figure 6.22: The early cell divisions produce identical cells, from zygote to blastula stage,

Early divisions

During the first week of development, the zygote undergoes 3 **cleavage divisions**, where mitosis occurs in a synchronized fashion ([see a cool video here](#)). The cells duplicate along a single plane, 3 times, producing an 8-cell embryo. Synchronizing mitosis requires cell-to-cell communication between gap junctions to coordinate cell cycle checkpoints. Synchronized mitosis leads to the embryo developing evenly. The fact that multiple cells are dividing in the same direction means these cells have some polarity, even if the embryo as a whole has no visible top or bottom yet. As mitosis continues, the number of cells continues to double, but the cells do not divide along the same plane. This solid ball of cells is called a **morula**. As mitosis continues, the cell count doubles and the cells continue to get smaller, until the solid ball of cells becomes a hollow ball of cells named a **blastula**. From the zygote to the blastula stage, none of the cells appear any different except in size. A fancier way to say this is that there are no changes to morphology. During the blastula stage, a group of cells named the **embryonic disc** move away from the others. The fate of the embryonic disc is to become the embryo, while the outer cells, known as the **trophoblast**, are fated to become extra-embryonic structures such as the placenta. The blastula implants in the endometrium about a week after fertilization and continue developing.

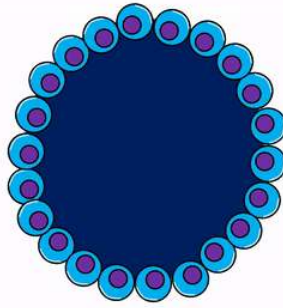


Figure 6.23: Illustration of gastrulation and differentiation of the three basic germ layers

Gastrulation

It is not birth, marriage, or death, but gastrulation, which is truly the most important time in your life.—Lewis Wolpert.

Gastrulation overview

During **gastrulation**, some cells invaginate, forming a layer of cells on the inside and a layer of cells on the outside of the embryo. The significance of this is that this is the first instance where embryonic cells are different from one another. But, wait, if all the blastula cells are identical, why did only *some* of them migrate? Mitosis does indeed generate 2 cells with identical DNA, but not necessarily identical *cytoplasm*. If an egg has mRNA for a transcription factor clustered into one spot in the cytoplasm, the first cleavage division produces two non-identical cells, one with the transcription factor mRNA and one without. Later, when the mRNA is translated, it could induce that cell to migrate inwards, while other cells stay on the outside. This is a maternal effect, because the mRNA for the transcription factor is not transcribed from the zygotes' DNA, but is made by mom's ovary cells and deposited into the egg. Let's return to gastrulation: the end result of gastrulation is an embryo with two layers (outside and inside). Imagine holding a tennis ball and pushing your thumbs inwards, forcing some of the outer rubber to fold inwards. The tube that forms becomes the gastrointestinal tract, and the opening (can you guess?) becomes the anus. The embryo therefore now has a distinct tail direction and head direction. Fig. 6.23 represents the way a sea urchin gastrulates. Human embryos look different because it is only the embryonic disc that undergoes gastrulation, not the entire blastula. But the 2 layers are the same, human gastrulas simply look more squashed. Oh, and sea urchins don't form anus-first. The reason sea urchin gastrulation looks simpler than human gastrulation is that human blastulas reserve some cells (the trophoblast) to form a placenta and amnion, while sea urchins dump their eggs into the sea. We are sacrificing a little accuracy here to focus on important concepts shared across species. If you are curious, watching movies of these processes [in humans](#) is not possible, but it is for organisms like [xenopus laevis](#) (frogs).

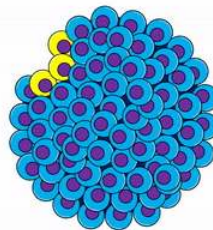


Figure 6.24: Superficial view of ectodermal cells undergoing gastrulation at Hensen's node, which travels in an anterior -to-posterior direction, creating the primitive streak.

Next, cells from the outer layer migrate between the two layers. This gives the embryo 3 layers, which are the 3 **embryonic germ layers** (Fig. 6.23). The outer layer of **ectoderm** and the inner layer of **endoderm** remain epithelial in appearance, while the middle layer of cells (**mesoderm**) undergoes a morphological change, and becomes mesenchyme. This migration begins at a single location called **Hensen's node**, which moves down the embryo forming the **primitive streak** behind it (Fig 6.24). The side of the embryo with the node is fated to become the dorsal side, and the streak divides the embryo into a left-half and a right-half. You have now witnessed, albeit in cartoon form, how something (all the basic body directions) arose from nothing. Don't let the significance of this sneak past un-noticed. Scientists have a hard time explaining what there was before the universe formed, or how the Earth went from having no life to having life. Embryologists, by contrast, explain how embryos change from having no shape to having shape in great detail (we've had the benefit of directly watching and manipulating this process).

3 embryonic layers	Cell fate
Ectoderm	Epithelium of skin and oral mucosa, neural tissue
Mesoderm	Connective & muscle tissue
Endoderm	Epithelial lining of hollow organs

Table 6.2: The three germ layers develop during gastrulation

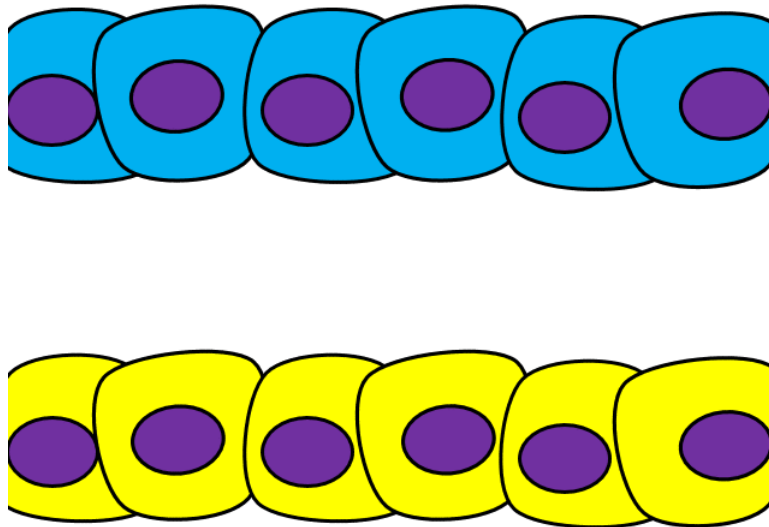


Figure 6.25: During gastrulation, an epithelial-to-mesenchymal transition creates the mesoderm layer.

Epithelial-to-mesenchymal transition

The process by which ectodermal cells quit being ectoderm and migrate to the middle and form mesoderm is called an **epithelial-to-mesenchymal transition** (EMT). The epithelial cells of the ectoderm must first lose their cell-to-cell contacts with neighboring cells, which makes them less-epithelial. They lose their polarity, de-differentiate into a stem cell, then differentiate into mesenchymal stem cells. This process is not only required to form mesoderm during gastrulation, it occurs during neural crest migration, wound healing, and cancer metastasis. Cells in the mesoderm (the name for the middle layer) form mesenchyme (a type of tissue), while the other two layers remain epithelial. There is a reverse process, called a **mesenchymal-to-epithelial transition** (MET), which also occurs during wound healing. EMT allows epithelial stem cells from a healthy region of oral mucosa to turn into mesenchymal stem cells, migrate away from the healthy region into a damaged region. MET then allows the mesenchymal stem cells to turn back into epithelial stem cells, which divide and differentiate into new keratinocytes. Because this mimics what happens during gastrulation, we say “wound healing **recapitulates** (states again) development”.

Differentiation of ectoderm

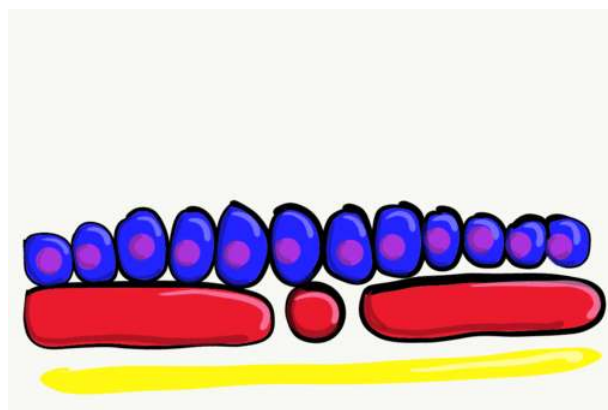


Figure 6.26: Illustration of neurulation from a cross section of an embryo. Legend: blue = ectoderm, red = mesoderm, yellow = endoderm, light-blue = neuro-ectoderm, green = neural crest cells.

Neurulation

Gastrulation gave the embryo its first polarity, as well as the beginning of the GI tract. One of the next organ systems to develop is the central nervous system. The basic processes used in **neurulation** are re-used when other hollow organs form. First, underlying mesoderm known as the **notochord** sends morphogens to nearby ectodermal cells. This induces the nearby region of ectoderm to differentiate into **neuro-ectoderm**, followed by local proliferation. Because these epithelial cells cannot easily spread side-to-side, they invaginate. Ultimately, invaginating neuro-ectodermal cells lose their contacts with the ectoderm and fuse to other neuro-ectodermal cells, creating a new structure called the **neural tube**. This tube develops into the brain and spine. If you are wondering about the notochord, its cell fate is to mostly undergo apoptosis, although some remains as the nucleus pulposus of the vertebral discs. In tiny fish-like chordates with no backbone called amphioxys, the notochord persists. This won't be the last time you see a human embryo make a structure that goes away or changes entirely.

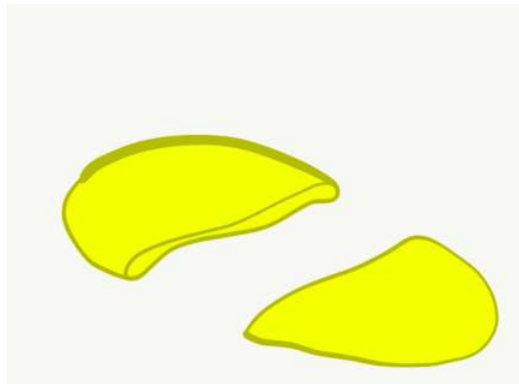


Figure 6.27: A second illustration of neurulation, focusing on the anterior-to-posterior direction of neural tube folding. Legend: Blue = ectoderm, light-blue = neuro-ectoderm, red = mesoderm, yellow = endoderm.

The neural tube begins folding from the anterior portion of the embryo, and zips up in an anterior-to-posterior direction. The zippering is not perfect, the anterior end is also growing wider as it is folding to form the primitive brain. For all of the developmental processes we do and don't cover, the anterior end is usually the region that develops first, followed by more posterior regions. Zipping the neural tube requires adequate levels of folic acid, so it is important that mother's melanin has protected this vitamin, otherwise incomplete closure of the neural tube may result (spina bifida).

Figure 1. The sites of origin, migration, and arrival of cranial neural crest cells. (A) Embryonic neural tube showing the mesencephalon, metencephalon, and rhombomeres, with the dorsal face of tube coloured to show the location of neural crest before migration. (B) Sagittal view of embryo, showing paths of migration of cranial crest cells. (C) Sagittal view of adult human, showing the origins of various cranial crest derivatives.

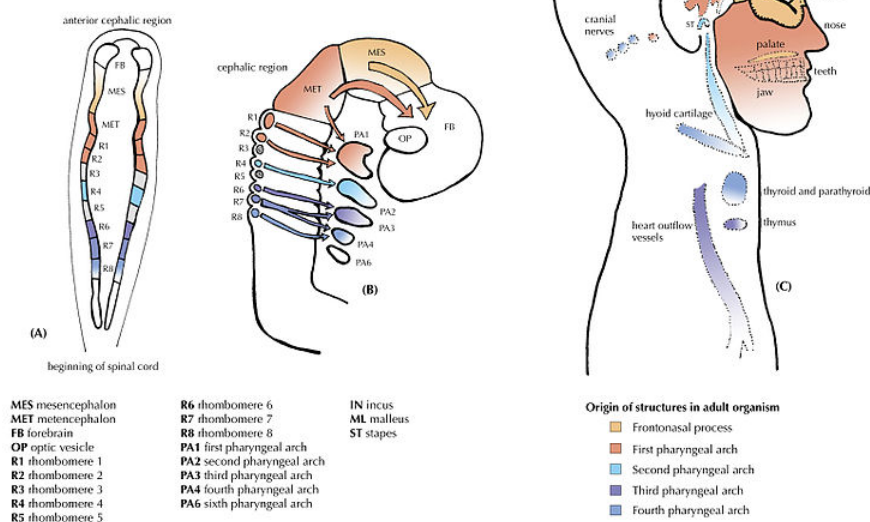


Figure 6.28: Origin of cranial neural crest cells and their target migratory locations. Images credit: "Cranial Neural Crest Cells – migration.jpg" by BiolCNC is licensed under [CC BY-SA 3.0](https://creativecommons.org/licenses/by-sa/3.0/)

Some of the neuro-ectoderm cells do not fuse. Instead, these cells undergo an epithelial-to-mesenchymal transition and migrate away from the neural tube. These cells are **neural crest cells**, they are fated to become a number of important cells and tissues throughout the body including melanocytes, odontoblasts, dental pulp, cementoblasts, and the neuro-mesenchyme of the pharyngeal arches. These cells are sometimes referred to as the fourth embryonic tissue (in addition to the three that arise during gastrulation), which suggests some people think neural crest are important. Even after neural crest cells migrate to new and distant tissues, they often retain visible signs of their neural lineage, such as the dendrites on melanocytes or the odontoblastic process of odontoblasts. To make migration easier, neural crest cells express an enzyme called a **matrix metalloproteinase** (MMP). This enzyme digests proteins found in the extra-cellular matrix. By now we hope remember the phrase *wound healing recapitulates development*, matrix metalloproteinase enzymes are re-used to heal certain type of **tooth wounds**. When neural crest cells reach the pharyngeal arches, they secrete the morphogens FGF and BMP, which antagonize each other, producing a striped pattern along the ectoderm. Some regions are induced to form tooth buds, the in between regions produce oral mucosa.

Differentiation of mesoderm

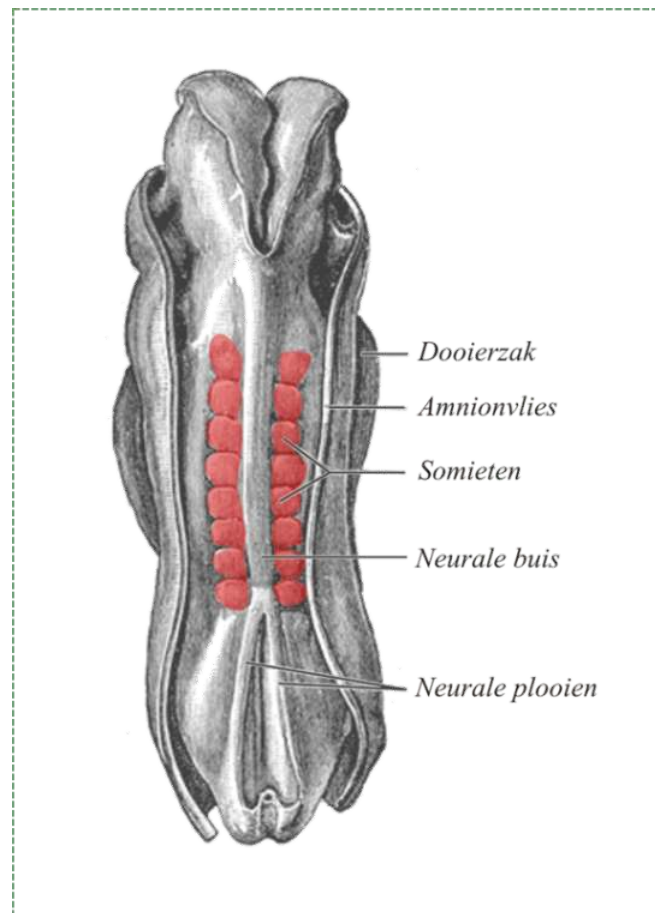


Figure 6.29: Somites in a neurula-stage embryo. Image credit: Plate 20, by Henrey Grey is in the Public Domain CC0

Somite formation

During the same time as neurulation, mesoderm undergoes differentiation. Mesoderm starts off as an amorphous layer of mesenchyme. Then, regions of mesenchyme pinch off in repeating spherical structures called **somites**, forming segments along the anterior-to-posterior axis of the embryo. The fate of the somites is to become solid organs, either repeating units of connective tissue such as the ribs and vertebrae, or repeating units of muscle tissue such as the rectus abdominus and intercostal muscles. The formation of each somite involves a mesenchymal-to-epithelial transition, some mesenchymal stem cells differentiate into an epithelium that separates one somite from the next.

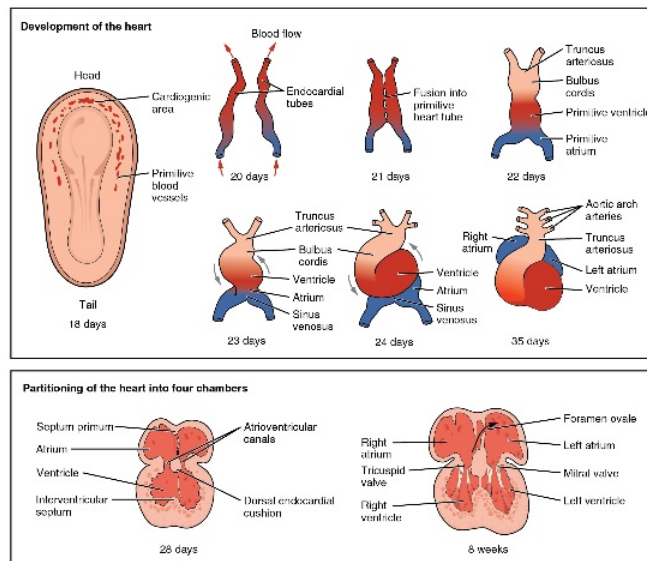


Figure 6.30: Development of the heart from two heart tubes. Image credit: illustration by OpenStax, is licensed CC BY 3.0

Formation of the heart

In the third week, mesoderm also begins to develop into blood, the heart and the circulatory system. Mesenchymal stem cells first differentiate into blood islands. Next, angiogenesis begins. Two large blood vessels fuse– with some help from neural crest cells– to form the primitive heart, which begins beating.

Differentiation of endoderm



Figure 6.31: Different embryonic stages highlighting the endoderm. Legend: yellow = endoderm, red = liver, green = gall bladder. Image credit: “Time line of mouse liver development emphasizing it” by Zorn, A.M., [Liver development](#), [StemBook](#) is licensed under CC BY 3.0

Formation of the pharynx

The endoderm that migrates interiorly during gastrulation extends its way towards the anterior end of the embryo. This forms the **primitive foregut**, which later becomes the pharynx, oral cavity and nasal cavity. At this time, however, there are no openings for the mouth and nostrils.

Figure 6.32: You, negative 8 months old.

4th week

We are now only up to the 4th week of development. A lot has happened! At this time, the embryo continues to undergo local regions of proliferation, patterning, fusion and the other processes covered at the beginning of the chapter. This leads to the formation of rudimentary (basic) structures. When a structure first becomes visible from the surface of an embryo, it is often called a **placode** (an ectodermal thickening). The cells in a placode are fated to become an organ, but their overall shape is no more than a raised bump or shallow pit. By the 4th week, head-related organs listed in Table 6.3 begin to develop.

Structure	Fate
Eye placodes	primitive eyes
Otic placodes	primitive ears

Nasal placodes	primitive nose
Pharyngeal arches	so many things they get their own chapter
Oro-pharyngeal membrane	primitive mouth (not open yet)

Table 6.3: primitive structures formed during the 4th week of development

Clinical applications of early development

Disturbances in these early stages of development are usually so severe the embryo does not survive. However, clinical conditions exist related to these early stages and to oral health.

Figure 6.33: An example of body plan duplication. Image credit: “Polydactyly 01 Lhand AP.jpg” by Drgnu23 is licensed under [CC BY-SA 3.0](#)

Homeobox gene mutations

Mutations to homeobox gene can cause *entire structures* to be missing, or to develop in the wrong location. Early experiments on flies led to observations of legs where antennae should be located, or antennae where wings should be located. There are a few homeobox-related genetic disorders in humans. [Homeobox-related disorders](#) are rare because the mutations are usually lethal, but when they aren’t the conditions are often severe. A small list is found in Table 6.4

Syndrome	Homeobox gene involved	Symptoms include
Axenfeld-Rieger Syndrome	FOXC1, PITX2	Mostly eye-related, sometimes hypodontia and microdontia
Autosomal dominant syndactyly	HOXD13	Syndactyly (fused fingers or toes)
Guttmacher syndrome	HOXA13	Polydactyly (extra fingers or toes)
Ectodermal dysplasia	HOXC13	<i>See below</i>

Table 6.4: A few examples of homeobox mutations in humans.

Figure 6.34: Lack of apoptosis leads to syndactyly. Image credit: “Hand of a child with Apert syndrome.” by Gzzz is licensed under [CC BY-SA 4.0](#)

Apert syndrome

Apert syndrome is caused by a mutation in the receptor for the morphogen FGF. It is categorized by a wide range of symptoms, including cranial deformations and syndactyly. FGF is involved in the formation of the pharyngeal arch apparatus (covered in the next chapter), which explains the craniofacial abnormalities. Relevant to this chapter is the ability of FGF (like many *growth* factors) to inhibit apoptosis. Having a mutation that causes an FGF receptor to be on all the time inhibits apoptosis in the hand and foot paddles. Regions of apoptosis are required to produce fingers and toes, hence this mutation leads to syndactyly. Partial disturbances to FGF and BMP signals can lead to partial syndactyly, or webbing of the fingers or toes.

Figure 6.35: Figure 185: “Michael Berryman, actor” by Stefan Borggraeffe is licensed under [CC BY 4.0](#)

Ectodermal Dysplasia (part 1 of 2)

Problems with the induction of neural crest cells during neurulation leads to disturbances in the formation of teeth, hair follicles, salivary glands and other structures. What these have in common is they are all specialized structures of the ectoderm, induced by neural crest cells to differentiate. In a healthy embryo, ectodermal cells receive morphogens which activate or inactivate the correct transcription factors to trigger differentiation into neural crest cells. Neural crest cells migrate to distant regions of the body, determine their location by interacting with morphogens in the ground substance, and release other morphogens to induce regions of ectoderm to differentiate into sweat glands, salivary glands, tooth buds or hair follicles. Mutations in any of the genes for these morphogens, their receptor proteins, or down-stream transcription factors may lead to a condition named **Ectodermal Dysplasia**. This group of syndromes is rare, with only 7,000 cases worldwide, but there are at least 40 different genes have been implicated in these syndromes. Compare that to Sickle Cell Disease, which currently affects over 100,000 *Americans* (predominantly African

Americans, Hispanic Americans, Greek Americans, Turkish Americans and Italian Americans), all due to mutations in a single gene, Hemoglobin-Beta. The point of this comparison is to highlight *when* these genes are expressed. The induction of ectodermal stem cells to proliferate and differentiate into different appendages is complex and occurs during embryogenesis. Mutation in the morphogens, the morphogen receptors, the second messengers or transcription factors and downstream genes that are activated to induce differentiation are all possible targets that cause ectodermal dysplasia. Mutations in these genes in a neural crest cell leads to a disruption in any of the subsequent cells induced by this new cell type, similar to the way tackling the ball carrier in soccer (football) disrupts the gameplay of any of his or her potential passing targets. By comparison, only red blood cells express hemoglobin-beta, and they are terminally differentiated cells, they do not become any other cell type. Obviously red blood cells are an important cell type, one that can be found early in embryogenesis, but the symptoms of Sickle Cell Disease present less of a spectrum than the types of diseases we have been discussing, such as Ectodermal Dysplasia.

Figure 6.36: Gaten John Matarazzo III, actor and CCD activist. Image credit: Gaten John Matarazzo III, by Gage Skidmore, is licensed CC BY SA 3.0

Cleido-cranial Dysostosis

Cleido-cranial dysostosis (CCD) is a congenital disorder caused by a mutation to a transcription factor required for the differentiation of bone and teeth. It is required to trigger osteo-chondro-progenitor cells to exit the cell cycle and differentiate into osteoblasts. It is also re-used to induce the differentiation of odontoblasts. Furthermore, after teeth have formed, this transcription factor is re-used to activate the expression of a Matrix metalloproteinase enzyme, which is necessary for remodeling of the alveolar sockets. Without this enzyme, retention of deciduous teeth occurs. Dental implants or dentures (such as the ones the actor and CCD-philanthropist Gaten John Matarazzo III received in Fig. 6.36) are the preferred treatment. In addition, a person with CCD may have small clavicles and changes to shape of the skull– bones that form by intra-membranous ossification. This illustrates two major concepts in development. First, many structures form one way, but are remodeled later to serve a different function (teeth form by folding inwards, they later move outwards). Secondly, many different patterns in embryology are re-used (recapitulated), such as the removal of tissue during neural crest migration and the removal of tissue during tooth eruption.

Figure 3, by Beatriz A. Ro Isdas-Junco et al, is licensed CC BY 3.0

Stem cell therapies

When cells terminally differentiate, they permanently inactivate un-needed genes by methylation and storage around histones. Researchers are learning ways to [reverse this process](#), and guide differentiated cells to revert to a stem-cell state. This raises the possibility of promoting regeneration of tissues that [do not otherwise regenerate](#), removing the need for tissue grafting or transplantation. Because some dental tissues do not regenerate well, there is potential for these technologies to be applied to the oral cavity, such as growing biological [dental implants](#) instead of using metals and ceramics. However, more interest has been placed on acquiring mesenchymal stem cells [from dental tissues](#). For instance, stem cells isolated from maxillary third molars have been used in clinical trials to improve healing and reduce the need for transplanted tissue in [maxillofacial surgery](#). Because of their potential to differentiate into a wide array of cells, great interest has been placed on collecting dental stem cells to treat diseases unrelated to the oral cavity. With the correct morphogen and plenty of dental stem cells, it may be possible to reverse the damage caused by neurodegenerative disease such as Alzheimer's Disease (AD) and Parkinson's Disease (PD), spinal trauma, myocardial infarct (heart attack), and Muscular Dystrophy (MD). The lineage of the cells that produce dentin, pulp, cementum and the periodontium will help explain the link to neurodegenerative disorders. What else will you do with extracted third molars? Unfortunately, there is no legal definition in the U.S. for what constitutes safe, effective stem cell therapy. Even if good rules are put in place, [medical-tourism](#) does not abide. In addition to honest and good research in stem cell therapies (the [Institute for Stem Cell and Regenerative Medicine](#) at the University of Washington is a good example), there are a large number of dubious-to-downright-quack stem cell clinics operating in the [grey market](#), using [risky, untested methods and making absurd claims](#) to take as much money as possible from vulnerable people. So, who's who? Therapies that use or boost a patient's own stem cells have the best possible chance of providing positive outcomes with minimal risk of side-effects– especially if that therapy is targeted, such as by adding a specific morphogen to boost a specific result. On the other hand, throwing exogenous stem cells at a problem– cells from other people, other animals, or from non-animal sources– carries the biggest risk of serious adverse effects with minimal potential for benefit. Keep in mind that 19 out of 20 (or so) potential drugs that are shown scientifically to work in animal models fail in human trials. That means there is scientific evidence in publication for treatments that we know don't work, but other people can cherry-pick the positive information and use it to sell a product.

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1.7: Pharyngeal Arches

- Overview of the pharyngeal arches
- Development of the external structures
 - Fronto-nasal process
 - 1st pharyngeal arch
 - maxillary process
 - 2nd-6th pharyngeal arch
 - Pharyngeal grooves
 - Pharyngeal pouches
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 - Pituitary gland
 - Salivary glands
 - Palate
 - Tongue
 - Tonsils
- Clinical application of pharyngeal arch development
 - Cleft lip/palate

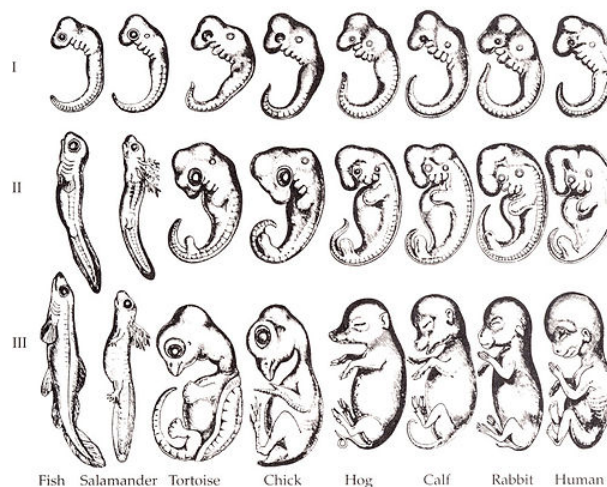


Figure 7.1: The mechanisms of early development are highly conserved, as seen in the similarity of these vertebrate embryos. Image credit: [Embryo drawings](#), by Ernst Haeckel, is in the Public Domain CC0

Overview of the pharyngeal arches

Ontogeny recapitulates phylogeny —[Ernst Haeckel](#)

Nothing in biology makes sense except in the light of evolution—Theodosius Dobzhansky

The human embryo is taking shape. It might seem odd that we look a bit wormy at this early stage, and next we morph into something fishy in appearance. The phrase *ontogeny recapitulates phylogeny* is hallowed among developmental biologists. It means, more or less, our early embryonic stages look like our evolutionary lineage. That is what the famous drawing (Fig. 7.1) shows: trace our evolutionary lineage (over millions of years), it looks like our embryonic lineage (the 9 months before birth). In this chapter, we see the human embryo grow gills arches, and re-shape those gill arches into other structures: a mandible and maxilla, ears, and salivary glands (now that we don't have easy access to saline to lubricate every bite of food). Why not just form a mandible and ears, why go through the middle gill arch step? Why do we begin resembling worms, then fish, then only much later, little people? The better question, from the perspective of an evolutionary biologist, is why would we *stop* developing the way our ancestors did? Would that somehow help us avoid predation, have more offspring or have superior offspring? The answer is apparently either no, or its not worth the risks involved. Therefore, we rarely see new structures arise in evolution *de novo* (from

the beginning), instead we observe structures changing slightly over time. This leads to different species having similarly shaped structures with potentially different functions, or **homologues**. A bat's wing, a whale's flipper, a horse's leg and your arm have the same basic skeletal pattern: 1 bone, 2 bones, 4 bones (the thumb is different), and are referred to as homologues. Their size, shape and purpose are different, but you don't need an entirely new design, it is more efficient to morph a leg into a flipper. Some homologues have very different functions, such as human lungs and fish swim bladders. Conversely, the wings of a fly, chicken and bat, despite their similar function, are not homologous, nor is the panda's (6th) thumb a homologue of the human pollex. This all becomes apparent when you study the lineage of different species and compare it to their development.

Homologues also exist within a single species. That is why your arm and leg share the same skeletal pattern, they too are homologues. Sharing instructions for different structures in the human body cuts down on the amount of DNA that needs to be built and copied with each cell division. Small changes in DNA can lead to big changes in morphology. Therefore, it is both faster and takes less space to tweak a design that works than create a whole new design *de-novo*. Human development is rife with examples of recapitulation, where the same basic process is re-used with small changes.

Keep in mind we did not evolve from modern-day worms or fish, but the most recent relative a trout and ourselves share resembled the fish more than us, and the most recent relative a trout and worm share resembled the worm more than the trout. Now reverse that: our embryonic stages initially look strikingly like some sort of parasitic worm, then more fishy, then kind of lizardy, and finally mammally. Another way to say that is *ontogeny recapitulates phylogeny*.



Figure 7.2: Brachial arches supported by cartilage. Image credit "Gill arches supporting the gills in a pike" by Uwe Gille is licensed under [CC BY 3.0](https://creativecommons.org/licenses/by/3.0/)

If the link between of the evolution and development seems complicated, it is. The purpose of this overview section is to conceptually prepare you for a complicated series of steps that go through unexpected transitions. Evolution explains *why* the transitions happen, your job is to learn *what* those transitions are.

Before we start, however, we would like to point out one more level of complexity. You need to differentiate between structures that have similar names, including the pre-maxillary segment and inter-maxillary segment, the mandibular arch and maxillary process, the maxillary process and the inter-maxillary segment, the facial prominences and pharyngeal arches, pharyngeal grooves and pharyngeal pouches, and more. Furthermore, most of these structures have multiple names, such as the pharyngeal arches, gill arches and branchial arches, which can make comparing this text to others tricky.

If at any time you feel like you need more detail or a different description, let us remind you of this excellent web resource:

- The [Embryology Education](#) page
 - by Dr. Mark Hill at the University of New South Wales, Sydney, Australia

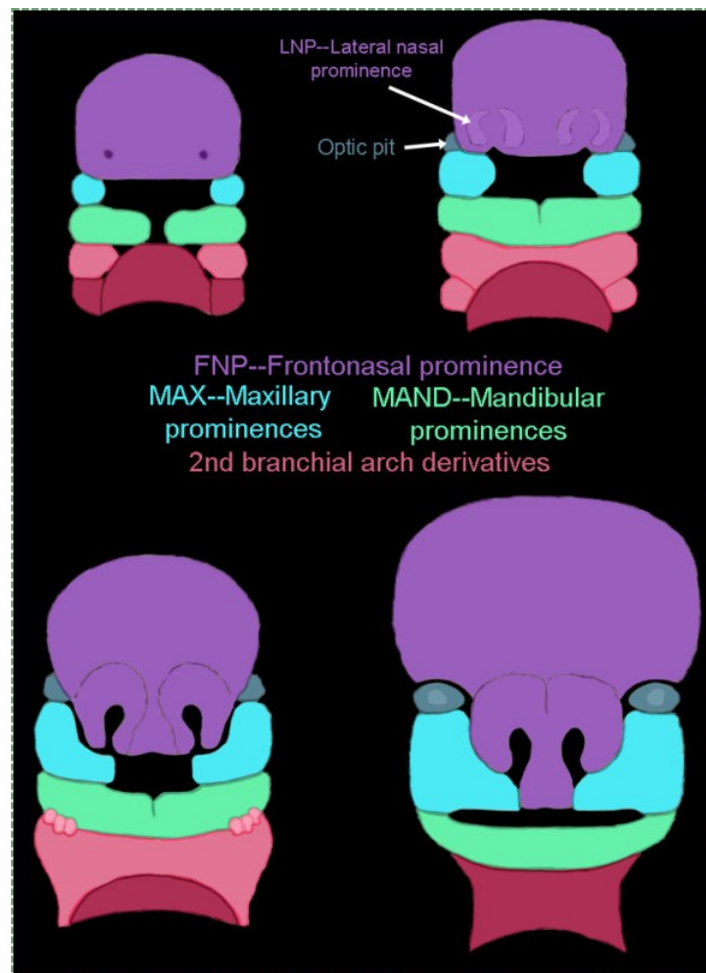


Figure 7.3: The pharyngeal arches, and the body parts into which they develop. Image credit: [Illustration of the seven facial prominences that give rise to specific regions of the face](#), by Kristina Aldridge, is licensed CC BY 4.0

Let us return our focus to human embryos. At 4 weeks, the face begins to develop. It is composed of several parts (or **prominences**), listed in Table 7.1. To use the word prominence means these are not necessarily the same type of thing, just things we can see. Some prominences are homologues of gill arches (gill arches are in Fig. 7.2, their human homologues are in Fig. 7.3 upper left). They are quickly remodeled (Fig 7.3, lower right). These **pharyngeal arches** (or branchial arches, although the name branchial arches should be limited to vertebrates with gills), are paired segmental bulges on the lateral borders of the primitive pharynx. Each pair of arches grows medially across the face (the ventral side) and fuses with its partner. One pair of *processes* (not arches) tries to fuse, but run into a bump called the fronto-nasal process, and fuse with it instead. If we were lampreys, the arches wouldn't fuse at all, and remain 6 gill arches. If we were bony fish, we would remodel 3 arches and have a mandible, inner ear structures and 3 gill arches. As humans, the pharyngeal arches are fated to become a number of cool things, including teeth, but not gill supports.

The facial prominences
Mandibular (1 st) arch
Maxillary process
Fronto-nasal process
Hyoid (2 nd) arch

Table 7.1: The facial prominences include two pharyngeal arches.

Development of the face (external)



Figure 7.4: Illustration of the pharyngeal arch apparatus at week 6, with a cutaway to show how they line the ventro-lateral walls of the pharynx. Note: the bumps around the pharynx are pharyngeal arches, and are not to be confused with the more caudal (posterior) bumps that are somites.

Formation of the pharyngeal arches

At the 4th week, the primitive gut has not fused with ectoderm, so no oral or nasal cavities exist. Where the mouth will form is visible as a depression in the ectoderm known as the **stomodeum** (the primitive mouth). A thin membrane, the **oro-pharyngeal membrane** (or bucco-pharyngeal membrane), separates the stomodeum from the end of the primitive foregut that becomes the pharynx. The primitive foregut is lined by endoderm.

The pharyngeal arches form under the instruction of homeobox genes. Activation of homeobox genes (in an anterior-to-posterior pattern) induces a program of other genes to be transcribed, including morphogens which induce growth of all 3 germ layers. This causes outward budding, creating **pharyngeal grooves** between each pair of pharyngeal arches on the outside of the embryo. On the inside of the primitive pharynx, opposite of the pharyngeal arches, localized growth forms invaginations known as the **pharyngeal pouches**. If you remember GAP, this mnemonic may help you to remember the names of these structures from external to internal: Groove, Arch, Pouch. Or, if you prefer the name pharyngeal cleft rather than groove, the mnemonic becomes CAP. These structures appear one pair at a time, from anterior to posterior, and their fate is listed in Table 7.2. Because the arches are quickly remodeled into other structures, we say that they are *transient* structures, but their brief existence explains how and why the adult structures in Table 7.2 have the morphology that they do.

Arch #	Name	Ectoderm and neuro-ectoderm fate	(associated groove)	Mesoderm and neuro-mesenchyme fate	Endoderm (pouch) fate
1 st	Mandibular arch	Maxillary process – > upper lip epidermis	n/a (this is not an arch)	Dermis, maxilla, zygomatic, palatine, vomer	n/a (this is not an arch)
		Lower lip epidermis, Trigeminal nerve (CNV).	External acoustic meatus	Dermis, mandible, malleus, incus	Eustachian tube
2 nd	Hyoid arch	Epidermis, Facial nerve (CNVII)	disappears	Dermis, most of the hyoid bone, stapes	Palatine tonsils
3 rd		Epidermis, Glossopharyngeal nerve (CNIX)		Dermis, the rest of the hyoid bone	Thymus, Parathyroid glands
4 th		Epidermis, Vagus nerve (CNX)		Dermis, Thyroid cartilage, epiglottis	Parathyroid, Thyroid glands

5 th		disappears			
6 th		Epidermis, Vagus nerve (CNX)	disappears	Dermis, the other laryngeal cartilages	Larynx tissues

Table 7.2: The pharyngeal arches and their fate, separated by the germ layers.

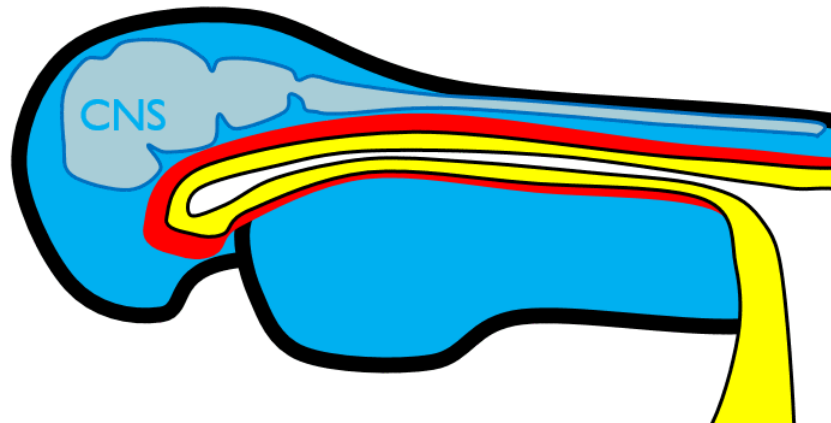


Figure 7.5: Growth of the paired arches, as well as budding of the maxillary process off the mandibular arch, animated from a lateral view, early week 4 embryo. Legend: blue = ectoderm, red = mesoderm, yellow = endoderm, arrows = neural crest cell migration.

The 1st pharyngeal arch

Next, neural crest cells guide modification of the pharyngeal arches. Neural crest cells first migrate from the head into pharyngeal arch mesoderm, undergo an epithelial-to-mesenchymal transition, and differentiate into special mesenchymal stem cells called **neuro-mesenchymal stem cells**. The neuro-mesenchymal stem cells release different morphogens which induce localized growth within the **mandibular arch** (the first pharyngeal arch), forming the **maxillary process**. The rest of the mandibular arch grows medially, as does the maxillary process, to form the upper and lower jaw. They grow above and below the stomodeum, which at this time is still not open to the primitive pharynx.

Tissue also grows on either side of the nasal placodes. Two growths in the middle, the **medial nasal processes**, fuse to form the **inter-maxillary segment** (or globular process). The growths on the sides form **lateral nasal processes**, which become the alae of the nose.

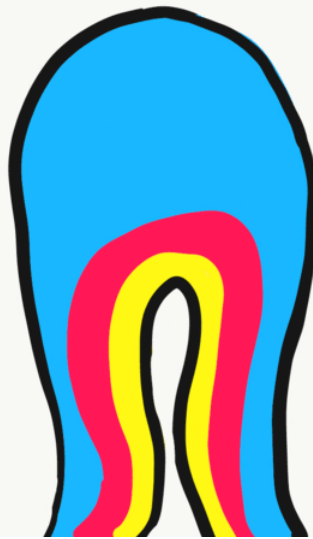


Figure 7.6: Fusion of the pharyngeal arches. Growth occurs in a lateral-to-medial direction, while fusion occurs in an anterior-to-posterior direction. Illustrated from the anterior view, early week 4. Legend: Blue = ectoderm, red = mesoderm or neuro-mesenchyme, yellow = endoderm

Fusion of the 1st pharyngeal arch

The mandibular arch forms on the lateral edges of the embryo during the 4th week of development and grows medially. The two halves fuse by the end of the 4th week of development, creating a single structure that becomes the mandible and nearby tissues. For the two halves of the mandibular arch to grow medially, mesenchyme is removed. This requires induction of the gene for the enzyme **hyaluronase**, which digests Hyaluronic Acid found in ground substance. This allows epithelial cells to fuse with epithelial cells from the other half of the arch. Fusion requires matching cell-adhesion molecules and desmosomes. The mesoderm of one arch also fuses with mesoderm of its partner, which requires matching the correct integrin to fibronectin.

Later, neuro-mesenchyme of the first pharyngeal arch differentiate into a cartilaginous structure known as **Meckel's cartilage**. Parts of Meckel's cartilage undergo endochondral ossification to become part of the mandible and middle ear bones, the rest undergoes apoptosis. The mandible forms from 4 separate parts beginning in the 10th week of development, which is more complicated than most bones. The body develops by intra-membranous ossification formed from the dense connective tissue derived from mesoderm. The rami (including coronoid and condylar processes) grow by endochondral ossification of Meckel's cartilage, derived from neuro-mesenchyme. This creates a cartilage-containing synovial joint between the ramus and temporal bones (the **temporo-mandibular joint**), and a fibrous joint between the two halves of the body which fully ossifies.

Fusion of the maxillary processes and inter-maxillary segment

The pair of maxillary processes grow medially in the 4th week, but run into the inter-maxillary segment and fuse with it by the 10th week of development. The upper lip, therefore, is formed of three parts (the left and right maxillary processes, and the inter-maxillary segment) while the lower lip just two (the left and right mandibular arches). The **philtrum** is the middle section derived from the medial nasal process. It does not serve a function in humans, it just happens to be there because of how we develop (kind of like the choice of wood used in the record player shelf). Many anatomy textbooks have a deep love of describing the functions of organs based on their adult form. Some that have tried to describe the function of the human philtrum based on its shape and location. They should learn to love development (**ontogeny**) and evolution (**phylogeny**)—anatomy makes more sense that way.



Figure 7.7: Closer view of the fate of the pharyngeal grooves (exterior) and pharyngeal pouches (interior). Legend: Blue = ectoderm, red = mesoderm or neuro-mesenchyme, yellow = endoderm

Fate of the pharyngeal grooves and pharyngeal pouches

If you were a fish, most of the pharyngeal grooves would develop into gills. As creatures of the land, grooves are either filled in or develop into other useful structures. Between the mandibular and hyoid arches, the first pharyngeal groove invaginates further and forms a tube that becomes the external acoustic meatus. It is lined by ectoderm. The other pharyngeal grooves disappear. On the opposite side, within the primitive pharynx, the pharyngeal pouches invaginate and grow towards the grooves. The first pharyngeal pouch elongates into a tube that is fated to become the Eustachian tube, connecting the pharynx to the middle ear (the internal acoustic meatus forms when bone tissue grows around cranial nerve VIII, connecting the inner ear to the brain). The Eustachian tube is lined by endoderm. The mesoderm and neuro-mesenchyme between these two tubes forms middle ear structures, including the malleus, incus and stapes bones.

The other pharyngeal pouches invaginate and form tonsillar and glandular tissue. Tonsils are lined by endoderm-derived epithelium, but the white blood cells of the germinal centers migrate there from bone marrow stem cells (which, like most connective tissues, are derived from mesoderm).

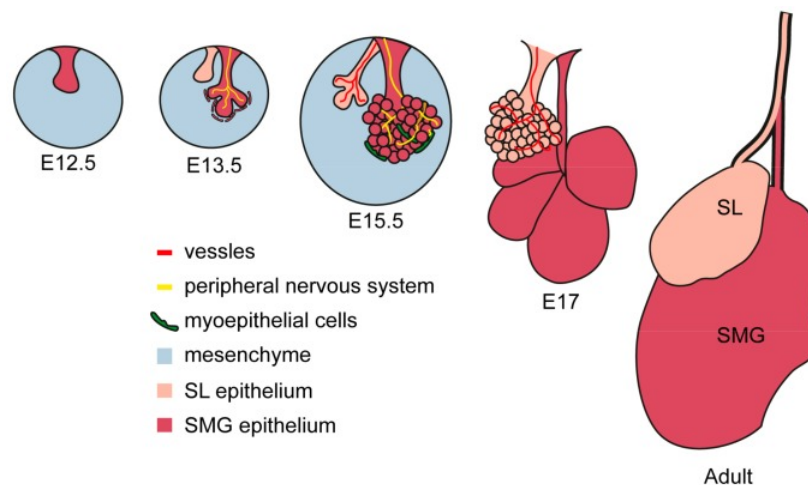


Figure 7.8: Development of sublingual (SL) and submandibular (SMG) salivary glands (in mice). Image credit: "Embryonic development of murine SMG and SL glands." by Cristina Porcheri and Thimios A. Mitsiadis is licensed under CC BY-SA 4.0

Development of the salivary glands

The salivary glands develop in a process that begins similarly to the formation of the teeth and neural tube. Placodes form on the ectoderm starting between weeks 4 through 12, under the control of morphogens including members of the FGF family. Salivary gland placodes grow and invaginate from there. Eventually, stem cells differentiate into a number of different epithelial cell types. The ducts are predominantly simple cuboidal epithelia. These epithelial cells have different functions based on their distal-to-

proximal location along the duct. The differentiation of salivary gland cells along a proximal-to-distal axis is guided by planar cell polarity morphogens, including members of the Wnt family. Some cells in the acini differentiate into myo-epithelial cells. Myo-epithelial cells are ectodermal by lineage, and therefore epithelial, despite looking and acting like smooth muscle cells. Myo-epithelial cells express genes mostly used only by muscle cells. This involves removing histones and DNA methylation from genes shut down in cells as they initially adopted an epithelial fate.

Development of the palate and other internal structures



Figure 7.9: Lateral view of an embryo, week 4, showing the opening of the mouth, division of the oral and nasal cavities, and the invagination of the pituitary gland.

Formation of the pituitary and mouth

Inside the stomodeum, a single invagination of ectoderm forms, along the medial portion of the roof (so far, all of the processes and pouches have been left/right pairs). This invagination is named **Rathke's pouch**. It grows and meets a downward budding of neuro-ectoderm. These two fuse to form the pituitary gland. The ectoderm forms the glandular half (adenohypophysis), and the neuro-ectoderm forming the infundibulum and neural half (neurohypophysis) of the pituitary gland. Rathke's pouch fills in as the two halves of the pituitary fuse, but it is possible a small depression will remain.

At the same time, the oro-pharyngeal membrane undergoes apoptosis. This connects the primitive pharynx and stomodeum, forming the primitive **oro-nasal cavity**. Finally, the mouth and anus are connected! The oral mucosa therefore develops from ectoderm of the stomodeum, while the pharynx and ventral surface of the tongue are derived from endoderm.



Figure 7.10: Illustration of the fusion of the palate, inferior view. Legend: im: inter-maxillary segment, ps: palatal shelves, ns: nasal septum.

Formation of the palate

Shortly after the lips begin forming, the **palate** begins to form as well, dividing the primitive oro-nasal cavity into a more mature oral cavity and nasal cavities. The palate has 3 parts that fuse with each other, and with the nasal septum. The **primary palate** grows from the inter-maxillary segment, and two **palatal shelves** grow from the maxillary processes.

Structure	Lineage	Forms during	Fuses with
Primary palate (pre-maxillary segment)	inter-maxillary segment (globular process)	6 th week	Secondary palate: 9 th week
Secondary palate (Palatal shelves)	Maxillary process	7 th week	The other palatal shelf: 9 th week Primary palate: 9 th week Nasal septum: 12 th week

Table 7.3: Summary of the development of the palate.

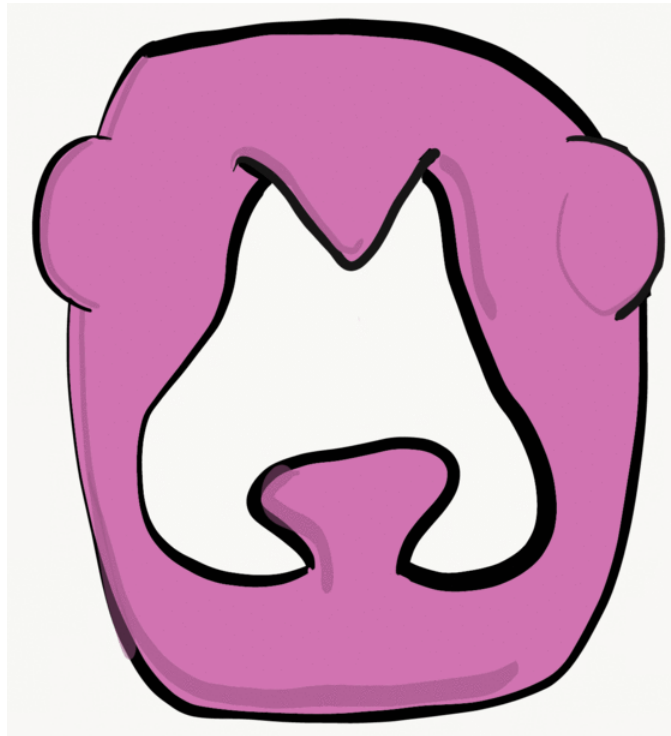


Figure 7.11: Fusion of the palatal shelves (purple) with the nasal septum, anterior cross-sectional view. Note the developing tongue moves out of the way before fusion of the palatal shelves occurs.

The first part of the palate to form is the primary palate, which develops from the inter-maxillary segment. When it forms initially, it partially divides the future oral and nasal cavities. Next, two palatal shelves grow off of the maxillary processes (Fig. 7.11). The palatal shelves first grow inferiorly, then change direction and grow medially. At this time, the developing tongue must move out of the way. This allows the palatal shelves to meet and fuse with the primary palate, as well as each other. The fusion happens in an anterior-to-posterior direction. All of this growth is directed by morphogens, including Fibroblast Growth Factors, Bone Morphogenetic Proteins, and Sonic Hedgehogs.

Maxillary incisors develop from the primary palate, while maxillary canines, pre-molars and molars develop from the secondary palate. At the site where the primary palate and the two palatal shelves fuse, a small hole remains, the **incisive foramen**, which houses the nasopalatine artery and vein as well as a branch of the trigeminal nerve. The oral mucosa above this foramen has a bump named the incisive papilla, which shares more in common with olfactory epithelium than it does mucosal epithelium (it is the homologue of the vomeronasal organ found in many vertebrates). Where the two palatal shelves fuse leaves a ridge named the **median palatine suture**. This creates a visible ridge on the overlying oral mucosa called the (median) palatine raphe.

Keep in mind that we are referring to the entire palate. Much later, anterior portions of the palate mesoderm undergo endochondral ossification and form the palatine bones and the palatine processes of the maxilla (the hard palate). The rest of palatal mesoderm differentiates into muscle tissue, forming the soft palate. Time out for spelling: this is the palate, not an artist's palette of colors, nor a pallet used in shipping, not even a plate on which we place a tasty dinner. Therefore, foodstuffs shipped on a pallet, cooked by a chef with a harmonious palette, served to us on a plate, will be enjoyed for their flavor when they hit our palate because we have a refined palate (an appreciation for flavor). Got it? English is fun.

The **nasal septum** grows inferiorly at this time. It fuses with the completed palate around the 12th week of development. This creates paired nasal cavities. Initially, mesoderm differentiates into the ethmovomerine cartilage, and then partially undergoes endochondral ossification to generate a bony portion (portions of the ethmoid and vomer bones) as well as cartilaginous portion. Ossification begins from a lateral pair of ossification centers, therefore the early septal bones develop as 2 layers (lamella) which fuse to form a single bony septum.



Figure 7.12: Illustration of the pharyngeal arch apparatus at week 6, with a cutaway to show the pharynx.

Development of the tongue

The tongue is a hybrid structure, it forms from multiple parts making its development complicated. Tongue development begins during the 4th week, after the pharyngeal arches fuse. The tongue develops from first 4 pharyngeal arches (skipping the 2nd, except for a connection to CNVII). Formation of the tongue involves proliferation, followed by fusion, followed by apoptosis of some tissue to give the tongue mobility.

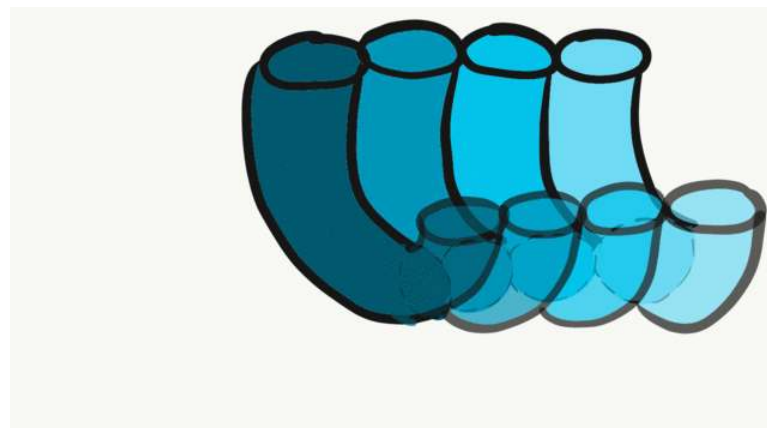


Figure 7.13: Development of the tongue from 3 of the first 4 pharyngeal arches.

At the 4th week, the pharyngeal arches fuse along the floor of the future oral cavity. A single triangular-shaped **tuberculum impar** proliferates off the 1st arch, followed by two **laterallingual swellings**. Because they come from the first pharyngeal arch, their inner lining is not endoderm like the other arches, but ectoderm from the oro-pharyngeal membrane. As these swellings grow, the 3rd and 4th arch develop a swelling named the **copula**, which grows over the 2nd arch. The oral mucosa of the anterior 2/3 of the tongue is derived from the ectoderm of the 1st pharyngeal arch, the posterior 1/3 from the endoderm of the 3rd pharyngeal arch, and the base of the tongue from endoderm of the 4th pharyngeal arch. Fusion of these structures occurs during the 8th week. The **median lingual sulcus** forms, where the left and right lateral lingual swellings fuse. The **sulcus terminalis** forms where the 1st and 3rd pharyngeal arches fuse. This border between the anterior and posterior portion of the tongue is pronounced due to the different lineages of the surface mucosa.

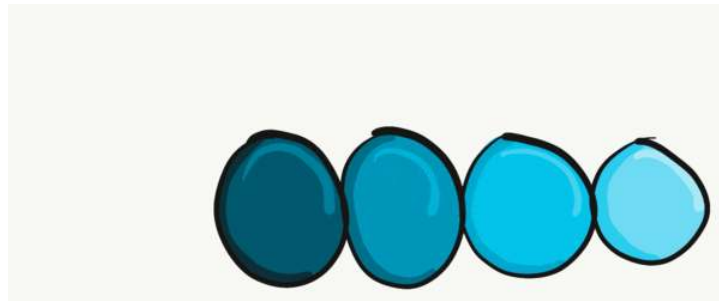


Figure 7.14: Apoptosis is important for the development of tongue mobility.

Apoptosis of tongue tissue on the ventral side leaves the tongue attached at the base, and freer to move around. Apoptosis does not remove all the tissue on the anterior portion, a small amount of mucous membrane remains, named the **lingual frenulum**. An invagination forms posterior to the sulcus terminalis and grows deeper, forming the thyroid gland. This process is similar to the way the anterior pituitary forms. It leaves behind a small depression named **foramen cecum**, which is a confusing name because foramen means hole, but this foramen fills in most of the way, making it more of a pouch. Similar to Rathke's pouch, it serves no purpose in humans, it's a remnant of the way epithelial tissue proliferates.

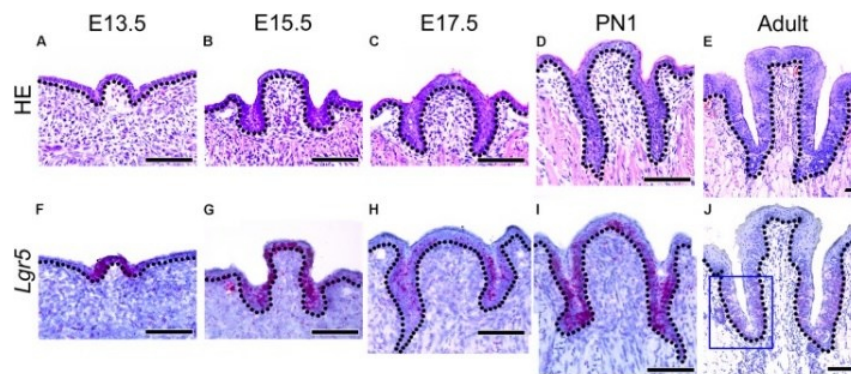


Figure 7.15: Invagination of lingual papillae. Image credit: "Morphology of developing CVP and expression patterns of Lgr5 and FGF10 during CVP development" by Sushan Zhang et al is licensed under CC BY 4.0 / cropped

Development of the lingual papillae

The lining of the oral cavity is derived from ectoderm, the pharynx from endoderm, and the lining of the tongue is a mixture of the two. The filiform and fungiform papillae develop from invaginations of the ectoderm, while the foliate and circumvallate from invaginations of endoderm. The growth and differentiation of the papillae is guided by morphogens secreted by underlying neuro-mesenchyme, including members of the FGF and Wnt families. Keratinocytes develop from an ectodermal precursor, while taste buds (including those in the soft palate and pharynx) are induced to develop from ectodermal or endoderm precursors starting the 8th week of development. Older evidence suggests taste bud differentiation depended on neural connections, but newer evidence suggests taste buds develop in response to the Sonic Hedgehog morphogen. By adulthood, both keratinocytes and taste bud cells continue to develop from a shared epithelial stem cell, and both are replenished throughout life. Whether the lineage of this stem cell is endodermal, ectodermal or both is unclear.

The connective tissue (lamina propria, sub-mucosa, and vasculature) of the tongue is derived from neuro-mesenchyme. The skeletal muscle tissue is derived from somatic mesoderm, guided by morphogens secreted from the neuro-mesenchymal stem cells of the neuro-mesenchyme.

Clinical applications of pharyngeal arch development



Figure 7.16: Example of commissural lip pits. Image credit: [Bilateral congenital lower lip](#) by Vela Desai is licensed CC BY-SA 4.0

Lip pits

Incomplete fusion of the pharyngeal arches leads to a number of conditions, some more severe than others. Two benign conditions include a **lower labial pit**, which forms when the two mandibular arches fail to fuse completely.



Figure 7.17: Commissural lip pit. Image credit: ["Commissural Pit"](#) by National Human Genome Research Institute is in the [Public Domain, CC0](#)

Commissural lip pits may form between the maxillary processes and mandibular arches. These are examples of cosmetic variations rather than congenital malformations.



Figure 7.18: Left unilateral cleft of the lip. Image credit: own work by James Heilman, MD is licensed CC BY-SA 4.0

Cleft lip

Incomplete fusion of either maxillary process with the inter-maxillary segment leads to the formation of a **cleft lip**. This can occur either on the left, right (unilateral) or both (bilateral) borders of the philtrum, although a left unilateral cleft lip is the most common. Cleft lips are more common and more severe in male children. A cleft lip may be accompanied by a cleft palate.

Cleft lip and palate occur in about 1 in 1000 births, making them a relatively common congenital malformation. Risk factors include older mothers, mothers who smoke during pregnancy or take certain medications (e.g. some anti-consultants). There are many genetic risk factors for cleft lip and palate, some examples are listed in Table 7.4.

Gene name	Class of gene	Function
IRF6	Transcription factor	Induced during development of mesoderm.
MSX1	Homeobox transcription factor	Limb patterning
BMP4	Morphogen	Induction and patterning of bone tissue, teeth and limbs
FGF10	Morphogen	Induction and patterning of connective tissue
Hyal2	Digestive enzyme	Digests hyaluronic acid prior to fusion of the lip or palate
p63	Transcription factor	Controls desmosome protein expression during fusion of the lip or palate

Epithelial Cadherin 1	Cell adhesion molecule	Allows epithelial cells to connect during fusion of the lip or palate
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Table 7.4: A partial list of genes that, when mutated, contribute to the formation of cleft lip/palate.

A cleft lip can cause difficulty with nursing, as it hinders the formation of a good seal around a nipple. With [proper instruction](#), babies with cleft lip can be breast-fed or bottle-fed using a regular bottle. A cleft lip may cause problems with learning speech. Learning to speak requires [sound mimicry](#), and because a cleft lip alters vocal sounds, it interferes with successful mimicry. Speech and hearing therapy help alleviate these problems. An increased risk of oro-nasal infections is also a concern. The preferred treatment for cleft lip is to seal the gap with surgery at 10 weeks of age. Surgery can leave behind a scar, but otherwise is highly successful.



Figure 7.19: Example of a cleft palate. Image credit: A 16 year old girl with unilateral complete cleft palate by Ghulam Fayyaz is licensed CC BY-SA 4.0

Cleft palate

Incomplete fusion of the primary palate and/or the palatal shelves leads to a **cleft palate**. A cleft palate may or may not be accompanied by a cleft lip. Cleft palate is more common in females.

Cleft palate causes difficulty with nursing, because a child cannot create suction with an opening from the oral cavity into the nasal cavity. There are a number of [specialty bottles](#) that help babies with cleft palate bottle feed. Similar to cleft lip, a cleft palate can lead to difficulty learning speech. Disruption of palate formation may lead to shape changes in the Eustachian tube, which in turn alters the ability to regulate middle ear pressure and leads to an increased risk of hearing loss.

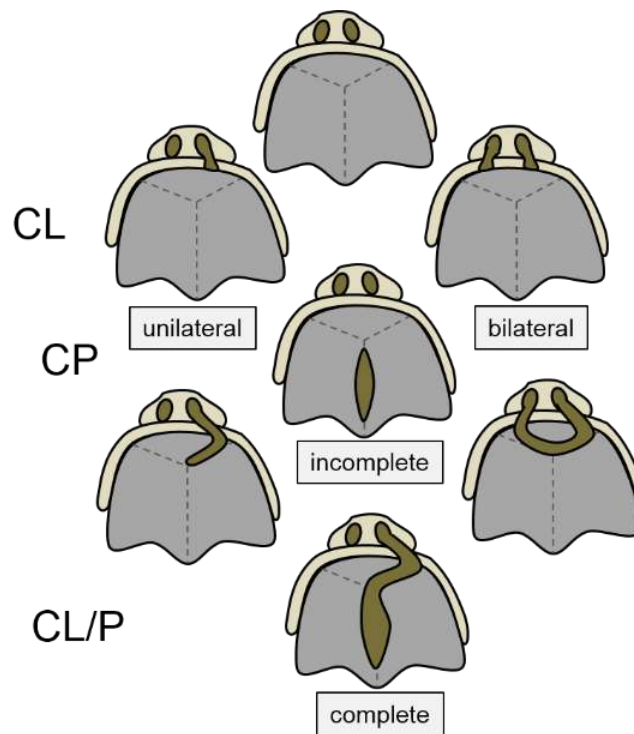


Figure 7.20: Summary of the different varieties of cleft lip/palate. Legend: CL = cleft lip, CP = cleft palate, CL/P = cleft lip and palate.

The preferred treatments for cleft palate include [Naso-Alveolar Molding \(NAM\)](#), followed by several surgeries. NAM involves screwing or taping an appliance to the maxilla at around 10 months of age to slowly pull in an antero-medial direction, towards the inter-maxillary segment. Using such an appliance reduces the amount of surgery required to correct the cleft, relying more on guided growth of the child's tissues. The device is adjusted by an orthodontist every two weeks for about a year. Multiple surgeries follow in the treatment of cleft palate— it is a complicated region, made all the more complex by the fact the child is growing fast. It is not ideal to wait for a child to stop growing for the same reason early intervention for cleft lip was important: orofacial clefts hinder speech development.

Orofacial clefts are categorized first as being a cleft lip (CL), a cleft palate (CP), or a cleft lip and palate (CL/P). A **unilateral cleft** lip or palate affects just the left or right side, while a **bilateral cleft** affects both sides. An **incomplete cleft** palate involves incomplete fusion between the primary palate and a palatal shelf, while a **complete cleft** also involves incomplete fusion between the two palatal shelves.



Figure 7.21: Example of a cleft uvula. Image credit: Own work by Adam6611 is in the Public Domain CC0

Cleft uvula

A cleft uvula is the least complicated form of cleft palate, and should be considered a cosmetic variation rather than a congenital malformation. A cleft uvula still closes off the nasopharynx during swallowing.



Figure 7.22: Example of a deviated septum. Image credit: [Nostrils before](#) by Jeff and Mandy G is licensed CC BY SA 2.0

Deviated nasal septum

If the nasal septum grows at an angle as it is developing, it leads to a **deviated septum**. In fact, it is rare for the septum to develop in a symmetrical fashion. 80% of people have some nasal septum deviation, usually without symptoms. Complications can arise because of nasal cavity physiology. The paired nasal cavities contain erectile tissue (areolar connective tissue) below the mucosa. This tissue undergoes a nasal cycle, alternating between one side swelling shut and the other remaining open for breathing. This prevents the nasal cavities from drying out from constant use. But for someone with a significant nasal septum deviation, it leads to difficulty breathing when the larger cavity swells shut. A relatively simple surgery called **septoplasty** can be done to increase the size of the smaller nasal cavity. Septoplasty is not the same as the plastic surgery procedure **rhinoplasty**, where the shape of the nose is altered.



Figure 7.23: Example of a palatal torus. Image credit: Photo by Kozlovsk is licensed CC BY SA 3.0

Palatal torus

Excessive growth of the palatal shelves can create a **palatal torus** (or torus palatinus), another example of a cosmetic variation. A palatal torus requires no treatment, as it usually does not cause any health-related issues, aside from complicating the fit to dentures. About 20-30% of the population has some degree of palatal torus. A **mandibular torus**, on the other hand, is not congenital. One can develop later in life, often as a response of bone tissue to bruxism.

Figure 7.24: Example of ankyloglossia. Image credit: Photo by Klaus D. Peter is licensed CC BY 2.0

Ankyloglossia

Ankyloglossia (tongue tie) is the persistence of tissue anchoring the tongue to the floor of the mouth. Most of the ventral side of the 1st pharyngeal arch should undergo apoptosis, leaving behind a small lingual frenulum. However, with inadequate apoptosis, a pronounced lingual frenulum results, limiting the mobility of the tongue. This causes problems with breastfeeding and learning speech, but it is correctable with a minor surgery (a lingual frenectomy). Ankyloglossia is a common congenital malformation, although there is significant amount of disagreement as to how prevalent it is, with [estimates ranging widely](#), from 1% to 25% of births (that is potentially a lot of surgical procedures). Similarly, there is disagreement as to how [necessary surgical intervention is](#), and this disagreement has been ongoing for well over 75 years.

Figure 7.25: Example of a pharyngeal cleft cyst. Image credit: [Patient with large right Pharyngeal Cleft Cyst protruding from neck, prior to excision](#) by BigBill58 is licensed CC BY SA 4.0

Pharyngeal cleft cyst

Pharyngeal cleft cysts form when incomplete fusion of neighboring pharyngeal arches leaves the remnant of a pharyngeal groove. These usually form a painless mass in the neck, until an infection occurs. They may be left untreated, or may be removed by surgery. This involves removing the extraneous ectodermal (epithelial) tissue trapped deeper in the neck. Whether surgery is or isn't performed may depend on how close the cyst is to the carotid artery, internal jugular vein or facial nerve.

Rathke's cleft cyst

Similar to pharyngeal cleft cysts, a cyst may form from incomplete obliteration of Rathke's pouch during formation of the pituitary gland. This leads to a mucus-filled cyst near the anterior pituitary. Due to its location, it may put pressure on the optic chiasm, leading to visual disturbances, otherwise it is asymptomatic. Drainage is the preferred treatment over removal, owing to how close it is to the pituitary gland.

Chapter 6 * Chapter 8

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1.8: Tooth Development

- Overview
- Tooth development
 - Initiation stage
 - Bud stage
 - Cap stage
 - Bell stage
 - Crown stage
- Root development
 - Root dentin formation
 - Cementum & pulp formation
 - Multitrooted teeth
 - Periodontal ligament and alveolar bone
- Tooth eruption
- Clinical application



Figure 8.1: Teeth begin to form during week 6, before the oropharyngeal membrane disintegrates, just after the pharyngeal arches begin to develop.

Overview of tooth formation

In this chapter we cover major concepts in tooth development, saving many of the details for subsequent chapters. The first big concept is timing. Both the primary and succedaneous teeth begin developing early in embryonic development—around weeks 5 or 6. To put this in perspective, that is *before* the mouth forms— the pharynx is still separated from the outside by the oropharyngeal membrane. The process of tooth development begins around weeks 5 or 6, when it finishes is complex. The crown develops during the embryonic period, the roots develop after birth, and the PDL and junctional epithelium form during tooth eruption.

Teeth develop in a fashion similar to hair follicles, they arise from invaginations of epithelium, derived from ectoderm. Instead of being called a tooth *follicle*, teeth grow from tooth buds. Like hair follicles, newer teeth erupt after older teeth are **exfoliated** (shed). Unlike hair follicles, tooth replacement only happens once (making the succedaneous teeth “permanent”).

Some teeth form before others, but the process of tooth formation is similar for all teeth. First, epithelial cells are induced to form a tooth bud. This happens because of the secretion of morphogens from neural crest cells below. You can’t see this under the microscope (not without more specialized tools than the H&E stain). As a result, this phase is often glossed over in histology books. That is a shame, because getting the correct number of teeth to form requires complex communication. Not only do the correct number need to form, their spacing must be determined at this time as well. There are more names for later events (usually named after long dead European men), and those names appear on license exams, so we cover those in detail as well. Lucky you.

Before going into detail, though, let’s cover big concepts related to formation of the teeth. First, regions of epithelial cells proliferate and invaginate to make room for new cells. Next, epithelial cells coordinate with underlying neuro-mesenchymal cells, and both tissues differentiate into the cells that make enamel, dentin, pulp, cementum, the PDL and alveolar bone. By 8 weeks (before the

palate has fused), there are 10 buds in each **dental lamina** (the regions fated to become the maxillary and mandibular alveolar ridges). The tooth buds of permanent teeth begin forming shortly after the primary buds. Both primary and permanent buds develop into crowns and become surrounded by the maxilla and mandible, once they ossify.

Enamel is produced by cells derived from the ectoderm, whereas dentin, cementum, pulp, PDL and alveolar bone are derived from neuro-mesenchyme. We did not say mesoderm (one of the three embryonic tissue layers), but mesenchyme (the tissue type)—the difference is important. While the hard tissues enamel, dentin and cementum all share characteristics with bone tissue, enamel has bigger differences because of its lineage. Perhaps the biggest difference is that people cannot *grow* new enamel after teeth erupt. Enamel can passively re-mineralize under the right conditions, but this does not involve the activity of human cells or enzymes (which are a lot more efficient at producing enamel). The enamel-producing cells are lost during tooth eruption. Dentin, pulp, cementum and periodontal ligaments contain cells throughout the life of a tooth, and have a much higher capacity to undergo remodeling throughout life and regenerate following trauma.

The mesenchyme is special and worth attention. Most mesenchyme is derived from mesoderm, but tooth connective tissues are derived from neural crest cells that join mesoderm. This leads to some unexpected similarities in the behavior of tooth and brain cells. This makes less sense to people who haven't learned embryology.

Lastly, enamel and dentin-producing cells are not induced to form separately. They induce one another in a reciprocal fashion. After this team-effort, the cells move apart from one another and do their own thing. Taken together, this reduces the chances that enamel or dentin could be produced in the wrong place or wrong time. They require a very special set of conditions that occurs only briefly during early embryonic development. Unfortunately, this also makes it harder for medical professionals to re-create these conditions later in life if more enamel or dentin would be beneficial.

Tooth formation

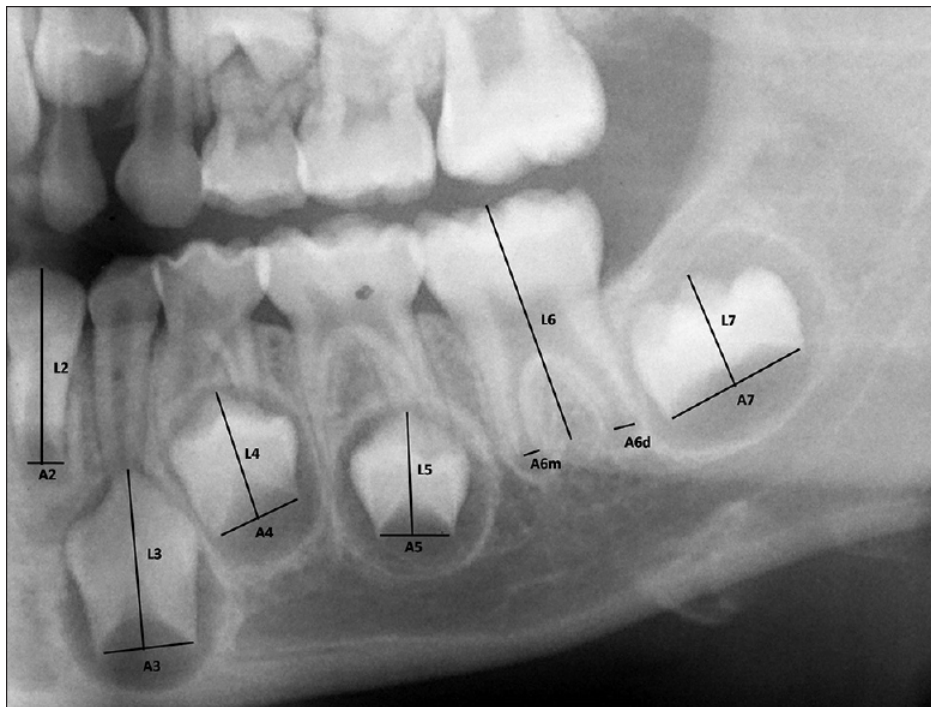


Figure 8.2: The crowns of all teeth (primary and succedaneous) form at roughly the same time, but the crown and root(s) of a single tooth develop at very different times. Image credit: “Cameriere’s method of measurement for the teeth with open apices” by Navaneetha Cugati, Ramesh Kumaresan, Balamanikanda Srinivasan, Priyadarshini Karthikeyan is licensed under [CC BY-NC 3.0](https://creativecommons.org/licenses/by-nc/3.0/)

Tooth formation, or **odontogenesis**, starts with primary teeth beginning to form around weeks 6-7, and permanent teeth around weeks 9-10. At week 6, the oral cavity is lined by ectoderm-derived epithelium, and deep to that is mesenchyme. For the skin and most of the oral cavity, the layer of mesenchyme is derived from mesoderm. Teeth, however, grow from ridges of ectoderm from the first pharyngeal arch (the mandibular arch) and the maxillary process, along the occlusal border of what becomes the mandible and maxilla. The mesenchyme layer here contains neural crest cells that migrated from the neural tube. Hence, we say the connective tissues of the teeth are derived from neuro-mesenchyme (or ecto-mesenchyme). Remember, because neural crest cells

came from the neural tube, and the neural tube invaginates from the ectoderm, these cells are ectodermal by lineage. They go through an ectodermal-mesenchymal transition and *resemble* mesenchyme derived from mesoderm under the microscope.

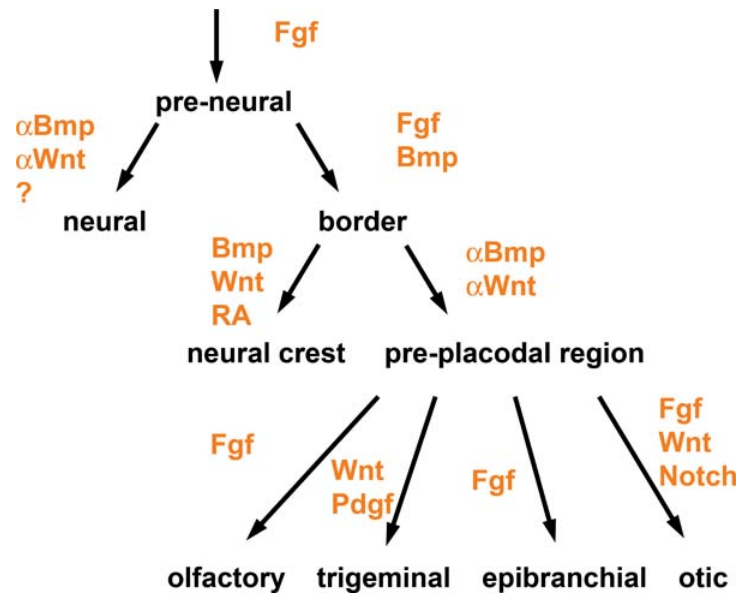


Figure 8.3: Morphogens involved in the induction of various placodes. Image credit: "Signaling pathways in placode formation." by Andrea Streit, [Stembook.org](https://stembook.org) is licensed [CC BY 3.0](https://creativecommons.org/licenses/by/3.0/)

Induction

The first step is poorly understood. Neuro-mesenchymal cells coordinate with one another through planar cell polarity signals to ensure the correct number of tooth buds form, and at the correct distance apart from one another. These neuro-mesenchymal cells secrete a number of morphogens forming complex gradients, and overlying ectoderm respond to these morphogen gradients.

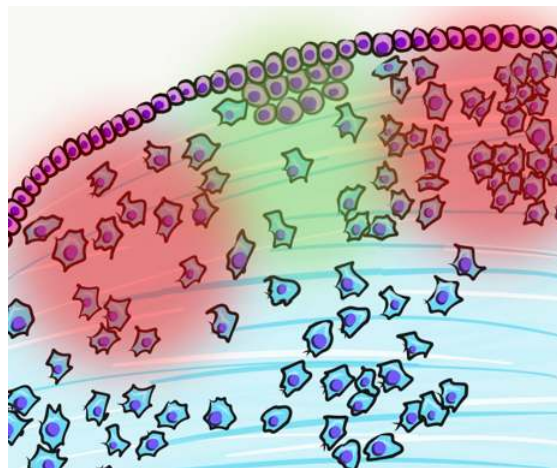


Figure 8.4: Illustration of FGF and BMP gradients.

Two important morphogens in tooth development are members of the Fibroblast Growth Factor and the Bone Morphogenetic Protein families of signaling molecules. As you might have guessed from their names, these chemicals aren't specific to tooth formation, they induce different cells to do different things at different times or locations. At this time and location, they induce ectoderm to differentiate into tooth buds instead of oral mucosa. Other morphogens inhibit placode formation, ensuring proper spacing exists between one placode and the next. One of the first visible responses in the ectoderm is proliferation—certain regions begin to grow thicker, and can at this time be called tooth placodes. Not every book (or exam) includes placode as its own stage, be aware. The placodes may be called the dental lamina or may be listed as a part of the dental lamina—fate mapping experiments can't easily be done on human embryos to accurately distinguish between these two technicalities. The important concept is most of the oral mucosa described in chapter 3 is derived from ectoderm and mesoderm, while the periodontium—plus Junctional Epithelium—is derived from ectodermal and neuro-mesenchymal cells of a tooth bud.

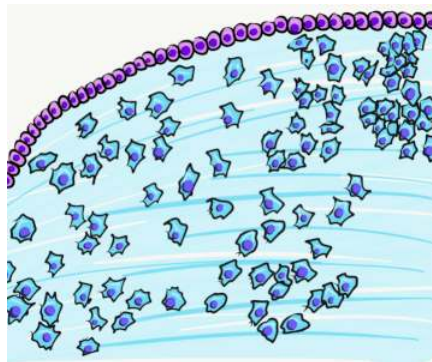


Figure 8.5: Animated overview of the early stages of tooth development: *Placode*, bud, cap, bell.

Bud stage

Proliferation of regions of ectoderm allows us to see the next stage of tooth development under the microscope, the **bud stage**. The name *bud* comes from the fact that tooth buds look like leaf buds on a plant. In the spring, you can see where leaves are fated to form on deciduous plants by the location of buds. Plant buds are not leaves yet, just bumps. Tooth buds begin to appear around week 6, and ultimately 10 tooth buds form on the maxillary processes and mandibular arch. Neuro-mesenchymal cells in the area also begin to proliferate. This makes the mesenchyme in a tooth bud appear different from the mesenchyme in regions that forms the sub-mucosa, those regions have more of the mucous ground substance and fewer cells.

Cap stage

Around week 10, the ectoderm continues to proliferate and it bumps into the cluster of neuro-mesenchymal stem cells below. This cluster of neuro-mesenchymal stem cells is called the **dental papilla**. The dental papilla forces the ectoderm of the tooth bud to grow around it, causing the ectoderm of the bud to resemble a cap (a hat), hence the name **cap stage**. At this time, the dental lamina has two prominent parts—an inner and an outer layer. The epithelial cells near the dental papilla are referred to as the **enamel organ**, because some of them differentiate into cells that produce enamel. The neuro-mesenchyme of the dental papilla forms dentin and pulp. The neuro-mesenchymal cells on the outside of the bell are called the **dental sac** (or *dental follicle*), which forms cementum, periodontal ligament and alveolar bone. All three together—the enamel organ, dental papilla and dental sac—are collectively called **tooth germ**.

At this time, the process repeats (or recapitulates). Cells of the dental lamina on the lingual side of the cap are induced to form another placode, and the succedaneous (permanent) teeth bud off the developing primary tooth germ. Imagine taking Fig 8.5, rotate it 90°, superimpose that onto the cap stage, and you have succedaneous tooth bud formation. Exceptions to this include the 2nd and 3rd molars, which do not succeed primary teeth. Their tooth buds develop from ectoderm similar to the primary dentition. The tooth germ of succedaneous teeth can move as they develop—tooth eruption does not always occur from the original lingual side, for instance the maxillary incisors generally erupt from the facial direction.

Bell stage

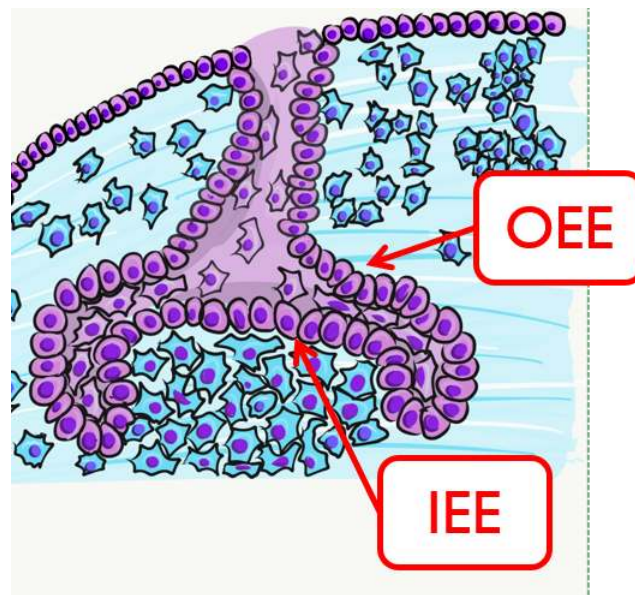


Figure 8.6: Bell stage with inner enamel epithelium (IEE) and outer enamel epithelium (OEE) labelled.

By the 11th-12th week, ectoderm and neuro-mesenchyme continues to proliferate. As the cap becomes larger, it resembles more of a bell-shape (bells on churches are usually larger than hats, may help you remember which stage comes before the other), hence the name **bell stage**. At this time, the enamel organ is two layers of cells named the **inner enamel epithelium** (IEE) and the **outer enamel epithelium** (OEE). The inner enamel epithelium is next to the dental papilla, with a basement membrane physically separating the two cell types. The IEE differentiates into ameloblasts and produce enamel, while the OEE is involved in tooth eruption and forms the junctional epithelium that bridges between the tooth surface and the oral mucosa.

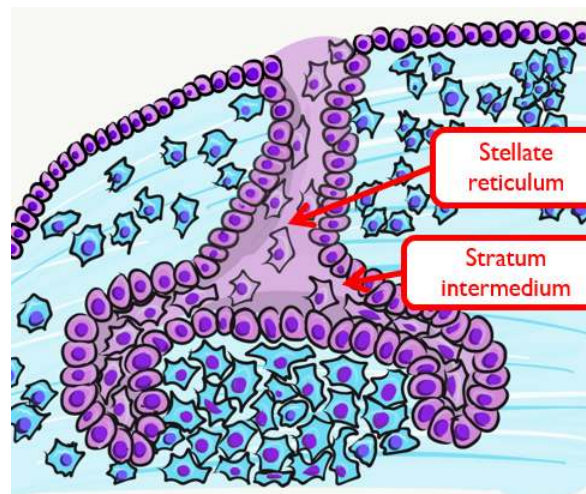


Figure 8.7: Cap stage with stellate reticulum and stratum intermedium labelled

Two more distinct regions of ectoderm develop at this time. The **stratum intermedium** is on the opposite side of the IEE from the dental papilla. These cells work with the IEE to form enamel. Past the stratum intermedium are ectodermal cells called the **stellate reticulum**, named for their star-shaped rather than cuboidal appearance. These cells help induce the IEE to differentiate and form enamel. These cells are found in the superficial but not the deeper regions of the growing tooth germ, this accounts for the production of enamel only in the crown and not the roots of teeth.

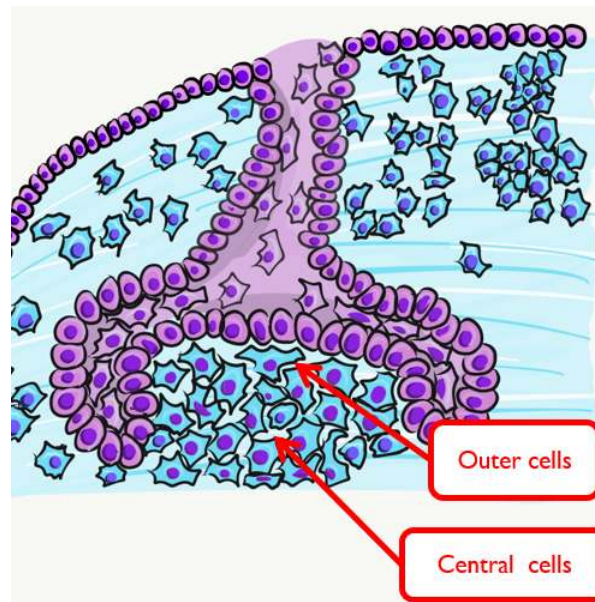


Figure 8.8: Cap stage with two regions of the dental papillae marked, the outer and central regions.

The dental papilla may be subdivided at this time into an outer region and a central region. The outer cells differentiate into odontoblasts, while the central cells form many cell types found in pulp. It is worth noting that cells are not ameloblasts or odontoblasts at this time. These cells are epithelial and neuro-mesenchymal stem cells, but based on their relative location, we know what they are fated to become in the near future, assuming they receive the correct morphogens.

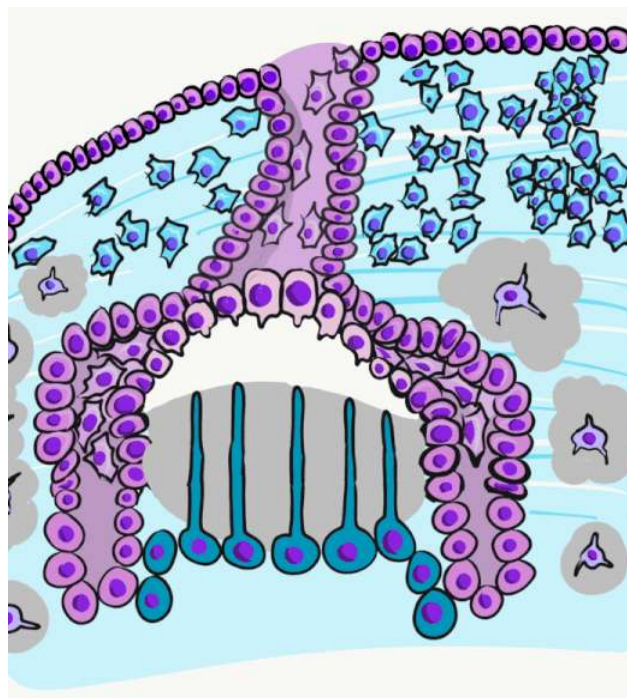


Figure 8.9: Animated representation of odontoblast and ameloblast differentiation, including the induction signals (red arrows) for each process.

A brief overview of the formation of ameloblasts and odontoblasts

During the bell stage, interactions between the IEE, stellate reticulum and outer cells of the dental papilla induce the differentiation of ameloblasts and odontoblasts. First, the basement membrane that physically separated the IEE from the neuro-mesenchymal cells of the dental papillae disintegrates. The IEE, now in contact with the neuro-mesenchymal cells and stellate reticulum, is induced to differentiate into **pre-ameloblasts**. The prefix pre- indicates that differentiation is not considered complete at this time,

but the IEE begins to look different: the cells elongate, changing from simple cuboidal epithelium to a simple columnar epithelium. These cells produce morphogens that induce neighboring neuro-mesenchymal cells to differentiate into **odontoblasts** (the cells that make dentin). Newly formed odontoblasts begin producing a squishy immature form of dentin that mineralizes later. This pre-dentin, along with signals from the stellate reticulum, triggers the differentiation of pre-ameloblasts into **ameloblasts** (the cells that make enamel).

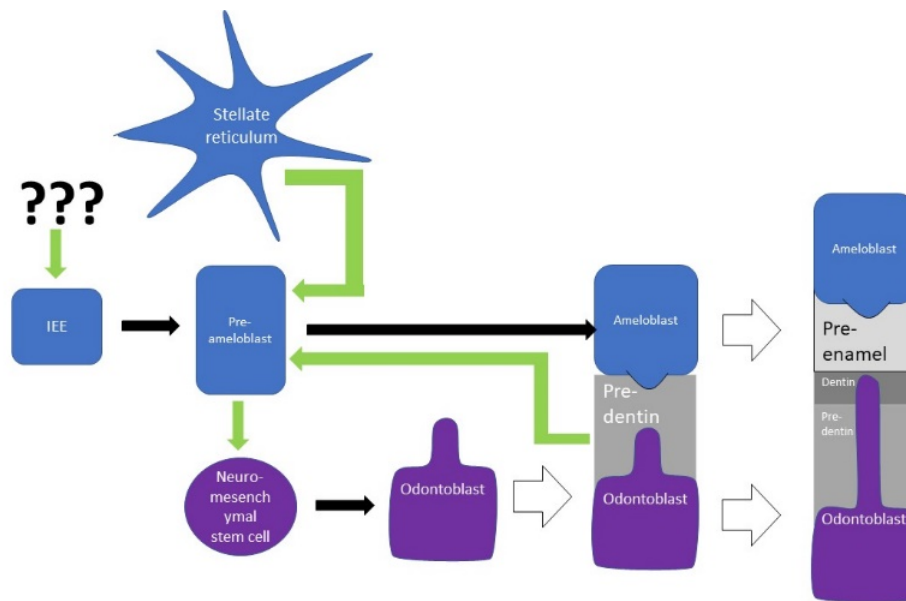


Figure 8.10: Diagram of the reciprocal induction of odontoblasts and ameloblasts. Legend: Green arrows = morphogen signal, Black arrows = differentiation, White arrows = ECM secretion.

In developmental biology, this dual effort is known as reciprocal induction, which is a common pattern. This is significant because it makes it hard to re-create one type of cell later in life (such as an ameloblast) without also re-creating a second cell type (such as an odontoblast) as well as re-creating the conditions they were in during reciprocal induction (such as creating pre-ameloblasts and stellate reticulum).

Dentinogenesis, the formation of dentin, therefore begins shortly before **amelogenesis**, the formation of enamel. This results in dentin being thicker than enamel. As odontoblasts secrete pre-dentin, their cell bodies are pushed deeper, but they leave a log tube-like extension called the **odontoblastic process** within the pre-dentin. By the time a tooth is done growing, the odontoblastic processes extend through nearly the entire layer of mature dentin. Ameloblasts, on the other hand, do not leave behind an extension. There is a little bump on each Ameloblast facing the enamel, and this bump is called **Tome's process**. There is important clinical significance related to odontoblastic processes coming up in subsequent chapters. Tome's process, on the other hand, is a name of a bump histologists use to identify ameloblasts during embryonic development.

Crown stage

After the bell stage is the late bell stage, or the **crown stage**. During this time the crown of the teeth form by the secretion of molecules by ameloblasts and odontoblasts. No new cells of these types form, but teeth continue to grow by the addition of ECM. Because the matrix mineralizes and hardens, these tissues must be added appositionally. This is somewhat similar to the growth of bone tissue, only the enamel and dentin-producing cells do not get trapped between layers of hard tissue, they are pushed in an apical or basal direction. The crown portion of each primary tooth is finished growing sometime within the first year or two of life. The timeline for the succedaneous teeth is more variable (a timetable may be found on Wikipedia).

Root Development



Figure 8.11: Histology of bell-stage tooth germ, with the IEE and OEE labelled. Image credit: "Latebellstage11-18-05.jpg" by Dozenist is licensed under CC BY 3.0 / labels added

The cervical loop

We jump years in time to watch the formation of the roots, after the crown is completed. The leading edge of the IEE and OEE are not separated by other cell types, together they are called the **cervical loop**. As the cervical loop continues to grow around the dental papillae and extend deeper, it is called **Hertwig's Epithelial Root Sheath (HERS)**. The pattern of HERS growth determines the shape of the root(s). HERS does not grow evenly around the dental papillae of larger teeth. Instead, HERS grows faster in some regions as it extends over the dental papilla, perhaps the way chocolate fudge drips over a ball of ice-cream unevenly. On larger teeth, this produces multiple roots.

Cementum formation

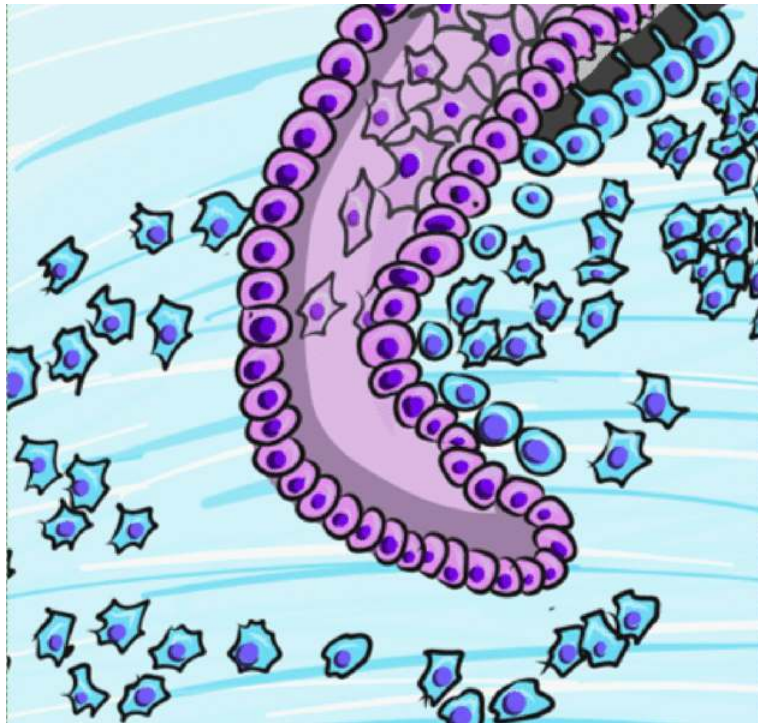


Figure 8.12: HERS and root formation.

HERS contains IEE and OEE, no stellate reticulum, which leads to key differences between the crown and roots of teeth. The IEE induce the formation of odontoblasts in the roots just as it did in the crown, but when odontoblasts form pre-dentin, the lack of a signal from stellate reticulum means the differentiation of ameloblasts does not occur. Hence, roots do not contain enamel. Instead, most of the IEE and OEE cells undergo apoptosis. A few are left behind, which get the name the **Epithelial Rests of Malassez (ERM)**. These leftover cells may have functions in the regeneration of damaged root tissues, although this is not currently well understood.

After the majority of the epithelial cells are removed, neuro-mesenchymal stem cells of the dental sac contact pre-dentin, and this induces them to differentiate into **cementoblasts**. These cells cover the root dentin in a relatively thin layer of cementum. Some regions of cementum have cementoblasts trapped within the ECM. This is cellular cementum, and the cells are called **cementocytes** once trapped within cementum. Other regions of cementum are acellular cementum. Formation of root dentin and cementum are covered in more detail in the next two chapters.

Tooth eruption



Figure 8.13: Tooth eruption highlighting epithelial fusion. Legend: OE = oral epithelium, REE = reduced enamel epithelium, JE = junctional epithelium.

We now jump forward in time yet again. Even though process of **tooth eruption** happens long after embryological development, it should be considered a developmental process. Because this process involves cells of the IEE and OEE, we are discussing it here, rather than splitting up processes chronologically.

After the roots develop, neuro-mesenchymal stem cells of the dental sac that did not come into contact with dentin differentiate into fibroblasts and osteoblasts. These form the PDL and alveolar bone. Therefore, development of the PDL occurs during tooth eruption, much later than the formation of dentin and enamel.

First, the force or forces that cause teeth to erupt are [not agreed upon](#). It is not even clear if it is a pushing or a pulling force, or a combination of the two. Whatever the cause or causes of tooth eruption are, movement of the crowns into the oral cavity is known as **active eruption**. One might imagine that as a root grows deeper and bumps into the edge of a bony socket, further lengthening of the root would force the crown upwards. This is similar to jumping: with your legs bent and feet on an immovable floor, rapid extension of your legs pushes you upwards. This is known as the *root formation theory*. To say this causes tooth eruption, it would have to be both *necessary and sufficient* (a phrase commonly used in biological research). Because sometimes root-less teeth erupt, this suggests root growth is not *necessary* for eruption.

We know that bone remodeling plays a role, which involves coordinated activity between osteoblasts and osteoclasts. We have already covered some of the evidence for this when we discussed how permanent teeth fail to erupt in people with cleidocranial dysostosis. A faulty matrix metalloproteinase enzyme leads to the inability to degrade ECM proteins, leaving permanent teeth trapped within their bony crypt. This suggests bone remodeling is *necessary*, but not necessarily *sufficient*. For the primary teeth, when growing roots encounter mineralized bones tissue, this puts hydrostatic pressure on the cells of the dental organ. In response, the stellate reticulum secretes morphogens that induce bone resorption, including **RANKL**, a [morphogen that induces osteoclast differentiation](#), and Parathyroid Hormone, a hormone that activates osteoclast activity. The stellate reticulum, found at the surface of the developing crown enamel, therefor secretes a signal that clears a path for the tooth to erupt. Furthermore, as the roots grow, they put pressure on bone tissue in the alveolar sockets. Pressure on bone tissue triggers release of bone-simulating morphogens (BMPs) which leads to the deposition of bone tissue, *pushing* teeth outwards. Bite forces may also contribute to BMP secretion.

Further evidence that pressure is necessary comes from observation that teeth may fail to erupt in the absence of healthy periodontal ligament. In fact, pressure *created* by fibroblasts of the PDL may trigger the stellate reticulum to secrete morphogens

that trigger osteoclast differentiation as well as bone tissue to release osteoblast-stimulating morphogens. Fibroblasts, after all, not only secrete collagen but can remove it as well, thus shortening of collagen fibers would put pressure on both the tooth root and alveolar socket. Polarization of PDL fibroblasts is observed before tooth eruption, suggesting that remodeling of PDL collagen fibers inserted in cementum may actively *pull* the tooth outwards.

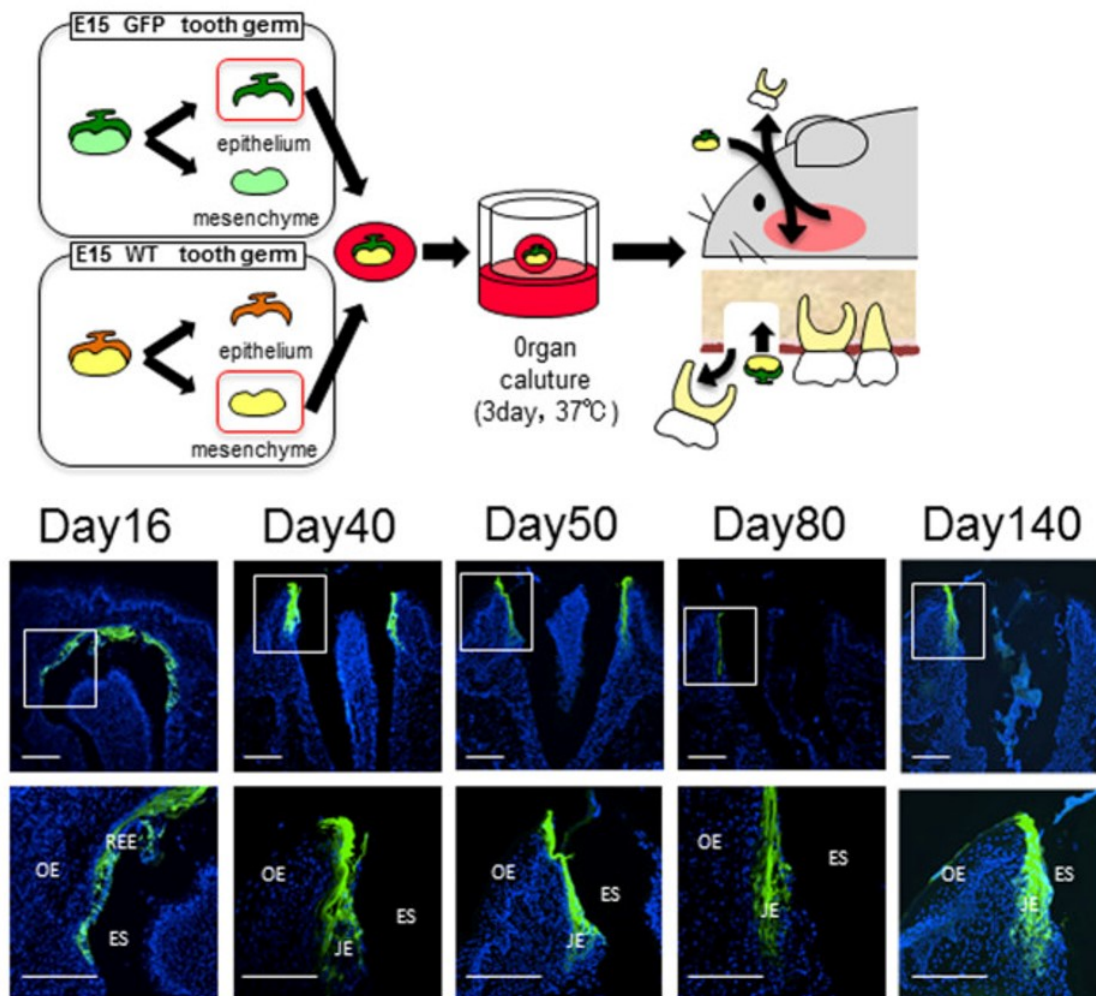


Figure 8.14: JE is produced on a bio-engineered tooth from the REE (green tracer). Legend: OE = oral epithelium, JE = junctional epithelium, ES = Enamel. Image credit: "The JE attached to the bioengineered tooth was derived from the odontogenic epithelium." by Sara Yajima-Himuro, et al is licensed under CC BY 3.0

Changes in the gingiva also occur for tooth eruption to proceed. Remodeling of oral mucosa to allow teeth to erupt is known as **passive eruption**. Old tissue is removed to clear a path for a tooth to erupt, but do not think of this like digging a tunnel for cars to pass through. The oral mucosa must be *connected* to the tooth as it erupts, otherwise oral bacteria could enter the sub-mucosa and trigger an infection or inflammatory response. Before eruption begins, the crown of the tooth contains 2 layers of epithelial cells on the surface of the enamel. This includes the ameloblasts and OEE. Collectively, these two layers of epithelial cells are called the **Reduced Enamel Epithelium (REE)**. Like the invagination and separation of ectodermal cells during the formation of the neural tube, the REE is separated from the epithelial cells of the oral mucosa. During tooth eruption, the REE re-joins the oral epithelium, forming desmosomes and other cell-cell junctions with oral epithelial cells, but maintaining contact with enamel. Many REE cells undergo apoptosis, but those that remain become the junctional epithelium—a very special epithelium that maintains hemidesmosome contacts on both sides of the tissue: apical (enamel) and basolateral (sub-mucosa).

Some of the REE may not fuse with the oral epithelium, and remain partially stuck to the surface of a newly erupted tooth. This is known as the **primary enamel cuticle**, or the more old-fashioned name Nasmyth's membrane. It can easily be removed by gentle brushing or mastication.

Because the REE is lost with tooth eruption, no new enamel can be produced by human cells afterwards (amelogenesis is faster and more organized than passive re-mineralization because it involves a protein scaffold which assists in crystal formation). Furthermore, the lack of REE means that gingiva cannot form junctional epithelium with dental implants. Understanding how junctional epithelium forms in the first place may improve our ability to trick oral mucosa cells into becoming more like junctional epithelium.

Clinical considerations

Initiation stage complications

Aberrations in the initiation of tooth germ leads to the formation of too few or too many teeth.



Figure 8.15: An example of partial anodontia (hypodontia). Image credit: "Incontinentia pigmenti presenting as hypodontia in a 3-year-old girl: a case report" By Kitakawa et al, Journal of Medical Case Reports 2009, is licensed CC BY 2.0

Anodontia

Failure in the initiation stage results in missing teeth, or **anodontia**. This may include inadequate release of morphogens by neuro-mesenchymal stem cells, mutations in the morphogen receptor proteins, or teratogens that interfere with the binding of morphogens to their receptors and/or activation of second messenger systems. Partial anodontia (also called **hypodontia**), where one or a few teeth are missing, is most common. The most commonly missing teeth are: permanent maxillary lateral incisors, 3rd molars, and mandibular 2nd pre-molars.



Figure 8.16: A patient with ectodermal dysplasia. Image credit: Ectodermal dysplasia, by Pratheeba227 – photographed at Govt Vellore Medical College, is licensed CC BY SA 3.0

Ectodermal dysplasia (part 2 of 2)

Ectodermal dysplasia is covered in chapter 6 where we discuss the induction of neural crest cells. When signals from neural crest cells are reduced, regions of ectoderm that are supposed to grow faster than others do not get the boost in growth they need, including hair follicles, finger and toenails, tooth buds, sweat glands and salivary glands. This may cause some or all of these structures to not develop at all, or to develop in reduced number or size.

For those who cannot afford and/or are too young for dental implants, complete or partial anodontia may be visible, as well as microdontia (Fig. 8.16). Children do not receive dental implants because implants do not grow in size to match a growing jawline. Multiple sets of dentures are an option (again, for those who can afford the treatment). Now is a good time to double-check you can distinguish between hypodontia and microdontia. If you haven't had Latin or a good medical terminology class, remember a hypo-dermic needle goes lower than the dermis, while micro-biology is the study of really small stuff.



Figure 8.17: An example of hyperdontia. Image credit: "Fig. 5" by Toby Hughes et al., [J. Dental Anthropology](#) is licensed under [CC BY 4.0](#)

Hyperdontia

Hyperdontia, of the formation of extra (supernumerary) teeth, occurs when initiation of tooth germ occurs where it shouldn't. This is often a genetic condition. The most common extra teeth are between the central incisors (**mesiodens**), distal to the maxillary 3rd molar (4th molar or **distomolar**) and the premolar region of either dental arch (**perimolar**).

Bud and cap stage complications

Aberrations in the bud stage of tooth development may lead to teeth that are too large (**macrodontia**) or too small (microdontia). This may affect all the teeth, or a single tooth.



Figure 8.18: An example tooth gemination. Image credit: "Periapical radiography of superior central incisive permanent geminated." by Katia Simone Alves dos Santos et al., [Intl J Morphology](#) is licensed under [CC BY 4.0](#)

Gemination

Gemination (twinning, as in the constellation Gemini) occurs when a single tooth germ is partially divided in two. This may occur if the growing tooth germ bumps into a small dense region in the dental arch. Under healthy conditions, teeth grow within loose mesenchyme tissue, the mesenchyme later differentiates into osseous tissue and calcifies. A geminated tooth, coming from a single tooth germ, is larger than average but has a single pulp cavity, hence this patient has an instance of macrodontia (but not hypodontia).



Figure 8.19: An example of two teeth fusing. “milk.teeth.fusion.jpg” by Sarefo is licensed under [CC BY-SA 3.0](https://creativecommons.org/licenses/by-sa/3.0/)

Fusion

Toothfusion (joining) of two tooth germs into a single germ can also cause a larger-than-average tooth to develop. In this case, macrodontia is accompanied by hypodontia (one fewer tooth). The fused tooth has two separate pulp cavities. This occurs when initiation of two tooth germs occurs too closely to one another, or when external pressure forces two tooth germs closer together. Tooth germs can fuse during early stages of tooth development because the tooth germ is soft epithelial and mesenchymal tissues. Getting too close together during later stages, when enamel and dentin are calcified, leads to plain-old tooth crowding (malocclusion).

It is worth a mention that the fusion of teeth involves two separate buds growing into what appears to be one large structure when their secretions meet. Syndactyly, the fusion of toes or fingers, is not really a fusion but a lack of separation due to diminished or absent apoptosis signals.

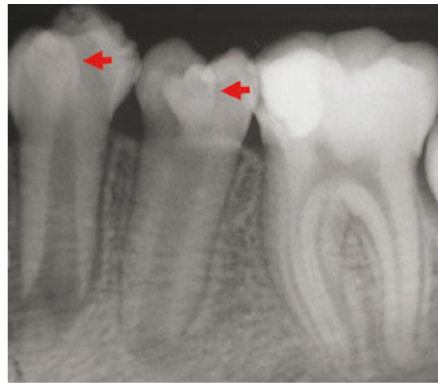


Figure 8.20: A radiograph of dens in dente (arrows). Image credit: “Radiographic view of Teeth” by Eduardo Borie E, et al, Intl J Morphology is licensed under [CC BY 4.0](#)

Dens in dente

Dens in dente (tooth within a tooth, or *dens invaginitus*) occurs when a small region of the enamel organ grows too fast and invaginates into the dental papilla during the cap stage. This sub-region of the enamel organ continues to develop, creating a miniature tooth within the main tooth. This can complicate a root canal surgery, but otherwise only makes for interesting radiographs.

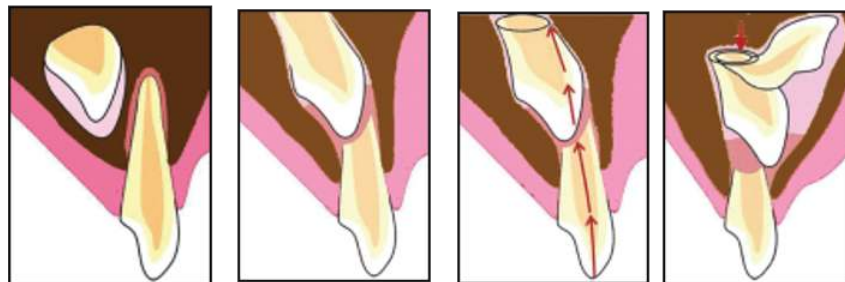


Figure 8.21: Example and illustration of root dilaceration. Image credit: “Figs 1-5” by Pawanjit Singh Walia, Intl J Clinical Ped Dentistry is licensed under [CC BY 3.0](#)

Dilaceration

Dilaceration, or a bend in the shape of a tooth, occurs when there is an impediment to the growth of HERS. A delay in tooth eruption can cause dilaceration. The older part of the tooth (the crown), which has calcified, will have grown at a different angle from the newer, softer root(s) of the tooth if the tooth bumps into calcifying osseous tissue and is forced to change its direction of growth. Alternatively, trauma to a deciduous tooth can be transduced to the deeper succedaneous tooth, causing dilaceration of the permanent tooth (Fig 8.21).

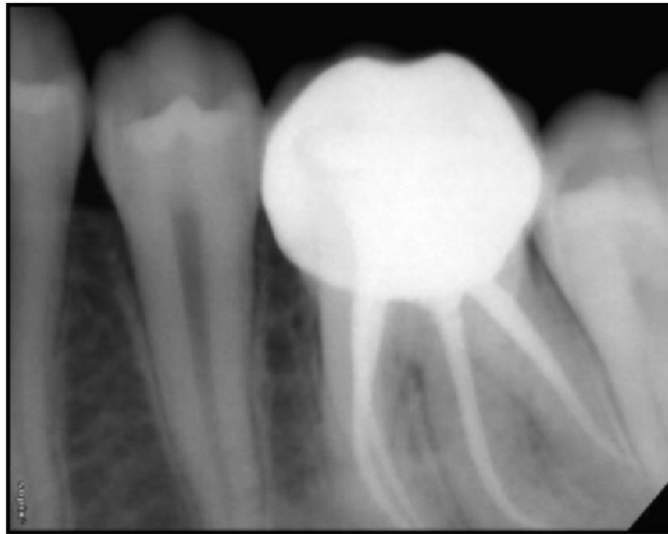


Figure 8.22: Radiograph of a root canal involving more roots than normal. Image credit: “Postoperative IOPA of 36 and 46.” by Mukdishree Mahendra et al, Case Reports in Dentistry is licensed under CC BY 3.0

Supernumerary roots

Teeth may develop extra, or **supernumerary roots**. As with dens in dente, this may complicate root canal surgery, but otherwise has limited clinical significance. When the shape of the tooth is altered but no pathology results, clinicians and textbook authors may note there is an alteration to the morphology of the tooth.

Eruption complications



Figure 8.23: Teething. Image credit: Own work By Daniel Schwen – is licensed under CC BY-SA 4.0

Teething

During tooth eruption, several tissues undergo significant remodeling, including the REE, oral epithelium and bone tissue. This involves removal of ECM by the secretion of digestive enzymes, and removal of cells by apoptosis. However, enzymes aren't smart enough to only remove ECM, they tend to kill cells as well. For instance, when the enamel organ releases proteinases to clear a path for the erupting tooth, this triggers inflammation as cell membrane proteins are destroyed along with ECM, leading to cell death. Inflammation can cause discomfort, tenderness or edema in the affected area. Rubbing a baby's gums can reduce pain

signals from being sent by nociceptors (similar to rubbing your knee after you hit it on something). Alternately, cooling the area reduces inflammation. Placing a teething ring in the refrigerator (not freezer) can be used to induce cooling in the affected area. If those techniques do not provide adequate comfort, a children's dose of over-the-counter anti-inflammatory medication may be administered.

It is not recommended to give babies the stronger over-the-counter pain-killing medication lidocaine. This medication is safe for use by adults to treat oral cavity pain, such as that caused by ulcers. However, it is significantly harder to measure a safe dose for a small baby than a large adult. Furthermore, because the nervous system of a newborn is still developing, there is always a risk that these medications will interfere with development, much the same way medications can act as teratogens during embryonic development. Developing the correct connections between axons and dendrites, whether in embryonic development or in childhood, involves [multiple morphogens and electrical activity](#) of neurons, as illustrated by the seminal work of neuroscientist Mu-Ming Poo. Lidocaine blocks electrical activity.

The use of homeopathic teething tablets or gels is not recommended. These products provide no measured benefit, and their contents are unregulated. Furthermore, in 2010 the FDA forced the recall of one such product (a rare event thanks to legal protections for the alternative and/or complementary medicine industry) because it contained belladonna (deadly nightshade). Teething necklaces or other jewelry are also not recommended because they provide no measured benefit but introduce a choking hazard.



Figure 8.24: Impacted third molar. Image credit: "Impacted Wisdom Tooth aka Lower Right Third Molar 48 RVG IOPA Xray" by Nizil Shah is licensed under [CC BY-SA 4.0](#)

Primary failure of eruption

Primary failure of eruption (PFE) is the partial or complete failure of a tooth to erupt (otherwise known as an [impacted tooth](#)) despite a healthy eruption pathway. While there are likely many causes of PFE, one major heritable cause is a mutation that disrupts [Parathyroid Hormone signaling](#) from the tooth germ to alveolar bone tissue. Parathyroid hormone activates osteoclasts and inhibits osteoblasts, therefore inhibiting parathyroid hormone signals leads to increased bone deposition. This leads to **ankylosis** (stiffening or immobilizing) of the tooth prior to eruption, making extracting difficult or impossible, especially as time progresses. Impacted teeth may cause inflammation and pain, or may not be detected until an x-ray is performed. The 3rd molars are the most commonly impacted teeth. Complications include crowding of neighboring teeth, infection, malocclusion, and—rarely but significantly—[neoplasia](#), making extraction important.

Mechanical failure of eruption

A tooth may also become impacted *after* erupting partially because of ankylosis of the tooth, which is termed **mechanical failure of eruption (MFE)**—which seems odd because it pairs a numerical name with a descriptive name). In contrast to PFE, there is a physical barrier to the eruption pathway. Absence of the PDL indicates fusion of tooth to alveolar bone and can indicate MFE. With MFE, more so than PFE, orthodontic or other therapies may allow the impacted tooth to erupt eliminating the need for extraction and replacement.

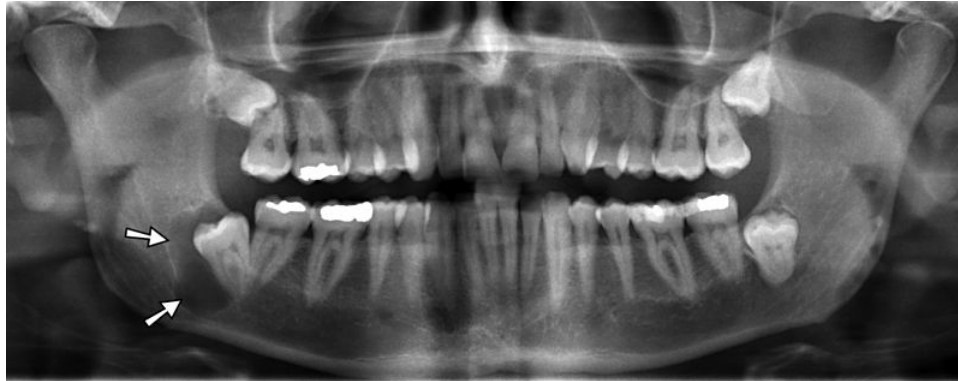


Figure 8.25: Example of a dentigerous cyst. Image credit: Jaw cyst By: Coronation Dental Specialty is licensed under CC BY 3.0,

Dentigerous cyst

An impacted tooth can produce a cyst around the crown, called a **dentigerous cyst**. As the motionless tooth attempts to digest its way through oral epithelium, fluid accumulates between the crown and REE. The cyst may continue to grow in size, causing pain and trauma to the jaw bone (usually the mandible). Extraction of the impacted tooth will solve the issue, or orthodontic facilitation of tooth eruption may be an option as well. Rarely, the REE in a dentigerous cyst develops into a benign tumor known as an ameloblastoma. The main risk of this cancer is that similar to that of the cyst—it can cause pressure which may cause bones to break or to grow malformed. The reason this tumor is rare is that the REE contains differentiated (non-mitotic) cells. Because these epithelial cells are anchored to one another by desmosomes, this type of tumor rarely metastasizes.



Figure 8.26: A Bohn's nodule is keratin, not a tooth. Image credit: Own work by West Exchange is licensed under CC BY 3.0

Bohn's nodules

Bohn's nodules (referred to as epithelial rests of Serres in older texts) are another gingival cyst similar to Epstein pearls. They are masses of keratin trapped in the gingiva, produced by the REE rather than oral epithelium. Sometimes remnants of minor salivary glands are also called Bohn' nodules, despite a lack of keratin. Their presence along the alveolar ridge may cause parents to mistake them for erupting teeth. They more commonly occur along the maxillary ridge. Like Epstein pearls, these cysts are transient—they go away on their own, usually within 3 months, and produce few in any disturbances.

Lastly, if you are interested in the history of embryology and long-dead European men, we offer the following. Antoine Étienne Serres collaborated with the embryologist Johann Friedrich Meckel, who named the cartilage in the first pharyngeal arch that develops into the mandible (Meckel's cartilage). Together, they attempted to unify embryology and the lineage of different species (late called *evolution by heritable selection* by Charles Darwin and the less-well-known Alfred Russel Wallace). Their theory was later developed by Ernst Haeckel into the famous phrase “ontogeny recapitulates phylogeny”, which we have quoted earlier.

Sadly, while Haeckel is celebrated for his work as an embryologist (including in this book), he also published racist pseudoscience which influenced the [eugenics movements in the U.S. and Nazi Germany](#). This may seem like a distraction, but the idea of a biological definition of race is [still popular in the U.S.](#) and elsewhere, despite having been [clearly disproved](#) by the human genome project. Furthermore, the rate of untreated caries is triple for African Americans children compared to their Caucasian peers in the U.S. thanks to ongoing systemic racism. This disparity persists into adulthood, affecting the likelihood of [receiving endodontic therapy](#) and other dental procedures. This is not due to differences in genes (nature), it is due to [inequality in dental care](#) (nurture).

Chapter 7 * Chapter 9

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1.9: Enamel Development

- Overview
- Amelogenesis
 - Initiation
 - Apposition
 - Mineralization
- Clinical considerations

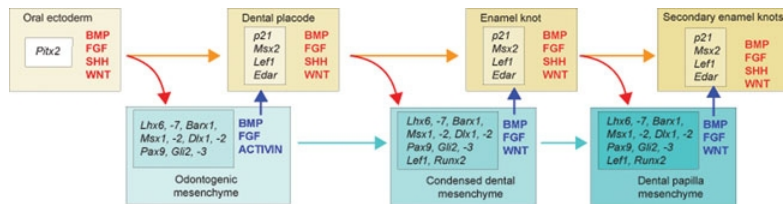


Figure 9.1: An illustration of the large number of and complicated interplay between morphogens involved in amelogenesis. Image credit: The sequential and reciprocal regulatory signaling between epithelium (red) and mesenchyme (blue) by Thesleff, I. and Tummers, M, [Stembook.org](https://www.stembook.org/), is licensed under [CC BY 3.0](https://creativecommons.org/licenses/by/3.0/)

Overview

Even though dentin formation occurs before enamel formation, we start with enamel formation. This way, the chapter on dentin formation can remain close to pulp, which is important because they share the same lineage from neuro-mesenchyme. Enamel is produced by cells derived from the ectoderm. This leads to key molecular difference between the extra-cellular matrix of enamel versus that of dentin, cementum and bone tissue. Another major difference we see is that enamel-producing cells are lost during tooth eruption, whereas the cells that produce dentin, cementum and pulp remain within a vital tooth. One major focus of studying embryology is to learn about how stem cells differentiate into adult cell types, in case we ever find the need to make adult cells de-differentiate and then re-differentiate into a cell type that needed. Re-creating ameloblasts would might not be terribly useful, because enamel is made from the surface of the crown, unless you could protect them with some sort of covering. But ameloblasts and odontoblasts undergo reciprocal signaling, meaning you can't get odontoblasts without ameloblast signals, and you can't get ameloblasts without odontoblasts, it takes a team effort. In this chapter, we mention two or three morphogens (ones we've seen before), and one transcription factor. Hopefully Fig. 9.1 illustrates that this process is more complex.

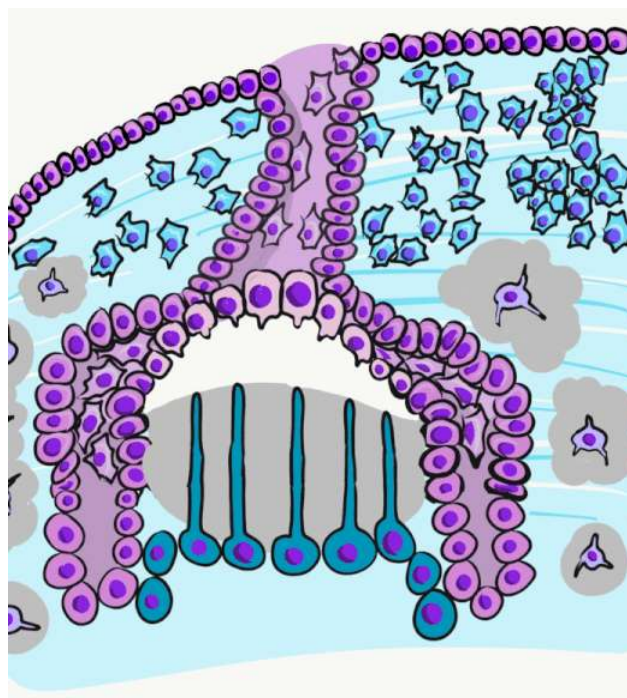


Figure 9.2: Animated representation of odontoblast and ameloblast formation.

Initiation

This paragraph should be review. Amelogenesis starts at the bell stage of tooth development. At this stage, ectodermal cells invaginate to produce two layers of simple cuboidal cells called the Inner Enamel Epithelium (IEE) and Outer Enamel Epithelium (OEE). Between the IEE and OEE, more epithelial cells are scattered, namely the stratum intermedium and stellate reticulum cells. Collectively, these cells are known as the Enamel Organ. Let us now add details to what we learned in the previous chapter. The enamel organ grows around a cluster of neuro-mesenchymal stem cells known as the dental papilla. Initially, a basement membrane separates the two. The first visible sign of differentiation occurs when the cuboidal IEE cells next to the dental papillae elongate, becoming more columnar in shape. Their nuclei move to the apical side of the cell, while organelles needed for secretion accumulate on the basal side. We therefore say the pre-ameloblasts undergo polarization. It is not known what triggers this change, a good first guess would be one of the different planar cell polarity morphogens. The ability to undergo mitosis halts for these cells at this time, meaning no new ameloblasts can form after the bell stage. The disappearance of the basement membrane allows these cells to move more easily, due to the loss of hemi-desmosome attachments. The pre-ameloblasts secrete a number of short-range morphogens which induce neighboring neuro-mesenchymal cells to differentiate into odontoblasts and begin secretion of pre-dentin. This puts pre-ameloblasts in direct contact with pre-dentin. Integrins on the pre-ameloblasts now contact a different form of collagen found in pre-dentin (type I), as opposed to the type in the basement membrane (type IV). In addition, newly formed odontoblasts secrete several morphogens, including BMPs. Collectively, these signals are the first signal in the differentiation of ameloblasts. The second signal comes from the opposite direction, the stellate reticulum. One such signal produced by stellate reticulum cells includes a member of the Fibroblast Growth Factor (FGF) family. This leads to the activation of transcription factors in the pre-ameloblasts, which up-regulate the expression of genes involved in the secretion of enamel. Transcription factors involved in amelogenesis include MSX-2, a homeobox gene, and RUNX2, a transcription factor which is also involved in osteoblast differentiation. Visible under the microscope, pre-ameloblasts change shape again, becoming shorter, and develop a bump on the basal side known as **Tomes' process**. A summary of some molecules involved is in Table 9.1. The names are less important than the general order of events: from morphogen to receptor to transcription factor to protein expression.

Morphogen examples	Receptors	Transcription factors	Genes up-regulated
Collagen (dentin) BMP (Odontoblasts)	Integrins BMP-R	MSX-2 DLX1 RUNX2	Enamelin Amelogenin Ameloblastin Matrix Metalloproteinase
FGF (Stellate Reticulum)	FGF-R		

Table 9.1: a partial list of molecules involved in ameloblast differentiation.



Figure 9.3: A thin section highlights enamel's colorless state. Image credit: "18036" is in the Public Domain, CC0 / cropped

Apposition

After changing morphology, ameloblasts secrete enamel matrix from Tomes' process. This ECM is similar to the ECM of bone tissue, with some key differences. Like bone matrix, enamel matrix is first secreted in a softer form, consisting mostly of proteins and water, and it mineralizes later. Enamel matrix contains CaPO_4 crystals and organic components. Enamel, after it matures, has a higher mineral percentage, making it the hardest substance in the human body. Unlike bone tissue, however, enamel matrix has very little or no collagen. Instead, ameloblasts secrete similar proteins known as **Amelogenins** and **Enamelins**. These proteins act as nucleation centers to help induce calcium and phosphate to form crystals. The important fact to remember here is that collagen is a protein made by Fibroblasts (and other cells derived from mesenchyme), which come from the mesoderm or from neuro-mesenchymal cells. Ameloblasts lineage, on the other hand, is from the ectoderm. Presumably the connective tissue genes like collagen are methylated and packed away around histones, and are not available for transcription. The shape of the enamel organ in the bell stage determines the shape of the crown. No new ameloblasts form because they lose the ability to undergo mitosis once they differentiate into pre-ameloblasts. Furthermore, no other stem cells can come into contact with both dentin and stellate reticulum once a thick layer of enamel pushes the two apart, so no new ameloblasts can form by differentiation, either. Because of how the pre-ameloblasts lined up in the bell stage, mostly pointing towards the oral cavity, enamel is thicker in the masticatory surfaces and thinner in the cervical areas.



Figure 9.4: Enamel is deposited appositionally, as seen by the Lines of Retzius. Image credit "Tooth of *Paranthropus robustus* SKX 21841 from Swartkrans" by Didier Descouens is licensed under CC BY 3.0 / red box added

Enamel is secreted by ameloblasts from Tomes' process, laying down a new layer of pre-enamel over the older layers, pushing the ameloblasts further away from dentin. Ameloblasts, like many human cells, do not have a uniform speed. About every 4-12 days there is a change in the enamel deposition rate. As a result of deposition of enamel speeding up and slowing down, visible bands of lighter and darker enamel (less dense and denser) are visible when viewed in cross section. These visible bands are known as **Lines of Retzius** (or striae of Retzius, Fig. 9.4). One such band, the **neonatal line**, is particularly dark because the ameloblasts rested during the day we were born. After birth, the edges of the layers may be visible on the surface of teeth, especially the anterior teeth,

in which case they are called **perikymata**. Over time, chemical and physical abrasion wears perikymata away. Counting the number of perikymata and multiplying by a number between 4 and 12 yields an estimate of how long it took the crown of that tooth to develop. This has no use in dental hygiene. It does represent a pattern unique to each individual, and thus may act like a fingerprint, with possible applications in forensics and [biometric identification](#) (a tooth print instead of a fingerprint).

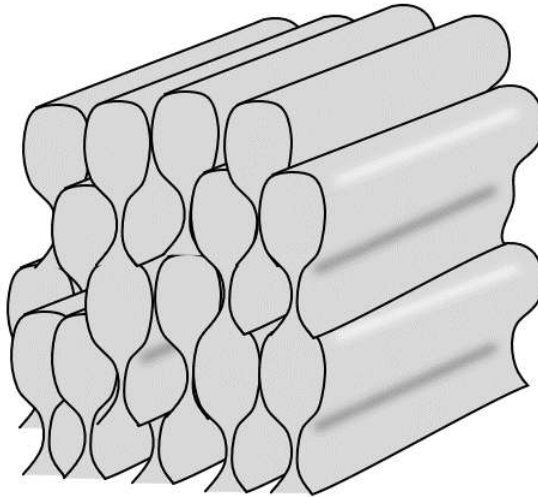


Figure 9.5: Enamel rods are each produced by one small group of ameloblasts.

A layer of ameloblasts produce horizontal layers of enamel, new on top of old, but one ameloblast produces a single **enamel rod** (or prism) in a perpendicular direction. Because the rods are rounded, and not square, there are gaps between them. These gaps aren't empty, they are filled with a slightly different form of enamel, called **inter-rod enamel** (while the enamel within rods is called **rod enamel**).



Figure 9.6: Hunter-Schreger bands are the enamel produced by a group of ameloblasts, and are lines that run perpendicular to the DEJ. Image credit: "18036" is in the Public Domain, CC0 / *cropped*

If you look closely to Fig 9.6, you might notice **Hunter-Schreger bands (HSB)**, which are lines running perpendicular to the lines of Retzius. Each line represents the contributions of a small group of ameloblasts. If it's a little hard to see, that could be because each enamel rod is microscopic. What you are looking for is a pattern similar to the fore edge of a textbook (all the pages as a group). Ameloblasts do not work in perfectly straight lines, the bands tend to curve slightly. Furthermore, different regions have different curve angles and patterns. This increases the tensile strength of enamel. If you have ever chopped wood, you know it is easy to do if you hit the axe right along the grain. Hit at an angle, and it can hurt you instead. The bending of Hunter-Schreger

bands ensures there is no single grain along which a tooth could split. Cracks develop, but they tend to not extend the whole way through. The planar cell polarity signals that guide rod organization have been identified ([Antagonists](#) of Wnt morphogens). As a fun side-note, or perhaps of relevance to those planning on pursuing a career as a [veterinary dental hygienist](#) at your local zoo, the HSB of hyenas occurs in a zig-zag pattern, increasing the tensile strength of their enamel to allow them to chew through bones. Dentin has curves (plural) which get specific names.

Maturation

Enamel is first secreted as a protein-rich gooey substance called **pre-enamel**. Two major protein components of enamel are the proteins amelogenin and ameloblastin. Ameloblasts also secrete a digestive enzyme. This enzyme, a matrix metalloproteinase, digests collagen and makes it easier for ameloblasts to migrate through the ground substance of overlying mesenchyme. This is necessary because as ameloblasts deposit pre-enamel from Tomes' process, it pushes the cells outwards. If you recall, matrix metalloproteinases are also involved in the proper migration of tissues during formation of the palate. Matrix metalloproteinases serve a second role by processing enamel proteins made by the ameloblasts after they are secreted into the extracellular matrix. This helps to ensure there is a delay between secretion of pre-enamel and the mineralization of enamel. Without a delay, ameloblasts could become trapped by their own enamel, halting amelogenesis. Once pre-enamel has been laid down and mineralizes, ameloblasts switch tasks and cause the enamel to mature. Some of the proteins are removed, replaced by fluids secreted by the ameloblasts, which then crystallize. We can therefore think of some of the protein secreted by ameloblasts as a scaffold that ensures enamel can be made quickly, at about 4 μM per day. Fluoride-based re-mineralization of tooth enamel is not measured using a small ruler, suggesting the rate of re-mineralization is too slow to see under the microscope. Our increased understanding of scaffold proteins has propelled researchers and companies to develop better tools for assisting in enamel re-mineralization, which mimic the ability of amelogenins and ameloblastin proteins to nucleate the formation of calcium hydroxyapatite crystals from a solution containing calcium and phosphate. These products do not need to contain proteins, for instance [xylitol](#) and certain glass compounds nucleate calcium hydroxyapatite crystals. Xylitol has the dual benefit of replacing simple sugars in foods, removing the substrates which harmful oral bacteria use for energy to secrete enamel-dissolving acids. A number of different electrolytes and molecules are incorporated into enamel during the maturation stage. One important electrolyte is Fluoride (F^-). Fluoride ions are negatively charged, and incorporate into hydroxyapatite crystals by replacing a small number of hydroxide (OH^-) ions. This has important clinical implications. First, a small amount of Fluoride increases the strength of enamel. Secondly, Fluoride ions found in either saliva or incorporated into enamel neutralize acids released by bacteria, reducing enamel demineralization and preventing caries. Excessive Fluoride levels, however, affect the activity of ameloblasts during amelogenesis, reducing their removal of protein scaffolds. This may create a visual change in the form of white lesions on the surface of teeth, where the enamel has a higher protein and lower crystalline content, known as **dental fluorosis**. Topical application, from toothpaste or gels, allows Fluoride to incorporate into the surface layers of enamel, which has considerable benefits. But the greatest potential benefit is when Fluoride exposure occurs during the apposition and maturation stages of amelogenesis, early in embryonic development.

Clinical considerations

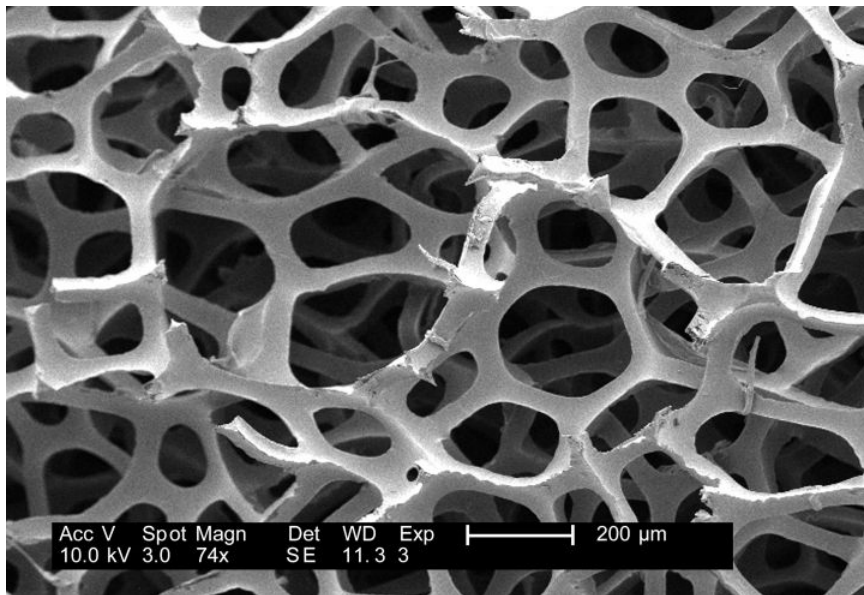


Figure 9.7: Acid etching demineralizes rod and interrod enamel at different rates, producing a rough surface suitable for bonding. Image credit: [This work](#) by Janice Carr, USDCDCP is in the [Public Domain](#), CC0

Surface etching

Because rod enamel is slightly different from interrod enamel, applying an acid to the crown surface wears the two at different rates—a process called **surface etching**. This creates an uneven surface, providing more surface area to create a stronger bond between the tooth and a surface sealant (think about how dermal papillae and rete pegs interdigit with one another for a stronger connection). Treatment with a weak acid may also remove debris at the surface, known as a smear layer, which otherwise interferes with bonding of an adhesive.



Figure 9.8: Oral manifestation of bulimia by [Jeffrey Dorfman](#) is licensed under [CC BY 4.0](#)

Erosion (or corrosion)

Prolonged exposure to a weakly acidic environment, or a shorter exposure to a highly acidic environment, can cause **erosion** of tooth enamel. Positively charged H^+ ions of an acid react with negatively charged OH^- and PO_4^- ions of calcium hydroxyapatite crystals, dissolving them. Bulimia and acid reflux bring highly acidic hydrochloric acid of the stomach in contact with teeth, causing erosion. Xerostomia, caused by the abuse of drugs such as methamphetamines, the use of certain medications, chemotherapy, or associated with old age, reduces the protective buffering ability of saliva to protect tooth enamel. Acidic foods or sugary foods may also contribute to enamel erosion. The simple sugars in many foods are nutrients oral bacteria metabolize and use to produce energy, which they then expend to create and secrete enamel-eroding acids.

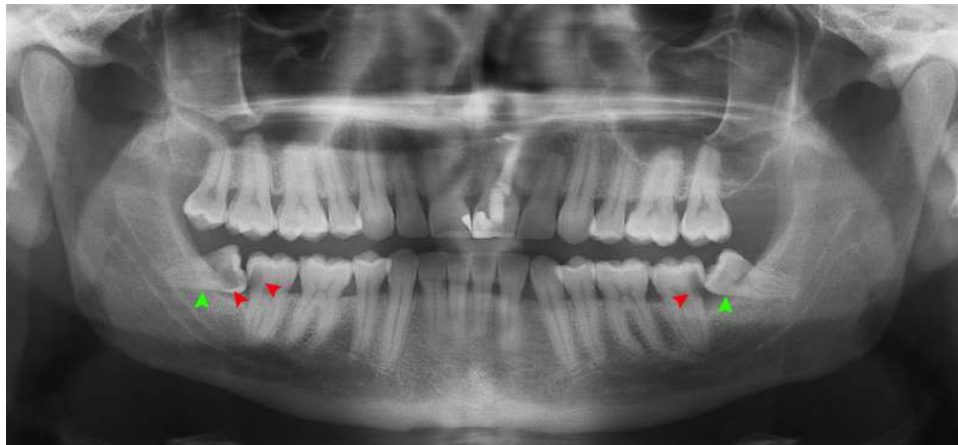


Figure 9.9: Dental caries (red arrows) caused by impacted 3rd molars (green arrows). Image credit: “Impacted wisdom teeth caries” by [Coronation Dental Specialty Group](#) is licensed under [CC BY-SA 3.0](#)

Early detection of erosion (a **dental caries**, or tooth decay) can be done during a regular examination. Early signs of enamel erosion includes the appearance of a white lesion on the surface of enamel, which may be softer to the touch, and appear more radiolucent on a dental radiograph (Fig. 9.9). Treatment often involves removal of the carious tissue followed by replacement with a bio-compatible material.

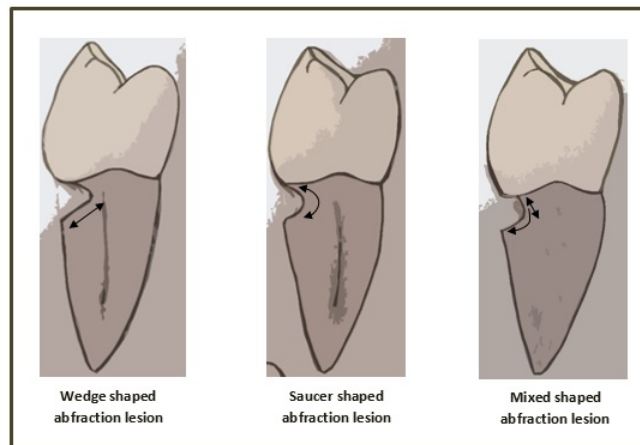


Figure 9.10: Three common patterns of tooth abfraction, all along the dentin-enamel junction. Image credit: Own work, By Ebd2015 is licensed under [CC BY 3.0](#)

Abfraction

When non-carious tooth material is lost by force, it is called **abfraction**. Abfraction frequently occur along the **dentin-enamel junction** (DEJ). Chewing causes flexion forces at the cervical region of the tooth. Treatment of this type of lesion should involve reducing the stress that caused it in the first place, such as parafunctional habits or tooth occlusion. Because enamel is stronger than dentin, dentin is frequently lost in response to force transmitted through the enamel to the DEJ.

Developmental disturbances



Figure 9.11: An example of the pitting and white lesions caused by enamel hypoplasia. Image credit: Symmetrical enamel hypoplasia of grade I on permanent incisors in a CD patient by Maurizio Procaccini et al, [Head & Face Medicine](#) is licensed under [CC BY 2.0](#)

Enamel hypoplasia

Reduced enamel formation during embryonic development is called **enamel hypoplasia**. This occurs to primary and/or succedaneous teeth. Hypoplasia in an embryo is often triggered by health changes in the mother, such as a gastrointestinal disorder or a syphilis infection during pregnancy. If ameloblasts cannot acquire enough minerals during the time they are active, upon tooth eruption the surface enamel may contain white lesions, or exhibit pits and grooves. The enamel is more brittle and susceptible to erosion and abfraction. Furthermore, increased sensitivity to temperature and increased incidence of dental caries and periodontal disease are expected. Because amelogenesis is complete long before birth, there is currently no way to significantly assist in enamel production after tooth eruption occurs. Therefore, preventative treatment becomes much more important, and full-coverage crowns might be required.



Figure 9.12: An example of a patient with amelogenesis imperfecta. Image credit: Amelogenesis imperfecta by 신연아 is licensed under [CC BY 3.0](#)

Amelogenesis imperfecta

Another cause of enamel hypoplasia is the genetic condition **amelogenesis imperfecta**. There are a number of different genes that, when mutated, cause this disease. For instance, mutations in the genes for amelogenin and enamelin lead to forms of amelogenesis imperfecta by reducing the ability of scaffolds to rapidly nucleate hydroxyapatite crystal formation. Another gene mutation that can be inherited is in a matrix metalloproteinase. Because amelogenesis imperfecta is caused by different mutations to a number of different genes, the symptoms occur on a spectrum, from mild to severe.



Figure 9.13: An enamel pearl. Image credit: Own work, By Wickcity is licensed under CC BY-SA 4.0 / *cropped*

Enamel pearl

The IEE within HERS does not normally differentiate into ameloblasts because of the lack of morphogens from stellate reticulum. However, a small region of the IEE might mistakenly differentiate into ameloblasts along the root and produce a small amount of enamel over cementum, known as an **enamel pearl**. There is no harm in having pearls, other than they may be confused with a calculus during a scaling procedure. If the wrong type of dental scaler is used, the instrument tip may break off when it encounters the much harder enamel pearl. They can be identified by radiography, because enamel is more radiopaque than cementum. Enamel pearls generally occur on the furcations of the multi-rooted molars.

Chapter 8 * Chapter 10

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1.10: Dentin-Pulp Complex Development

- Overview
- Dentin
 - Induction
 - Apposition
 - Maturation
 - Types of dentin
- Clinical considerations of dentinogenesis
- Pulp
 - Anatomy
 - Microscopic features
- Clinical considerations of pulp

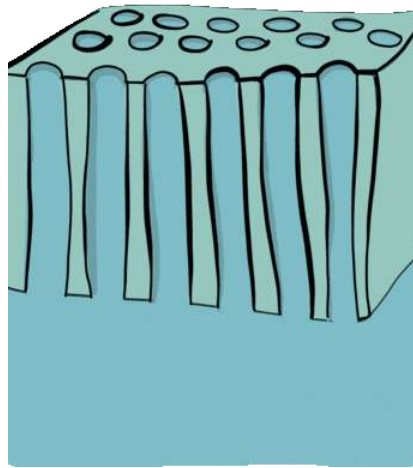


Figure 10.1: Illustration of dentinal tubules. Order of appearance: odontoblasts, nerve endings, overlying gingiva and enamel.

Overview

Dentin and pulp are in this chapter together because of their shared lineage: they are derived from the neuro-mesenchyme of the dental papilla. Even though dentin resembles enamel superficially, by the end of this chapter you should see similarities between dentin and pulp. The two are often referred together as the **dentin-pulp complex**. One key characteristic of dentin is that the extracellular matrix contains long tunnels that run through the whole layer, and these are filled with a little fluid and a cytoplasmic extension of a dentin-producing cell, the odontoblast. This has significance when it comes to dental hypersensitivity, as well as in the repair of dentin following damage.

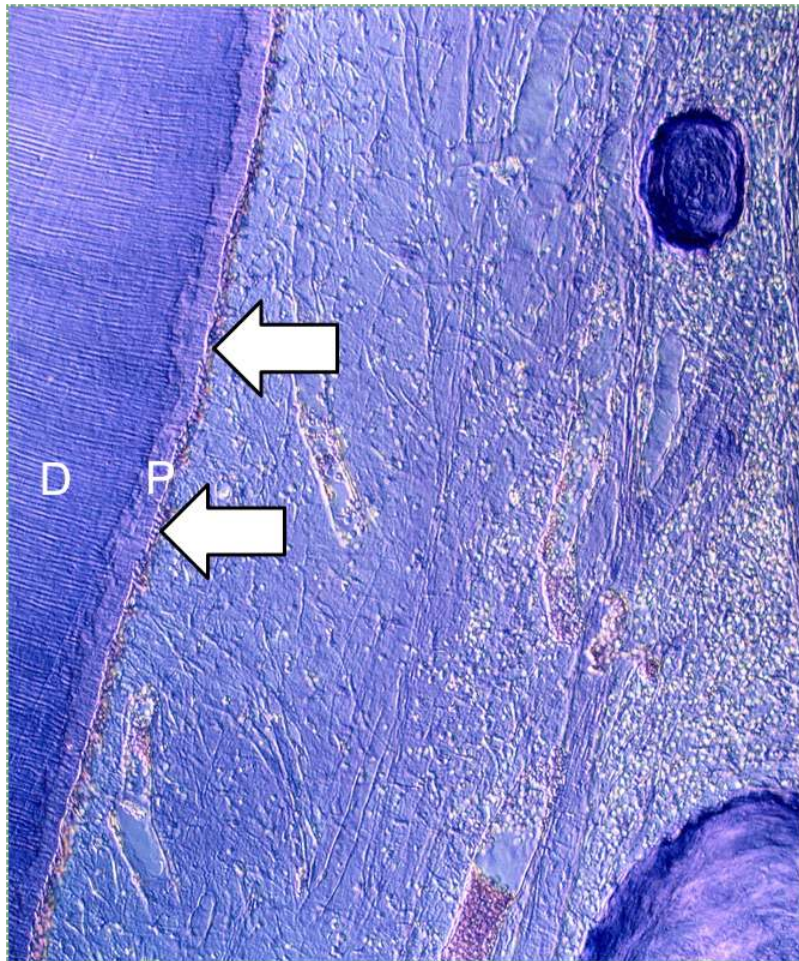


Figure 10.2: Important regions during dentinogenesis. Legend: P = pre-dentin, D = mature dentin, arrows indicate cell bodies of odontoblasts found at the edge of the pulp. Image credit: [Histological section of tooth](#) by Doc. RNDr. Josef Reischig, CSc. is licensed under [CC BY-SA 3.0](#).

Dentin formation

Dentinogenesis is the process of dentin formation (and should not be confused with the word odontogenesis). Dentinogenesis begins during the bell stage of tooth development. Odontoblasts (arrows in figure 228) are the cells that produce dentin, first by secreting proteins including collagen that act as a scaffold. Mineralization around the scaffold occurs later. The initial protein-rich material is **pre-dentin** (P in Fig. 10.2), after it mineralizes it is called dentin (D in Fig. 10.2).

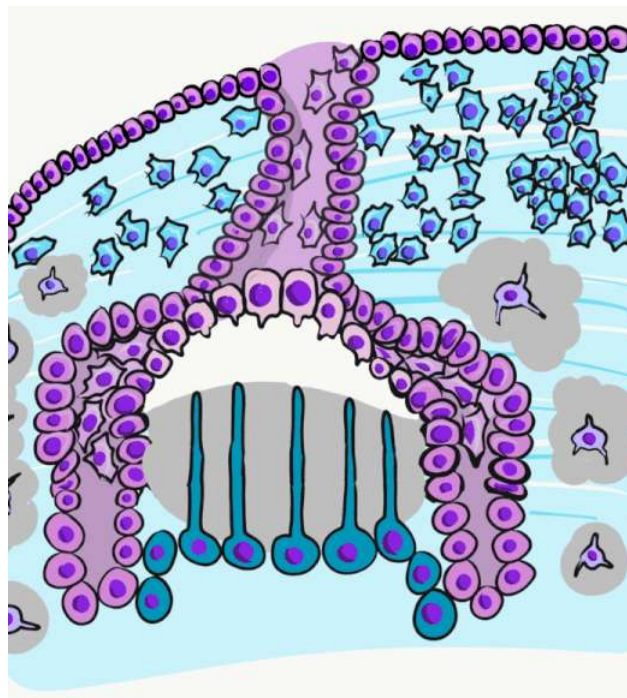


Figure 10.3: Animated view of amelogenesis and dentinogenesis.

Induction

Cells of the IEE differentiate into pre-ameloblasts and secrete morphogens, including a Bone Morphogen Protein. The outer layer of neuro-mesenchymal cells of the dental papilla receive these morphogens and differentiate into odontoblasts. This involves a morphological change, as the relatively shapeless neuro-mesenchymal stem cells line up with one another, forming what looks more like a simple cuboidal epithelium, complete with apical-to-basal polarity. This sort of polarization is unusual for a connective tissue, but odontoblasts are not derived from mesoderm, but neuro-mesenchyme. It is very common for neurons and glial cells to be polarized, and the odontoblasts do not lose this ability. Inside the odontoblasts, large amounts of endoplasmic reticulum and Golgi apparatus are forming in preparation for the secretion of large amounts of protein.

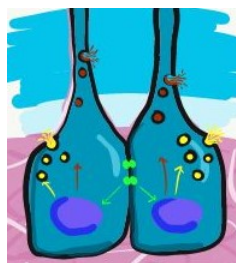


Figure 10.4: Polarization of odontoblasts, including cell-to-cell junctions that trigger activation of genes for proteins secreted into dentin. Legend: Green = cell-cell junctions help establish cell polarity by triggering intra-cellular signals. Yellow= secretion of collagen-filled vesicles to form pre-dentin (light blue layer) occurs at the apical surface of the cell body. Brown = secretion of enzyme-filled vesicles from the odontoblastic process triggers mineralization of pre-dentin into dentin (darker blue layer). Pink = pulp.

Apposition

Newly formed odontoblasts begin secreting proteins that are found within mature dentin and act as a scaffold during the mineralization of dentin. This protein-rich substance is pre-dentin (the lighter blue layer in Fig 230). It is mostly collagen plus a few other dentin-specific proteins. Pre-dentin later mineralizes, producing calcium hydroxyapatite crystals similar to enamel and bone tissue (darker blue layer in Fig 230). As layers of dentin mineralize, odontoblasts continue secreting new pre-dentin, pushing the layer of odontoblast cell bodies deeper into the jaw. Unlike ameloblasts, odontoblasts leave behind an odontoblastic process in the dentin they secrete. This arm-like extension contacts nearly every layer of dentin that odontoblast creates. Because dentin mineralizes around the odontoblastic process, a **dentinal tubule** runs through nearly the entire length of dentin. If you removed the odontoblasts, dentin would be perforated by millions of these tiny tubes. Collagen and dentin-specific proteins are secreted by the

side of the odontoblast cell body facing the enamel (yellow vesicles, Fig 230), which ensures that the cell body does not become trapped and instead touches a thin layer of pre-dentin. Proteoglycans and enzymes like Matrix Metalloproteinase are secreted a short distance up the odontoblastic process (less than 1mm, red vesicles in Fig 230) and trigger mineralization of dentin.

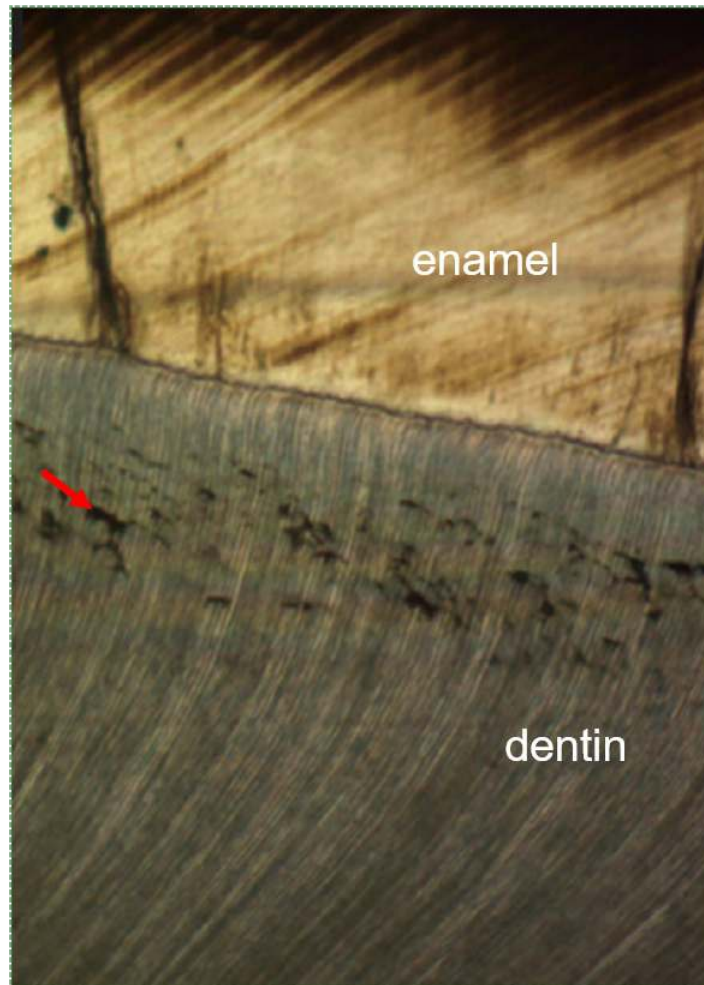


Figure 1a by Monaogna Vangala is licensed under [CC BY-SA 3.0](https://creativecommons.org/licenses/by-sa/3.0/) / labels added

Dentin mineralizes in one of two ways, in globs or in lines. The first dentin that is produced mineralizes in globs because collagen is secreted in circular globs. **Globular dentin** mineralizes into mature dentin. Some regions do not mineralize, and are called **inter-globular dentin**. Low levels of phosphate during the first trimester can increase the amount of inter-globular dentin. After the first collagen fibers are secreted in globs, the fibers can be lengthened by odontoblasts. This dentin mineralizes more uniformly, one layer at a time, in a process called **linear mineralization**. Collagen fibers run parallel to each other in regions that mineralize linearly. The lines that should be clearly visible in the layer of dentin in Fig. 10.5 are dentinal tubules, the tubes in which the odontoblastic process are found. Notice that they do not run in a straight line. Odontoblasts, like ameloblasts, grow in a slightly curved direction, and this increases the strength of dentin. The large curves are called the **primary curvature**—if you zoomed out you would see a sinusoidal (back and forth) shape. This curvature is more pronounced in the crown than the root. If you zoom in on a single odontoblastic process, you might see areas where it curves back the opposite direction a little. That is called a **secondary curvature**. In contrast, the bending of enamel rods is called “wavy”, there are no names for bigger and smaller curves.

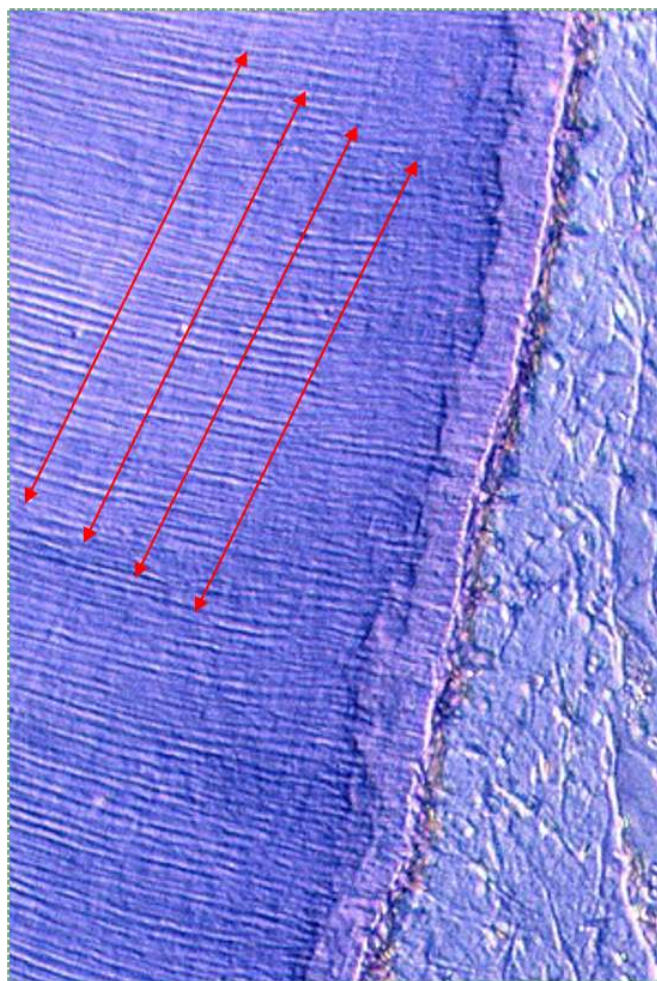


Figure 10.6: Imbrication lines of Von Ebner (in direction of red lines). Image credit “[histological section of tooth](#)” by Doc. RNDr. Josef Reischig, CSc. is licensed under CC BY-SA 3.0 / red lines added

Similar to the formation of enamel, odontoblasts undergo cyclic patterns of more and less rapid pre-dentin deposition. As a result, light and dark bands found in dentin are visible, called the **Imbrication lines of Von Ebner** (or the incremental lines of Von Ebner), which are comparable to the Lines of Retzius seen in enamel. Don’t ask how Retzius saw lines in enamel but missed the same pattern in dentin a few millimeters away, leaving that to Von Ebner to discover a hundred years later. People get credited for *re-discovering* things all the time, such as Christopher Columbus. These should both be renamed incremental lines in enamel and the incremental lines in dentin, anyway. Particularly prominent imbrication lines can, furthermore, be called **contour lines of Owen**. This includes the neonatal line, similar to the one found in enamel. These represent big changes in nutrition that lead to changes in the density of dentin produced that day. You can read part of [Owen’s treatise on the comparative anatomy of the tooth](#), someone scanned 30 pages if you are curious what it was like to learn about histology without pictures.

Maturation

Unlike enamel, dentin does not undergo significant changes after it mineralizes. As a result, some textbooks call the mineralization of pre-dentin into dentin as the “maturation” step, while others (including this one) have chosen to use the words apposition and maturation consistently. Why do ameloblasts take an extra step removing some scaffolding after mineralization occurs, and odontoblasts do not? One possibility is ameloblasts are epithelial cells, and epithelia generally secrete very little ECM, and are not very good at it. Odontoblasts differentiate from neuro-mesenchymal stem cells. It is likely during their epithelial-to-mesenchymal transition they unpacked genes that make them more efficient at creating ECM. A second possibility is that the increased percentage of minerals in mature enamel can only occur after protein scaffolding is removed, leaving behind a higher percentage of minerals. The reason is not as important as the illustration of another difference between enamel and dentin formation that can be traced to different cell lineages.

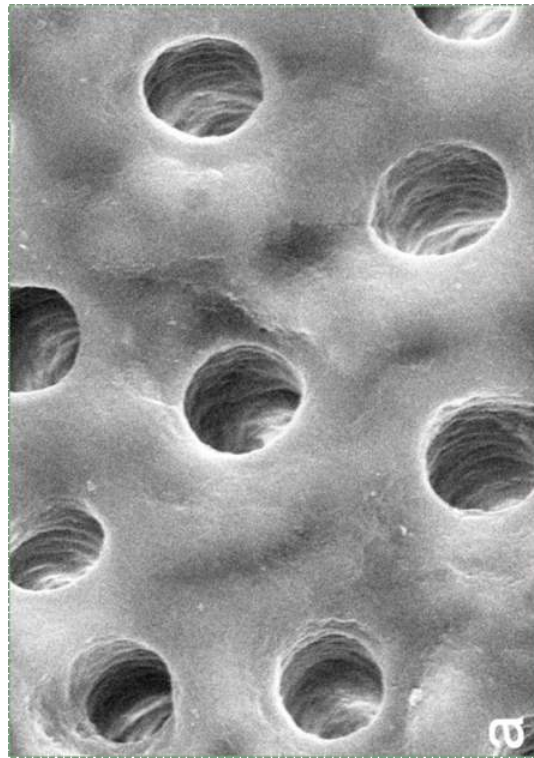


Figure 10.7: Dentinal tubules without odontoblastic processes. Image credit: [Dentinal tubule occlusion of dentine discs after treatment](#) by Peiyan Yuan is licensed under CC BY-SA 3.0 / *cropped and animated labels added*

In Fig. 10.7, dentinal tubules should be easily visible. In a living tooth, each would contain an odontoblastic process. Not all regions of dentin are the same, and there are several ways of classifying different types of dentin. One way is based on how close the dentin is to the dentinal tubules. The thin white area of dentin immediately surrounding each dentinal tubule is called **peri-tubular dentin**, while the rest is **inter-tubular dentin**.

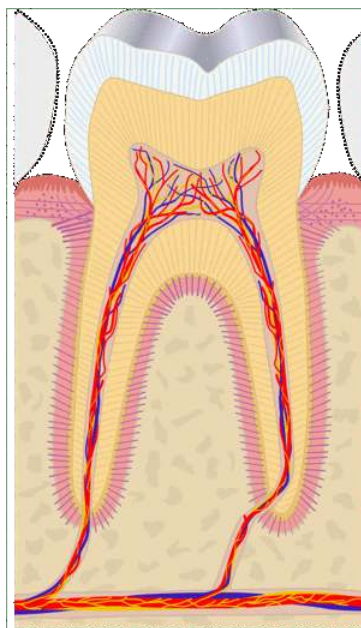


Figure 10.8: Types of dentin by location. Image credit: [“cross sections of teeth”](#) by Gorak Tek-en is licensed under CC BY 3.0 / *animation and text added*

Another way to classify types of dentin is on its location relative to the pulp cavity. **Mantle dentin** forms first, and is a thin layer (15-30mm) closest to the enamel. Mantle dentin contains few dentinal tubules, they are filled in during the maturation stage. Mantle dentin is where globular mineralization occurs. The collagen fibers that remain in mantle dentin run perpendicular to the

DEJ. The rest is **circum-pulpal dentin**, which mineralizes linearly, leaving dentinal tubules intact. Mantle and circum-pulpal dentin have slightly different levels of mineralization and protein content. The collagen fibers in circum-pulpal dentin run parallel to the DEJ.



Figure 10.9: Types of dentin by time of production. Original image “cross sections of teeth” by Gorak Tek-en is licensed under CC BY 3.0 / animation and text added

Yet another way to classify dentin is based on when it is formed relative to the apical foramen. If this seems redundant to mantle versus circum-pulpal dentin, you are mostly right. **Primary dentin** is dentin formed before completion of the apical foramen, therefore it is formed prior to tooth eruption. **Secondary dentin** is formed after the completion of the apical foramen (after tooth eruption). Unlike mantle versus circumpulpal dentin, there is no major histological difference between primary and secondary dentin. Much later, a tooth may suffer damage. Odontoblasts in the pulp produce **tertiary dentin** and repair the damage. This may also be called **reactionary dentin**. Formation of this form of dentin involves secretion of matrix metalloproteinases from dentinal tubules. These enzymes are also used during the formation of dentin in embryogenesis, so this is another example of wound repair recapitulating embryonic development. During embryogenesis, however, dentin is formed appositionally. Tertiary dentin mineralization occurs from a dentinal tubule, causing the tubules to become occluded. Therefore, fewer dentinal tubules are found in reactionary dentin. Furthermore, it is the parallel dentinal tubules that give primary and secondary dentin their yellow-ish hue. With reduced or no tubules, reactionary dentin becomes more translucent (see Fig 238).

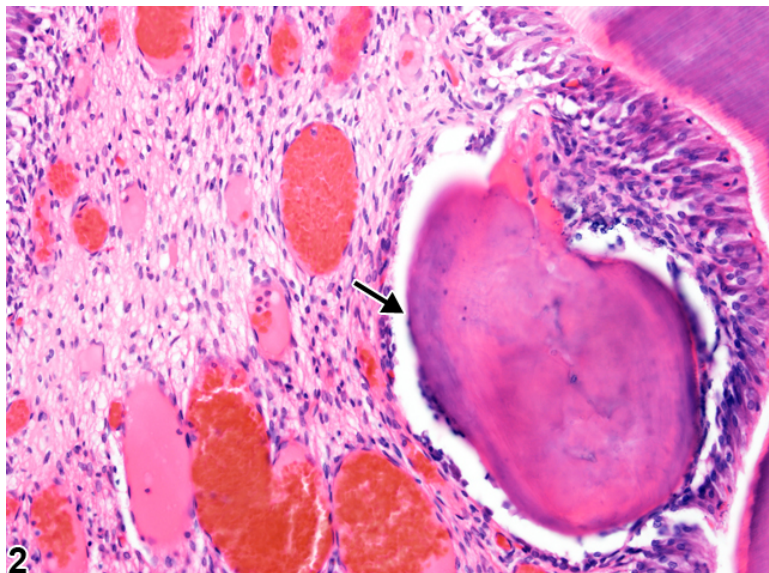


Figure 10.10: Osteodentin (arrow) inside of the pulp cavity. Image credits “Tooth, Pulp – Osteodentin in a male F344/N rat from a chronic study” by Cora MC, Travlos GS. 2014. , National Toxicology Program is in the Public Domain, CC0

If a large enough injury occurs as to expose the pulp chamber, possibly destroying odontoblasts, a much more robust response is needed. Mesenchymal stem cells in the pulp are induced by morphogens to differentiate into new odontoblasts and form a type of tertiary dentin called **reparative dentin**. This form of dentin does not form the same way dentin does during development, which began at the dentin-enamel junction and moved deeper. Instead, to form reparative dentin, odontoblasts and fibroblasts migrate throughout the injured area (after a hematoma forms) and secrete proteins and electrolytes, trapping many of the cell within. No dentinal tubules are created when odontoblasts secrete dentin in all directions. For this reason, reparative dentin is sometimes referred to as **osteodentin**, because of its resemblance to bone tissue more than tubular dentin.

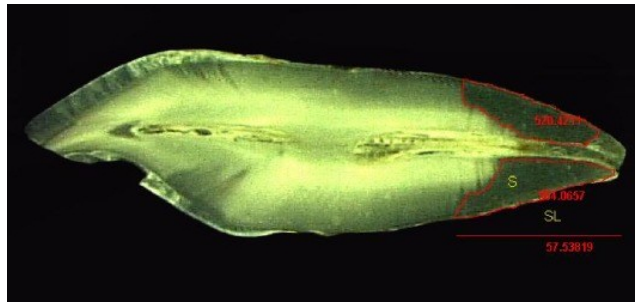


Figure 10.11: Sclerotic dentin (S). Image credits “Stereomicroscope image (5X) of tooth with measurement of Sclerotic Dentin Area (S) and Length of Sclerotic Dentin (SL)” by Selvamani M, et al, *Journal of International Oral Health* is licensed under CC BY-SA 4.0 / *cropped*

Lastly, if there are more fibroblasts than osteoblasts repairing the damage, the end result is more of a calcified scar than dentin, some textbooks call this **sclerotic dentin**. Sclerotic dentin is mostly collagen and calcium hydroxyapatite crystals.

Type of dentin		Location	Features
Peri-tubular		Walls of tubules	
Inter-tubular		Between walls	
Mantle		Thin border next to DEJ	No tubules Collagen perpendicular to DEJ
Circum-pulpal		Rest of tooth	Tubules Collagen parallel to DEJ
Primary		Formed before apical foramen	Made by original odontoblasts
Secondary		Formed after apical foramen	Contains tubules
Tertiary	Reactionary	Formed after small injury	Made by new odontoblasts Fewer tubules
	Reparative and sclerotic	Formed after significant injury	Made by new odontoblasts and fibroblasts Cell trapped in calcified tissue (osteodentin)

Table 10.1: Summary of the types of dentin.

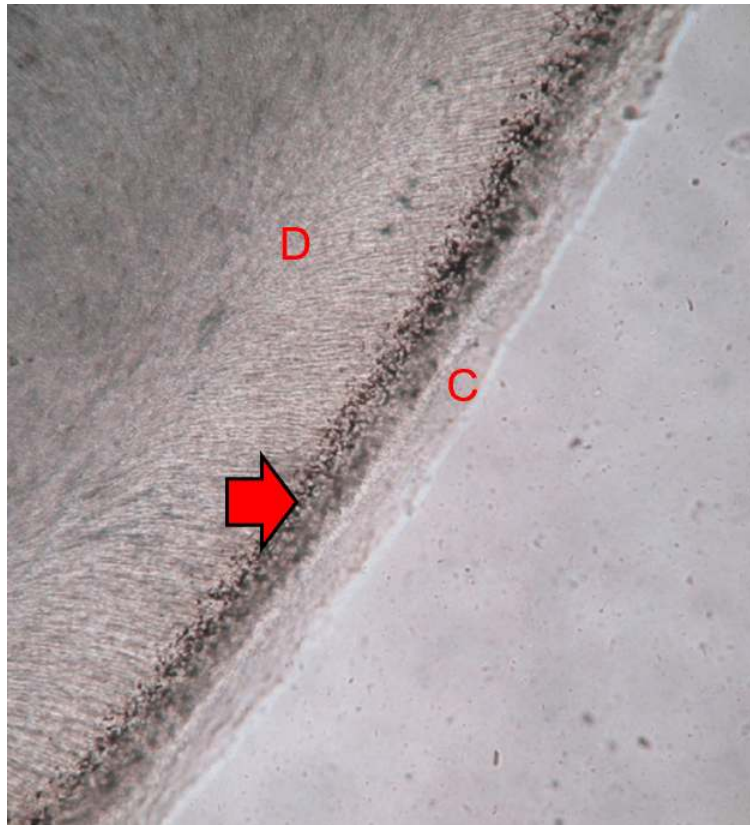


Figure 10.12: Tome's granular layer (arrow) found in root dentin (D), just deep to cementum (C). image credit "Tomes granular layer " by Shaik Mohamed Shamsudeen is licensed under CC BY-SA 3.0 / letters and arrow added

Root dentin

Roots do not contain mantle dentin, but they do contain a superficial layer of dentin that is visually distinct from deeper regions. Close to the border with cementum, spots are visible in a band of dentin known as **Tomes' Granular Layer**. It has no known clinical significance; it is simply a grainy area. It is useful for orienting yourself when looking at histological sections of teeth. Those grain are not the nucleuses of cells, nor are they inter-globular dentin. Older data suggested the grains represent loops of dentinal tubules, but re-analysis using more advanced microscopes suggests the grains are large bundles of collagen fibers.

Clinical considerations of dentinogenesis

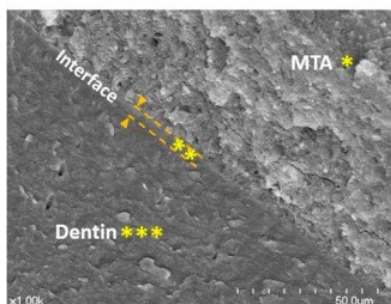


Figure 10.13: Electron micrograph of the interface between dentin and a mineral aggregate (MTA) used in the a root canal therapy to boost replacement of resorbed root dentin. Image credit: "Scanning electron microscopic images that are representative of the root canal dentin and mineral trioxide aggregate (MTA) interface" by Yoo, Yeon Gee et al is licensed under CC BY 3.0

Mineral aggregates

If there is a large amount of damage to dentin, the production of tertiary dentin may be too slow. For cases such as treating root resorption or for root end filling during endodontic therapy, artificial ECM can be used. Unlike some of the artificial tissues discussed in gingival healing, hard substances generally not make good scaffolds, as the density of the matrix inhibits migration of stem cells. The goal of using mineral aggregates is to provide the necessary materials required by odontoblasts without inhibiting

their movement during dentinogenesis. One such compound, Mineral Trioxide Aggregate (MTA), was developed in California by Dr. Mahmoud Torabinejad. MTA contains a purified version of Portland cement plus calcium-containing minerals. The addition of mineral aggregates speeds up the formation of tertiary dentin. MTA slowly releases calcium hydroxide, which provides a raw material for mineralization of tertiary dentin, as well as attracting phosphate from the blood or ECF. Author's note: the authors currently live in or near Portland, Oregon, but Portland cement was invented in Portland, England. Do not apply Portland cement from your hardware store to teeth, it is caustic and may contain arsenic or other heavy metals. The Portland cement used in dentistry is purified.



Figure 10.14: The appearance of dentin should be glistening and moist. Image credit: "Dentin appearance" by Pinkmanggis is licensed under [CC BY-SA 4.0](https://creativecommons.org/licenses/by-sa/4.0/)

In one type of dental restoration (a tooth filling), a wet resin is bonded (glued) to dentin and hardened using a special light. To bond resin to dentin, one should take into account the chemical structure of dentin. The hydroxyapatite crystals and collagen of the ECM of dentin contain some water molecules, giving dentin a moist appearance. Many bonding agents do not adhere well to a wet surface, and require dentin to be dried first. This is usually done with a volatile solvent such as ethanol. Mineral acids may be used to remove an amount of the mineral ECM of dentin, leaving behind the more porous collagen framework which can improve bonding. This is similar to acid-etching of enamel, but there are no rods and inter-rods to take advantage of in dentin. There is interest in the research community for developing bonding agents that adhere to wet surfaces (all living human tissues are wet). One promising area is the study of [mollusk mucus](#). Mucus is secreted *from* the body, but it is similar to ground substance *in* human connective tissues, especially mesenchyme. Mucus secreted by mollusks like limpets adhere to wet surfaces very tightly, and has the potential to stimulate tertiary dentin formation ([link to pdf download](#)).

Morphogens

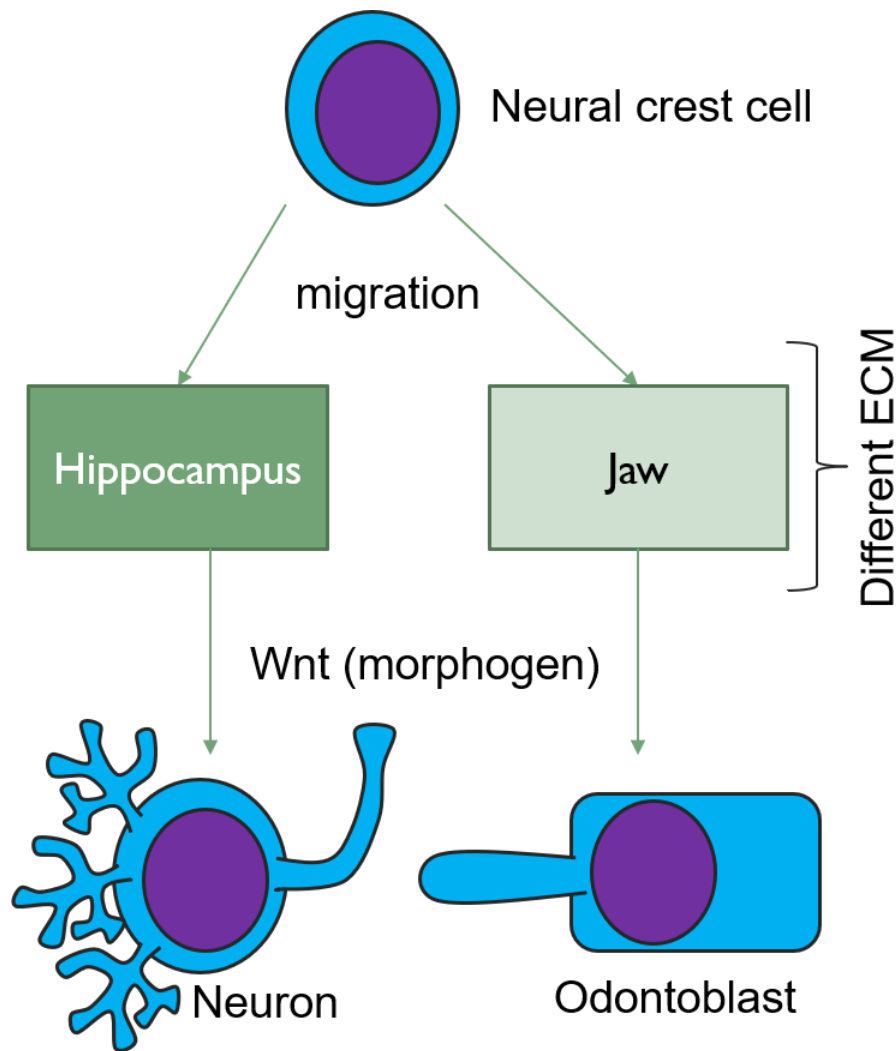


Figure 10.15: Wnt morphogens induce the differentiation of different cell types in different environments. The formation of secondary and tertiary dentin requires morphogens to induce the differentiation of mesenchymal stem cells into new odontoblasts. As we learn more about these morphogens, it opens up the possibility to administer these morphogens and speed up natural healing processes, potentially reducing the need for inorganic cements or caps. One such morphogen is a Wnt (the W stands for wingless, because when the right one is mutated in flies it prevents the induction of wing placodes.) In the brain, Wnts induce the differentiation of neural crest cells into specific types of neurons and glia. But when neural crest cells migrate to the face and become the pharyngeal arches, the same Wnt induces them to differentiate into odontoblasts. The difference arises because of a second morphogen. This second signal comes from the ECM, and ECM is very different in the developing brain than it is in developing neuro-mesenchyme of the pharyngeal arches. The significance of this is that a drug used to treat Alzheimer's Disease (AD), Tideglusib, is [used off-label in dentistry](#) to boost tooth repair. We suspect it is very rare to randomly test psychoactive drugs for their use in dentistry. Prior knowledge of shared lineages, however, makes new avenues of treatment more obvious.



Figure 10.16: Erosion of enamel and dentin. Image credit: Own work By Klaus Limpert, Scuba-limp is licensed under CC BY-SA 3.0

Attrition and erosion

Because dentin has a lower mineral content than enamel, it is lost more quickly than enamel. If mantle dentin is lost, this exposes dentinal tubules in the underlying circum-pulpal dentin. This increasing the amount of surface area for which acids can act upon, which speeds up dentin erosion further. Surface exposure of dentinal tubules also leads to increased sensitivity of the teeth (see below).

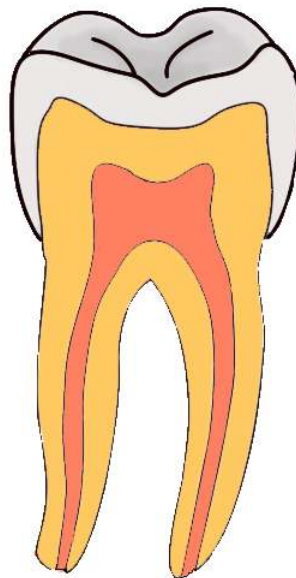


Figure 10.17: Caries in enamel versus dentin. Image credit Tooth By ADuran is licensed CC BY-SA 3.0

Dentin caries

Once a caries spreads through enamel, when it reaches the DEJ it spreads at an increased rate. The triangle pattern of enamel loss is quickly repeated in a new triangle of a **dentin caries**. It is possible, but not agreed upon, that a small enough enamel caries might

spread to the DEJ without causing visible changes, and upon reaching the DEJ the rate of tooth loss accelerates. Whatever the exact cause, a caries can start below enamel and spread through dentin. This **hidden caries** is obviously harder to detect than one at the surface of a tooth.

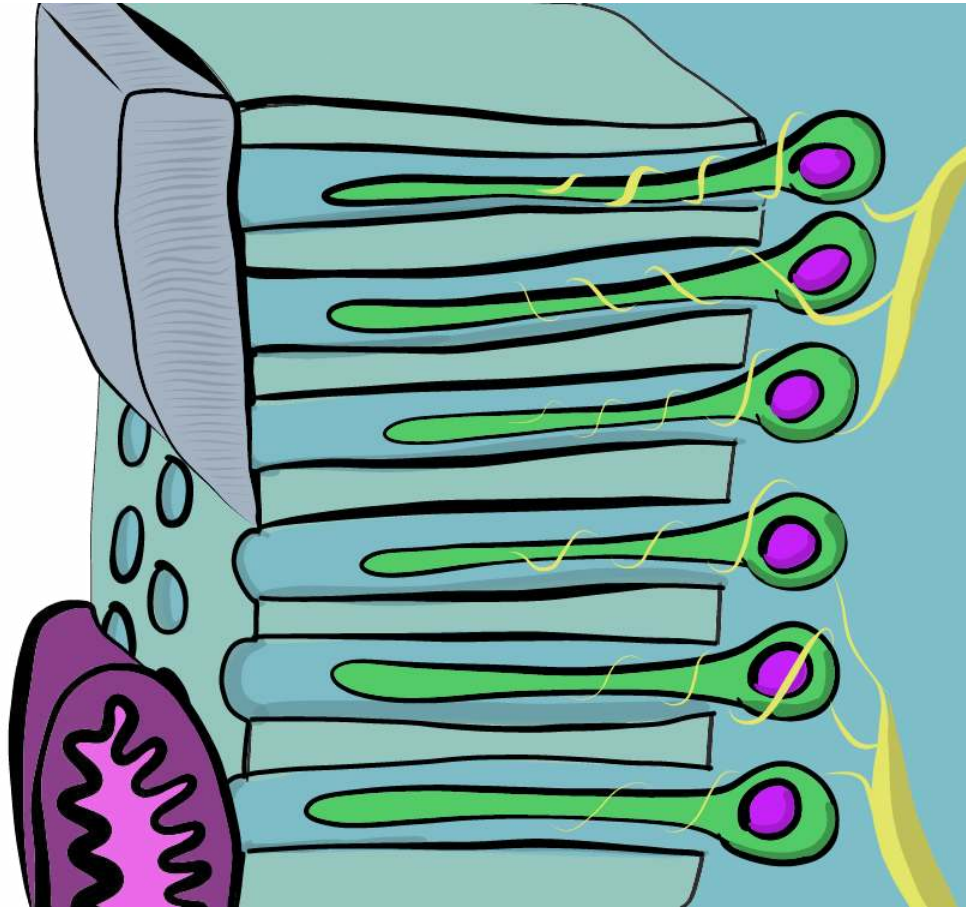


Figure 10.18: Tooth hypersensitivity due to exposed dentinal tubules.

Tooth hypersensitivity

The loss of enamel or cementum covering dentin may expose dentinal tubules. In the crown, the few millimeters of mantle dentin at the outer surface contain few open dentinal tubules, but as that dentin is lost many more tubules in the circum-pulpal layer are exposed. These tubules extend all the way to the root pulp, where many nerve endings are located. Changes in the environment of the oral cavity, such as temperature, pH, alcohol level or osmolarity changes can now affect the temperature, pH, alcohol level or osmolarity of the pulp ECF. Odontoblastic processes do to entirely fill up the dentinal tubule, there is a small amount of ECF between the process and peritubular dentin whose chemical composition can change. To ECF can be detected by nerve endings that relay painful stimuli to the brain. This is called tooth hypersensitivity, or more accurately **dentin hypersensitivity (DH)**. Treatments for DH include special toothpastes, mouthwashes or chewing gums that deposit minerals into exposed dentinal tubules and occlude them. Such products likely contain salts and fluoride. Alternately, varnish mineral aggregates such as Portland cement might be applied to the affected area. Conversely, with age, odontoblasts add layers of peritubular dentin inside dentinal tubules, causing the tubules to become narrower. As this happens, teeth become less sensitive. This may not sound so bad until you take into consideration the role that **proprioception** has in preventing excessive occlusal forces. Cranial nerve V is one of the larger cranial nerve, it transmits a lot of important sensory information to the brain from the teeth. With diminished sensitivity comes an increased risk of occlusal trauma.



Figure 10.19: The importance of proper dental hygiene procedures. Image credit “Dental details” by Senior Airman Kristi Emler, US Air Force is in the [Public Domain, CC0](#)

Dentin may become exposed due to loss of cementum or enamel, or if the edge of enamel does not meet/overlap cementum. Furthermore, gingival recession exposes the thin layer of cementum to environments it is not designed to handle, making dentin exposure along the neck of the tooth the most likely area for DH. Improper technique on the part of dental hygienists or dentists may inadvertently remove protective layers of cementum as well. *There is no reason to believe this is the case in figure 10.19, government agencies are simply a great place to find copyright-free images.*



Figure 10.20: Dentin resorption (arrow). Image credit “An irregular radiolucency in the coronal third to middle third of the root” by Fang-Chi Li is licensed under [CC BY-SA 3.0](#)

Dentin resorption

During the shedding of primary teeth, **dentin resorption**—the loss of dentin due to cells re-absorbing minerals—assists in the loss of attachment between the tooth root and alveolar bone. This is mediated by odontoclasts. Compared to their relative the osteoclast, which have baseline activity throughout life maintaining bone tissue as part of a remodeling unit. Odontoclasts are not always present, their differentiation from mesenchymal stem cells as well as their activity is regulated by different morphogens. Dentin resorption can be improperly triggered in cats, causing feline odontoclastic resorptive lesions. There is a significant amount known about this process in cats. In humans, dentin resorption occurring at any time other than the shedding of primary teeth is **idiopathic** (of unknown cause). We do understand that the dentinal tubules found throughout dentin create a large amount of surface area for odontoclasts to adhere to and trigger demineralization. This is similar to what we observe in spongy bone: spongy bone is lost at a faster rate than compact bone because it has a higher amount of surface area, thus symptoms of osteoporosis appear first in bones

which higher amounts of spongy bone, like the mandible. Dentin resorption can be triggered from the pulp-cavity side and be referred to as internal resorption. Conversely, external resorption occurs from the DEJ or CEJ side.

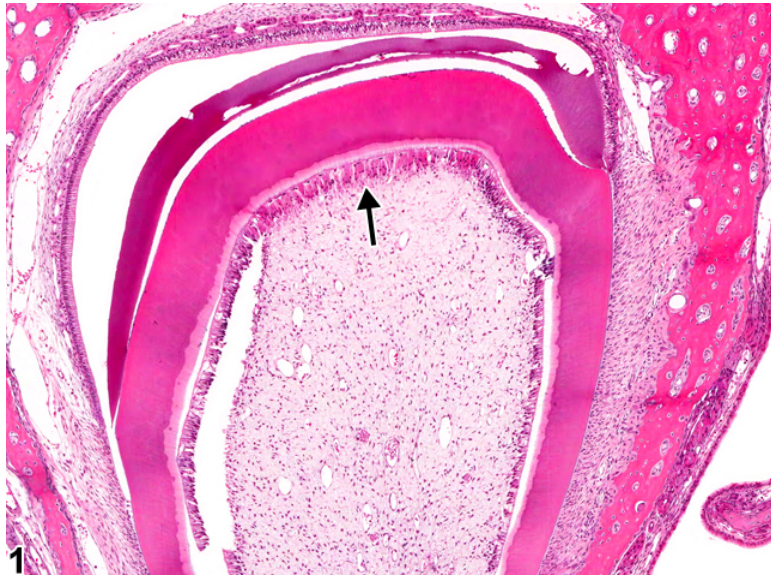


Figure 10.21: Histology of pulp. Arrow indicates odontoblastic layer. Image credit “Tooth, Odontoblast necrosis” by Cesta MF, Herbert RA, Brix A, Malarkey DE, Sills RC (Eds.), [National Toxicology Program Nonneoplastic Lesion Atlas](#) is in the [Public Domain, CC0](#)

Pulp overview

The dentin and pulp both develop from neuro-mesenchymal cells of the dental papilla. Odontoblasts make a more specialized tissue, containing just one terminally-differentiated cell type. The rest of the neuro-mesenchymal cells make a more generic tissue, areolar connective tissue, which contains numerous cell types, including adult stem cells. It is here that blood vessels, nerve fibers and lymphatic vessels have space and support to grow.

Histology of pulp

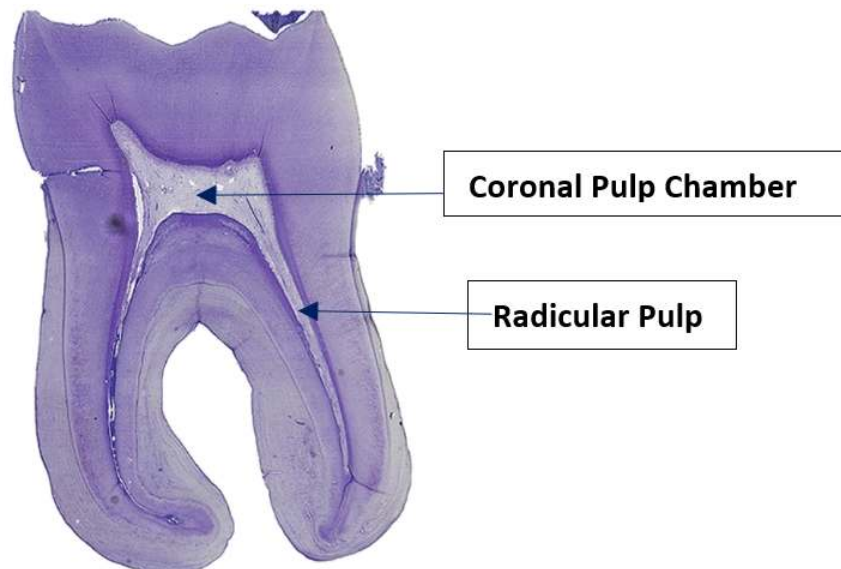


Figure 10.22: Coronal and radicular pulp. Image credit “Longitunal tooth 5” by Natdental is licensed under [CC BY-SA 4.0](#)

Pulp can be divided into **coronal pulp** and **radicular pulp**. The coronal pulp is in the crown of the tooth and contains smaller **pulp horns** beneath the cusps. Radicular pulp is in the roots, and may extend into **accessory canals**. Accessory canals connect the pulp to connective tissue external to the tooth, traveling laterally rather than out the apical foramen. Accessory canals form when HERS runs into a blood vessel and is forced to grow around it. Otherwise, radicular pulp terminates at the apical foramen.

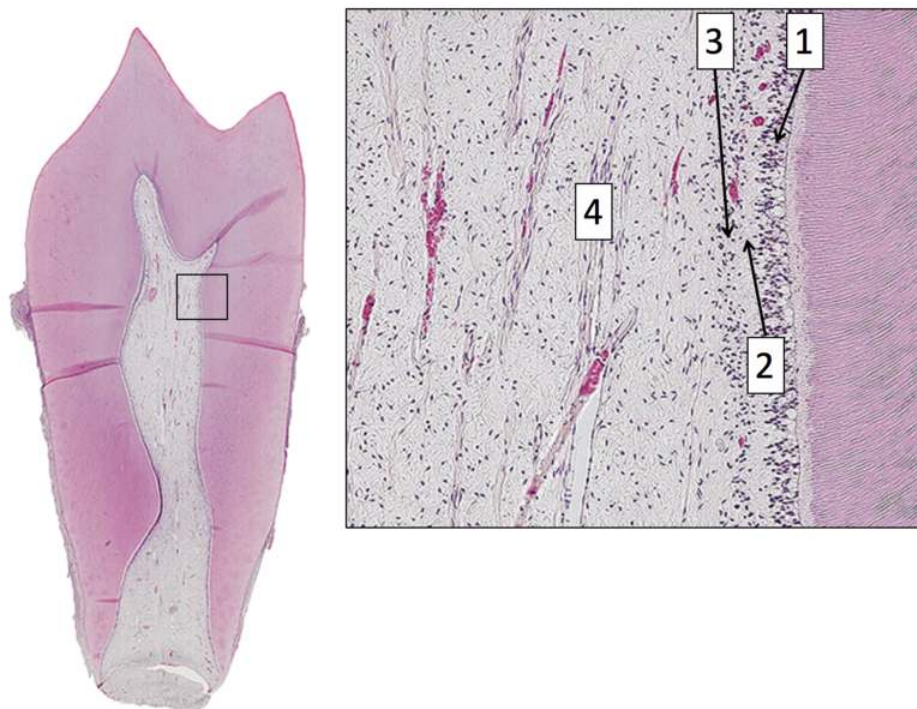


Figure 10.23: Layers of the pulp. Legend: 1) odontoblast layer 2) cell-free layer 3) cell-rich layer 4) pulp core. image credit "Pulp Histology © School of Dentistry, University of Dundee" by Dododopamine is licensed under [CC BY-SA 4.0](#)

Pulp layers

Pulp is a single tissue, but there are four layers that appear distinct from one another under the microscope. The first is the **odontoblast layer**, which is closest to the dentin. Odontoblasts initially form a single layer of cells along the broad region of the DEJ. But as they add dentin the pulp cavity becomes smaller, and the cell bodies crowd together, ultimately forming a thicker layer of cell bodies at the periphery of the narrower pulp cavity wall. Deep to the odontoblasts is what is called the **cell-free zone**. This zone contains cells, but they are not visible under a traditional H&E stain. Deep to that is a **cell-rich zone**, composed primarily of fibroblasts, mesenchymal stem cells, white blood cells and other connective tissue cells. Lastly is the **pulp core**, which contains the same types of cells, but more ground substance spaces them apart. The pulp core is where most of the blood and lymphatic vessels are located.

Clinical considerations of pulp



Figure 10.24: Gumboil caused by an underlying periapical abscess. Image credit "abcès parulique" by Damdent is licensed under [CC BY-SA 3.0](#)

Periapical abscess

If damage to dentin exposes the pulp to oral bacteria, an infection of the pulp may occur. This usually triggers inflammation of pulp tissue, known as **pulpitis**. Death of pulp tissue can lead to accumulation of pus within the pulp chamber, which is called a **periapical abscess**. The release of inflammatory molecules resulting from tissue damage can lead to swelling of the overlying gingival tissue, often referred to as a **gumboil**. A gumboil may also form due to an infection of periodontal tissues, in which case it

would not be a periapical abscess, it would be a **periodontal abscess**. It is possible to remove necrotic pulp surgically. If some healthy pulp remains, regeneration of the pulp can occur. This is because of the high degree of vascularization of areolar connective tissue and the high mitotic potential of mesenchymal stem cells.



Figure 10.25: Endodontic therapy (root canal). Image credit Tooth #30 By DRosenbach at English Wikipedia is in the Public Domain CC0

Endodontic therapy

A periapical abscess likely requires the removal of the infected pulp tissue and replacement by a material that is similar in density and elasticity, a procedure known as **endodontic therapy**. The preferred material used is gutta-percha, a naturally-occurring latex polymer that is the sap of trees in Malaysia. It was once widely used as an insulator for electronics, but has been replaced by synthetic polymers, except in endodontic therapy. It is radiopaque because of an additive, barium sulfate. Otherwise, latex does not show on radiographs, which would make it difficult to confirm gutta percha had completely filled the pulp cavity.



Figure 10.26: Discoloration of a non-vital tooth after endodontic therapy. Image credit “Discolored maxillary left central incisor tooth” by Anandkumar Patil is licensed under CC BY-SA 4.0

Removal of living pulp tissue removes all cells from the tooth, including odontoblasts. This tooth is referred to as **non-vital**. A non-vital tooth cannot make repairs to dentin, which leads to increased brittleness and the accumulation of stains over time. Stain molecules may invade from the enamel side, or molecules from pulp necrosis may invade dentin by traveling up open dentinal tubules.



Figure 10.27: Pulp vitality testing. Image credit Own work By Coronation Dental Specialty Group is licensed CC BY-SA 3.0

Pulp vitality testing

Painful stimuli at the surface of the teeth are detected by nerve endings located within the pulp. **Pulp vitality testing** takes advantage of this to estimate the health of the tooth pulp. An electrical stimulus is applied at the surface of teeth followed by self-reporting of the discomfort level experienced by the patient (ideally, medical tests should not be painful). Loss of nerve endings reduces tooth sensitivity, and indicates pulp tissue has undergone fibrosis or necrosis. However, a reduction in the diameter of dentinal tubules also reduces the ability of electrical signals at the surface to be detected within the pulp. Conversely, loss of enamel and exposure of dentinal tubules may increase sensitivity without any changes to pulp vitality.

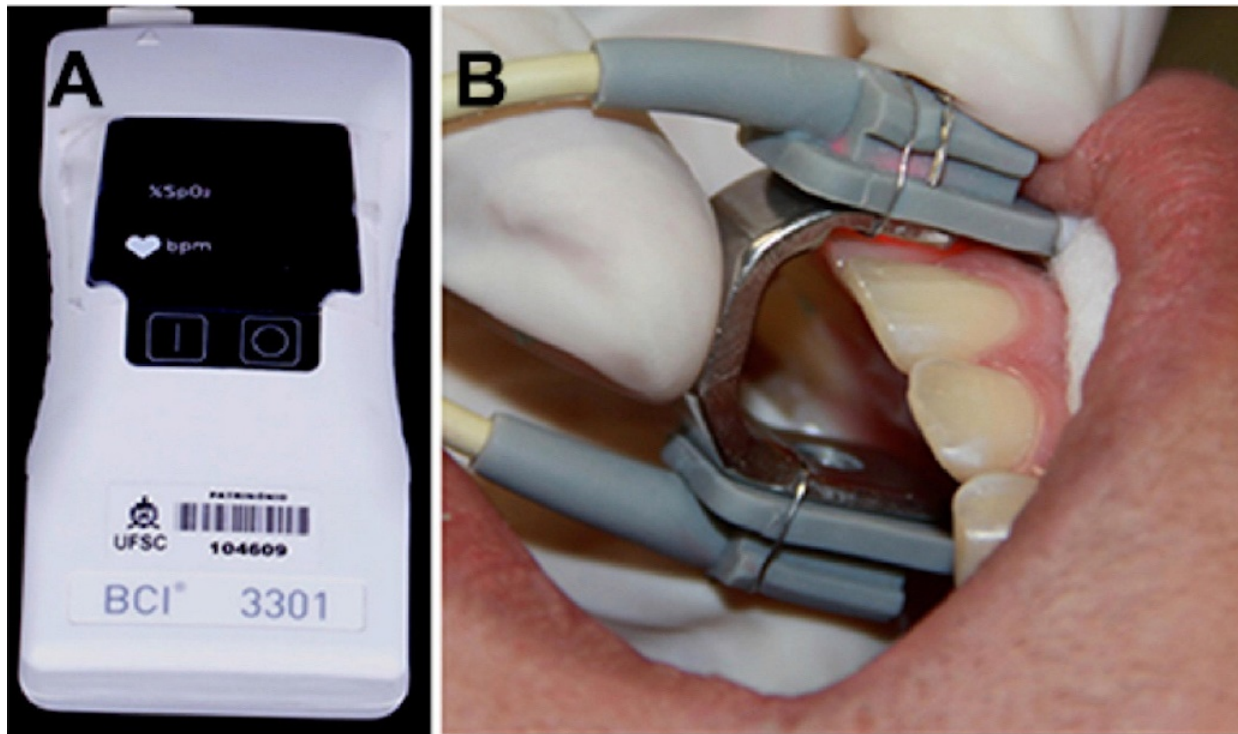


Figure 10.28: A pulse oximeter (A) and dental adapter (B used instead of pulp vitality testing). Image credit: “Pulse oximeter and sensor with specially manufactured adapter” by Lorena Ferreira Lima, et al is licensed under [CC BY 4.0](https://creativecommons.org/licenses/by/4.0/)

Because different patients report pain differently, and because factors besides the health of the pulp affect the degree of discomfort reported, false-positive and false-negative results are possible with pulp vitality testing. Other less-invasive tests exist, including pulse oximetry and laser doppler flowmetry. These measure the vascular supply to each tooth, which correlates with the vitality of the tooth. Pulse oximetry takes advantage of the color change hemoglobin undergoes as it picks up oxygen. Laser doppler flowmetry takes advantage of the doppler shift waves exhibit when they bounces off moving objects (such as flowing red blood cells) but not stationary ones– similar to the way the sound of a motorcycle changes pitch when it is rapidly travelling toward you or away from you.

Age-related changes

Because a vital tooth contains a layer of living odontoblasts, dentin becomes gradually thicker with age. This is most noticeable in the fine pulp horns, as a result, the pulp horns recede with age. Furthermore, as with many cells, the mitotic ability of mesenchymal stem cells within the pulp decreases with age. As a result, older pulp tends to contain more scar tissue (collagen fibers) and has decreased regenerative capability and diminished sensitivity.

Chapter 9 * Chapter 11

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1.11: Periodontium Development

- Overview
- Cementum
 - Development
 - Types of cementum
- Alveolar bone
- Periodontal ligament
 - Cells
 - Fiber groups
- Gingiva
- Clinical considerations

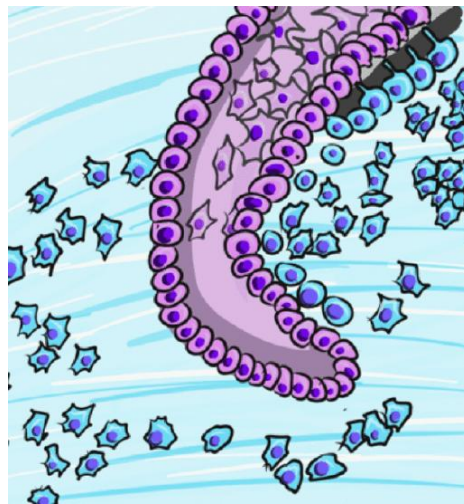


Figure 11.1: HERS and cementum formation

Overview

The **periodontium** includes cementum, periodontal ligament, alveolar bone and gingival tissues. The gingiva contain a stratified squamous epithelium which develops from the ectoderm of the pharyngeal arches. Underlying connective tissue layers develop from mesoderm (as does most of the skull). The ECM of these tissues contains collagen and elastin fibers. Cementum, PDL and alveolar bone develop from the neuro-mesenchymal stem cells of the dental sac. These three tissues share extracellular components, making for a strong connection, thanks to their shared lineage. Like mesodermal-derived connective tissues, these tissues have a lot of collagen, but instead of elastin these tissues produce a special fiber type called **Oxytalan**. Oxytalan fibers can be found in a few other places in the human body, such as the aorta, whose lining is also derived from neural crest cells. Hertwig's Epithelial Root Sheath plays an important role in the induction of these tissues, even if it is mostly fated to undergo apoptosis.



Figure 11.2: Cross section of a tooth to show hard tissues, including cementum. Image credit “What is inside a human tooth” by Mohamed A.M. Ahmed is licensed under CC BY-SA 4.0 / cropped

Cementum development

Cementum forms a thin layer on the roots of teeth, attaching the teeth to the alveolar bone by way of the PDL. The mineral content of cementum is lower than that of dentin, making it appear more radiolucent than dentin, but more radiopaque than the PDL. The surface of cementum will feel grainier than enamel when probed with instruments.

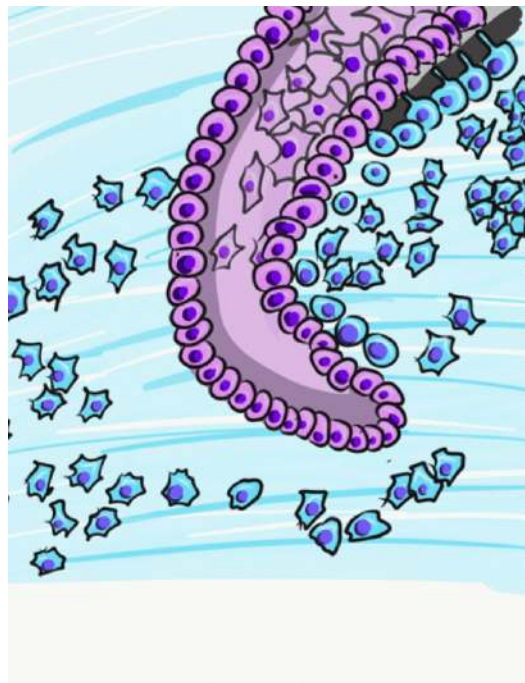


Figure 11.3: Disintegration of HERS and the formation of cementum.

After crown formation is nearly complete, HERS grows apically, separating the dental papilla from the dental sac. The IEE of HERS induces the differentiation of odontoblasts, which begin dentinogenesis. Without stellate reticulum, the IEE cannot be reciprocally induced to form ameloblasts. Instead, the IEE and OEE secrete morphogens onto the surface of dentin, including members of the FGF and Wnt family, but after that, these cells mostly undergo apoptosis. A few epithelial cells will remain and become trapped within the PDL, known as the Epithelial Rests of Mallasez. Some of the epithelial cells of HERS may undergo an epithelial-to-mesenchymal transition and become cementoblasts. With HERS out of the way, pre-dentin made by root odontoblasts now comes into contact with neuro-mesenchymal stem cells of the dental sac. Contact with pre-dentin and with morphogens secreted by HERS induces the neuro-mesenchymal stem cells of the dental sac to become **cementoblasts**. These cells, like odontoblasts, align up next to one another, behaving more like a tissue derived from ectoderm (an epithelium) than a connective tissue. **Cementogenesis** is the process of forming cementum, its name follows the pattern of amelogenesis and dentinogenesis.

Cementoblasts secrete the protein components of cementum. This is frequently referred to as **cementoid**, which breaks from the pattern we used for pre-enamel and pre-dentin. Layers of cementoid are laid down appositionally, and soon mineralize, at which time it is called cementum. Some cementoblasts become trapped in cementum, after which they are referred to as **cementocytes**. Other cementoblasts remain near the surface of the cementum. These cells continue to lay down layers of cementoid throughout life, and can become more active during times of injury and repair to cementum. Because of the shared lineage between cementoblasts and odontoblasts, plus the similarity of their ECM, the **cemento-dentinal junction** (CDJ) is less distinct than the DEJ. In fact, the CDJ was once considered to be imaginary ([reviewed here](#)).

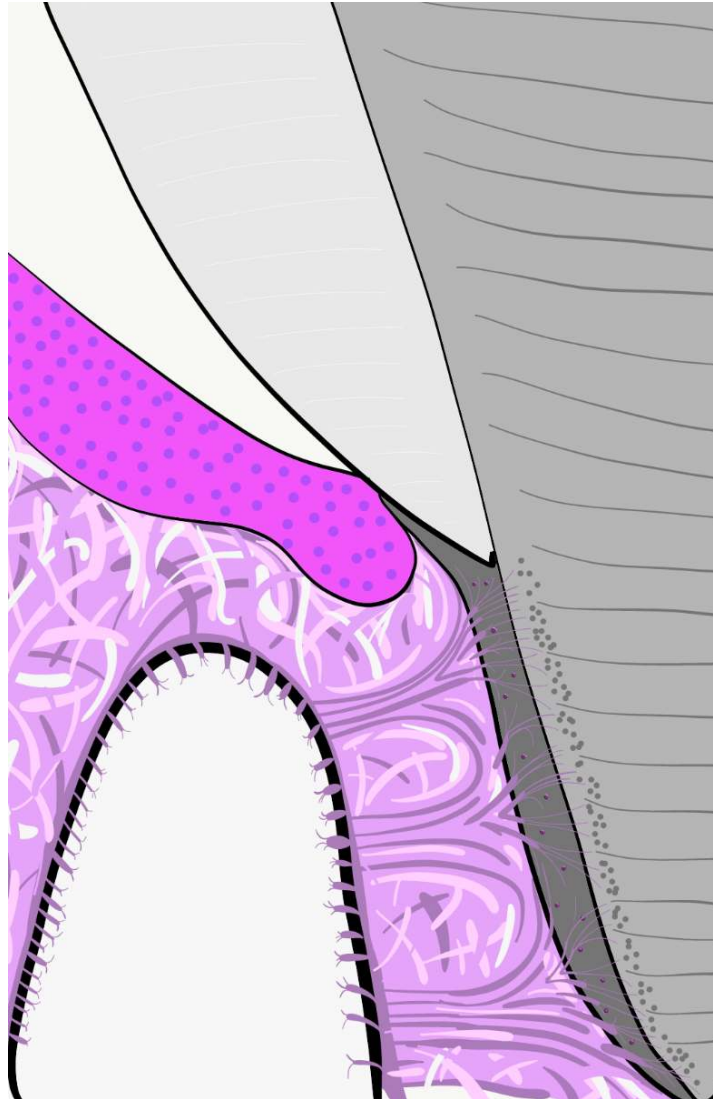


Figure 11.: Cartoon of the periodontium, highlighting bundles of collagen fibers that extend from Tomes' granular layer through to alveolar bone.

One major protein in cementum is collagen, which extends out of cementum into Tomes' granular layer of root dentin, and in the other direction becomes the collagen fibers of the PDL, which in turn become Sharpey's fibers in alveolar bone. Rather than thinking about alveolar bone, PDL, cementum and dentin as distinct tissues that are bonded to each other, we should instead think of them as a gradient, thanks to their shared lineage. This makes for a much stronger connection than could be produced by bonding 4 separate tissues, especially ones that are too thin for a good amount of rete peg or dermal papillae-like digitations. Other proteins secreted by cementoblasts include two glycoproteins (bone sialoprotein and osteopontin) which help collagen adhere to calcium hydroxyapatite crystals. Cementoblasts also secrete enzymes that catalyze the formation of crystals, similar to those active in dentinogenesis.

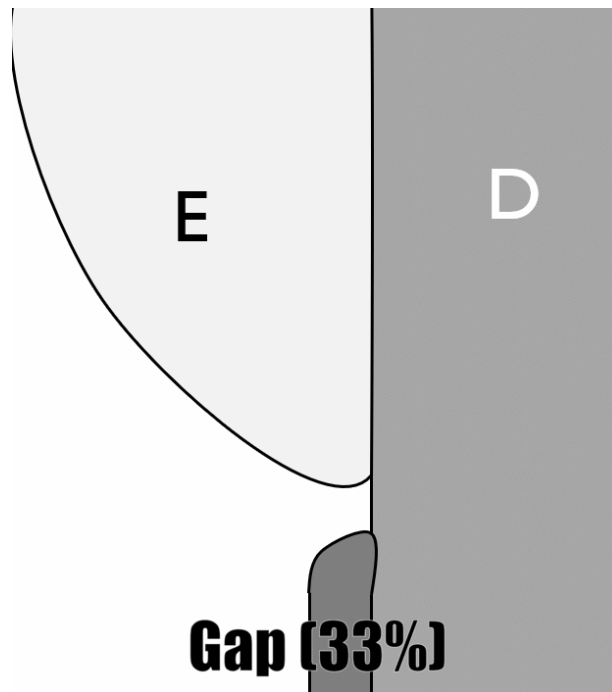


Figure 11.5: patterns of cementum at the CEJ

Types of cementum by area of the root.

For most teeth, the apical one third of the root contains cellular cementum, and acellular cementum covers the rest. About 52% of the time, cementum completely covers root dentin, all the way up to the enamel. Roughly 33% of the time there is a gap between the cementum and enamel, with underlying dentin exposed. The rest of the time, roughly 15%, cementum extends over the enamel a short distance. These percentages are not for individual teeth, but for any area of the cervical region on a single tooth. Therefore, a single tooth may have all three patterns. The gap pattern is the most significant because of the increased risk of dentin hypersensitivity. Cementum found overlapping enamel contains no collagen fibers, and does not connect to the PDL.

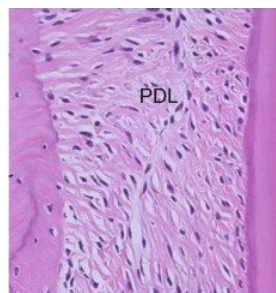


Figure 11.6: Histology of the PDL. Image credit "Immunohistochemistry of CXCL12 in PDL of rat molars." by Yashiro Y et al is licensed under CC BY-SA 4.0 / cropped

Periodontal ligament development

The PDL, like other ligaments, is a dense regular connective tissue, composed primarily of collagen fibers in the ECM, plus the special oxytalan fibers. These fibers are made by fibroblasts that differentiate from neuro-mesenchymal stem cells of the dental sac. In figure 260, fibroblast nuclei stain purple. From this image, you should be able to determine which side of the PDL is anchored to bone tissue versus anchored to cementum/dentin based on whether those tissues have cells within lacunae or not. Otherwise, the pink color is mostly collagen found in all 4 of these tissues.

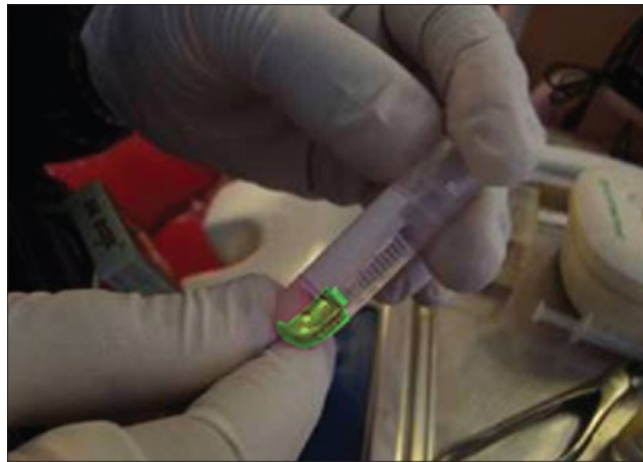


Figure 11.: Collection of stem cells from an extracted tooth (the fluorescent green color lets you know this is science!). Image credit: "Sample embedded in tooth preservation cocktail and nourishment media" by PM Sunil et al is licensed under CC BY-NC-SA 4.0

Like other ligaments, the PDL contains osteoblasts (mostly at the border with alveolar bone), and osteoclasts, but unlike other ligaments the PDL also contains cementoblasts and odontoclasts. Furthermore, the stem cells found in the PDL are slightly more differentiated than a typical mesenchymal stem cell, and may be referred to as Periodontal Ligament Stem Cells (PDLSCs). These stem cells can differentiate into fibroblasts, osteoblasts, odontoblasts or cementoblasts given the correct morphogen. This allows the PDL to play a role in the remodeling and repair of bone, cementum and dentin. Whether they are truly distinct from the neuro-mesenchymal stem cells we discussed in the dental sac, dental papilla/dental pulp is unclear, but perhaps relevant to the use of [stem cells from extracted teeth](#) to promote the healing of other tissues.

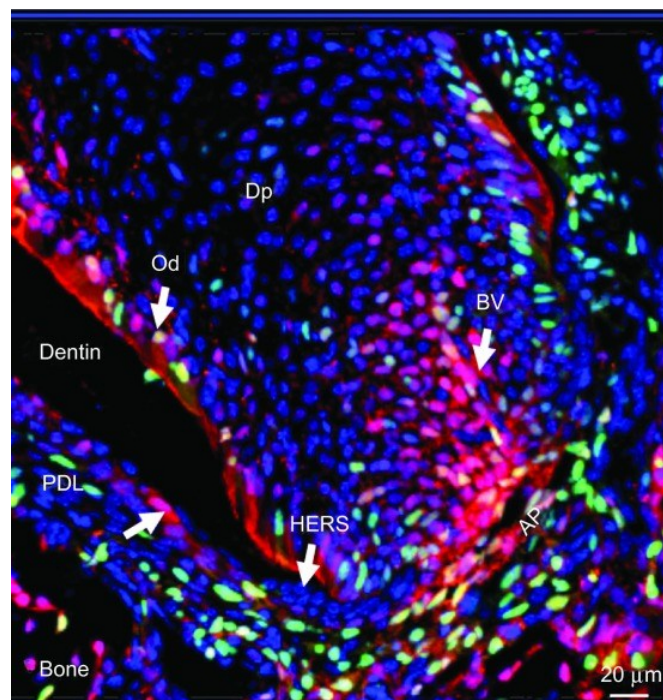


Figure 11.8: Confocal image of developing mouse tooth. Legend: BV = blood vessel, Od = odontoblasts, Dp = dental pulp, PDL = periodontal ligament. Colors: purple = DNA (nuclei), red and green = experimental trans-genes. Image credit: "Lineage studies by mapping Cre activity in the root region of a second molar in a 2-week-old mouse by confocal microscopy using the Olympus FV 1000." by Rakian A, et al is licensed under CC BY-NC-ND 4.0

Unlike cementum and dentin, the PDL is vascular. Fibroblasts in the PDL secrete morphogens such as [Vascular Endothelial growth Factor \(VEGF\)](#) to promote angiogenesis. The expression of this gene is controlled by morphogens of the BMP family, which when combined with other morphogens (including FGF), trigger neuro-mesenchymal stem cells to differentiate into fibroblasts. BMPs are also involved in the differentiation of odontoblasts, which is why other morphogen signals are necessary to specify fibroblast

differentiation. Fibroblasts in the PDL, which have a neuro-mesenchymal lineage, have a higher capacity to undergo renewal (new ones forming from stem cells) and trigger tissue repair than the fibroblasts derived from mesoderm found in other ligaments. Oddly, the tissues that can best be repaired by cells from the PDL are bone and cementum, not the PDL itself. PDL fibroblasts are distinct from mesodermal fibroblasts in that they express some genes in common with cell types that share their neuro-mesenchymal lineage. For instance, PDL fibroblasts share features with cementoblasts, expressing RUNX2 and cementoid proteins, as well as neurons, expressing Neuronal Cell Adhesion Molecules (NCAMs) and N-cadherin (a neuronal desmosome protein) (scientific review article).

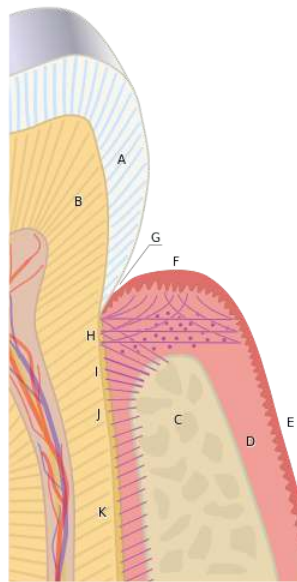


Figure 11.9: The periodontium. Legend: A. Enamel, B. Dentin, C. Alveolar bone, D. Gingiva, E. Oral epithelium, F. Free gingival margin, G. Gingival sulcus, H. Principal gingival fibers, I. Alveolar crest fibers of periodontal ligament, J. Horizontal fibers of periodontal ligament, K. Oblique fibers of periodontal ligament. Image credit: “the periodontium” by Goran tek-en is licensed under [CC BY-SA 4.0](#)

Development of the PDL begins during tooth eruption. PDL Fibroblasts secrete collagen fibers that become embedded in cementum and extend outwards (principal fibers). This process begins at the CEJ and continues apically as the root grows. The other end of collagen fibers is embedded within alveolar bone later, activated by tooth occlusion. This results in greater mobility of tooth roots during eruption. PDL collagen fiber bundles run in different directions, which can be classified as alveolar crest, horizontal, oblique, (peri) apical, interradicular (on multirooted teeth) and trans-septal. You should learn about these different collagen fiber bundles in a different class. **Gingival fibers** are also collagen fiber bundles, but anchor cementum to the gingiva. These are also categorized based on their location and fiber orientation. Depositing collagen in the correct orientation requires morphogens of the planar cell polarity class. One example is a membrane protein called CD44 which binds to Hyaluronic acid, collagen and fibronectin. These same general types of morphogens are involved in the polarization of odontoblasts following their induction by the IEE. Changes to polarity morphogens are likely necessary for re-orientation of fibroblasts during tooth occlusion and the production of collagen bundles in different directions. When you learn about these fibers, ask your instructor whether the guidance of fibroblast polarity represents the recapitulation of an embryological process.

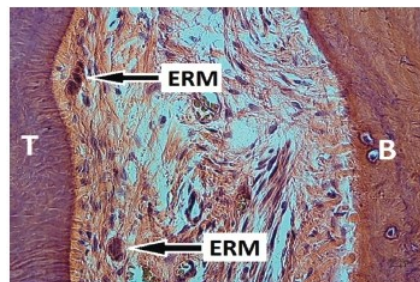


Figure 11.10: Histology of the epithelial Rests of Mallasez (ERM). T = tooth, B = alveolar bone. Image credit ” “Photomicrograph of the periodontal ligament showing clusters of epithelial cell rests” by HD Miniggio and EJ Raubenheimer is licensed under [CC BY-NC 4.0](#)

Remnants of HERS may linger after root formation is finished. These cells, the Epithelial Rests of Mallasez, *may* be involved in repair of cementum and the PDL (the evidence is not entirely clear at this time). While these cells are epithelial, derived from ectoderm, they may undergo an epithelial-to-mesenchymal transition before differentiating into cementoblasts or fibroblasts, if the correct morphogens are produced. Evidence for this comes primarily from studying transgenic mice, which must be intentionally injured and sacrificed (killed) before repair to tooth tissues can be measured. Obviously, this cannot be repeated in humans.

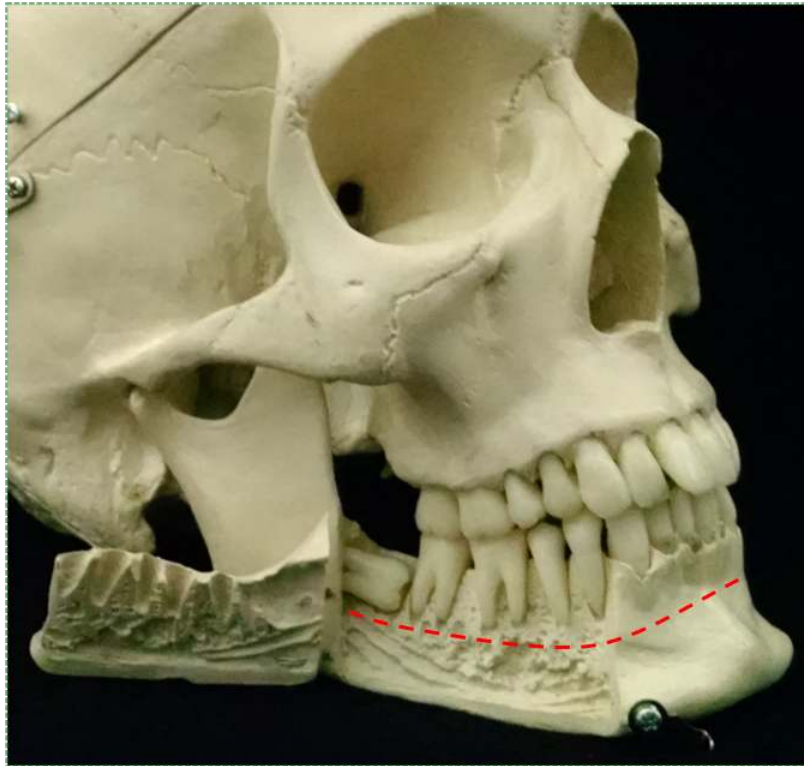


Figure 11.11: Alveolar bone (roughly above dotted red line)

Alveolar bone

The vault of the skull and most of the face develops by intramembranous ossification (including the maxilla). Thus, the sutures contain dense connective tissue (unless they have fully ossified). The base of the skull, on the other hand, develops by endochondral ossification. The development of the mandible is more complicated. First, neuro-mesenchyme forms two bands of cartilage (Meckel's cartilage). The posterior portion is fated to become the ramus of the mandible plus the malleus and incus bones, while the anterior portion disappears. Before it does, the body of the mandible forms around it by intramembranous ossification. Later, the condylar process, coronoid process and mandibular symphysis develop by endochondral ossification. That is *how* the skull forms. What it *forms from* does not match as nicely. Most bones are derived from mesoderm, including the entire appendicular skeleton, plus most of the axial skeleton. The exceptions are the facial bones (including the hyoid) and inferior parts of the cranium, which are derived from neural crest cells. In developmental biology classes, this is not a minor issue: very ancient fish (either long extinct, or modern-day lampreys) have no lower jaw, but they do have seven branchial (pharyngeal) arches. Vertebrates, including you and bony fish, remodel some of the branchial arches into other structures, such as a powerful lower jaw and ears. If you are curious, the lower jaw of a shark (and even the sturgeon) is not a mandible, it is Meckel's cartilage (so sharks are a closer family member of yours than a lamprey, but more distant relative than a goldfish). This brings us to alveolar bone. The alveolar ridge of both the mandible and maxilla develop from neuro-mesenchyme of the dental sac. Neuro-mesenchymal stem cells in this area are induced to differentiate into osteoblasts by morphogens (including members of the BMP family). This in turn activates transcription factors such as members of the RUNX family and MSX homeobox family, which up-regulate bone-specific proteins. From a development standpoint, it is important to understand that the basal bone of the mandible and maxilla are induced by signals from Meckel's cartilage, while the induction of alveolar bone formation comes from the developing tooth germ. Alveolar bone contains a number of small **Volkman's canals**, through which the PDL connects deeper into bone tissue. This is the same name used for perforating canals that run perpendicular to Haversian canals in compact bone, which contain blood vessels.



Figure 11.12: Bite wing of one of the authors teeth, noting PDL and lamina dura. Note: he grew up drinking well water that contained no fluoride.

Like all bones, the outer portion of alveolar bone is compact bone and sandwiched between that is spongy bone. The compact bone portion is referred to as the **lamina dura** on a radiograph, as it shows up more radiopaque than the spongy bone deep to it (or the PDL superficial to it). The **interdental septum** is a triangular-shaped area between two teeth, which should be of uniform height between all teeth. The **interradicular septum** is the bone tissue between roots

Clinical applications



Figure 11.13: Hyper-cementosis. Image credit: “Periapical radiograph showing radiopaque halo around the root of tooth #36” by Antonione Santos Bezerra Pinto is licensed under CC BY-NC-SA 4.0

Hyper-cementosis

Because cementoblasts are retained at the surface of cementum (within the PDL), cementum has the ability to undergo remodeling and repair, similar to dentin but different from enamel. Excessive cementoblast function can result in **hyper-cementosis**. This more frequently occurs at the root apex due to excessive occlusal forces on a tooth, although it may also be caused by growth factor disturbances such as gigantism/acromegaly or Paget's disease.



Figure 11.14: Concrescence examples. Image credit : "Second and third molars joined by cement, in teeth with and without hypercementosis." by Consolaro A et al is licensed under [CC BY 4.0](#)

Concrescence

Enough hyper-cementosis can adhere two teeth together by the roots, which is called **concrescence**. This should illustrate one reason why a radiograph is necessary prior to tooth extraction. This most commonly occurs between the second and third upper molar.



Figure 11.15: Cemental caries. Image credit: "Photograph showing root caries in 33 & 34." by B Gupta is licensed under [CC BY-SA 4.0](#)

Cemental caries

Gingival recession is worrisome in part because it exposes the roots of teeth to the environment that only enamel is exposed to under healthy conditions. Because of its lower mineral content, **cemental caries** spread faster than in enamel. Erosion quickly passes to dentin, and if untreated, to pulp. Dental pain may not arise until infection of the pulp sets in, at which time treatment is generally limited to tooth extraction rather than prevention and maintenance. Early detection of cemental caries usually involves radiographs or use of a dental mirror and explorer.

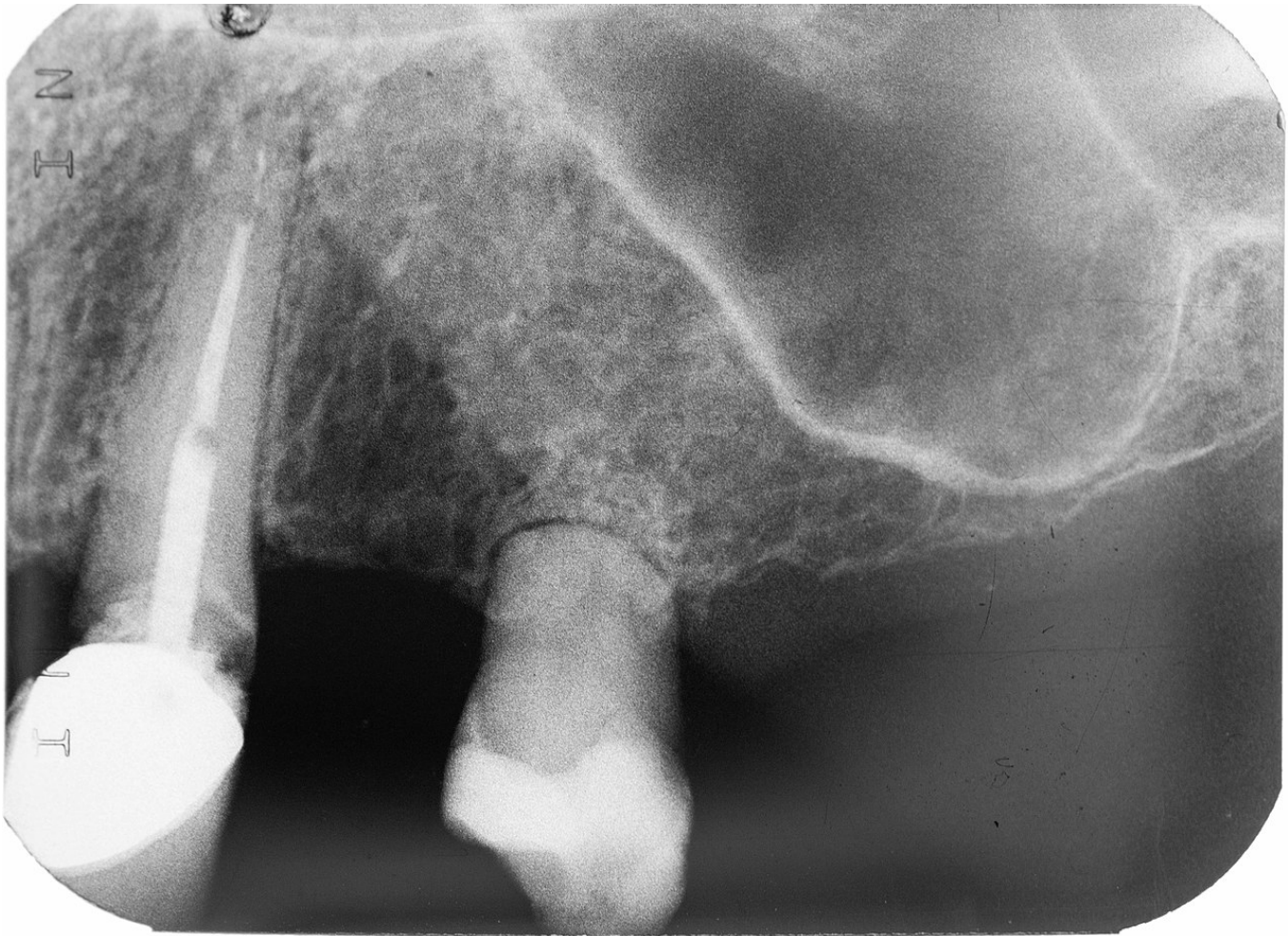


Figure 11.16: Root resorption. Image credit: Own work By Bin im Garten is in the Public Domain CC0

Root resorption

Severe trauma may induce **root resorption** over time. Both dentin and cementum have the capability to be repaired in response to mild or moderate trauma. This is because living odontoblasts are located in the outer layer of the pulp, and cementoblasts in the PDL. If necessary, mesenchymal stem cells in the pulp or PDL may be induced to differentiate into these cells and lay down more extra-cellular matrix. However, reparative cementum does not contain Sharpey' fibers and will not contribute to tooth attachment to bone tissue.



Figure 11.17: Lateral maxillary incisor has undergone root resorption, evident by its pinkish appearance. Image credit: "Mummery" by DRosenbach is licensed under [CC BY 3.0](https://creativecommons.org/licenses/by/3.0/)

Severe trauma, on the other hand, can induce the differentiation of mesenchymal stem cells into cementoclasts or odontoclasts, leading to the loss of cementum and dentin. Loss of these tissues from the roots of teeth is **root resorption**. Loss of dentin from deep layers is **internal resorption**. As dentin is lost, the space is filled in by vascular pulp tissue, possibly causing the tooth to have a more pinkish coloration. This tooth may be referred to as a pink tooth of Mummery (named for the anatomist who first described the condition, it has nothing to do with a pantomime). If internal resorption is caused by chronic inflammation, removal of inflamed pulp by endodontic therapy may halt the condition. **External resorption** is the loss of cementum and dentin from the superficial side of the root. External resorption may be transient and resolve on its own, owing to the regenerative capability of cementum

thanks to the cementoblasts in the PDL. Chronic inflammation or tooth ankylosis may trigger more severe resorption, where lost tooth tissues are replaced with bone tissue.

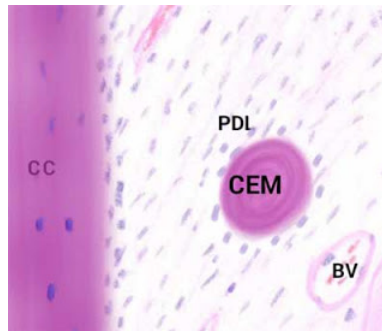


Figure 11.18: Cementicle. Labels: PDL = periodontal ligament, CEM = cementicle, BV = blood vessel, CC = cellular cementum. Image credit: "Cementicles" by Mandana Donoghue is licensed under [CC BY-NC-ND 4.0](https://creativecommons.org/licenses/by-nc-nd/4.0/)

Cementicles

Masses of acellular cementum may develop in abnormal locations, especially later in life, and are referred to as **cementicles**. These may be free (within the PDL but unattached to the tooth), attached (on the surface of the layer of cementum) or embedded (once attached, but now surrounded by the cemental layer of the root). It is thought these develop when cementoblasts encounter debris such as a blood clot (thrombus) lodged in a nearby capillary. This disrupts appositional deposition of cementum and instead creates a nucleus around which a cementicle forms.

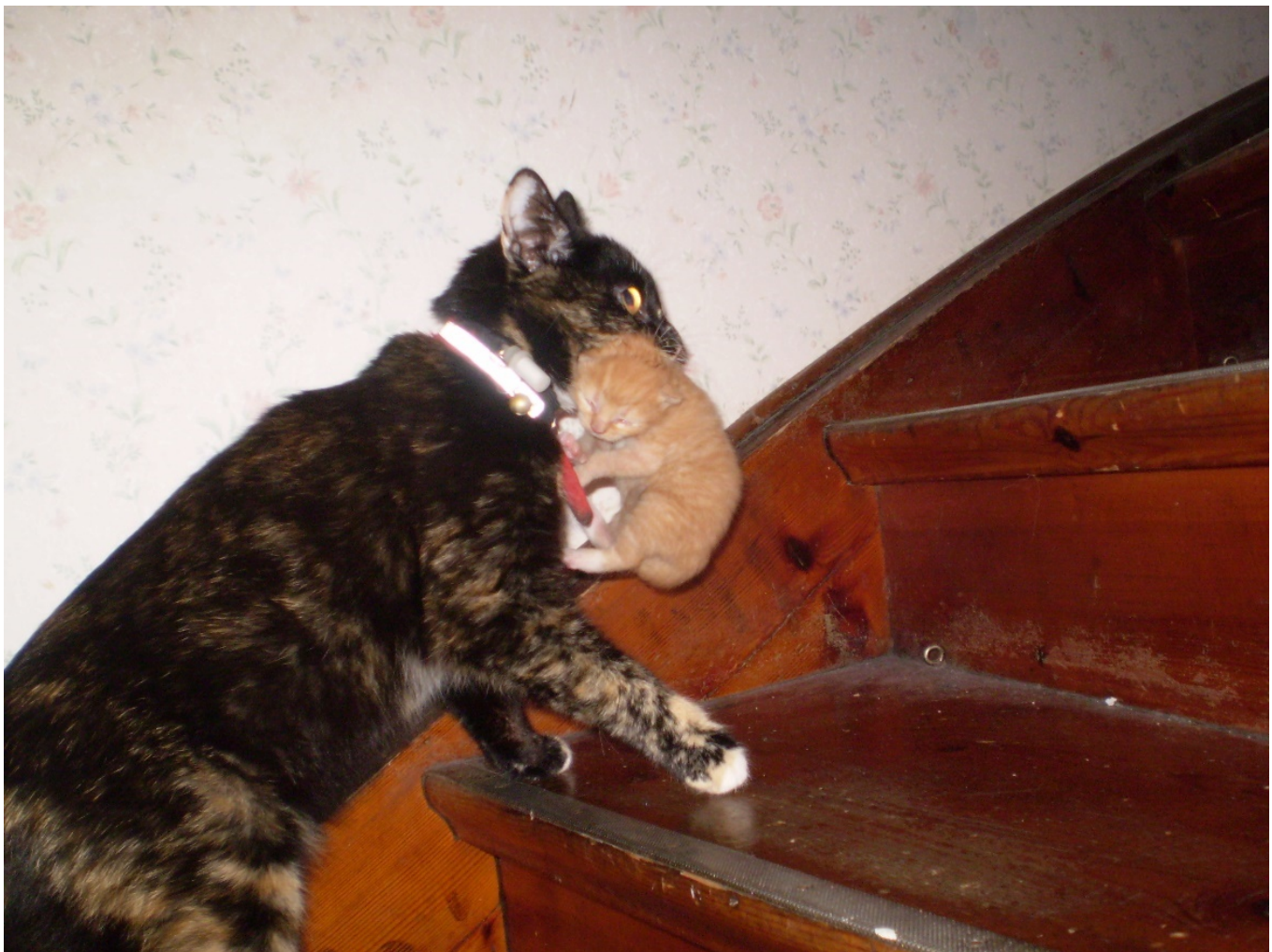


Figure 11.19: Adorable example of the importance of tooth proprioception. Image credit: "Tortoiseshell cat carrying her kitten up a flight of stairs. She grips the kitten's scruff in her teeth." by [Margo Akermark](https://www.flickr.com/photos/margoakermark/) is licensed under [CC BY 2.0](https://creativecommons.org/licenses/by/2.0/)

Tooth discomfort following endodontic therapy

After endodontic therapy, the pulp—including nerve endings— is replaced with a non-living polymer. Nevertheless, tooth discomfort may still occur. This is because the PDL is still present and living. Besides functioning to attach the tooth roots to bone and gingival tissue, the PDL is involved in proprioception, or the sensation of where the body is located. Nerve endings in the PDL relay information to the [somatosensory cortex](#) about where the teeth are located and when they experience occlusal forces. This is very important in reducing or avoiding occlusal forces during mastication and speech. The jaws exert some of the largest amount of force the human body can generate, but also exhibit the greatest amount of control. It is routine for people to bite down through substances of various density and thickness without teeth hitting one another thanks to a large region of somatosensory cortex dedicated to signals from the teeth. Dental implants, however, do not enjoy this sensitivity.



Figure 11.20: Mesial drift. Image credit: “Class II human Molar relationship.” by Dr. Vipin C. P. is in the [Public Domain](#), [CC0](#)

Mesial drift

Mesial drift (or physiological drift) is the tendency of teeth mesial to the first molar the first to migrate in a mesial direction with age. It is a natural phenomenon caused by the remodeling of alveolar bone tissue, which occurs asymmetrically. With age, teeth tend to wear down at their contact points, which would lead to gaps between the teeth. Mesial drift leads teeth to move closer together and prevents gaps from forming despite wear. Too much movement can lead to crowding. The molars, on the other hand, tend to drift distally. Missing teeth can accelerate drift, causing neighboring teeth to bend inwards, changing the bite plane. This, in turn, can lead to abnormal forces in the temporo-mandibular joint, causing pain, discomfort and reduced range of motion.



Figure 11.21: Loss of alveolar bone. Image credit: Own work by By Bin im Garten is licensed under CC BY-SA 3.0

Loss of alveolar bone

The loss of alveolar bone leads to an uneven level of the inter-radicular septa or inter-dental septa. The consequence of this is a reduced connection between tooth and bone, which can lead to tooth mobility and loss. This is often first noted in the reduced opacity of the alveolar crest region(s). Under healthy conditions, alveolar bone—especially spongy bone—undergoes bone remodeling. Chronic inflammation, such as result of periodontitis, inhibits the rate of osteoblast function without reducing the rate of osteoclast function. This is likely due to the fact osteoclasts are related to white blood cells, their lineage is from the bone marrow—and white blood cells tend to be more active during inflammation, unlike most other cells in the body.

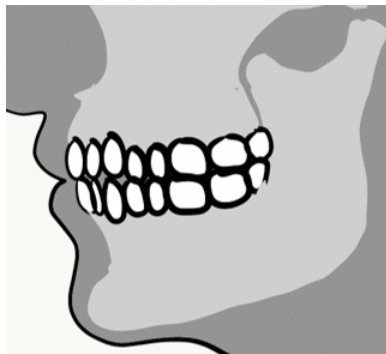


Figure 11.22: Illustration of fenestration and dehiscence of alveolar bone tissue, exposing underlying tooth roots.

Fenestration and dehiscence

With significant alveolar bone loss, portions of tooth roots may become exposed. A small window of root may become exposed, known as a **fenestration** (for the Latin word for window). If the exposed root area connects all the way to the CEJ, the region is known as a **dehiscence** (a general term for a wound that cannot close). This is distinct from gingival recession or periodontal pockets, where junctional epithelium migrates to a region more apical on the root of the tooth, exposing cementum. A fenestration or dehiscence may still be covered by oral mucosa, but the lack of epithelial attachment means oral bacteria may come into contact with cementum and bone tissue, neither of which is designed to resist infection the way partially keratinized avascular epithelia are. This occurs much more frequently on the facial side than the lingual side.

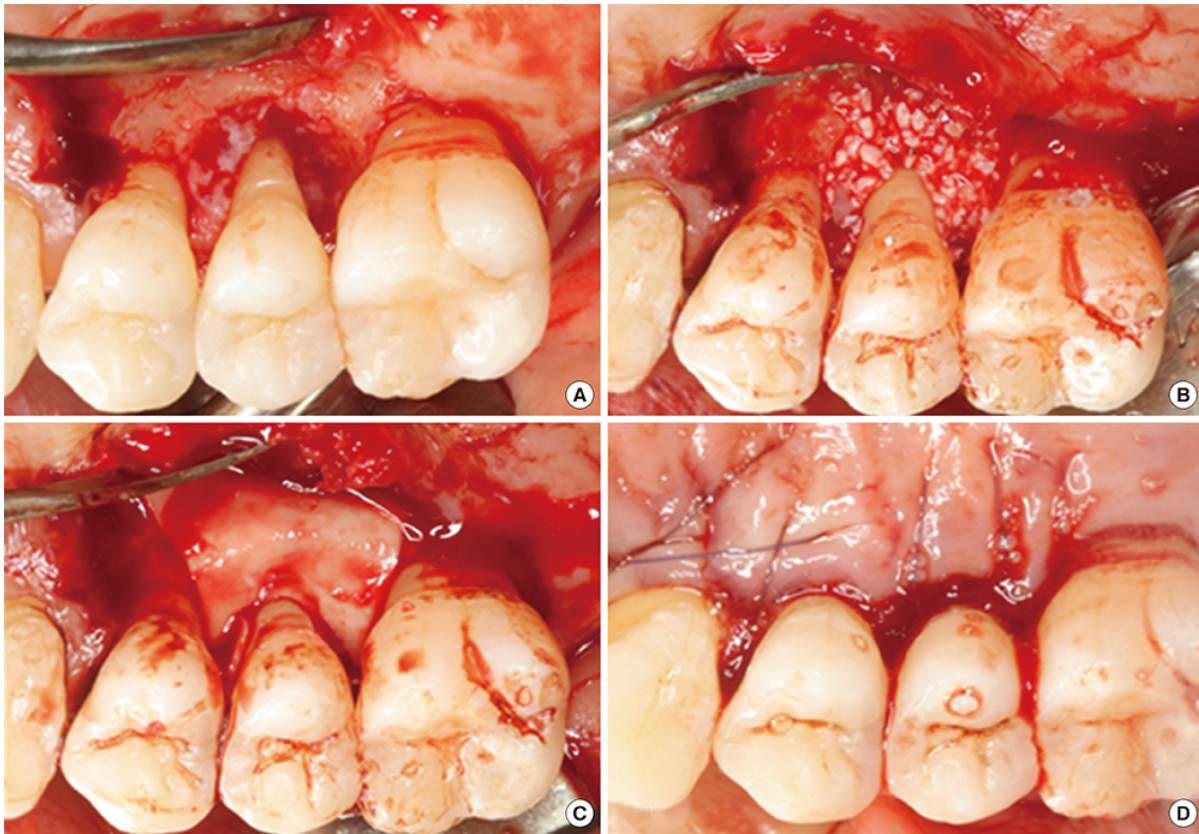


Figure 11.23: Guided tissue regeneration. Legend: A) bony defect after root planing and debridement. B) Bone graft C) Collagen-membrane D) Gingival flap sutured over graft materials. Image credit: "Class II human Molar relationship." by Young Mi Chung is licensed under [CC BY-NC-SA 4.0](#)

Guided Tissue Regeneration

Surgery can help the body to repair bone loss, including alveolar bone, by filling in the damaged area with a scaffold. The proper scaffolding material allows mesenchymal stem cells to migrate across and differentiate into osteoblasts. Surgeries involving this method are referred to as Guided Bone Regeneration or **Guided Tissue Regeneration (GTR)**. First, a membrane must be placed over the injured area. Old-fashioned membranes, such as cotton gauze bandages, are a barrier between pathogens in the environment and exposed vascular tissues. They also keep other tissues, such as epithelia or scar tissue, from filling up the lost region with the wrong type of tissue. Healing of the injury will occur from the edges of the wound. Newer membranes can be made of molecules that are recognized by mesenchymal stem cells and used as a scaffold over which the cells migrate. These membranes allow for healing to occur across the entire injured area, rather than be limited to the edges. These membranes may be organic (Carbon-containing molecules similar to collagen) or inexpensive inorganic polymers. Furthermore, these membranes may be gelatinous, molded into the injured area, or harder and 3-D printed to fit into the injured area. Bio-active membranes add morphogens that promote the differentiation of mesenchymal stem cells into osteoblasts, rather than fibroblasts (which would produce scar tissue). Similarly, morphogens (such as VEGF) may be included to promote angiogenesis, speeding up the healing process. Antimicrobial agents may also be embedded within the polymer. One consideration when choosing a membrane is whether it is resorbable, or whether it will need to be removed (requiring another surgery). Both organic and synthetic polymers may be resorbable or not. For instance, cotton is organic, but is not resorbed readily by the human body, whereas poly-lactic acid (PLA) membranes (which are also organic, but synthesized in a laboratory) are readily absorbed during the healing process and are replaced by human tissue. Guided tissue regeneration is conceptually very different from replacing damaged tissues with an inert material, such as latex in endodontic therapy, or a filling made of metal, porcelain or plastic—the goal is not to fill in a gap, but to promote the healing of a gap.



Figure 11.24: Changes to an edentulous alveolar ridge. Image credit: Own work by JASFUS assumed in the Public Domain CC0

Resorption of the alveolar ridge

Following tooth extraction or loss, regions of the alveolar ridge not anchored to PDL and tooth roots undergo resorption. Basal bone should be unaffected. This occurs because all bone tissue undergoes remodeling, and a lack of tension slows down the activity of osteoblasts, but not osteoclasts. The opposite is why exercise maintains healthy bones, which includes the alveolar ridge.

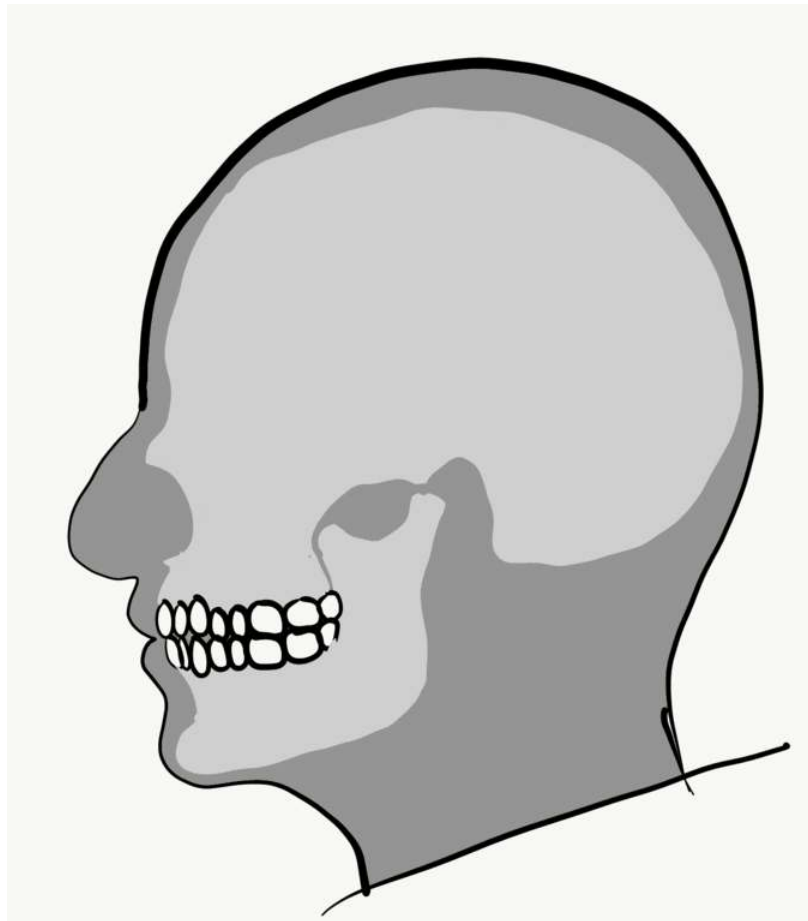


Figure 11.25: Changes to the vertical dimension of the maxilla and mandible in an edentulous mouth.

Loss of alveolar bone reduces the vertical dimension of the mandible and/or maxilla. Without teeth to occlude, the mandible and maxilla can move closer together. This causes the mandible to protrude forward, leading to a “popeye chin”.

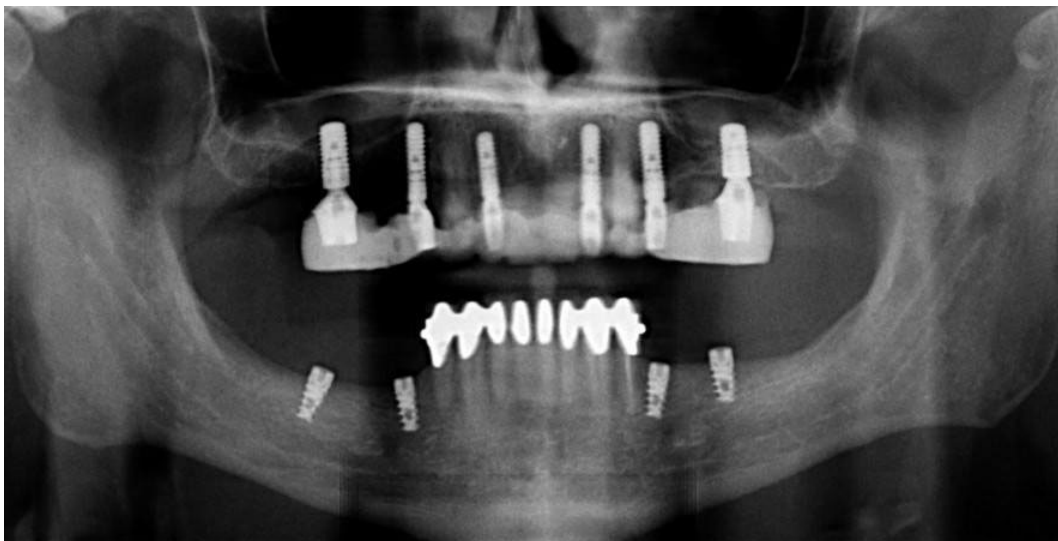


Figure 11.26: Dental implants. Image credit: Own work By Danjhon – Own work, is licensed under CC BY-SA 4.0

Dental implants

Dental implants can prevent loss of alveolar ridge by transmitting force onto bone tissue during mastication. Osteoblasts respond to force by increasing the deposition of calcium and phosphate, increasing bone density and maintaining bone health. If bone loss has already occurred, a bone grafting or guided tissue regeneration procedure may need to be done long enough before the implants are

installed to allow for regeneration of lost bone tissue. Similarly, gingival grafting may be involved if gingival tissue has receded significantly.



Figure 11.27: The core (and abutment) and crown portions of a dental implant. Image credit: Own work by Logicwhatelse is licensed under CC BY-SA 4.0

Dental Implant technology

Each dental implant consists of a core, abutment and crown portion. The core is made of titanium and is implanted into bone tissue. To get the shape and size of the core to properly fit the alveolar ridge of the patient may involve [computed tomography](#) (CT scans) to image bone tissue and computer-aided design (CAD) to specify the core's dimensions. The abutment connects the core to the crown by a screw or dental cement. The crown is usually made of ceramic. Titanium is strong, durable and unlikely to trigger tissue rejection by the patient's immune system. It is more or less invisible to the cell surface receptors white blood cells use to detect antigens. Unfortunately, it is also invisible to other cells in the body, and as a result does not adhere well to the connective tissues it is embedded in. Knowledge of histology helps with this: coating the core with Hyaluronic Acid improves the connection between the core and the patient's connective tissue. Another important connection that is lost with tooth extraction is Junctional Epithelium (JE). Because the junctional epithelium of each tooth is derived from the IEE during tooth eruption, new JE does not form on an implant. At best, oral mucosa may adhere to titanium via hemi-desmosomes, creating peri-implant mucosa. Coating a ring of the core with morphogens (such as FGF) can mimic the induction of JE from IEE, improving the connection between oral mucosa and the implant.

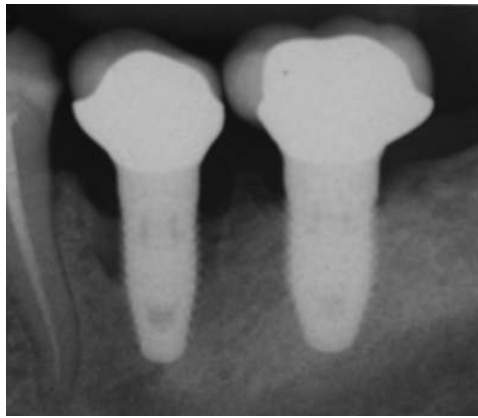


Figure 11.28: Peri-implantitis leading to bone loss. Image credit: Bone loss following peri-implantitis in a smoker by Coronation Dental Specialty Group is licensed under CC BY-SA 3.0

Peri-implantitis

Failure to create a complete junction between the oral mucosa and the implant exposes underlying connective tissue to the oral microbiome, frequently leading to peri-implantitis. Because the oral microbiome does not go away, chronic inflammation occurs, leading to loss of tissue around the affected implant, including gingival and bone tissue.

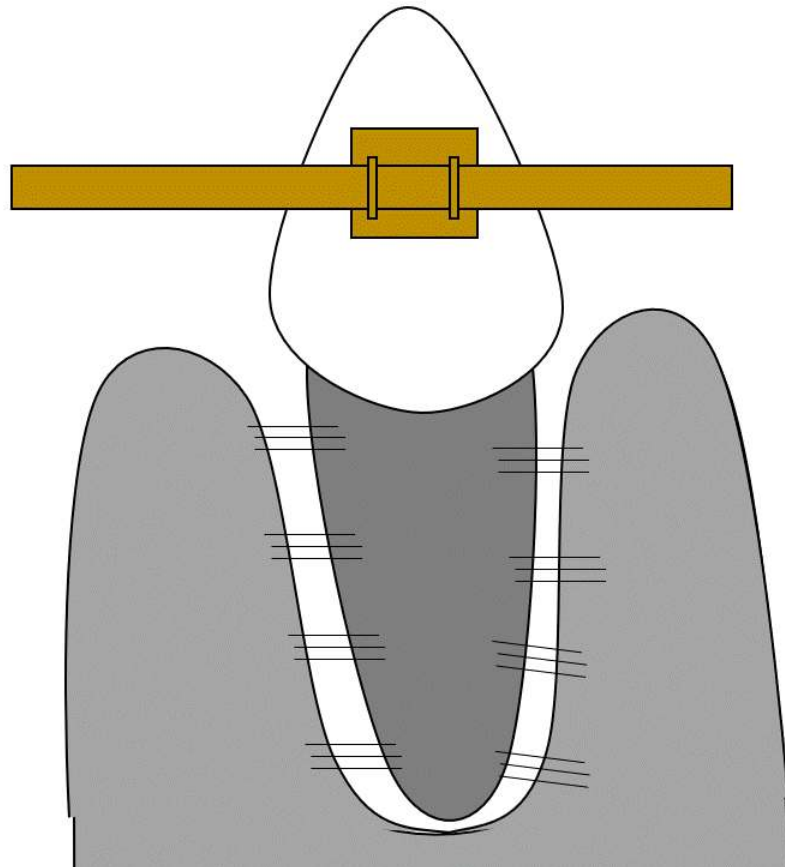


Figure 11.29: Illustration of the change in force applied to bone tissue from the PDL during orthodontic movement.

Orthodontia

The same mechanism that leads to both proper bone health and loss of alveolar bone tissue are taken advantage of with **orthodontia**. Pressure applied to a tooth can cause the PDL on one side to slacken, and on the other side to tense up. The side with lower amount of force will tend to exhibit bone resorption. Higher tension triggers bone deposition on the opposite side. As a result, the location of the alveolar socket shifts in the jawline, without altering the overall dimensions of the socket itself. It is

important that the correct amount of force be applied—to much stress can lead to bone resorption all around, increasing the size of the alveolar socket and increasing tooth mobility. Common treatments for osteoporosis include drugs that inhibit the activity of osteoclasts, such as bisphosphonates. Because the entire remodeling unit is required for orthodontia, patients using these medications cannot undergo orthodontic therapy. Another factor to consider with orthodontia is the activity of odontoclasts and cementoclasts. The morphogens that induce odontoclast differentiation and activity (RANKL) during orthodontic movement *inhibit* the activity of cementoclasts and odontoclasts. For this reason, there is significantly less resorption of cementum and dentin than there is bone tissue during orthodontic movements, even though the change in force across the PDL should be the same for bone and tooth root tissues. It is possible to experience root resorption with orthodontic treatment, especially as the orthodontic force increases, although the causes of root resorption are complex and multi-factorial.



Figure 11.30: Widening of the PDL space. Image credit: “Widening of periodontal ligament space in tooth 36” by CH Chuh is licensed under [CC BY-NC-SA 4.0](https://creativecommons.org/licenses/by-nc-sa/4.0/)

Widening of the PDL space

With occlusal trauma, fibroblasts within the PDL respond with increased activity, leading to a widening of the PDL. For example, in endodontic therapy, a wider PDL space is temporarily generated on the side opposite of the direction of force, but this triggers bone deposition and returns the PDL space to its original width. A wider PDL space can be visualized on a radiograph, and may be accompanied by a thickening of the lamina dura, bone loss, and/or hyper-cementosis. Other conditions besides occlusal trauma may lead to a widening of the PDL space, such as the use of certain medications. The term wider PDL *space* means that neighboring tissue is lost. It does not necessarily indicate PDL fibroblasts have made more collagen fibers, only that a larger radiolucent gap exists between the root and the lamina dura. This space may contain more ground substance or pus, for instance. This brings us to another example, which may seem contradictory to the first: reduced force can also lead to widening of the PDL space. For example, conditions that negatively affect bone health lead to changes in chewing behavior which in turn reduces occlusal force, followed by bone loss and a widening of the PDL space. Similarly, chronic inflammation of nearby tissues, such as pulpitis, can trigger widening of the PDL space. Whether these conditions stimulate production of collagen by fibroblasts is unlikely because pulpitis and osteoporosis are associated with increased tooth mobility. Common treatments usually aim to reduce the insult, such as using a nightguard if bruxism is suspected. Dental implants help distribute the forces applied to remaining teeth in a partially edentulous mouth, reducing changes to the PDL space and maintaining tooth attachment.

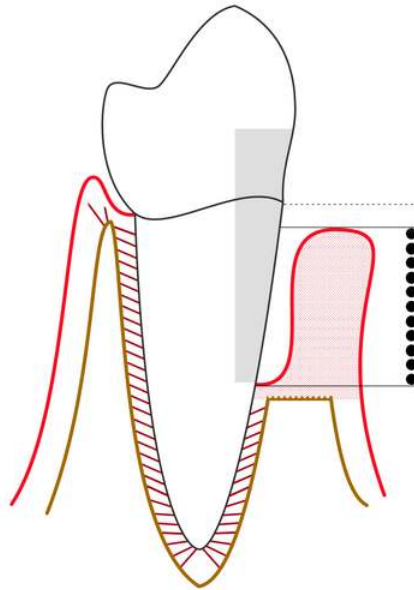


Figure 11.31: Illustration of a healthy pocket versus severe periodontitis, including fibers of the PDL. Image credit: “Line diagram of a tooth showing the gingiva, bone, periodontal ligament with a scale showing the pocket depth of severe periodontitis” by Ian Furst is licensed under [CC BY-SA 4.0](#)

Loss of the PDL apparatus

Loss of attachment between cementum and alveolar bone promotes tooth loss. Two common causes of **PDL loss** are smoking and bacterial biofilms located within periodontal pockets. With smoking, it is here that nicotine may play a role as a toxin. Nicotine activates nicotinic acetylcholine receptors whose activity modulates blood flow, mitosis, chemotaxis and cell attachment to ECM. These are all necessary for a fibroblast to repair or regenerate the ECM of dense regular connective tissue. Bacterial biofilms contain toxins which can cause similar changes in fibroblast activity—but these toxins generally only diffuse 1-2mm through ECM, which means biofilms not located near the DEJ will be too far away to reach fibroblasts of the PDL (Fig. 11.31).

Figure 11.32: PDL regeneration schematic. Figure Legend: a) loss of bone and soft tissue during periodontitis. b and f) Long Junctional Epithelium (LJE) repair. c and g) ideal repair. E) Guided Tissue Regeneration schematic. C = cementum, NC = new cementum, D = dentin, NB = new bone, NPLF = new PDL fibers. Arrowhead = apical end of JE. Image credit: “Periodontal regeneration.” by Jin Liu, et al is licensed under [CC BY 4.0](#)

Regeneration of the PDL following periodontitis

We have discussed regeneration of bone tissue using bioactive scaffolds (guided tissue regeneration), as well as promotion of epithelial attachment to dental implants using ECM protein coatings. The use of similar bioactive membranes to promote regeneration of the PDL has only been [studied in animals](#) at this time, not humans. **PDLRegeneration** is conceptually more complicated—which is perhaps the reason why PDL regenerates poorly, and why we have made less progress on PDL regeneration than bone or oral mucosa. The poor ability of the PDL to regenerate is unusual given it contains stem cells and ample vascularity, two factors that make other connective tissues regenerate well (like pulp, bone and the sub-mucosa of the oral cavity). First, the PDL is polarized, it is not enough to simply boost fibroblast activity. Cells must be oriented correctly to create the proper attachment. Unfortunately, the polarity signals involved in this process are poorly understood at this time.

Figure 11.33: Like the programming of traffic lights, development involves timers (such as cyclins) and signal buttons (morphogens), internal electric changes (gene transcription changes) that lead to externally visible changes (morphogenesis). Like a well-planned city traffic light system, there is significant cross-talk between developing structures in a human embryo not illustrated here. Image credit: “FSA of a traffic light system” by Pluke is in the Public Domain, CC0 / colors added

Secondly, the two connections of the PDL form at different times: collagen fibers are created within cementoid during growth of the roots, but formation of the collagen fibers that connect to alveolar bone do not develop until tooth eruption. Again, the signals involved are poorly understood, and it remains possible that they could be mutually exclusive. We’ve covered the same morphogens doing different things at different times throughout this book. Without knowing more specifics, let us offer a metaphor: the two connections of the PDL could be like car traffic traveling in two directions. For every green light you travel through, cross-traffic must have a red light. With proper timing, you might be able to hit all green lights as you travel down the street, but the traffic light you drove through a minute ago is probably now turning red. It may not be possible to simultaneously get a green light occurring both at the light you are at and the light you were at 5 blocks ago at because urban planners realize that would be a horribly inefficient system.

Don’t worry about the red light 5 blocks behind you, you have the green light to go apply this knowledge to your clinical coursework. If the light turns yellow, yellow means go faster.

Chapter 10 *

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