Must-knows

Resolution Smallest distance at which two points are still seen as separate; d = 0.61λ / NA. Shorter wavelength or higher NA gives finer resolution.

Limits Eye ~0.2 mm; light microscope ~0.2 µm; TEM ~1–3 nm in tissue sections (theoretical far less).

Magnification Total = objective × eyepiece (40× × 10× = 400×). Magnifying past the resolution limit is empty magnification: no new detail.

Immersion oil Oil (n ≈ 1.515, like glass) between coverslip and 100× objective raises NA to ~1.4, so resolution improves.

Worked example Green light λ 550 nm, NA 1.4: d = 0.61 × 550 / 1.4 ≈ 240 nm = 0.24 µm.

Units 1 mm = 1000 µm; 1 µm = 1000 nm. RBC ~7.5 µm; ribosome ~25 nm (TEM only).

Bright-field parts Light source, condenser (with diaphragm), stage, objective, eyepiece; image from light absorbed by stained tissue.

Working routine Find and focus with the low-power objective first; bring a high-power lens to the coverslip watching from the side, then focus by moving it away.

Electron microscopy Electrons have a far shorter wavelength than light; lenses are electromagnetic coils; vacuum column; image seen on a screen or camera.

TEM contrast Heavy metals (osmium, uranium, lead) scatter electrons: bound areas look dark (electron-dense), others pale (electron-lucent).

Fluorescence Fluorochrome absorbs short-wavelength light and emits longer-wavelength light (Stokes shift); fluorescein green, rhodamine red, DAPI blue.

Modern solutions Confocal and multiphoton optical sectioning, super-resolution (Nobel 2014), cryo-EM (Nobel 2017), digital whole-slide imaging.

Basophilia and acidophilia Basic dyes (haematoxylin) bind acidic groups, e.g. nucleic acid phosphates; acidic dyes (eosin) bind basic proteins of cytoplasm and collagen.

METHOD Light microscopes

Bright-field Principle transmitted light absorbed by stained section Best for routine stained (H&E) sections

Phase contrast Principle converts differences in refractive index into light and dark Best for living, unstained cells (cell culture)

Dark-field Principle only light scattered by the specimen enters the objective Best for bright objects on black: spirochaetes, crystals

Polarising Principle polariser + analyser; birefringent structures shine Best for collagen, skeletal muscle A band, crystals, amyloid (Congo red)

Fluorescence Principle UV/blue excitation; longer-wavelength emission seen Best for fluorochrome-labelled antibodies, DNA dyes

Confocal (laser scanning) Principle pinhole rejects out-of-focus light; point scanning Best for optical sections and 3D reconstruction of thick tissue

METHOD Electron microscopes

Transmission (TEM) Image formed from electrons passing through the section Specimen ultrathin section 40–90 nm, heavy-metal stained Shows ultrastructure: organelles, ribosomes, membranes

Scanning (SEM) Image formed from secondary electrons from the surface Specimen whole specimen coated with gold/metal Shows 3D surface of cells and tissues

Freeze-fracture Image formed from metal replica of fractured frozen tissue Specimen cleaved along lipid bilayer Shows inner membrane faces (P and E), intramembrane proteins

Cryo-EM Image formed from electrons through frozen sample Specimen molecules in vitreous ice, unstained Shows near-atomic protein structure (Nobel 2017)

MORE Resolution: what decides it

Wavelength (λ) Effect shorter λ gives finer d Note blue/UV better than red; electrons best

Numerical aperture (NA = n sin α) Effect higher NA gives finer d Note dry objective ≤0.95; oil ~1.4

Refractive index (n) of medium Effect oil raises n Note air 1.0, water 1.33, oil 1.515

Magnification Effect does not change d Note beyond ~1000× NA is empty magnification

MORE Scale: what each instrument can see

Red blood cell Size ~7.5 µm Seen with LM

Bacterium Size ~1–2 µm Seen with LM

Mitochondrion Size ~0.5–1 µm wide Seen with LM (barely, special stains); detail by TEM

Cell membrane Size ~7.5–10 nm Seen with TEM

Ribosome Size ~25 nm Seen with TEM

METHOD Modern and super-resolution methods

Super-resolution in general How it beats the limit or adds breaks the 0.2 µm diffraction limit Resolution / use Nobel Prize Chemistry 2014 (Betzig, Hell, Moerner)

Whole-slide scanning (digital pathology) How it beats the limit or adds entire slide digitised at high power Resolution / use virtual microscopy, telepathology, AI analysis

Cryo-EM How it beats the limit or adds frozen-hydrated molecules, image averaging Resolution / use near-atomic structure; Nobel 2017 (Dubochet, Frank, Henderson)

MORE Common fluorochromes

Fluorescein (FITC) Binds / used for conjugated to antibodies Emission colour green

Rhodamine / Texas red Binds / used for conjugated to antibodies Emission colour red

DAPI / Hoechst Binds / used for DNA (nuclei) Emission colour blue

METHOD Other imaging and labelling techniques

Autoradiography Principle radioactive precursor (e.g. ^{3}H-thymidine) incorporated; emulsion develops silver grains Shows where and when molecules are synthesised

Immunohistochemistry (indirect) Principle unlabelled primary, then labelled secondary antibody against it Shows same, with signal amplification

Must-knows

Routine sequence Fixation → dehydration → clearing → embedding (infiltration) → sectioning → mounting → staining → coverslipping.

Fixation Stops autolysis and bacterial decay, preserves structure; formalin (buffered 4% formaldehyde) cross-links proteins via methylene bridges.

EM fixation Glutaraldehyde (protein cross-links) then osmium tetroxide (fixes and stains lipid membranes); embed in epoxy resin.

Dehydration and clearing Graded alcohols (70% → 100%) remove water; xylene mixes with both alcohol and paraffin, so wax can enter.

Embedding Paraffin (~56–60 °C) or resin infiltrates the tissue and gives it a firm consistency for thin sectioning.

Section thickness Paraffin 3–10 µm (microtome); semithin resin ~1 µm (toluidine blue); ultrathin 40–90 nm (ultramicrotome, glass/diamond knife).

Frozen sections Cryostat; no alcohol or xylene, so lipids and enzymes survive; minutes-long intraoperative diagnosis.

Processing losses Paraffin processing dissolves lipids (empty adipocytes); water-soluble glycogen can wash out.

H&E Haematoxylin (basic, with mordant) stains acidic structures blue-purple: nuclei, rER-rich cytoplasm. Eosin (acidic) stains basic proteins pink: cytoplasm, collagen.

Histochemistry Chemical reaction in the section localises a substance: PAS (glycogen, basement membrane, mucins), Feulgen (DNA), Perls (ferric iron), enzyme reactions.

Immunohistochemistry Labelled antibody shows where an antigen (protein) lies; direct or indirect (labelled secondary antibody amplifies signal).

In situ hybridisation Labelled DNA/RNA probe binds a complementary sequence in the section (FISH if fluorescent).

Decalcification Bone and calcified tissue are softened with acid or EDTA before cutting on the microtome.

Tissue context Four basic tissues: epithelial, connective, muscle, nervous; all derive from ectoderm, mesoderm and endoderm by differentiation.

MORE Steps of paraffin processing

1 Fixation Agent 10% neutral buffered formalin Purpose prevent autolysis, preserve structure

(Decalcification, if bone) Agent acids (formic, nitric) or EDTA Purpose remove calcium so tissue can be cut

2 Dehydration Agent graded alcohols (ethanol 70→100%) Purpose remove water

3 Clearing Agent xylene (or toluene) Purpose replace alcohol; miscible with paraffin; tissue turns translucent

4 Infiltration and embedding Agent molten paraffin wax, set in a block Purpose firm support for sectioning

5 Sectioning Agent rotary microtome, steel knife Purpose sections 3–10 µm

6 Mounting Agent float on warm water, pick up on glass slide Purpose flat section on slide

7 Dewaxing and rehydration Agent xylene, then descending alcohols to water Purpose allow water-based dyes in

8 Staining Agent e.g. H&E Purpose contrast for structures

9 Dehydrate, clear, coverslip Agent alcohol, xylene, resin mountant Purpose permanent slide

METHOD Light vs electron microscopy preparation

Fixative Light microscopy buffered formalin TEM glutaraldehyde, then osmium tetroxide

Block size Light microscopy ~1–2 cm across, a few mm thick TEM ~1 mm^3

Embedding medium Light microscopy paraffin TEM epoxy resin (Epon, Araldite)

Instrument Light microscopy microtome TEM ultramicrotome

Knife Light microscopy steel TEM glass or diamond

METHOD Light vs electron microscopy preparation (cont.)

Thickness — Light microscopy: 3–10 µm; TEM: 40–90 nm (semithin survey 0.5–1 µm)

Support — Light microscopy: glass slide; TEM: copper grid

Stain / contrast — Light microscopy: dyes (H&E); TEM: heavy metals: uranyl acetate, lead citrate

METHOD Fixatives

Formaldehyde (formalin) — Action: cross-links proteins; Use: routine LM, IHC

Glutaraldehyde — Action: stronger cross-links (two aldehyde groups); Use: EM primary fixation

Osmium tetroxide — Action: binds unsaturated lipids; adds electron density; Use: EM secondary fixation; stains myelin black

METHOD Routine and special stains

Haematoxylin — Shows: nuclei, rER, acidic matrix (basophilic); Colour: blue-purple

Eosin — Shows: cytoplasm, collagen, RBCs (acidophilic); Colour: pink-red

Masson's trichrome — Shows: collagen / muscle and cytoplasm / nuclei; Colour: blue or green / red / black

Orcein, Verhoeff, resorcin-fuchsin — Shows: elastic fibres; Colour: brown / black / purple

Silver impregnation (Gomori) — Shows: reticular fibres (type III collagen), nerve fibres; Colour: black (argyrophilic)

Toluidine blue — Shows: semithin sections; mast-cell granules metachromatic; Colour: blue; granules purple-red

Alcian blue — Shows: acidic mucins, sulfated GAGs; Colour: blue

Oil Red O, Sudan black — Shows: neutral lipid (frozen sections); Colour: red / black

Congo red — Shows: amyloid; Colour: red; apple-green birefringence in polarised light

METHOD Histochemical reactions

PAS (periodic acid-Schiff) — Detects: glycogen, neutral mucins, basement membrane, glycoproteins; Note: magenta

PAS + diastase — Detects: confirms glycogen; Note: diastase (amylase) digests glycogen; loss of stain = glycogen

Feulgen — Detects: DNA; Note: magenta; quantitative; RNA unstained

Perls' Prussian blue — Detects: ferric iron (haemosiderin); Note: blue

Enzyme histochemistry — Detects: phosphatases, dehydrogenases, peroxidase; Note: frozen sections of unfixed or lightly fixed tissue; coloured or dense product at the site

MORE Dye binding terms

Basophilic — Meaning: binds basic dye; rich in acidic groups; Examples: nuclei (DNA phosphate), plasma-cell cytoplasm (rRNA of rER), cartilage matrix

Acidophilic (eosinophilic) — Meaning: binds acidic dye; rich in basic proteins; Examples: cytoplasm, mitochondria, collagen, RBCs

Metachromasia — Meaning: dye changes colour on binding dense polyanions; Examples: mast-cell heparin with toluidine blue (purple-red)

METHOD Molecular localisation methods

Direct immunohistochemistry — Probe: labelled primary antibody; Detects: antigen

Indirect immunohistochemistry — Probe: unlabelled primary + labelled secondary anti-antibody; Detects: antigen, amplified signal

In situ hybridisation / FISH — Probe: labelled complementary nucleic acid; Detects: specific DNA or mRNA

MORE Artefacts

Parallel scratches across the section — Cause: nick in the knife edge

Folds, wrinkles — Cause: poor flotation or mounting

Shrinkage spaces (cell retraction) — Cause: fixation and dehydration

Empty adipocytes, empty spaces — Cause: lipid dissolved in processing

Mnemonics

Mnemonic — Fix, Dehydrate, Clear, Embed, Cut, Stain = order of routine paraffin processing

Must-knows

Four basic tissues Epithelial, connective, muscle and nervous tissue. Histology = tissues and how they build organs; 'histo' = tissue or web.

Origin from all three germ layers Epithelia arise from ectoderm, mesoderm and endoderm (e.g. epidermis, endothelium, gut lining).

Defining features Tightly packed polyhedral cells with little extracellular matrix, polarised (apical, lateral, basal domains), resting on a basement membrane. Avascular: fed by diffusion from capillaries in the lamina propria.

Functions Covering, lining, protection (epidermis, urothelium), absorption (intestine), secretion (stomach, glands), sensation, transport (cilia), contractility (myoepithelial cells).

Naming rule Number of layers (simple / stratified) + shape of the cells of the most superficial layer (squamous, cuboidal, columnar). Stratified squamous is further split by keratinisation.

Pseudostratified is simple Every cell rests on the basement membrane but not every cell reaches the lumen; nuclei lie at several levels so it looks layered. Short basal cells are stem cells.

Basement membrane Basal lamina (epithelial product: type IV collagen, laminin) plus reticular lamina (connective tissue product: type III collagen). Selective barrier; controls polarity, proliferation and differentiation.

Apical specialisations Microvilli (actin core, absorption), stereocilia (long microvilli, epididymis), cilia (9+2 axoneme on basal bodies, motile), glycocalyx (carbohydrate coat).

Goblet cell Unicellular exocrine gland secreting mucin (heavily glycosylated protein): PAS-positive, pale in H&E; apical mucin mass pushes the nucleus to the basal pole.

Stratified squamous: keratinised vs not Non-keratinised: living nucleated surface cells on moist surfaces (mouth, oesophagus, vagina, cornea). Keratinised: dead anucleate keratin-filled surface cells (epidermis).

Transitional epithelium (urothelium) Lines renal calyces, pelvis, ureter, bladder, proximal urethra. Relaxed 4–5 layers with dome-shaped (umbrella) surface cells, some binucleate; stretched 2–3 layers, flat surface cells.

Gland development Surface epithelium proliferates into the connective tissue. Exocrine: connection persists as a duct. Endocrine: connection is lost, product enters capillaries.

Modes of secretion Merocrine = exocytosis, no cytoplasm lost (most common). Apocrine = product plus apical cytoplasm (mammary lipid). Holocrine = whole cell breaks down (sebaceous).

Renewal Epithelia renew continuously by mitosis of basal / stem cells; holocrine glands replace lost cells from peripheral basal cells.

TYPES Classification of covering epithelia

Simple squamous Layers and cell shape one layer, flat cells, flat nuclei bulging into lumen Where endothelium (vessels, heart), mesothelium (pleura, peritoneum, pericardium), lung alveoli, parietal layer of Bowman's capsule, thin limb of loop of Henle, corneal endothelium, inner and middle ear Function exchange (gases, fluid), filtration, lubrication, low friction

Simple cuboidal Layers and cell shape one layer, cells as tall as wide, round central nucleus Where kidney tubules, thyroid follicles, small exocrine ducts, ovary surface Function secretion, absorption, barrier

Simple columnar Layers and cell shape one layer, tall cells, oval nuclei near the basal pole Where stomach surface and gastric pits, small intestine and colon (with goblet cells), gallbladder (microvilli, no goblet cells); ciliated form in uterine tube Function absorption, secretion, protection

Pseudostratified columnar Layers and cell shape one layer, all cells on basement membrane, nuclei at several levels Where trachea and bronchi (ciliated, with goblet cells = respiratory epithelium), nasal cavity; epididymis and ductus deferens (stereocilia) Function secretion, moving mucus by cilia, protection

Stratified squamous non-keratinised Layers and cell shape many layers, cuboidal basal to flat nucleated living surface cells Where oral cavity, oesophagus, vagina, ectocervix, anal canal, corneal epithelium, inner lips Function protection on moist surfaces, some secretion

Stratified squamous keratinised Layers and cell shape many layers, surface cells dead, anucleate, filled with keratin Where epidermis of skin Function protection against abrasion, water loss, microbes

Stratified cuboidal Layers and cell shape two (or more) layers of cuboidal cells Where ducts of sweat glands, developing ovarian follicles Function protection, secretion

Stratified columnar Layers and cell shape several layers, superficial cells columnar Where conjunctiva, large excretory ducts, parts of male urethra Function protection, secretion

Transitional (urothelium) Layers and cell shape relaxed 4–5 layers (basal cuboidal, intermediate polygonal, dome-shaped umbrella surface cells); stretched 2–3 layers, flat surface cells Where renal calyces and pelvis, ureter, bladder, proximal urethra Function protection, distension, barrier to urine

CELLS Cell shapes

Squamous Proportions width greater than height Nucleus flat, elongated Example endothelium, alveoli

CELLS Cell shapes (cont.)

Cuboidal Proportions height ≈ width ≈ depth Nucleus round, central Example thyroid follicle, kidney tubule

Columnar Proportions height greater than width Nucleus oval, near basal pole Example intestine, stomach

SURFACE Apical surface specialisations

Microvilli Core / composition actin filaments anchored in terminal web Size and motility ≈1 µm long, 0.08 µm wide; non-motile; brush (striated) border Where enterocytes, proximal convoluted tubule

Stereocilia Core / composition actin filaments (very long microvilli, not cilia) Size and motility long, non-motile; branched in epididymis Where epididymis, ductus deferens; sensory hair cells of inner ear

Cilia Core / composition axoneme of 9 peripheral doublets + central pair (9+2) of microtubules, dynein arms; arise from basal bodies Size and motility 5–10 µm long, 0.2 µm wide; motile, beat in one direction Where respiratory tract, uterine tube

Glycocalyx Core / composition carbohydrate chains of membrane glycoproteins and glycolipids Size and motility thin coat on apical membrane Where all cells, prominent on enterocytes

CELLS Cell domains and junctions

Apical domain What it is free surface facing the lumen Function absorption, secretion, specialisations

Lateral domain What it is surfaces facing neighbouring cells Function cell–cell junctions

Basal domain What it is surface on the basement membrane Function anchorage, receptors, ion transport (basal folds)

Tight junction (zonula occludens) What it is most apical; claudins, occludin seal the membranes Function blocks paracellular passage; separates apical and lateral membranes

Desmosome (macula adherens) What it is cadherins linked to keratin intermediate filaments Function strong spot adhesion; 'spines' of stratum spinosum

Gap junction What it is connexons (connexins) forming channels Function passage of ions and small molecules between cells

SURFACE Basement membrane

Basal lamina Made of / by type IV collagen, laminin, nidogen, perlecan; made by epithelial cells Note 20–100 nm; seen by EM (lamina lucida + lamina densa)

Functions Made of / by selective filter, scaffold for regeneration Note controls polarity, proliferation, differentiation

SURFACE Epidermis layers (keratinised stratified squamous), deep to superficial

Stratum basale Cells one layer of cuboidal to low columnar cells on basement membrane Key feature mitosis (stem cells)

Stratum spinosum Cells several layers of polygonal cells Key feature desmosomes give 'spiny' (prickle) look

Stratum granulosum Cells 3–5 layers of flattened cells Key feature keratohyalin granules

Stratum lucidum Cells thin layer of flat eosinophilic cells Key feature only in thick skin (palms, soles); eleidin, pale/bright

Stratum corneum Cells many layers of dead anucleate flat cells Key feature filled with keratin

SURFACE Germ layer origin

Ectoderm (outer) Epithelia derived epidermis, corneal epithelium, oral and nasal epithelium, distal anal canal

Mesoderm (middle) Epithelia derived endothelium, mesothelium, kidney tubules, reproductive tract

Endoderm (inner) Epithelia derived lining of gut and respiratory tract, liver, pancreas, thyroid

TYPES Gland classification

Ducts Type Exocrine Feature duct to surface or lumen Example sweat, sebaceous, salivary

Ducts Type Endocrine Feature ductless, secretes hormones into blood Example thyroid, adrenal, pituitary

Cell number Type Unicellular Feature single secretory cell Example goblet cell

Cell number Type Multicellular Feature many cells, secretory portion + duct Example sweat, salivary glands

Duct Type Simple Feature unbranched duct Example sweat gland

Duct Type Compound Feature branched duct Example salivary glands, pancreas

Secretory portion Type Tubular Feature tube of about duct diameter (simple, coiled or branched) Example intestinal crypts; sweat gland (coiled); gastric glands (branched)

Secretory portion Type Acinar (alveolar) Feature rounded sac-like end piece Example sebaceous gland (branched acinar), exocrine pancreas (compound acinar)

Secretory portion Type Tubuloacinar Feature both tubules and acini Example submandibular gland

MORE Modes of secretion

Merocrine (eccrine) How product leaves exocytosis of vesicles at apical surface Cell fate intact, keeps secreting Example most glands: eccrine sweat, salivary, pancreas

Apocrine How product leaves product released with part of apical cytoplasm Cell fate apical region reforms Example lipid of mammary gland

Holocrine How product leaves whole cell fills with product and breaks down Cell fate dies; replaced by mitosis of peripheral basal cells Example sebaceous gland

Mnemonics

Mnemonic Come Let's Get Sun Burned = Epidermis superficial to deep: corneum, lucidum, granulosum, spinosum, basale

Mnemonic Mero = merely exocytosis, Apo = apex, Holo = whole = Merocrine loses nothing, apocrine loses apical cytoplasm, holocrine loses the whole cell

Must-knows

Three components Cells + extracellular matrix (ground substance and fibres). Matrix dominates; cells are scattered in it.

Origin: mesenchyme All connective tissues arise from embryonic mesenchyme (mostly mesoderm). Fibroblasts, chondroblasts, osteoblasts, adipocytes and haematopoietic stem cells descend from it.

Functions Support and binding, nutrition of avascular epithelia by diffusion through ground substance, defence (immune cells), protection, fat storage, repair.

Loose vs dense Loose: many cells, much ground substance, thin loosely woven fibres. Dense: few cells, little ground substance, thick collagen bundles; regular (one direction) or irregular (many directions).

Resident vs wandering cells Resident (fixed): fibroblasts, myofibroblasts, macrophages, adipocytes, mast cells, mesenchymal cells. Wandering (from blood): lymphocytes, plasma cells, neutrophils, eosinophils, basophils, monocytes.

Fibroblast Commonest cell; synthesises collagen, elastin, GAGs, proteoglycans and glycoproteins. Active form: large, pale oval nucleus, basophilic cytoplasm; resting fibrocyte: smaller, dark slim nucleus.

Macrophages and mast cells Macrophages come from blood monocytes; phagocytic, antigen-presenting. Mast cells: granules of histamine and heparin, metachromatic; release on IgE cross-linking (immediate hypersensitivity).

Ground substance Hydrated, viscous gel of GAGs, proteoglycans and multiadhesive glycoproteins; lubricant and barrier to microorganisms; fine granular in TEM, lost in routine sections.

Tissue fluid and oedema Plasma-like fluid filtered from capillaries by hydrostatic pressure, returned by colloid osmotic pressure and lymphatics. Excess in loose CT ground substance = oedema.

Collagen hierarchy Tropocollagen (3 alpha chains, triple helix, 300 nm) -> fibril (64-67 nm cross-banding) -> fibre -> bundle. Type I is the commonest (~90% of body collagen).

Elastic and reticular fibres Elastic: elastin core + fibrillin microfibrils, thin branching network; orcein / resorcin-fuchsin. Reticular: type III collagen, argyrophilic (silver, black) and PAS-positive, made by fibroblasts (by reticular cells in lymphoid organs and marrow).

White vs brown fat White: unilocular, one droplet, peripheral flattened nucleus (signet ring), energy store. Brown: multilocular, central round nucleus, many mitochondria with UCP1 (thermogenin) -> heat.

Why fat looks empty Alcohols and xylene of paraffin processing dissolve lipid; frozen section + Sudan or osmium shows it.

Embryonic connective tissue Mesenchyme: stellate cells, abundant viscous ground substance, almost no fibres. Mucous tissue (Wharton's jelly of umbilical cord): rich in hyaluronan, few fibres.

CELLS Cells of connective tissue proper

Fibroblast Origin / resident or wandering mesenchyme; resident How it looks elongated, pale oval nucleus with nucleolus; basophilic cytoplasm with RER and Golgi Function makes fibres and ground substance

Fibrocyte Origin / resident or wandering inactive fibroblast; resident How it looks smaller, spindle-shaped, dark slim nucleus, little cytoplasm Function maintenance; reactivates in repair

Myofibroblast Origin / resident or wandering fibroblast; resident How it looks fibroblast with actin bundles and smooth muscle actin Function wound contraction

Macrophage Origin / resident or wandering blood monocyte; resident (histiocyte) How it looks irregular surface with processes, indented nucleus, many lysosomes Function phagocytosis, antigen presentation, cytokines

Mast cell Origin / resident or wandering bone-marrow progenitor; resident How it looks large oval, central nucleus hidden by many basophilic metachromatic granules; near small vessels Function releases histamine, heparin, leukotrienes, ECF-A

Adipocyte Origin / resident or wandering mesenchyme; resident How it looks white: one droplet, peripheral nucleus; brown: many droplets, central nucleus Function fat storage, insulation, heat (brown), leptin

Undifferentiated mesenchymal cell Origin / resident or wandering mesenchyme; resident How it looks small, stellate, near vessels (pericytes) Function stem cell for new CT cells in adults

Plasma cell Origin / resident or wandering B lymphocyte; wandering How it looks oval, eccentric nucleus with clock-face chromatin, basophilic cytoplasm, pale Golgi halo Function secretes antibodies

Reticular cell Origin / resident or wandering specialised fibroblast; resident How it looks stellate, processes along fibres Function makes reticular (type III) fibres of lymphoid organs, marrow

Pigment cell (melanocyte) Origin / resident or wandering neural crest; resident How it looks branched, brown-black melanin granules Function melanin from tyrosine by tyrosinase; skin, choroid of eye

Lymphocyte Origin / resident or wandering blood; wandering How it looks small, round dark nucleus, thin rim of cytoplasm Function immune responses

Neutrophil Origin / resident or wandering blood; wandering How it looks multilobed nucleus, faint granules Function phagocytosis of bacteria (acute inflammation)

Eosinophil Origin / resident or wandering blood; wandering How it looks bilobed nucleus, large orange-red granules Function parasites, allergy

Basophil Origin / resident or wandering blood; wandering How it looks lobed nucleus hidden by large dark blue granules Function histamine release, like mast cell

Monocyte Origin / resident or wandering blood; wandering How it looks large, kidney-shaped nucleus, grey-blue cytoplasm Function becomes macrophage in tissue

CELLS Fibres of the extracellular matrix

Collagen Made of type I collagen (tropocollagen triple helix) Light / EM look thick, unbranched, wavy bundles; EM: fibrils with 64-67 nm banding Stain eosin pink; blue with Mallory/trichrome Property / where great tensile strength, little stretch; tendon, dermis, bone

Reticular Made of type III collagen, heavily glycosylated Light / EM look thin, branched delicate network Stain silver (black, argyrophilic), PAS+ Property / where supporting stroma of lymph nodes, spleen, marrow, liver, basement membranes

CELLS Fibres of the extracellular matrix (cont.)

Elastic — Made of: elastin core + fibrillin microfibrils — Light / EM look: thin, straight, branching, form networks or fenestrated sheets — Stain: orcein, resorcin-fuchsin, Verhoeff — Property / where: stretch and recoil; arteries, lung, elastic ligaments

MATRIX Collagen types (main ones)

I — Form: thick fibrils, fibres — Where: skin (dermis), bone, tendon, ligaments, dentin, fibrocartilage

II — Form: thin fibrils — Where: hyaline and elastic cartilage, vitreous body

III — Form: reticular fibres — Where: skin, lung, blood vessels, lymphoid organs, smooth muscle

IV — Form: sheet-like network — Where: basal lamina

V — Form: fibril-forming, with type I — Where: with type I; placenta

VII — Form: anchoring fibrils — Where: dermal-epidermal junction; binds basal lamina to dermal collagen

MATRIX Components of ground substance

Glycosaminoglycans (GAGs) — Nature: long unbranched polysaccharides of repeating disaccharides, strongly negative, bind water — Examples / role: hyaluronan (not sulfated, no core protein), chondroitin, dermatan, heparan, keratan sulfate

Proteoglycans — Nature: core protein + sulfated GAGs — Examples / role: aggrecan (cartilage), decorin, syndecan, perlecan; give gel and turgor

Multiadhesive glycoproteins — Nature: proteins with binding sites for cells (integrins) and matrix — Examples / role: fibronectin, laminin; anchor cells to matrix

Tissue fluid — Nature: plasma-like filtrate with fewer proteins — Examples / role: carries nutrients and wastes; excess = oedema

TYPES Classification of connective tissues

Embryonic CT — Ground substance: viscous gel, few fibres — Members: mesenchyme; mucous (gelatinous) tissue

Connective tissue proper — Ground substance: semi-liquid gel — Members: loose (areolar); dense regular; dense irregular

Specialised: adipose, reticular, elastic — Ground substance: gel — Members: white (unilocular) and brown (multilocular) fat; reticular tissue; elastic tissue

Cartilage — Ground substance: firm, rubbery — Members: hyaline, elastic, fibrocartilage

Bone — Ground substance: calcified, very hard — Members: woven (immature), lamellar (mature): compact and spongy

Blood and lymph — Ground substance: liquid (plasma) — Members: blood cells and platelets in plasma

TYPES Types of connective tissue proper and related tissues

Mesenchyme — Structure: stellate cells joined by processes, abundant ground substance, almost no fibres — Where: embryo — Function: gives rise to all CT

Mucous (gelatinous) tissue — Structure: fibroblasts in hyaluronan-rich jelly, sparse type I and III fibres — Where: umbilical cord (Wharton's jelly), pulp of young teeth — Function: cushions cord vessels

Loose (areolar) — Structure: many cells of all types, much ground substance, thin collagen + elastic fibres in all directions — Where: lamina propria, papillary dermis, hypodermis, around vessels, mesentery — Function: binding, diffusion, defence, oedema site

Dense irregular — Structure: thick collagen bundles interwoven in many planes, few fibroblasts — Where: reticular dermis, organ capsules, submucosa of gut — Function: resists stretch from many directions

Dense regular — Structure: parallel collagen bundles with rows of flattened fibroblast (tenocyte) nuclei — Where: tendons, ligaments, aponeuroses, cornea stroma — Function: resists pull in one direction

Elastic tissue — Structure: thick parallel elastic fibres with some collagen — Where: ligamenta flava, suspensory ligament of penis, wall of large arteries — Function: stretch and recoil

Reticular tissue — Structure: reticular cells and type III fibre meshwork; free cells in spaces — Where: lymph nodes, spleen, bone marrow, liver sinusoids — Function: supporting stroma for free cells

White adipose — Structure: unilocular cells in lobules separated by CT septa, rich capillaries — Where: subcutaneous, visceral (omentum, mesentery), buttocks, thighs, breast — Function: energy store, insulation, padding, endocrine

Brown adipose — Structure: multilocular cells, very vascular, sympathetic nerves — Where: newborn: interscapular, neck; adult: supraclavicular, paravertebral, mediastinal, para-aortic, suprarenal — Function: non-shivering heat

TYPES White vs brown adipose tissue

Lipid droplets — White (yellow, unilocular): one large droplet fills the cell — Brown (multilocular): many small droplets

Nucleus — White (yellow, unilocular): flattened, peripheral (signet ring) — Brown (multilocular): round, central

Cell size — White (yellow, unilocular): large, 50-150 µm — Brown (multilocular): smaller, polygonal

Mitochondria — White (yellow, unilocular): few — Brown (multilocular): very many, with UCP1 (thermogenin)

Colour cause — White (yellow, unilocular): yellow from dietary carotenoids — Brown (multilocular): brown from cytochromes of mitochondria and rich blood supply

Function — White (yellow, unilocular): energy store, insulation, padding, leptin secretion — Brown (multilocular): heat production (uncoupled oxidation)

Distribution — White (yellow, unilocular): most fat in adults: subcutaneous, visceral; in newborns too (e.g. buttocks) — Brown (multilocular): newborns (~2-5% of body weight); adults: supraclavicular, paravertebral, mediastinal

H&E look — White (yellow, unilocular): empty polygonal spaces with thin pink rims — Brown (multilocular): pale, foamy (vacuolated) cytoplasm, many capillaries

METHOD Stains and how CT elements look

Collagen fibres — Method: H&E — Result: pink (eosinophilic)

Reticular fibres — Method: silver impregnation; PAS — Result: black; magenta

Elastic fibres — Method: orcein, resorcin-fuchsin, Verhoeff — Result: brown-red; dark purple; black

Mast cell granules — Method: toluidine blue — Result: purple-red (metachromasia, from heparin)

Fat — Method: frozen section + Sudan or osmium tetroxide — Result: orange-red or black droplets

Mnemonics

Mnemonic — Type 1: bONE; Type 2: carTWOlage; Type 3: reTHREEcular (reticular fibres, vessels); Type 4: floor (basement membrane) = Main collagen types and where they are

Must-knows

Three cartilage types Hyaline, elastic and fibrocartilage; told apart by the kind and amount of fibres in the matrix. Hyaline is the commonest and forms most of the fetal skeleton.

Cartilage is avascular No vessels, nerves or lymphatics; chondrocytes are fed by diffusion from perichondrial capillaries (or synovial fluid). Low metabolic rate, so it heals poorly.

Perichondrium Dense connective tissue sheath: outer fibrous layer (collagen I, fibroblasts), inner chondrogenic layer (chondroblast precursors). Absent from articular cartilage and fibrocartilage.

Chondrocytes and isogenous groups Chondroblasts trapped in matrix become chondrocytes, one per lacuna; mitosis yields isogenous groups. Superficial cells flattened parallel to the surface, deep cells larger and rounder.

Hyaline matrix zones Capsular (pericellular) matrix richest in sulfated GAGs and most basophilic; territorial matrix around each group; interterritorial matrix between groups stains paler.

Cartilage growth Interstitial: mitosis of chondrocytes inside the matrix (young cartilage, epiphyseal plates). Appositional: chondroblasts from the inner perichondrium add matrix on the surface.

Bone = cells + mineralised matrix Cells: osteoprogenitors, osteoblasts, osteocytes, osteoclasts. Matrix: type I collagen (organic) with hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ laid along and between the fibrils.

Osteoblast lineage Osteoprogenitor -> osteoblast (secretes osteoid toward the bone surface) -> osteocyte in a lacuna, or flattened bone lining cell.

Osteocyte network One osteocyte per lacuna; processes in canaliculi joined by gap junctions pass nutrients and signals; fluid flow in the lacunocanalicular system lets them sense load.

Osteoclast Huge (up to ~100 um), up to ~50 nuclei, acidophilic; fused monocyte-macrophage cells. Resorbs bone in Howship lacunae via a ruffled border sealed off by a clear zone.

Woven vs lamellar bone Woven (primary): irregular collagen, many osteocytes, less mineral; first in development and repair, persists at sutures, tooth sockets, tendon insertions. Lamellar (secondary) forms adult compact and spongy bone.

Osteon (Haversian system) Structural unit of compact bone: 4-20 concentric lamellae around a central canal, bounded by a cement line; collagen changes direction in neighbouring lamellae. Volkmann canals link central canals crosswise.

Periosteum and endosteum Periosteum: outer fibrous + inner osteogenic layer, anchored by Sharpey fibres, absent at articular surfaces. Endosteum: thin single cell layer lining marrow cavity, trabeculae and canals.

Bone biomechanics Collagen gives tensile strength and flexibility, mineral gives hardness and stiffness. Shaft is stiffest along its long axis; trabeculae follow lines of stress.

TYPES Cartilage types

Hyaline Matrix fibres fine type II collagen fibrils, masked by ground substance Perichondrium yes (not on articular surfaces) Where articular surfaces, costal, nasal, larynx (thyroid, cricoid, most of arytenoid), trachea, bronchi, epiphyseal plates, fetal skeleton Key features glassy, homogeneous, basophilic; isogenous groups; calcifies with age

Elastic Matrix fibres dense elastic fibre network + type II collagen Perichondrium yes Where auricle, wall of external acoustic meatus, auditory tube, epiglottis, corniculate and cuneiform cartilages, vocal process of arytenoid Key features yellowish fresh; flexible support; elastic fibres shown by orcein (brown) or resorcin-fuchsin; does not calcify

Fibrocartilage Matrix fibres thick type I collagen bundles + some type II Perichondrium no Where intervertebral disc (anulus fibrosus), pubic symphysis, menisci, articular discs (temporomandibular, sternoclavicular), some tendon and ligament insertions Key features small chondrocytes single or in rows between bundles; mostly acidophilic; withstands compression and tension

CELLS Cartilage cells and matrix regions

Perichondrium, fibrous layer Description outer dense connective tissue, type I collagen, fibroblasts Note carries the capillaries that feed the cartilage

Perichondrium, chondrogenic layer Description inner cellular layer of chondroprogenitor cells Note source of appositional growth

Chondroblast Description matrix-secreting cell at the surface, not yet in a lacuna Note becomes a chondrocyte once enclosed

Chondrocyte Description cell in a lacuna; flattened superficially, large and round deep Note shrinks from the lacunar wall in paraffin sections

Isogenous group Description cluster of chondrocytes from one parent cell Note sign of interstitial growth

Chondron Description chondrocyte or isogenous group + its pericellular matrix Note functional unit

Capsular (pericellular) matrix Description thin rim lining each lacuna; richest in sulfated GAGs; type VI collagen Note stains most intensely

Territorial matrix Description basophilic matrix around each isogenous group Note high sulfated GAG content

Interterritorial matrix Description matrix between groups; fewer proteoglycans, type II collagen Note stains paler

MATRIX Hyaline matrix molecules

Type II collagen Role fine fibrils, ~40% of dry weight; resist tension

MATRIX Hyaline matrix molecules (cont.)

Aggrecan — Role: proteoglycan with chondroitin and keratan sulfate; binds water, resists compression; makes matrix basophilic

CELLS Bone cells

Osteoprogenitor cell — Origin: mesenchymal stem cells; Look: flattened, spindle-shaped, pale oval nucleus, scant cytoplasm; Where: inner periosteum, endosteum; Function: divides; differentiates into osteoblasts

Osteoblast — Origin: osteoprogenitor cell; Look: cuboidal to columnar, basophilic (much RER), in a single layer; Where: bone surfaces where bone forms; Function: secretes osteoid (type I collagen, proteoglycans, osteocalcin) toward the bone; starts mineralisation (alkaline phosphatase, matrix vesicles)

Osteocyte — Origin: osteoblast enclosed in matrix; Look: less RER, smaller Golgi, condensed chromatin; many processes; Where: one per lacuna; processes in canaliculi; Function: maintains matrix; senses load; gap junctions with neighbours

Bone lining cell — Origin: inactive osteoblast; Look: flat, thin; Where: quiescent bone surfaces (part of endosteum); Function: maintains surface; can reactivate

Osteoclast — Origin: fusion of monocyte-macrophage precursors (haematopoietic); Look: giant (up to ~100 um), up to ~50 nuclei, acidophilic; Where: Howship lacunae on bone surfaces; Function: resorbs bone: H+ pumps dissolve mineral, cathepsin K and MMPs digest collagen

MATRIX Bone matrix composition

Organic (osteoid) — Components: ~90% type I collagen; proteoglycans; glycoproteins osteocalcin, osteonectin, osteopontin, bone sialoprotein; Gives bone: tensile strength, flexibility

Inorganic — Components: hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ along and between collagen fibrils; also carbonate, citrate, Mg, Na, K; Gives bone: hardness, stiffness

TYPES Bone tissue types

Woven (primary) — Structure: irregular collagen, many osteocytes, lower mineral (less dense on X-ray); Where: embryo, fracture repair; adult sutures, tooth sockets, tendon insertions

Lamellar (secondary) — Structure: collagen in parallel sheets (lamellae, 3-7 um); Where: all adult compact and spongy bone

Compact (cortical) — Structure: dense, osteons + circumferential and interstitial lamellae; Where: shafts of long bones, outer and inner tables of flat bones

Spongy (cancellous) — Structure: network of trabeculae with marrow spaces; usually no osteons; fed from marrow; Where: epiphyses, metaphyses, diploe, vertebral bodies

MORE Structures of compact bone

Osteon (Haversian system) — Description: 4-20 concentric lamellae around a central canal, parallel to the long axis

Concentric lamellae — Description: collagen parallel within a lamella, at a different angle in the next

Central (Haversian) canal — Description: lengthwise canal with vessels, nerves, loose connective tissue; lined by endosteum

Perforating (Volkmann) canal — Description: crosswise canal joining central canals and the surfaces; not ringed by lamellae

Lacunae — Description: spaces holding osteocytes; in compact and spongy bone

Canaliculi — Description: fine channels running radially between lacunae and to the central canal

Cement line — Description: outer boundary of each osteon; mineralised matrix with few collagen fibres

Interstitial lamellae — Description: angular remnants of older osteons partly resorbed in remodelling

Outer circumferential lamellae — Description: around the whole shaft, deep to the periosteum

Inner circumferential lamellae — Description: around the shaft at the endosteal surface, facing the medullary cavity

MORE Coverings and marrow

Periosteum, fibrous layer — Make-up: outer dense connective tissue, type I collagen, fibroblasts; Note: covers bone except articular surfaces

Periosteum, osteogenic layer — Make-up: inner cellular layer, osteoprogenitor cells; Note: bone growth in width and fracture repair

Sharpey (perforating) fibres — Make-up: type I collagen bundles from periosteum into outer circumferential lamellae; Note: anchor periosteum, tendons, ligaments

Endosteum — Make-up: thin, mostly one layer of osteoprogenitor and lining cells; Note: lines marrow cavity, trabeculae, central and perforating canals

Red marrow — Make-up: haematopoietic cells on a stroma of reticular cells and fibres; Note: high activity; spongy bone of flat bones, vertebrae, epiphyses

Yellow marrow — Make-up: mostly adipocytes; Note: low activity; adult diaphyses; can revert to red marrow

MORE Bones by shape and parts of a long bone

Long bone — Meaning: longer in one dimension; shaft and two ends; Example or note: tibia, femur, metacarpals

Short bone — Meaning: length about equal to width; Example or note: carpals, tarsals

Flat bone — Meaning: thin plate: two compact tables + diploe; Example or note: skull vault, sternum, scapula

Irregular bone — Meaning: complex shape; Example or note: vertebrae

Epiphysis — Meaning: end of a long bone; spongy bone with red marrow, thin compact shell; Example or note: covered by articular hyaline cartilage

Metaphysis — Meaning: flared zone between diaphysis and epiphysis, next to the growth plate; Example or note: mostly spongy bone

Diaphysis — Meaning: shaft: thick compact bone around the medullary cavity; Example or note: nutrient artery enters via nutrient foramen

Epiphyseal plate / line — Meaning: hyaline growth cartilage; replaced by a line of bone after closure; Example or note: site of lengthwise growth

Mnemonics

Mnemonic — Osteoblasts Build, osteoClasts Chew (Carve) = blasts form bone, clasts resorb it

Must-knows

Blood is a connective tissue — Cells (formed elements) suspended in a fluid matrix, plasma. Its fibres, fibrin threads, appear only when blood clots. pH 7.35–7.45; about 38 °C.

Volume — About 5 L in an adult (men 5–6 L, women 4–5 L), roughly 7–8% of body weight.

Centrifuged blood — Plasma ~55%, packed erythrocytes ~45% (haematocrit), buffy coat <1% (leukocytes + platelets) between them.

Erythrocyte — Biconcave disc ~7.5 µm, no nucleus or organelles; ~97% of dry content haemoglobin, plus carbonic anhydrase. ATP from anaerobic glycolysis, so it uses none of the O2 it carries. Lives ~120 days.

Haemoglobin — HbA = 2 α + 2 β globin chains, each with a haem whose Fe2+ binds one O2: up to 4 O2 per molecule, reversibly.

Reticulocyte — Newest red cell, ~1% of circulating RBCs, slightly larger, keeps residual ribosomes (shown by brilliant cresyl blue). A raised count means faster marrow output.

Neutrophil — Commonest leukocyte (50–70%), 12–15 µm, 3–5 nuclear lobes; pale specific granules + azurophilic granules. Phagocytoses bacteria; blood half-life ~6–7 h, 1–4 days in tissue, then apoptosis.

Eosinophil and basophil — Eosinophil: bilobed nucleus, orange-red granules; fights helminths, phagocytoses antigen-antibody complexes, falls with corticosteroids. Basophil: rarest (<1%), dark granules with heparin + histamine, mast-cell-like.

Monocyte — Largest leukocyte (12–20 µm), indented/kidney-shaped nucleus, grey-blue cytoplasm. Leaves blood after 1–3 days and becomes a macrophage (mononuclear phagocyte system, incl. osteoclasts); does not return.

Lymphocytes — Small 6–8 µm, round dark nucleus, thin blue rim. B cells mature in marrow → plasma cells (humoral immunity); T cells mature in thymus (cellular immunity, most blood lymphocytes); NK cells kill infected/tumour cells without prior sensitisation.

Platelets — Anucleate fragments of megakaryocyte cytoplasm, 2–4 µm, 150 000–400 000/µL, ~10 days. Peripheral hyalomere (marginal microtubule bundle) + central granulomere (granules, mitochondria, glycogen).

Diapedesis — Leukocytes leave postcapillary venules by squeezing between endothelial cells (selectin rolling → integrin adhesion → transmigration); they use blood only for transport.

Haemopoietic hierarchy — Pluripotent stem cell → common myeloid progenitor (erythrocytes, platelets, granulocytes, monocytes, mast cells) or common lymphoid progenitor (B, T, NK). Red marrow is the adult site.

MORE Composition of blood

Plasma — Amount ~55% of blood volume — Note water ~92%, proteins ~7%, other solutes ~1% (by weight)

Erythrocytes (haematocrit) — Amount ~45% of volume; ~4–6 million/µL — Note women ~3.9–5.5, men ~4.1–6.0 million/µL

Buffy coat — Amount <1% of volume — Note leukocytes + platelets

Leukocytes — Amount 4 500–11 000/µL — Note granulocytes (neutrophil, eosinophil, basophil) and agranulocytes (lymphocyte, monocyte)

Platelets — Amount 150 000–400 000/µL — Note fragments, not cells

MORE Plasma proteins

Albumin (most abundant) — Made by liver — Function main source of colloid osmotic (oncotic) pressure; carrier of hormones, drugs, fatty acids

α and β globulins — Made by liver — Function carriers of iron (transferrin), haemoglobin (haptoglobin), lipids; add to oncotic pressure

γ globulins (immunoglobulins) — Made by plasma cells — Function antibodies; the one major plasma protein not made by liver

Fibrinogen (smallest share of the main groups, ~4–7%) — Made by liver — Function converted to fibrin threads of the clot

Prothrombin and other clotting factors — Made by liver — Function coagulation cascade (thrombin forms fibrin)

Lipoproteins — Made by liver — Function transport of triglycerides and cholesterol

CELLS Leukocytes

Neutrophil (granulocyte) — Diameter 12–15 µm — % of leukocytes 50–70% — Nucleus 3–5 lobes; drumstick (Barr body) in women; band = unsegmented young form — Cytoplasm / granules pale lilac-pink specific granules (lysozyme, lactoferrin, collagenase, alkaline phosphatase) + azurophilic lysosomes — Life blood ~6–7 h, tissue 1–4 days — Main role phagocytosis of bacteria; H2O2 and other oxidants

Eosinophil (granulocyte) — Diameter 12–15 µm — % of leukocytes 1–4% — Nucleus bilobed — Cytoplasm / granules large orange-red specific granules with crystalloid core (major basic protein, eosinophil peroxidase, histaminase) — Life 1–2 weeks — Main role kills helminths; phagocytoses antigen–antibody complexes; rises in allergy, falls with corticosteroids

Basophil (granulocyte) — Diameter 12–15 µm — % of leukocytes 0.5–1% (rarest) — Nucleus bi- or irregularly lobed, hidden by granules — Cytoplasm / granules coarse dark blue-purple granules: heparin, histamine, leukotrienes (SRS-A) — Life several months — Main role vasodilation and allergy (IgE receptors); supplements mast cells

Lymphocyte (agranulocyte) — Diameter small 6–8 µm, large up to 18 µm — % of leukocytes 20–30% — Nucleus round, dense, heterochromatic, nearly fills cell — Cytoplasm / granules thin rim of pale blue cytoplasm (free ribosomes), few azurophilic granules — Life days to years — Main role specific immunity

Monocyte (agranulocyte) — Diameter 12–20 µm (largest) — % of leukocytes 3–8% — Nucleus oval, indented or kidney-shaped, eccentric, finer paler chromatin — Cytoplasm / granules grey-blue, fine azurophilic granules (lysosomes), often vacuolated — Life blood 1–3 days, then months as macrophage — Main role becomes macrophage; removes debris and microbes with lysosomal enzymes; antigen presentation

— B lymphocyte — Diameter small — % of leukocytes ~15–20% of lymphocytes — Nucleus as lymphocyte — Cytoplasm / granules surface immunoglobulin — Life memory cells years — Main role matures in marrow; becomes antibody-secreting plasma cell (humoral immunity)

— T lymphocyte — Diameter small — % of leukocytes ~75–80% of lymphocytes (most) — Nucleus as lymphocyte — Cytoplasm / granules T-cell receptor — Life memory cells years — Main role matures in thymus; helper (CD4), cytotoxic (CD8), regulatory (slide 41: 'suppressor') and memory cells; cell-mediated immunity

— NK cell — Diameter larger — % of leukocytes ~5–10% of lymphocytes — Nucleus as lymphocyte — Cytoplasm / granules large granular lymphocyte (perforin, granzymes) — Life — — Main role kills virus-infected and tumour cells without prior sensitisation

CELLS Erythrocyte membrane skeleton

Spectrin Position / link network on cytoplasmic (inner) face of membrane Role flexibility and biconcave shape; lets cell deform through capillaries

Actin (short filaments) + tropomyosin Position / link junctional complexes joining spectrin tetramers Role nodes of the network

Ankyrin Position / link binds spectrin to band 3 Role anchors network to membrane

Band 3 Position / link transmembrane protein Role Cl−/HCO3− exchanger; ankyrin anchor

Protein 4.1 Position / link binds spectrin–actin junctions to glycophorin C Role second anchor

CELLS Platelet zones and granules

Hyalomere (peripheral, pale blue) Contents marginal bundle of microtubules, actin, myosin Role keeps disc shape; contraction for clot retraction

Granulomere (central, purple) Contents α, δ, λ granules, mitochondria, glycogen Role secretion and energy

α granules (largest, most numerous) Contents fibrinogen, PDGF, platelet factor 4, von Willebrand factor, other clotting factors Role clotting, vessel repair

δ (dense) granules Contents serotonin, ADP, ATP, Ca2+ Role vasoconstriction, platelet recruitment

λ granules Contents lysosomal enzymes Role clot removal

Open canalicular system Contents invaginations of plasma membrane Role rapid release of granule contents

CELLS Haemostasis by platelets (in order)

1 Primary aggregation What happens platelets adhere to exposed collagen (via vWF) and form a plug

2 Secondary aggregation What happens released adhesive glycoproteins and ADP recruit more platelets; plug enlarges

3 Coagulation What happens clotting cascade: prothrombin → thrombin; fibrinogen → fibrin mesh trapping cells

4 Clot retraction What happens platelet actin and myosin contract; clot shrinks

5 Clot removal What happens plasminogen → plasmin (activators from endothelium) digests fibrin

MORE Bone marrow

Red marrow Feature haemopoietic; all marrow at birth; in adults in flat bones (skull, ribs, sternum, pelvis), vertebrae and proximal epiphyses of femur and humerus

Yellow marrow Feature mostly adipocytes; replaces red marrow with age; reverts to red after severe bleeding or hypoxia

Stroma Feature reticular cells + reticular fibres (type III collagen), macrophages

Parenchyma Feature haemopoietic cords between sinusoids

Sinusoids Feature discontinuous endothelium; new cells cross the wall to enter blood

Matrix Feature collagen I and III, fibronectin, laminin, proteoglycans; hemonectin binds blood cells to matrix

Functions Feature blood cell production; destruction of old RBCs; iron storage in macrophages (haemoglobin breakdown)

Megakaryocyte position Feature lies on sinusoid wall, pushes proplatelets into lumen

Section look Feature empty round spaces = adipocytes (fat dissolved); islands of haemopoietic cells; giant megakaryocytes

MORE Haemopoietic lineages

Erythropoiesis Progenitor common myeloid → MEP (CFU-E) Stages proerythroblast → basophilic → polychromatophilic → orthochromatophilic erythroblast → reticulocyte Product erythrocyte

Granulopoiesis Progenitor common myeloid → GMP (CFU-G) Stages myeloblast → promyelocyte → myelocyte → metamyelocyte → band → segmented Product neutrophil, eosinophil, basophil

Monocytopoiesis Progenitor common myeloid → GMP (CFU-M) Stages monoblast → promonocyte → monocyte Product monocyte → macrophage, osteoclast, myeloid dendritic cell

Thrombopoiesis Progenitor common myeloid → MEP (CFU-Meg) Stages megakaryoblast → promegakaryocyte → megakaryocyte (endomitosis, polyploid) Product platelets (cytoplasm fragments)

Mast cell line Progenitor common myeloid Stages precursors leave marrow, mature in connective tissue Product mast cell

Lymphopoiesis Progenitor common lymphoid Stages lymphoblast → prolymphocyte → lymphocyte; T precursors mature in thymus Product B, T, NK cells; B → plasma cell

CELLS Maturation stages: red and granulocyte series

Proerythroblast Nucleus large, nucleoli, fine chromatin Cytoplasm basophilic (ribosomes)

Basophilic erythroblast Nucleus smaller, condensing Cytoplasm strongly basophilic

Polychromatophilic erythroblast Nucleus condensed, checkerboard Cytoplasm grey-lilac (Hb + ribosomes)

Orthochromatophilic erythroblast (normoblast) Nucleus small, pyknotic; extruded at this stage Cytoplasm pink (Hb)

Reticulocyte Nucleus none Cytoplasm pink, residual ribosomes; ~1 day more in marrow/blood

Myeloblast Nucleus large, nucleoli Cytoplasm basophilic, no granules

Promyelocyte Nucleus round, nucleoli Cytoplasm azurophilic (primary) granules appear

Myelocyte Nucleus round to slightly flattened Cytoplasm specific granules appear; lineage (N, E, B) now recognisable; last stage that divides

Metamyelocyte Nucleus kidney-shaped indentation Cytoplasm specific granules

Band (stab) cell Nucleus curved, unsegmented band Cytoplasm specific granules; up to a few % in blood

Segmented granulocyte Nucleus lobed Cytoplasm mature

Mnemonics

Mnemonic Never Let Monkeys Eat Bananas = Leukocytes from commonest to rarest: neutrophil, lymphocyte, monocyte, eosinophil, basophil

Mnemonic 60, 30, 6, 3, 1 = Approximate differential count (%) in the same order

Must-knows

Three muscle types Skeletal (striated, voluntary), cardiac (striated, involuntary), smooth (no striations, involuntary). Striation = sarcomeres of neighbouring myofibrils lying in register.

Muscle vocabulary Sarcolemma = cell membrane; sarcoplasm = cytoplasm; sarcoplasmic reticulum (SR) = smooth ER storing Ca2+; a skeletal 'fibre' = one whole multinucleated cell.

Origin and development Mostly mesoderm. Skeletal fibre = syncytium from fusion of many myoblasts into a myotube (central nuclear chain), whose nuclei then move to the periphery.

Satellite cells Quiescent mononuclear myogenic stem cells between sarcolemma and external lamina; on injury they proliferate and fuse to repair or form fibres.

Sarcomere Z disc to Z disc, ~2.5 um at rest = one A band + half of each flanking I band. The contractile unit of striated muscle.

Sliding filament model Filaments keep their length; thin filaments slide toward the M line. Sarcomere, I band and H zone shorten; A band constant.

Triad vs diad Skeletal: triad = T tubule + 2 terminal cisternae, at each A-I junction (2 per sarcomere). Cardiac: diad = T tubule + 1 cistern, at the Z line.

Ca2+ controls contraction ACh at the motor end plate -> action potential along sarcolemma and T tubules -> SR releases Ca2+ -> Ca2+ binds troponin C -> tropomyosin shifts, uncovering the myosin-binding sites on actin -> cross-bridge cycling.

Intercalated discs Step-like dark transverse lines at cardiac cell ends, at the level of a Z line. Transverse part: fasciae adherentes + desmosomes; lateral part: gap junctions (functional syncytium).

Purkinje fibres Modified cardiomyocytes in the subendocardial layer: larger, pale, glycogen-rich, sparse peripheral myofibrils; conduct faster than ordinary myocardium.

Smooth muscle contractile apparatus No myofibrils or sarcomeres; actin and myosin form an oblique lattice anchored to dense bodies (cytoplasmic) and dense plaques (sarcolemmal), linked by intermediate filaments.

TYPES Skeletal vs cardiac vs smooth muscle

Cell shape Skeletal long unbranched cylinder Cardiac short, branched, joined end to end Smooth spindle (fusiform), tapering ends

Size Skeletal 10-100 um wide, up to many cm long Cardiac ~15 um wide, 85-100 um long Smooth 5-10 um wide, 20 um (small vessels) to 500 um (pregnant uterus)

Nuclei Skeletal many, flattened, peripheral, deep to sarcolemma Cardiac 1 (or 2), central, oval Smooth 1, central, elongated (cigar); corkscrew when contracted

Striations Skeletal yes Cardiac yes Smooth no

Myofibrils / sarcomeres Skeletal yes Cardiac yes Smooth no; oblique lattice on dense bodies

Cell junctions Skeletal none between fibres Cardiac intercalated discs (fascia adherens, desmosome, gap junction) Smooth gap junctions

T tubules / SR Skeletal triads at A-I junction; extensive SR Cardiac diads at Z line; wider T tubules, less SR Smooth no T tubules; caveolae; sparse SR

Control Skeletal voluntary (somatic motor nerve) Cardiac involuntary, intrinsic pacemaker + autonomic Smooth involuntary: autonomic, hormones, stretch

Contraction Skeletal strong, quick, discontinuous Cardiac strong, quick, rhythmic, continuous Smooth weak, slow, sustained; fatigue-resistant

Connective tissue Skeletal epi-, peri-, endomysium Cardiac endomysium rich in capillaries Smooth external lamina + reticular fibres

MORE Organisation of skeletal muscle

Whole muscle What it is many fascicles Wrapping / contents epimysium (dense CT) Note continues into tendon; deep fascia superficial to it

Fascicle What it is bundle of fibres Wrapping / contents perimysium Note larger arteries, veins, nerves run here

Muscle fibre What it is one multinucleated cell Wrapping / contents endomysium (reticular fibres, capillaries, nerve twigs) + external lamina Note nuclei between fibres = fibroblasts, endothelium

Myofibril What it is chain of sarcomeres, ~1-2 um wide Wrapping / contents wrapped by SR network Note dots in TS; mitochondria in rows between

Myofilament What it is thick (myosin) and thin (actin) Wrapping / contents - Note thick ~15 nm x 1.6 um; thin ~8 nm x 1.0 um

SURFACE Bands and lines of the sarcomere

Z disc (line) Contains alpha-actinin; anchors thin filaments, titin Appearance thin dark line bisecting I band In contraction Z discs move closer

I band Contains thin filaments only Appearance light; isotropic in polarised light In contraction narrows; almost gone at full contraction

A band Contains thick filaments (+ overlapping thin) Appearance dark; anisotropic (birefringent) In contraction constant length (= thick filament)

H zone Contains thick filaments only (no thin-filament overlap); its centre is the bare zone, without myosin heads Appearance paler zone in middle of A band In contraction narrows; disappears at full contraction

M line Contains myomesin, creatine kinase cross-linking thick filaments Appearance thin dark line bisecting H zone In contraction stays central

Overlap zone (TS) Contains each thick among 6 thin (hexagon); each thin among 3 thick Appearance rosettes in EM In contraction widens

Sarcomere Contains Z to Z: A band + 2 half I bands Appearance ~2.5 um resting In contraction shortens

MORE Proteins of the striated contractile apparatus

Actin (G -> F) Location thin filament: 2 F-actin strands in a helix Role has myosin-binding sites

Tropomyosin Location in grooves of F-actin helix Role covers myosin-binding sites at rest

MORE Proteins of the striated contractile apparatus (cont.)

Troponin (TnT, TnC, TnI) Location on tropomyosin, thin filament Role TnT binds tropomyosin, TnC binds Ca2+, TnI inhibits actin-myosin binding

Myosin II Location thick filament (~200-500 molecules, bipolar) Role 2 heavy + 4 light chains; head = actin-binding site + ATPase; hinge lets head pivot

Light / heavy meromyosin Location trypsin fragments of myosin Role LMM = rod (tail); HMM = heads + short rod

Titin Location Z disc to M line, along thick filament Role elastic spring; centres thick filament; passive elasticity

Nebulin Location along thin filament from Z disc Role sets thin filament length

Alpha-actinin Location Z disc (and smooth muscle dense bodies) Role anchors thin filaments

PROCESS Excitation-contraction coupling in skeletal muscle

1 Event ACh released from axon terminal (blocked by botulinum toxin) Structure neuromuscular junction (motor end plate)

2 Event ACh binds receptors -> sarcolemma depolarises; acetylcholinesterase ends signal Structure junctional folds of sarcolemma

3 Event action potential spreads into the fibre Structure sarcolemma -> T tubules

5 Event Ca2+ binds troponin C; tropomyosin shifts, exposing the myosin-binding sites on actin Structure thin filament

6 Event myosin heads bind actin, power stroke pulls thin filament toward M line; ATP detaches head Structure cross-bridges

7 Event relaxation: ATP-dependent Ca2+ pump returns Ca2+ to SR Structure SR membrane

MATRIX Skeletal muscle fibre types

Type I (slow oxidative, red) Look small, dark red: much myoglobin, many mitochondria, rich capillaries Metabolism / speed aerobic, slow twitch Use fatigue-resistant, posture, endurance

Type IIb/IIx (fast glycolytic, white) Look large, pale: little myoglobin, few mitochondria, much glycogen Metabolism / speed anaerobic glycolysis, fast twitch Use short bursts of force, fatigue quickly

Mixed muscles Look most human muscles contain all types Metabolism / speed proportions differ by muscle Use fibre type set by its motor nerve

MORE Cardiac muscle specialisations

Fascia adherens Where transverse part of intercalated disc Feature anchors actin of terminal sarcomere Function transmits pull cell to cell

Desmosome (macula adherens) Where transverse part of intercalated disc Feature anchors intermediate filaments Function keeps cells from pulling apart

Gap junction Where lateral part of intercalated disc Feature ionic channels Function spreads excitation: functional syncytium

Diad Where at Z line Feature T tubule + one SR cistern Function Ca2+ release

Mitochondria Where between myofibrils Feature ~40% of cytoplasm Function continuous aerobic ATP supply

SA node Where wall of right atrium Feature small modified cardiomyocytes Function pacemaker

AV node -> AV bundle (His) -> bundle branches Where atrial septum / interventricular septum Feature modified cardiomyocytes Function conduct impulse to ventricles

Purkinje fibres Where subendocardial layer of ventricles Feature large, pale, glycogen-rich, peripheral myofibrils, sometimes 2 nuclei Function fast conduction to ventricular myocardium

Heart wall layers Where endocardium, myocardium, epicardium Feature myocardium thickest Function cardiac muscle in myocardium

CELLS Smooth muscle cell structure

Dense bodies Feature cytoplasmic; contain alpha-actinin (like Z discs) Function anchor thin filaments

Dense plaques Feature dense bodies attached to inner face of sarcolemma Function transmit force to cell membrane

Thin and thick filaments Feature oblique crisscross lattice; thick filaments side-polar Function contraction; cell shortens and bulges, nucleus corkscrews

Intermediate filaments Feature desmin (vimentin in vascular SM) linking dense bodies Function spread pull across the whole cell

Caveolae Feature flask-shaped invaginations of sarcolemma Function Ca2+ entry (T tubule equivalent)

Gap junctions Feature between neighbouring cells Function coordinated contraction (visceral / single-unit)

External (basal) lamina Feature around each cell + reticular fibres Function binds cells into a working unit

Organelles Feature mitochondria, Golgi, RER at the two poles of the nucleus Function energy; secretion of collagen, elastin, proteoglycans

Arrangement Feature middle of one cell next to tapering ends of neighbours; in vessels circular or low helix Function narrows lumen / propels contents

METHOD Identifying muscle in sections

Skeletal Longitudinal section long parallel fibres, cross-striations, many flattened peripheral nuclei Transverse section large polygonal profiles of even size, peripheral nuclei, none central Clue myofibrils as fine dots in TS

Cardiac Longitudinal section branching fibres, central nuclei, step-like intercalated discs Transverse section irregular profiles of varied size, single central nucleus in pale area; many capillaries Clue pale perinuclear zone

Smooth Longitudinal section overlapping spindles, elongated central nuclei, wavy when contracted Transverse section small round profiles, only some with a nucleus; little CT Clue no striations

Purkinje fibres Longitudinal section broad pale fibres deep to endocardium Transverse section large pale cells, central nucleus, myofibrils at periphery Clue glycogen-rich

Mnemonics

Mnemonic H zone, I band shrink; A stays = In contraction H and I narrow, A band constant ("HI shrink, A is Always the same")

Mnemonic dArk = Anisotropic; lIght = Isotropic = A band dark and anisotropic, I band light and isotropic

Mnemonic Epi-Peri-Endo = Wrappings from surface to single fibre: epimysium, perimysium, endomysium