Stress-strain measurements by strip extensiometry

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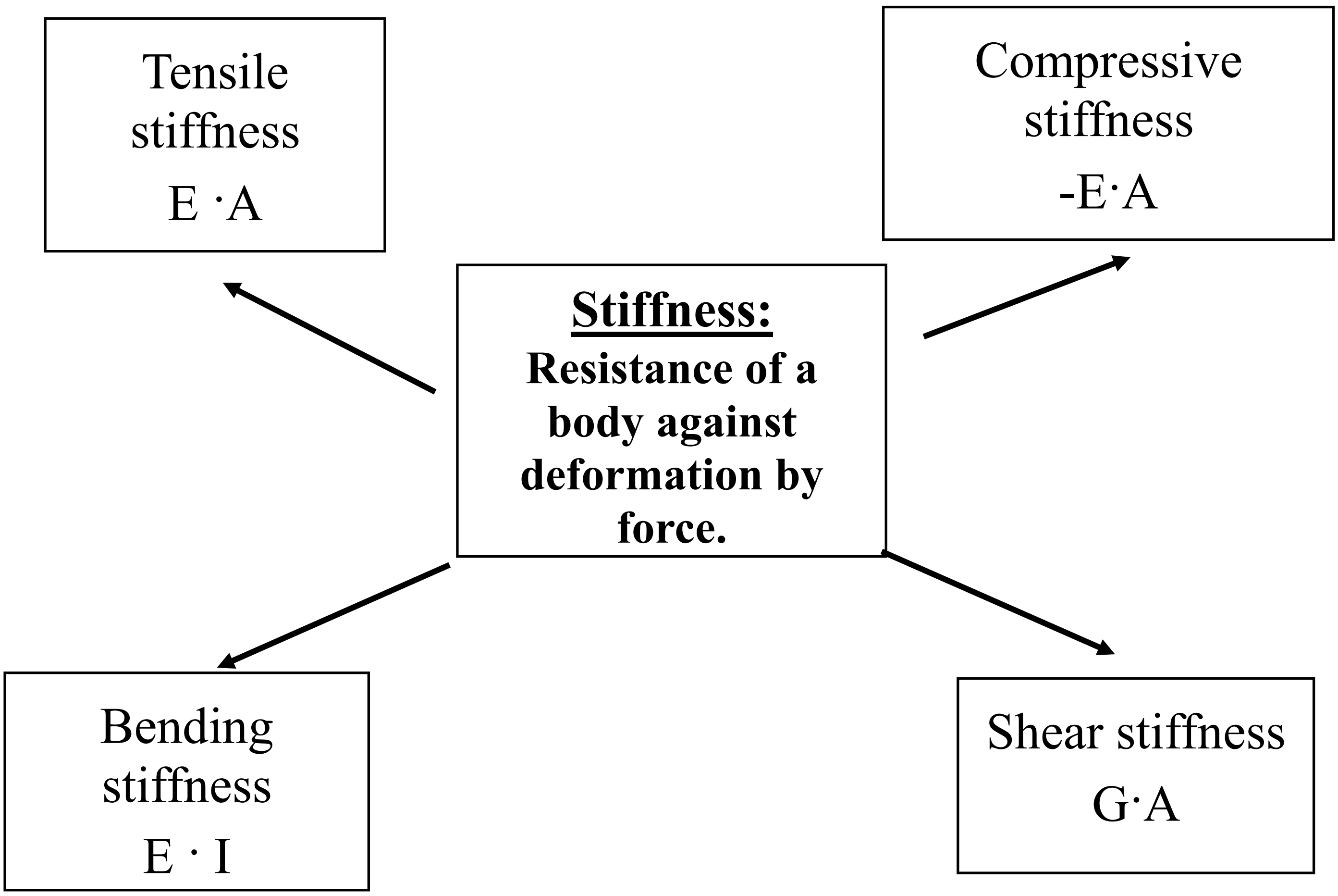
Biomechanical properties can be determined by applying a force and measuring the response to it (deformation).

Biomechanical properties are:

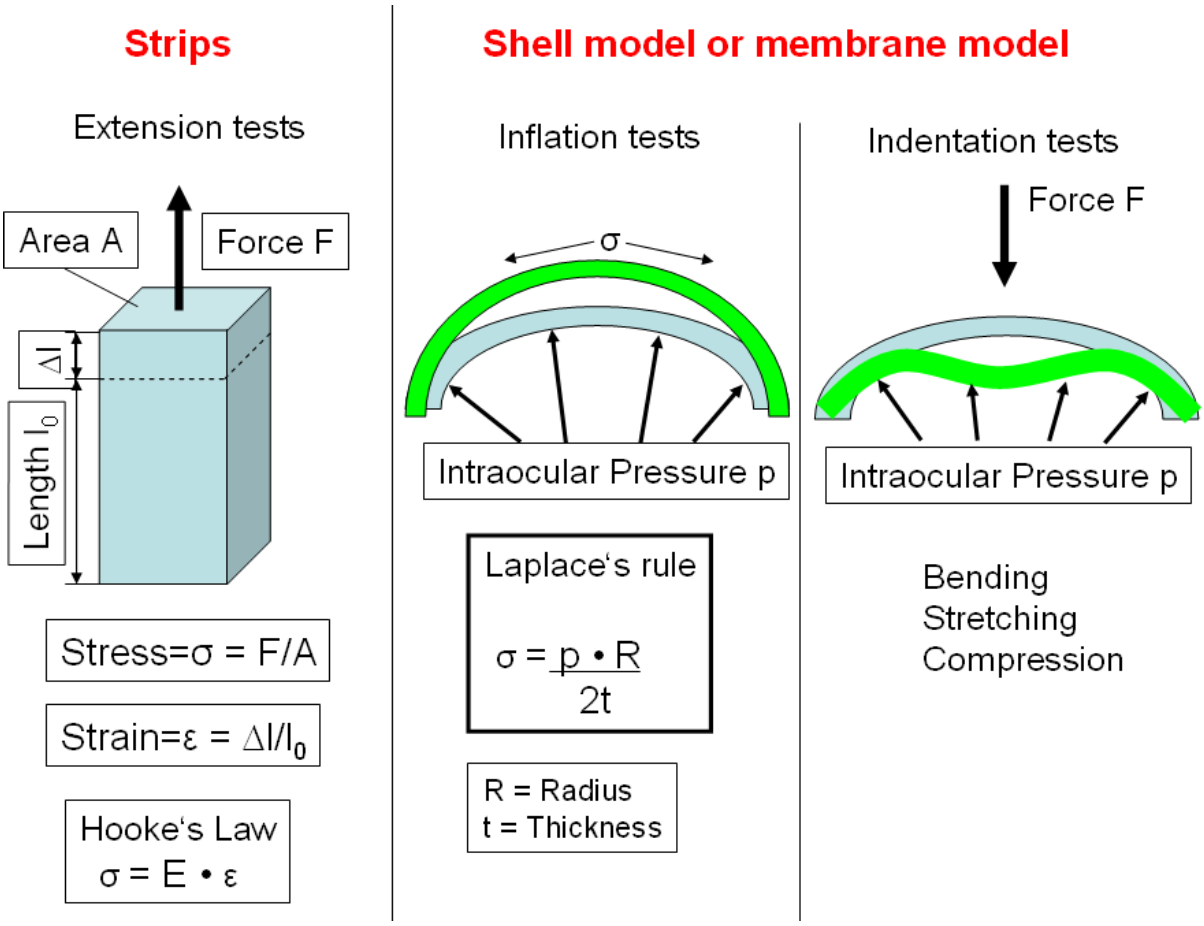
- Young's modulus or modulus of elasticity E
- shear modulus G,
- compression modulus,
- cohesive strength

Stiffness is not a poor biomechanical property



Stiffness is determined by material properties and geometry

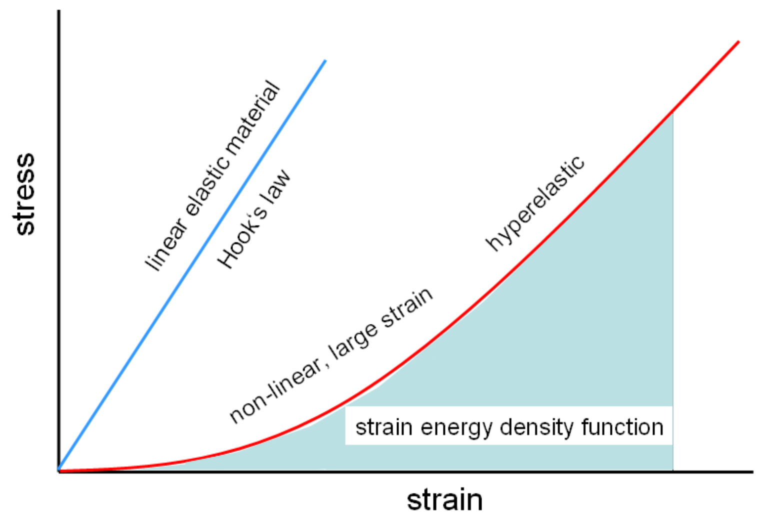
Strip extensiometry and whole globe experiments



Hyperelasticity

Hook's law is valid for linear elastic materials or for small strains.

Biological tissues are nonlinear elastic and show a high strain. „hyperelastic"

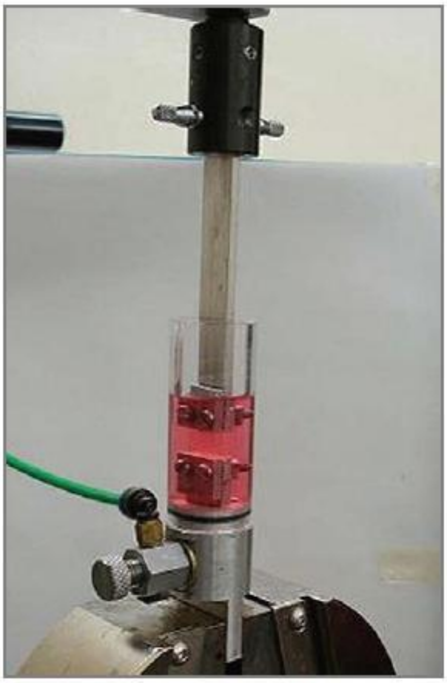


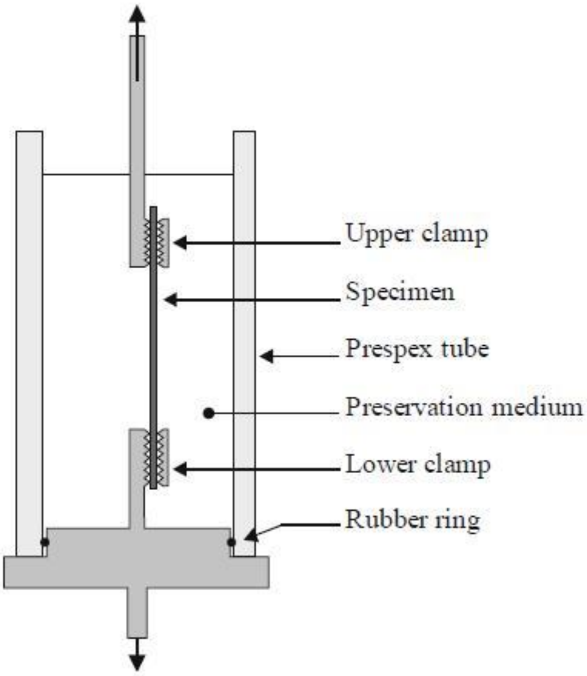
Hyperelastic behaviour can be described by *strain energy density function*.

The energy stored in a body due to deformation is called the *strain energy*.

The strain energy per unit volume is called the strain energy density and is the area below the stress-strain curve up to the point of deformation.

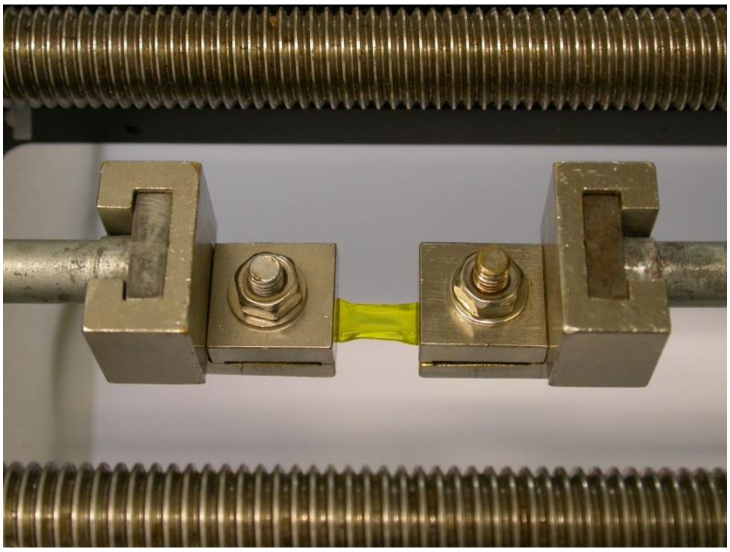
Uniaxial strip extensiometers (vertical version)



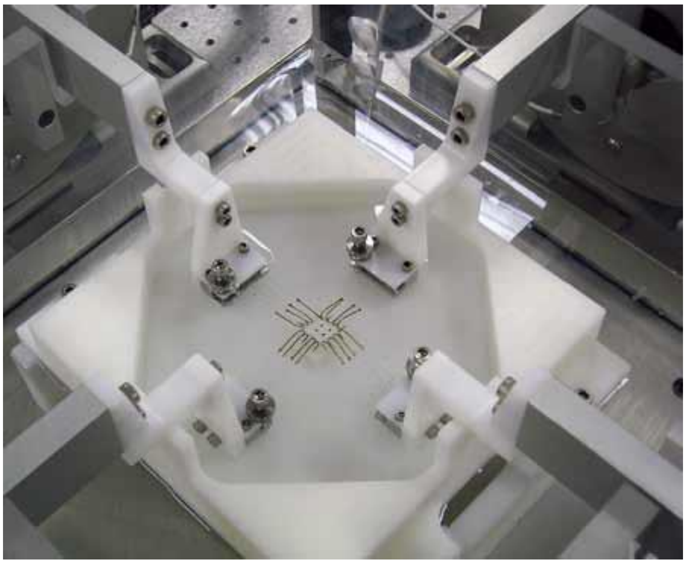


Elsheikh A. Strain-rate sensitivity of porcine and ovine corneas.*Acta of Bioengineering and Biomechanics 2011;13,25-36*

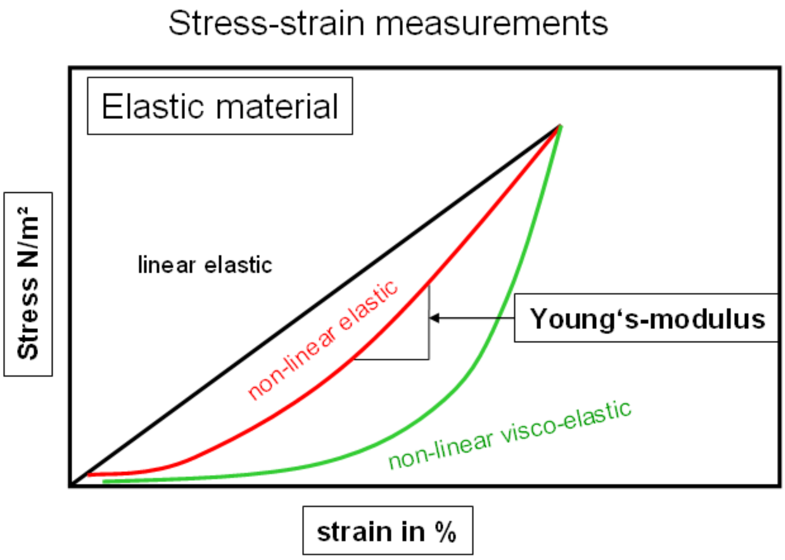
Uniaxial strip extensiometers (horizontal version)



Planar biaxial Testing



What can be investigated by strip extensiometry?



Young‘s modulus is the slope of the stress-strain curve.

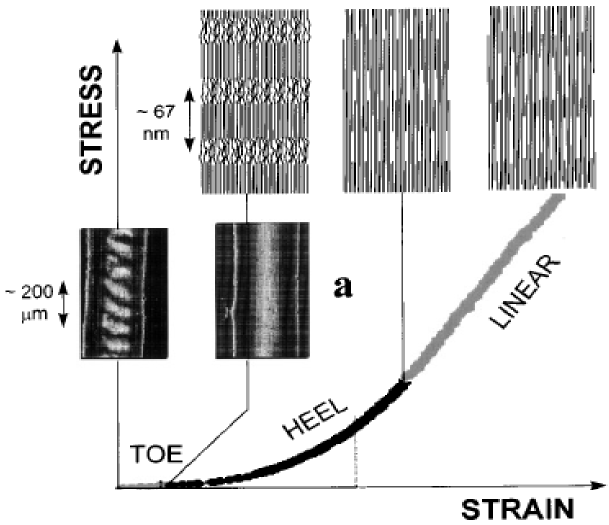
In biological materials the relationship between stress and strain is nonlinear, thus a series of Young‘s modulus approximation must be made for different stress levels (tangent modulus, secant modulus)

($1N/m^2 = 1Pa$ (Pascal))

The stress-strain values can be fitted by the equation:

$\sigma=A(\exp(B\varepsilon) -1)$ and the Young‘s modulus can be derived from **$E= A \cdot B \cdot \exp(B\varepsilon)$**

What can we read from a stress-strain curve?



There is an initial plateau region where the sample has not been straightened.

After it is straightened, the mechanical resistance of the sample consistently increases because of the continous recruitment of collagen fibers.

Toe modulus and heel modulus

Nonlinear behaviour is due to waviness and sliding of collagenous fibers.

The stress-strain relationship can be divided into two distinctive phases:

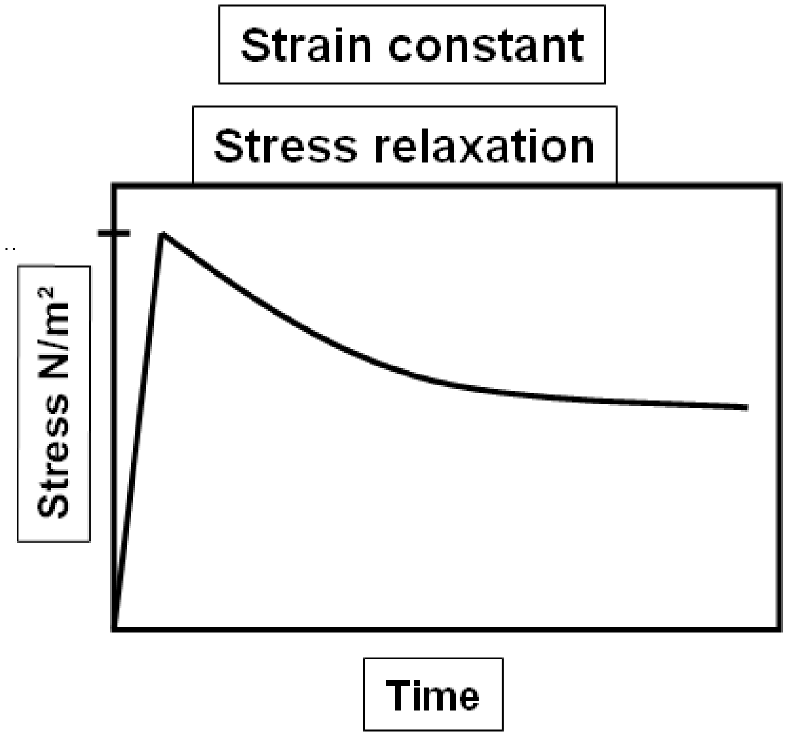
1. A matrix regulated phase with low stiffness also called the toe or toe/heel region. In this phase the collagen fibril layers remain loose and are unable to contribute much to the stress. The collagen undulates carrying load like an uncoiling spring. No stretching at the micro-fibril level is observed.
2. A collagen regulated phase with much higher stiffness. The fibril layers become taut.

(Fratzl: Fibrillar structure and mechanical properies of collagen. J Struct Biol 1997;122:119-122

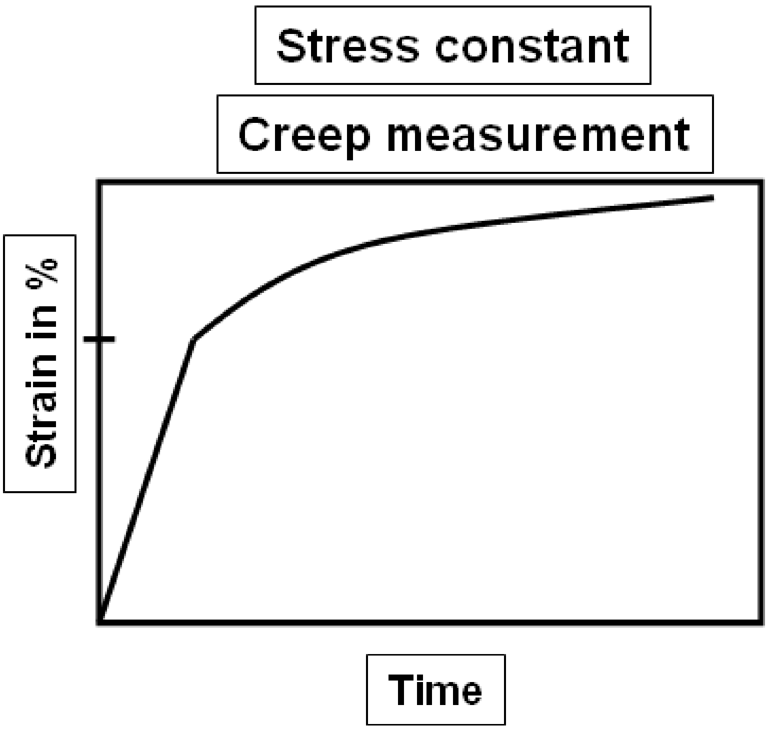
Liu: A mechanical model of the cornea considering the crimping morphology of collagen. IOVS 2014)

Viscoelastic properties

Length is held constant and the force required to maintain this length is measured over time.



Applying a constant stress (force) and measuring the extension over time.



Preparing the samples

- Cut the samples from the

    same location, same orientation

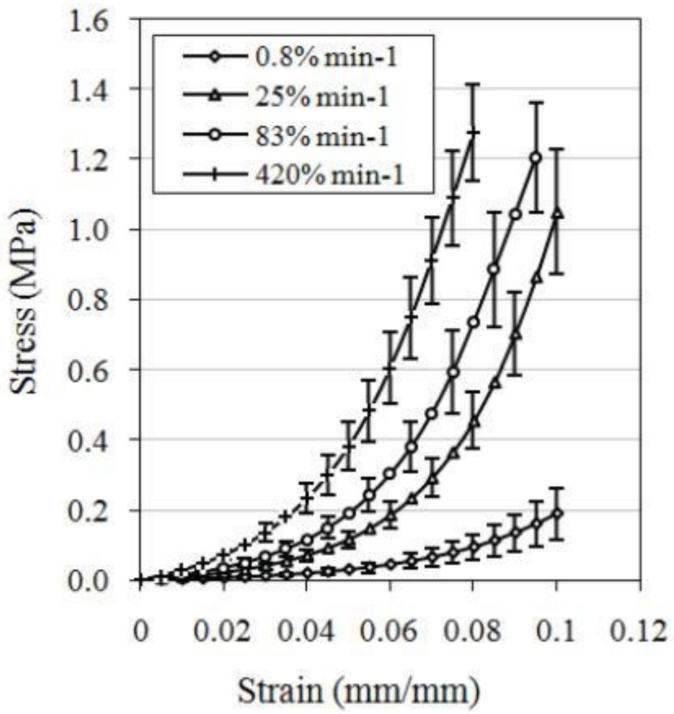
- Choose the same geometrical parameters (length, width)

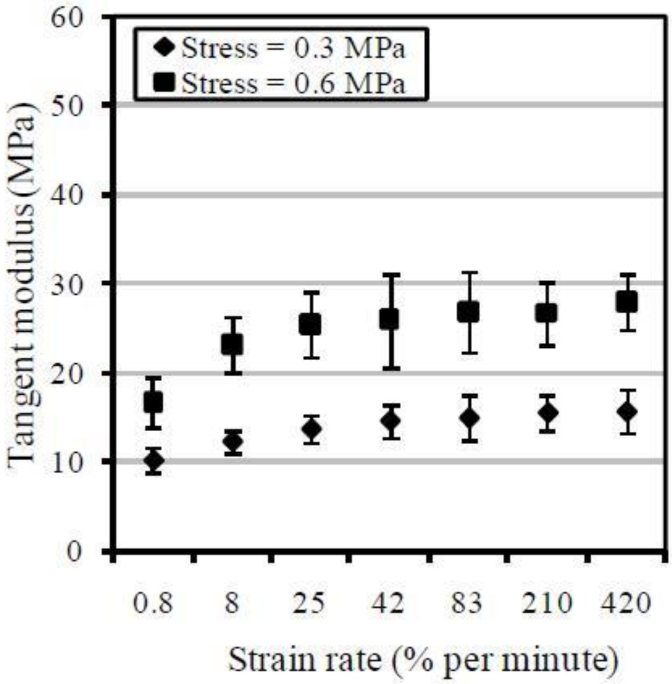
- For very thin samples (lens capsule, amniotic membrane, choroid, retina, pericard) use a carrier (cigarette paper)

- Use the same preload (prestress)

- Use the same strain rate

Influence of strain rate

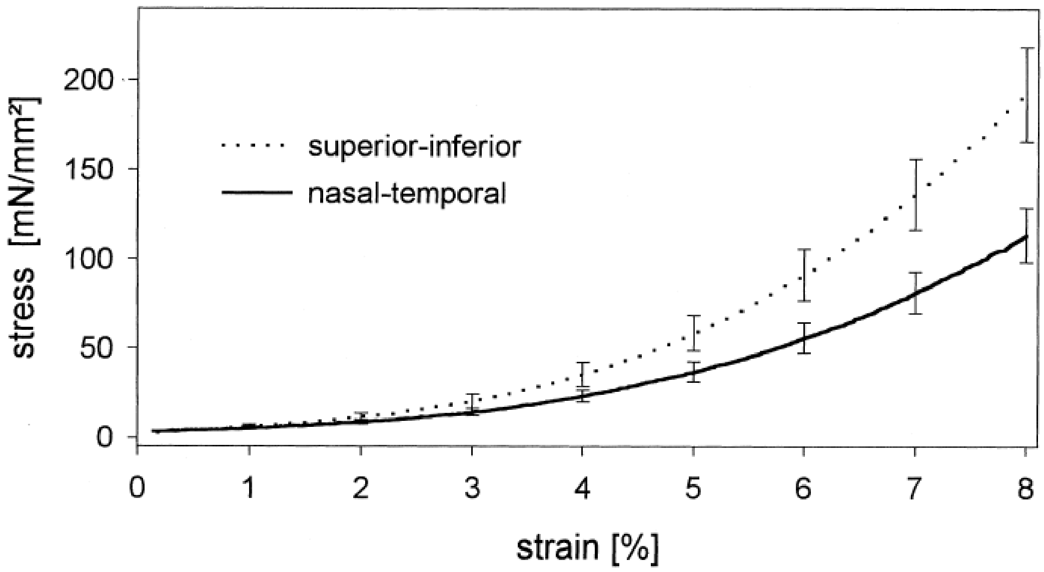




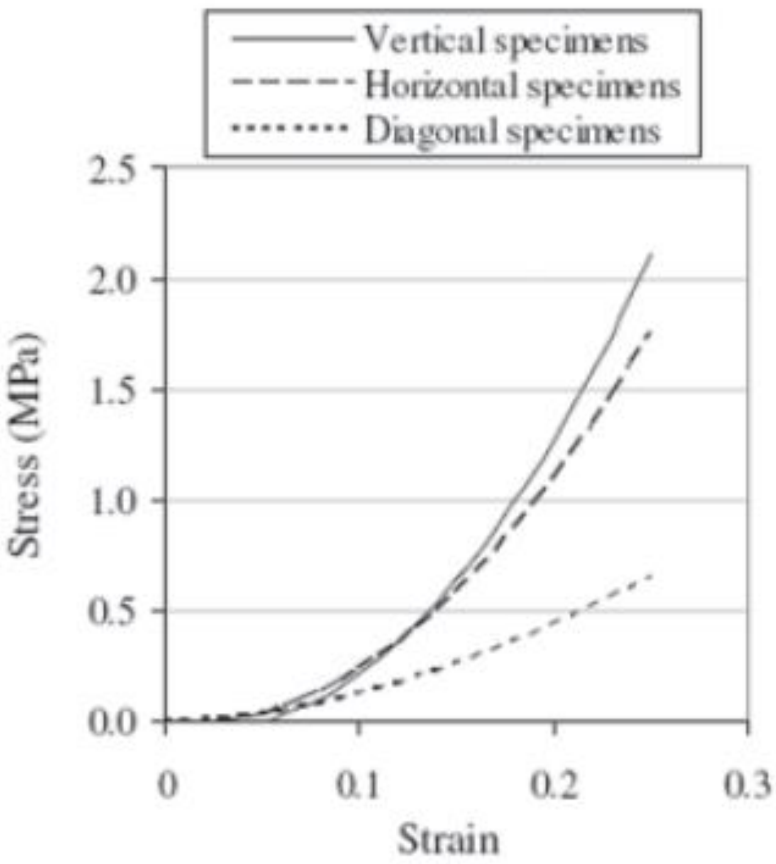
Elsheikh: Strain-rate sensitivity of porcine and ovine corneas. Acta Bioeng Biomech 2011

Orientation of the tissue

Stromal fibrils have a preferential orientation in the superior-inferior and temporal-nasal direction.

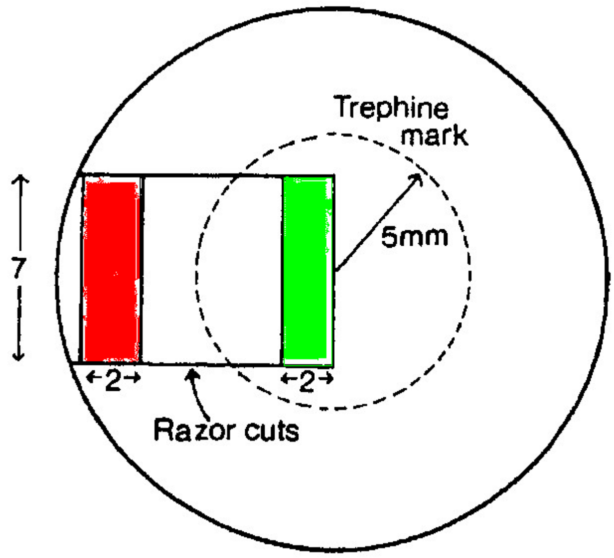


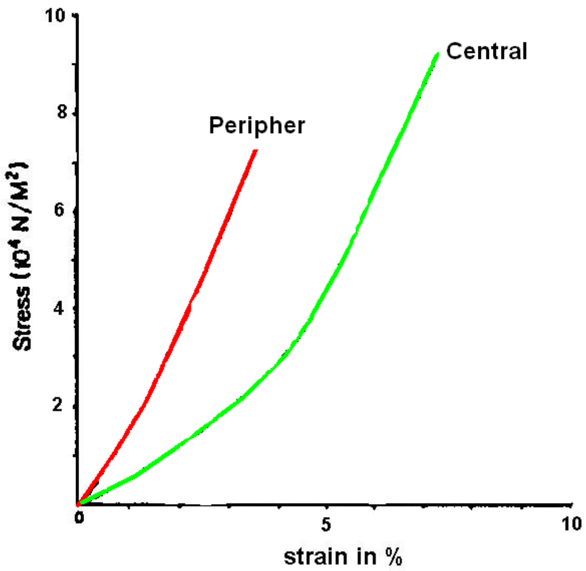
Kampmeier: Thermal and biomechanical parameters of porcine cornea. Cornea 2000



Elsheikh: Experimental assessment of corneal anisotropy. JRS 2008

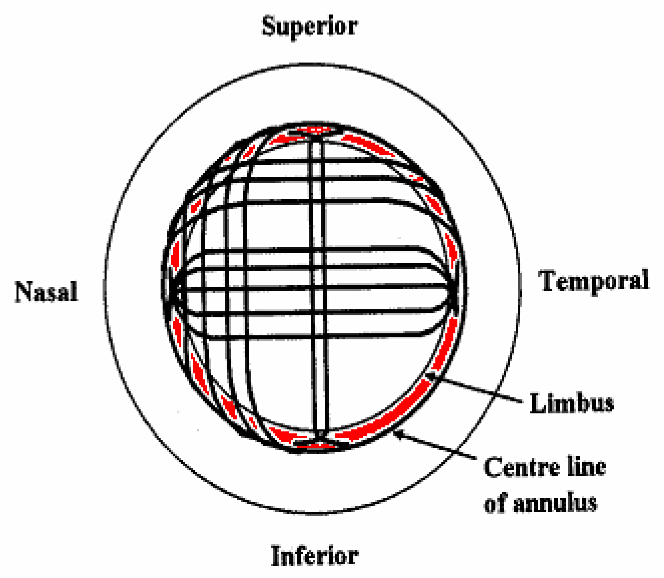
Location of the tissue





The peripheral cornea (in circular direction) is stiffer than the central one.

Circumferentially running fibers form a circular ligament (Meek)

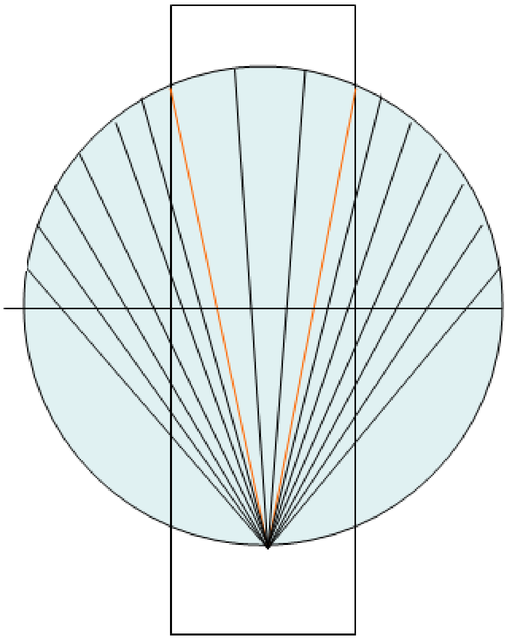


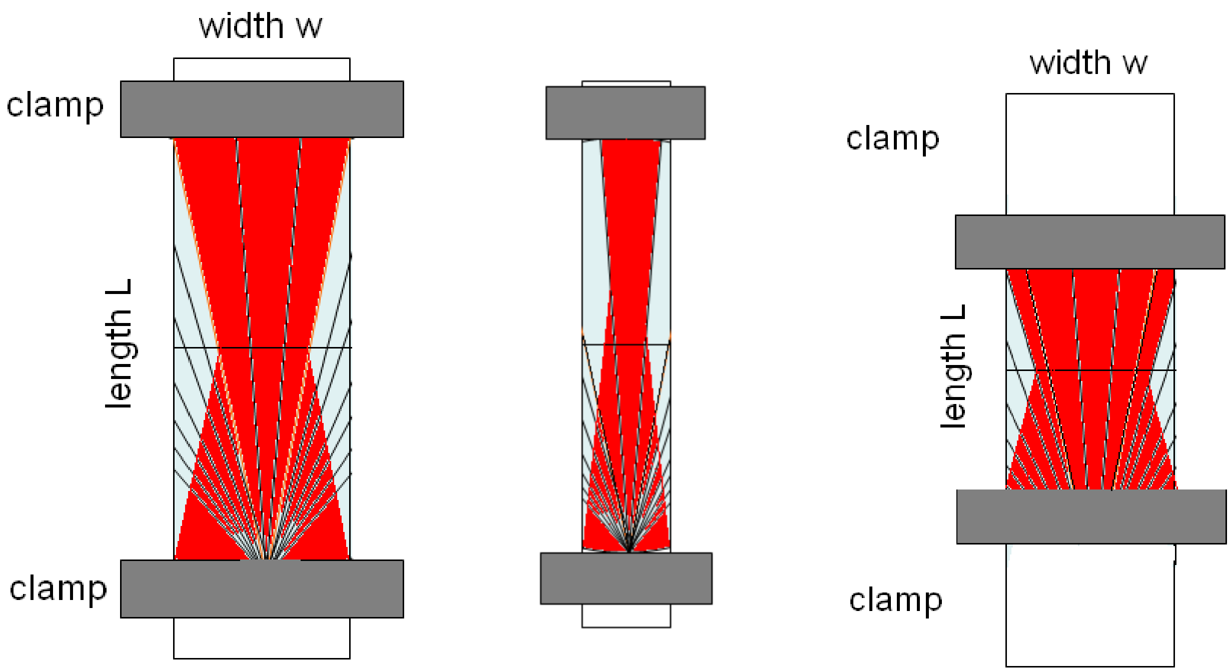
Reichel/Miller: The elastic modulus of central and perilimbal bovine cornea. Ann Ophthalmol 1989

(Meek, KM: Organization of collagen fibrils in the corneal stroma in relation to mechanical

properties and surgical practice. J Refract Surg. 1999

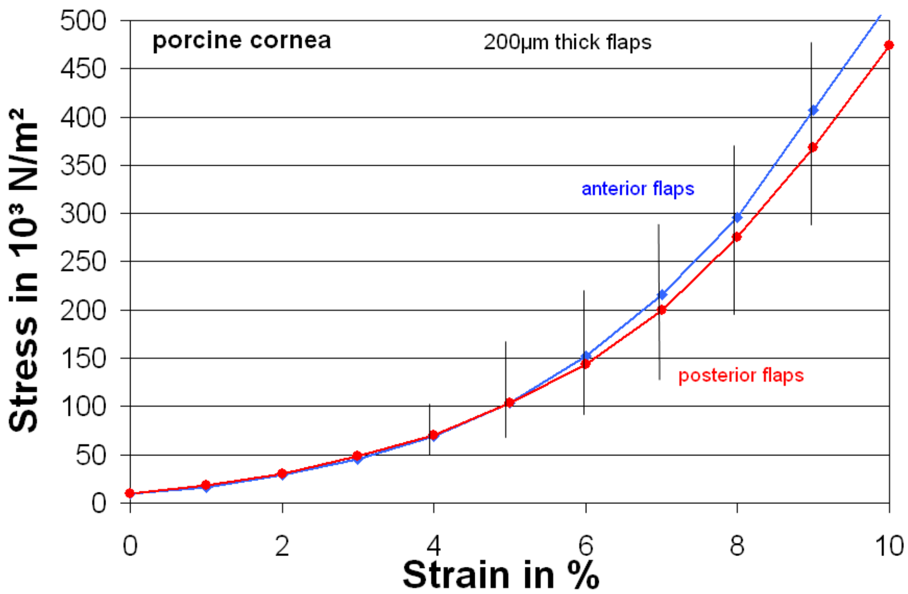
Same width and same length: because some load bearing fibers are cut due to preparation.

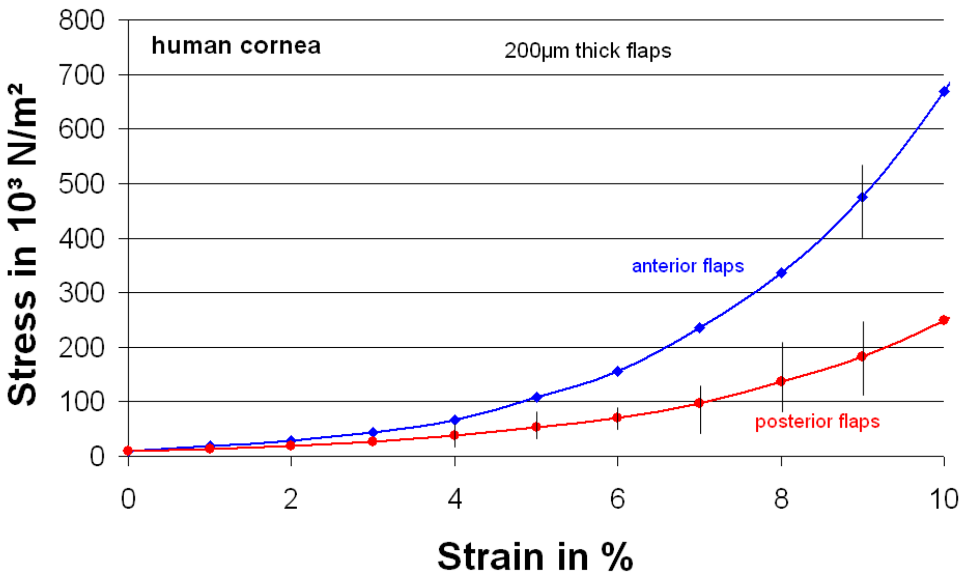




Assuming an equal distribution of the collagen fibers the number of oblique load-bearing fibers can be estimated. d= diameter of the cornea

Depth-dependance of Young's modulus



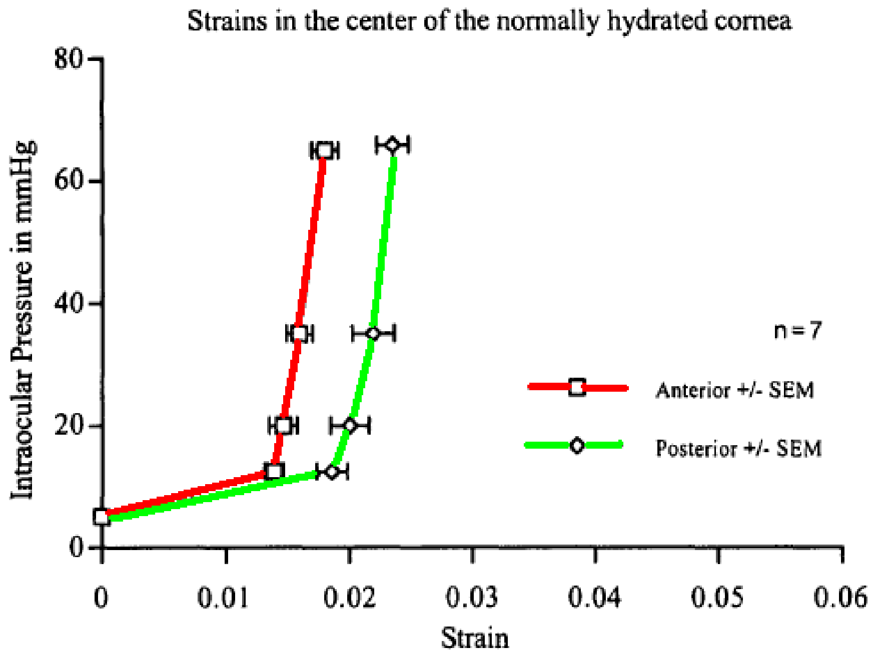


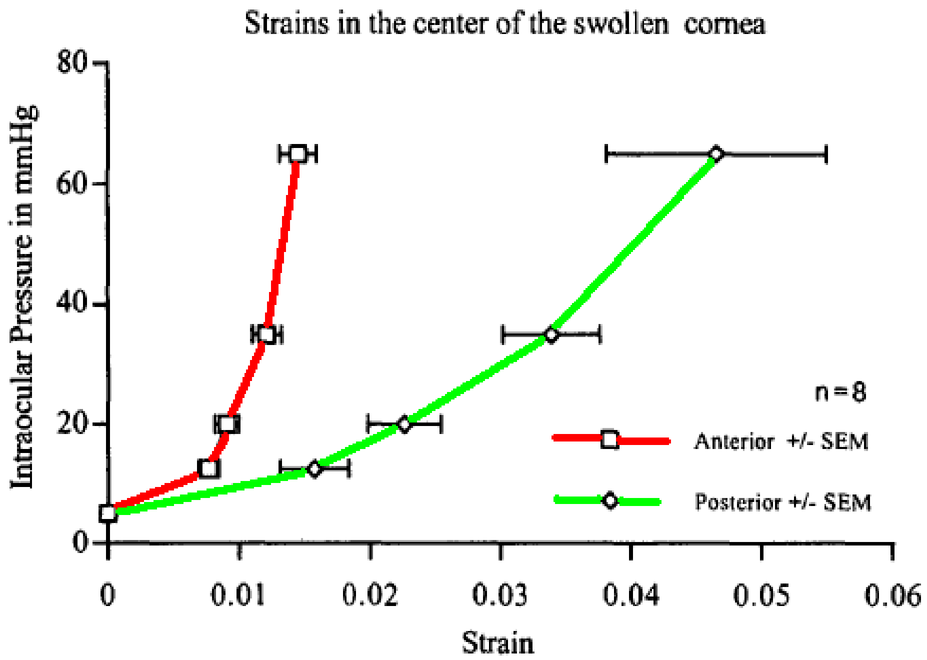
	Young's Modulus of Flaps of Porcine Cornea in MPa	Young's Modulus of Flaps of Human Cornea in MPa
Anterior control flaps	2.9	3.6
Posterior control flaps	2.8	1.3

Kohlhaas/Spoerl: Biomechanical evidence of the distribution of crosslinks in cornea. JCRS 2006

Influence of hydration

Water is enriched with GAGs and causes collagenous fiber bundle swelling, separation, and promotes fibril sliding. These factors affect the mechanical properties.

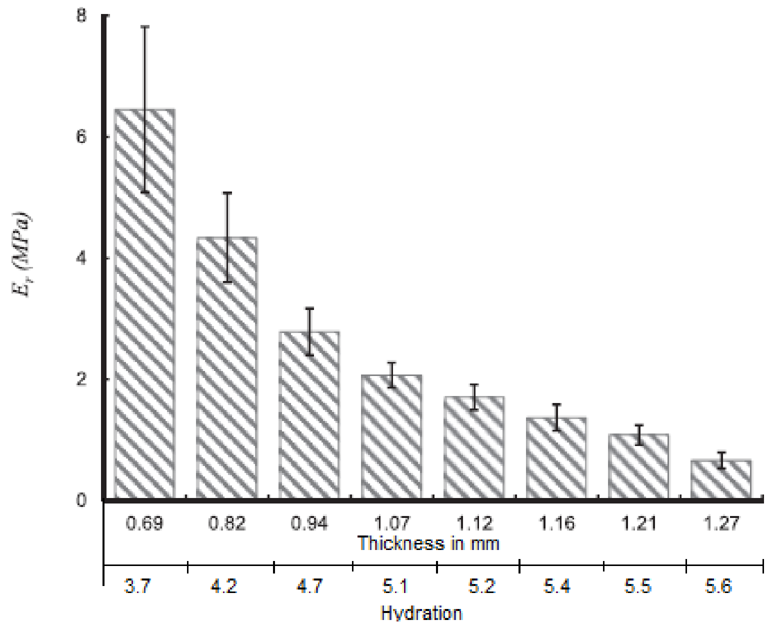


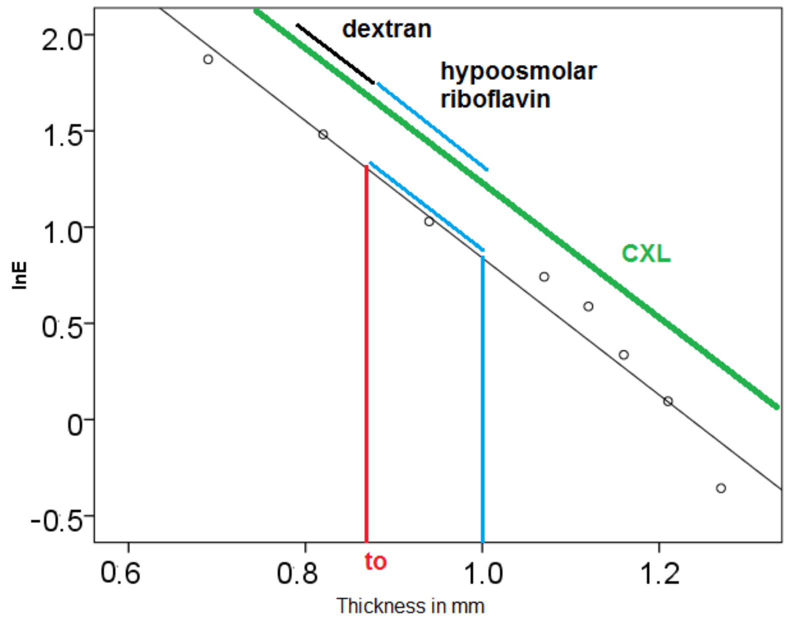


Hennighausen: Anterior—posterior strain variation in normally hydrated and swollen rabbit cornea. IOVS 1998

Compensation of hydration effect

E= Eo exp(t/to)





Hatami-Marbini, H. Hydration dependent biomechanical properties of the corneal stroma.

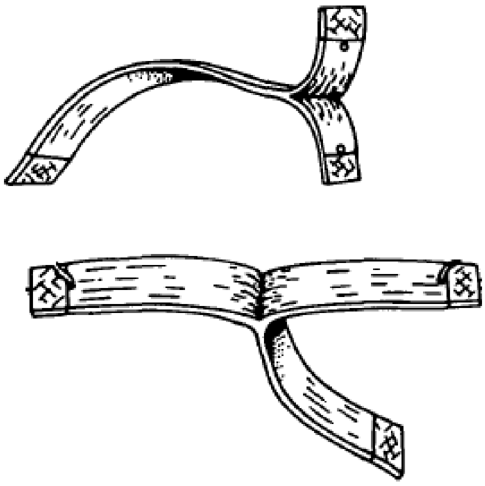
Experimental Eye Research 116 (2013) 47-54

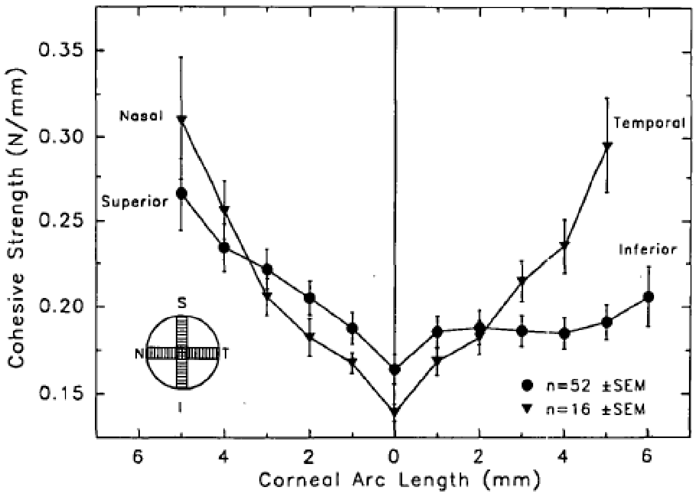
For comparison of stress-strain values or Young's modulus, the corneas should have the same hydration. That will be realized by incubation of the samples in 15 % dextran solution before biomechanical measurement.

Cohesive tensile strength

The cohesive tensile strength of interlamellar adhesion is measured by the tearing force to seperate the stroma along an interlamellar plane.

Interlamellar cohesive strength characterizes the molecular binding of proteoglycans and the interwoven collagen lamellae.

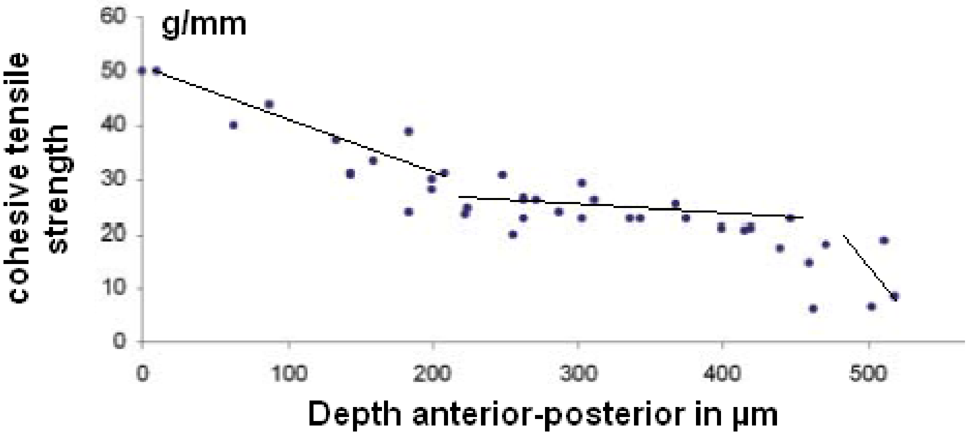




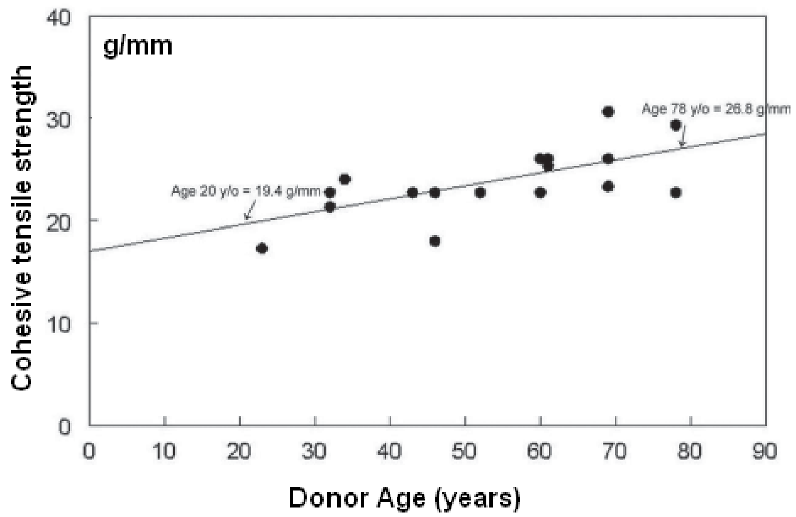
Interlamellar cohesive strength varies with location in the human cornea.

Smolek: Interlamellar cohesive strength in the vertical meridian of human eye bank corneas, IOVS,1993

Depth-dependent cohesive tensile strength

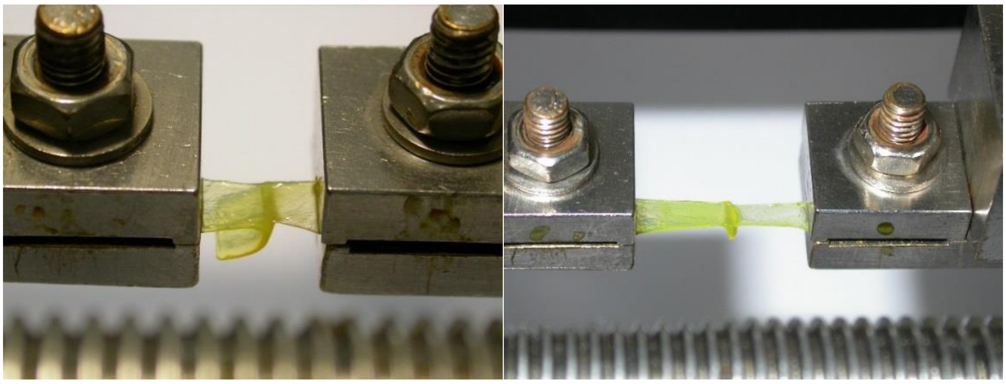


The anterior 40% of the corneal stroma has significantly higher cohesive tensile strength than the posterior 60%.



Randleman: Depth-dependent cohesive tensile strength in human donor corneas. JRS, 2008

Cohesive tensile strength and CXL



Sample group	Controls	Crosslinking	Crosslinking with swelling	24 h swelling only	48 h formaldehyde	72 h α-amylase
Cohesive force (N/mm) (mean±SD)	0.24±0.025	0.256±0.032	0.253±0.049	0.232±0.024	0.267±0.034	0.166±0.034
Change (%)	–	+5.7	+4.5	−4.4	+10	−31.5
p Value	–	<0.310 (NS)	<0.067 (NS)	<0.382 (NS)	<0.067 (NS)	<0.0001*

*Significant difference.

- 400 µm thick flaps of porcine cornea
- the splitting plane was created at 50% depth (200µm)
- after digestion of proteoglycans intralamellar cohesive force is lower

- In 200µm depth the CXL effect is low regarding interlamellar cohesion

Limitations of strip extensiometry

- Flattening of the initial curved form of the specimen

- Damage of structure due to cuts
  - Orientation of strips is important
- Mean value of the entire sample
  - Measuring local differences is not possible
- Methods with intact corneal samples provide more physiological data

Advandages of strip extensiometry

- Easy preparation and measurement
- Investigation of influencing factors like

  * age, glucose, hydration, collagenase,
  * drugs (prostaglandins, steroids)
  * crosslinking (ribo+UV, genipin, nitroalcohol),
  * hormones (estrogen, cortisol)
  * collagen orientation, several layers
  * pathologies (diabetes, keratoconus)
  * surgical techniques (Lasik, PRK, SMILE)
  * comparison of different species (rabbit, porcine, human)

Summary

Strip-extensiometry is suitable for comparison, for testing influencing factors

It should be used as first line method

Definied conditions should be used to measure only the biomechenical changes.