Connective tissues

Fibrous proteins – structure, metabolism
Bones – structure, formation
Connective tissue

**Cells**
- Fibroblasts (generalized connective tissue, skin, tendons...)
- Chondroblasts (cartilage)
- Osteoblasts (bones)

**Insoluble protein fibres**
- Collagen (~30% body proteins)
- Elastin

**Matrix**
- Proteoglycans
  - Glycoproteins
  - Phosphoproteins
  - Proteolipids

**Extracellular matrix**
- Adhesive proteins
  - Fibronectin
  - Elastonectin
  - Laminin.....
Composition of connective tissues

Contains also blood and lymph vessels and various transient cells – macrophages, mast cells, ... 

Adipose tissue – specialized form of connective tissue – adipocytes cluster between the protein fibres
Fibrous proteins

- **Collagen** — cartilage, bone, tendon, cornea
- **Elastin** — aorta, alveols, ligaments

- Typical primary structure — high content Gly, Pro
- Posttranslational hydroxylation — Hyp, (Hyl)
- Fiber formation
- Cross-links between chains and fibres
- High stability
**Primary structure**

**Collagen** - 1 chain (α) \((\text{Gly}-X-Y)_{333}\) – cca 1000 AA

mostly **Pro** (cca 100), **Hyp** (cca 100)

1-2 % **Hyl** (5-50 AA), **Ala** - relatively high quantity, no **Trp**

0,5 -10 % **carbohydrate** residues (attached to **Hyl**)  

\[\text{Gly-Pro-Met-Gly-Pro-Leu-Gly-Pro-Hyp-Gly-Ala-Hyl-Gly-Pro-Ala-Gly-Lys-Hyp} \]

\[\text{Gly-Pro-Lys-Gly-Pro-Ala-Gly-Glu-Hyp-Gly-Pro-Hyp-Gly-Pro-Hyp-Gly-Ala-Hyp} \]

**Elastin** – 1 chain (tropoelastin)- cca 850 AA, rich in nonpolar AA (95 %) – **Gly** (1/3), **Ala**, **Val**, **Pro**; few **Hyp**, no **Hyl**, no carbohydrate residue

\[\text{Gly-Gly-Val-Pro-Gly-Val-Gly-Val-Pro-Gly-Val-Gly-Ala-Pro-Gly-Val-Gly-Val-Ala} \]

\[\text{-Ser-Gly-Val-Hyp-Gly-Val-Gly-Val-Pro-Gly-Ala-Gly-Val-Lys-Ala-Ala-Ala-Lys-Pro-} \]

**Sequences**

- **Lys-Ala-Ala-Ala-Lys** - ,  - **Lys-Ala-Ala-Lys** - important for the formation of **elastin fiber network**
Secondary and tertiary structure of collagen

**triple-stranded superhelix**

3 α-chains (left-handed helix, 3 AA/turn)
- may differ in primary structure
- **rigid conformation** – Pro, Hyp hinder free rotation around -N-Cα-bond
- limited stabilization of helix by intra-molecular hydrogen-bonds

**tropocollagen** – cca 3000 AA
- right-handed **triple-stranded superhelix**
- nonhelical regions at C and N termini
- **hydrogen bonds between chains**
- stabilization of superhelix
  - Gly-NH – OC-Pro (X),
- **side chains** of AA at X,Y-positions
  - hydrophobic and ionic interactions between tropocollagen molecules – important for aggregation into microfibrils
Collagen microfibrils

4-8 tropocollagen molecules – aggregation in a highly ordered manner – interaction of AA residues at X- and Y-positions

Gaps/holes - sites for deposition of hydroxyapatite crystals in the formation of bones
## Collagen types

>16 different types in human body, encoded by 28 genes (12 chromosomes)

<table>
<thead>
<tr>
<th>TYPE</th>
<th>TISSUE DISTRIBUTION</th>
<th>COMPOSITION</th>
<th>PROPERTIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibril-forming</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>Skin, bone, tendon, blood vessels, cornea</td>
<td>((\alpha_1(I))_2\alpha_2(I))</td>
<td>no Cys, no –S-S-, most abundant</td>
</tr>
<tr>
<td>II</td>
<td>Cartilage, intervertebral disk, vitreous body</td>
<td>((\alpha_1(II))_3)</td>
<td>no Cys, no –S-S-, high extent of glycosylation</td>
</tr>
<tr>
<td>III</td>
<td>Blood vessels, fetal skin</td>
<td>((\alpha_1(III))_3)</td>
<td>stabilization by –S-S-</td>
</tr>
<tr>
<td>Network-forming</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>Basement membrane</td>
<td>((\alpha_1(IV))_2\alpha_2(IV))</td>
<td></td>
</tr>
<tr>
<td>VII</td>
<td>Beneath stratified squamous epithelia</td>
<td>((\alpha_1(VII))_3)</td>
<td></td>
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<tr>
<td>Fibril-associated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IX</td>
<td>Cartilage</td>
<td>(\alpha_1(IX)(\alpha_2(IX))_2)</td>
<td></td>
</tr>
<tr>
<td>XII</td>
<td>Tendon, ligaments, some other tissues</td>
<td>((\alpha_1(XVII))_3)</td>
<td></td>
</tr>
</tbody>
</table>
Formation of hydroxyproline, hydroxylysine
= posttranslational hydroxylation of selected Pro or Lys residues

Substrate: polypeptide chain (α-chain, tropoelastin)

Enzymes:
prolyl hydroxylase (Pro → 4-OH-Pro)
prolyl 3-hydroxylase (Pro → 3-OH-Hyp)
lysyl hydroxylase (Lys → 5-OH-Lys)

involved only in collagen synthesis

Cofactors:
Fe^{2+}, α-ketoglutarate, ascorbate (= vitamin C), O_2

Localization: ER

! Hydroxylation occurs before triple helix formation
Role of hydroxylation

**OH-Pro** - interchain bonding, stabilization of triple superhelix
**OH-Lys** - site for attachment of carbohydrate residue
- involved in intermolecular collagen crosslinks

**Impaired hydroxylation** – nonstable triple helix, reduced cross-linking – altered collagen – altered function of connective tissues

**Ascorbate deficiency** - low activity of prolyl and lysyl hydroxylase → scurvy

**Low lysyl hydroxylase activity** in skin
- decreased collagen fibril diameter
- changes in mechanical properties
  ↓
  **hyperextensible (stretchy) skin**
  (Ehlers-Danlos syndrom, type IV)
Glycosylation

= attachment of **galactose**
or disaccharide **glucosylgalactose** to specific **OH-Lys**

- occurs only in **collagen** (not in elastin)

**collagen = glycoprotein**

<table>
<thead>
<tr>
<th>Carbohydrate portion:</th>
<th>skin 0.5 %</th>
<th>cartilage 4%</th>
<th>base membrane &gt; 10 %</th>
</tr>
</thead>
</table>

Enzymes:

**galactosyltransferase:**

\[
\text{UDP-galactose} + \text{OH-Lys}^- \rightarrow \text{galactosyl-O-Lys}^- + \text{UDP}
\]

**glucosyltransferase:**

\[
\text{UDP-glucose} + \text{galactosyl-O-Lys}^- \rightarrow \text{glucosylgalactosyl-O-Lys}^- + \text{UDP}
\]

Function of carbohydrate portion:

? role in **organization of fibrils**, interaction with aqueous environment, resistance against proteolytic cleavage
Cross-linking

- formation of **covalent cross-links** between tropocollagen molecules, microfibrils, fibrils involving Lys and/or OH-Lys side chains

Cross-links within microfibrils

Cross-links between fibrils

1. step: oxidative deamination of Lys/OH-Lys \( \varepsilon \)-amino group
   \( \rightarrow \) allysine residue (aldehyde)

   Enzyme: **Lysyl oxidase** (cofactors- pyridoxal phosphate, \( \text{Cu}^{2+} \))

2. step: spontaneous condensation of allysine +lysine or allysine + allysine residues \( \rightarrow \) linear or cyclic cross-links
Cross-links in collagen

**Cyclic cross-links** – "mature cross-links" – between fibrils

- **Pyridinoline cross-links**
  - 2 OH-Allys + OH-Lys- or Lys-
  - more stable, responsible for insolubility and strengths of collagenous tissue
  - present mainly in bone – released during bone resorption – excreted in urine
  - specific markers of bone resorption (namely L-Pyr)

- **Pyrrole cross-link**
  - 2 -Allys + OH-Lys-

**Linear (ketoimine) cross-link**
- Lys – Allyls-
**Structure of elastin fiber**

Basis: single linear polypeptide chain – *tropoelastin*

**Mature elastin**: polypeptide chains connected by covalent cross-links into a fiber network

responsible for tissue elasticity, stretchability, capable of stretching in two dimensions

- **Elastin fiber**
  - amorphous and fibrilar components
  
- **elastin**
  - **fibrillin** (glycoprotein)

surrounds amorphous elastin
Elastin cross-links

- Formed between 2 polypeptide chains
- Provided by specific lysine rich regions:
  - Lys-Ala-Ala-Ala-Lys-, Lys-Ala-Ala-Lys-
- Derived from 4 Lys residues

\[
\text{Lysyl oxidase} \rightarrow \begin{align*}
\text{3 -Allys-} & \quad \text{1 - Lys-} \\
\text{condensation} & \\
\text{Desmosine Isodesmosine}\end{align*}
\]
Collagen biosynthesis

Unusual - many posttranslational modifications:

1. Hydroxylation -Pro-, -Lys-
2. Glycosylation OH-Lys-
3. Folding of procollagen peptide into triple helix
4. Conversion of procollagen to tropocollagen
5. Self-assembly into fibrils
6. Cross-links formation

Location:
ER, Golgi system (1-3), extracellular space (4-6)

Cells:
fibroblasts, chondroblasts, osteoblasts
Disorders in collagen metabolism

1. Enzyme defects in biosynthesis - procollagen proteinase, lysyl oxidase
   - Ehlers-Danlos syndrome - stretchy skin, loose joints, bruiability of tissues

2. Cofactor deficit –
   - Cu\(^{2+}\) - impaired cross-linking (lysyl oxidase = copper dependent enzyme)
     - Marfan’s syndrome – skeletal and cardiovascular abnormalities
     - Menkes’ (kinky-hair) syndrome – poor copper absorption in GIT-
       neurological, connective tissues, hair abnormalities

Ascorbate – impaired hydroxylation, accumulation of collagen polypeptides in ER, impaired assembly of collagen fibrils

Scurvy - defective blood vessels, fragility → frequent hemorrhages; defective formation of bones and teeth, poor wound healing
Disorders in collagen metabolism

3. Mutation – alteration of primary structure - Gly → X – abnormal structure of tropocollagen, abnormal fibrils

   Osteogenesis imperfecta – fragility and bending of bones, defects in dentin, tendon, ligaments, skin

4. Excessive biosynthesis – increased formation of fibres – excessive fibrosis - liver, lung – severe limitation of tissue function

   Diabetes mellitus – changes in collagen metabolism- poor wound healing, atrophy of the skin, thickening of the basement membranes
Collagen degradation

Collagenases — a specific proteinases (fibroblasts, macrophages, leucocytes...)

- extracellular cleavage
- intracellular cleavage — lysosomes (20-40% of newly synthesized polypeptide chains; leucocytes — fagocytosis)

Metalloenzymes — Zn in the active site, Ca$^{2+}$ required as activator

Matrix metalloproteinases MMPs — fibroblast collagenase = MMP1

Specificity: —Gly-Leu(Ile)— peptide bond in triple helix (three quarters of the distance from N-terminus) — 2 fragments — cleaved by other proteinases and MMPs

Human MMPs — a family of 23 proteinases — endopeptidases - cleavage of extracellular matrix proteins and proteoglycans

- contribute to: growth, development, wound healing pathologies - arthritis, cancer
Elastin metabolism

**Biosynthesis:** fibroblasts, chondrocytes, smooth muscle cells – lung, skin, cartilage, vessels walls

- proelastin
- tropoelastin → elastin → elastic fibres
- cross-linking by fibrillin

**Elastin adhesion to cell surface:** elastonectin

**Tumor cells:** ↑ elastonectin  → ↑ interaction with connective tissue → metastases

! Soluble elastin – trapping of tumor cells, inhibition of proliferation of highly metastatic cells via binding to membrane elastin receptor

? prevention of metastasis after tumor surgery

**Degradation:** turnover relatively low, ↑ emphysema, pancreatitis, advanced atherosclerosis

**Elastases** – pancreas, PMN, macrophages, platelets, smooth muscle cells

Some **MMPs**
Bone - composition

Bone = modified connective tissue

<table>
<thead>
<tr>
<th>Cells</th>
<th>Organic matrix</th>
<th>Bone mineral</th>
<th>H₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 %</td>
<td>Osteoid</td>
<td>55 %</td>
<td>15 %</td>
</tr>
</tbody>
</table>

Osteoblasts
- production of organic matrix

Osteocytes
- mineralization of osteoid

Osteoclasts
- bone resorption

Osteoprogenitor cells

Collagen I (95 %)
- Osteocalcin
- Osteonectin
- Proteoglycans (chondroitin sulfate)
- Phosphoproteins
- Sialoproteins
- Proteolipids

Noncollagenous proteins = oseomucoid

Hydroxyapatite crystals –
\[ \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 \] (95 %)

Fluorapatite - teeth
\[ \text{Ca}_{10}(\text{PO}_4)_6\text{F}_2 \]

Amorphous precursors
- \( \text{CaHPO}_4 \)
- \( \text{Ca}_3(\text{PO}_4)_2 \)
- \( \text{MgCO}_3, \text{Na}_2\text{CO}_3 \)
- \( \text{MgP}_2\text{O}_7, \text{Na}_4\text{P}_2\text{O}_7 \)
Bone formation - osteosynthesis

1. Formation of organic matrix – osteoblasts

   high proteosynthetic activity → secretion of tropocollagen + noncollagenous proteins → matrix transformation into insoluble fibrilar network

2. Mineralization – osteoblasts → osteocytes (nourish the bone, 90 % of bone cells)

   - Premineralization - intracellular process – protein bound Ca\(^{2+}\) + phosphate → Ca\(_3\)(PO\(_4\))\(_2\), CaHPO\(_4\).2H\(_2\)O → precipitation, transport into extracellular space

   - Nucleation – interaction of amorphous calcium phosphates at the specific sites of collagen helix (? holes) → formation of hydroxyapatite micro-crystals → oriented growth of macrocrystals length wise the collagen fiber (= epitaxes)

   nucleation centre
Bone resorption - osteolysis

= Degradation of bone components – osteoclasts

- release of lysosomal enzymes → cleavage of noncollagenous proteins, activation of collagenase

- synthesis of hyaluronic acid → Ca+ binding
- release of organic acids (citrate, lactate) and H+ from carbonic acid → dissolution of mineral (hydroxyapatite)

after mineral destruction

- collagen breakdown - collagenase, other MMPs, lysosomal proteinases

Bone turnover = remodeling

Dynamic, continuous process of bone resorption (osteoclasts) and bone formation (osteoblasts) – basic multicellular units

Adult skeleton – renewed ~ every 10 years
Bone cells differentiation

Osteoprogenitor cells – precursors of other bone cell transformation
stimulated/inhibited hormonally

osteoblasts

osteoclasts

Stimulation:
calcitonin
estrogens
growth hormone
inorganic phosphate
mechanical stress

Inhibition:
PTH (parathyroid hormone)
Vitamin D metabolites
calcitonin
estrogens
glucocorticoids

PTH (parathyroid hormone)
thyroxine
vitamin D metabolites
growth hormone
Calcium and phosphate homeostasis

- prerequisite of normal bone metabolism
- influencing factors: diet, vitamin D and its metabolites, PTH, calcitonin

**Diagram:**

- **Diet:** Cholecalciferol (D3) → **Skin:** Cholecalciferol (D3) → **Blood:** 25-(OH)D3 → **Kidney:** 1,25-(OH)2D3 → **Bone:** Induction of Ca2+ transport → **Intestine:** Inhibition of Ca2+ transport

- **Low serum Ca2+**
  - Parathyroid gland → PTH → Kidney → ↑ Ca2+ reabsorption and HPO42- excretion → ↑ serum Ca2+

- **High serum Ca2+**
  - Thyroid gland → Calcitonin → Kidney → Inhibition of Ca2+ resorption → ↓ serum Ca2+
Osteoporosis

- most common metabolic bone disease, common in postmenopausal women
- loss of bone mass – both mineral component and organic matrix

increased bone resorption, defects in bone formation

Biochemical markers

<table>
<thead>
<tr>
<th>Bone formation</th>
<th>Bone resorption</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Serum:</strong></td>
<td><strong>Serum:</strong></td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>Acid phosphatase</td>
</tr>
<tr>
<td>(bone specific, rich in osteoblasts)</td>
<td>(bone specific, lysosomal enzyme of osteoclasts)</td>
</tr>
<tr>
<td><strong>Osteocalcin</strong> – major noncollagen protein</td>
<td><strong>Urine</strong> - collagen breakdown products</td>
</tr>
<tr>
<td><strong>N- and C-terminal procollagen 1 extension peptides</strong></td>
<td><strong>N- and C- telopeptides</strong></td>
</tr>
<tr>
<td>(by products of collagen biosynthesis)</td>
<td><strong>Pyridinoline cross-links</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Hydroxyproline</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Hydroxylysine glycosides</strong></td>
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