Micro- and nano-mechanics of osteoarthritic cartilage: The effects of tonicity and disease severity

---Presented by
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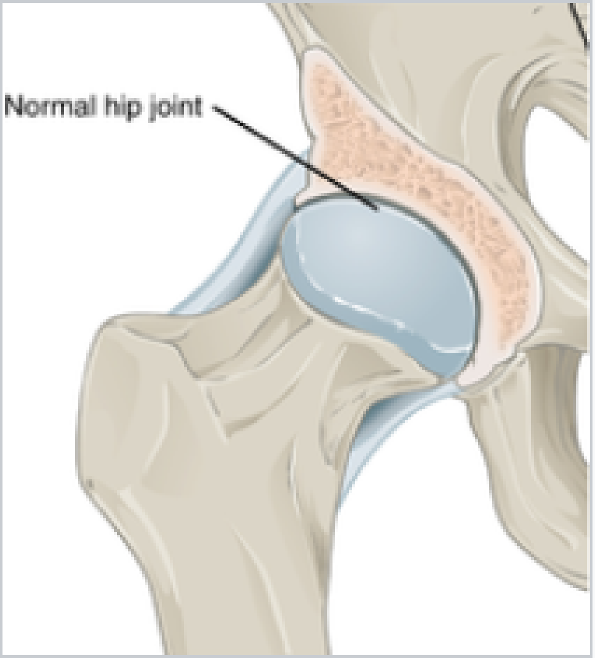
osteoarthritic cartilage ：骨性关节炎软骨
Tonicity ：肌肉弹性

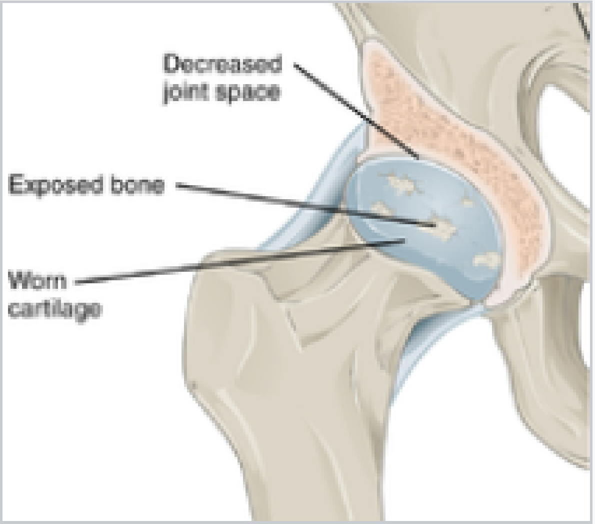
Contents

OA (osteoarthritics)

- A type of joint disease that results from breakdown of joint cartilage and underlying bone.
- The most common symptoms are joint pain and stiffness. Other symptoms include joint swelling, decreased range of motion.
- It is characterized by the loss of proteoglycans(PGs) and hence GAG content via enzymes as well as failure of the collagen network and subsequently loss of cartilage stiffness.



Normal hip joint



Hip joint with osteoarthritis[32]

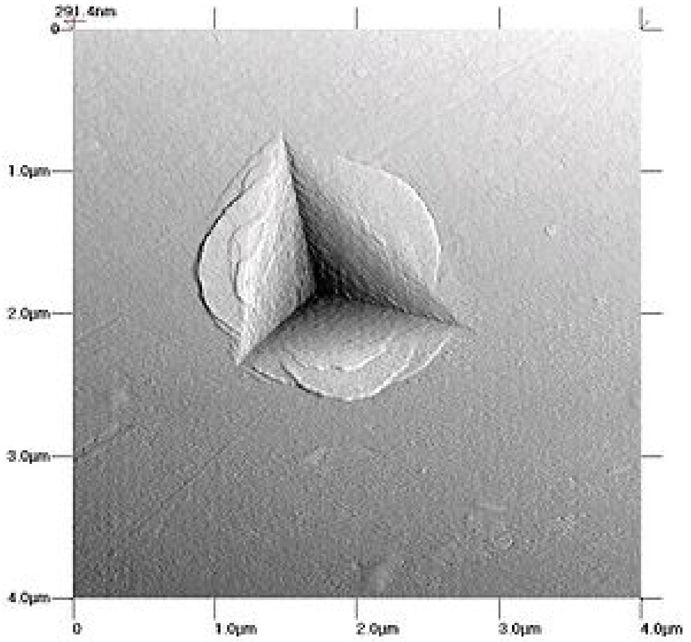
ed from : https://en.wikipedia.org/wiki/Osteoarthritis

Objectives

- Understand the response of OA cartilage to changes in ionic strength (tonicity) and correlate it with
  - The mechanical properties of cartilage
  - measured at the micro- and nano-scale
  - from indentation experiment;
  - The severity of the disease

Nanoindentation(纳米压痕)

- Nanoindentation is a variety of indentation hardness tests applied to small volumes. Indentation is perhaps the most commonly applied means of testing the mechanical properties of materials;



In a traditional indentation test (macro or micro indentation), a hard tip whose mechanical properties are known (frequently made of a very hard material like diamond) is pressed into a sample whose properties are unknown. The load placed on the indenter tip is increased as the tip penetrates further into the specimen and soon reaches a user-defined value. At this point, the load may be held constant for a period or removed. The area of the residual indentation in the sample is measured and the hardness, H, is defined as the maximum load, P_{max}, divided by the residual indentation area, A_r:

$$H = \frac{P_{\max}}{A_{\mathrm{r}}}.$$

For most techniques, the projected area may be measured directly using light microscopy. As can be seen from this equation, a given load will make a smaller indent in a "hard" material than a "soft" one.

Speciemens Preparation

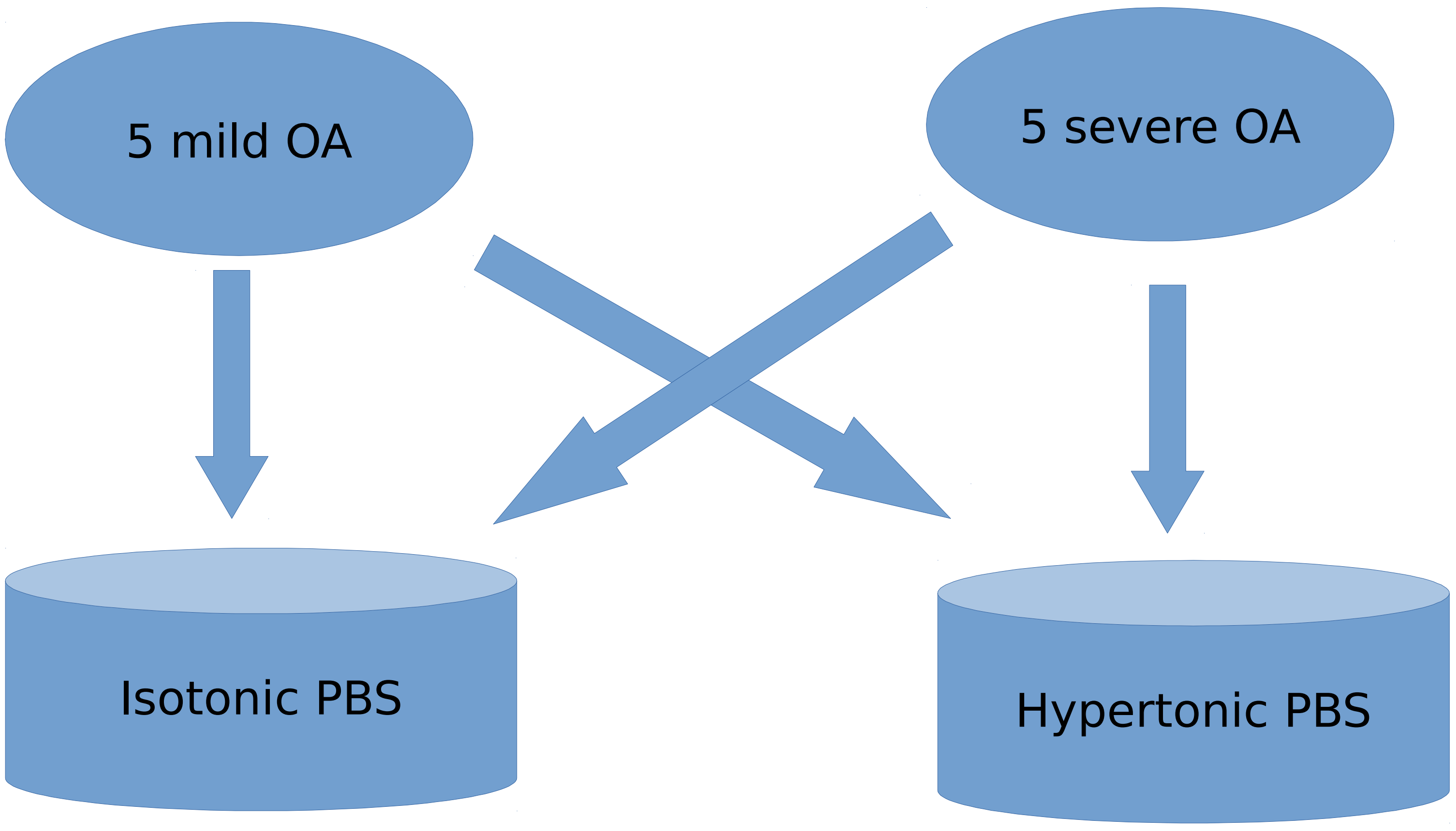
- Source: Total knee which was replaced in surgeries

5 mild OA

5 severe OA

Speciemens Preparation

- Source: Total knee which was replaced in surgeries



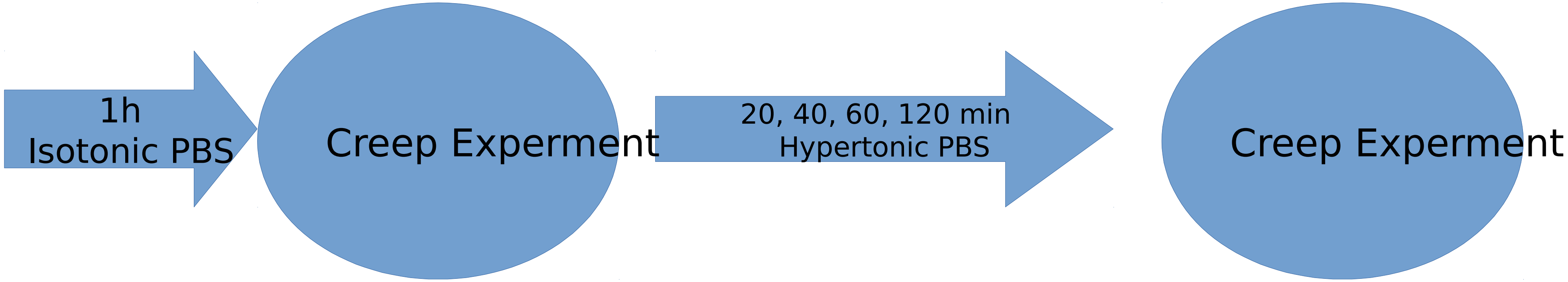
Micro-scale Indentation experiment --- Method

Mild OA

1h Isotonic PBS → Creep Experment → 20, 40, 60, 120 min Hypertonic PBS → Creep Experment

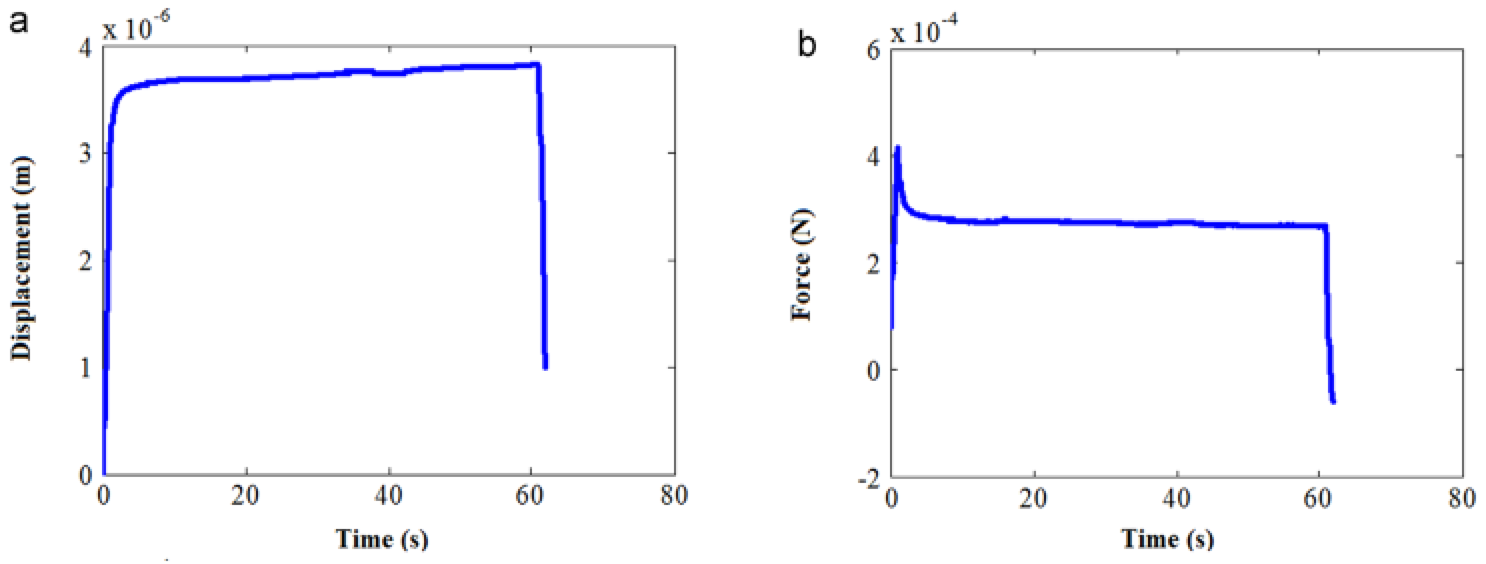
Micro-scale Indentation experiment --- Method

Mild OA



Two or more locations each sample
Every location measure 3 times

Micro-scale Indentation experiment --- Method



$$h^{\frac{3}{2}}(t) = A_0 - \sum A_i \exp(-t/\tau_i)$$

$$C_0 = \frac{8A_0\sqrt{R}}{3P_{max}}$$

$$C_i = \frac{8A_i\sqrt{R}}{(RCF_i)3P_{max}}$$

$$(RCF_i) = \frac{\tau_i}{\tau_R}\left[\exp(\tau_R/\tau_i) - 1\right]$$

$$G = \frac{1}{2 \times (C_0 - \sum C_k)}$$

$$E = G \times 2 \times (1 + \nu)$$

Assumption:
Viscoelastic creep
Poisson’s rate = 0.5
Incompressibility

Young’s Modulus
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Mircro-stiffness

Micro-Scale Indentation experiments --- Result

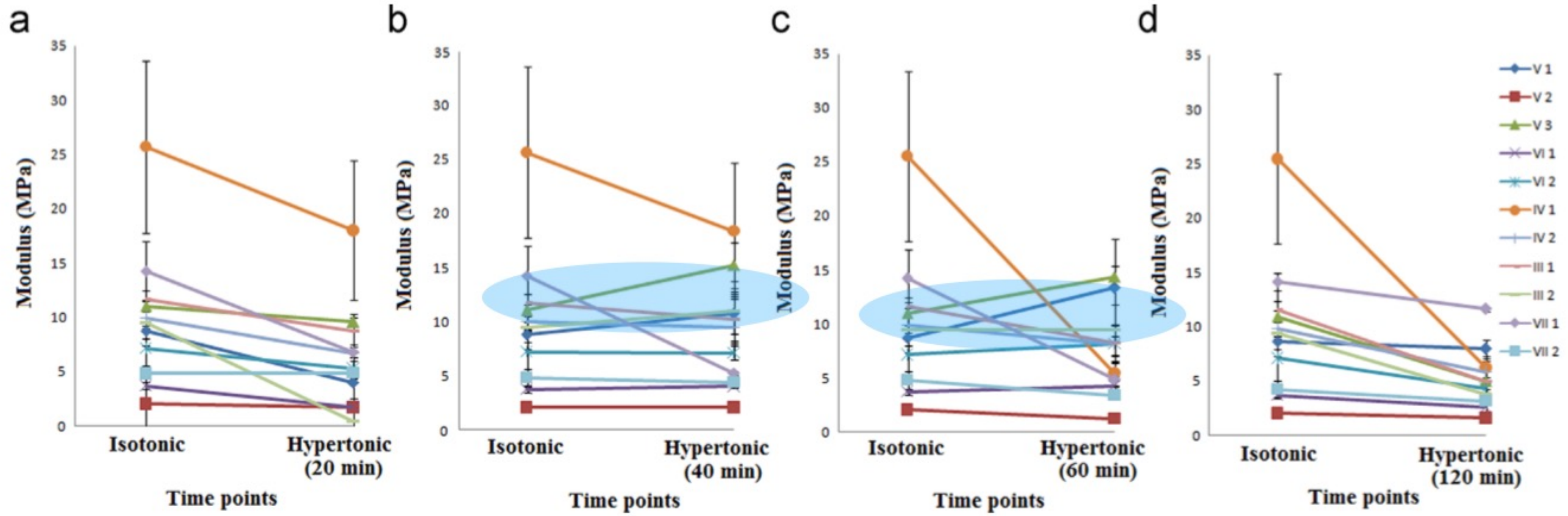


Fig. 3 – Micro-stiffness of mild OA cartilage samples at two different ionic strengths of the PBS solution at different time points: first isotonic PBS then after 20 min incubation in hypertonic PBS (a), after 40 min (b), after 60 min (c), after 120 min (d).

tonicity dependence

Table 1 – Semi-equilibrium modulus at micro-scale.

Time points	E (MPa)	SD
Isotonic	9.88	6.08
Hypertonic (20 min)	7.00	4.40
Hypertonic (40 min)	8.89	4.75
Hypertonic (60 min)	7.42	2.75
Hypertonic (120 min)	5.23	2.67

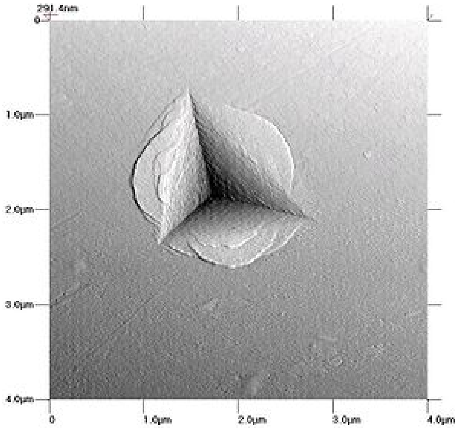
E: average micro-stiffness; SD: standard deviation.

micro-scale elastic modulus decrease in hypertonic solution
Each sample has its own dynamic behavior response to tonicity.

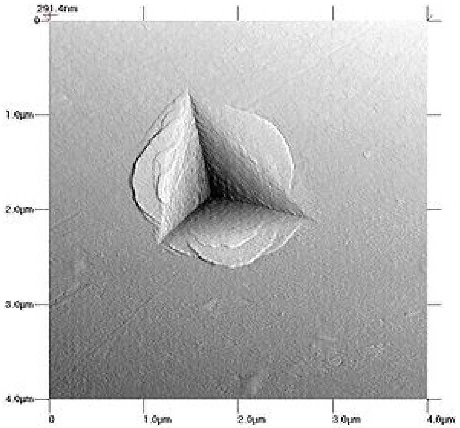
Nano-scale Indentation experiment --- Method

OA Sample

2h in
Isotonic PBS



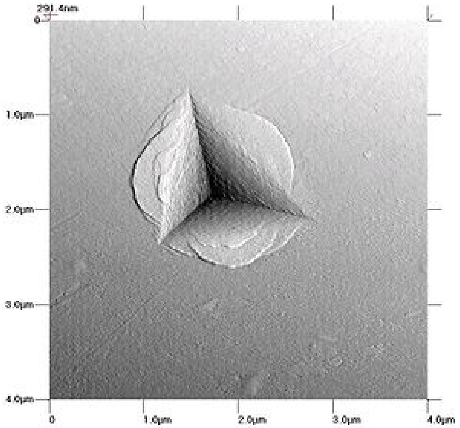
2h in
Hypertonic PBS



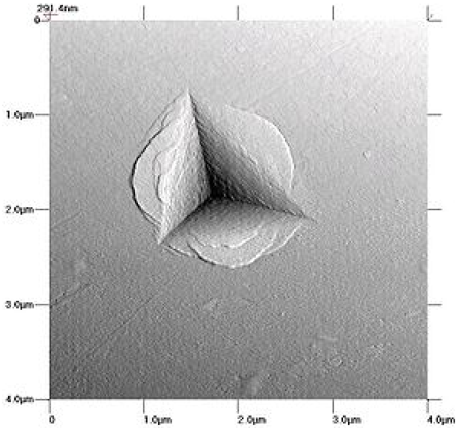
Nano-scale Indentation experiment --- Method

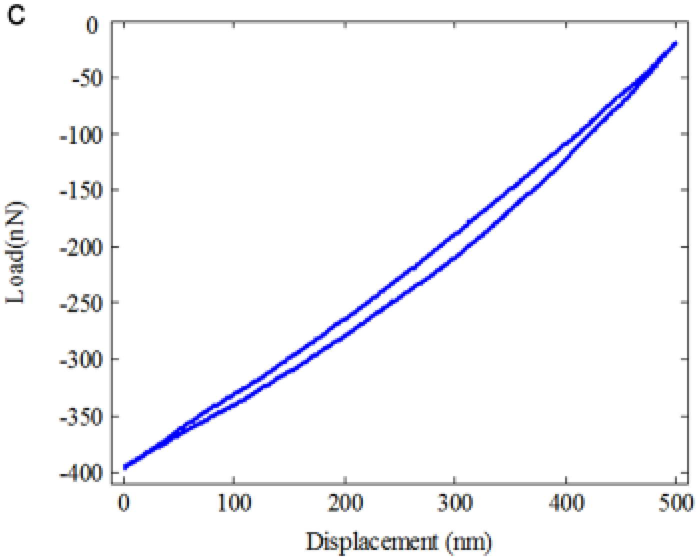
OA Sample

2h in Isotonic PBS



2h in Hypertonic PBS

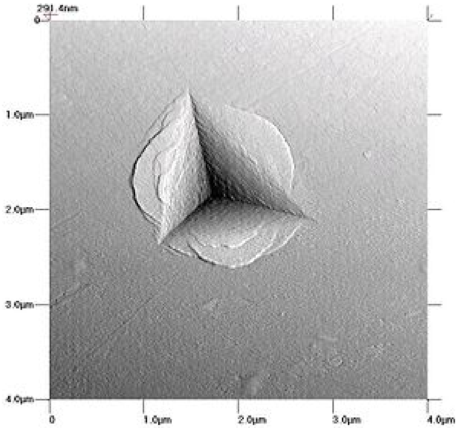




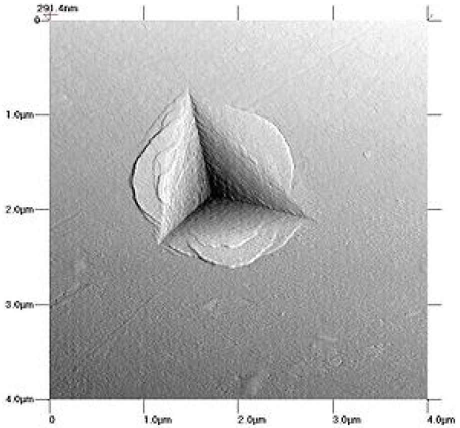
Nano-scale Indentation experiment --- Method

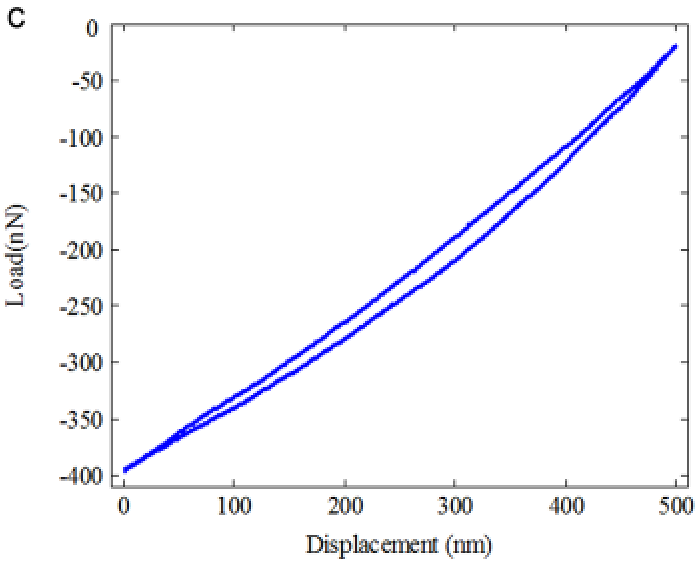
OA Sample

2h in Isotonic PBS



2h in Hypertonic PBS



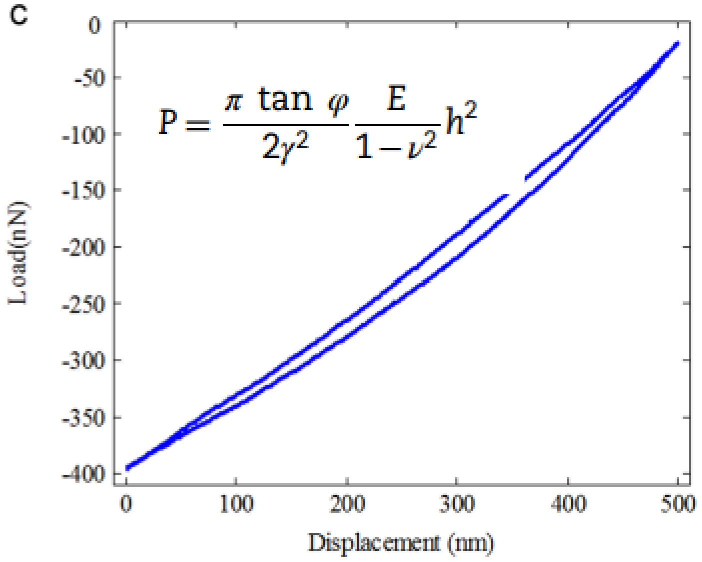


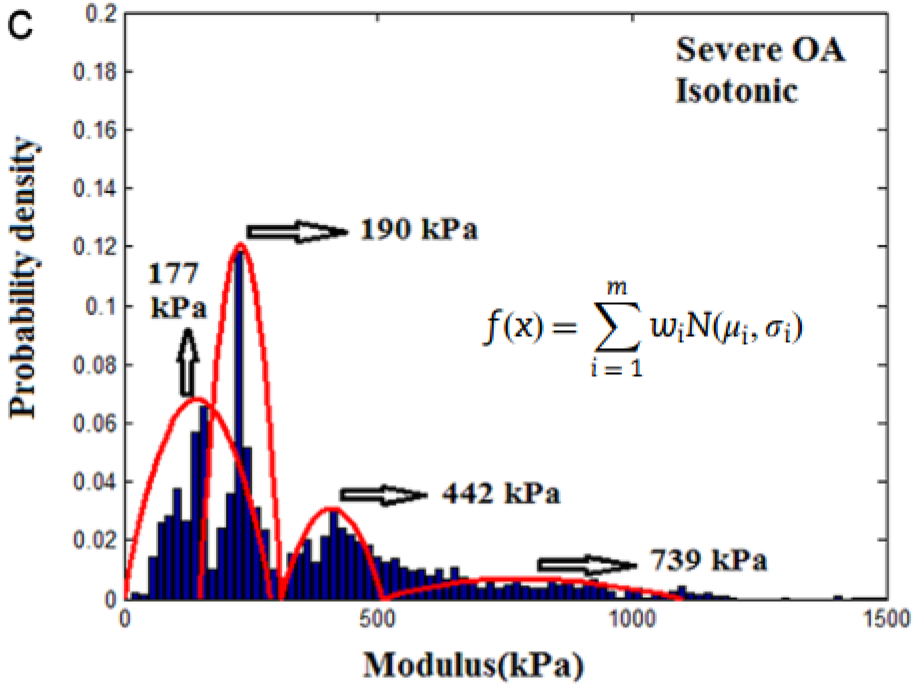
$$P = \frac{\pi \tan \varphi}{2\gamma^2} \frac{E}{1-\nu^2} h^2$$

Elastic moduli

Nano-scale Indentation experiment --- Method

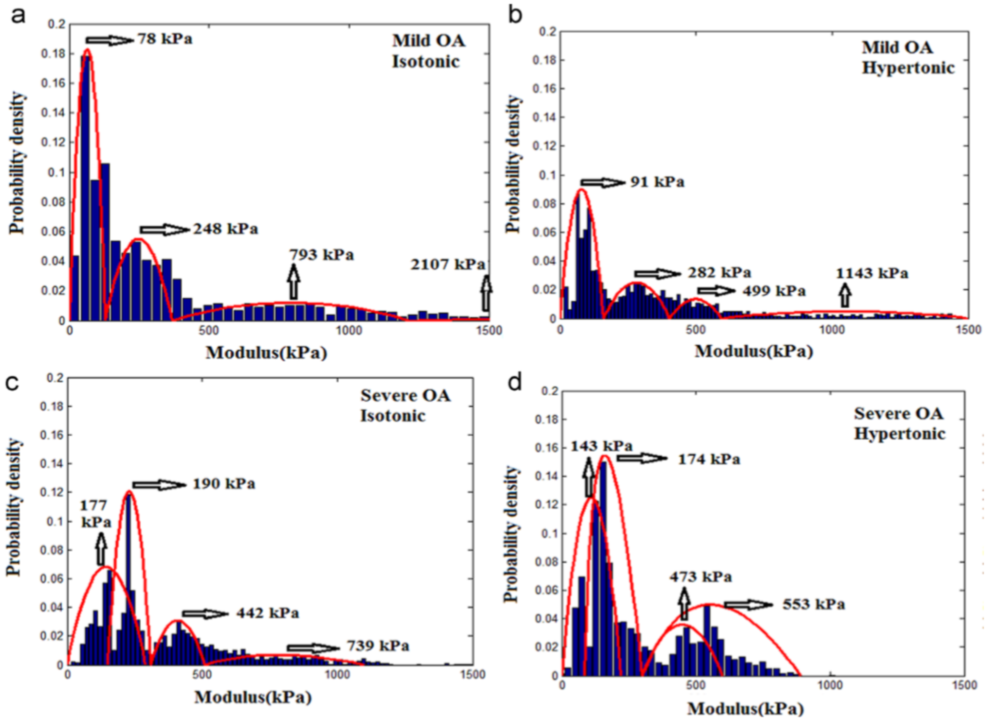
5000





4-Components Gaussian mixture model with Long-Likehood Criterion

Nano-scale Indentation experiment ---Result



1st peak: associated with GAGs
3nd peak:associated with collagen network

Table 2 – Maximum likelihood estimates of the parameters of the Gaussian finite mixture models when 4 Gaussian components were used.

Sample name	μ_1 (kPa)	μ_2 (kPa)	μ_3 (kPa)	μ_4 (kPa)	σ_1 (kPa)	σ_2 (kPa)	σ_3 (kPa)	σ_4 (kPa)	w_1	w_2	w_3	w_4
mOA_N	78	248	793	2107	36	98	317	772	0.37	0.34	0.21	0.08
mOA_H	91	282	499	1143	33	99	160	185	0.39	0.30	0.23	0.08
sOA_N	177	190	442	739	67	66	87	300	0.30	0.25	0.24	0.21
sOA_H	143	174	473	553	56	1056	167	21	0.57	0.002	0.37	0.06

reverse
>2times
17%↑ 14%↑ 37%↓ 46%↓
19%↓ 9%↓ 6%↓ 34%↓

Mild OA in normal PBS solution: mOA_N.
Mild OA in hypertonic PBS solution: mOA_H.
Severe OA in normal PBS solution: sOA_N.
Severe OA in hypertonic PBS solution: sOA_H.
μ_i: mean; σ_i: standard deviation; w_i: weighting factor of every constituent.

Finite Gaussian components represent the nano-scale mechanical properties of the collagen network
no direct evidence for first mixture component represents the nano-scale mechanical properties of GAGs

Contranst enhanced micro-CT(EPIC-μCT)---Method

$$\text{Average gray value} = \frac{\sum P_i x_i}{\sum x_i}$$

Just the mean in whole CT image

3.2 EPIC-µCT

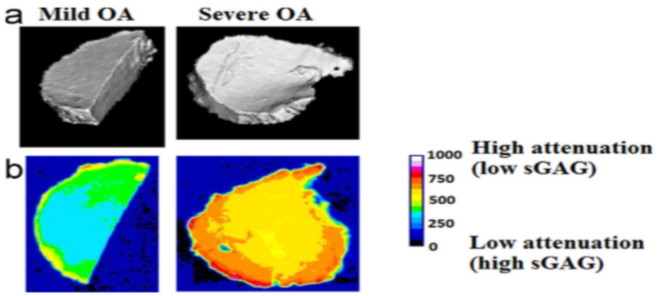


Fig. 6 – Micro-CT image of two specimens from mild and severe OA groups (a), EPIC-µCT images of two representative specimens from mild and severe OA groups (b). The distribution of ioxaglate through the x-ray attenuation values (arbitrary unit) is represented where extremely high ioxaglate concentration is shown with bright color and extremely low ioxaglate concentration is shown with blue. For every group, the representative specimen is the one that has the closest average gray value to the average gray value of its group. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

average of mean gray values
severe OA group: 335±57>>mild OA group : 252±19

GAG: mild OA > severe OA

dependency of the indentation assay to the test locati

3.3 Histology

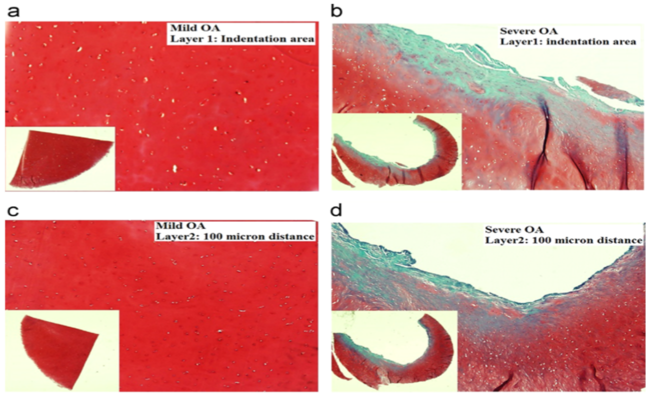


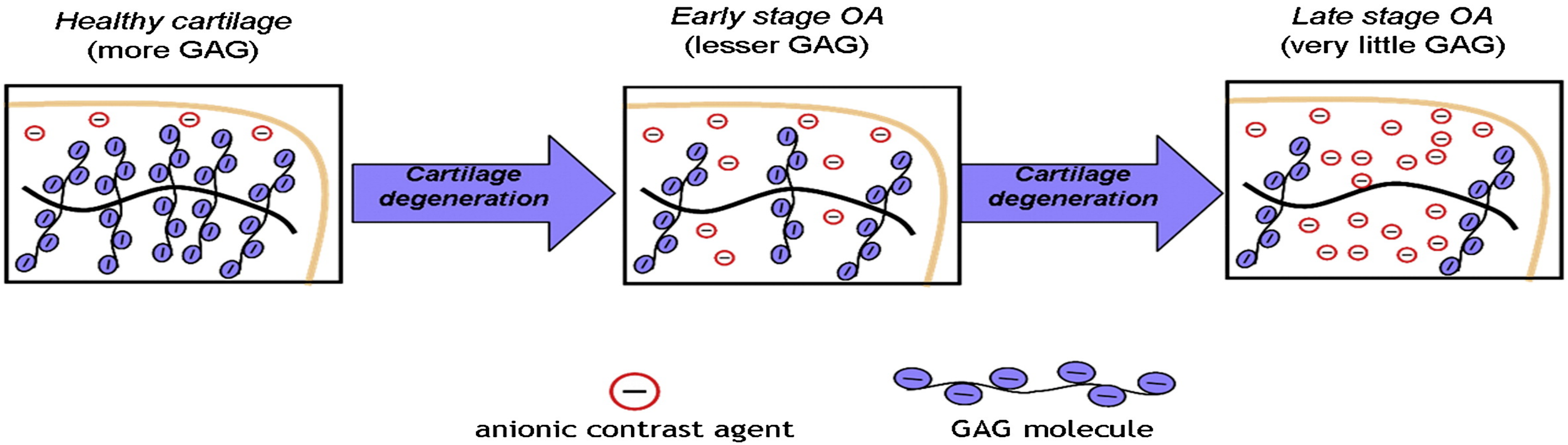
Fig. 7 – Histology of cartilage specimens stained by Safranin-O at two different intervals. mild OA at the same area with microindentation experiment (a), severe OA at the same area with microindentation experiment (b), mild OA at 100 µm distance from the first layer (c), severe OA at 100 µm distance from the first layer (d).

mild OA: nicely distributed
high concentration of GAG

severe OA: less GAGs content, noticeable
surface fibrillation and irregularity

DISCUSSION

Micro-mechanics of OA cartilage

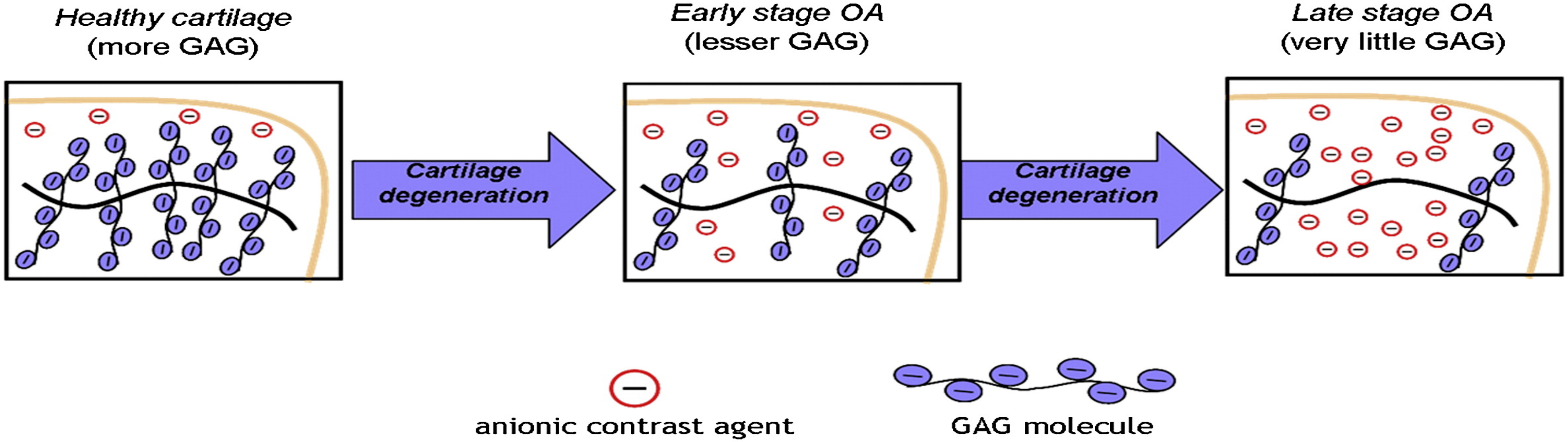


Decrease in semi-equilibrium modulus due to osmotic effects

- Samples with same **significant decrease**
- **Threshold** to equilibrium is different
- Each sample has **own dynamics in osmotic change response**

DISCUSSION

Micro-mechanics of OA cartilage (swelling is driven by GAGs)

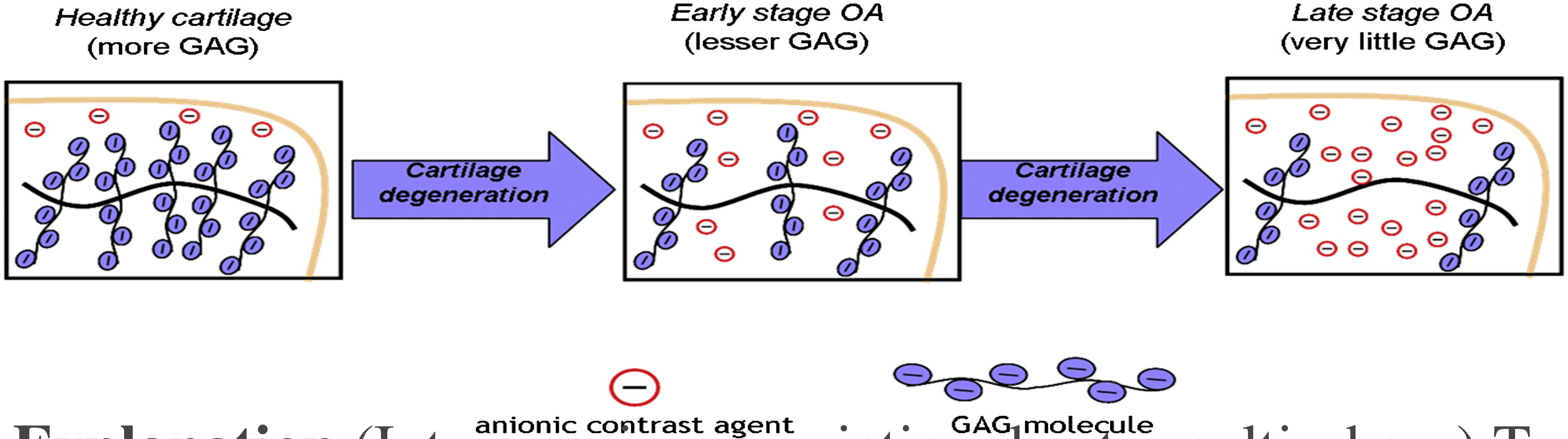


Explanation

- **Intra** specimen variation due to multi-phase
- **Inter** specimen variation

DISCUSSION

Micro-mechanics of OA cartilage (swelling is driven by GAGs)

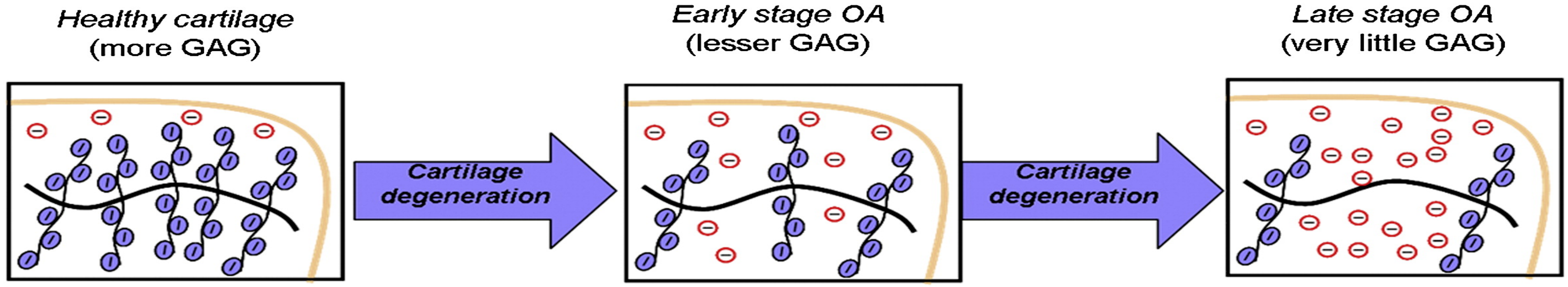


Explanation (Intra specimen variation due to multi-phase)-Two transport time scales

- **Movement of the water to tissues**
- **Diffusion of macromolecular**: such as PGs degradation products or intact molecules freed from the swollen collagen matrix out of the tissue

DISCUSSION

Micro-mechanics of OA cartilage (swelling is driven by GAGs)



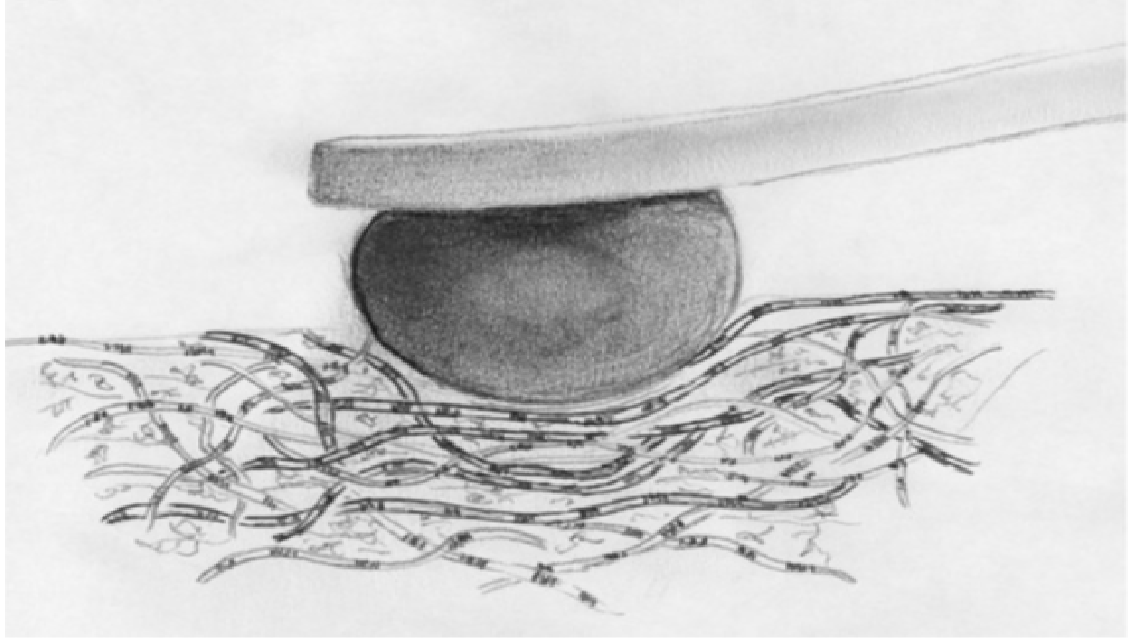
Explanation (Inter specimen variation)

- Samples from **different** patients, **different** stages, different level of GAGs
- **Mechanical properties** of articular cartilage **sensitive to topographic**

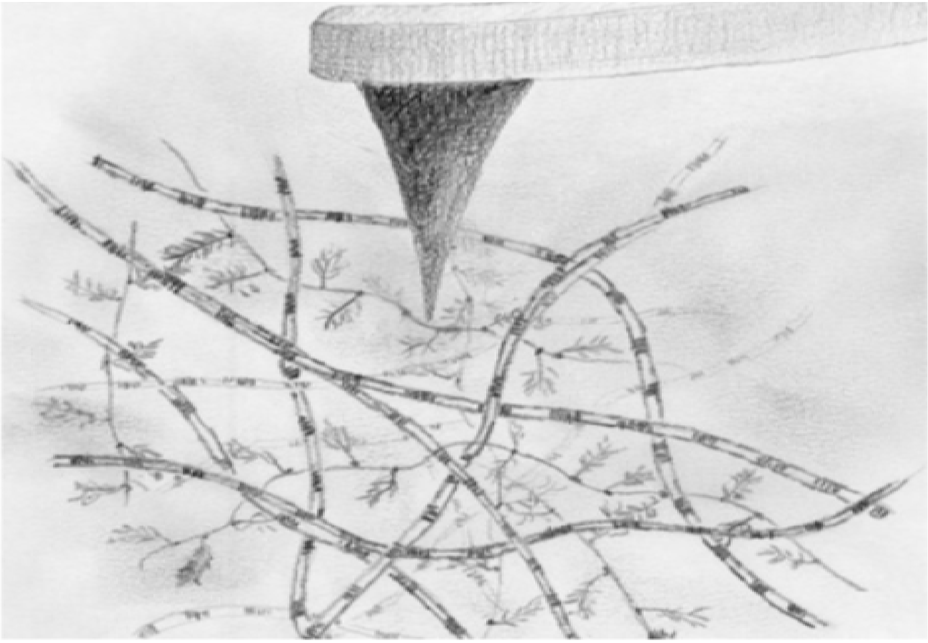
DISCUSSION

Nano Mechanics of OA cartilage: separate constituents with stimulus

icroscopy: **Two** very clear peaks, associated with the nano-scale elastic modulus



Micro: Hard borosilicate glass spheres, were glued onto tipless rectangular cantilevers



Nano: Pyramidal tips with a nominal tip on V-shaped cantilevers with a nominal

DISCUSSION

Nano Mechanics of OA cartilage: separate constituents with stimulus

licroscopy: **Two** very clear peaks, associated with the nano-scale elastic modulus

his paper: Several differences

Four components are needed to described, first and third peak: GAG and collagen

Additional constituent with **distinct mechanical properties**, make change in polymer conforma

DISCUSSION

ic modulus of cartilage depends on the ionic and non-ionic portio

- **Ionic:** GAG chains with their **negative charge** can interact with **positive** ions from the environment and distinguish themselves in certain ways
- **Non-ionic**: Collagen fibers not contain ionic part on their chains

DISCUSSION

Limitation

- **Neglect intrinsic interaction**
- **Inaccurate due to nano indentation techniques themselves**
- **Measure at surface, not represent bulk (surface are proved to be good indicators of OA)**

Thanks

Reference

- https://www.hysitron.com/techniques-properties/mechanical-properties/creep
-