Elastic Network Models of Lung Tissue Diseases and Their Treatments

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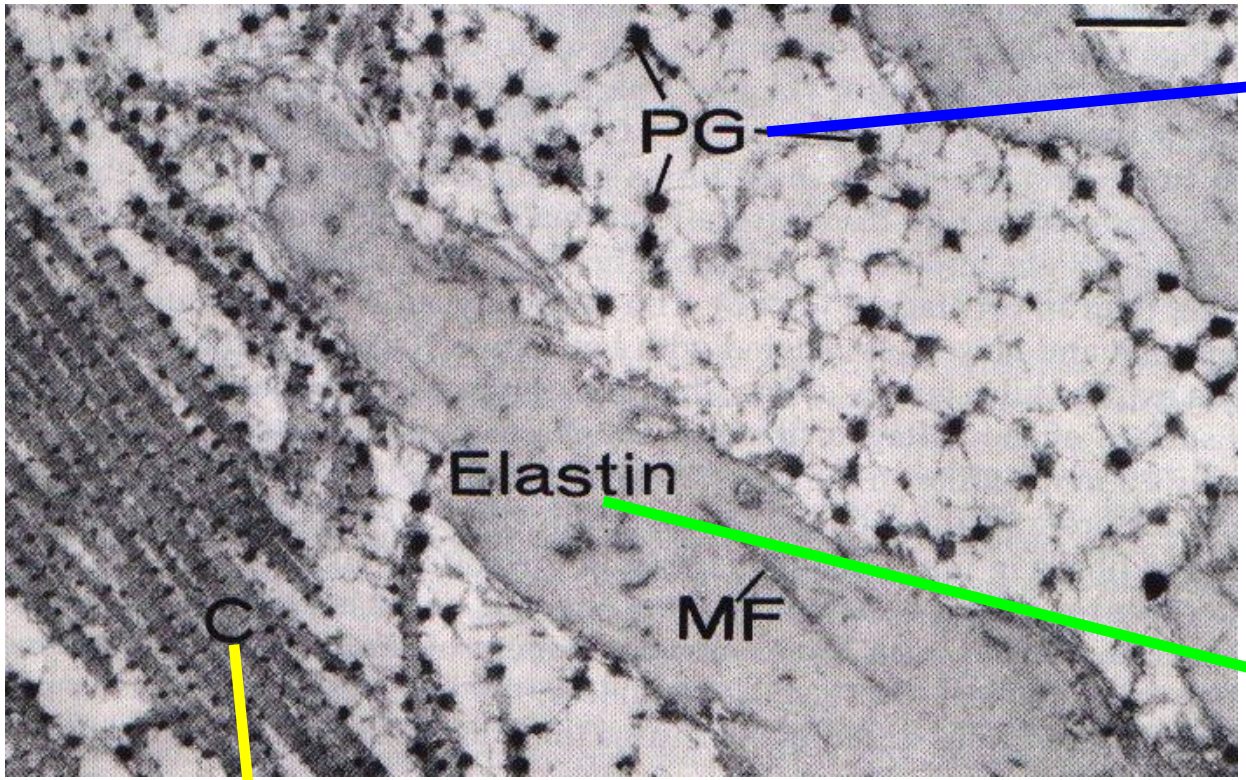
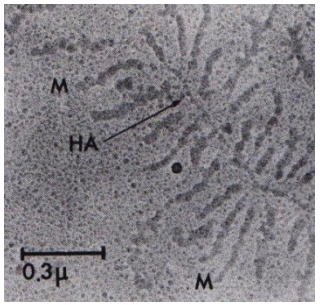
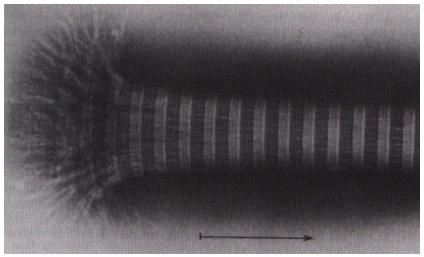
Mechanical Properties of ECM Components

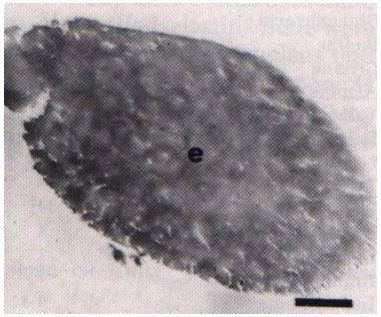




Proteoglycan:
- Protein core surrounded by GAG branches.
- Resists compression and may link collagen and elastin fibers



Collagen Fibril:
- Superhelix (3 α helices).
- Absolute stiffness of molecule is 5 GPa while absolute stiffness of fibril is 5 MPa.



Elastin Fiber Cross-section:
- Composed of helical regions interspersed with lysine-rich non-helical regions.
- Absolute stiffness of 600- 700 kPa.

Collagen Image and Proteoglycan Image from "Extracellular Matrix Biochemistry", K. Piez and A. Reddi, Elsevier Press, New York, 1984.
Main Image and Elastin Image taken from "Cell Biology of Extracellular Matrix", Elizabeth Hay (ed.), Plenum Press, New York 1981.

Failure of biological tissues

- When micro-scale damage exceeds a critical amount, the consequences can reach the macroscopic level eventually leading to failure.

- Capillary stress failure in the lung during severe exercise in athletes or in animals bred for high aerobic activity.

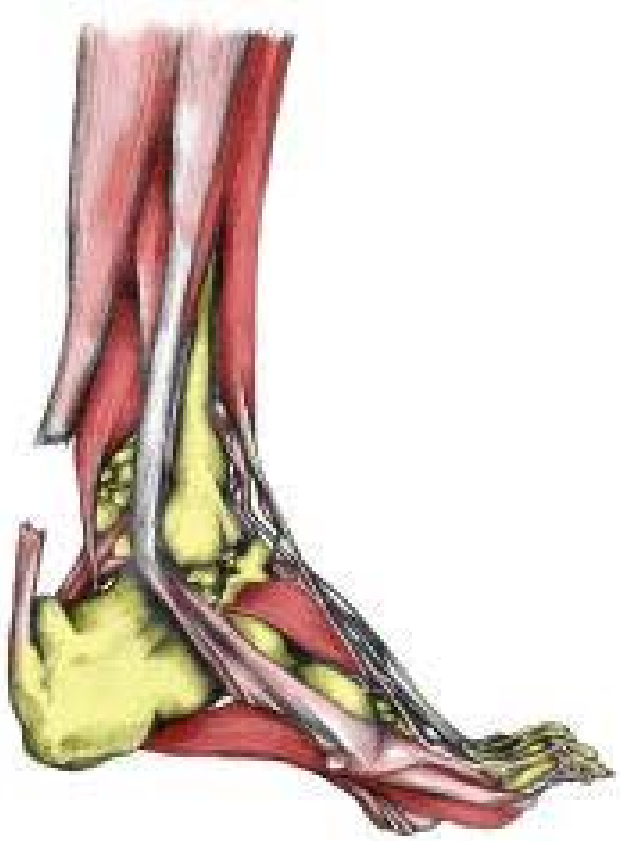
- Capillary failure can also be induced by mechanical ventilation of patients suffering
- from acute lung injury.

- During pregnancy, the membrane surrounding the fetus prematurely ruptures in 5-10% of pregnancies with an increased perinatal morbidity and mortality.

- In vascular aneurysm and pulmonary emphysema, the extracellular matrix undergoes proteolytic digestion and rupture.

- Implanted tissue engineered constructs such as prosthetic heart valves can also undergo digestion and subsequent failure.

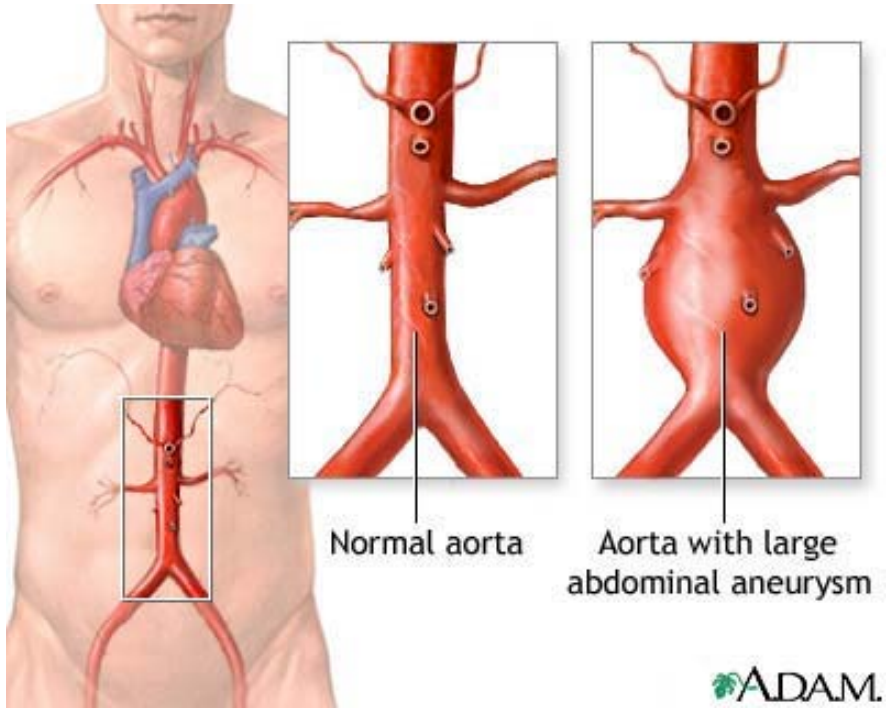
Sports injury



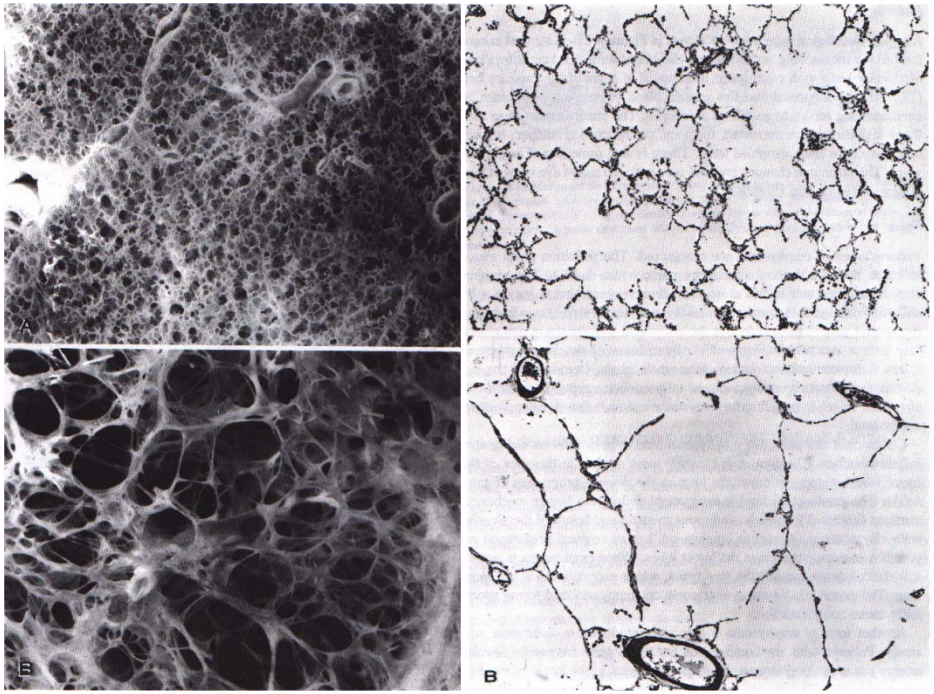
During extensive sport, or if the tissues are not allowed to recover from strenuous activity or when the tissue is not well hydrated, rupture can occur.

Tissue failure in aneurysm

Aneurysms: Decreased mechanical function of vessel wall due to digestion of elastin can lead to increase in local diameter and eventually a fatal rupture of the vessel without timely surgical intervention. By LaPlace law, the tension T in the wall is transmural pressure times radius of curvature. As the latter increases so does the tension which can accelerate digestion of elastin.



Emphysema



(J. West, Pulmonary Pathophysiology – The Essentials, 1992)

Normal

Emphysematous

Lung stiffness is 2-3 times smaller than in normal subjects, structure destroyed

These images show examples of normal alveoli on the top, and emphysematous regions on the bottom, and the difference in structure is evident. On the left are scanning electron microscopic images and on the right are 2D histologic sections. Emphysema is a pulmonary obstructive disease leading to destruction of alveolar walls and airspace enlargement, as seen here. Mechanically, emphysema results in a decreased tissue elastance, and because of the loss of elastic recoil, patients have trouble getting air out of their lungs and breathe at high volumes.

The most widely accepted view of the development of emphysema is that in imbalance of protease and anti-protease activity occurs within the lung. In normal conditions, the presence of anti-proteases act to inhibit the fiber-degrading proteases and prevent destruction. An increase in protease activity or a deficiency in anti-proteases can thus lead to abnormal fiber destruction. The role of inflammation has led to an expansion of this protease/anti-protease theory. Inflammatory cells release proteases in excess of the inhibitors leading to fiber destruction. Additionally, the lung continually undergoes cyclic loading during breathing, and after an initial degradation of the alveolar walls, these mechanical forces may be sufficient to cause rupture of the weakened walls.

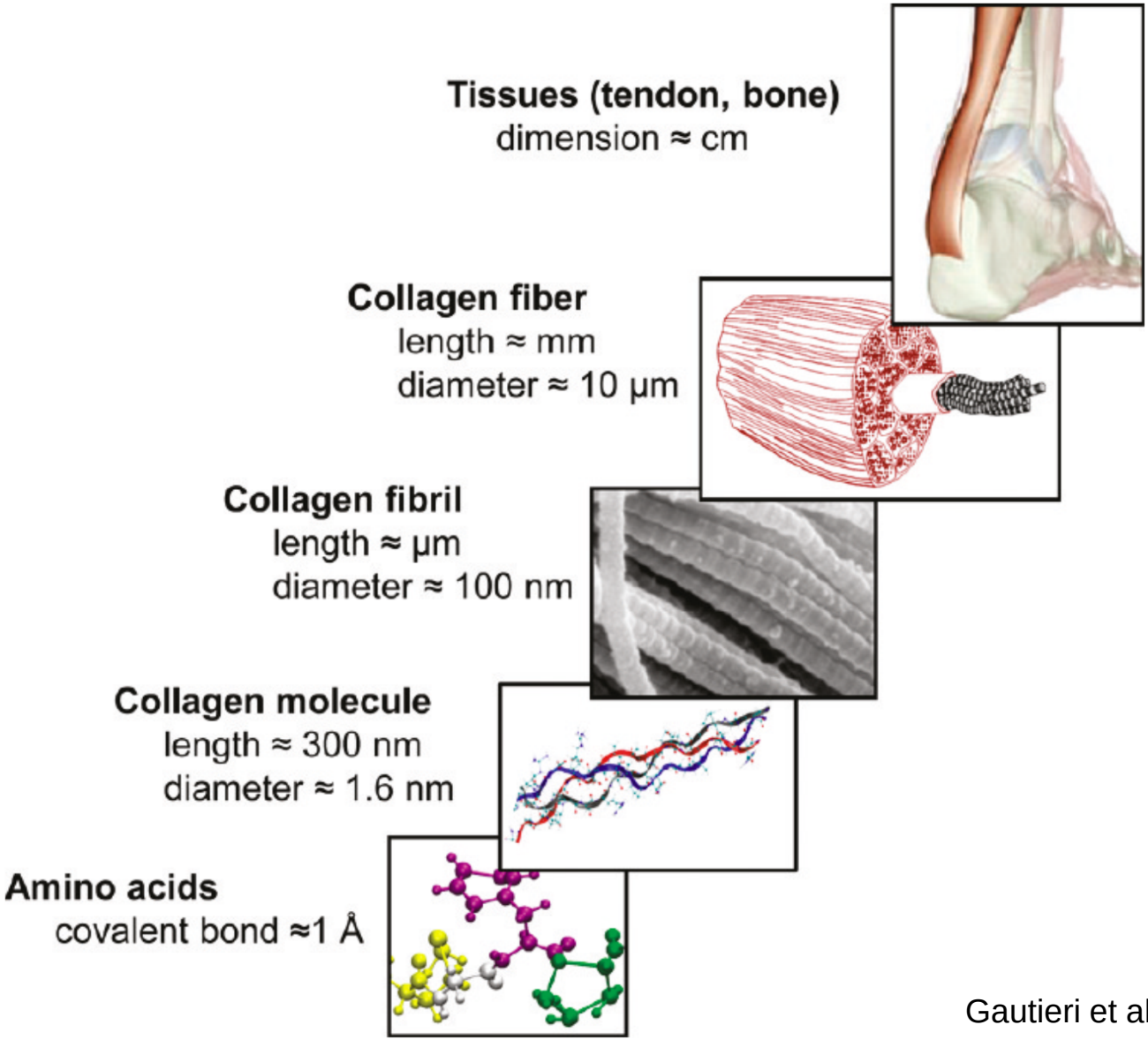
Questions

- What is the mechanism of failure in soft tissues?
- What roles the constituents including collagen, elastin, PGs play in failure?
- What is the role of the structural organization of the tissue in failure?
- How does failure contribute to disease progression?
- Can we utilize the understanding of network failure in treating diseases?

The multiscale nature of failure

- A container of Avogadro number of molecules show a smooth pressure-volume behavior.
- The same number of molecules arranged in tissue shows high variability:
  - The number of ways the molecules can be combined into tissues is astronomical
  - The failure of a single polymer is a stochastic process
  - Avogadro number of molecules do not fully average out molecular fluctuations
- Tissue failure is a multiscale phenomenon.

The hierarchical organization of collagen

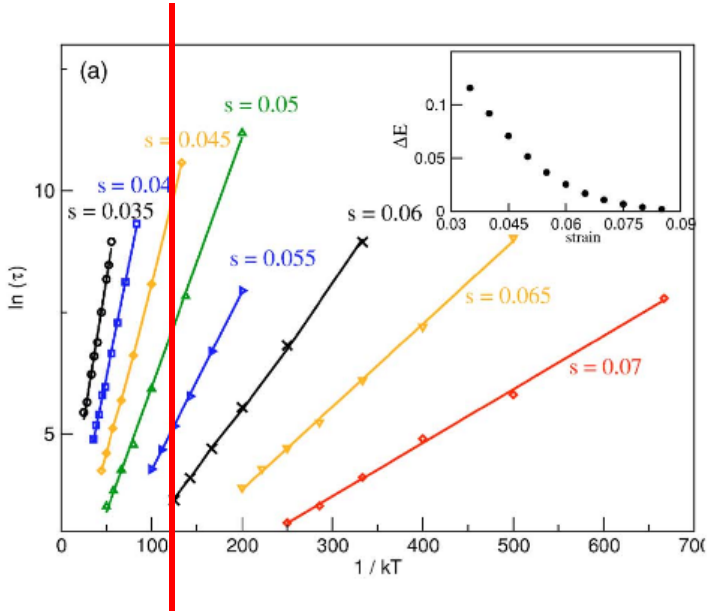


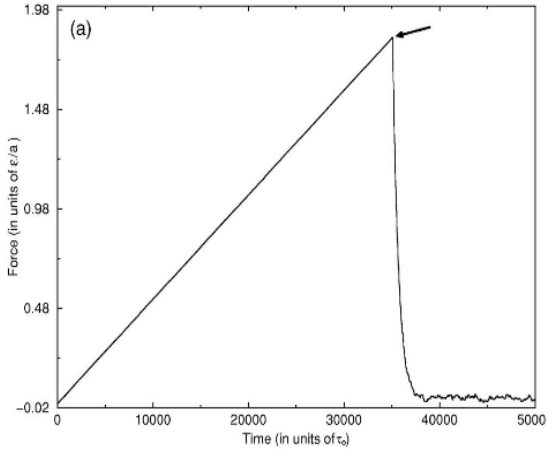
Gautieri et al. Nano Lett, 2011

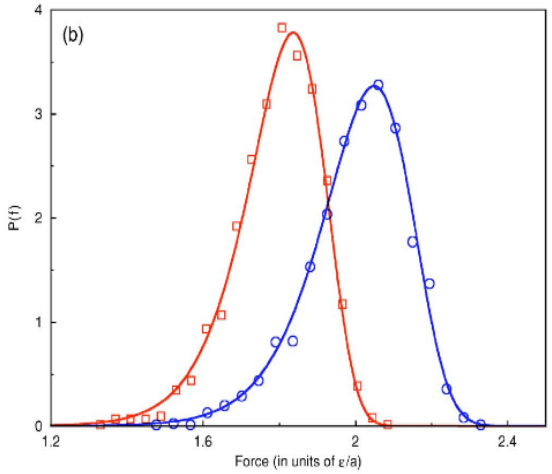
Failure of single molecules

- Two particles (atoms or molecules) are held together via a specific bond characterized by for example the Lennard-Jones potential.
- When the molecule is held at a given strain, rupture of bonds can occur as a result of thermal agitation.
- During stretching, the energy landscape of the bond gradually tilts and elongation proceeds by internal ruptures and subsequent unfolding events.
- Full rupture occurs when the bond stretch exceeds a critical bond strain, beyond which the likelihood of rebinding is negligible.
- The force at rupture (failure force) is a random variable.
- Thus, the rupture of a single molecular bond can generate a distribution of failure forces.

Single molecule failure simulations







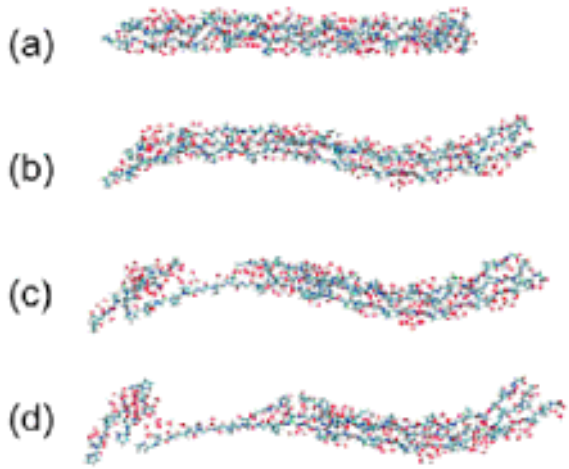
$$P(\tilde{f}) = \Omega^* \tilde{f}^{1/2} \exp\left[-E_0^* \tilde{f}^{3/2} - \frac{2}{3} \frac{\Omega^* F_c}{E_0^*} e^{-E_0^* \tilde{f}^{3/2}} \right]$$

The molecules are represented as a chain of atoms held together by the Lennard-Jones potential. The dependence of breakup time on temperature and strain. The energy barrier as a function of strain is shown in the inset. Different colors correspond to different applied strains.

(a) Typical force on the molecule during stretching. The arrow indicates rupture. (b) Normalized probability distribution of breaking force. Simulation results are indicated by symbols. These simulations were performed at T= 0.02 and v = 0.01 (squares, red) and v = 0.08 (circles, blue). Curves correspond to analytical result.

Dias et al. Phy Rev E (2005)

Failure of the collagen molecule



The failure occurs at around 50% strain and the peak force is 2.4×10^4 pN which corresponds to 11 GPa fracture stress

A single α chain fails around 37% strain and at a peak force of 0.7×10^4 pN.

The 3 α chains are roughly in parallel sharing the same strain and the total force is the sum of the forces on each.

Since the α chain fails at a lower strain and the 3 times the peak force of the α chain is smaller than the peak force on the triple helix, the triple helical configuration together with the H bonds add extra strength to the collagen.

Failure of network of molecules

- Failure strain in collagen fibrils range from 6 to 40% and failure stresses increase from 0.5 to 5 GPa with increasing cross-link density (Tang et al. J R Soc Interface, 2010).

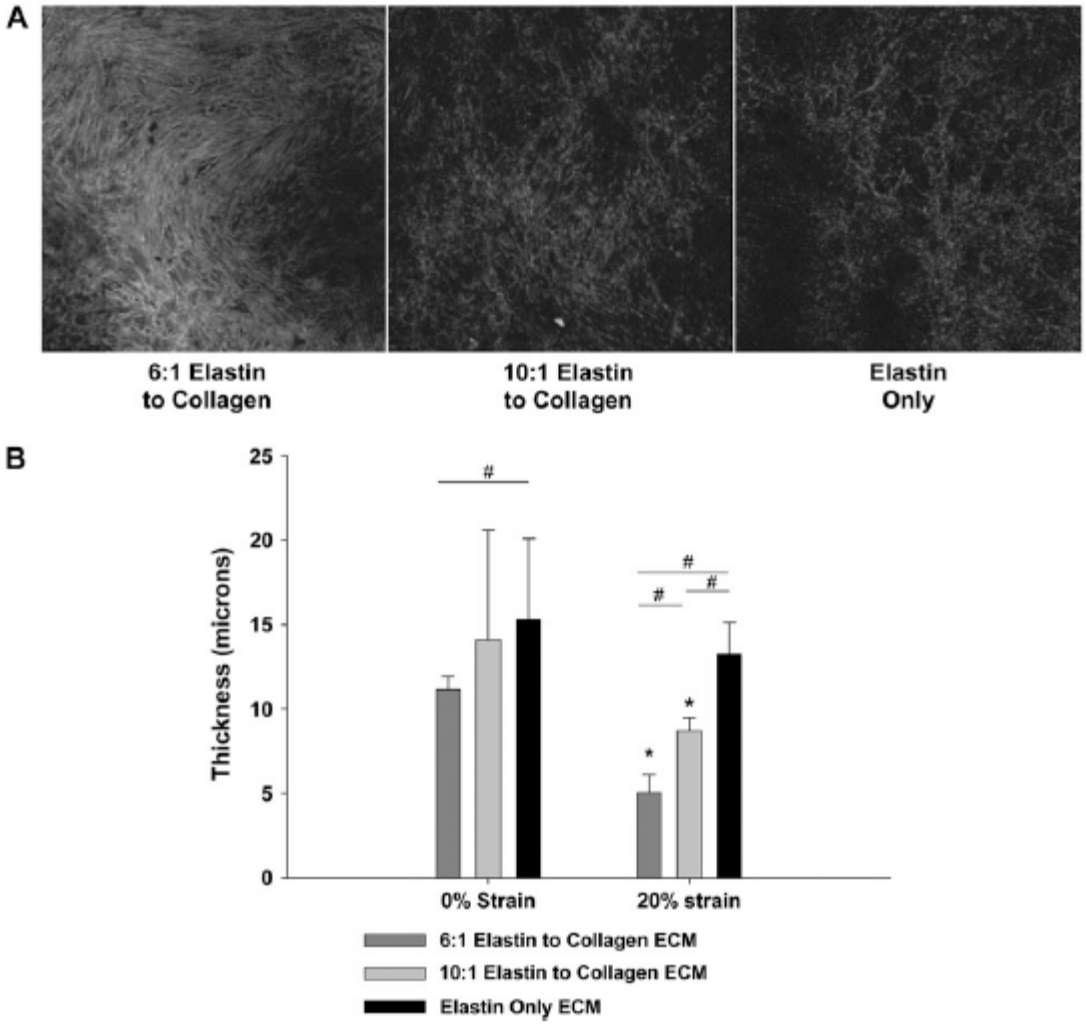
- In the normal range of temperatures and hydration levels, the failure stress of elastin is between 1 and 2 MPa and the failure strain is at least 200% (Lillie and Gosline, Int J Biol Macromol, 2002).

- Molecular dynamics simulations suggest that the GAG chains can take up significant strains (>800%) and can transfer stress from one collagen fibril to a neighboring one (Redaelli et al. J Biomech, 2003). However, the failure force between two hyaluronan and its binding protein is very small, only 40 pN (Liu et al. FEBS Lett, 2004).

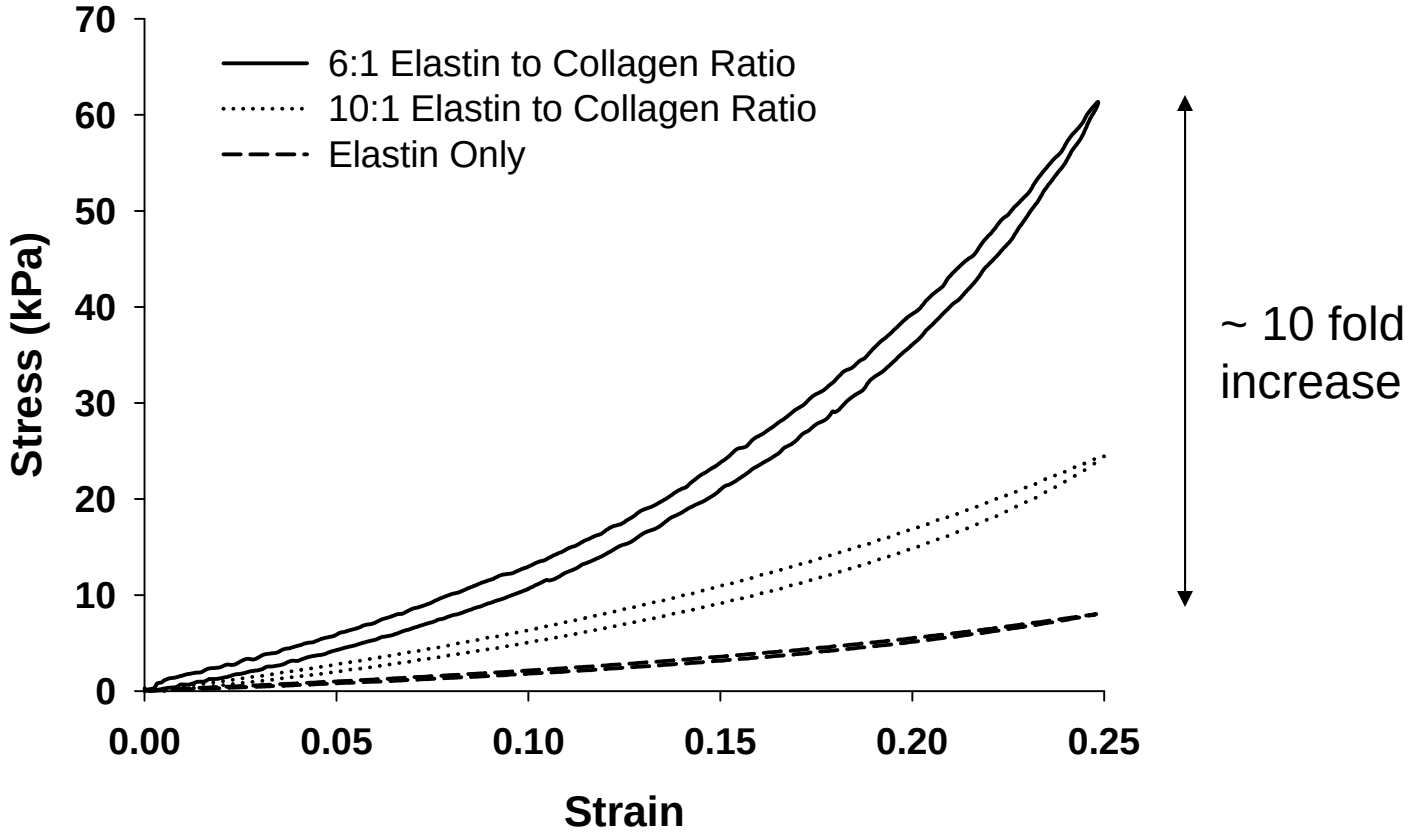
- These components also form a network and interact with each other.

- What is the mechanism of failure in a network?

ECM sheets


A
6:1 Elastin to Collagen
10:1 Elastin to Collagen
Elastin Only
B
Thickness (microns)
25
20
15
10
5
0
#
#
#
#
*
*
0% Strain
20% strain
6:1 Elastin to Collagen ECM
10:1 Elastin to Collagen ECM
Elastin Only ECM

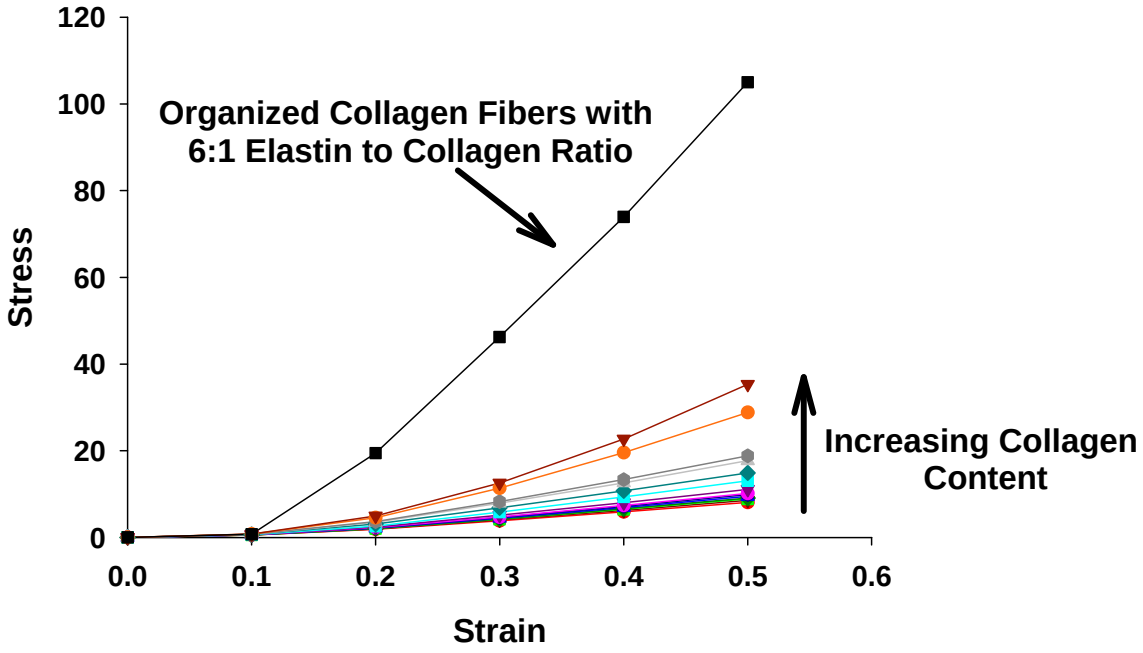
ECM sheet: Static Stress-Strain Curves



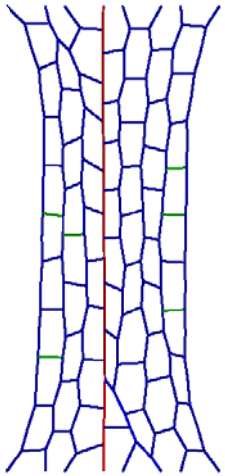
Adding collagen significantly increases the stiffness of the sheets.

Black et al. *Biophys. J*. (2008)

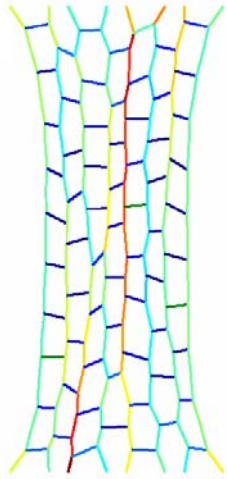
Network Model of ECM Sheet



The collagen percolates the network!



14%: collagen organized to a single fiber: two-phase.

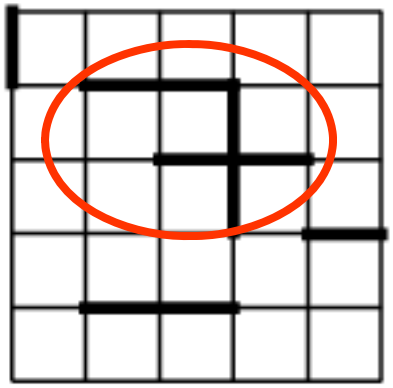


40%: collagen randomly distributed in the network: mixture

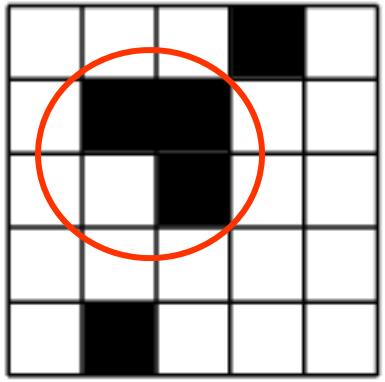
Black et al. *Biophys. J*. (2008)

What is Percolation?

p=0.4



bond percolation



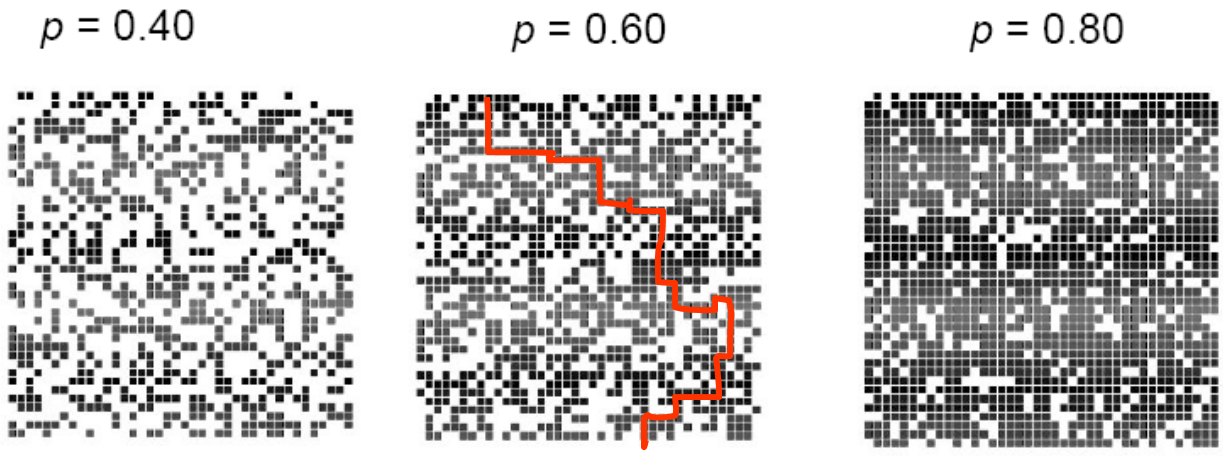
site percolation

p=0.2

• On a given network structure, each bond or each site is occupied by with a specified probability p.
• Neighboring occupied bonds or sites form contiguous clusters.

• Percolation concerns with the properties (size, connectivity) of clusters!
• When p=0, all sites (bonds) are empty with no connectivity.
• When p=1, all sites (bonds) are occupied with full connectivity.
• When p increases from 0 to 1, there is a critical p, called percolation threshold, p_c, when a cluster that spans the system first appears!

Why Percolation?



The connectivity of the structure suddenly changed at p_c=0.59.

Why is p_c interesting?

- Consider the forest fire problem!
- Each site is a tree and start a fire at on end.
- Ask: 1) What is the probability that the fire reaches the other end? Or 2) How long will the fire burn?
- Fire can propagate from tree to tree if trees are within a certain distance.
- This is modeled by neighbors: fire propagates within a cluster.
- When the density or concentration of trees is above p_c, the fire reaches the other end.
- The percolating path is tortuous!

Properties and Applications

Properties

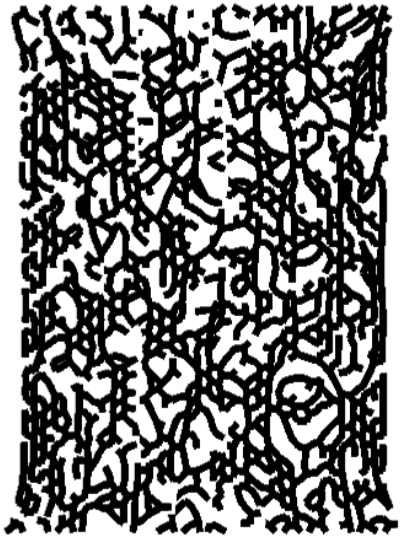
• At the percolation threshold, the properties are singular (infinite cluster, infinite burning time) with a qualitative change in behavior
• The spanning cluster forms a fractal with internal holes at many length scales
• Percolation is a kind of phase transition
• It's a simple geometric game, yet it is powerful.

Applications

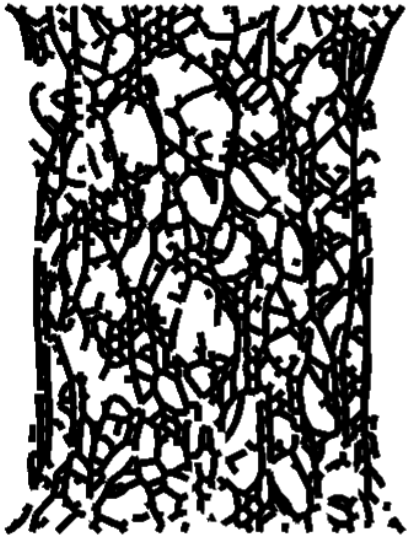
• Forest fire (including wind direction, susceptibility, etc)
• Porous media: Oil fields and drilling
• Infectious diseases: how infectious a flue strain has to be to cause a pandemic?
• Networks: error attack and tolerance in networks, how many nodes have to be eliminated such that the network stops functioning (electrical grid, internet)
• Elastic networks
• Gelation: understanding polymerization, boiling an egg, etc
• Immune system: antibody-receptor clustering (mast cells releasing His)
• Phase behavior of biological membranes (FRAP)
• Failure mechanics

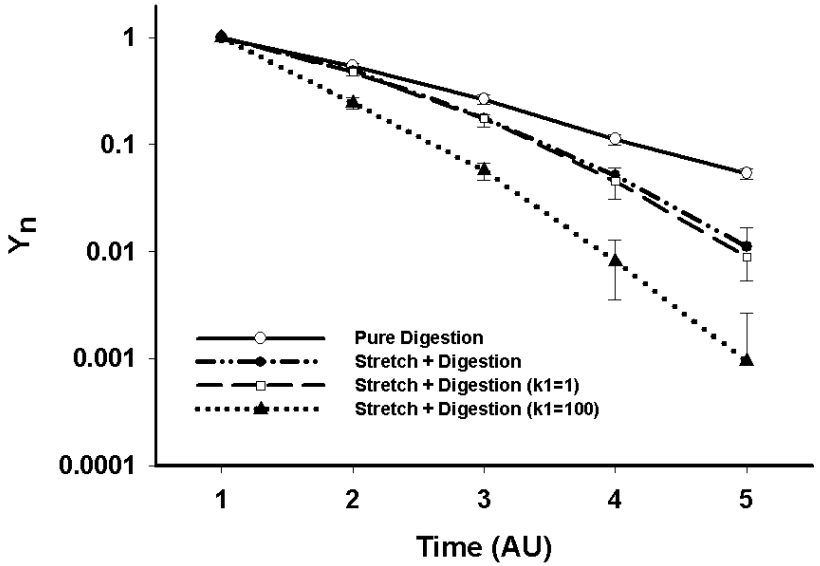
Single phase percolation model of degradation

A



B





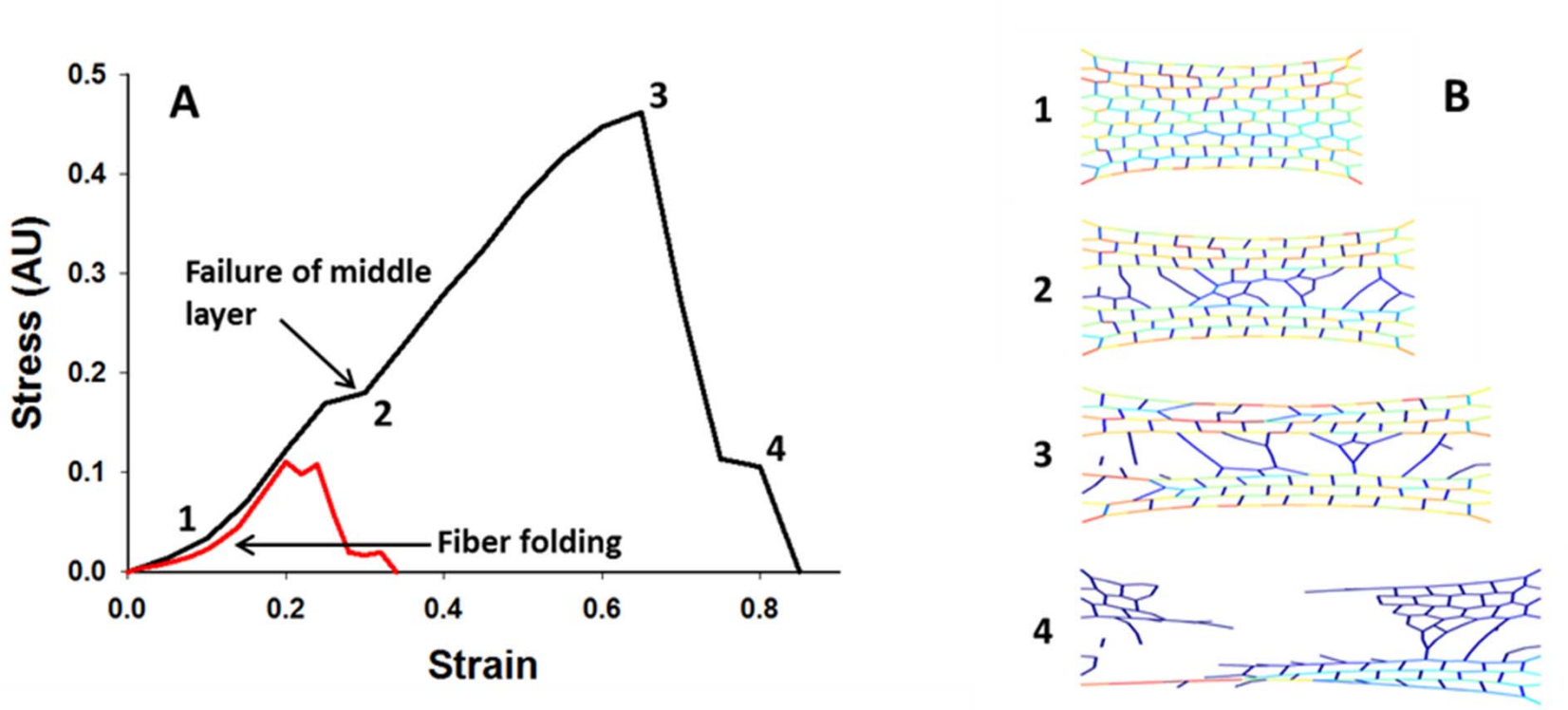
Unstretched digestion by random percolation of holes

Stretched digestion:
$P = Po + c\,\varepsilon$
By correlated percolation of holes

Stiffness decline

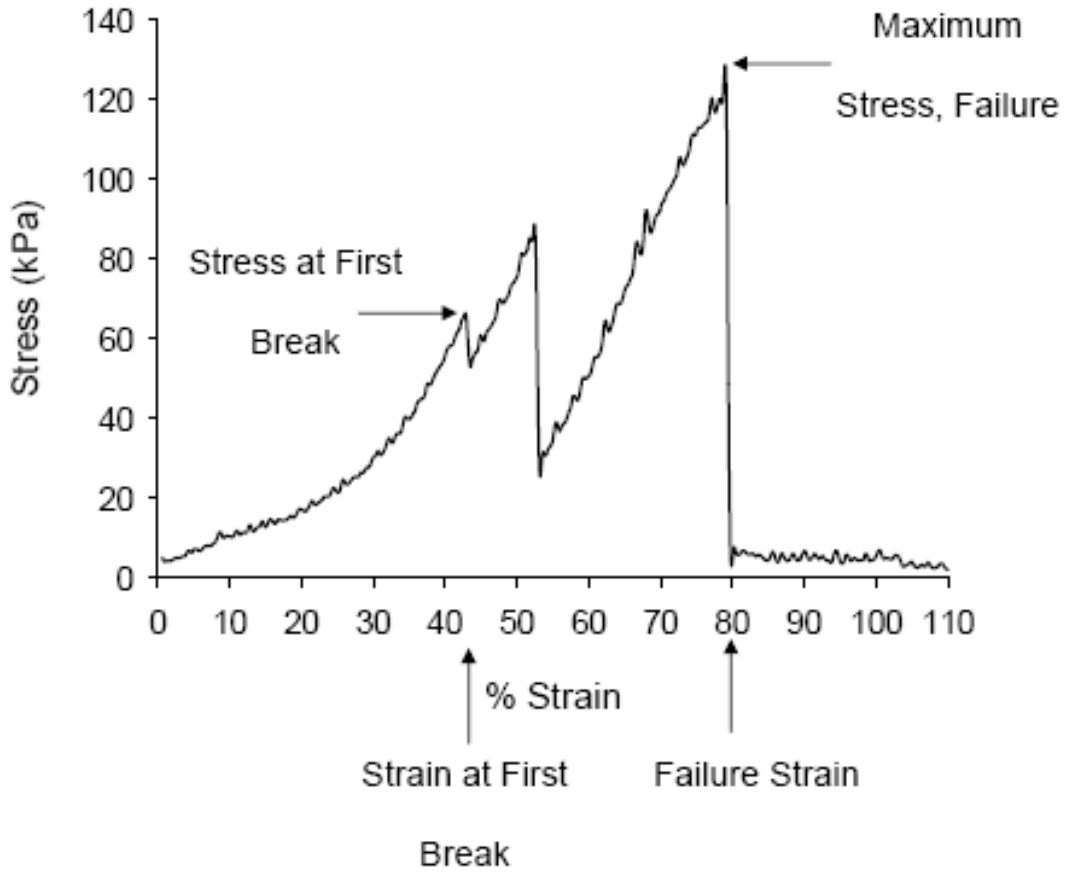
Percolation can be used in a single component material to describe the degradation process. Failure occurs at the percolation threshold.
Percolation of holes = depercolation of bonds

Two-phase component percolation



Stress-strain failure curves and network configurations of a two-phase model with a layer of percolating springs that have a failure strain 5 times lower than the rest of the springs. A) The black line corresponds to a network in which the layer is horizontal and the red line corresponds to a network in which the layer is vertical. Stretching proceeds in the horizontal direction. B) Snapshots of the network configurations corresponding to the black line in A at the points marked by numbers along the stress-strain curve. The colors are proportional to strain; blue is low and red is high strain.

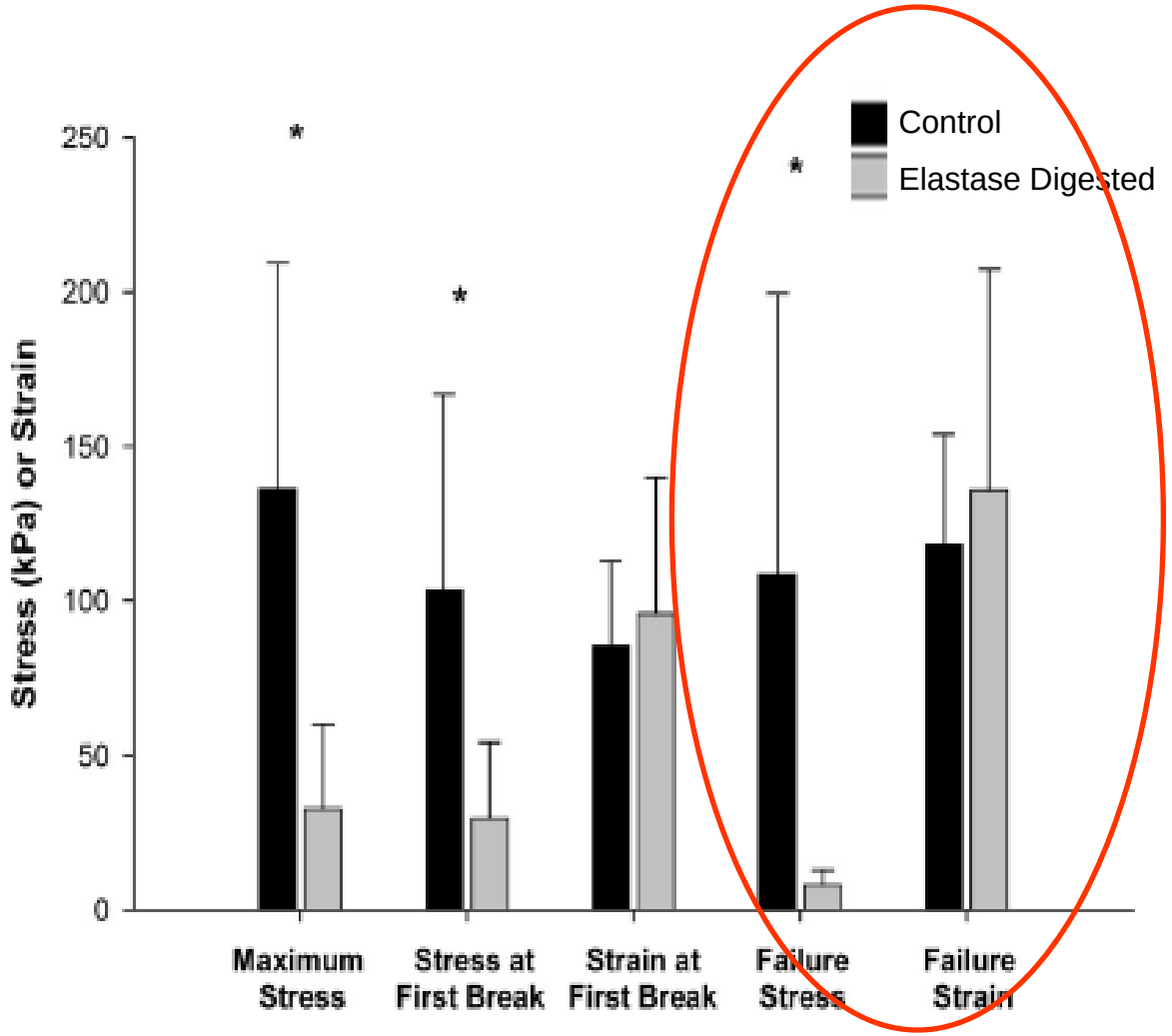
Failure Curve of ECM Sheets



(Black et al., JAP, 2005)

Effects of Digestion on the Failure of Engineered ECM

- Failure stress is reduced significantly by digestion
- Failure strain is not affected by digestion



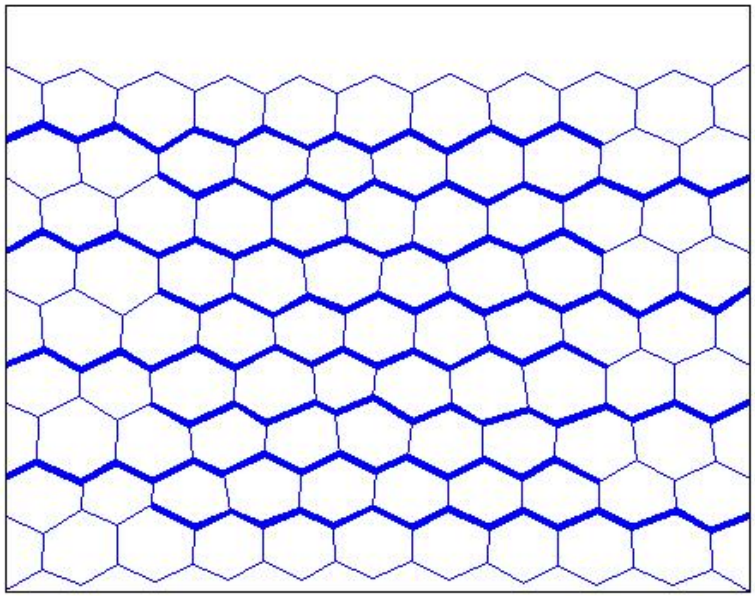
(Black et al., JAP 2005)

Unexplained Results from the Failure of the ECM

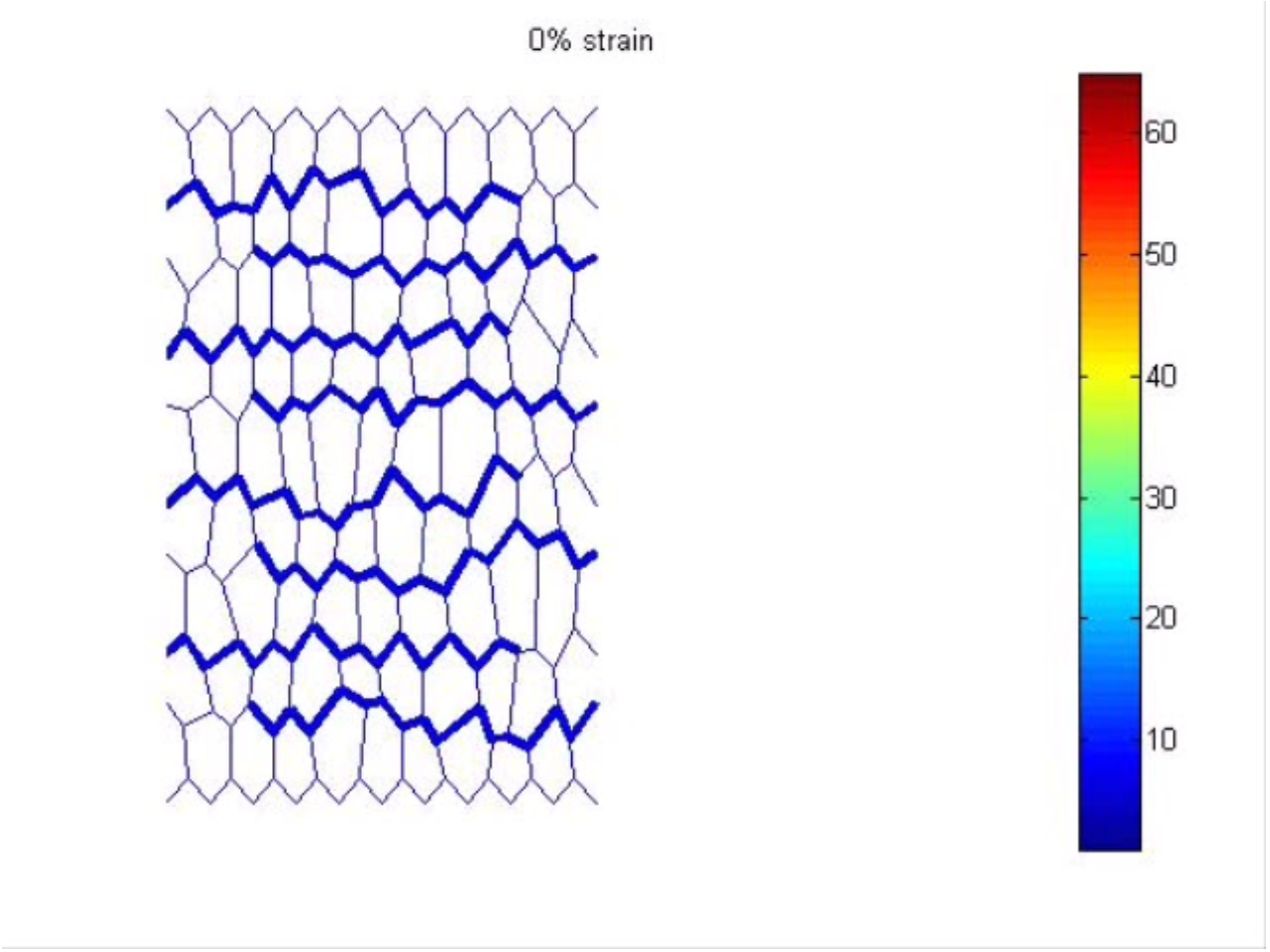
- Elastin's strain threshold is about 200% (Fung, 1993)
- If elastin percolates the tissue, one would expect the failure strain to be near that of elastin
- The experimentally obtained failure strain of constructs is about 90 – 120% (Black et al. 2005, Jesudason et al. 2007)
- Something else must be contributing to the failure!!
- ***Are PG bridges responsible for failure?***

Two-phase non-percolating model: The Zipper Network Model (ZNM)

- Elastin and PGs have varying mechanical properties:
  - Linear spring constant, k_0:
    - Elastin – 6
    - PGs – 2
  - Failure strain threshold:
    - Elastin – 200%
    - PGs – 0.5%
- Elastin does not percolate and is linked via PG bridges
- Model utilized to test:
  - Avalanching
  - Tissue degradation
    - Elastin digestion
    - PG digestion

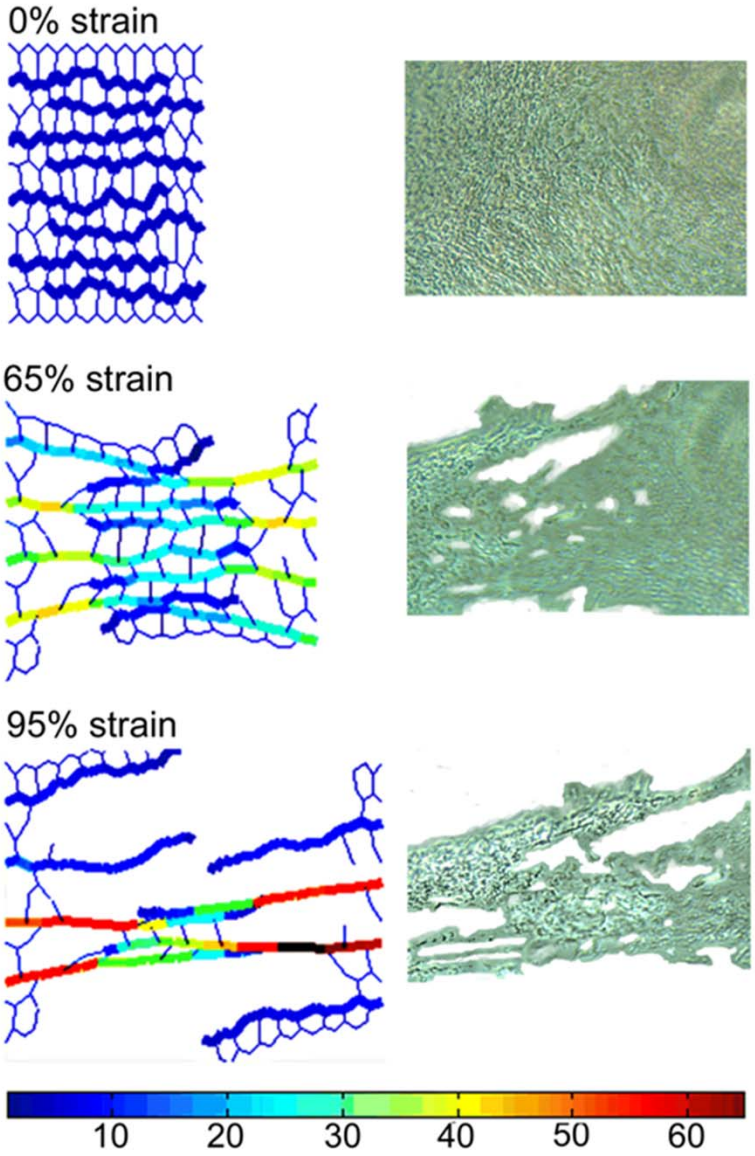


Simulation of Failure Mechanics



Based on color, elastin must carry the majority of forces.

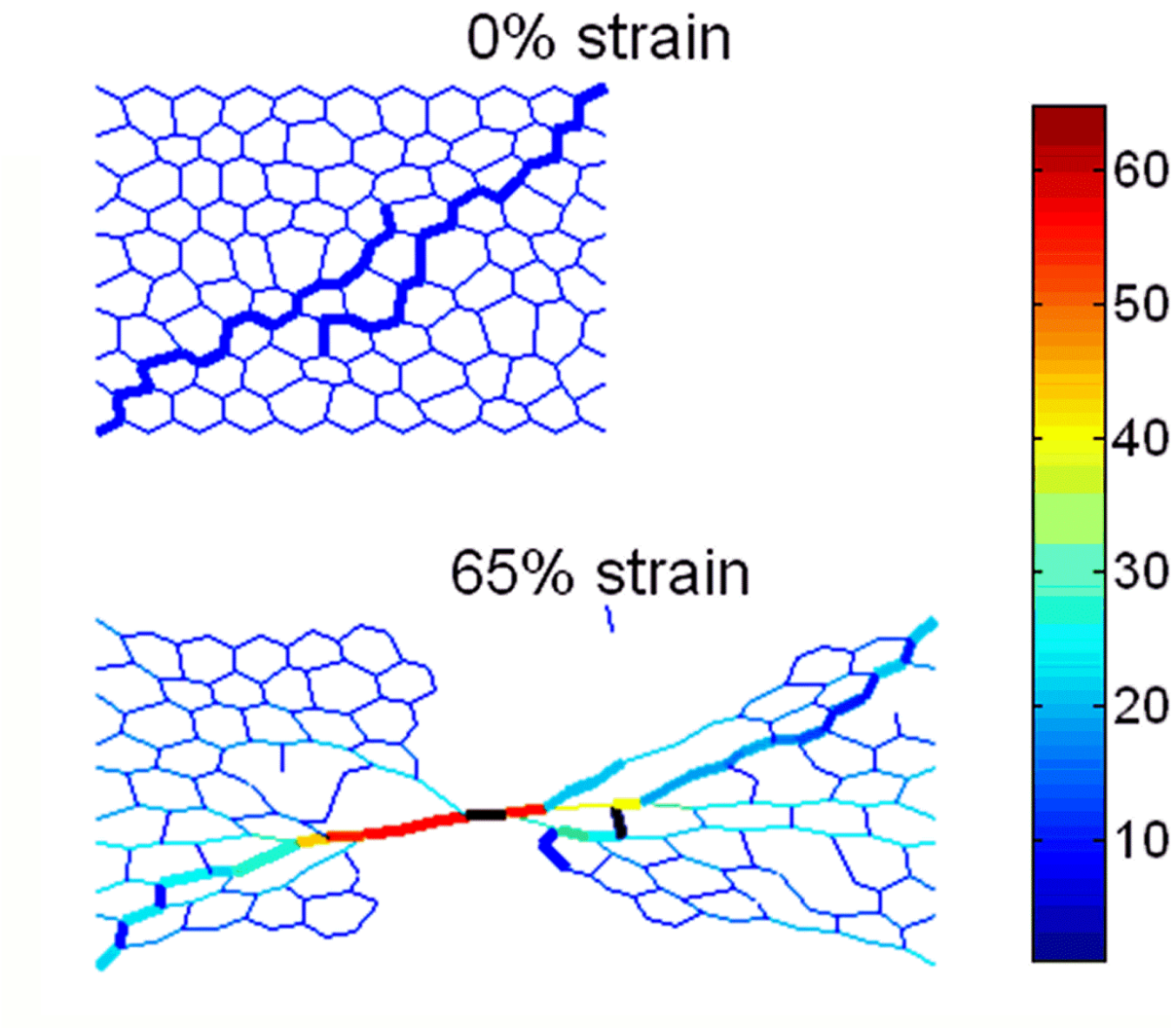
Comparison of ZNM with tissue structure



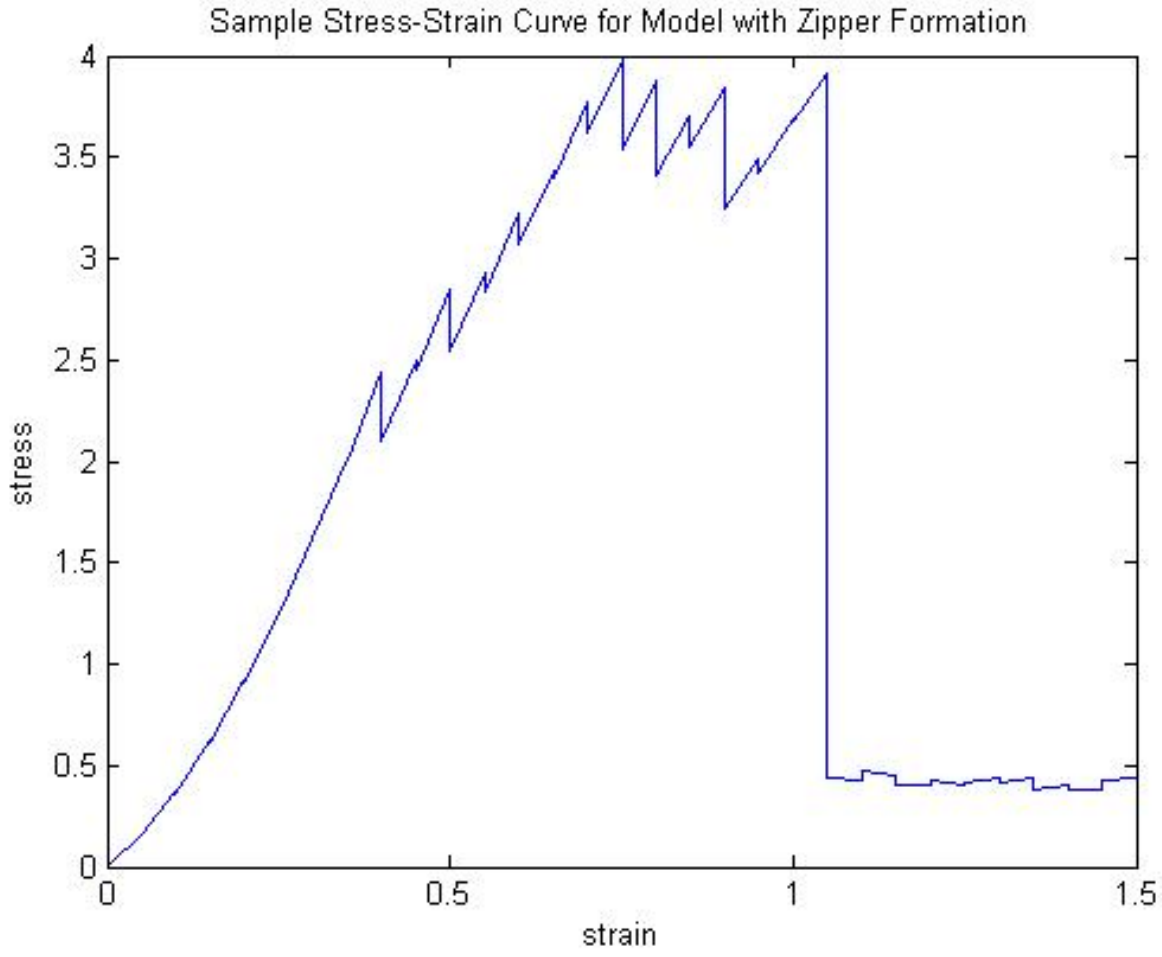
Left: Images of the ZNM being stretched to various strains. The elastin fibers are drawn as thick lines and the PGs as thin lines. Note that the elastin does not percolate across the network. The color scale shows the relative forces on each spring. Right: Phase contrast images of a region of an engineered tissue construct sample undergoing failure taken at strains comparable to those in the network model on the left panel.

(Ritter et al. PNAS, 2009)

When fibers are not parallel to strain

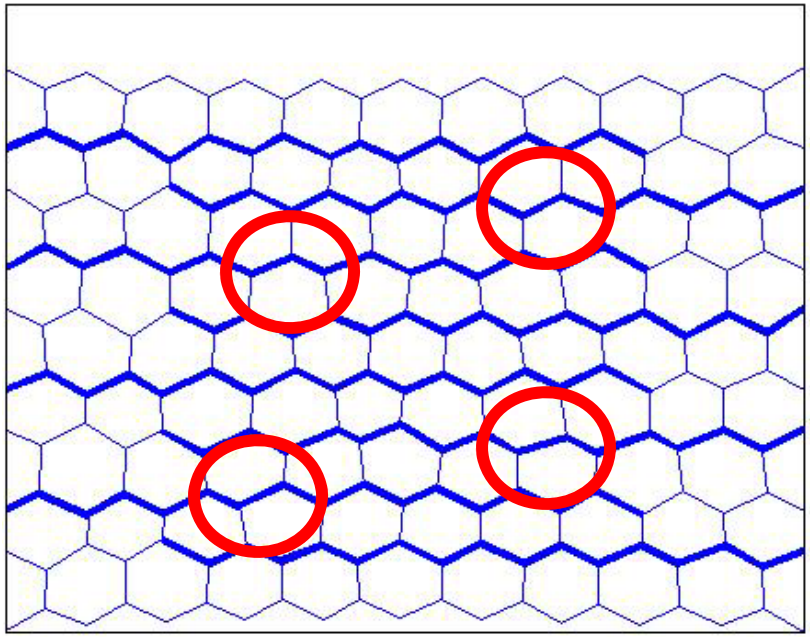


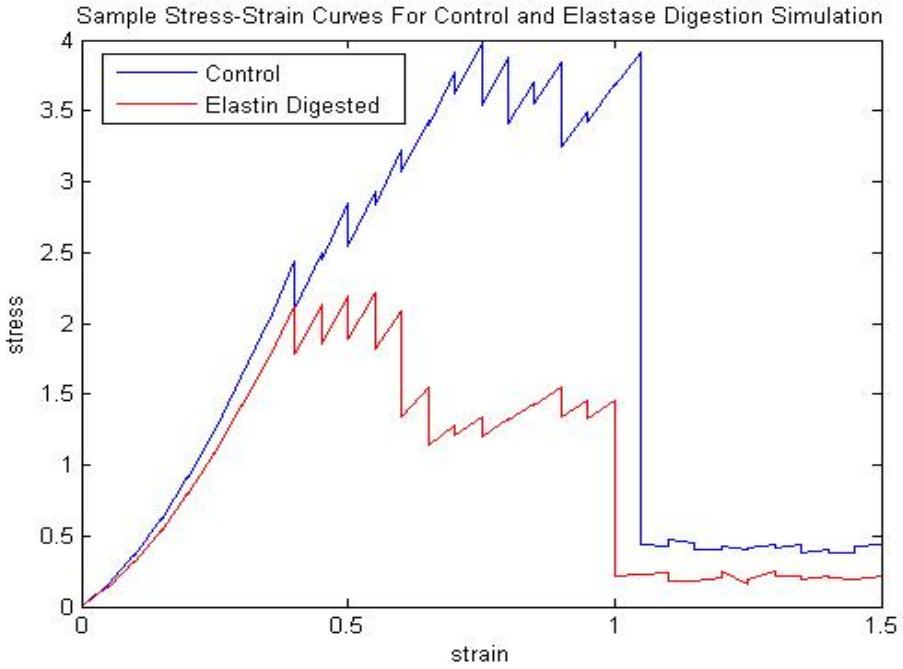
Simulation of Failure Mechanics



- Network fails around 100% strain while holding elastin to its known failure strain of 200%

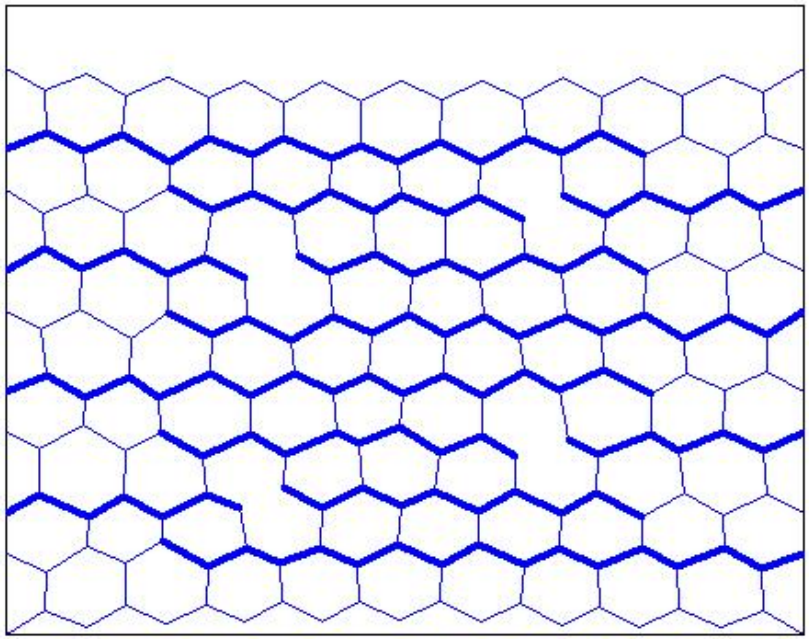
Simulating the Digestion of Elastin

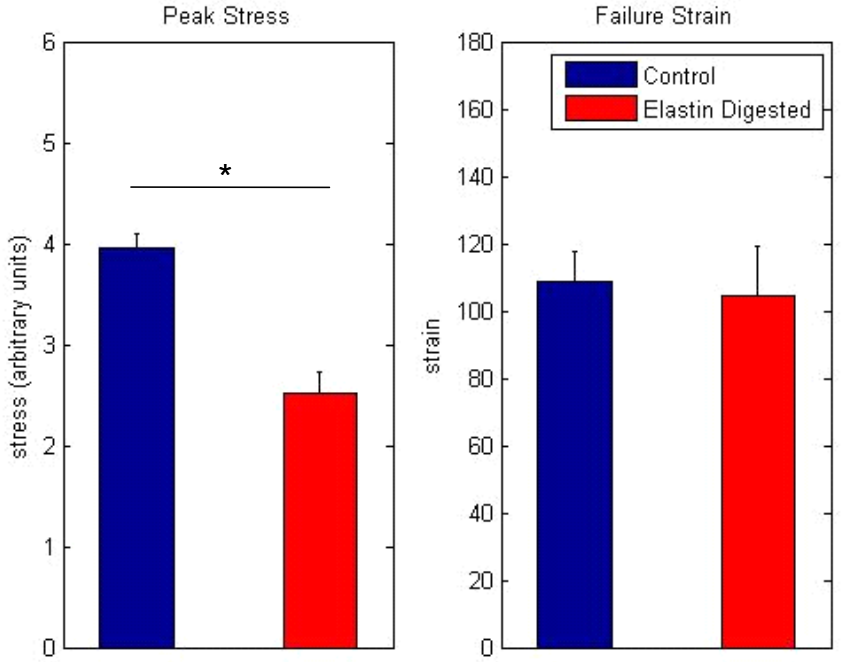




Failure curves and the corresponding peak stresses and failure strains of the ZNM. (A) Sample stress–strain curves for model simulations with and without simulated elastase digestion. Peak stress and stress at failure, or failure stress, are indicated by arrows. (B) Failure data comparing control and elastase digestion simulations. Failure stress but not strain is significantly reduced!

Simulating the Digestion of Elastin



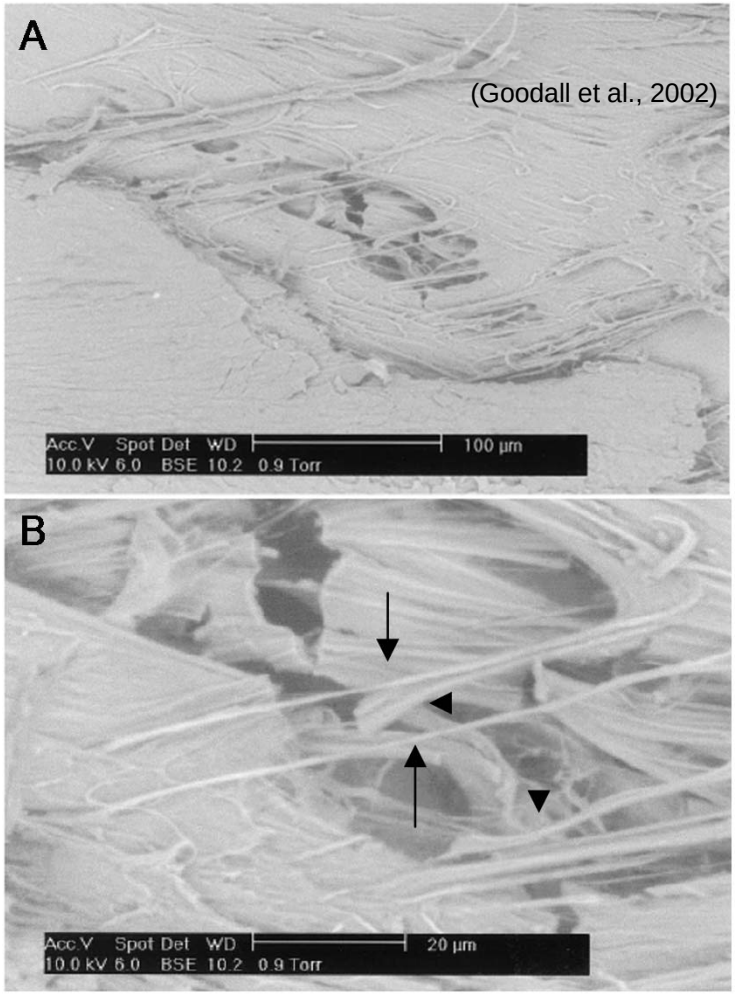


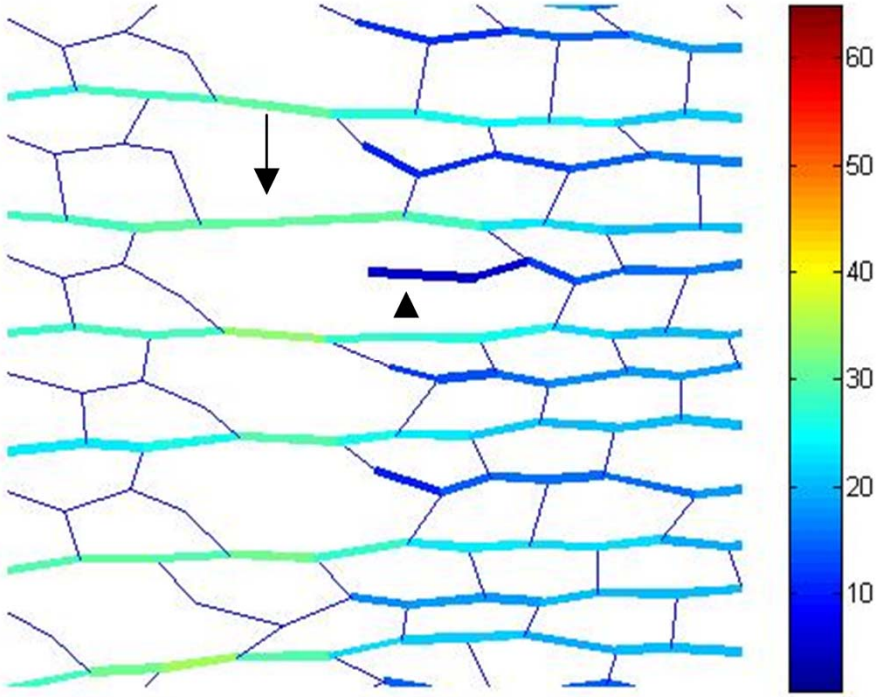
* Signifies significant difference between samples ($P < 0.001$)

Digestion leads to a significant drop in peak stress but no change in failure strain. Stress is in arbitrary units. *, $P < 0.001$.

Failure results are in excellent agreement with the data obtained by Black et al. (2005) and Goodall et al. (2002)

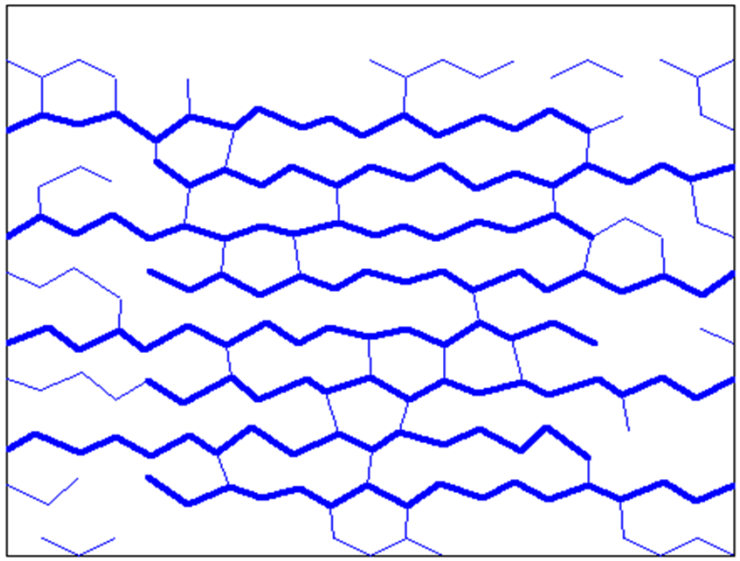
Comparison to Electron Scanning Microscope Image



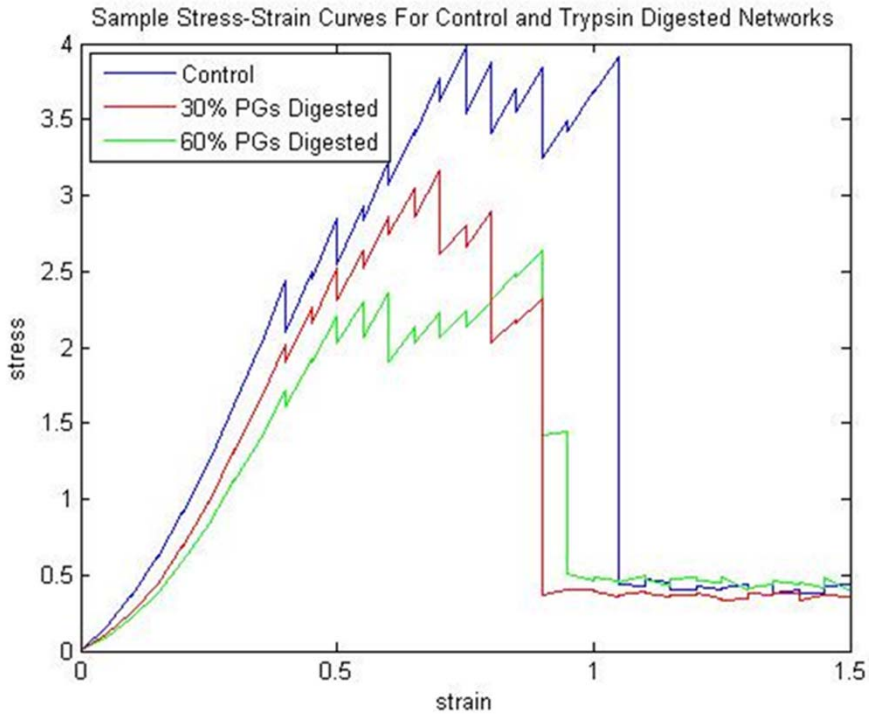


The model provides structure similar to vessels during failure!

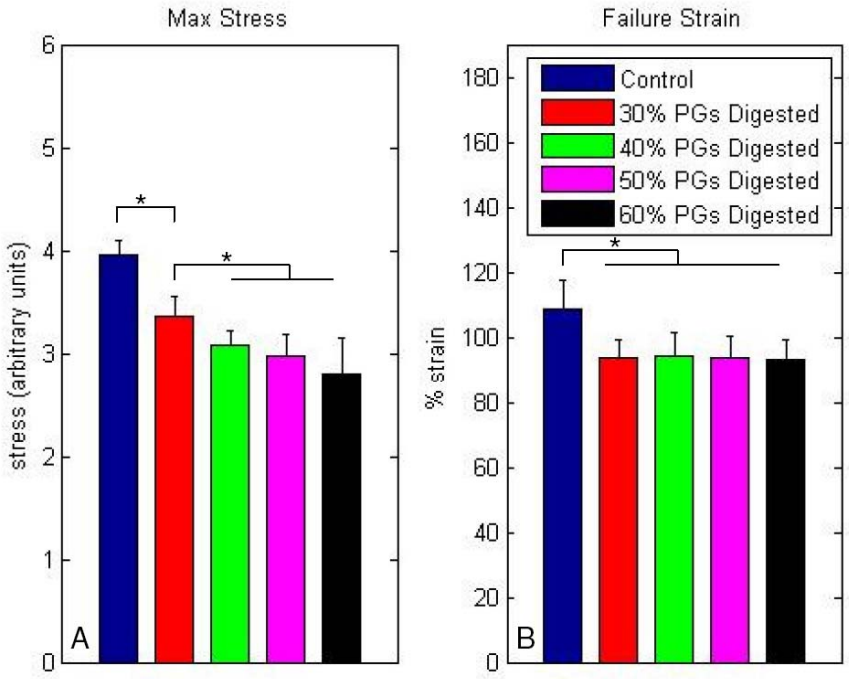
Simulating PG Digestion



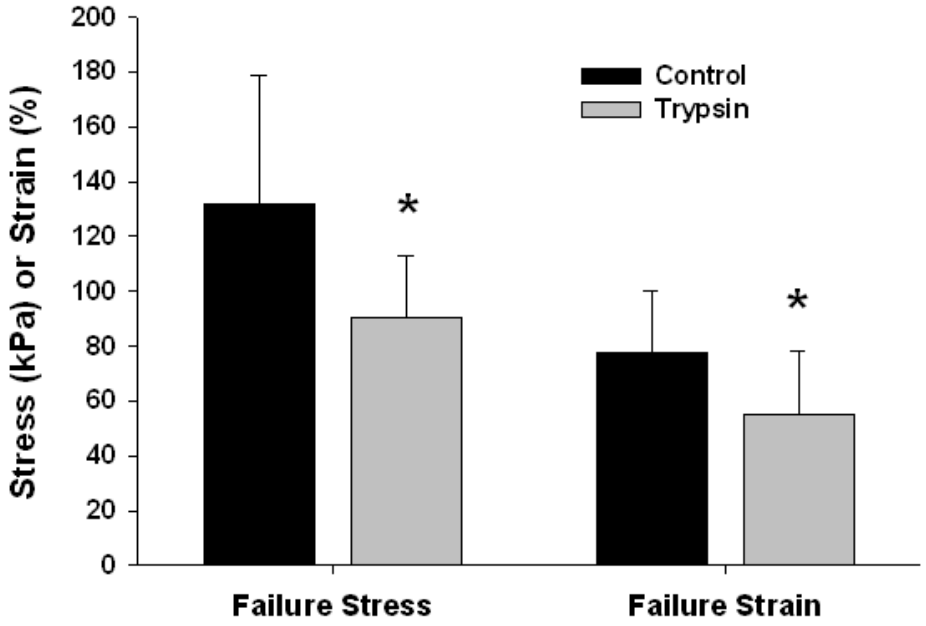
60% of PGs digested



Comparison of PG digestions



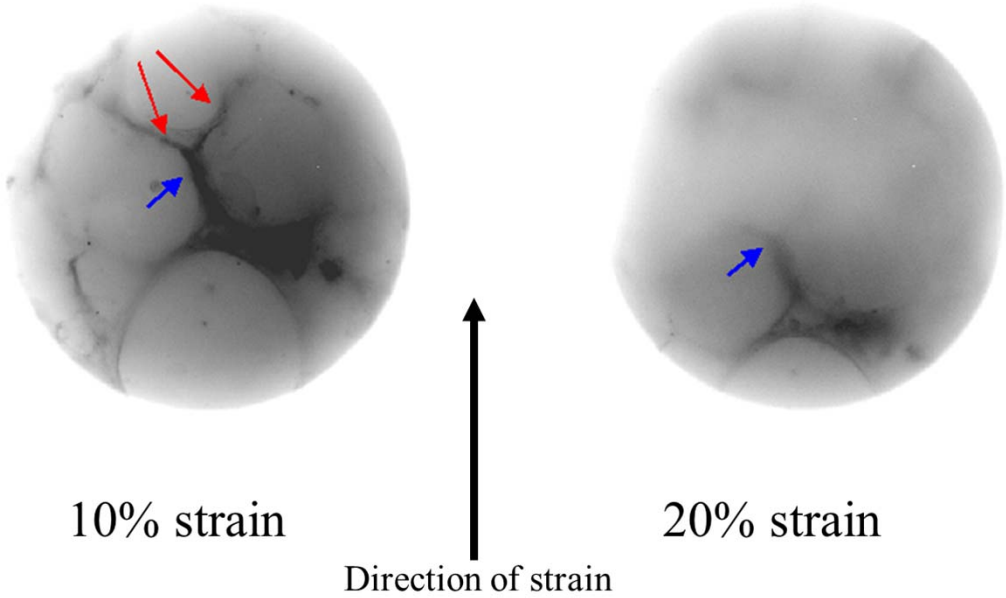
Model predictions



Biochemical analysis showed that digestion eliminated 61% of PGs.

Summary: 1) Elastin carries the load and determines failure stress;
2) PGs transmit load, fail and determine failure
3) Mechanism is a network failure

Evidence of Failure of the Alveolar Wall

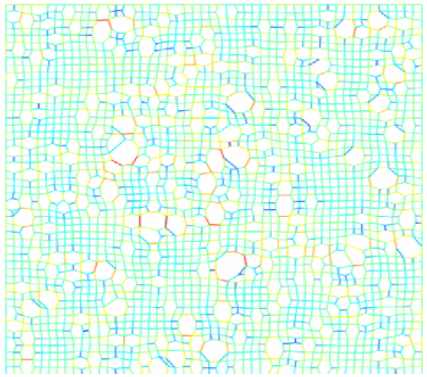


This slide shows the same region of collagen labeled tissue from a rat lung that had been treated with elastase at 10% strain. The treatment causes symptoms and structural changes similar to human emphysema. The blue arrow indicates an anatomical landmark in the tissue, a bifurcation. The two red arrows clearly show 2 fibers connecting to this node. When the sample was stretched to 20% strain, these two fibers are not seen. This indicates that the fibers most likely broke going from 10 to 20% strain. Indeed, further increasing the strain to 30% resulted in a complete failure of this sample, that is it separated into two parts. This we often observed in the emphysematous tissue, but not in the normal tissue. Note that a 30% uniaxial strain is estimated to be within the limits of the strains during an isotropic 3D straining of the lung.

Kononov et al. *Am. J. Resp. Crit. Care Med*. (2001).

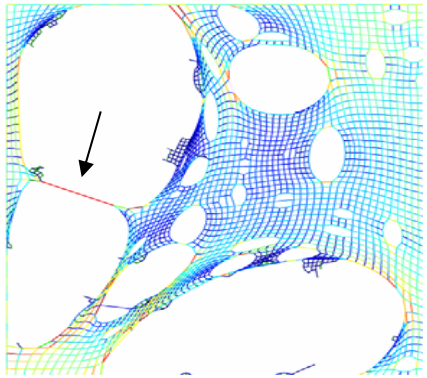
Whole Lung Model: Comparison with CT

A



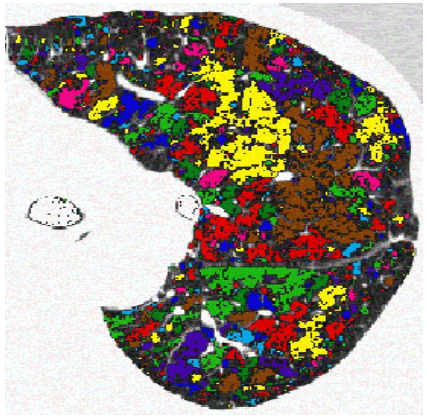
Chemical

B



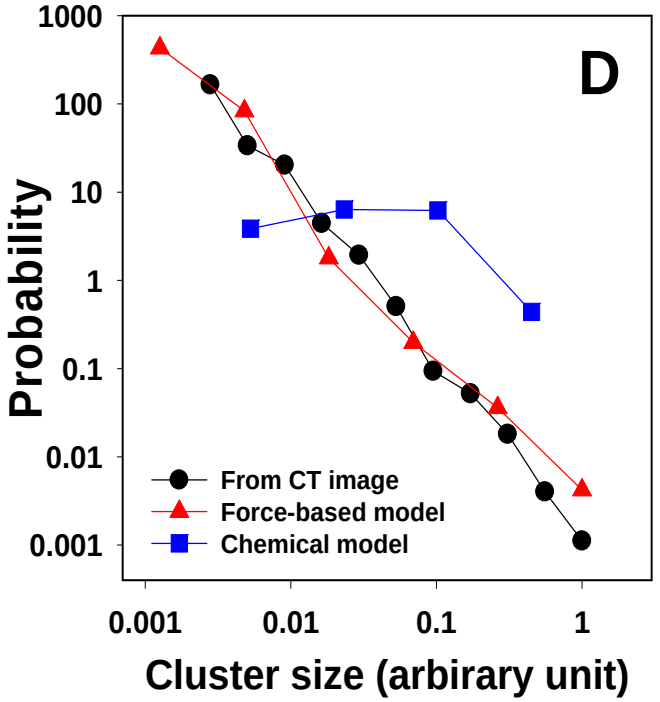
Force-based

C



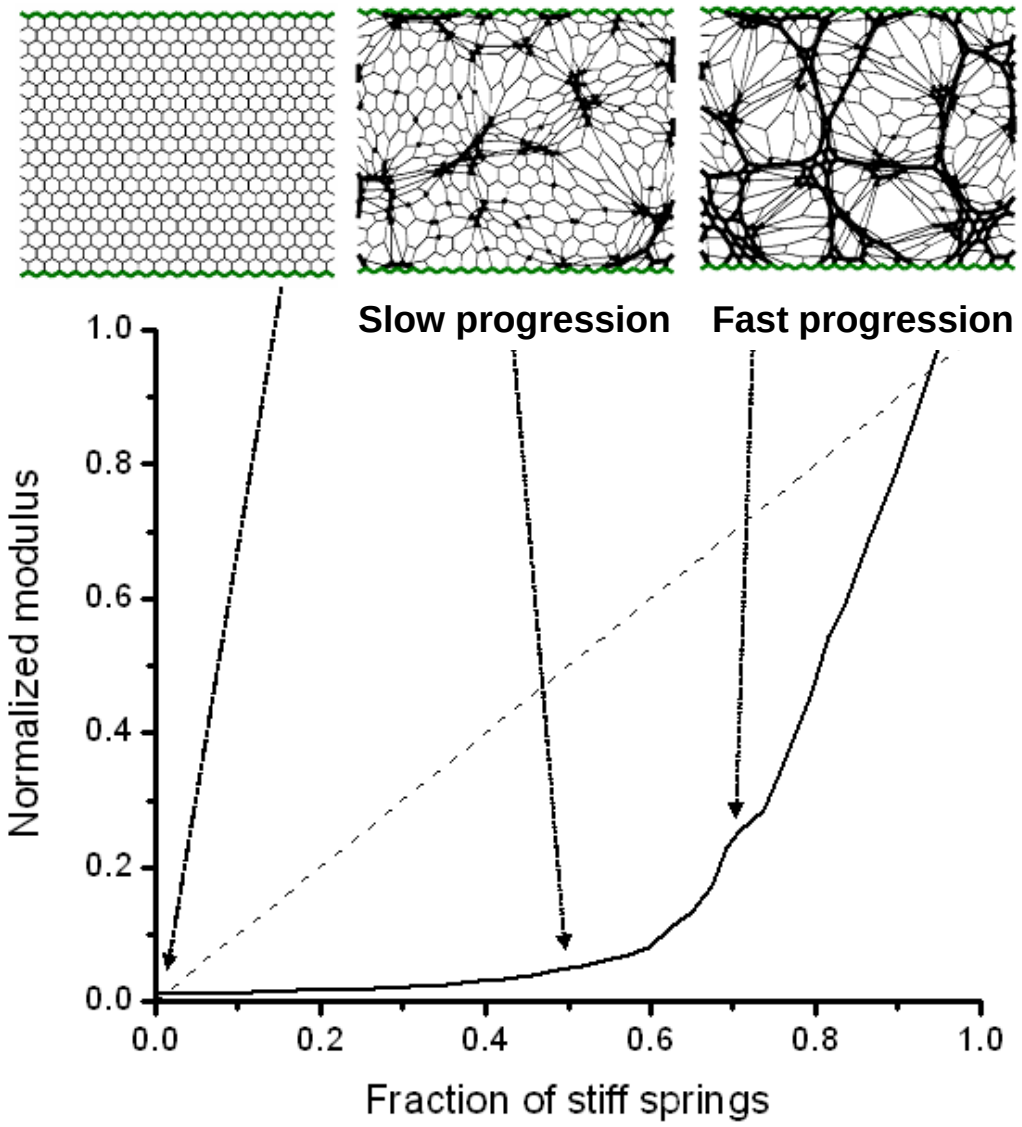
CT image

Mishima et al. PNAS (1999).



Suki et al. Am. J. Resp. Crit. Care Med. (2003).

Implications for Medicine: Fibrosis

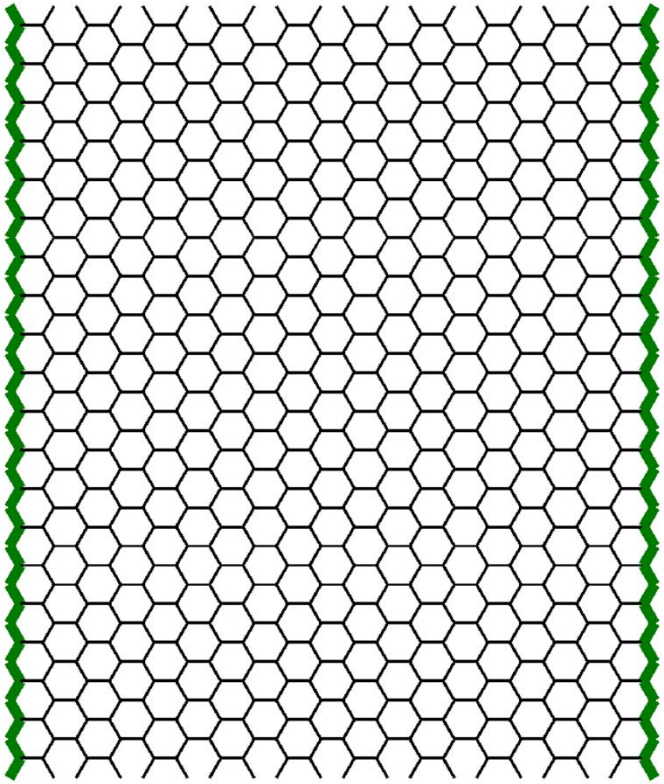


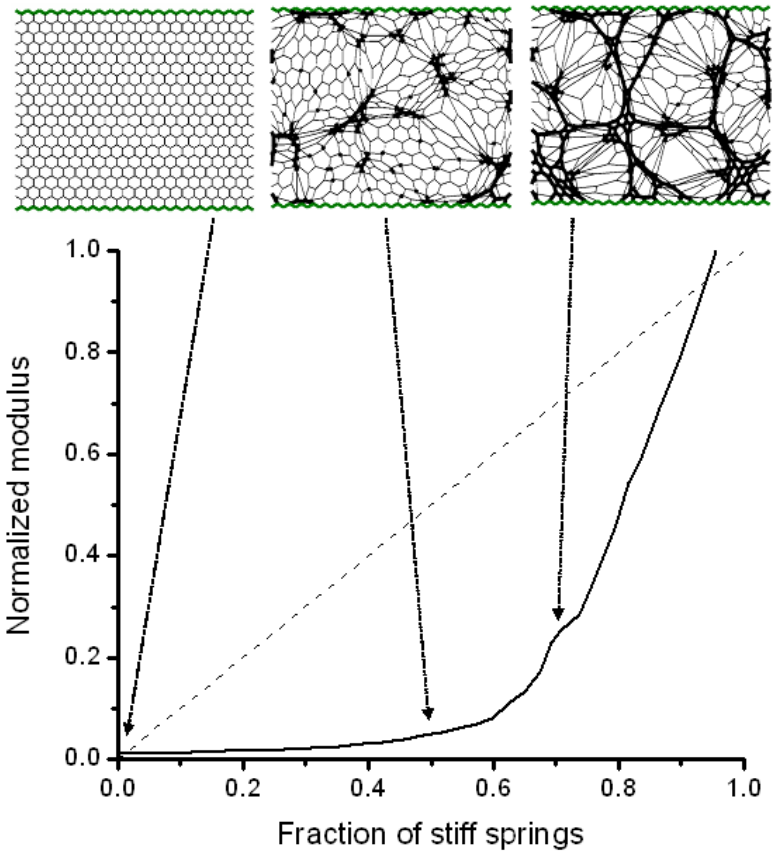
Phase transition when clusters of stiff springs percolate.

Before the rapid change in function, physiologic function appears almost normal!

Bates et al. Am. J. Respir. Crit. Care Med. (2007).

Progression of fibrosis

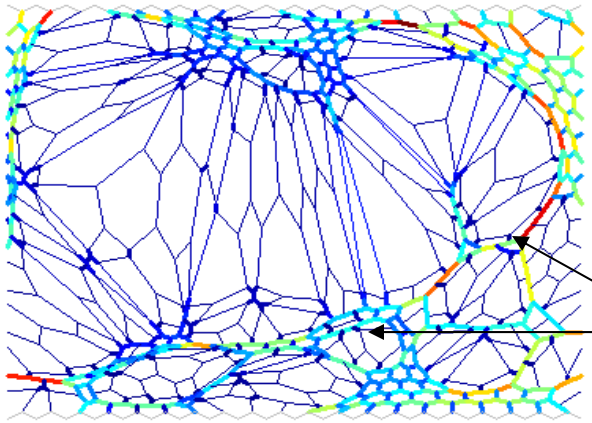




Notice the development and disappearance of heterogeneity!

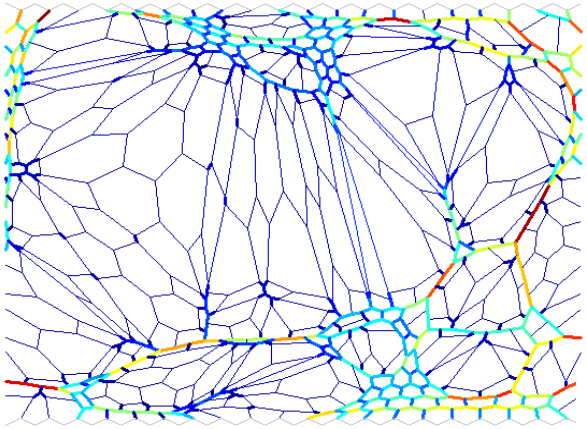
Repair of Emphysema and Fibrosis

- Progress in tissue engineering and stem cell biology gives hope to cure emphysema and fibrosis.
- The selection of treatment location is based on the judgment of the physician.
- The efficacy of treatments could be maximized by targeted delivery of drugs, cells, or engineered tissues to those sites that result in the greatest reversal of symptoms.
- How should the treatment be directed, and in what quantity?
- Computational modeling can help answer this question.



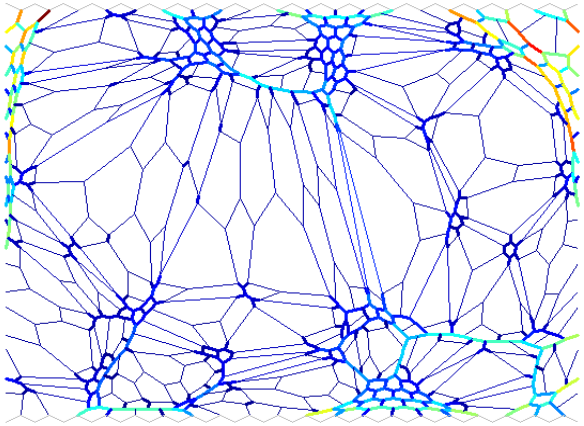
Initial network: concentration of stiff springs c=0.65 and stiffness B=26.6. (Red high force, dark blue low force)

Percolating cluster of stiff springs



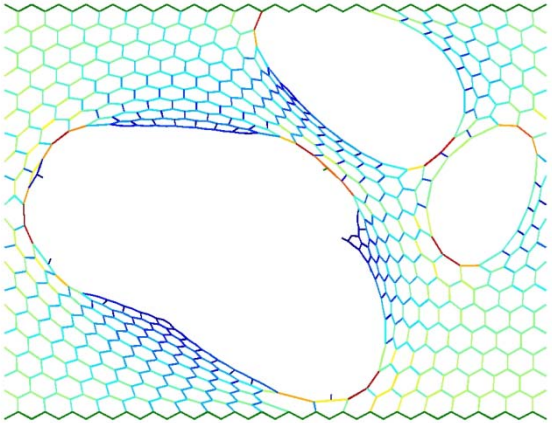
Random elimination of 45 springs:
(May mimic systemic drug treatment)
c=0.61
B=22.6 (15% decrease)
Percolation remains !

DEPERCOLATION

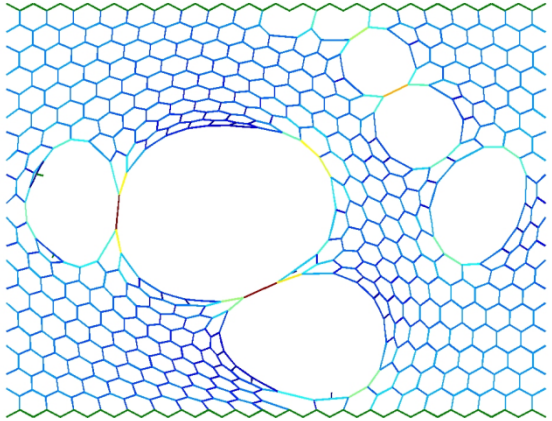


Targeted elimination of 22 springs:
c=0.63
B=16 (32% decrease)
Percolation eliminated !

Suki et al. (Drug Discovery Today, 2008)

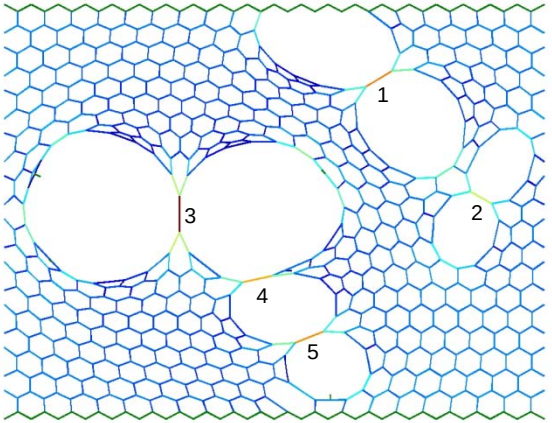


Initial network containing 312 holes with 3 emphysematous lesion. Mean area (A) is 1810±12650, *c*=0.034, and *B*=0.62. Note the low *B* and the large heterogeneity.
(Red high force, dark blue low force)



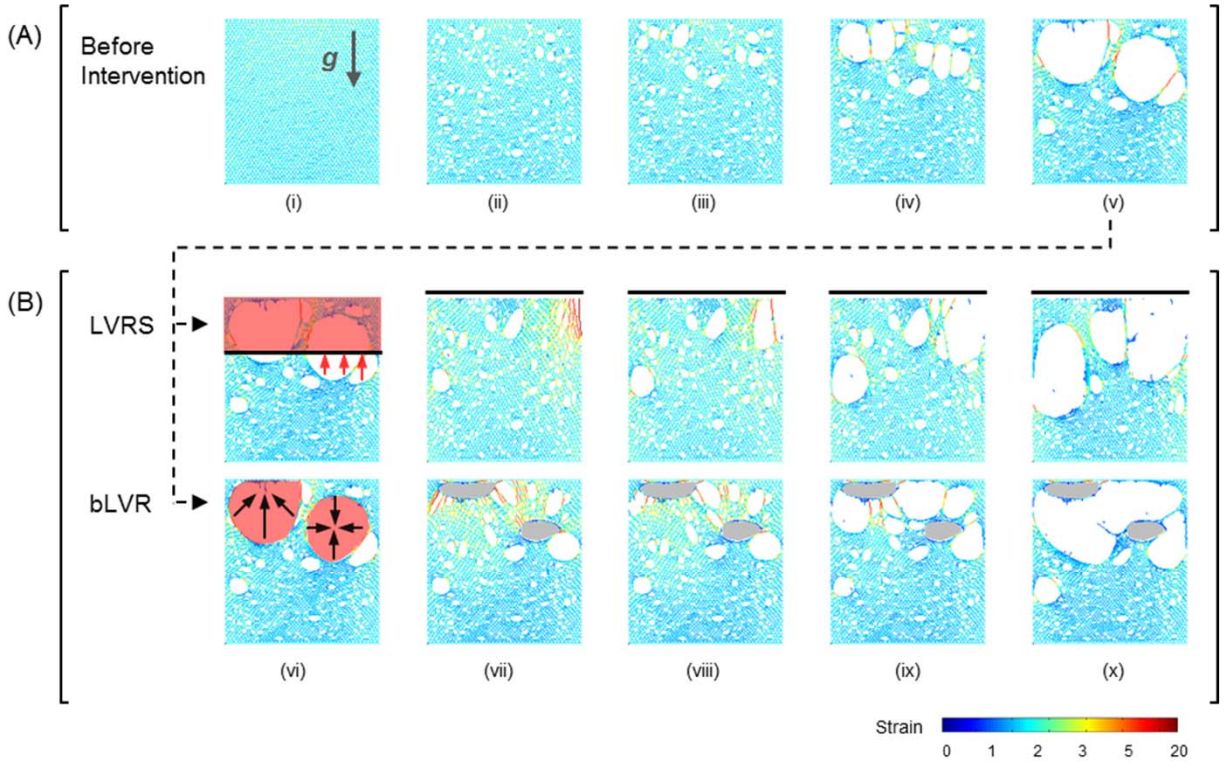
By inserting 8 springs randomly, we increase recoil and reduce heterogeneity to obtain *c*=0.027, *B*=0.82 and *A*=1751±6254.

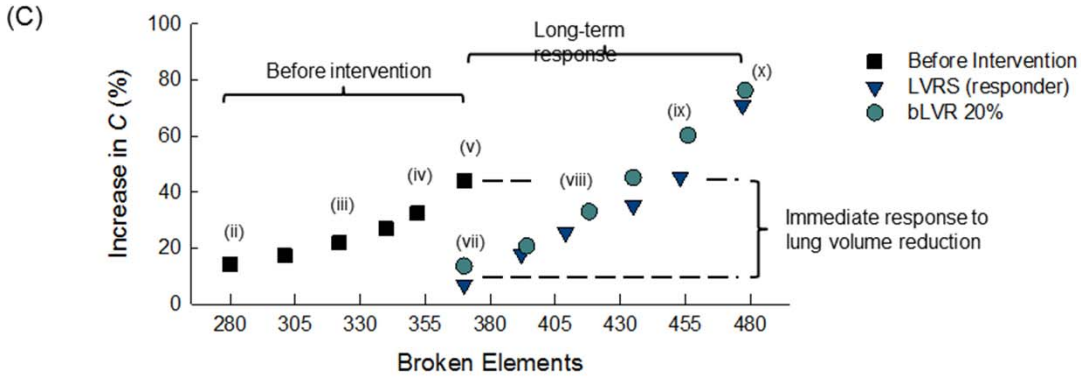
Suki et al. (Drug Discovery Today, 2008)



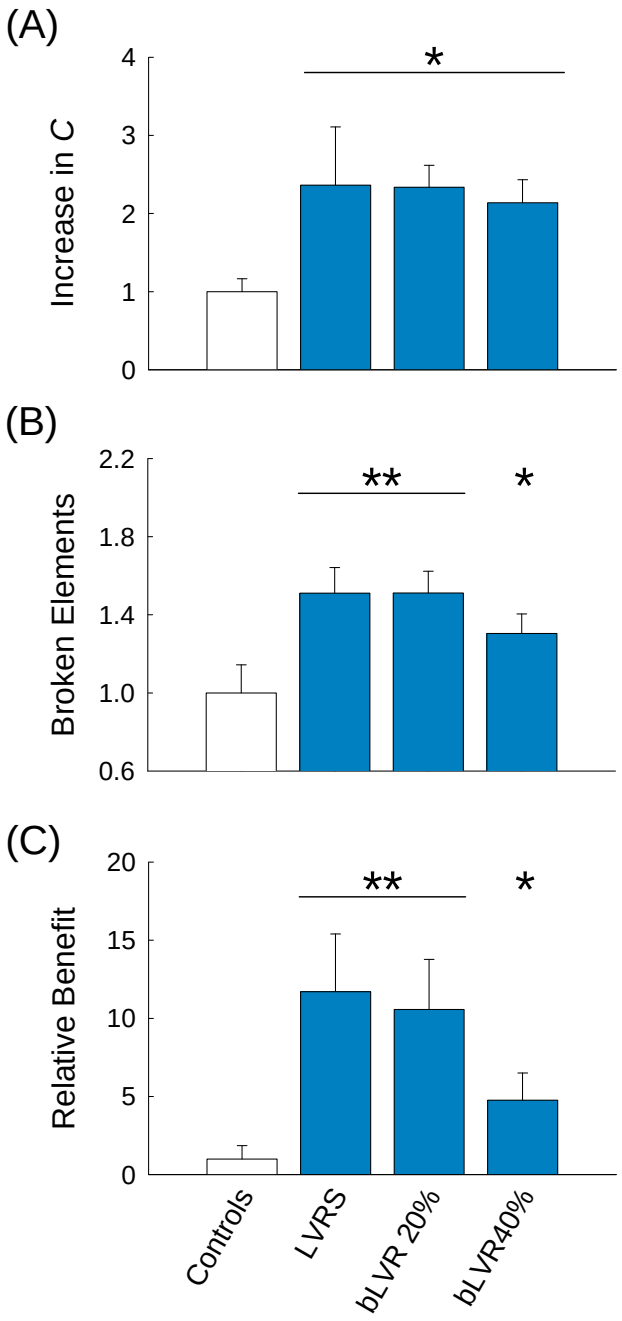
By inserting 5 springs (numbered 1 through 5) at targeted locations we obtain *c*=0.03, *B*=0.85 and A=1766±4909. This increase *B* beyond the random repair and eliminates more heterogeneity.
Breaking up the large cluster!

COMPARISON OF TWO TREATMENTS OF EMPHYSEMA





(A) Representative simulation of emphysema progression before intervention, and (B) comparison of lung volume reduction techniques are shown in a responder network. (i) Initial network configuration representing healthy tissue, direction of gravity indicated by arrow. (ii) Disease progression initiated by randomly breaking ~4% of elements. (iii-v) Gradual network destruction representing tissue failure during emphysema progression. (vi) Illustrations of lung volume reduction techniques initiated from the same disease condition. For LVRS, the upper 30% of the network (red shaded region) was removed and intersected regions were stretched (solid arrows) to form a continuous upper border (solid line). For bLVR, affected regions (red shaded areas) were reduced (dashed arrows) to 20% of their original sizes. (vii-x) Disease progression following either LVRS or bLVR, with reduced regions shown (grey shaded areas). Color bar represents strain distribution of individual elements. (C) Changes in compliance, *C* for the representative network shown.



(A) Lung volume reduction accelerated the rate of tissue failure as estimated by the increase in compliance after four steps of disease progression. (B) LVRS and bLVR reduction to 20% yielded the longest predicted survival estimated as the number of broken elements to reach a 60% increase in *C* from baseline. (C) Even greater improvements were observed for the relative benefit index, which represented a measure of quality of life. For all cases, mean values for LVRS and bLVR reduction to 20 or 40% (blue bars) were normalized by projected outcomes without treatment (open bars), error bars represent standard deviations. *Indicates values significantly larger than controls, **indicates values significantly higher than controls and bLVR reduction to 40%.

Summary

1. **Failure of biological tissues is a multiscale phenomenon;**
2. **Percolation can be used to describe degradation and failure;**
3. **Protein fibers control elasticity and failure stress;**
4. **Proteoglycans control failure strain;**
5. **Network breakdown can explain disease progression;**
6. **Network physiology can be used to gain insight into treatments.**