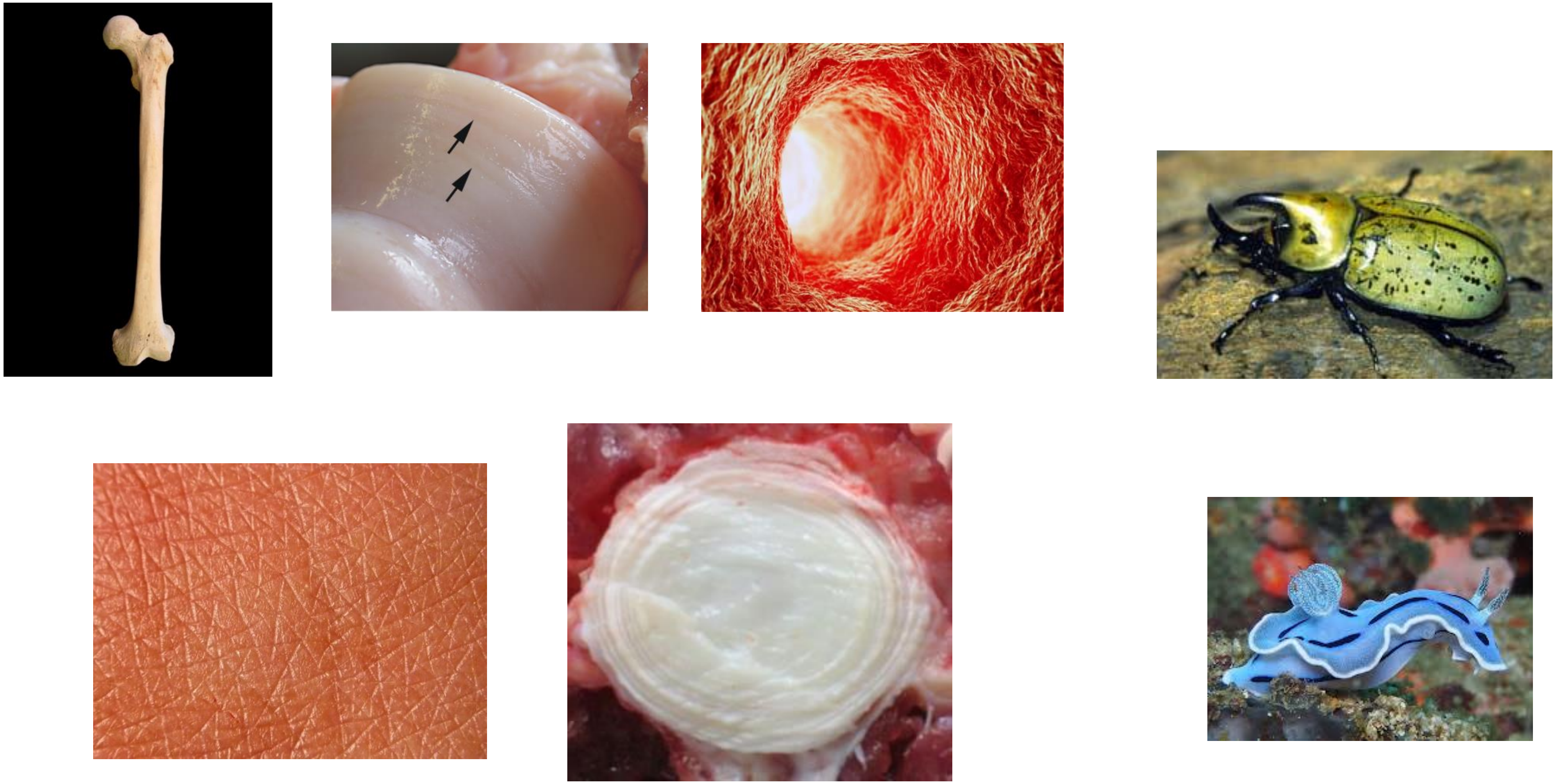
Mechanical behaviour of biological tissues

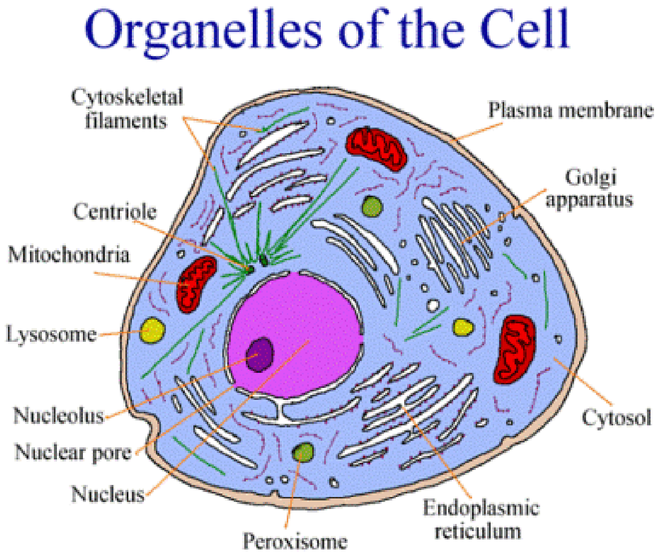
Further reading:
Gupta, Himadri S., and Hazel RC Screen. "Structural Building Blocks of Soft Tissues: Tendons and Heart Valves." *Material Parameter Identification and Inverse Problems in Soft Tissue Biomechanics*. Springer, Cham, 2017. 1-35.
Download at https://link.springer.com/chapter/10.1007/978-3-319-45071-1_1

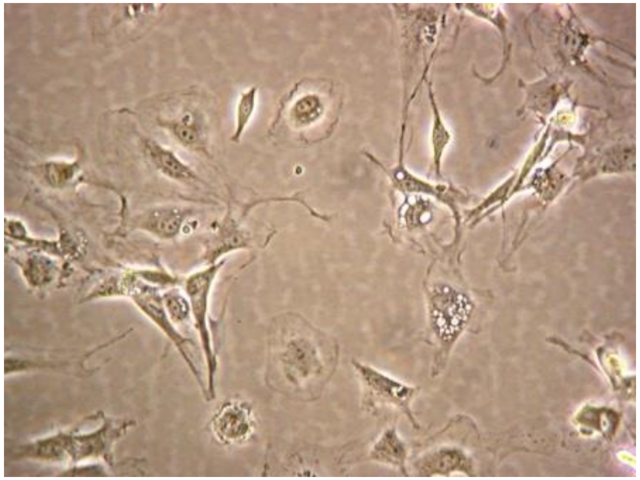
Mechanics – what's possible?

- Structures to move and support against gravity
- Swimming / flying against air and water pressure



What's Possible?





- Cell mechanics: ~5 kPa modulus; ~10μm diameter

Cells must make extracellular matrix

Building Blocks? Proteins, sugars & some mineral salts

What are the Building Blocks?

- Proteins

Polymers of amino acids

Generally fibrous

Structural Proteins:

- Keratins
- Collagens
- Silks

fibrous

- Elastins
- Resilin
- Abductin

rubbers

- Polysaccharides

Polymers of sugars

Generally space-filling / water stabilisation

- Chitin
- Cellulose

fibrous

- Glycosaminoglycans
- Pectins
- Mucus

gels

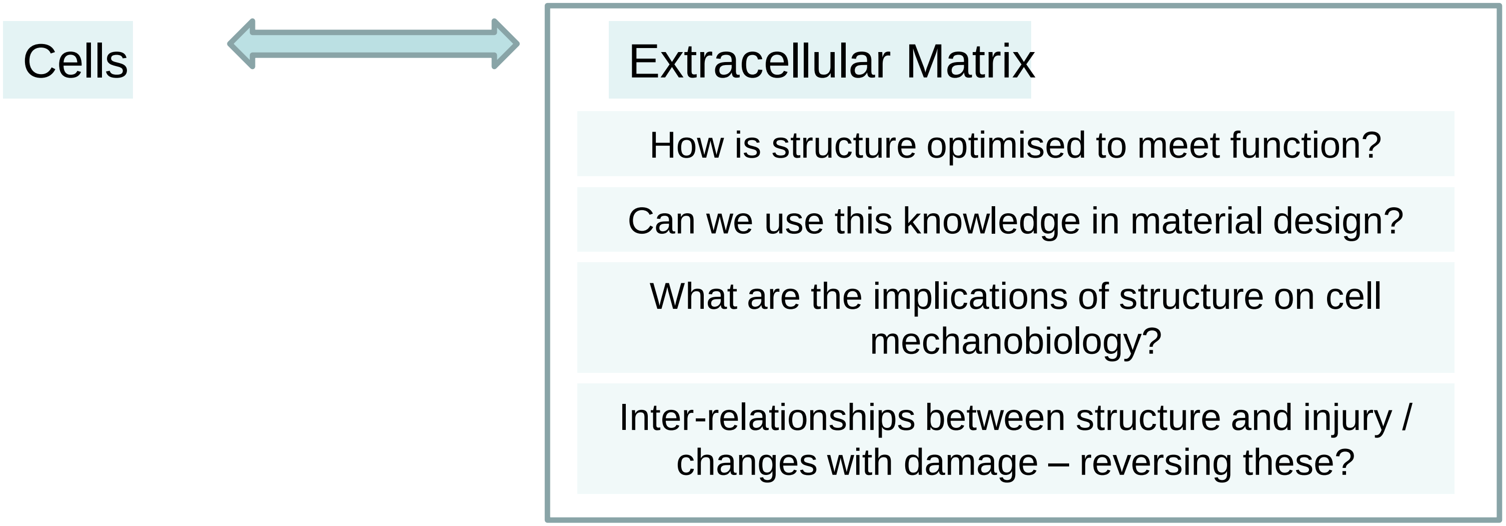
Most biological materials combine proteins and polysaccharides

Building biological materials

- Cells use this comparatively small list of components to make the vast range of mechanical properties we see across tissues.

•Biologist perspective

- How do cells make matrix?
- How do they order it and control turnover?
- Can we manipulate processes to grow healthy tissue (tissue engineering)?



Cells ↔ Extracellular Matrix

How is structure optimised to meet function?

Can we use this knowledge in material design?

What are the implications of structure on cell mechanobiology?

Inter-relationships between structure and injury / changes with damage – reversing these?

ECM in Tissue Mechanics

- From a Materials Science perspective
  - Range of different biological components combined to make a material with different phases
  - Fibres (one phase) .... in matrix (second phase)

Fibres to provide strength

Stabilise fibres and water to manage compression

Animal Tissues

- Collagen fibres
- Protein & polysaccharide matrix

Plant Tissues / Arthropods

- polysaccharide fibres
- Protein & polysaccharide matrix

Hydrated

Using Fibres

- Fibre Composite materials
  - Fibres to create strength
  - Embed in matrix to provide stability, viscous properties, toughness

⇩

- Hierarchical Materials
  - Cells make nano-scale matrix components
  - Often build fibre composites in multiple levels (hierarchical levels)

⇩

Facilitates the highly variable properties of different tissues

Creating Different Properties with fibres

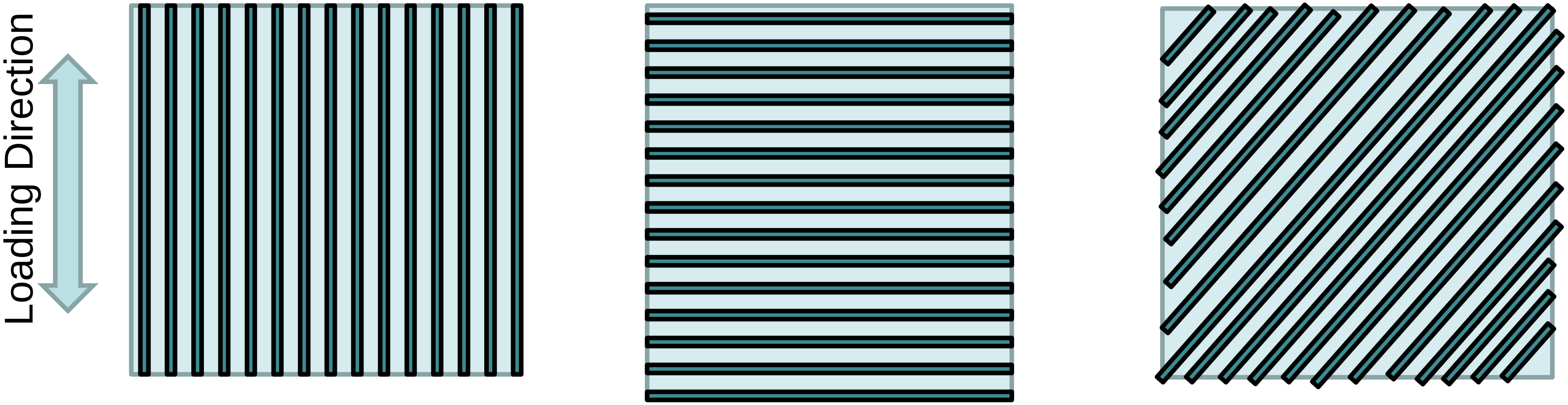
- Tensile Strength
- Elasticity
- Toughness
- Pre-stressed internal pressures
- Compression
- Stiffness

Organising Fibres – Strength, Elasticity & Toughness

<u>General Case</u>:

Fibre = stiff & elastic

Matrix = compliant & viscous



Stiff material
Elastic
Recover well

Viscous response
No elastic
stretch/recovery

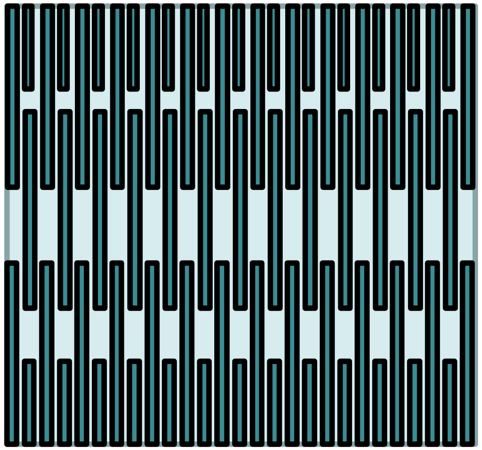
Combination?
Fibre rotation in load direction
More extensible than a
Less viscous than b

Organising Fibres – Strength, Elasticity & Toughness

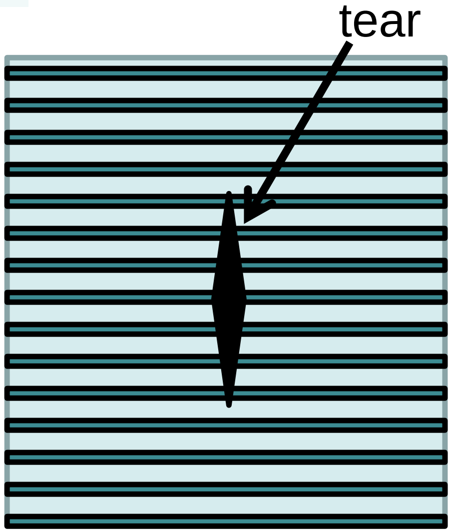
<u>General Case</u>:

Fibre = stiffer

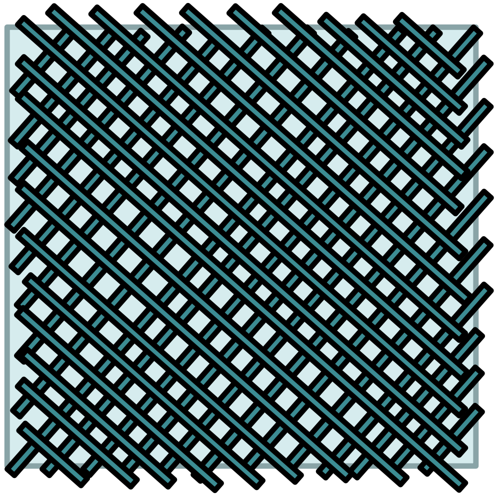
Matrix = more viscous



Reduce the influence of fibres

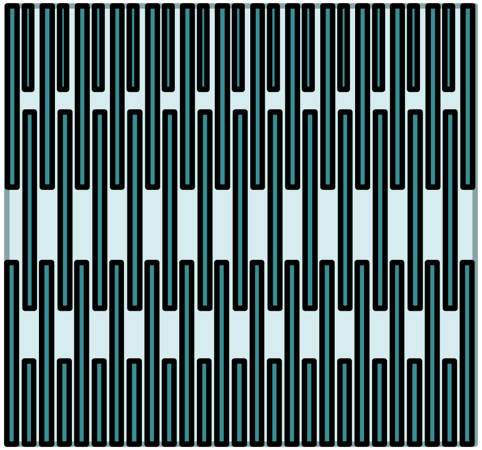


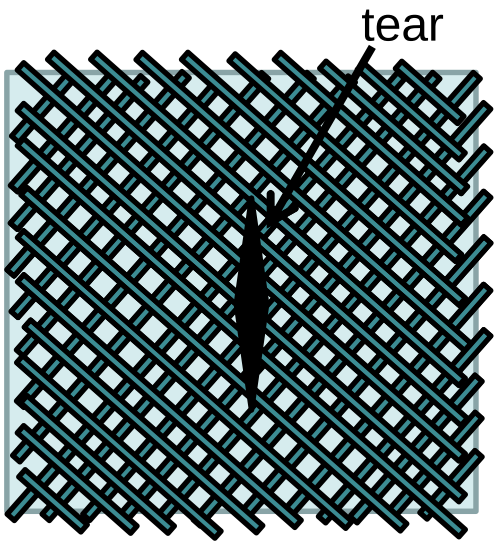
Tears don't propagate easily (tough)

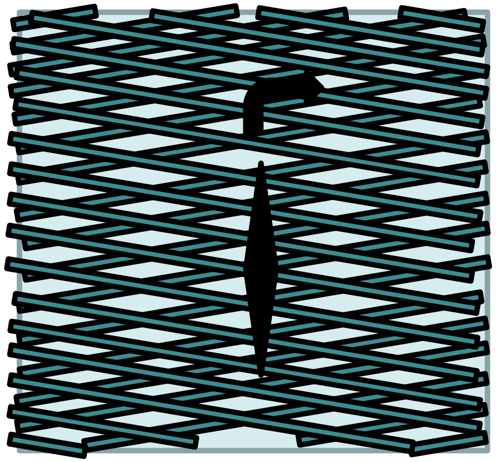


Strength in multiple directions
Balance fibre reorientation with tensile strength

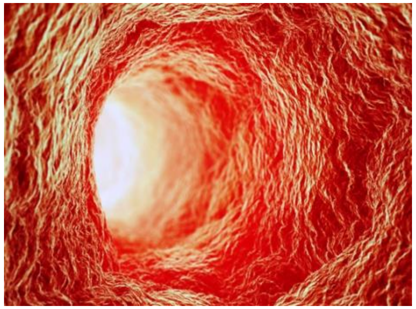
Organising Fibres – Strength, Elasticity & Toughness





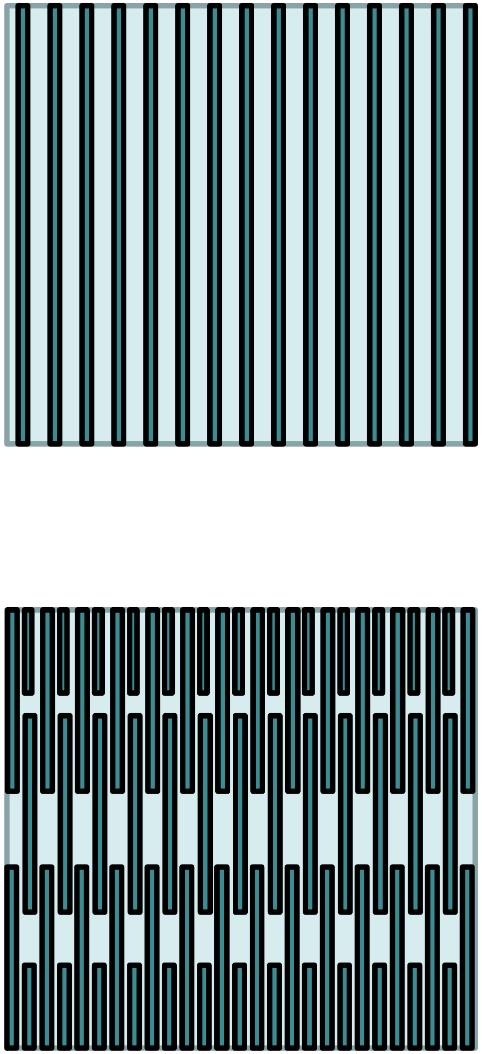


Extensible but tough



Circumferential elasticity & resilience most important

Fibre Composite Theory



Volume fraction equation

$$E_c = E_f V_f + E_m(1-V_f)$$

⇩

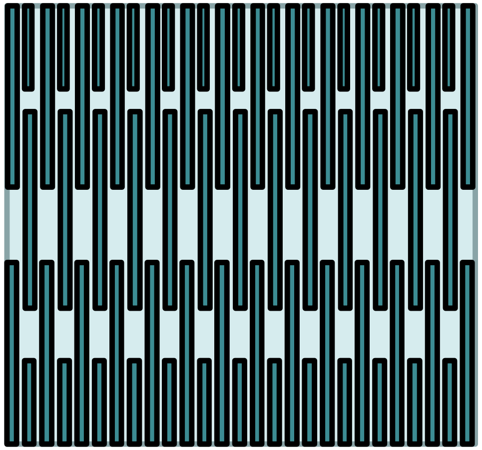
Difference in stiffness between the fibres & matrix = shear gradients along the matrix material

$$E_c = E_f \boxed{V_f} (z) + E_m(1-V_f)$$

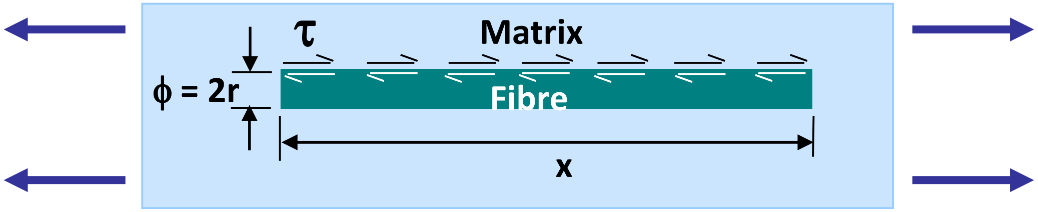
Complex “correction factor”: Fibres and matrix are never fully bonded. Accounts for this.

Highly dependent on fibre dimensions & relationship between G & E

Fibre Composite Theory



Relationship between G and E clearly critical

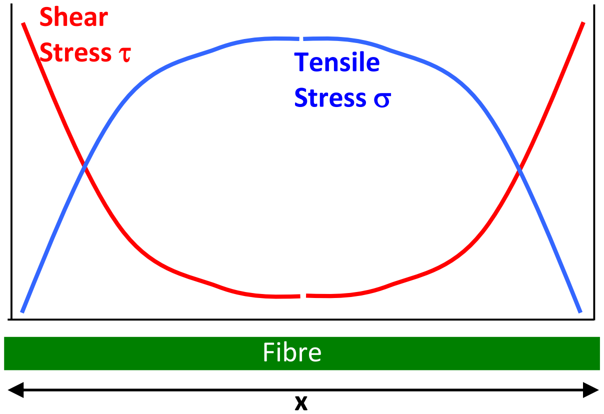


Fibre tensile force:

$$\mathbf{F}_{\mathrm{T}} = \pi r^2 \sigma_{\max}$$

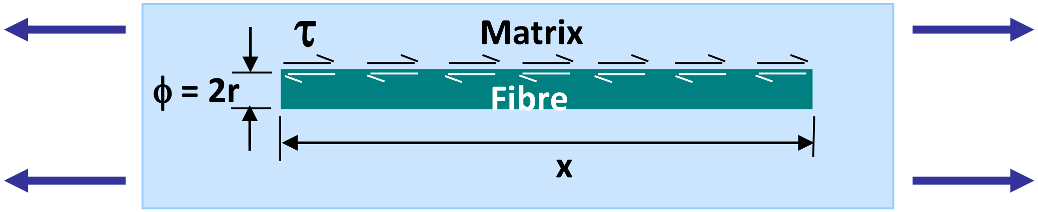
Fibre shear force:

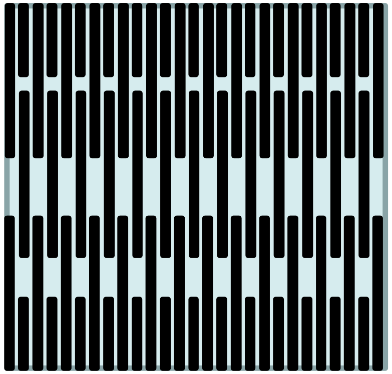
$$\mathbf{F}_{\mathrm{S}} = 2\pi r x \tau_{\max}$$



$$\boxed{\text{Composite Maximum Force} \qquad \mathrm{F} = f\left(\mathrm{F}_{\mathrm{T}} + \mathrm{F}_{\mathrm{S}}\right)}$$

Fibre Composite Theory





Discontinuous fibres

Force must be transmitted to fibres through shear along the fibre length

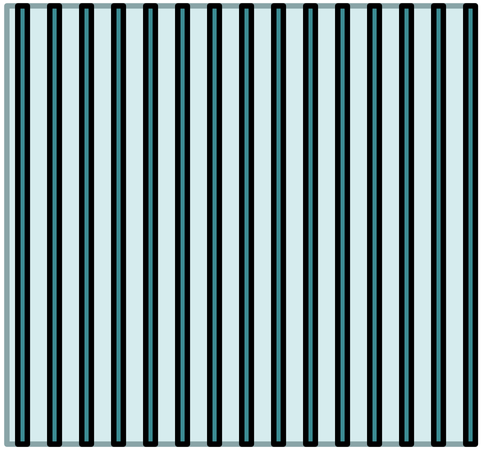
Strain is shared between the fibres and the matrix

Fibres must be long enough for the shear forces to be sufficiently large

critical fibre length = the minimum length necessary such that all load is transmitted from matrix to fibers

critical fibre length $l_c = \dfrac{\sigma r}{\tau}$

Designing hierarchical fibres



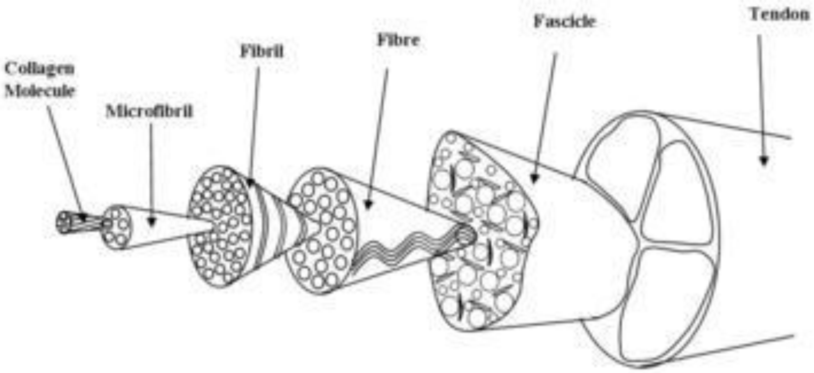
Relationships between fibres & matrix throughout the hierarchy

Tight bonds at low hierarchical levels

More homogenous strain distributions across sample

Less stress transfer at higher hierarchical levels

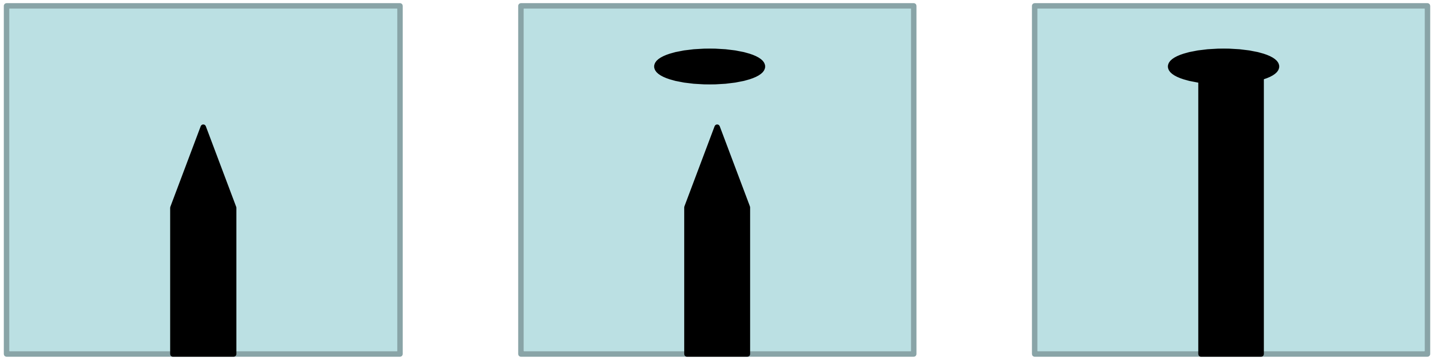
Notch insensitive materials
Don’t propagate damage



Other ways of providing toughness

- Holes

Cook-Gorden crack stopping mechanism (1964)



- Blunt the crack
- Cause it to deflect
- Create highly tortuous crack paths

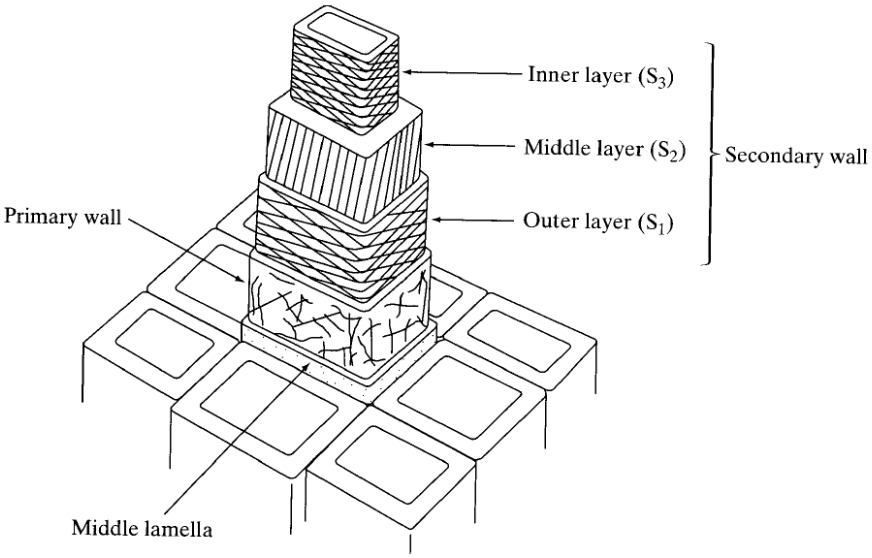
Cracks begin from stress concentrations

As they grow they remove strain energy from surrounds – prevent further cracks

Other ways of providing toughness

- Holes

Careful balance:
Too many holes = weaken structure
Some holes = toughen structure



Wood

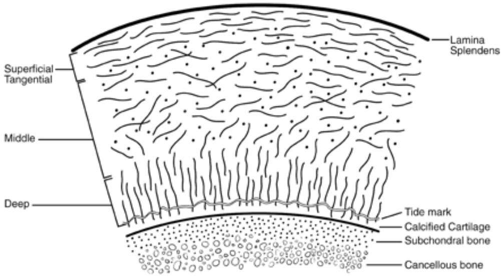
Fibre composite features:
- Fibre composite: Cells are the “fibres”
- Layers with different organisation of cellulose microfibres – prevent crack propagations

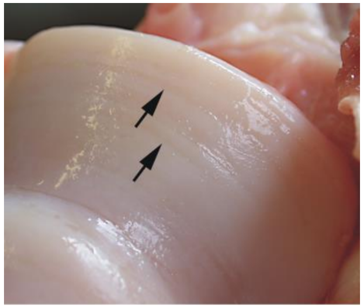
Holes for toughness:
- Vessels in the xylem for water transport

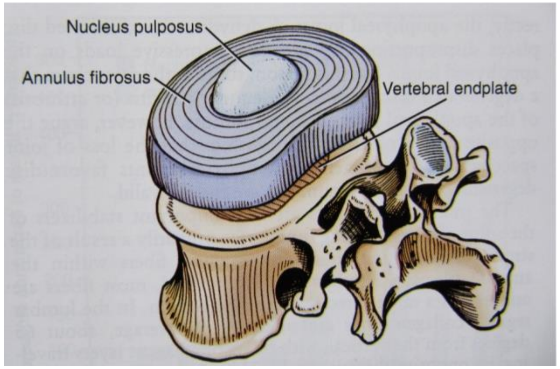
- Ash = Uniformly distributed = high toughness

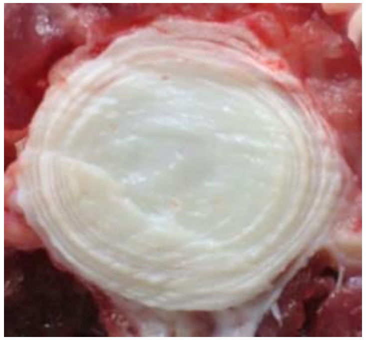
Organising Fibres – Prestress & Compression

- Use water to manage compression
  - Fibre wound containers
  - Fibre resists the tension from internal pressures
  - Matrix (gel) stabilises the water









Organising Fibres- Stiff Materials

- Fibres in matrix = pliant
  - Can carry loads along length
  - Can resist internal pressures

- Managing longitudinal compression?

Add a materials phase to stiffen the matrix

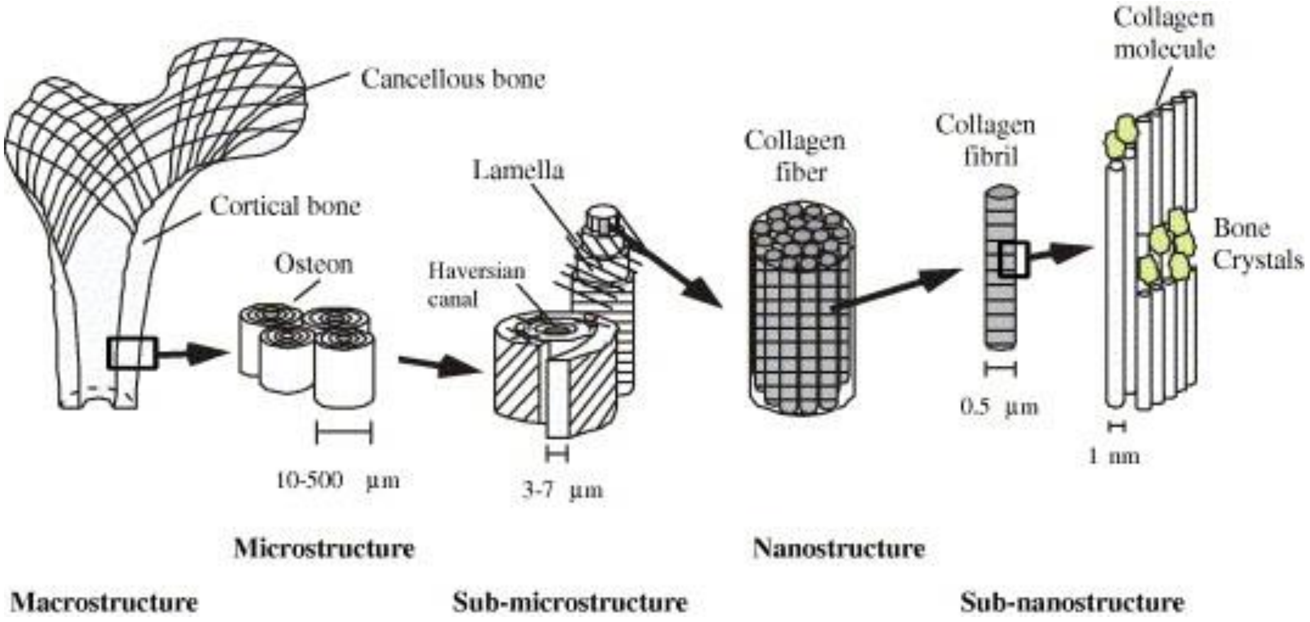
Correctly order fibres to directly resist compression
Package & secure them, so they can not reorientate

Biological Ceramics

- Stiffening Matrix Phases = Calcium Carbonate

Balancing greater stiffness and strength with reduced toughness

Bone / teeth ⇨ $Ca_3\ (PO_4)_3\ (OH)$ = Hydroxyapatite



Collagen scaffold with crystals for higher stiffness

Bone

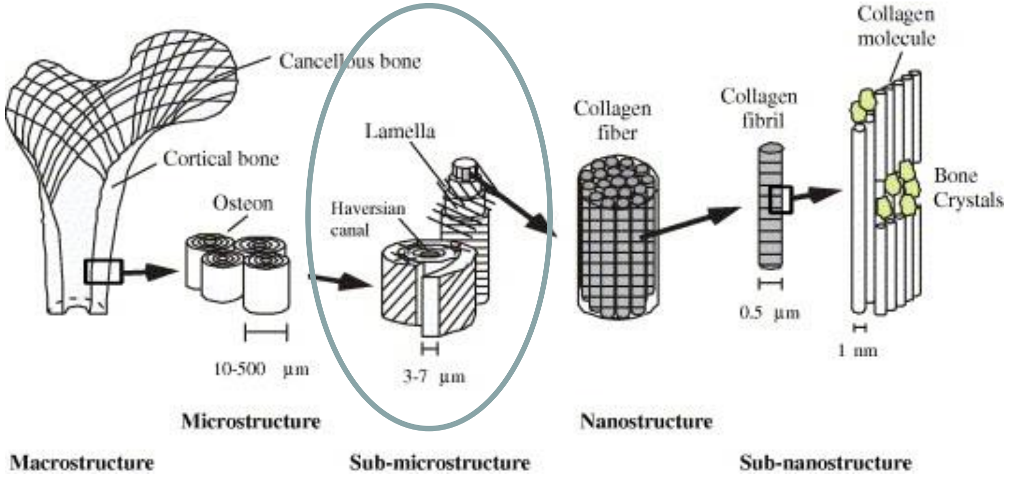
- How do you consider bone?

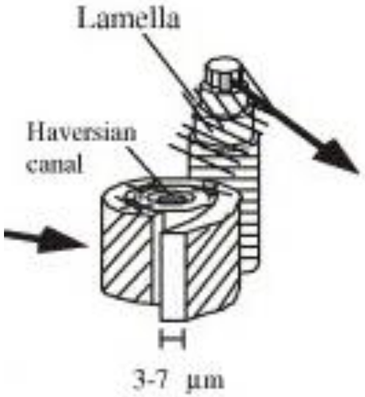
2 phase composite of crystal and collagen?

Bone has a range of crystal content
When is crystal content enough to be the main weight bearer?
Stress transfer between collagen and crystal?
How about other matrix components?

Fibre composite of collagen in matrix stiffened by mineral

How about the higher hierarchical levels?





Levels of complexity

Layers aligned in varying orientations

Organising Fibres- Stiff Materials

- Fibres in matrix = pliant
  - Can carry loads along length
  - Can resist internal pressures
- Managing longitudinal compression?

Add a materials phase to stiffen the matrix

Correctly order fibres to directly resist compression
Package & secure them, so they can not

Organising Fibres- Stiff Materials

- Correctly order fibres to directly resist compression
- Package & secure them, so they can not re-orientate

- Materials which can not afford to be too heavy
  - Insect skeletons (cuticle)
  - Plant cell walls

Increasing Hardness & Stiffness?

- Teeth: Layer hard enamel of top of tough dentin

Dealing with the material interface (very different stiffnesses)

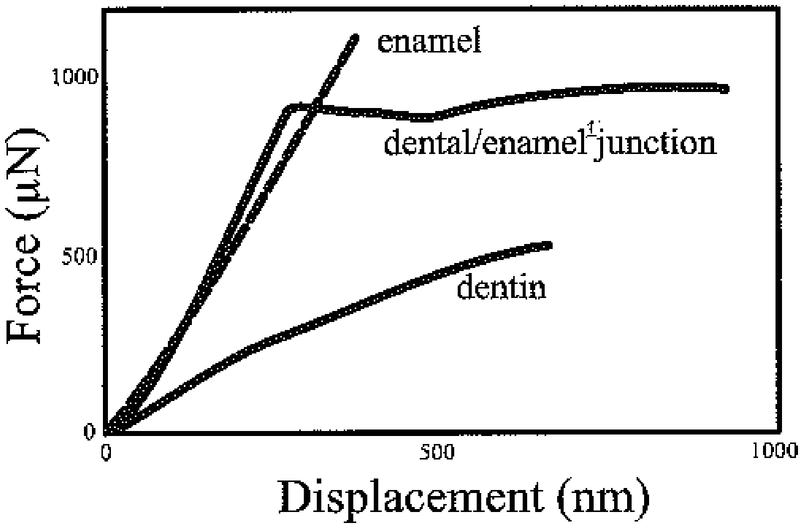
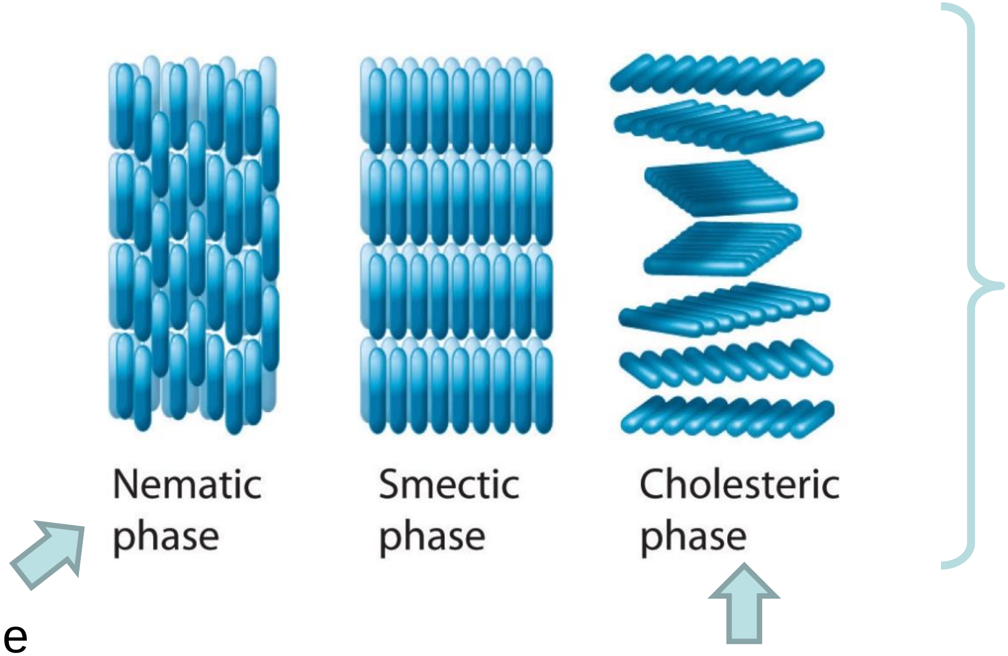


Figure 6.30. Force-displacement curves of enamel and dentin, in an effort to understand the mechanical nature of the interface between the two (Chan 2010).

Significant yield in the junction to facilitate mismatch

Self Assembly of Materials

- Cells make the molecules for each tissue
- How to they assemble to make tissues?
  - Liquid Crystals?
  - “Liquid” molecules assemble & orientate, to create structurally optimised self-assembled materials.



Readily form hierarchical assemblies: Ideal for toughness – energy dissipation through the differently ordered hierarchical levels

Bone

Annulus Fibrosis

An insight into the following lectures....

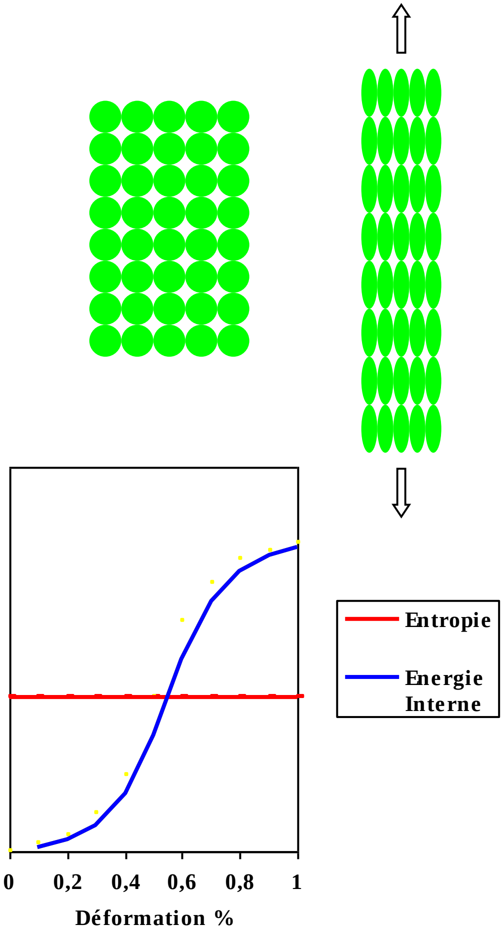
- How do we create specific material properties?
- How are the different constituent components used?
- How are they built is hierarchical levels to optimise behaviour?
- How are subtle variations in structure and composition used to good effect?
- How are transitions between materials optimised to meet functional need and connect different materials?

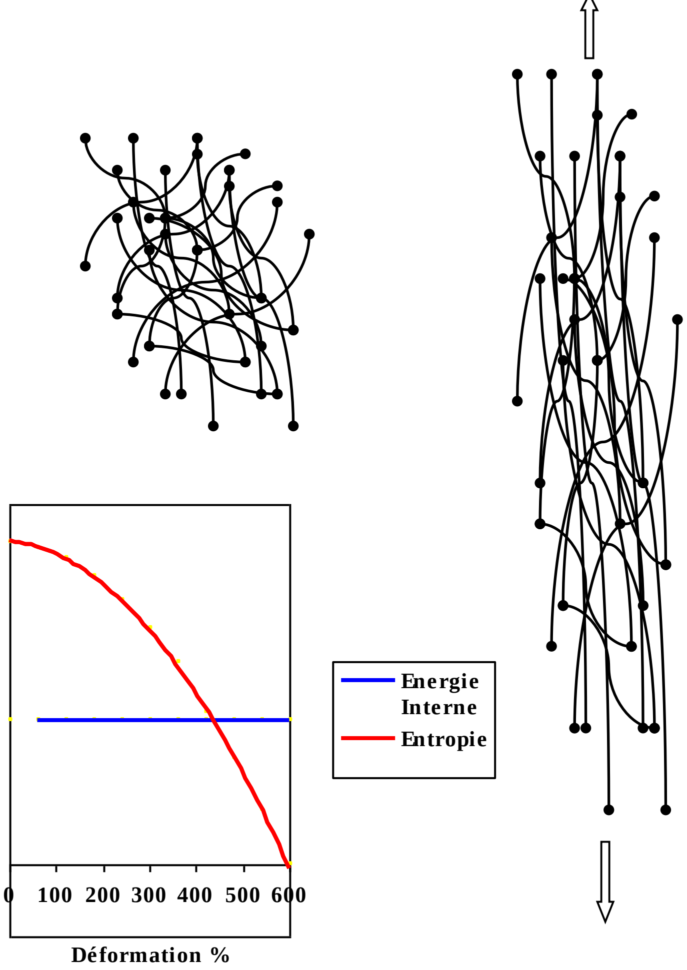
Overview

1. What are the building blocks?
   - How are these polymers built and manufactured?
   - What properties to these structural units convey?
2. How are the building blocks combined to create different structural tissues?
   - Optimising for different mechanical requirements
3. Why we study this and the latest research
   - Biomimetic material development
   - Understanding tissue health & disease – injury prevention
   - Mechanobiology – the mechanical cell cues for mechanotransduction

Elastic function of Soft biological tissues

Further reading:
Avril, Stéphane. "Hyperelasticity of soft tissues and related inverse problems." *Material Parameter Identification and Inverse Problems in Soft Tissue Biomechanics*. Springer, Cham, 2017. 37-66.
And:
https://www.docsity.com/pt/lecture04-mechanics/4739123/



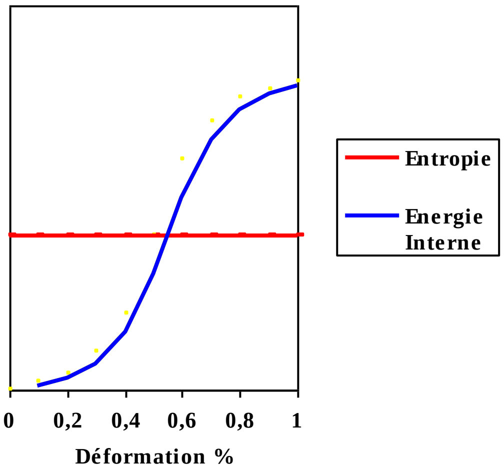


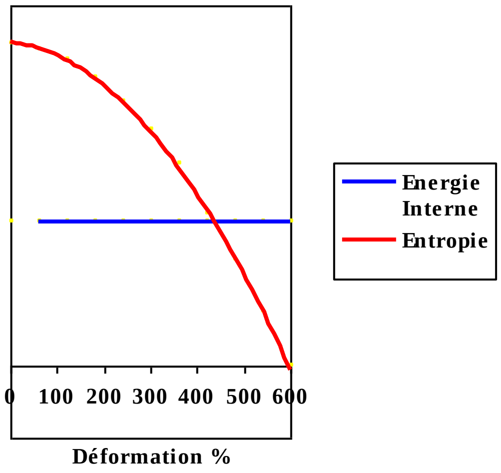
enthalpic
elasticity
(cristal)

entropic
elasticity
(biological tissue)

Thermodynamics (isothermal, reversible):

$\Delta E = W + Q$ and $Q = T\,\Delta S$





enthalpic elasticity

entropic elasticity

$\Delta E = W + \cancel{T\Delta S}$

$\cancel{\Delta E} = W + T\Delta S$

Composition of soft tissues histology

- Cellules: fibroblasts
- Ground substance
- Collagen (fiber)
- Plates et fibers of elastin

ORIENTED FIBROUS STRUCTURE

Parallel: tendons, ligaments

Biaxial: skin, arteries

Connective Tissue

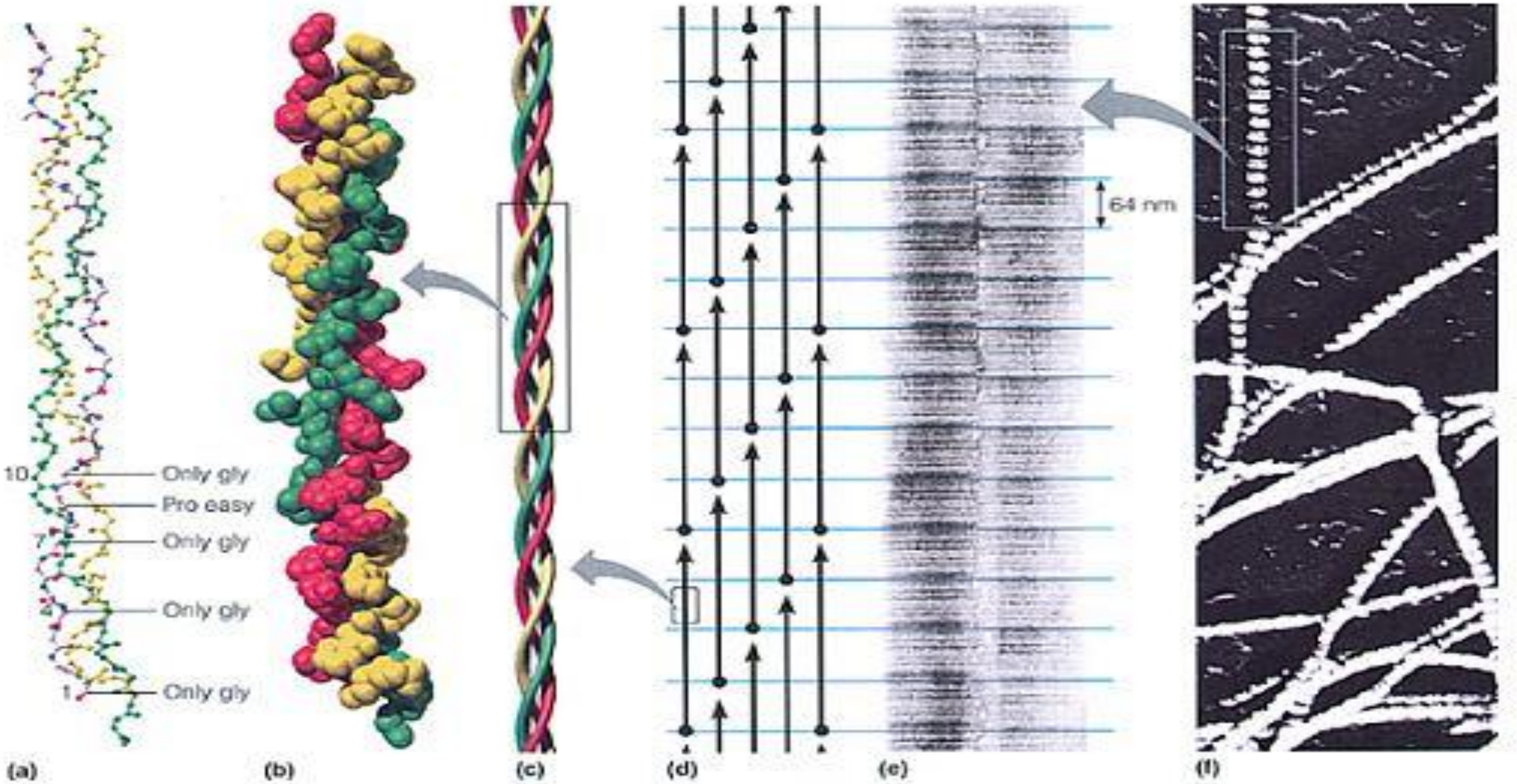
- Extracellular Matrix
  - Fibers
    - Collagen
    - Elastin
    - Reticular Fibers
  - Ground Substance
    - Blood Ultrafiltrate
    - Proteoglycans
    - Glycosaminoglycans

Mechanical Properties

- Fibers give tensile strength and recoil in the direction of the fiber

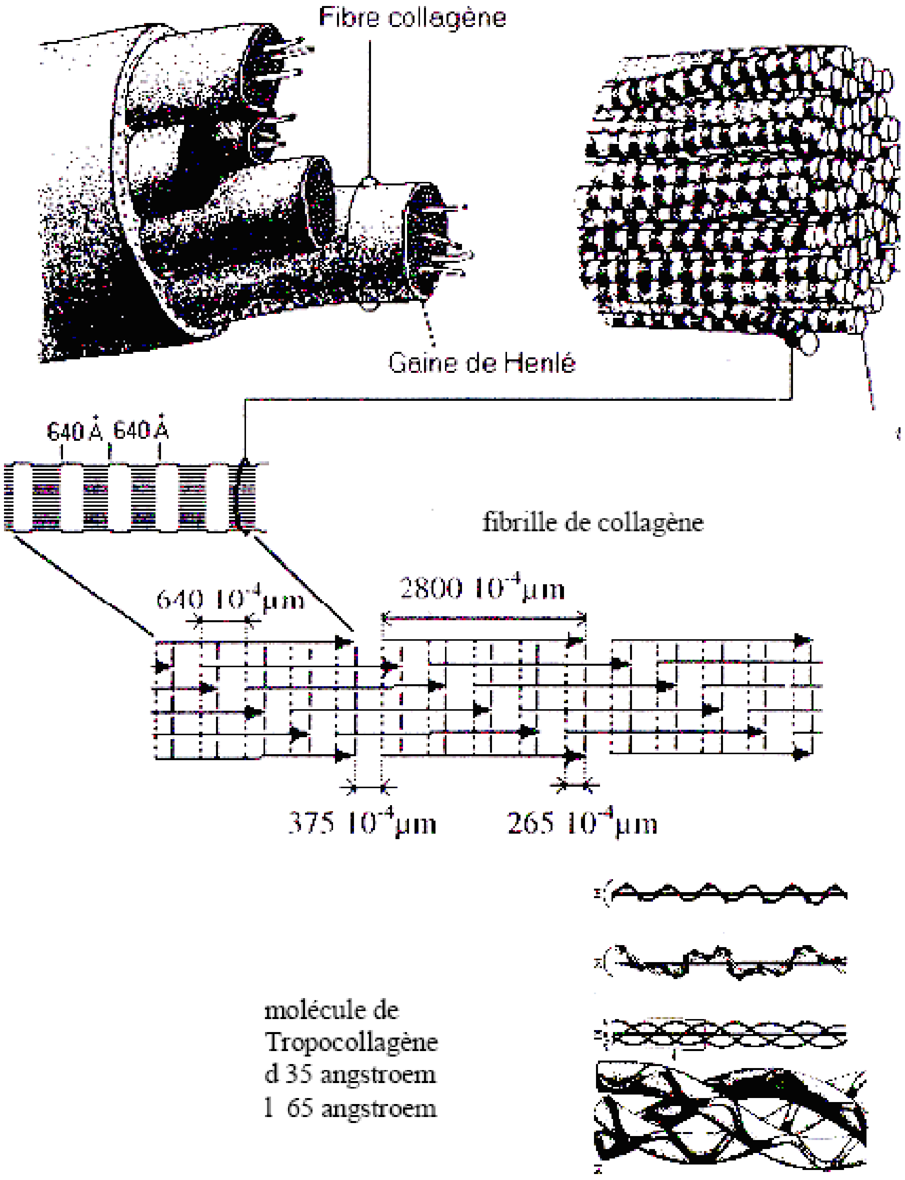
- Ground substance gives compressibility and expansion

Collagen


10
Only gly
Pro easy
7
Only gly
4
Only gly
1
Only gly
64 nm
(a)
(b)
(c)
(d)
(e)
(f)

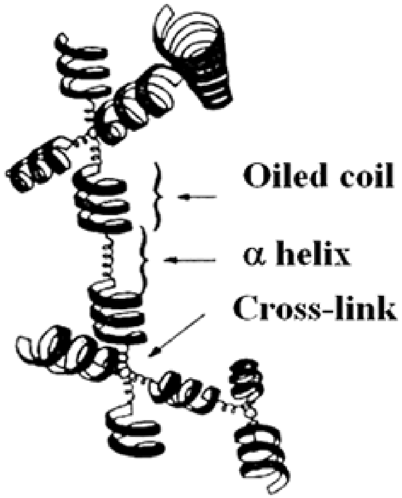
Le collagène

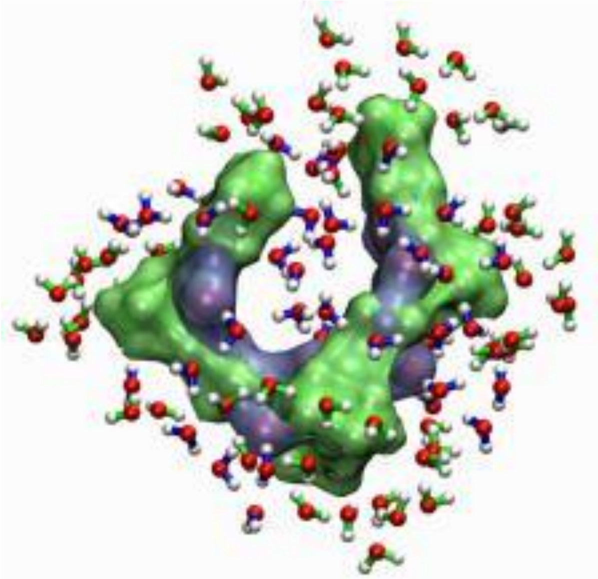
structure hiérarchique

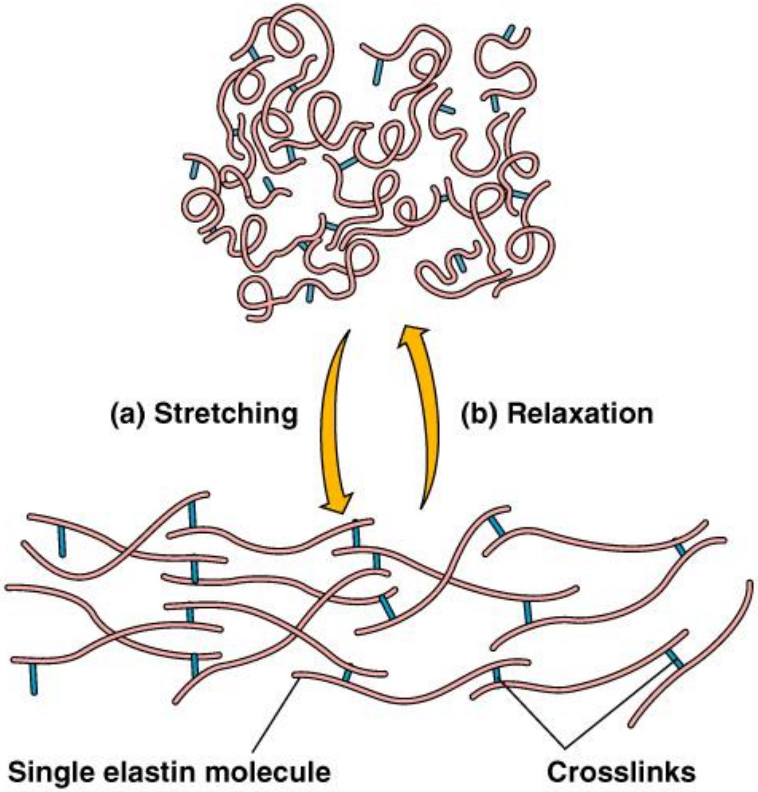


molécule de
Tropocollagène
d 35 angstroem
l 65 angstroem

Elastin







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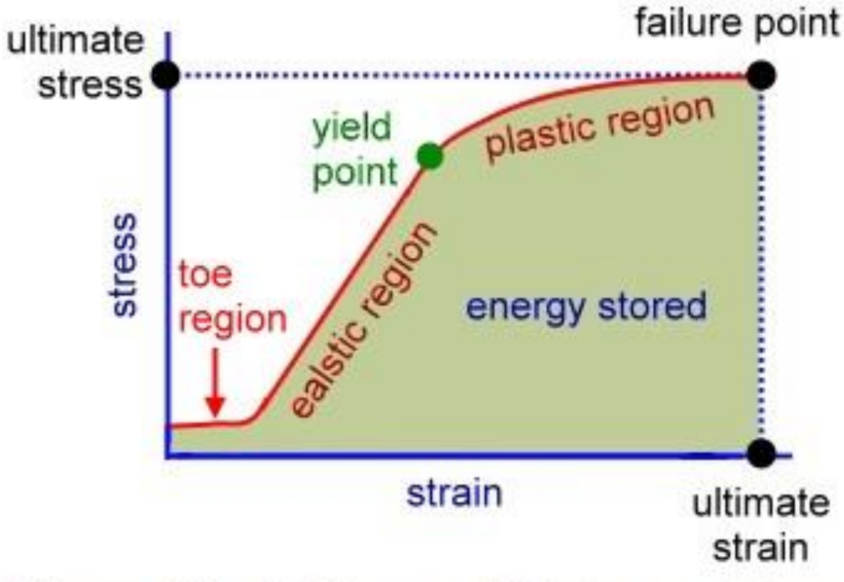
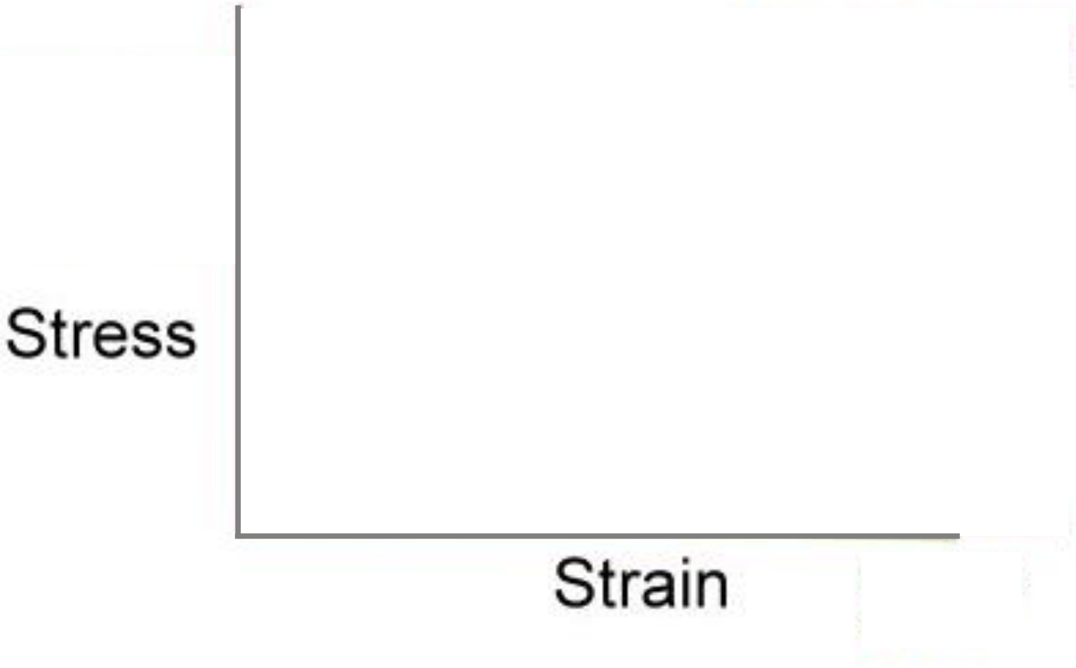
Mechanical Properties



Stress-Strain Curve of Collagen Fiber

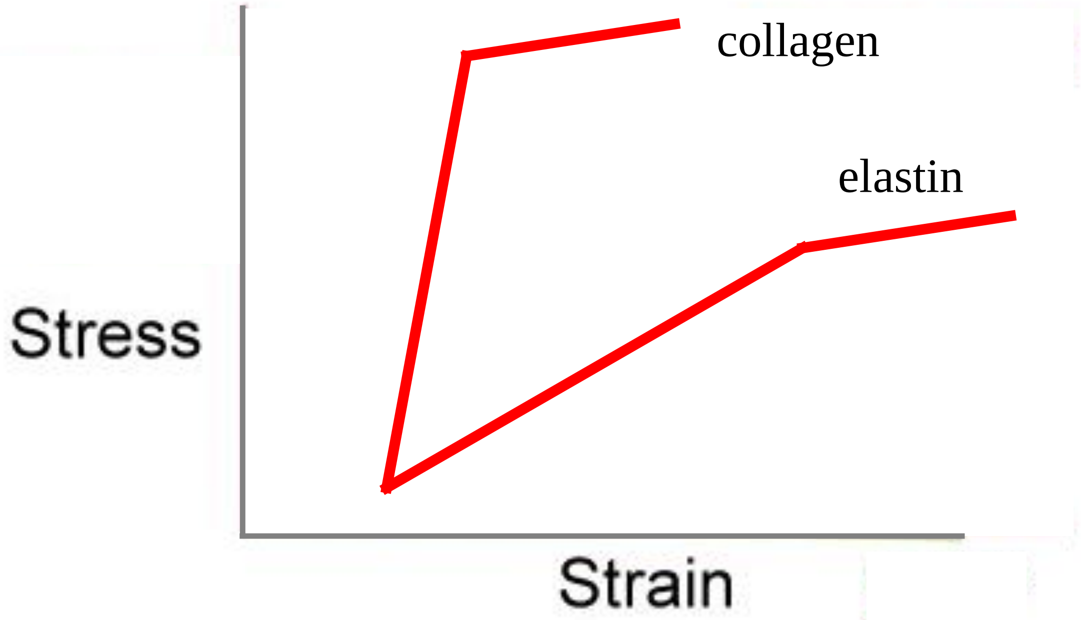
Slope of the “elastic region” determines whether a fiber provides more resistance or allows more recoil

Stress Strain Curve



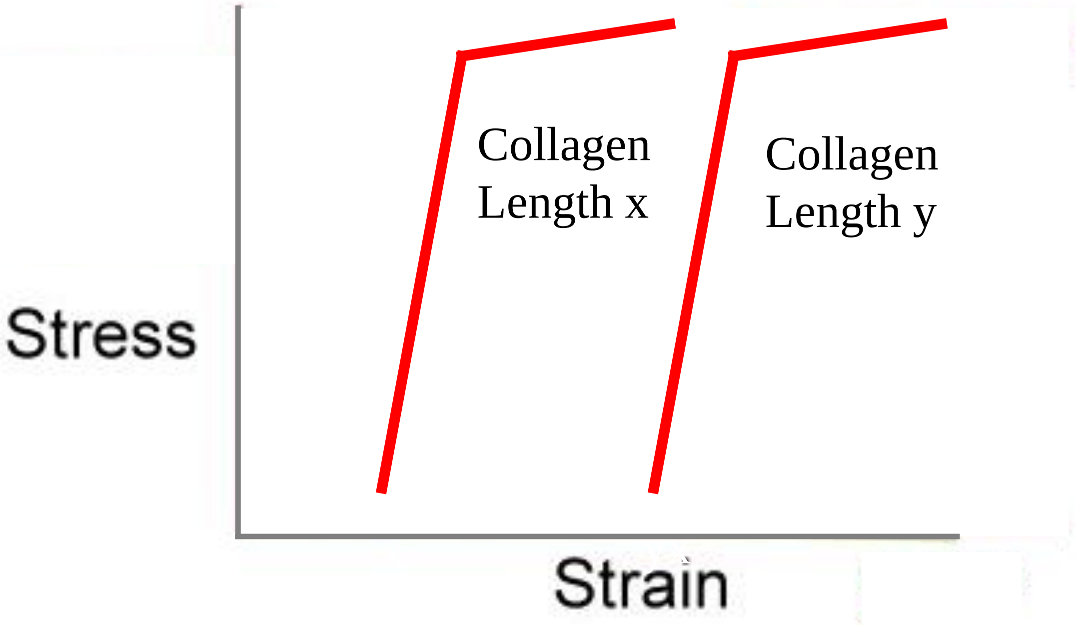
- Strain = elongation / equilibrium length
- Stress = applied force / cross-sectional area

Stress Strain Curve



- Collagen = Lots of stress, minimal elongation
- Elastin = Stress generates excellent elongation

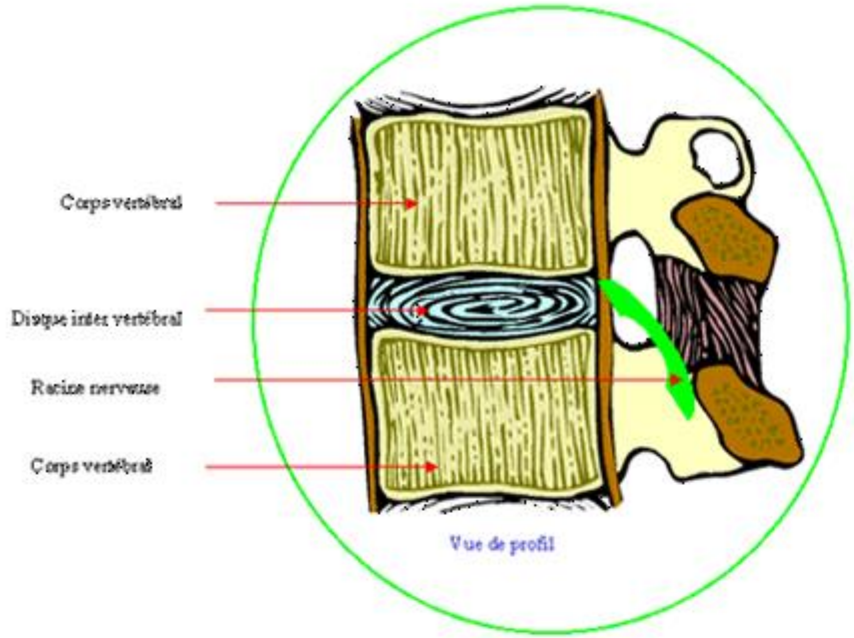
Stress Strain Curve

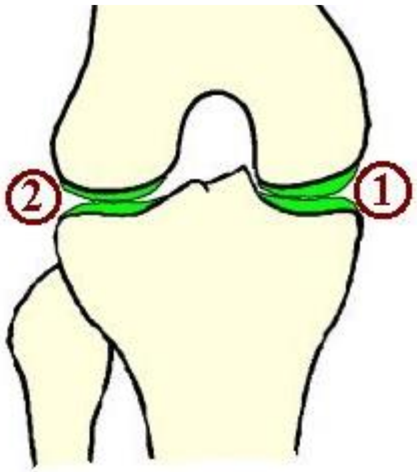


- Collagen = Lots of stress, minimal elongation
- Length of Fibers determine curve

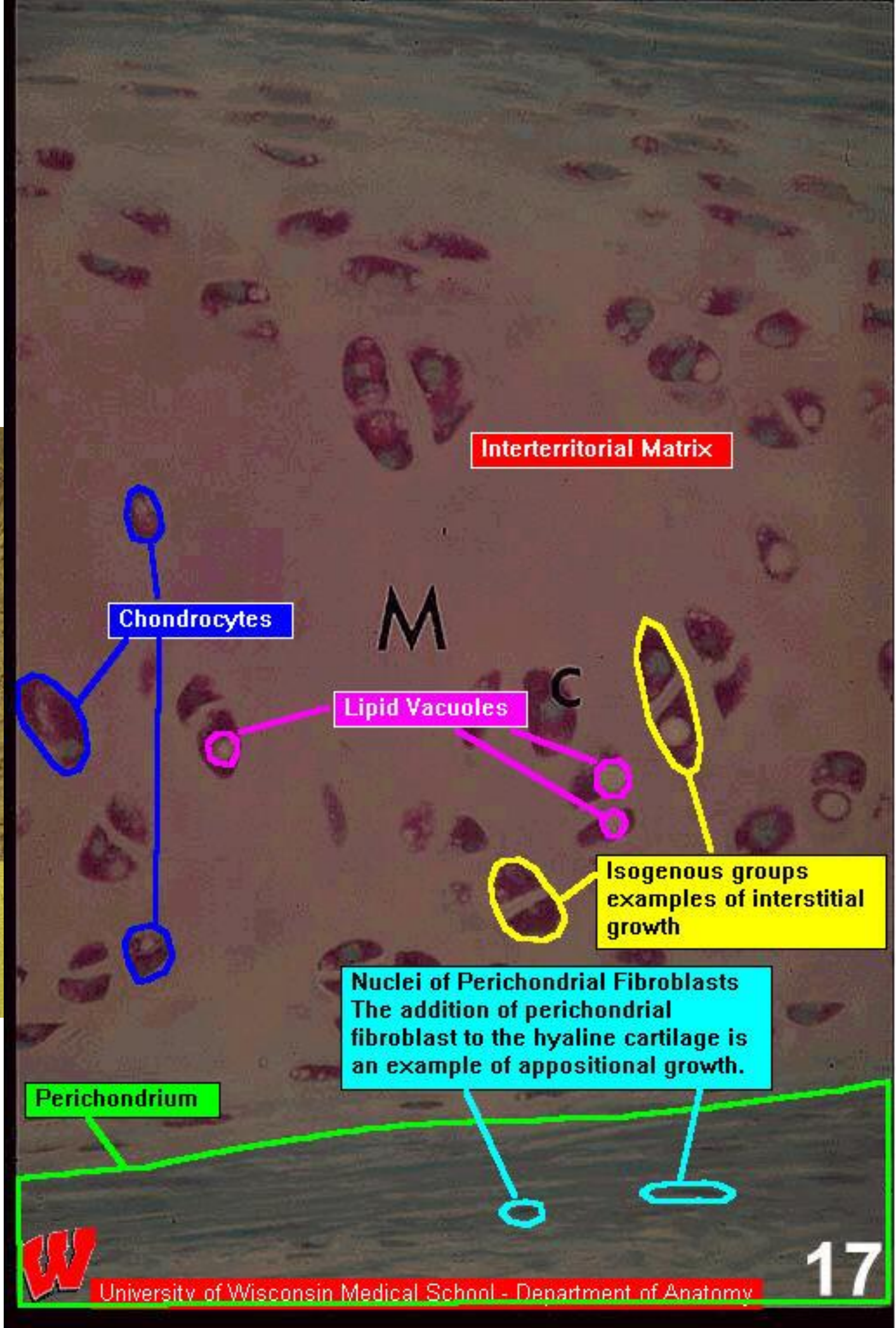
SCHÉMA D'UN DISQUE INTERVERTÉBRAL

cartilage





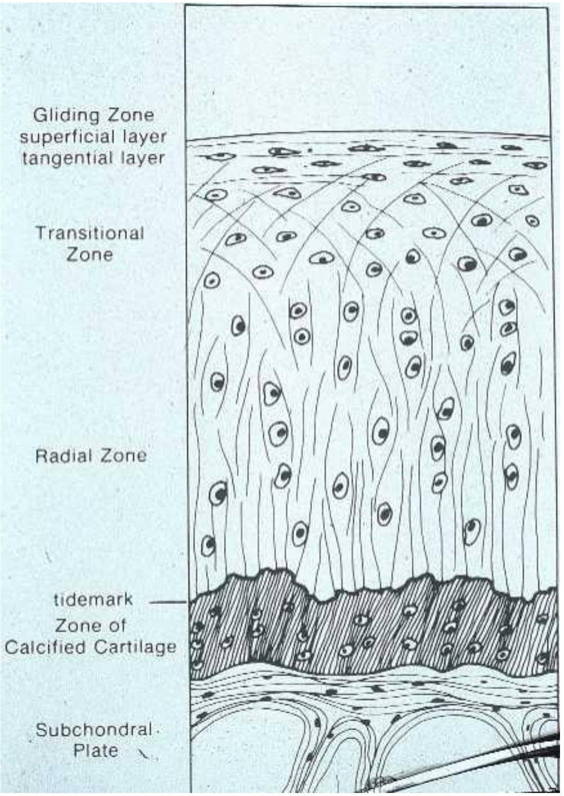
cartilage

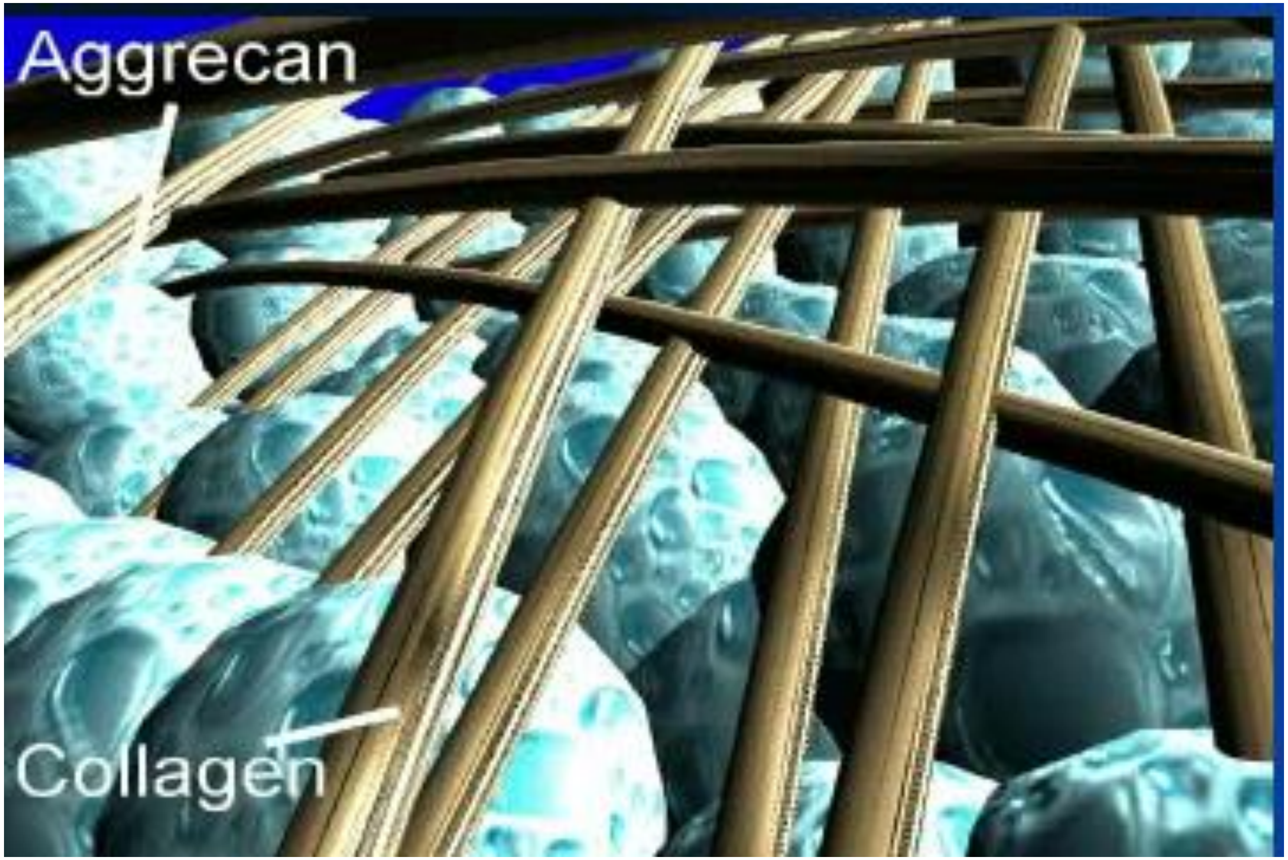


Isogenous groups examples of interstitial growth

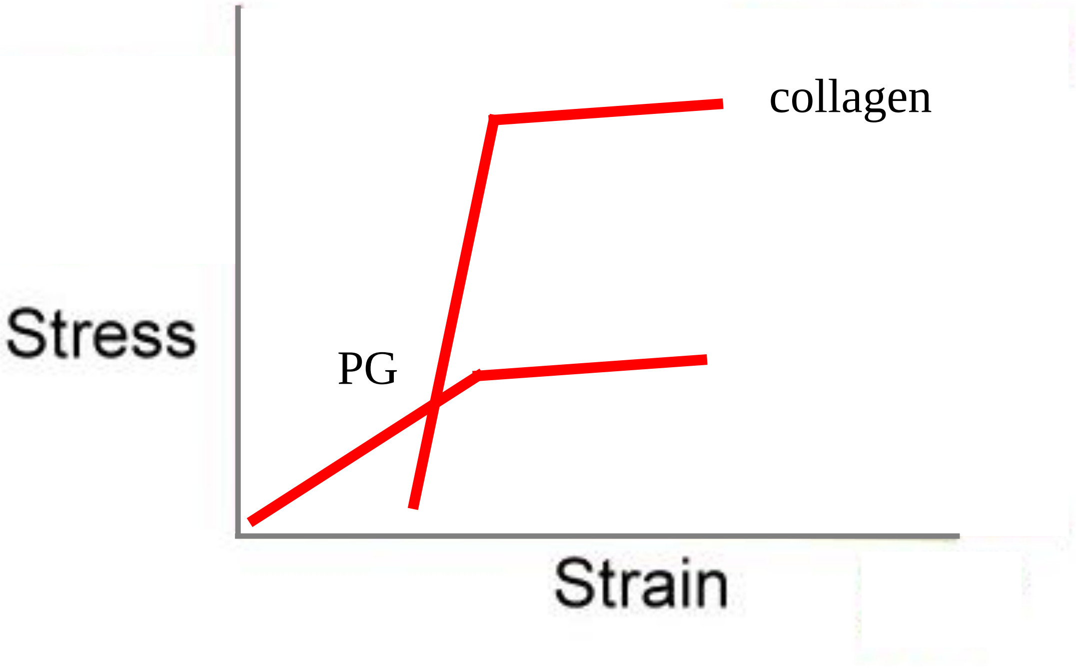
Nuclei of Perichondrial Fibroblasts The addition of perichondrial fibroblast to the hyaline cartilage is an example of appositional growth.

Cartilage Architecture



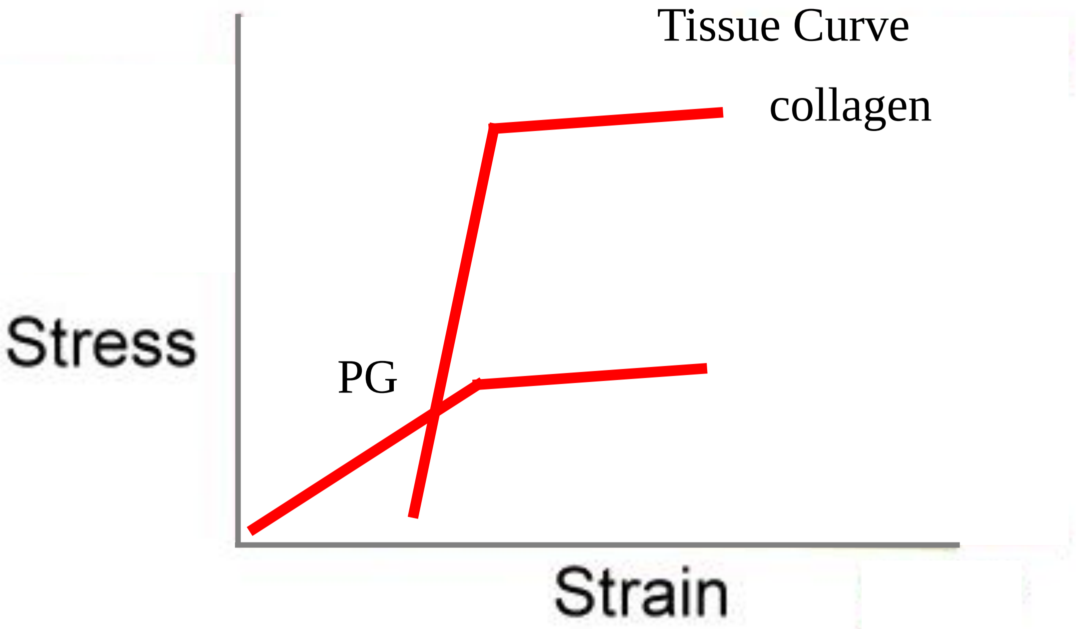


Stress Strain Curve



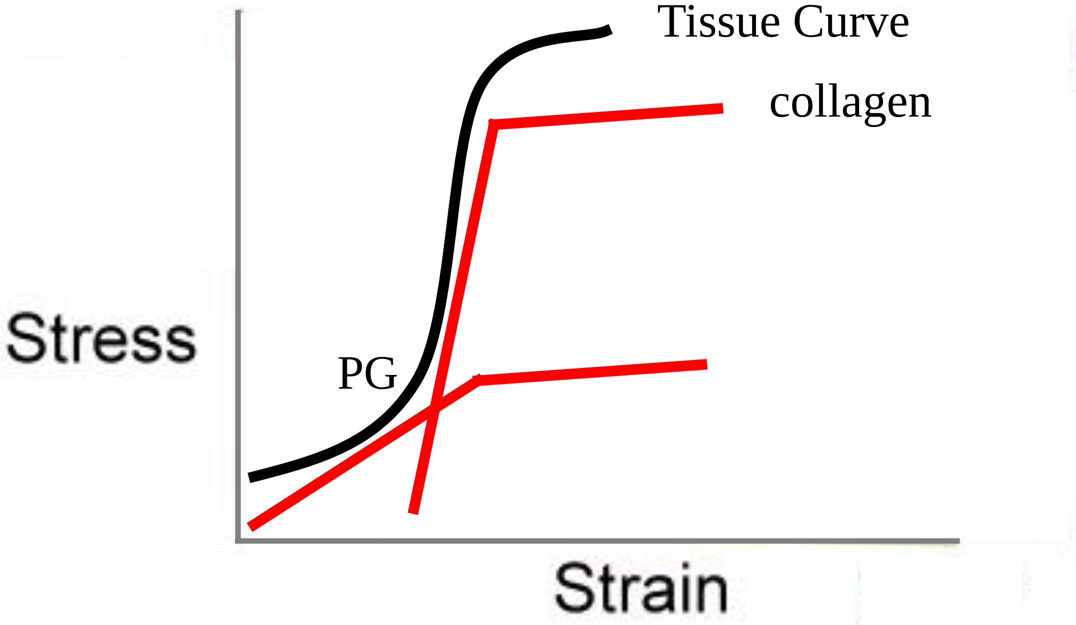
- Collagen, Proteoglycan different curves

Stress Strain Curve



- Total Curve satisfies two different needs:
- Absorb shocks, Resist tension

Stress Strain Curve



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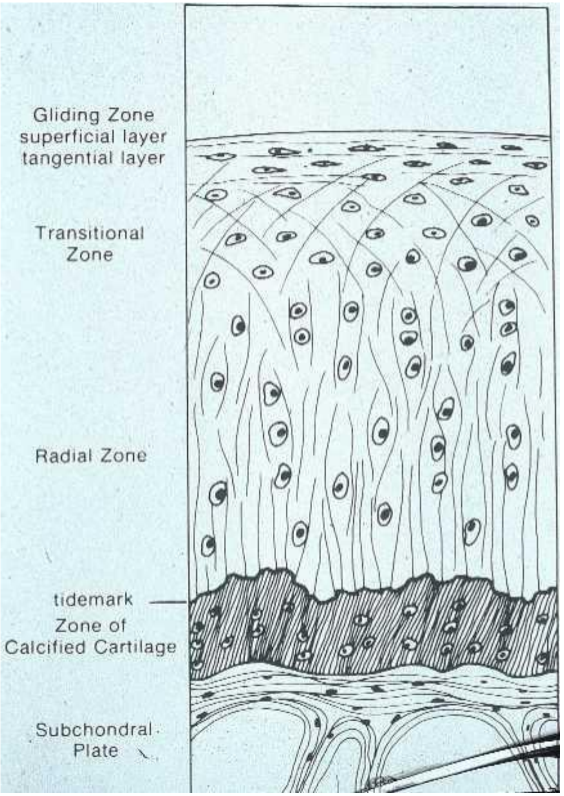
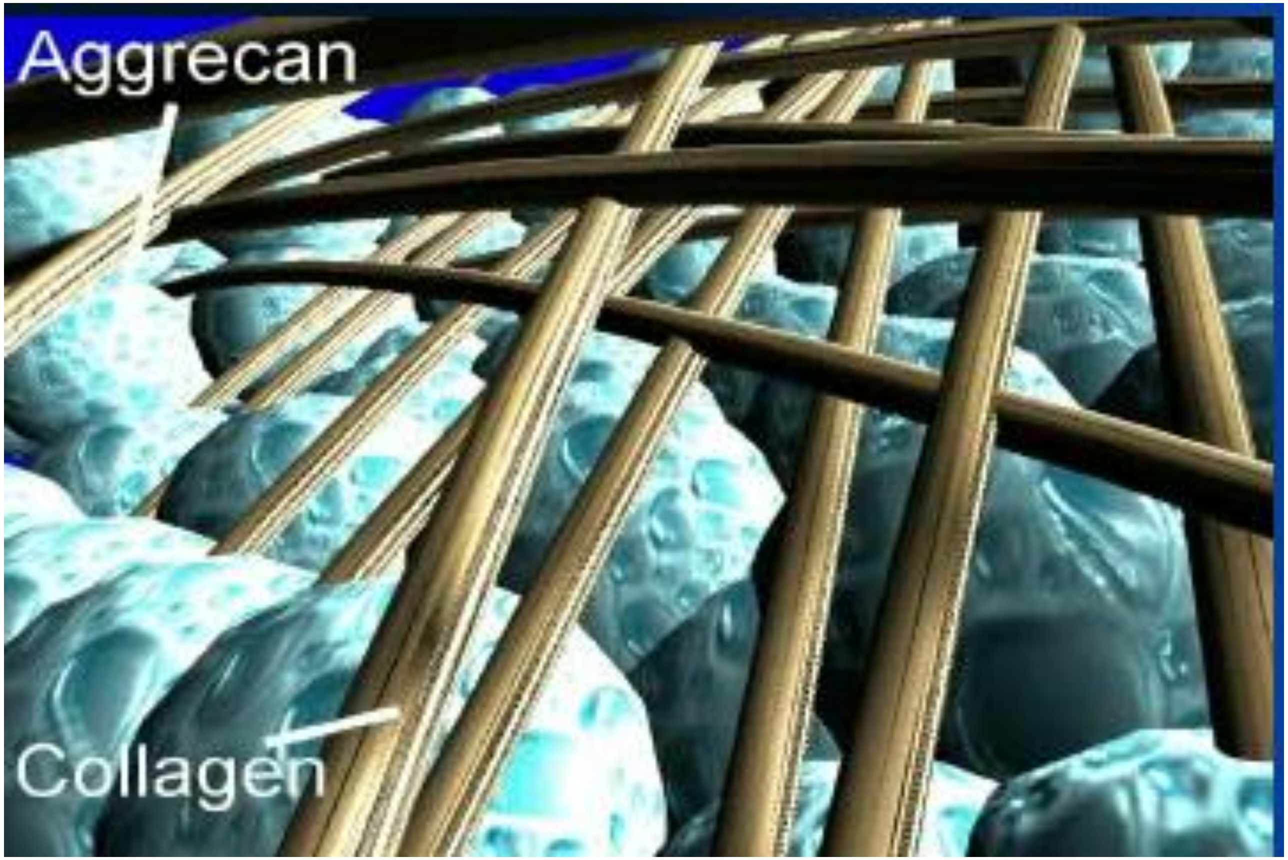
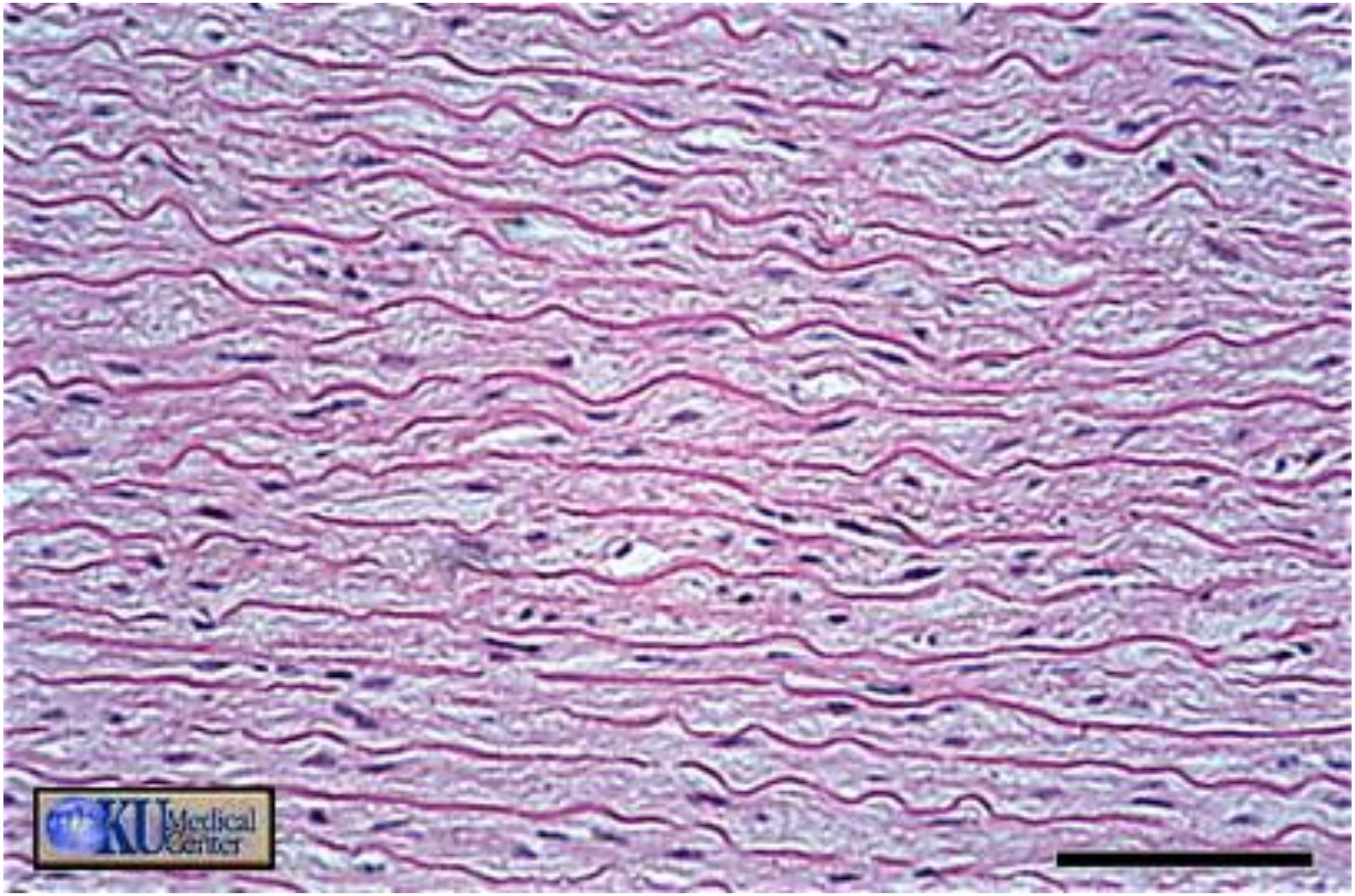
Cartilage Architecture

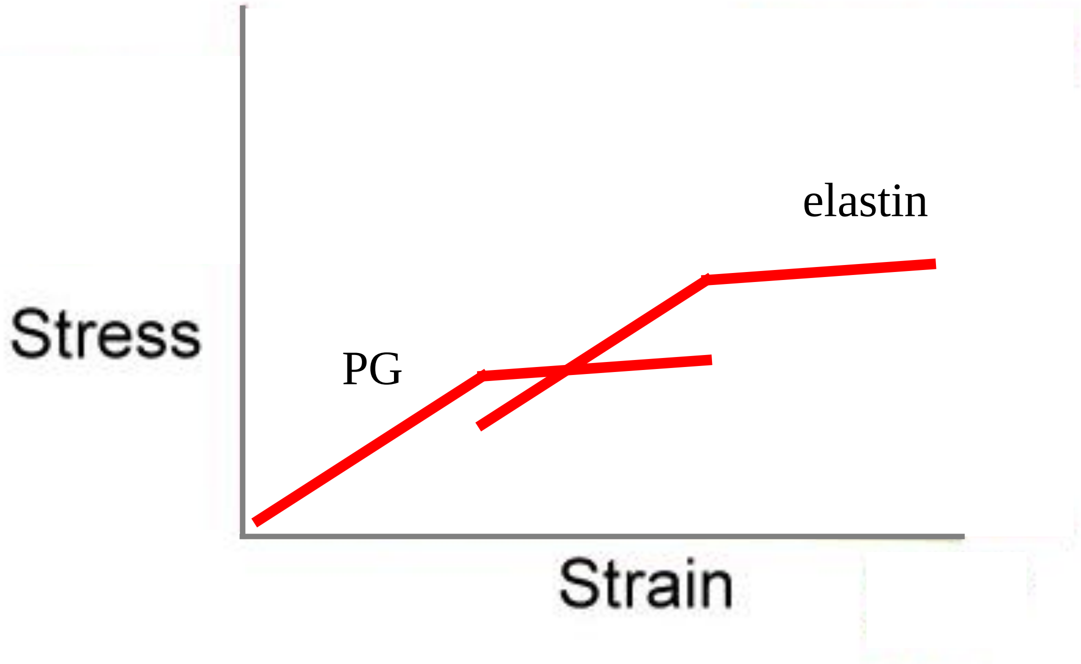




Elastic soft tissues

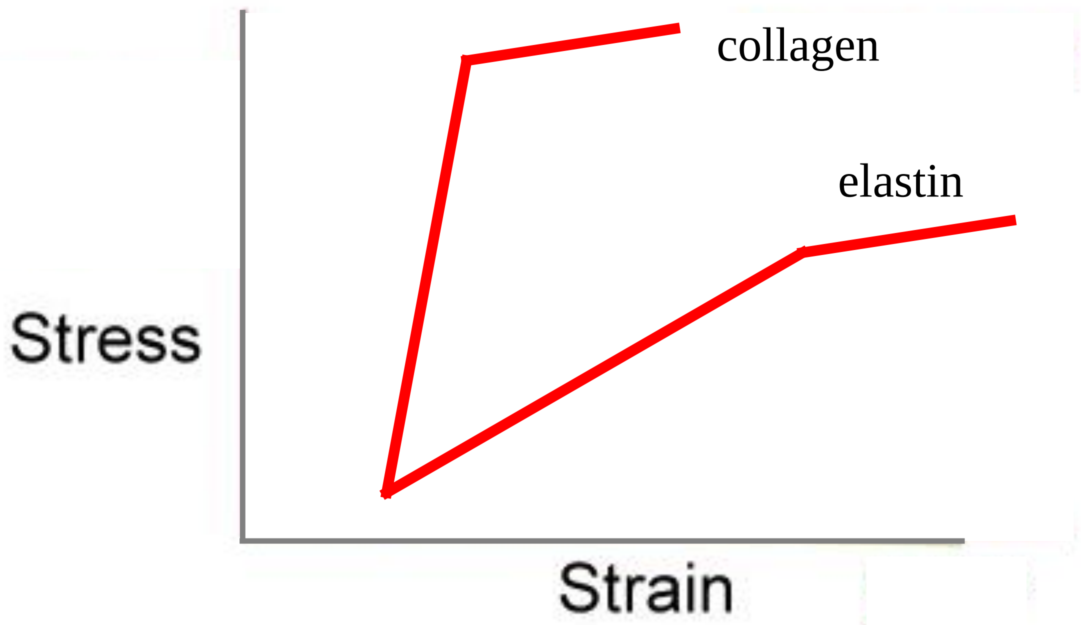


Stress Strain Curve



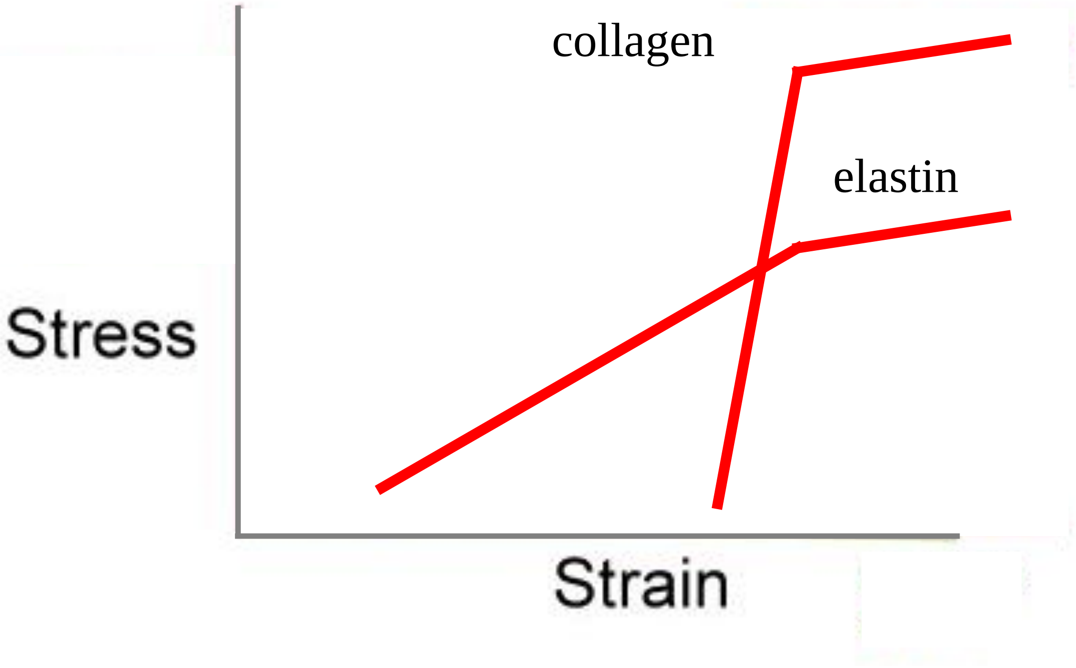
- Elastin, Proteoglycan similar profiles
- Difference is relative starting point

Stress Strain Curve



- Collagen = Lots of stress, minimal elongation
- Elastin = Stress generates excellent elongation

Stress Strain Curve



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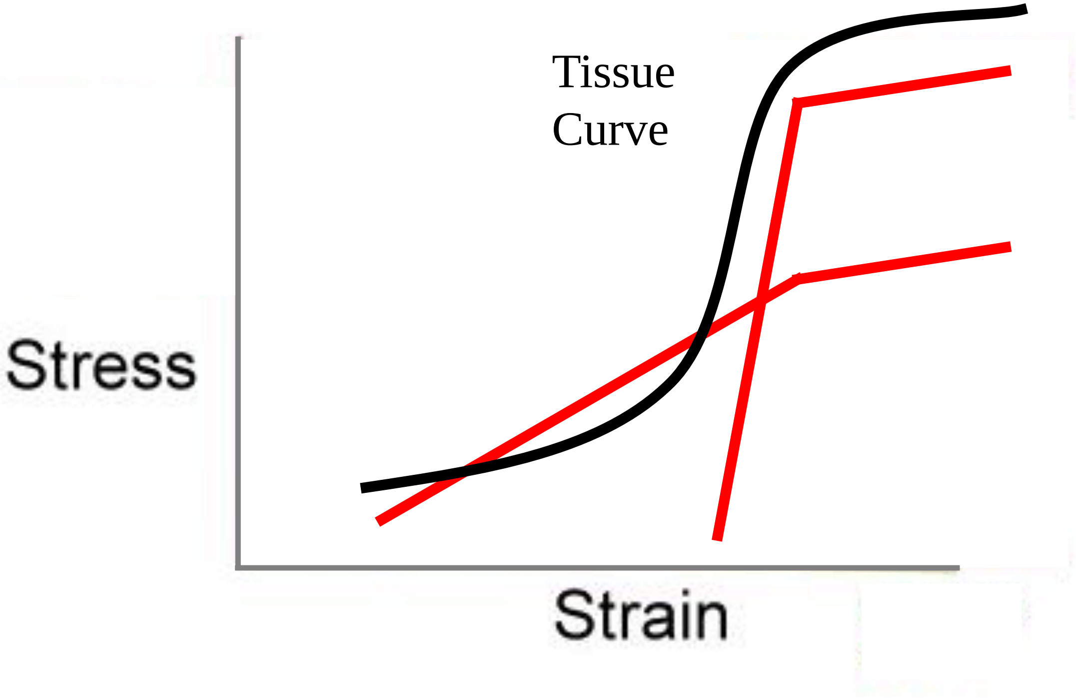
Stress Strain Curve



- Collagen = Lots of stress, minimal elongation
- Elastin = Stress generates excellent elongation

A closer look at tendon and ligaments

Further reading:
Gupta, Himadri S., and Hazel RC Screen. "Structural Building Blocks of Soft Tissues: Tendons and Heart Valves." *Material Parameter Identification and Inverse Problems in Soft Tissue Biomechanics*. Springer, Cham, 2017. 1-35.
Download at https://link.springer.com/chapter/10.1007/978-3-319-45071-1_1

Tendon

- Transmits muscle forces to the skeleton

- Provides a link from compliant muscle to stiff bone
- Acts as a level arm (reduces need for large muscles)
- Saves need to place muscles near joints
- Efficient transfer of forces
    - Limited extensibility
    - Some protection from impact loading
    - Can store energy to assist in locomotion

Ligaments

- Connect bone to bone to limit joint mobility

- Mechanical stabilise joints
- Prevent excess movement in a joint
    - Limited extensibility
    - Some protection from rapid overload (ie twisted ankle)

Understanding Structure-Function

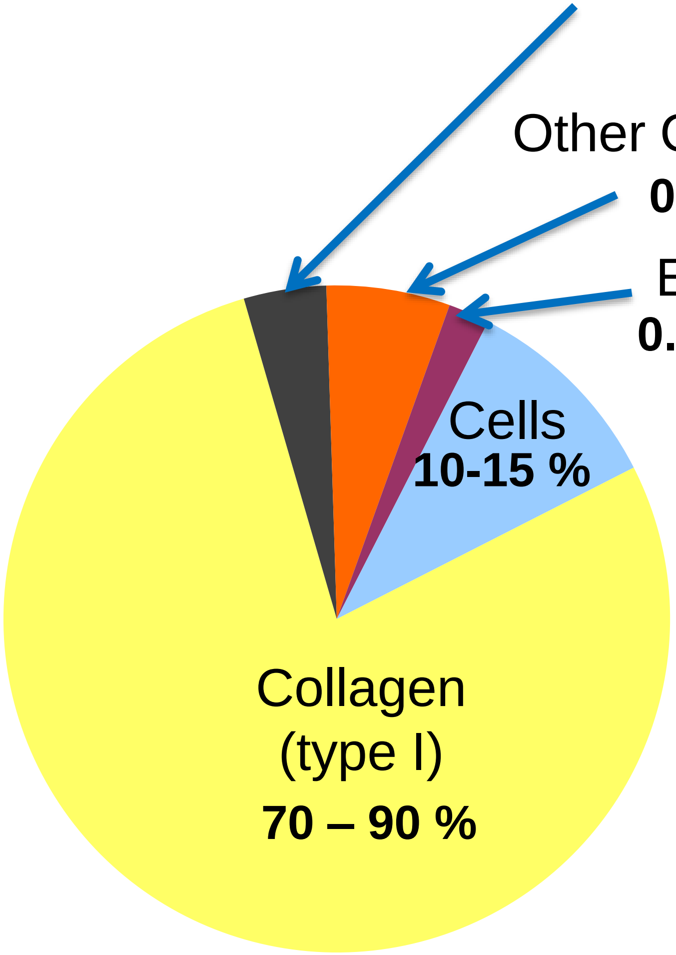
- What a tissue is made of (composition)
- How the constituent components are organised (organisation)

Structure

- How structure leads to mechanical behaviour

Tendon/Ligament Composition

Sample digest and compositional analysis



Proteoglycans
2 – 5 %

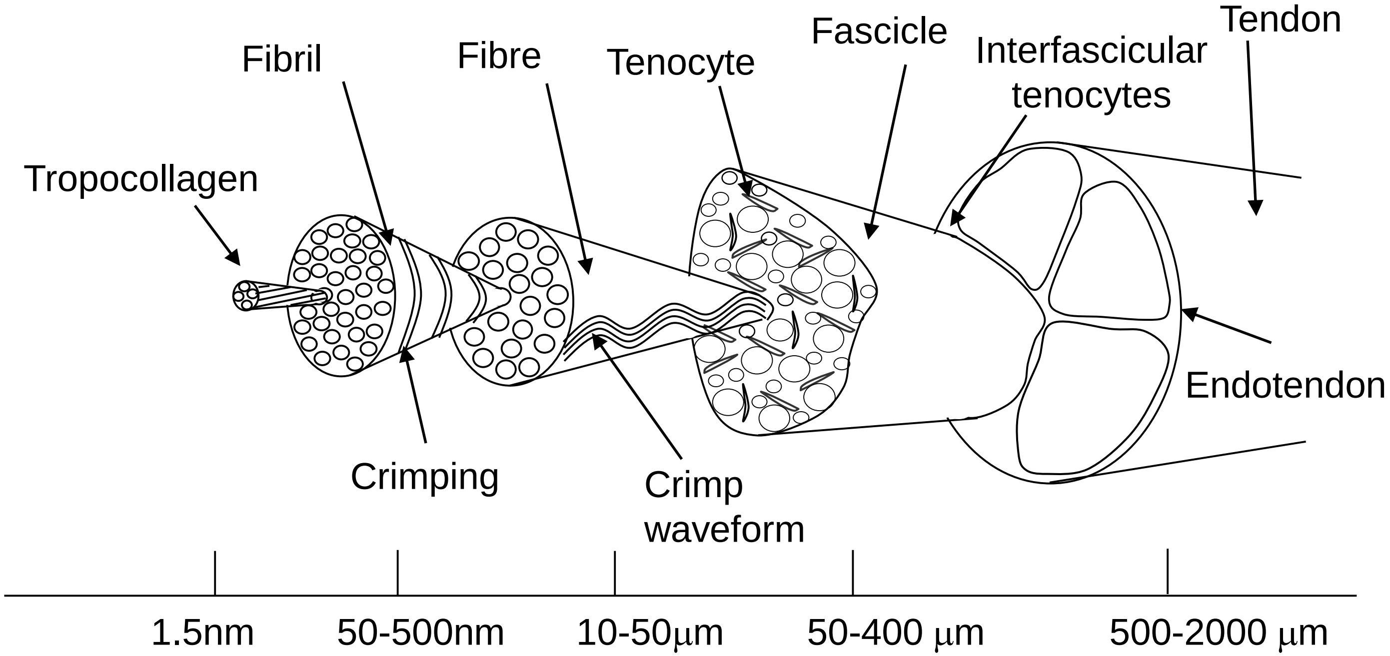
Other Glycoproteins
0.5 – 5 %

Elastin
0.5 – 3 %

- Decorin
- Biglycan
- Aggrecan
- Versican
- Fibromodulin
- Lumican
- Tenascin-C
- COMP (Cartilage Oligomeric Matrix Protein)
- Lubricin

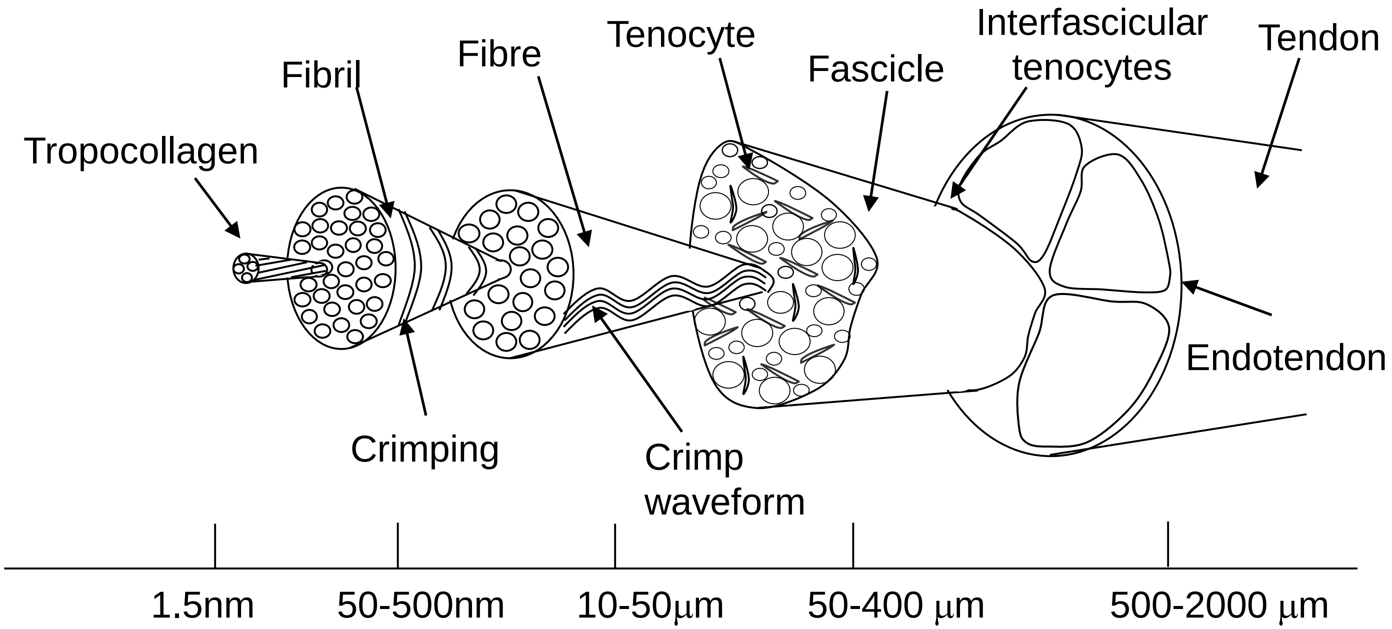
Thorpe et al (2013) Int J Exp Path **94,** 249-259.
Benjamin & Ralphs (1997) *Histol. Histopathol.* **12,** 1135-44.
Blevins (1996) *Curr. Opin. Orthop.* **7,** 57-61
Yoon & Halper (2005) J. Musculoskelet. Neuronal Interact. **5**, 22–34.
Smith (1997) Matrix Biol. **16,** 255–271
Riley (1996) Am. J. Pathol. **149,** 933–943

Tendon/Ligament Structure



- Aligned fibre composite material
  - – Multiple hierarchical levels of collagen
  - – Proteoglycanous matrix binding
  - – Interspersed with cells (tenocytes/ligamentocytes)

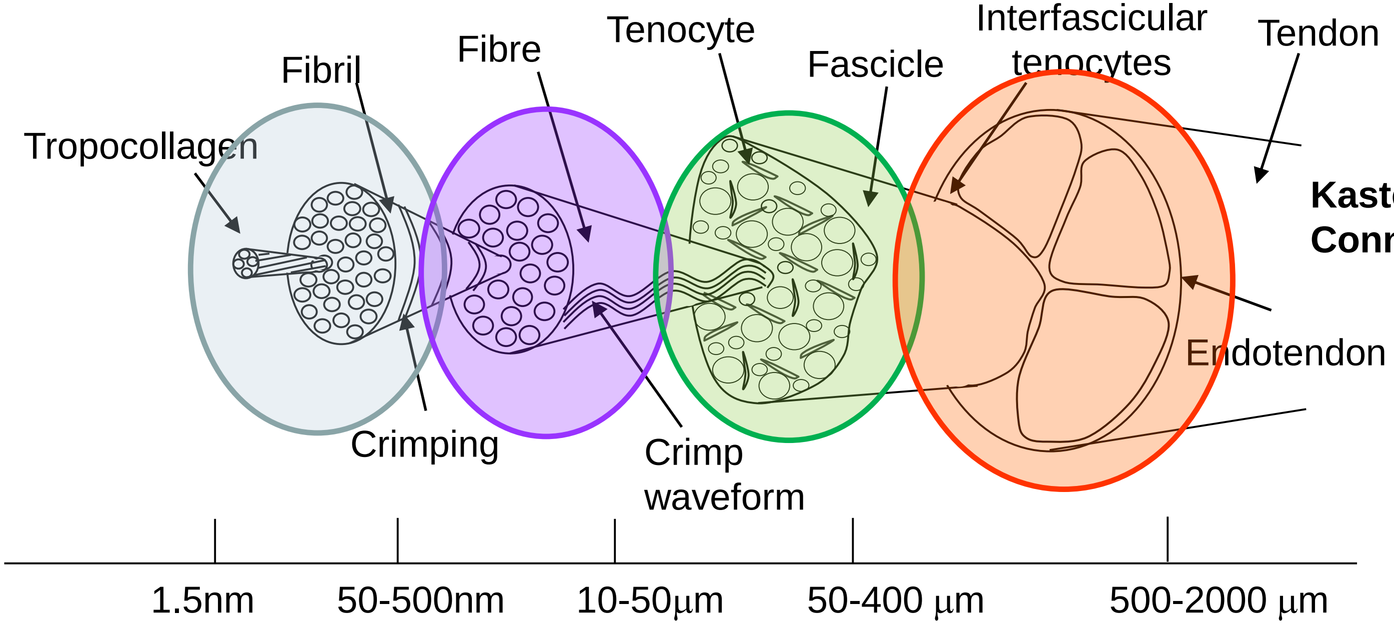
Tendon/Ligament Structure



Dyer,R.F. and Enna,C.D. (1976) Ultrastructural Features of Adult Human Tendon. *Cell Tissue Res.* **168,** 247-259.

Kastelic,J., Galeski,A., and Baer,E. (1978) The Multicomposite Structure of Tendon. *Conn. Tiss. Res.* **6,** 11-23.

Tendon/Ligament Structure



Kastelic et al., 1978
Conn Tiss Res 6;11-23

Collagen Molecules: 1.5 nm ø ; 300 nm length
Cross linked together to build fibrils

Ramachandran 1988
Int J Peptide Protein Res 31; 1-16

Collagen fibrils surrounded by decorin rich matrix
Matrix links adjacent fibrils to build fibres

Scott 2003. J Physiol Lond 553; 335-343

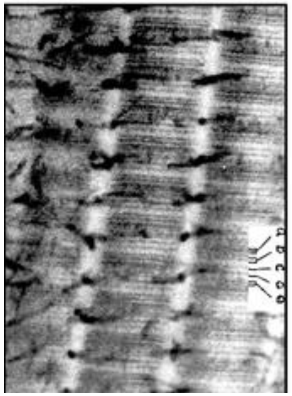
Collagen fibres interspersed by rows of cells
Surrounded by non-collagenous matrix builds fascicles

Screen et al., 2004. J Eng Med 218; 109-119

Fascicles: visible to eye, disect-able subunits
Surrounded by endotendon, bound together to make tendon

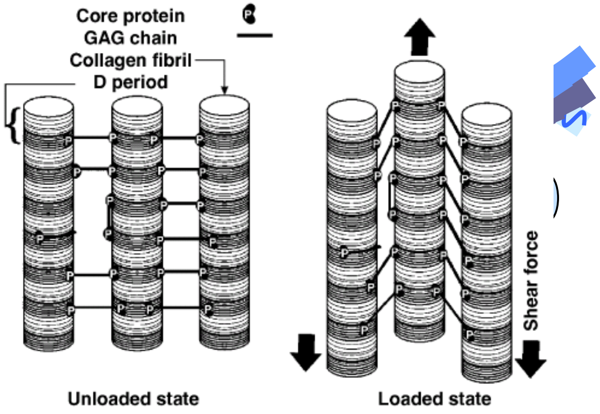
Kastelic et al., 1978
Conn Tiss Res 6;11-23

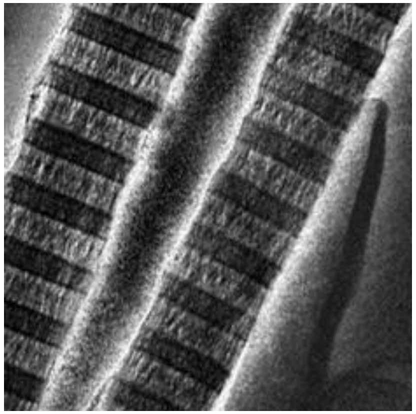
Tendon/Ligament Structure



Collagen Molecule

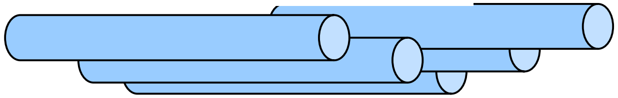
(∅ = 1.5 nm)



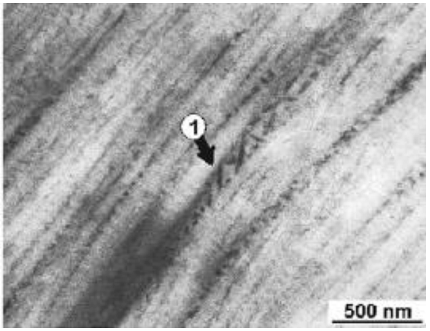


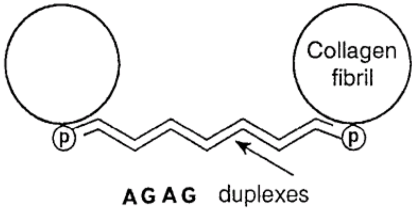
Microfibril

Orgel et al (2009)
PLOSone 4(9) e7028



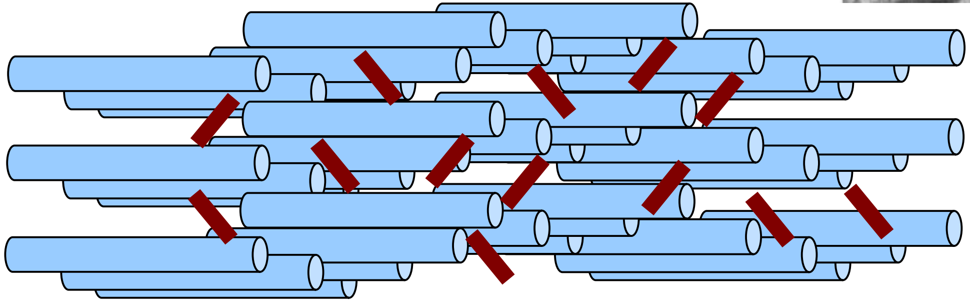
(∅ = 3.5 nm)





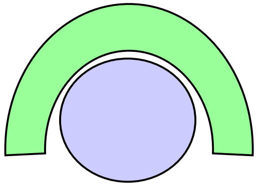
Scott JE (2003) J
Physiol 553(2): 335–43

Liao & Vesely (2007) J
Biomech 40(2), 390-9



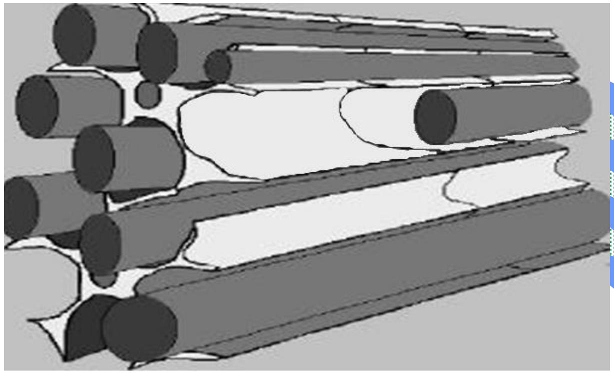
Fibril

(∅ = 50-500 nm)

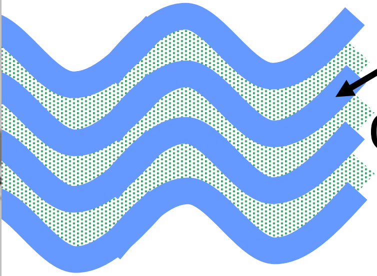


Vesentini et al (2005)
J Biomech 38(3), 433-43

Tendon/Ligament Structure



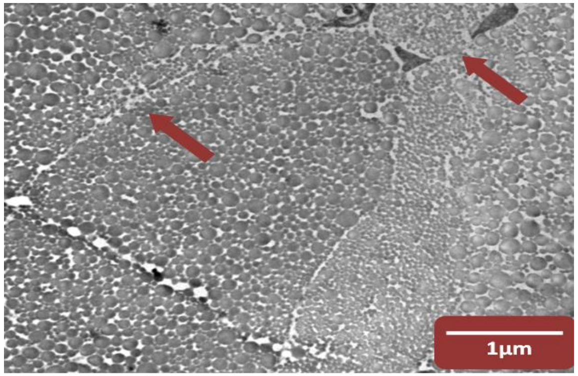
McNeilly et al. (1996)
J Anat 189(3); 593-600

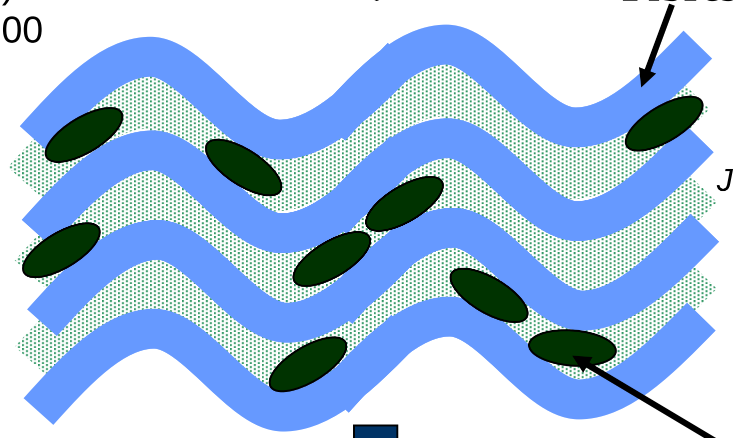


Fibrils

(crimped)

Toorani (2009) PhD Thesis





Fibres

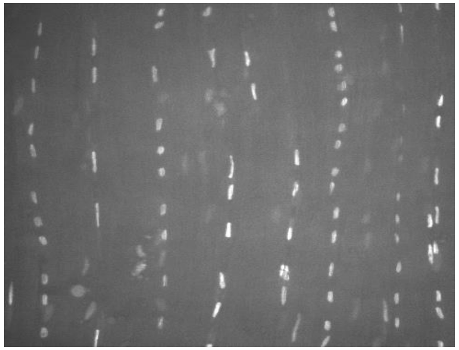
Tenocyte

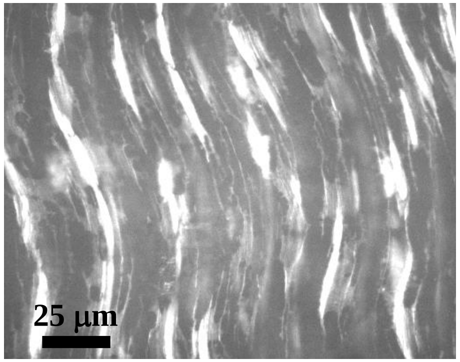
Fascicle

(∅ = 50-500 µm)

Screen et al. (2003)
Biorheol. 40, 361-8

Screen et al. (2004)
J. Eng. Med. 218, 109-19

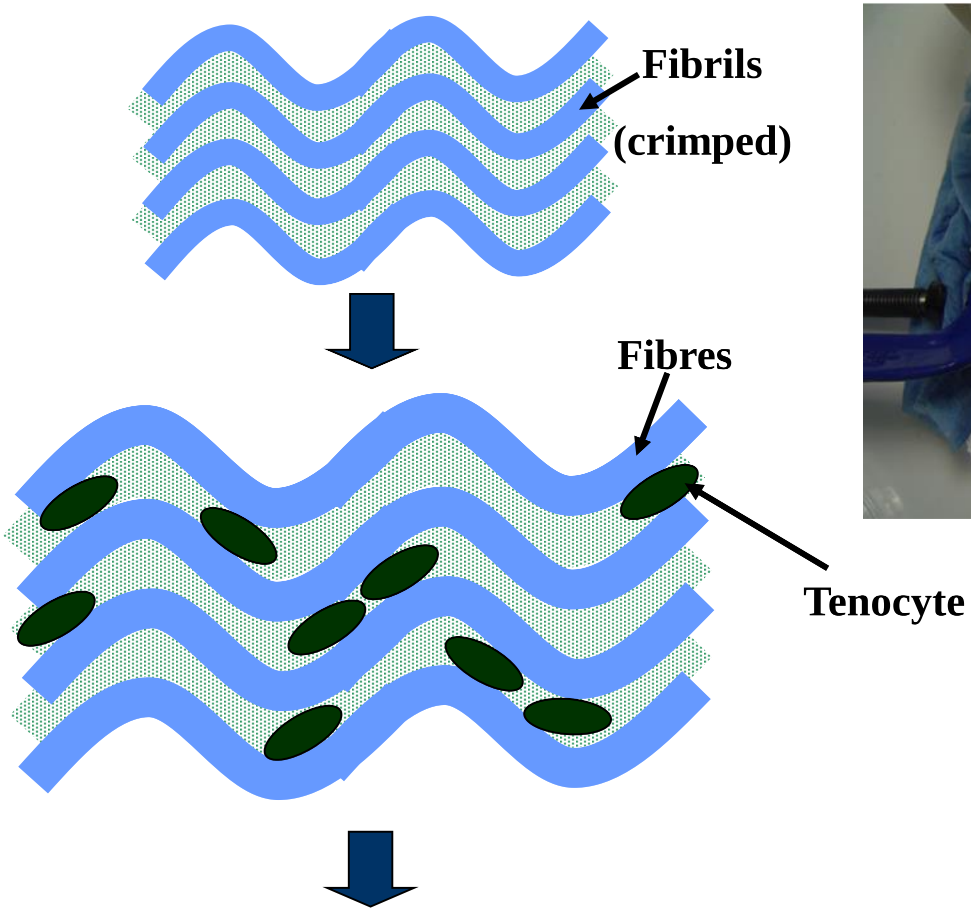




Tendon

(∅ = 5 - 20 mm)

Tendon/Ligament Structure



Fibre

(∅ = 10-50 µm)

Fibrils

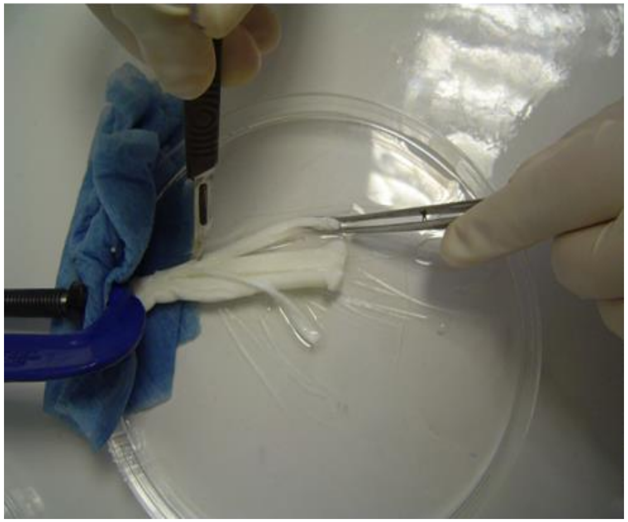
(crimped)

Fibres

Fascicle

(∅ = 50-500 µm)

Tenocyte



Tendon

(∅ = 5 - 20 mm)

Variations in Tendon/ Ligament Structure

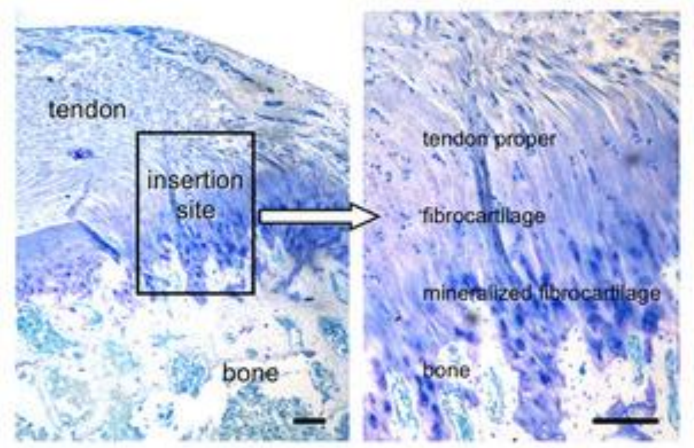
	Tendon	**Ligament**
Collagen	~90%	~80%
Other Components (ground substance)	~10%	~20%
Elastin	~0-3%	~3-10%
Orientation	Highly aligned in loading direction	More weave-like orientation
Organisation	Very organised	Little more random

Simon, SR. (1994) Orthopaedic Basic Science: American Academy of Orthopaedic Surgeons

Variations in Tendon/ Ligament Structure

Osseotendinous junction

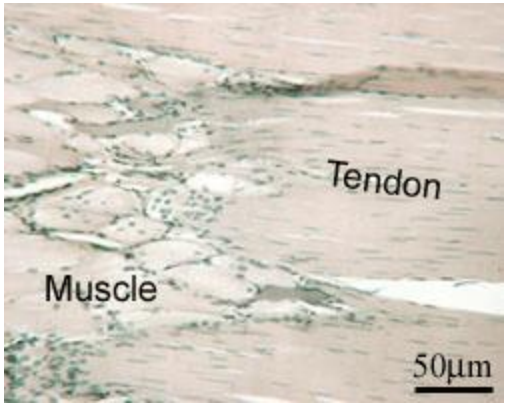
The mechanical properties of tendon are vastly different from those of bone:
Tendon tensile modulus ~1GPa
Bone tension and compression ~20GPa



Functionally graded transition to minimise stress concentrations
Scale bar = 200mm

- Linear increase in mineral content across junction
- Type II collagen transition
- Loss of collagen orientation

Myotendinous junction



Interdigitation of muscle & tendon fibres

Variations in Tendon/ Ligament Structure

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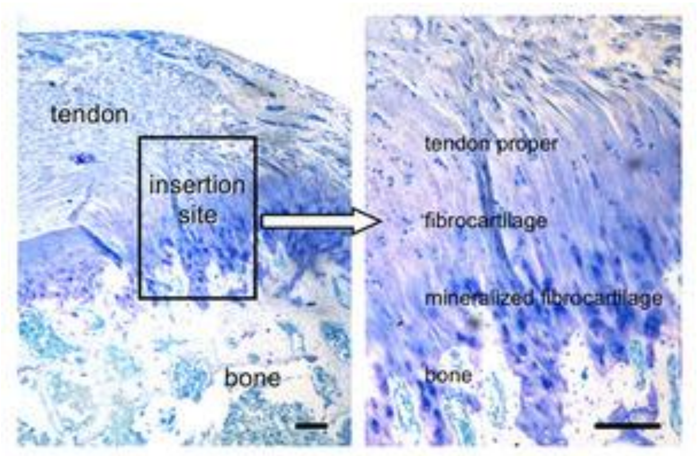

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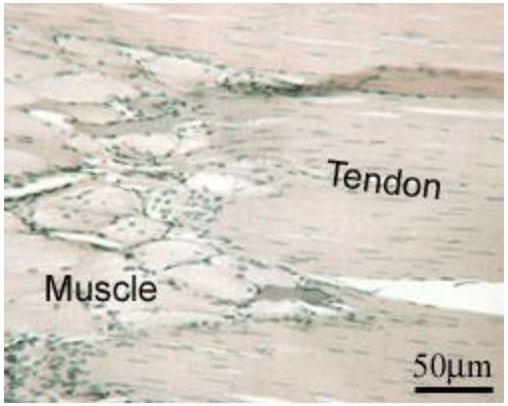
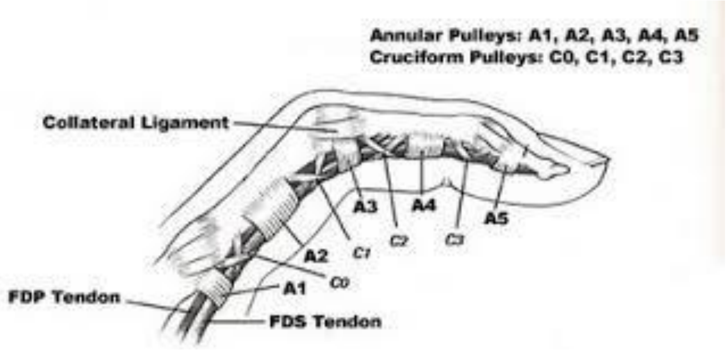


Interdigitation of muscle & tendon fibres

Osseotendinous junction = potential weak point in structure
Prone to injury

Variations in Tendon/ Ligament Structure

Tendons acting as pulleys



Fibrocartilaginous region

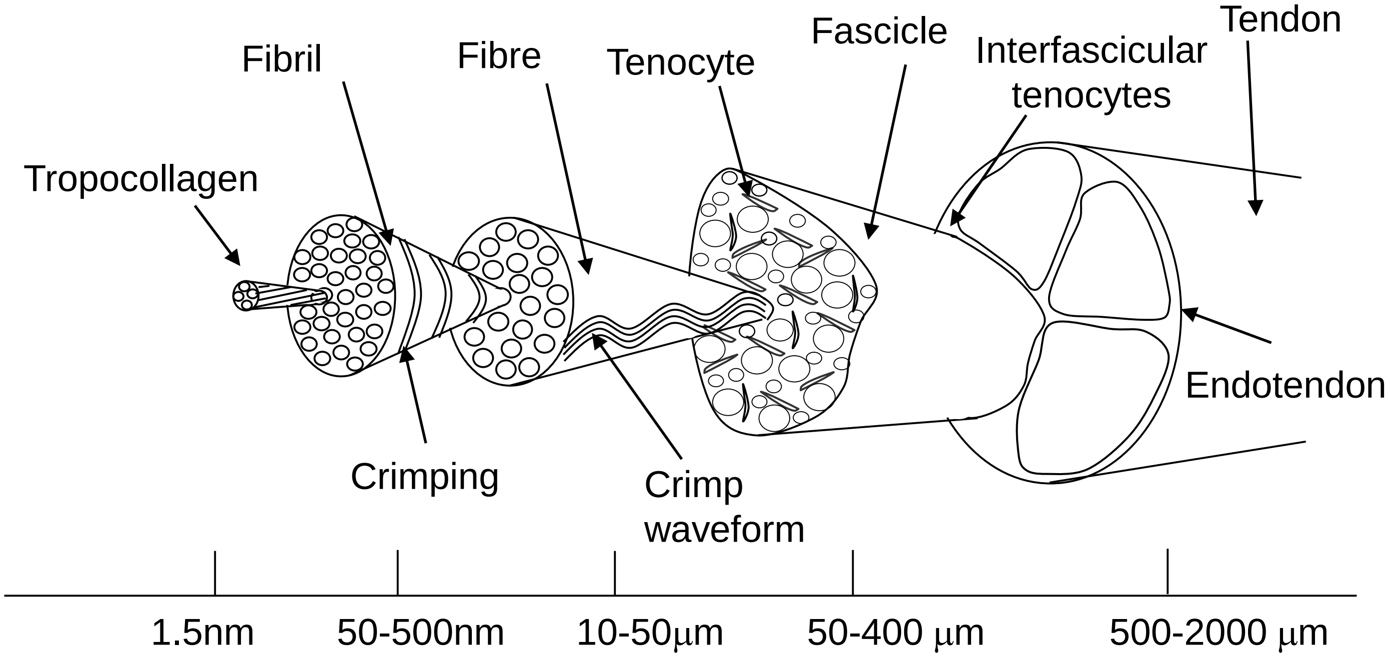
- Pronounced interweaving of collagen fibres: prevents tendon from splaying apart under compression.
- Aggrecan in matrix to allow tendon to imbibe water and withstand compression.
- Type II collagen - particularly in very heavily loaded tendons.
- Fibrocartilage is dynamic.
  - disappears if compression removed
  - Extent of fibrocartilage depends on extent of compression

Protecting the tendon from damage as it bends

Extreme solution

Benjamin & Ralphs (1998) J Anat 193:4; 481-94

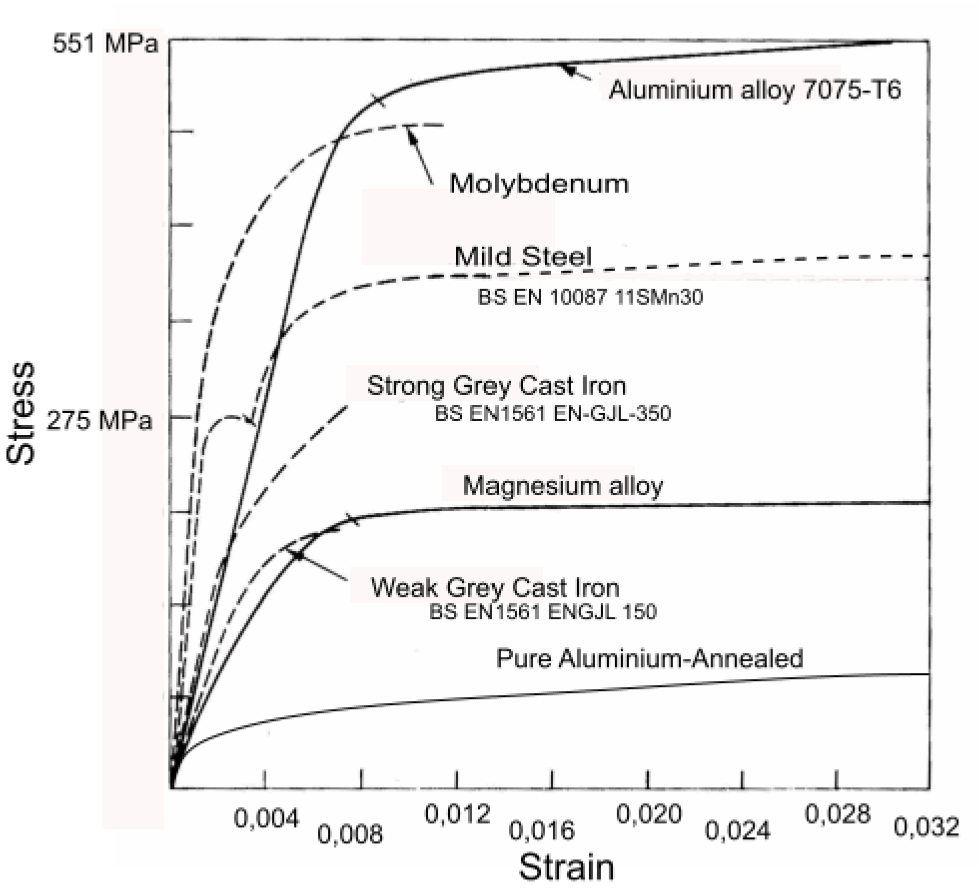
Tendon/Ligament Structure



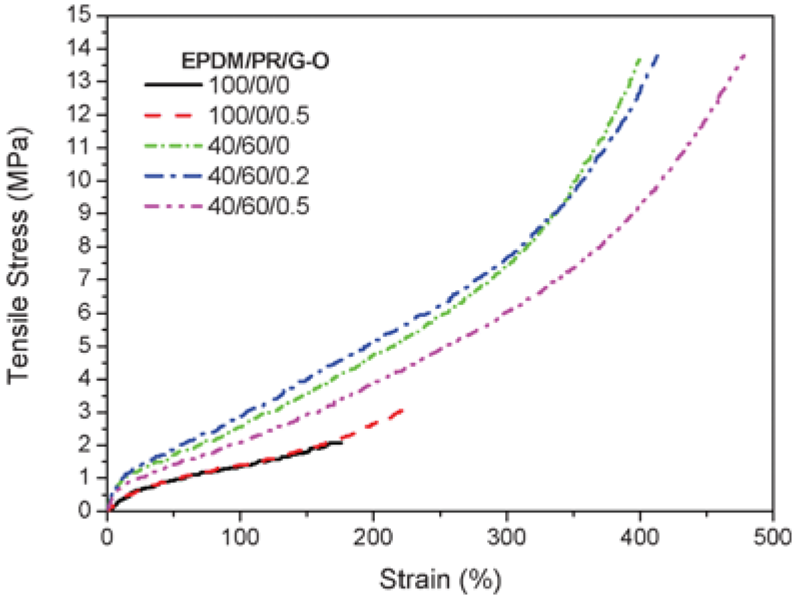
Compositionally and structurally simple!

Mechanical/Ligament Properties

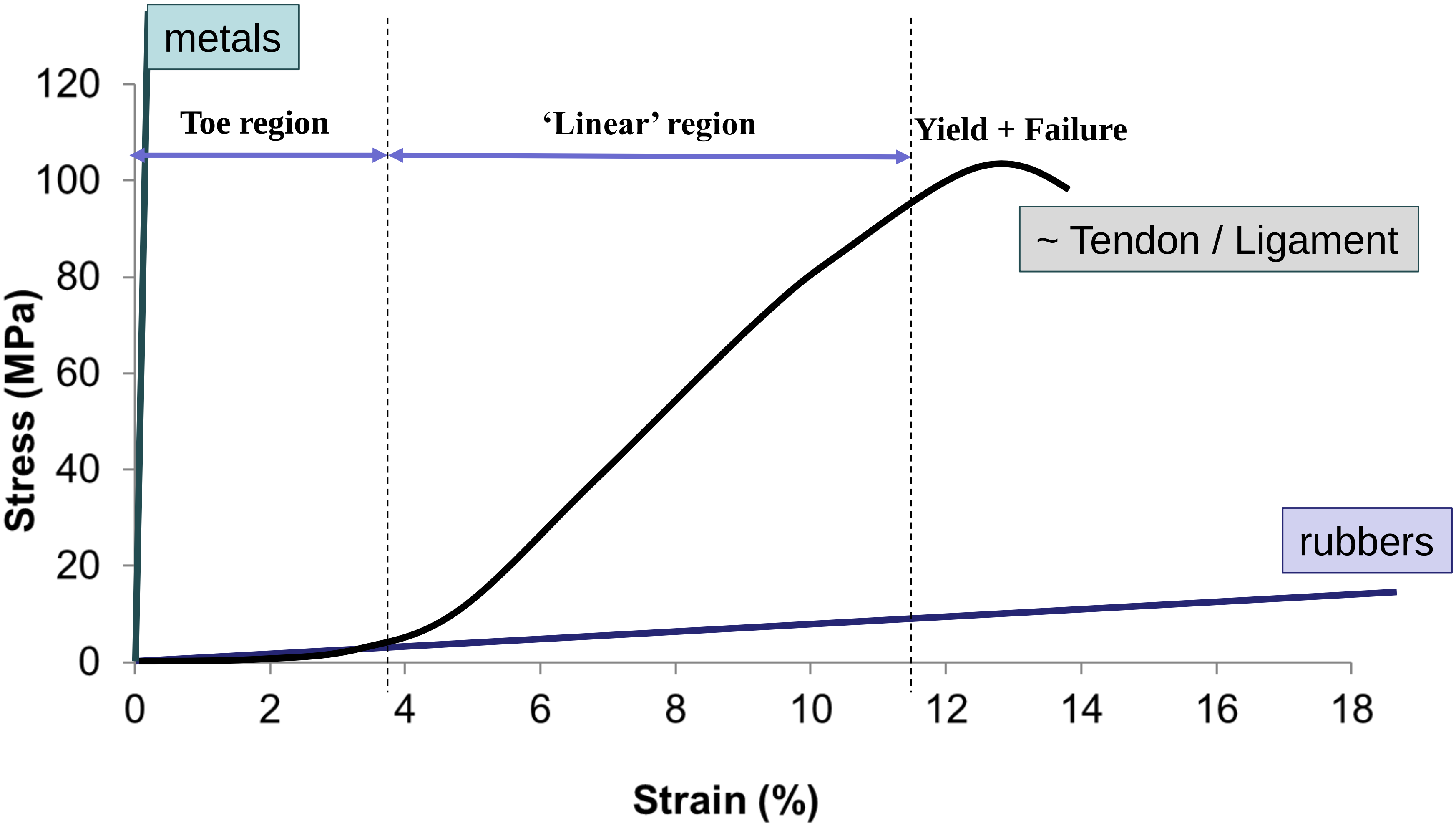
METALS:
www.roymech.co.uk



RUBBERS:
Chen et al 2012 RSC Adv **2**, 4683-4689

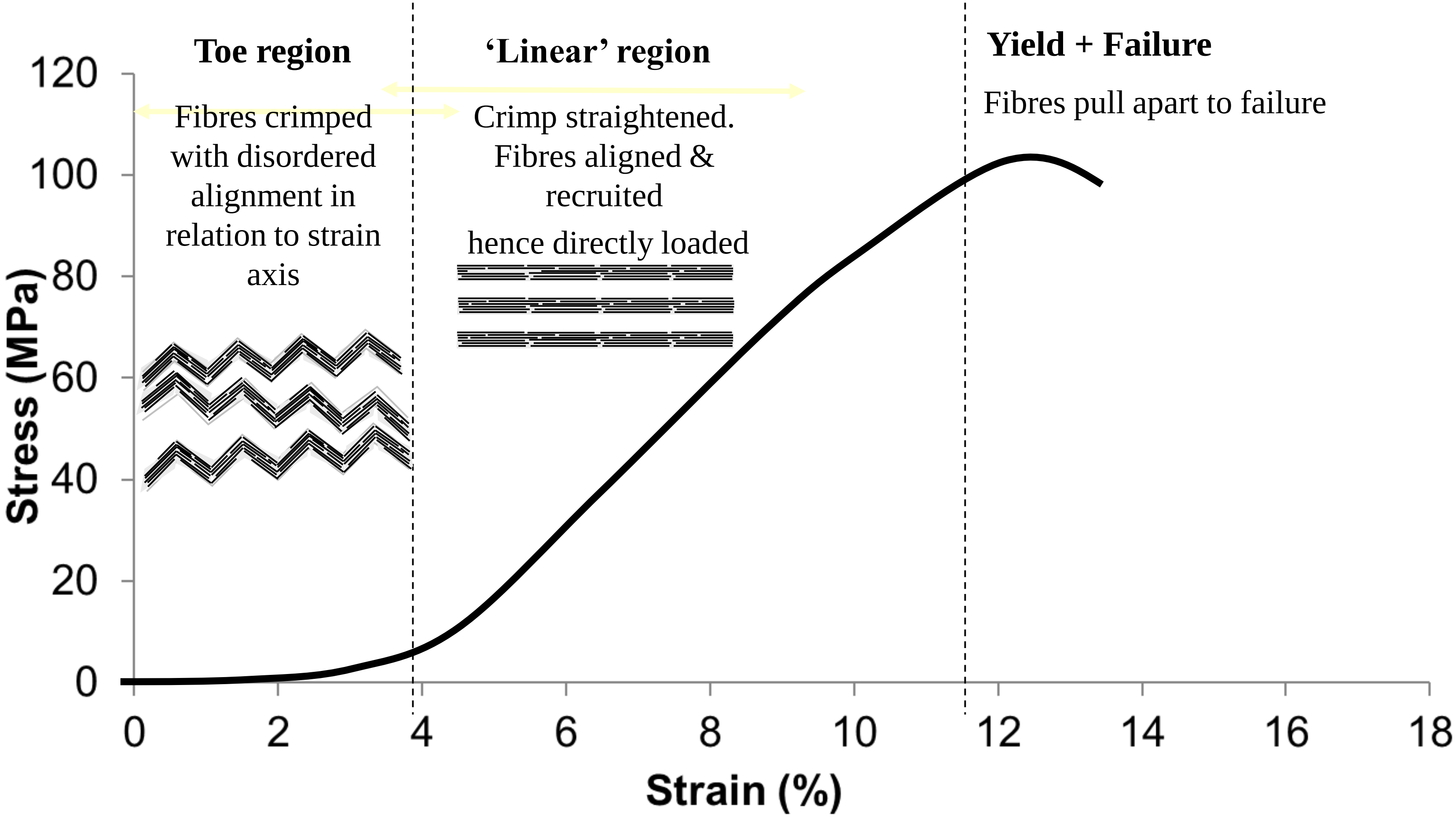


Mechanical Properties



Mechanical Properties

How does the structure of tendon create this mechanical behaviour?



Toe region

Fibres crimped with disordered alignment in relation to strain axis

‘Linear’ region

Crimp straightened. Fibres aligned & recruited

hence directly loaded

Yield + Failure

Fibres pull apart to failure

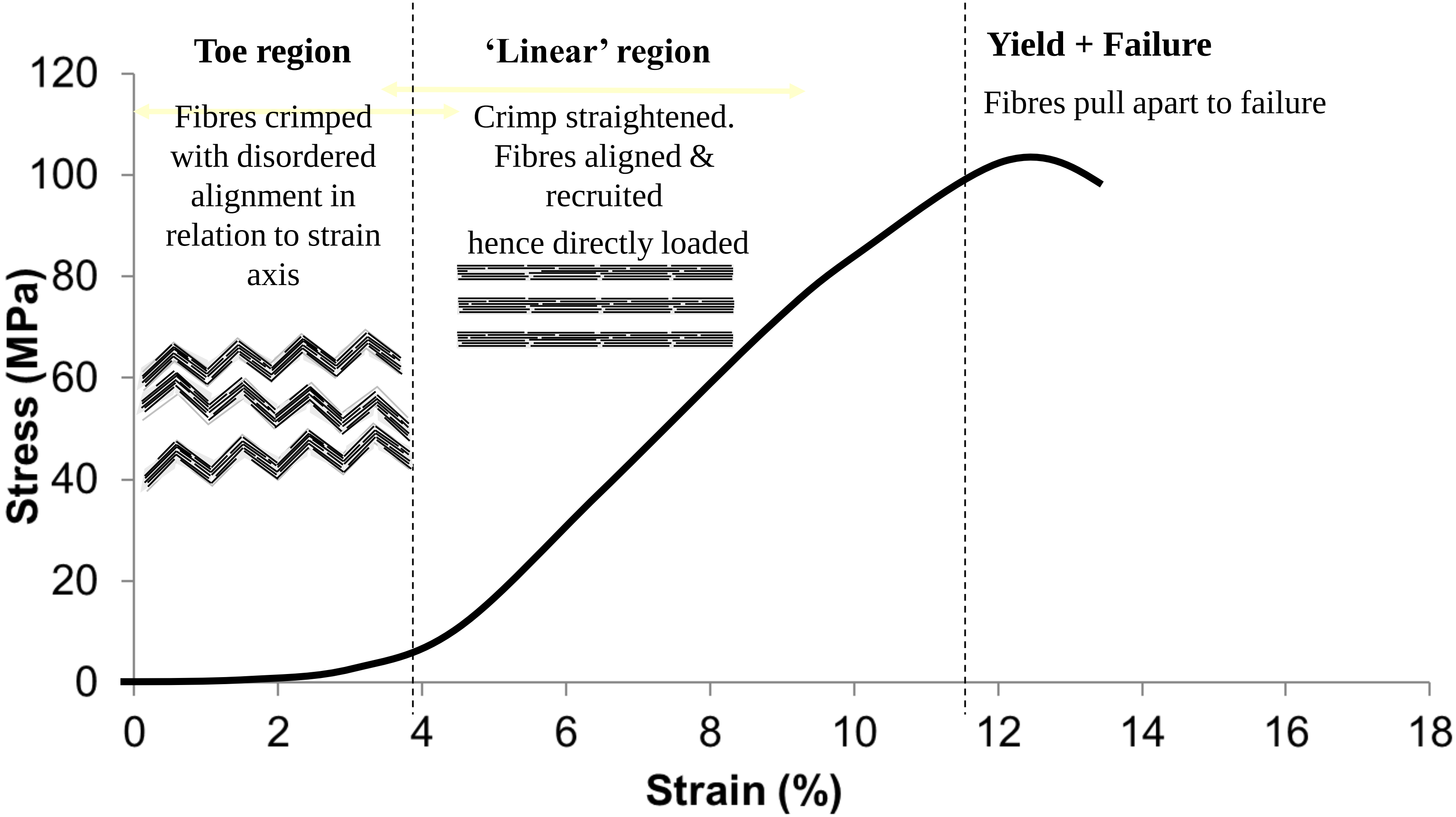
Screen et al. (2004)
J. Eng. Med. 218, 109-19

Atkinson et al. (1999)
J. Biomech 32, 1907-14

Kastelic et al. (1980)
J. Biomech 13, 887-893

Mechanical Properties

Methods for visualising & measuring mechanics at local (hierarchical) scales



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J. Eng. Med. 218, 109-19

Atkinson et al. (1999)
J. Biomech 32, 1907-14

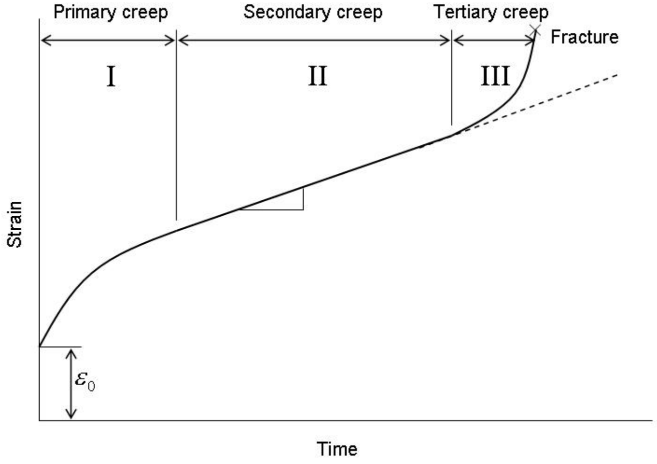
Kastelic et al. (1980)
J. Biomech 13, 887-893

…and Other Mechanical Properties

Viscoelastic (time dependent) behaviour

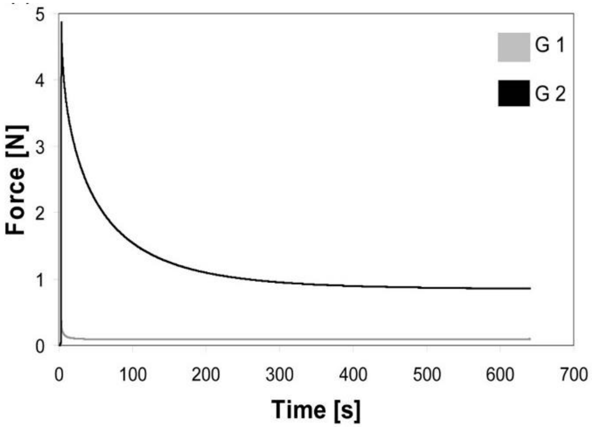
Creep:

Hold a tendon at a constant load and it stretches to failure



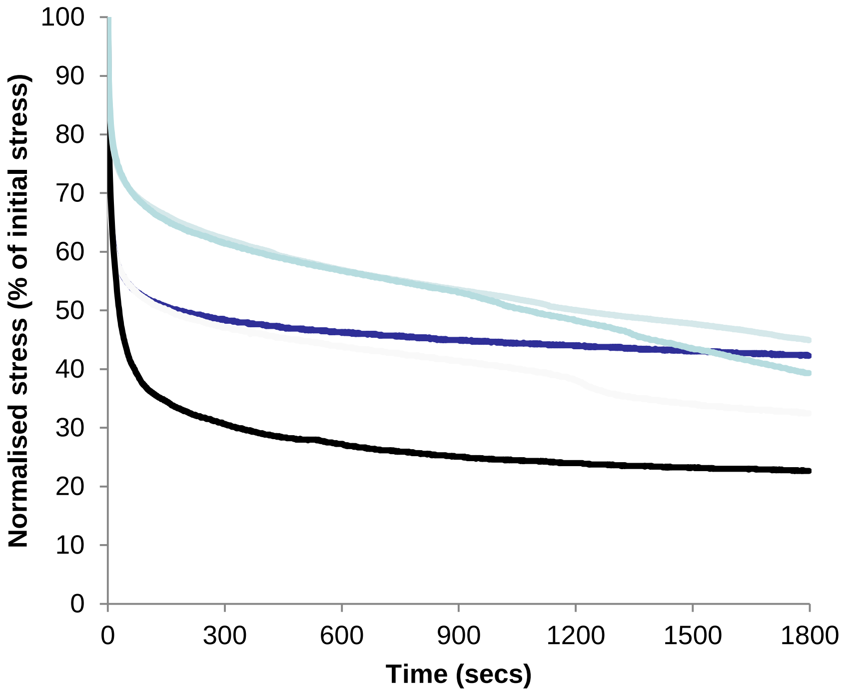
Stress Relaxation:

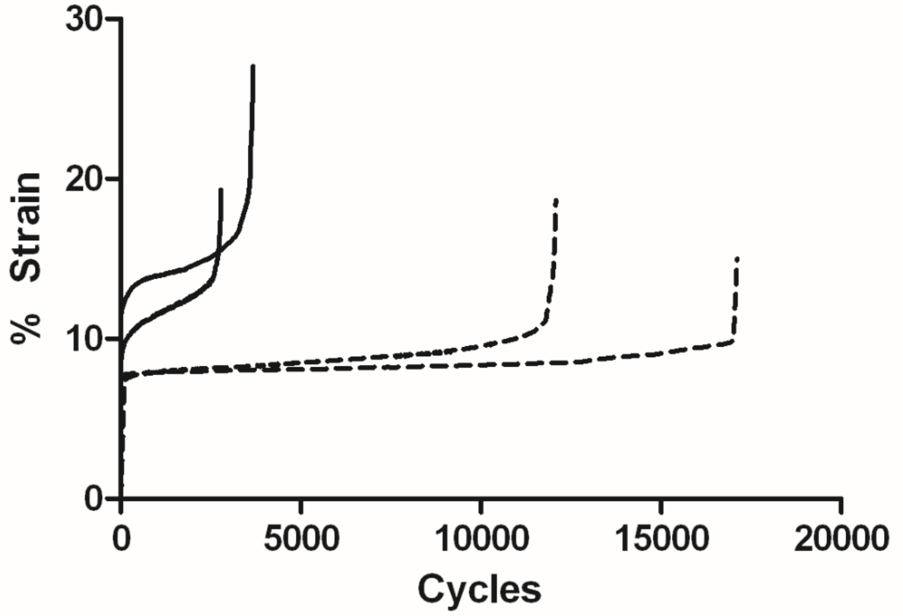
Hold a tendon at a constant extension and the force drops away



…and Viscoelastic Mechanical Properties

Viscoelastic (time dependent) behaviour



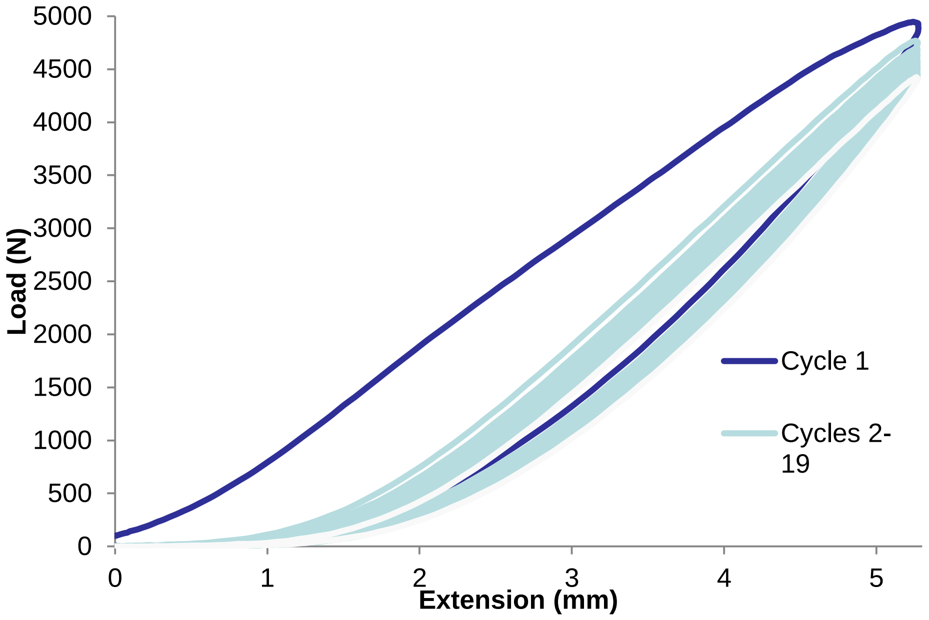


Typical stress relaxation curves

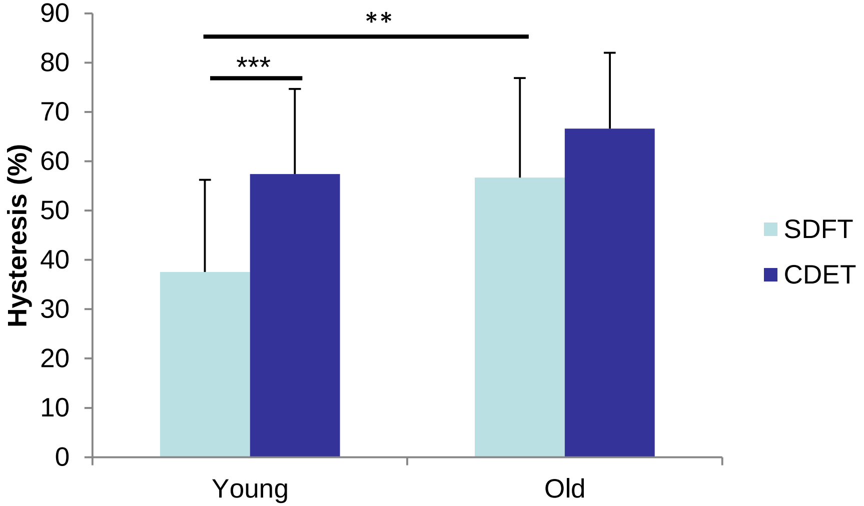
Creep cycles to failure: young and old tend

…and Viscoelastic Mechanical Properties

Viscoelastic (time dependent) behaviour

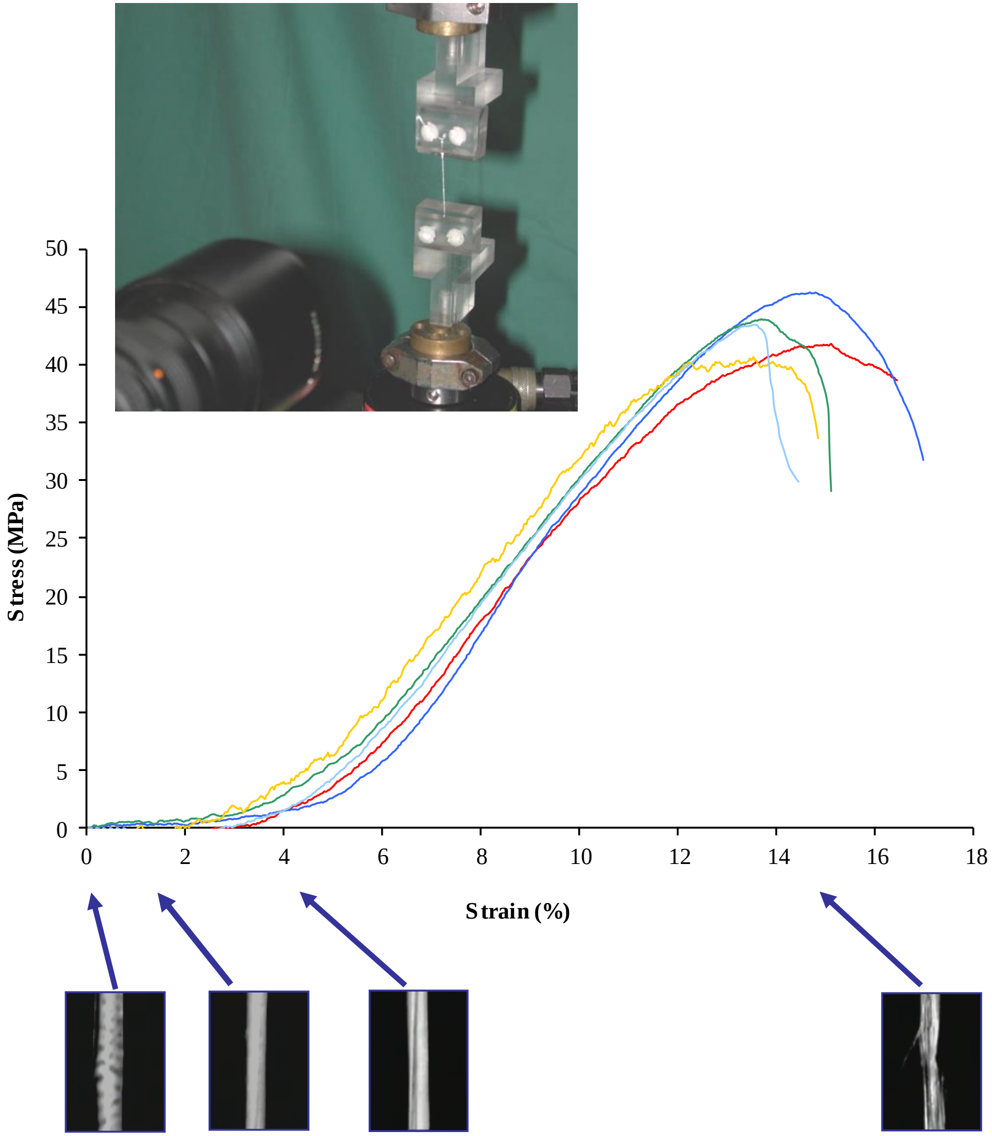


Cyclic loading – Pre-conditioning

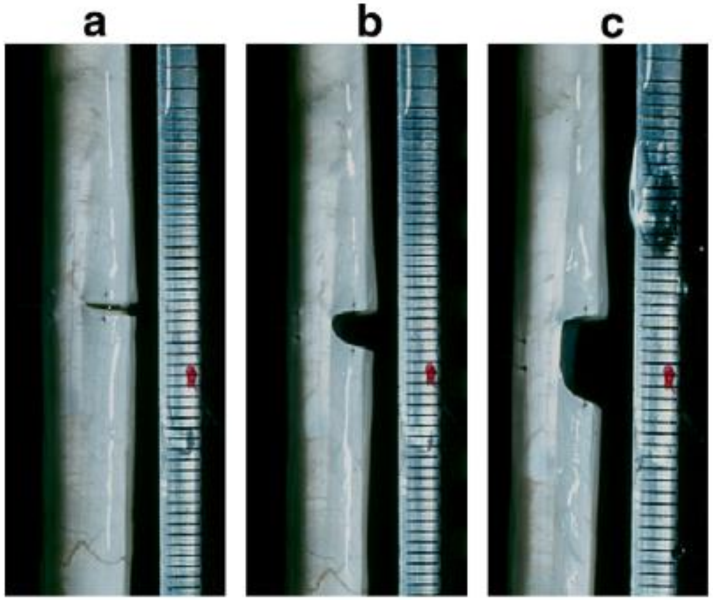


Hysteresis loss during cyclic loading in young & old tendons

Macro scale analysis



Toe Region	Collagen fibers straighten (less prominent than in ligaments because fibers begin more aligned)
Linear Region	
Irreversible Deformation	Collagen fibers slide past one another; permanent elongation
Macroscopic failure	Tensile failure of fibers and shear failure between the fibers Once maximum load is surpassed, complete failure occurs rapidly



Force 11 N 97 N 304 N

Fig. 8. A slit experiment on a sheep plantaris tendon: (a) A slit has been cut with a razor blade about half way through at the mid-point of the specimen's length. A small force has caused the slit to open a little. (b) A gaping mouth at a higher load. Stress is transferred across the tendon by shear, so that at points distant from the cut the full cross-section of the tendon carries some stress. The gape of the mouth is therefore less than the distance the clamps have moved apart. (c) With a higher load, the material has failed in shear where the crack tip was. The now unsupported portions on the right have pulled back allowing the newly formed vertical surface to be seen.

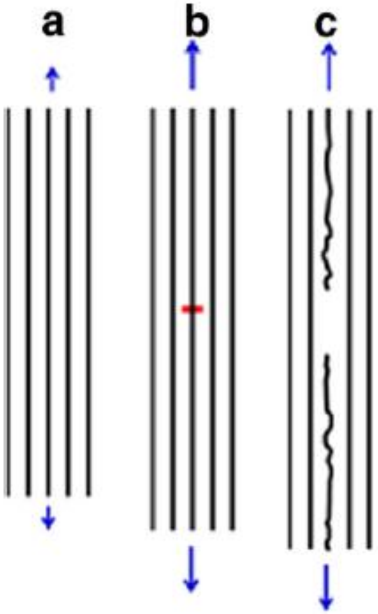


Fig. 9. An explanation of the formation of longitudinal cracks in tendon: (a) Vertical fibrous units, at the level of fibril or above, are separated by a weaker matrix. (b) The initial break is assumed to occur in a fibrous unit leading to high strain in shear in the matrix. The resulting region of failure runs along the two portions of broken fibrous unit which are now unsupported. A portion of the tendon is no longer functional, but the rest is unimpaired.