Connective Tissue

Components and Classification

Connective tissue forms a matrix that supports and connects other tissues and organs. Below is an overview of its components:

- Cells
  - Resident (fixed)
  - Transient (wandering)
- ECM
  - Fibers
  - Amorphous ground substance

Connective tissue types belong to three main categories:

- Connective tissue proper
  - loose
  - dense irregular
  - dense regular
- Embryonic connective tissue
  - mesenchyme
  - mucoid
- Specialized connective tissue
  - reticular
  - adipose
  - elastic
  - cartilage
  - bone
  - blood

Types of the cells of connective tissue

Fixed cells

Reside within connective tissue. Derived from mesenchyme.

Fibroblasts

Fibroblasts function in synthesis and degradation of the ECM. Therefore, DNA is in euchromatin form, and the RER and Golgi apparatuses are abundant. Fibro*cytes* are "retired" fibroblasts that do not synthesize much.

Reticular cells

Similar to fibroblasts, produce reticular fibers. Note: Reticular cells ≠ reticulocytes! Reticulocytes are immature RBCs.

Adipocytes

- **Univacuolar**
  - spherical cell
  - one large vacuole (stores triacylglycerols) takes up most of the space
  - cytoplasm, flattened nucleus and other organelles are pushed to the periphery of the cell
  - major component of white adipose tissue
    - energy storage
    - also functions in hormone production (adipokinins, leptins)
    - highly vascularized
- **Multivacuolar**
  - multiple smaller fat droplets
  - nucleus is more central
  - burns fat to heat via mitochondria, functions in thermoregulation
  - major component of brown adipose tissue
    - more abundant in fetuses, neonates and small children
    - found primarily between scapulae, in axilla, mediastinum, and retroperitoneum

Pigment (melanin) cells

- irregularly shaped
- arise from *neuroectoderm* rather than mesenchyme
- synthesize and store melanin

Schematic comparison of all fixed cells here.

Wandering cells

Formed in red bone marrow from hemocytoblasts, enter connective tissue via bloodstream.

Macrophages

- variable shape with protrusions
- kidney-shaped nucleus
- phagocytose debris, damaged, or foreign cells
- mainly in sparse connective tissue along capillaries

Plasma cells

- eccentrically placed nucleus, alternating heterochromatin and euchromatin
- large amount of cytoplasm and RER (intensive proteosynthesis)
- synthesize IgE, which binds to antigens
- usually in places where bacteria and foreign substances easily enter the body

Mast cells

- large, ovoid shape
- occur in the sparse collagen ligament of GIT and respiratory mucosa
- mediate allergic reaction
    1. receptors bind to IgE
    2. basophilic secretory granules with histamine and heparin released
    3. inflammation in surrounding tissue

Schematic comparison of wandering cells here.

Extracellular Component

Fibers

Collagen

Collagens include a family of proteins categorized into three main categories (fibril-forming, network forming, and anchoring). They are made up of individual fibrils of tropocollagen polymers. The thickness varies depending on the type.

The most well-known types and their locations are listed below. Mnemonic: **B**e **S**o **T**otally **C**ool, **R**ead **B**ooks!

- **collagen I** (**B**one, **S**kin**, T**endons)
    - ~1.5 nm diameter and 300 nm long
    - most abundant
    - 67 nm periodicity
- **collagen II** (**C**artilage - hyaline and elastic)
- **collagen III** (**R**eticulin of reticular fibers)
- **collagen IV** (**B**asal lamina)

Synthesis of collagen type I (most studied):

1. **procollagen α** chains produced by ribosomes in RER and imported into cisternae
2. Hydroxylases modify some AAs, requiring O_2, Fe^{2+}, and Vitamin C
3. three α chains are aligned, linked by disulfides, and folded into a **triple helix**
4. triple helix is exocytosed, N- and C- terminal domains are cleaved off to form **tropocollagen** (basic subunit which is differently assembled into different types of collagen)
5. tropocollagen is converted to collagen molecules, which self-assemble into polymeric collagen (this is driven by entropy)
6. Lysyl oxidase cross-links collagen molecules to further stabilize structure

Schematic of synthesis here (https://upload.medbullets.com/topic/102078/images/collagen.jpg)

Collagen is acidophilic and typically stains pink.

Elastic fibers

- 2 µm diameter elastic fibers
- elastin gets embedded into fibrillin scaffolds to form elastic fibers
- secreted by fibroblasts
- found interspersed with collagen in locations subject to tensile or shear stress, as well as in elastic lamellae

- allows structures to return to original shape

Reticular fibers

- 0.5-2 µm diameter
- consist mostly of collagen type III
- found in reticular connective tissue
  - hematopoietic tissue (bone marrow and spleen)
  - lymphatic tissue
  - surrounds adipocytes, smooth muscle, nerve fibers, small blood vessels

Ground substance

Ground substance is a highly-hydrated space-filler between cells and in the ECM. It consists mainly of three different types of molecules

- **glycosaminoglycans** (GAGs) aka mucopolysaccharides
  - long polymers of repeating disaccharide units
  - often sulfated
  - ex: hyaluronic acid, chondroitinsulfate, keratansulfate
- **proteoglycans** and their aggregates
  - consist of a core protein with covalently bond sulfated GAGs (predominant part)
  - e.g. aggrecan (in cartilage), perlecan (in basal lamina)
- **glycoproteins**
  - consist of a protein core with branched oligosaccharide chains
  - e.g. fibronectin, laminin

Connective tissue proper

- dominant fibrillar component, cells (mostly fibroblasts) rare
- collagen fibers arranged to paralell or interlaced bundles
- regular dense collagen c.t. (tendons)
- irregular dense collagen c.t. (capsules of organs, sclera)

Connective Tissue Proper	Composition	Structure	Locations
Loose (areolar)	ground substance and many cells	randomly distributed fibers (much GAGs, elastic fibers, some collagen I&III)	stroma in epithelial organs, lamina propria of tunica mucosa, tela submucosa, tunica adventitia and organ interstitium
Dense regular	few cells (mostly fibroblasts), mostly collagen	parallel collagen fibers	ligaments, tendons, aponeuroses, stroma of cornea
Dense irregular		randomly arranged collagen fibers	dermis, capsules of organs, sclera, GIT submucosa

Embryonic connective tissue

Mesenchyme

- mostly a matrix of collagen fibers with undifferentiated cells (both components are sparse)
- has progenitor cells for all adult connective tissue
- found in embryonic mesoderm

Mucoid

- originates from extra-embryonic mesoderm
- random fibroblasts and collagen embedded in matrix of hyaluronic acid and GAGs (chondroitinsulfate)
- ex: Wharton's jelly

Specialized connective tissue

Elastic connective tissue

- high amount of elastic fibers arranged in parallel, with a few collagen fibers
- sparse cells
- ex: ligamenta flava of spine and other elastic ligaments, elastic membranes of arteries

Reticular connective tissue

- reticular fibers produced by fibroblasts

- provides supporting scaffold and attachment site for immune cells
- found in bone marrow, secondary lymphatic organs, and some other tissues

Adipose tissue

Primarily made up of adipocytes (see above description)

Cartilage

Cartilage is a type of connective tissue that offers resistance yet is flexible and resilient (https://en.wikipedia.org/wiki/Resilience_(materials_science)).

The dominant constituent is ground substance, while collagen and elastic fibers are found in various proportions, depending on the type.

It is avascular, so nutrition has to be provided via diffusion from the synovial fluid or through the perichondrium.

The main cells are

- **chondroblasts**
  - actively synthesize cartilage matrix (ground substance, collagen and elastin)
  - therefore, DNA is in euchromatin form, RER and Golgi app. are abundant
  - cells group together in isogenous groups, chondrons, and territories
  - spindle-shaped cells closer to perichondrium, spherical shape further away
- **chondrocytes**
  - decreased synthetic activity but function in maintenance

Elastic and hyaline cartilage are encapsulated by **perichondrium**, which is a layer of dense connective tissue. It has two layers:

- fibrous layer
  - contains dense irregular connective tissue with elastic fibers and fibroblasts
  - functions in protection
  - rich vascularization and innervation for chondrogenic layer below
- chondrogenic layer
  - hosts mesenchymal cells, which proliferate and differentiate to chondroblasts
  - important in cartilage production

Classifications of cartilage

Schematic of the arrangement of cartilage cells and matrix in the individual types of cartilage can be found here (http://histologie.lfp.cuni.cz/education/Web_histo_schemata/eng/chon/imgs/cartilago1.jpg).

Hyaline

- consists of type II collagen and aggrecan
- cells are alone or in isogenous groups, embedded within basophilic territorial matrix
- can provide
  - structural support (in fetal skeleton, ribs, respiratory tract)
  - smooth surface for articulations

Scheme of the developing hyaline cartilage can be found here (http://histologie.lfp.cuni.cz/education/Web_histo_schemata/eng/chon/imgs/cartilago2.jpg). Note the basophilic territorial matrix (due to a higher concentration of proteoglycans), while the interterritorial matrix is acidophilic

Elastic

- consists of aggrecan and more noticeable fibrous component (type II collagen and elastic fibers)
- cells are regularly dispersed
- can provide
  - structural support of soft tissues (epiglottis and small laryngeal cartilages)
  - offer flexibility (external ear)

Fibrocartilage

- consists mostly of type I collagen with some type II
- isolated chondrocytes, also has fibroblasts
- resistant to compression, shearing, and tension (intervertebral discs, menisci, pubic symphysis, tendon insertions)

Bone

Structure

Bone is a solid connective tissue composed of mineralized ECM.

- **periosteum**: covers bone on outside
  - outer component has dense irregular collagen
  - inner component has osteoblasts and osteoprogenitor cells
  - Sharpey's (aka perforating) fibers fix it to the bone
- **endosteum**: covers bone on inside (part that faces medullary cavity)
  - thin layer of bone lining cells
  - cells include osteoblasts, osteoprogenitor cell

Primary bone (woven, fibrilar)

- scaffold of randomly oriented collagen fibers and dispersed bone cells
- temporary structure
- bone cells dispersed randomly
- found in skull sutures, dental cement
- otherwise replaced by secondary bone

Secondary bone (lamellar)

- Consists of hydroxyapatite and collagen I fibrils (3-5 µm) highly oriented in similar direction
- other proteins have structural or metabolic functions (ex. osteocalcin, osteonectin, osteopontin, bone sialoprotein)
- **trabecular (spongy) bone**
  - consist of trabecules (~300 µm) - interconnected rods and plates
  - nutrients reach via diffusion from bone marrow
- **compact bone** consists of
  - Haversian systems (aka osteons, ~100-400 µm) of 5-20 bone lamellae concetrically arranged around vertical Haversian (aka central) canals
    - Haversian canals (~20 µm) are passages for capillaries or postcapillary venules and occasional c.t. and nerve fibers, lined by endosteum
    - transverse Volkmann canals (aka perforating canals) connect Haversian canals
  - external and internal circumferential lamellae enclose the Haversian systems
  - interstitial lamellae are between the Haversian systems

Schemes for bone structure are found here (http://histologie.lfp.cuni.cz/education/Web_histo_schemata/eng/index_en.html)

Cells

- **osteoblasts**
  - derived from mesenchyme
  - roughly cuboidal shape
  - found on external and internal surface of bone
  - synthesize organic components of matrix (osteoid) and collagen type I fibers
  - therefore, have DNA in euchromatin form and abundant RER and Golgi apparatuses
  - secrete osteocalcin protein, which binds Ca^{2+} (together with glycoproteins) and concentrates it, leading to mineralization
- **osteocytes**
  - derived from mesenchyme
  - found enclosed in lacunae of mineralized bone matrix
  - connected to each other and other bone cells via cytoplasmic processes through canaliculi ossium
  - connections function as mechanosensors; stress elicits remedial responses from osteoblasts and osteoclasts
- **osteoclasts**
  - large, multi-nucleated cells (50-100 µm)
  - derived from fusion of monocyte-macrophage progenitors; development requires polypeptides from osteoblasts
  - found in in resorption lacunae (Howship's lacunae) - enzymatically etched cavities
  - part of the plasma membrane binds to the bone via a circular zone (sealing zone); the part that faces the subcellular pocket has many surface projections (ruffled border)
  - osteoclasts pump protons and enzymes into the pocket to digest bone tissue

Schematics of principal ultrastructural hallmarks of osteoblasts, osteocytes and osteoclasts can be found here (https://www.histology.leeds.ac.uk/bone/bone_cell_types.php).

Joints

Bones join each other at joints. Joints can be classified according to the freedom of motion that they allow.

Diarthroses (synovial joints) allow free movement of the bones. An articular capsule encloses the joint and the synovial fluid within. The outer layer of the capsule consists of a fibrous layer. The **synovium** forms the inner layer of the articular capsule (as well as bursae and tendon sheaths). It is well-vascularized and innervated. It can be further divided into two layers: *subintima,* which consists of connective tissue, and *intima*, which has thin folds extending into the joint cavity and cuboidal cells with microvilli.

The **synovial fluid** itself is high in hyaluronans, serves in the exchange of gases, nutrients and wastes, and allows for the passage of leukocytes.

Synarthroses severely limit movement. These include fibrous joints (sutures, gomphoses) and cartilaginous synarthroses (synchondrosis).

Amphiarthroses allow limited movement. These include fibrous joints (syndesmoses) and cartilginous ones amphiarthroses (symphyses).

Bone ossification

There are two main types of ossification:

- **intramembraneous ossification**
  - direct ossification of mesenchyme precursor
  - begins in ossification centers dispersed between network of developing capillaries
  - osteoid is secreted by osteoblasts, calcifies, and forms woven bone
  - eventually, ossification centers fuse with each other
  - method of formation of flat bones
- **endochondral ossification**
  - ossification of hyaline cartilage model
  - bone collar produced by osteoblasts prevents gas and nutrient exchange of underlying chondrocytes
  - the underlying chondrocytes hypertrophy, compress matrix and initiate its calcification
  - the hypertrophied chondrocytes die off, creating space for blood vessels
  - more osteoprogenitor cells arrive and start forming woven bone
  - method of forming most bones

Primary ossification centers form via endochondral ossification, and secondary ossification centers form in a similar way. Eventually, the woven bone is replaced by trabeculae or bone marrow. After the bones are ossified, the epiphyseal plate still remains and is responsible for growth.

Typical zonation of the **epiphyseal plate** (mnemonic: real people have career opportunities):

- reserve zone (hyaline cartilage)
- proliferating zone
- hypertrophic (and maturation) cartilage
- calcification (provisional) zone - has line of erosion
- ossification and remodelling zone

Growth stops after puberty due to release of sex hormones. However, the timing varies for different bones, which allows one to determine age in clinical or forensical practice.

Schemes for bone ossification and zones of the growth plate are found here (http://histologie.lfp.cuni.cz/education/Web_histo_schemata/eng/index_en.html) (see "Bone").

Bone remodelling

Bone remodelling is the continual process of renewing bone to adapt it to changing stress. Stress - disruption of the periosteum - attracts preosteoclasts, which mature and resorb bone in that region. This process is reversed by osteoblasts, which re-synthesize new bone. Since this process depends on signal pathways and regulators, disruption of the pathway can result in osteoporosis. Disruption of hormone levels (such as after menopause) or a lack of vitamin D are such examples that can lead to osteoporosis.

Scheme for bone remodelling here (https://www.orthopaedicsone.com/download/attachments/71434800/bone+remodel.png?version=1&modificationDate=1389455440000).

References

Mescher, A. and Junqueira, L., 2018. *Junqueira's basic histology*. New York: McGraw-Hill, pp. 96-159.

Vaňhara, Petr et al. *Guide To General Histology And Microscopic Anatomy*. 1st ed., Masaryk University Press, 2020, pp. 7-9.