Limbus
Delivery of blood to the anterior segment

- **oa**: ophthalmic artery
- **aca**: anterior ciliary artery
- **lpca**: long posterior ciliary artery
- **ma**: artery of rectus muscle
- **mc**: capillaries of rectus muscle
- **ec**: episcleral capillaries

- **→**: episcleral arterial circle
- **□**: major circle of iris + intra-muscular arterial circle
FIGURE 7. Diagram of the rat limbal microvasculature, illustrating the association of arteries (light outline) and veins (heavy outline). Limbal artery (arrows) is supplied by anterior ciliary arteries (ACA) and long posterior ciliary arteries (LPCA), providing collateral blood supply for the iris (I) and ciliary processes (CP). Venous plexus (asterisk) is connected to Schlemm’s canal (SC) through multiple collector channels (arrowheads) and drains into radial aqueous-containing veins (AV).

\[ ev = \text{episcleral vein} \]
Avascular cornea

Limbus (L)

Modified from van der Merwe and Kidson, 2014
Epithelial abrasion results in inflammation, platelet accumulation at the limbus, and neutrophil infiltration into the cornea (24h post-injury).
While neutrophils migrate into the cornea, platelets remain within the limbal region.
Topical rIL-20 decreases corneal inflammation in wildtype mice
Anterior Limiting Lamina (Bowman’s Layer)
Anterior Limiting Lamina (Bowman’s Layer)

- 8-10um thick
- Acellular
- Randomly woven type I collagen fibrils 20-30nm diam
- Balance of IL-1 (negative) and PDGF (positive) maintains acellularity
- IL-1 induces keratocyte apoptosis if get too close to epithelium
- Function?
  - rigid base for epithelium to conform to
  - prevents stroma-epithelialial contact
- Most species do not have an ALL
Anterior Limiting Lamina (Bowman's Layer)
8-10um thick
• Acellular
• Randomly woven type I collagen fibrils 20–30nm diam
• Balance of IL-1 (negative) and PDGF (positive) maintains acellularity (both produced by epithelium)
• IL-1 induces keratocyte apoptosis if get too close to epithelium
• Function?
  rigid base for epithelium to conform to
  prevents stroma-epithelium contact
• Most species do not have ALL
- Highly organized transparent tissue
- Allows passage of light and forms rigid framework
- 500um thick (central)
- 78% water, 1% salts, 21% macromolecules
- Flattened bundles of collagen = lamellae
- 242 lamellae/cornea, 2um thick, 2-260um wide
- Posterior lamellae orthogonal, anterior more oblique
- Fibrils are 27-35nm diameter
- Fibrils are regularly spaced 25-30nm apart
Stroma
• 71% dry wt. of cornea
• Helix of 3 polypeptide chains (= tropocollagen)
• Chains are called \( \alpha \)-chains, many different ones so many different collagens
• Presence of glycine rich collagenous domains dictates shape
• Collagen I \((\alpha_1\alpha_2\alpha_2)\) main component of lamellae
• Collagen V may help regulate fibril diameter
• Collagen VI may mediate keratocyte-matrix interactions
How is collagen made?

- **STEP 1:** Synthesis of $\alpha$-chains of pre-procollagen on ribosomes and endoplasmic reticulum
How is collagen made?

• **STEP 2:** Hydroxylation of proline residues to obtain hydroxyproline (an amino acid unique to collagen).
  – a reaction that substitutes a hydroxyl group, OH, for a hydrogen atom, H, in the proline
  – the hydroxylation reaction secures the chains in the triple helix of collagen
  – hydroxylation is catalyzed by the enzyme prolyl-4-hydroxylase
  – Vitamin C is essential for enzyme action, scurvy!

![Diagram of collagen structure](image-url)
How is collagen made?

• **STEP 3:** Hydroxylation of lysine residues to obtain *hydroxylysine*
  – hydroxylysine is needed to permit the cross-linking of the triple helices into the fibers
  – the enzyme peptidyl proline hydroxylase is essential
How is collagen made?

• **STEP 4:** Glycosylation of some hydroxylsine residues
  – glucose and galactose are added by enzymes galactosyl transferase and glycosyl transferase
  – may affect fibril size
How is collagen made?

- **STEP 5:** Assembly of the three alpha chains to form procollagen
  - formation of disulphide bonds between parts of the polypeptide chains known as *registration peptides* at the C-terminal
  - three chains associate, align and the triple helix forms in a zipper-fashion giving procollagen
How is collagen made?

- STEP 5 (cont):
  - PROCOLLAGEN
  - C-Terminal
  - N-Terminal
How is collagen made?

• **STEP 6:** Secretion of procollagen molecules by exocytosis into the extracellular space
• **STEP 7:** Cleavage of registration peptides in the extracellular space, by procollagen peptidases.
• The resulting molecule is collagen a.k.a. TROPOCOLLAGEN
How is collagen made?

- **STEP 8:** Self-assembly or polymerization of collagen molecules form collagen fibrils.
- **STEP 9:** Cross-linkage between adjacent collagen molecules stabilizes the fibrils.

\[ \text{collagen molecule} \]

---

**Diagram:**

[Diagram of collagen molecule and fibrils]
Collagen
<table>
<thead>
<tr>
<th>Type</th>
<th>Localization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td>Stromal fibrils</td>
</tr>
<tr>
<td>Type III</td>
<td>Scars</td>
</tr>
<tr>
<td>Type IV</td>
<td>Basement membrane</td>
</tr>
<tr>
<td>Type V</td>
<td>Stromal fibrils</td>
</tr>
<tr>
<td>Type VI</td>
<td>Stroma</td>
</tr>
<tr>
<td>Type VII</td>
<td>Anchoring fibrils</td>
</tr>
<tr>
<td>Type VIII</td>
<td>Descemet's membrane</td>
</tr>
<tr>
<td>Type XII</td>
<td>Stroma, basement membrane</td>
</tr>
<tr>
<td>Type XIII</td>
<td>Stroma</td>
</tr>
<tr>
<td>Type XVII</td>
<td>Hemidesmosomes</td>
</tr>
<tr>
<td>Type XVIII</td>
<td>Basement membrane</td>
</tr>
<tr>
<td>Type XX</td>
<td>Basement membrane</td>
</tr>
</tbody>
</table>
EXTRACELLULAR MATRIX

TWO MAJOR CLASSES OF MOLECULES

Glycosaminoglycans (GAGs)
- form hydrated porous gels

Fibrous Proteins
- structural (collagen)
- adhesive (fibronectin, laminin – basal lamina)
GAGs

Hyaluronan
- non-sulfated
- synthesized on PM

All other GAGs
- Sulfated
- synthesized in Golgi
- attached to protein core (i.e., proteoglycans)

GAG = long unbranched polysaccharide consisting of a repeating disaccharide unit
Proteoglycans

- Core protein + glycosaminoglycans (GAGs)
- GAGs = keratan sulphate (KS)
  dermatan sulphate (DS)
- Core proteins = decorin (DS)
  lumican (KS)
  keratocan (KS)
  mimecan (KS)
- Anterior decorin
- Posterior lumican
- Produced by keratocytes
### Table 1.3 Proteoglycan family in the cornea

<table>
<thead>
<tr>
<th>Glycosaminoglycans</th>
<th>MW</th>
<th>Disaccharides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heparan sulfate</td>
<td>5–12 kD</td>
<td>N-acetylgalactosamine glucuronic acid</td>
</tr>
<tr>
<td>Heparin</td>
<td>6–25 kD</td>
<td>N-acetylgalactosamine glucuronic acid</td>
</tr>
<tr>
<td>Dermatan sulfate</td>
<td>15–49 kD</td>
<td>N-acetylgalactosamine iduronic acid</td>
</tr>
<tr>
<td>Chondroitin 4,6-sulfate</td>
<td>5–50 kD</td>
<td>N-acetylgalactosamine glucuronic acid</td>
</tr>
<tr>
<td>Keratan sulfate</td>
<td>4–19 kD</td>
<td>N-acetylgalactosamine galactose</td>
</tr>
<tr>
<td>Hyaluronan</td>
<td>4–8000 kD</td>
<td>N-acetylgalactosamine glucuronic acid</td>
</tr>
</tbody>
</table>

**Sulfated**

<table>
<thead>
<tr>
<th>Core protein</th>
<th>Glycosaminoglycans</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumican</td>
<td>Keratan sulfate</td>
<td>Interaction with corneal epithelial cells</td>
</tr>
<tr>
<td>Keratocan</td>
<td>Keratan sulfate</td>
<td>Causes cornea plana</td>
</tr>
<tr>
<td>Memece</td>
<td>Keratan sulfate</td>
<td>Recently identified in bovine corneas</td>
</tr>
<tr>
<td>Decorin</td>
<td>Chondroitin or dermanate sulfate</td>
<td>Wound healing</td>
</tr>
</tbody>
</table>

**Non-sulfated**

70%

Lumican -/- cloudy cornea with ^ collagen fibril diameter and more variation

Decorin -/- clear cornea

Keratan sulfate...water sorptive/poor retention

Dermatan sulfate...low water sorptive/good retention
Important for transparency
Maintain spaces between fibrils
Responsible for stromal hydration
-ve charge so attract Na\(^+\) and therefore water will absorb excess fluid leading to loss of transparency
GAGs and Proteoglycans

- Regulate diffusion and flow of macromolecules through connective tissues

- Provide resilience
  - negative charge → cannot move closer in compression
  - negative charge → holds water → osmotic swelling pressure
PROTEOGLYCANS IN THE ECM
bind various secreted molecules
and modify their activity:

2 Examples

1) Fibroblast Growth Factor, FGF
binds to proteoglycans this binding is required
for the FGF activation of the FGF receptor

2) Transforming Growth Factor-β, TGF-β
binds to the core protein of decorin and other
Proteoglycans. This binding inhibits the activity
of TGF- β
Stromal Matrix Maintenance

- Constant (but slow) laying down of new material and degradation of old by keratocytes
- Turnover time for collagen 2-3 years
- Matrix metalloproteinases (MMP1, MMP2, etc.) degrade components...at least 28 MMPs
- Tissue inhibitors of matrix metalloproteinase (TIMPs) regulate action of MMPs.....at least 4 TIMPs
Fig. 1.9A Transmission electron microscopy of the human corneal stroma. A, A keratocyte localized between stromal lamellae. B, A higher-magnification view showing a keratocyte in relation to collagen fibers coursing in various directions.
Fig. 1.10A Transmission electron microscopy of the corneal stroma. A, Lamellar structure of collagen fibers and electron-dense gap junctions (1) between the cellular processes of keratocytes in the human cornea. B, Three-dimensional view of keratocytes in the rat cornea after digestion of collagen. Note the cellular network formed by keratocytes.
Another Structural molecule in the Stroma

- Fibrillin

EM micrographs of 5 mammal species; (a) C57BL/6 mouse, (b) Brown Norway rat, (c) New Zealand White rabbit, (d) Macque monkey, (E) Human. EFMBs are within lamellae in these examples, but they were also located between lamellae. Panels (B) and (C) show EFMBs sectioned obliquely. All images are the same magnification. (Scale bar = 200nm)
Another Structural molecule in the Stroma

- Fibrillin
Another Structural molecule in the Stroma

• Fibrillin
Other Cells in the Stroma

- Axons surrounded by Schwann cells
- Antigen presenting cells (macrophages, dendritic cells, langerhans cells)
- After injury - neutrophils
Transparency

- Light passes through stroma with minimal scattering (1%)...allows slit lamp viewing

- Early suggestions
  RI of stromal components identical (not true) differences in RI cancelled out....collagen is 1.4 Surrounding ECM is 1.365

- Maurice 1957 - “Lattice theory”
  Stroma is a perfect crystalline lattice
  Collagen fibrils are of equal diameter and equidistant
  Any scattered waves are cancelled by destructive interference
Maurice - Lattice theory
Goldman & Benedek 1967

Light cannot resolve structures substantially smaller than its wavelength

Fluctuations in RI occurring over dimensions smaller than one half of wavelength of light do not cause appreciable scattering

In stroma fibers are separated by small distances, fluctuation in RI going from fibers to surrounding matrix occurs over 50nm...i.e. much smaller than wavelength of light
Anything that alters the arrangement of collagen

Deposition of incorrect collagen (scar formation)
Edema (excess fluid)
Loss of Transparency

- Normal stromal hydration (78%) maintained by:
  - epithelial barrier
  - endothelial barrier
  - endothelial pump
- Stromal components have natural tendency to attract water
- Loss of proteoglycans can lead to aggregation of collagen fibrils
Loss of Transparency
Loss of Transparency

**NORMAL CORNEA**
- equal diameter collagen fiber
- equally distant from each other

**SWOLLEN CORNEA**
- distance between collagen fiber increase

**SWOLLEN CORNEA**
- distance between collagen fiber increase
- collagen fiber aggregation occurs
- loss of GAGs occur
Loss of Transparency

• Edema mostly occurs in posterior stroma due to looser arrangement of lamellae and presence of keratan sulphate

• Epi/endo must be restored to regain transparency
So why is the cornea transparent?

- because light passing through it is minimally scattered
- a combination of both Maurice and G&B theories
- probably some scatter is reduced due to destructive interference (Maurice)
- but the major factor is as suggested by G & B
Stromal wound healing

- Swelling of lamellae
- **Death of keratocytes** by apoptosis/necrosis
- Neutrophils arrive to clear debris
- "activated" keratocytes (fibroblasts) appear in 24 hrs
- Myofibroblasts contract and reduce wound size
Fibroblast

Myofibroblast
• "activated" keratocytes (fibroblasts) appear in 24 hrs
• Myofibroblasts (Bad/scatter light) contract and reduce wound size (Good)
Stromal wound healing

- Type III collagen scar formed (Good/Bad)
- Scar re-modeled over time
Posterior Cornea
Endothelium

- Single layer of interdigitating cuboidal cells
- Mediates flux of solutes and water across posterior cornea so regulates delivery of nutrients to cornea and maintains transparency
- Lots of organelles indicating metabolically active
- Gap junctions allow communication
- Tight junctions hold cells together
- “Hexagonal mosaic”
- Cell density decreases with age
- Cells enlarge & change shape with age (polymegathism & pleomorphism)
Endothelium

6-year-old
3015 cells/mm²

74-year-old
1944 cells/mm²
CV (SD/mean) >0.25 = polymegathism

**Figure 4-17** Morphometric analysis of the corneal endothelium demonstrating variability in cell density and coefficient of variation of cell size. Note that cell size can vary significantly with no change in cell density. CV, Coefficient of variation. (From Yee RW et al: Curr Eye Res 4:671, 1985.)
Endothelium - functions

Mediates flux of solutes and water across posterior cornea so regulates delivery of nutrients to cornea and maintains transparency

Barrier function
Pump function
Epinephrine – pupil dilation; safe for endothelium at pH 6.5-8 but toxic (as are many solutions) at pH 4.
• Tight junctions are macula occludens (rather than zonula occludens)
• Attach cells at focal areas
• Endothelial barrier is leaky
• Provides flow of nutrient from aqueous humour
Freeze Fracture
Freeze Fracture

1. Frozen cell

2. Freeze-fracture splits membrane

3. Replication preserves fracture face structure

4. SDS removes cellular material leaving surface membrane components attached to the replica

Platinum (angled to bring out details)

Carbon (stabilize sample)

Epitopes available for labeling
Intestinal Epithelium

(a) Apical membrane
Basolateral membrane
Kissing point
Cytoplasmic surface
Strand
Extracellular surface

(b) Mv
L

Strand
P-face

TJ strand
Cytoplasmic surface
Fracture
Extracellular surface

Groove
E-face
Corneal Endothelium
• “Endothelial fluid pump” pumps excess fluid from the stroma
• Transportation of ions (Na and bicarbonate) from stroma to aqueous humour generates osmotic gradient which draws water out of the stroma
• Pump components
  NaK ATPase
  Diffusion of bicarbonate
  Na - H exchanger
Pump Function

Acidifies extracellular fluid and promotes CO₂ entry
Corneal endothelial cell transports water out of the stroma by an ATP-driven ion pump mechanism.

**Figure 4-39** Model of ion and water movements across the corneal endothelium. Activity of the metabolic pump sets up an osmotic gradient, resulting in movement of fluid from the stroma to the aqueous humor balancing the leak of fluid from the aqueous humor into the stroma. CA, Carbonic anhydrase. (Based on models proposed in Jentsch TJ et al: *Curr Eye Res* 4:361, 1985; and Kuang K et al: *Exp Eye Res* 50:487, 1990.)

179 mEq/L total... 45 mEq/L bound to proteoglycans

Provides stream of nutrients
Corneal Endothelium “Pump-Leak”

The corneal endothelium maintains the stroma of the cornea relatively dehydrated by functioning as both a barrier to fluid movement into the cornea and an active pump that moves ions to draw water osmotically from the stroma into the aqueous humour. The “pump-leak” mechanism corresponds to a combined leaky barrier and fluid pump.
Other pump models......

Endothelial - wound healing

- Cells do not typically divide
- Damaged area (*guttae*) covered by enlargement of surrounding cells...*Fuch’s dystrophy*
- Activity of fluid pump may be increased
- Eventually decompensation occurs
Contact lenses $\rightarrow$ epithelial eicosanoids $\downarrow$

Polymegethism $\leftarrow$ Na$^+$/K$^+$-ATPase inhibition

Figure 4-43  Endothelial polymegethism and pleomorphism caused by 27 years of contact lens wear. A, A 46-year-old non-contact lens wearer; cell density = 3086 cells/mm$^2$; coefficient of variation (CV) = 0.27; hexagonality = 63%; B, A 46-year-old who has worn hard contact lenses for 27 years. Cell density = 1449 cells/mm$^2$; CV = 0.59; hexagonality = 42%. (Courtesy Dr. Scott MacRae.)

Figure 4-45  Contact lenses are capable of stimulating the arachidonic acid (AA) cascade in the corneal epithelium directly from the lenses and the protein they absorb from wear. The epithelial cells have a P-450 metabolic pathway that produces two eicosanoids [12(R)-HETE and 8(R)-HHTDE] resulting from contact lens wear. These two products have been shown to diffuse across the stroma and inhibit the endothelial Na$^+$/K$^+$-ATPase, which causes endothelial cell edema—resulting in polymegethism—and corneal swelling. (From Edelhauser HF: Cornea 19:263, 2000.)
Figure 4-19  Alizarin red-stained rabbit corneal endothelium 3 days after excimer laser ablation. Normal (A). Residual corneal thickness of 177 μm (B), 130 μm (C), and 91.25 μm (D). There is loss of the endothelial hexagonal cells, and the endothelial barrier function has been compromised. (From Edelhauser HF: Cornea 19:263, 2000.)