Term Paper Project

Enzymatic Targeting of the Stroma Ablates Physical Barriers to Treatment of Pancreatic Ductal Adenocarcinoma (PDA)

Cancer Cell
2012

Paolo P. Provenzano,[1] Carlos Cuevas,[4] Amy E. Chang,[1] Vikas K. Goel,[1] Daniel D. Von Hoff,[5] and Sunil R. Hingorani[1,2,3,*]
[1]Clinical Research Division
[2]Public Health Sciences Division
Fred Hutchinson Cancer Research Center, Seattle, WA 98109, USA
[3]Division of Medical Oncology, University of Washington School of Medicine, Seattle, WA 98195, USA
[4]Department of Radiology, University of Washington, Seattle, WA 98195, USA
[5]Clinical Translational Research Division, Translational Genomics Research Institute, Scottsdale, AZ 85259, USA

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Hyaluronan, fluid pressure, and stromal resistance in pancreas cancer

British J of Cancer
2013

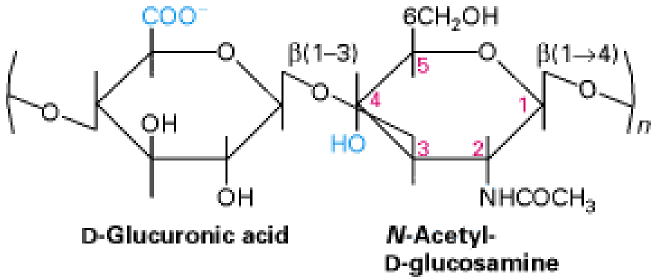
P P Provenzano[1,4] and S R Hingorani[*,1,2,3]

[1]Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA 98109, USA; [2]Public Health Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA 98109, USA; [3]Division of Medical Oncology, University of Washington School of Medicine, Seattle, WA 98195, USA

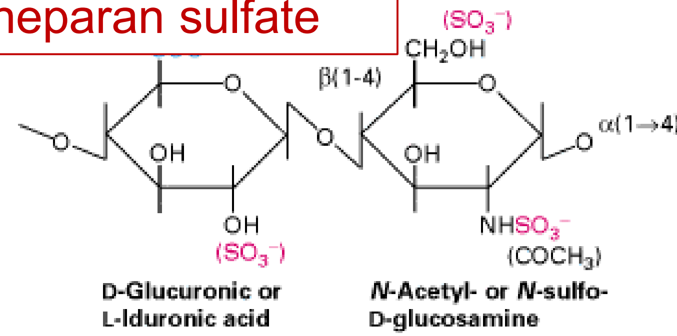
Family of Glycosaminoglycans (GAG Chains):

→ Glycosylation of "Core Protein (addition of sugar moieties)

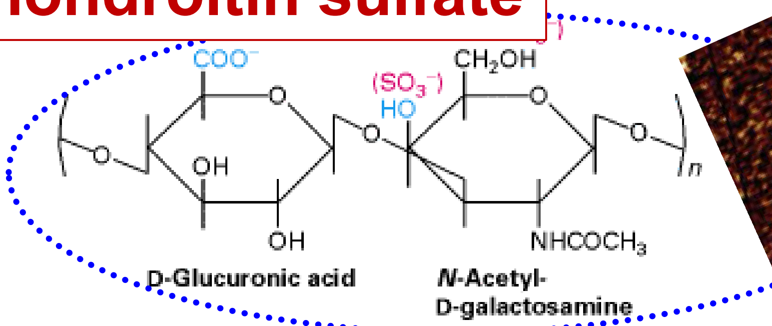
Hyaluronan (HA)

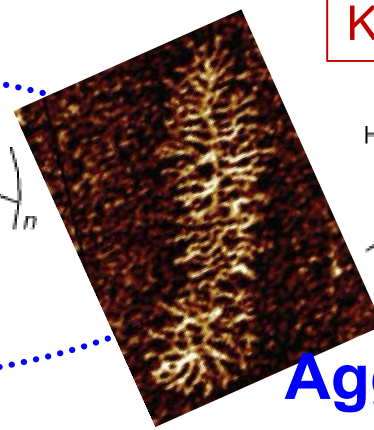


Heparin/heparan sulfate

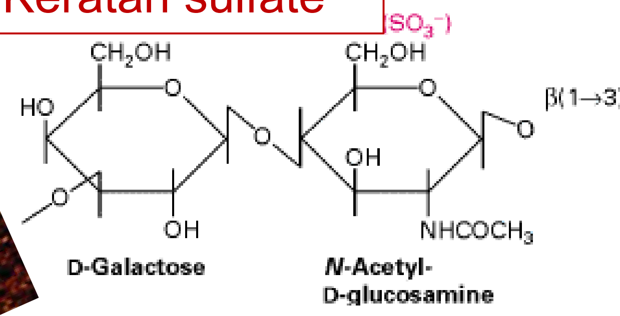


Chondroitin sulfate



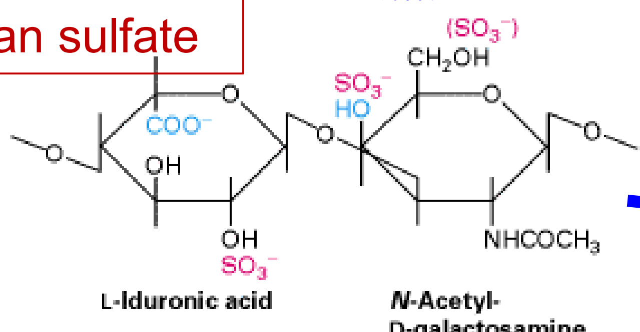


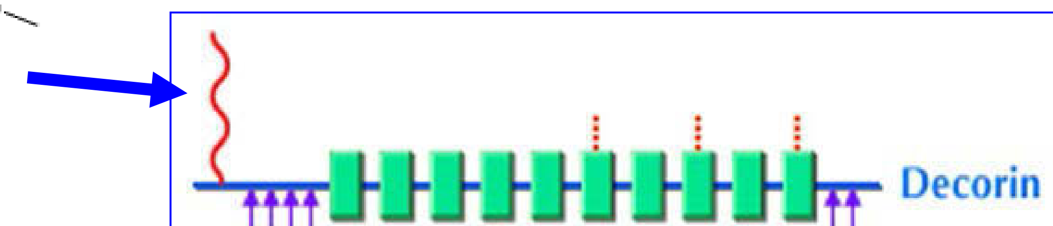
Keratan sulfate



Aggrecan

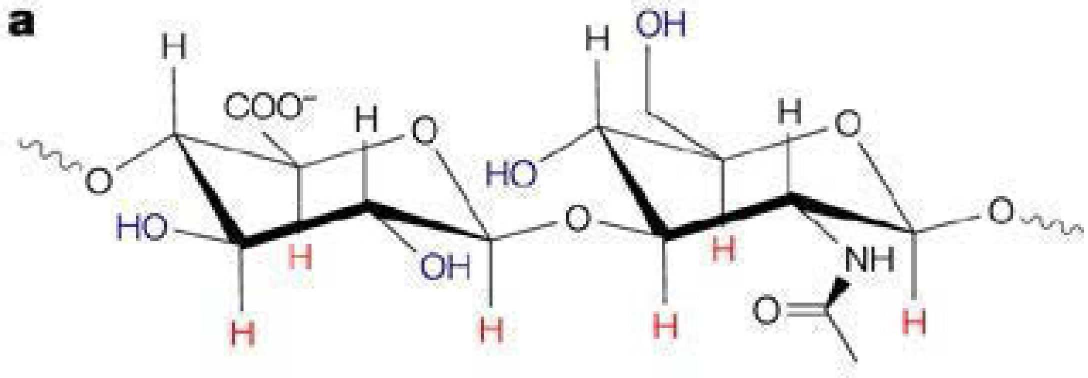
Dermatan sulfate

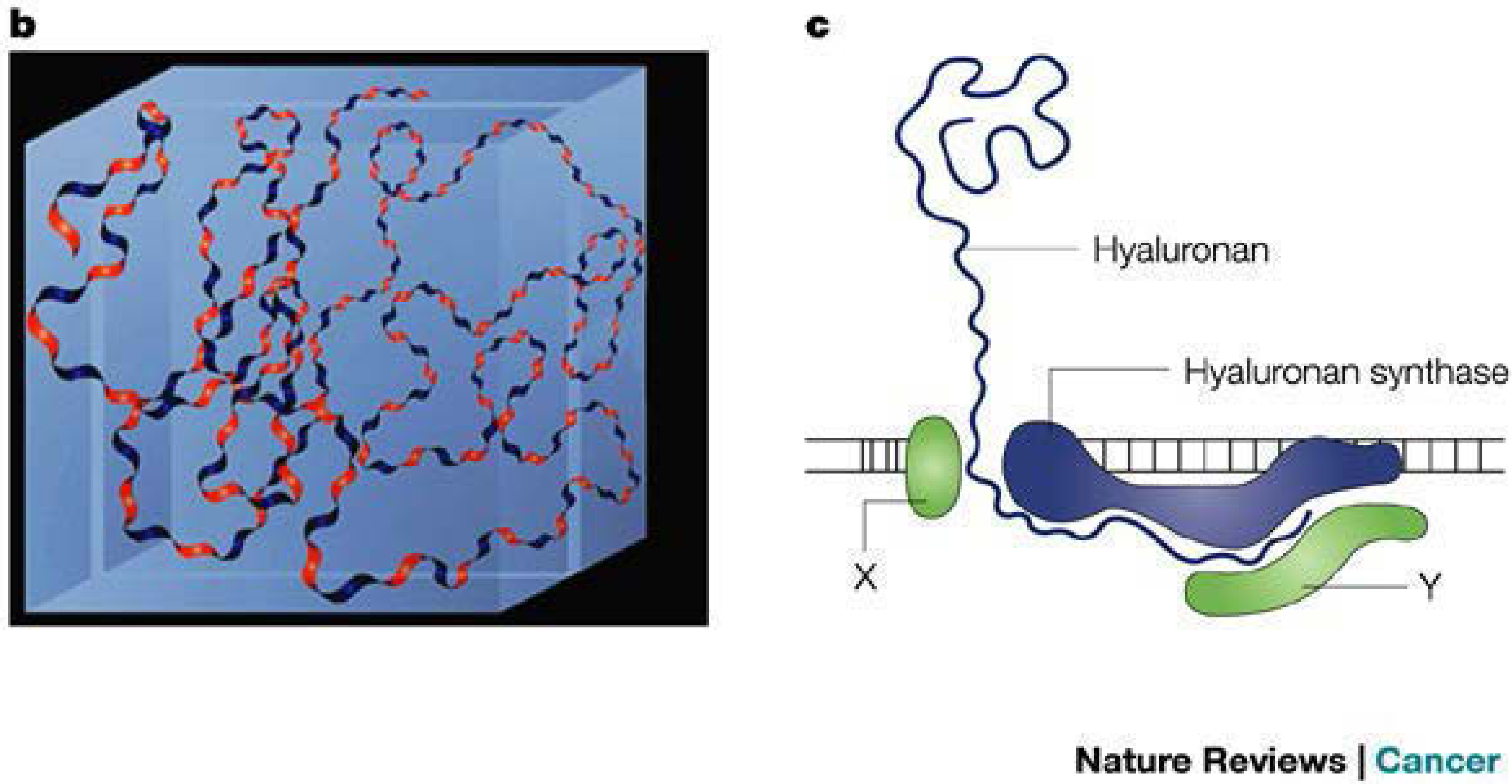




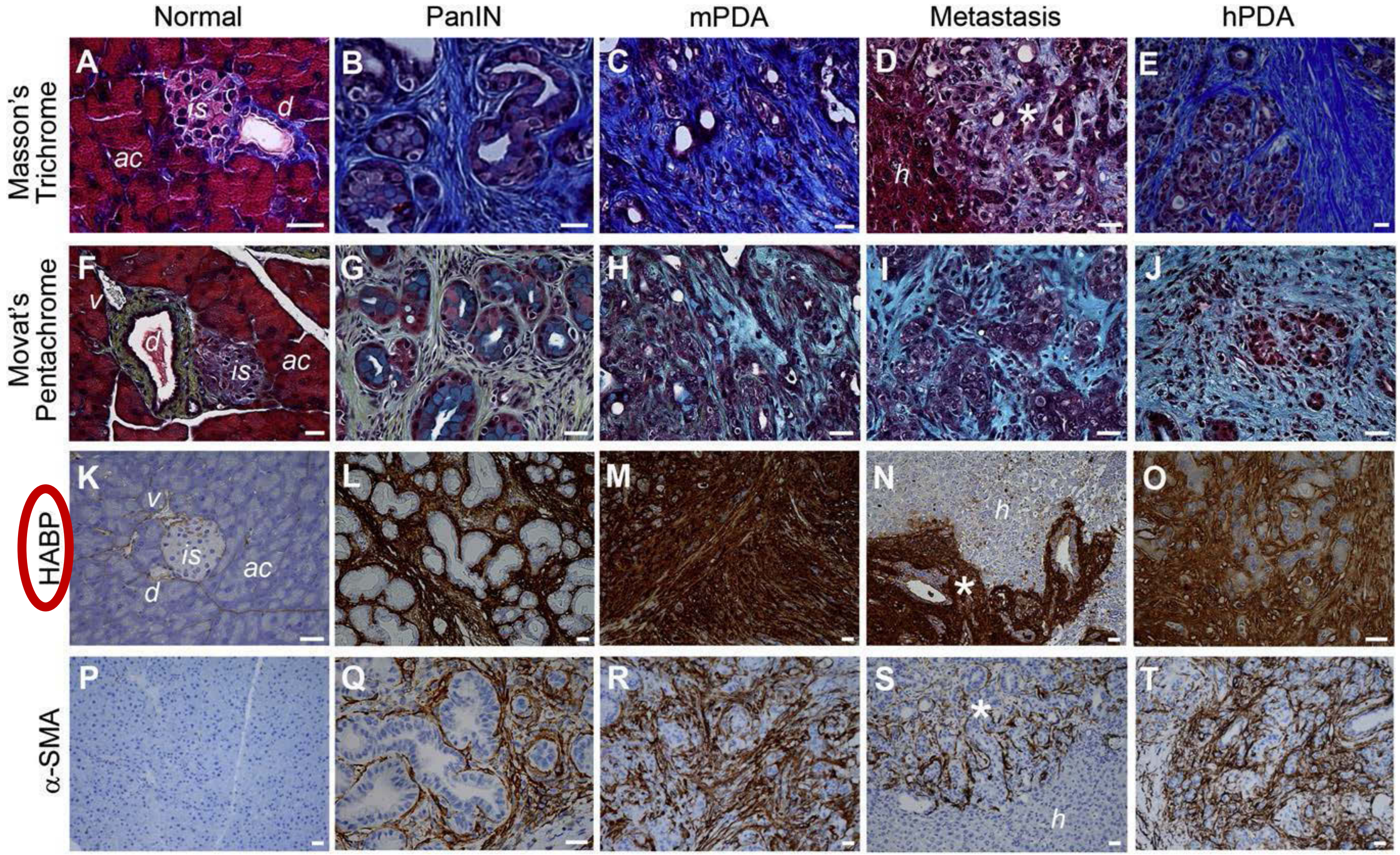
Dermatan sulfate

Cell Synthesis of HA





Courtesy of Macmillan Publishers Limited. Used with permission.
Source: Toole, Bryan P. "Hyaluronan: from extracellular glue to pericellular cue." Nature Reviews Cancer 4, no. 7 (2004): 528-539.



Source: Provenzano, Paolo P. et al. "Enzymatic targeting of the stroma ablates physical barriers to treatment of pancreatic ductal adenocarcinoma." Cancer Cell 21, no. 3 (2012): 418-429.

Figure 1. Evolution of the Desmoplastic Reaction in Murine and Human PDA

(A–E) Masson's trichrome histochemistry shows robust collagen deposition (blue) at all stages of disease.

(F–J) Movat's pentachrome histochemistry reveals collagen (yellow), GAGs and mucins (blue), and their colocalization (turquoise/green).

(K–O) Histochemistry with HA-binding protein (HABP) reveals intense HA content beginning with preinvasive disease (PanIN).

(P–T) Activated PSC express α-smooth muscle actin (α-SMA) and are abundant in preinvasive (Q), invasive (R) and metastatic mPDA (S) and hPDA (T), but not in normal pancreata (P). *ac*, acini; *is*, islet; *d*, duct; *v*, venule; *h*, hepatic parenchyma; *, metastatic lesions. Scale bars, 25 μm. See also Figure S1.

Study of Gemcitabine + PEGPH20 vs Gemcitabine Alone in Stage IV Previously Untreated Pancreatic Cancer

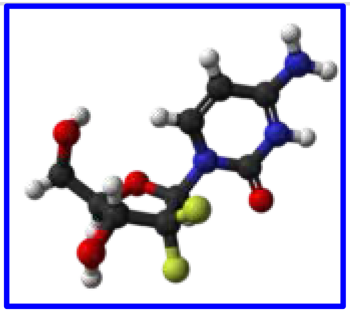
This study has been completed.

Sponsor:
Halozyme Therapeutics

Information provided by (Responsible Party):
Halozyme Therapeutics

ClinicalTrials.gov Identifier:
NCT01453153

First received: October 13, 2011
Last updated: October 12, 2015
Last verified: September 2013
History of Changes



263 Da

Full Text View | Tabular View | No Study Results Posted | Disclaimer | How to Read a Study Record

Purpose

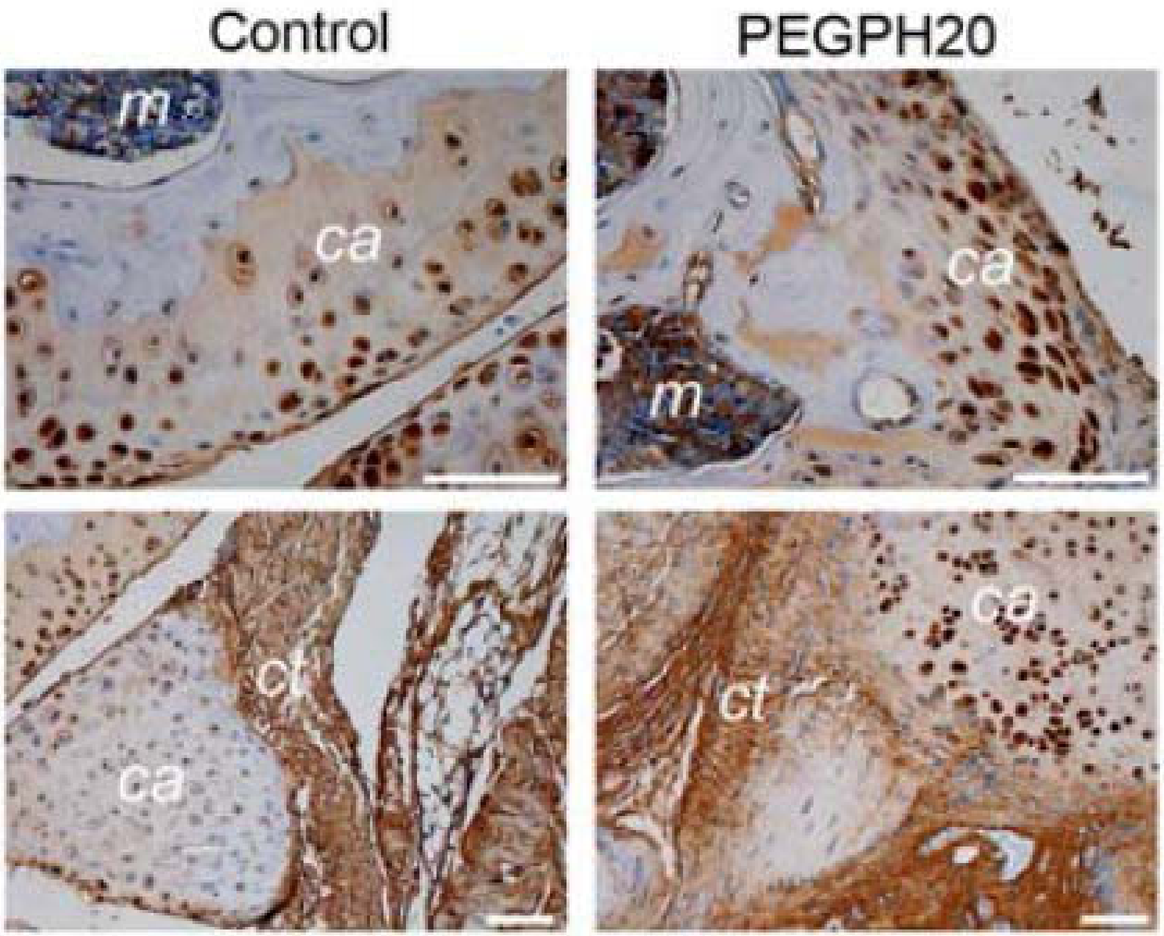
Phase 1B: Open label (all patients receive PEGPH20+gemcitabine), dose escalation, safety and tolerability study to determine the safe dose of PEGPH20 to use in combination with gemcitabine in Stage IV previously untreated pancreatic cancer patients.

Phase 2: Randomized, double blind study to compare the effect of overall survival of gemcitabine plus PEGPH20 vs gemcitabine plus placebo in Stage IV previously untreated pancreatic cancer patients.

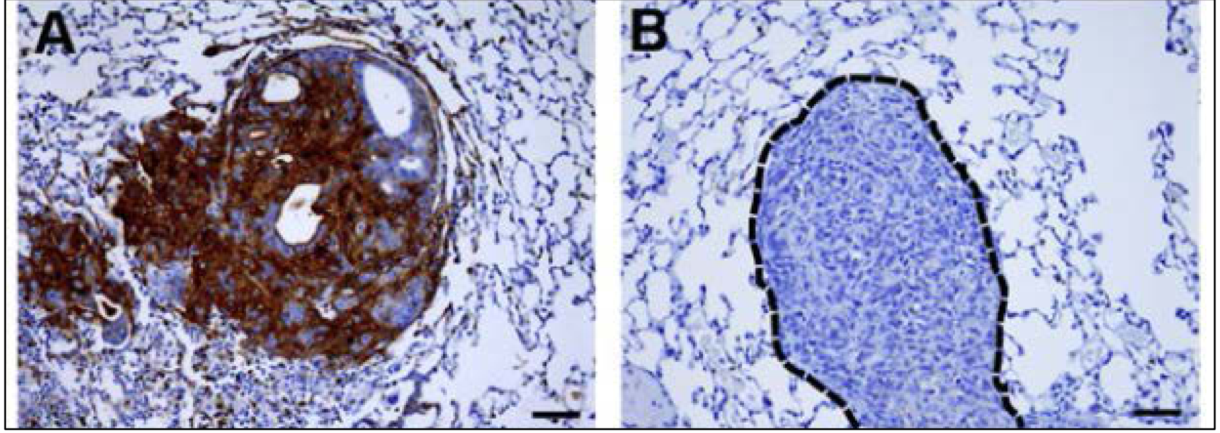
Condition	Intervention	Phase
Stage IV Pancreatic Cancer	Drug: Gemcitabine Drug: PEGPH20+ gemcitabine	Phase 1 Phase 2

- Halozyme's FDA-approved, *HYLENEX®* recombinant human hyaluronidase, rHuPH20, is administered subcutaneously and temporarily and reversibly degrades HA to facilitate the absorption and dispersion of other injected drugs or fluids and for subcutaneous fluid administration.
- However, rHuPH20 acts only locally at the injection site, is rapidly inactivated in the body, and does not survive in the blood.
- **An investigational PEGylated form of rHuPH20 (~60 kDa), PEGPH20 (~90 kDa), in under development by Halozyme to increase the half-life of the compound in the blood and allows investigation for intravenous administration.**

PEGPH20 can't remove HA from Cartilage ☺

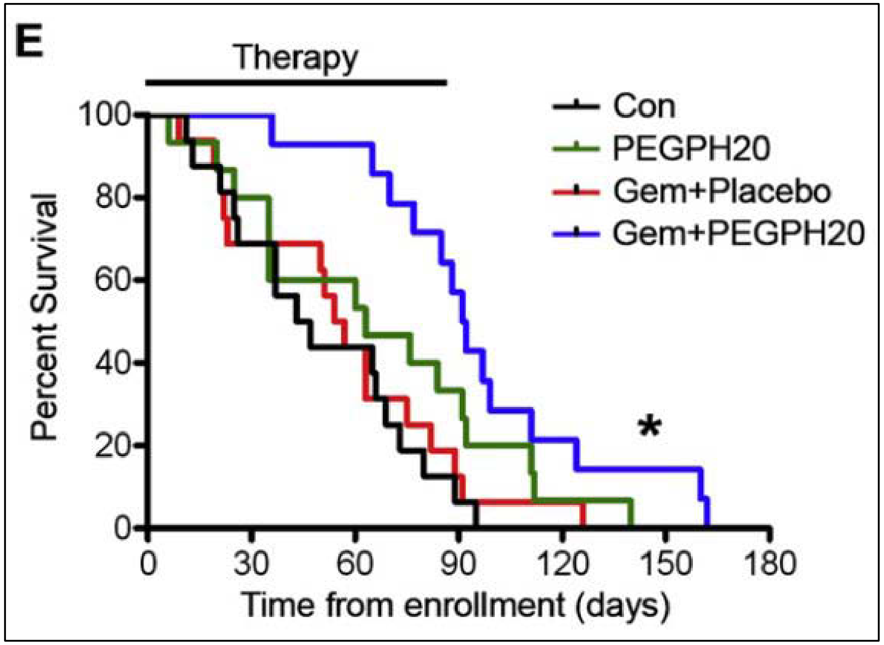


(F) HA expression is specifically retained within **cartilage** (***ca***) and the joint space. *m*, bone marrow; *ct*, connective tissue; Scale bars, 50 μm.



HA expression in lung metastases from untreated (A) and PEGPH20-treated (B) animals.

Figure 6. Gemcitabine+PEGPH20 Combination Therapy Decreases Metastatic Tumor Burden and Improves Survival



Survival curves from time of enrollment in control (Con; n = 16), Gem (n = 16), PEGPH20 (n = 15), and Gem+PEGPH20-treated KPC animals (n = 14).

Source: Provenzano, Paolo P. et al. "Enzymatic targeting of the stroma ablates physical barriers to treatment of pancreatic ductal adenocarcinoma." Cancer Cell 21, no. 3 (2012): 418-429.

Figures from Box 2 removed due to copyright restrictions.
Source: Jain, Rakesh K. "An indirect way to tame cancer."
Scientific American 310, no. 2 (2014): 46-53.

Provenanzo/Hingorani: **IFP** squeezes vessels shut

Dr. Jain's Argument

Jain: **Solid Stress** squeezes vessels shut, porous or not!

Source: Jain, Rakesh K. "An indirect way to tame cancer." Scientific American 310, no. 2 (2014): 46-53.

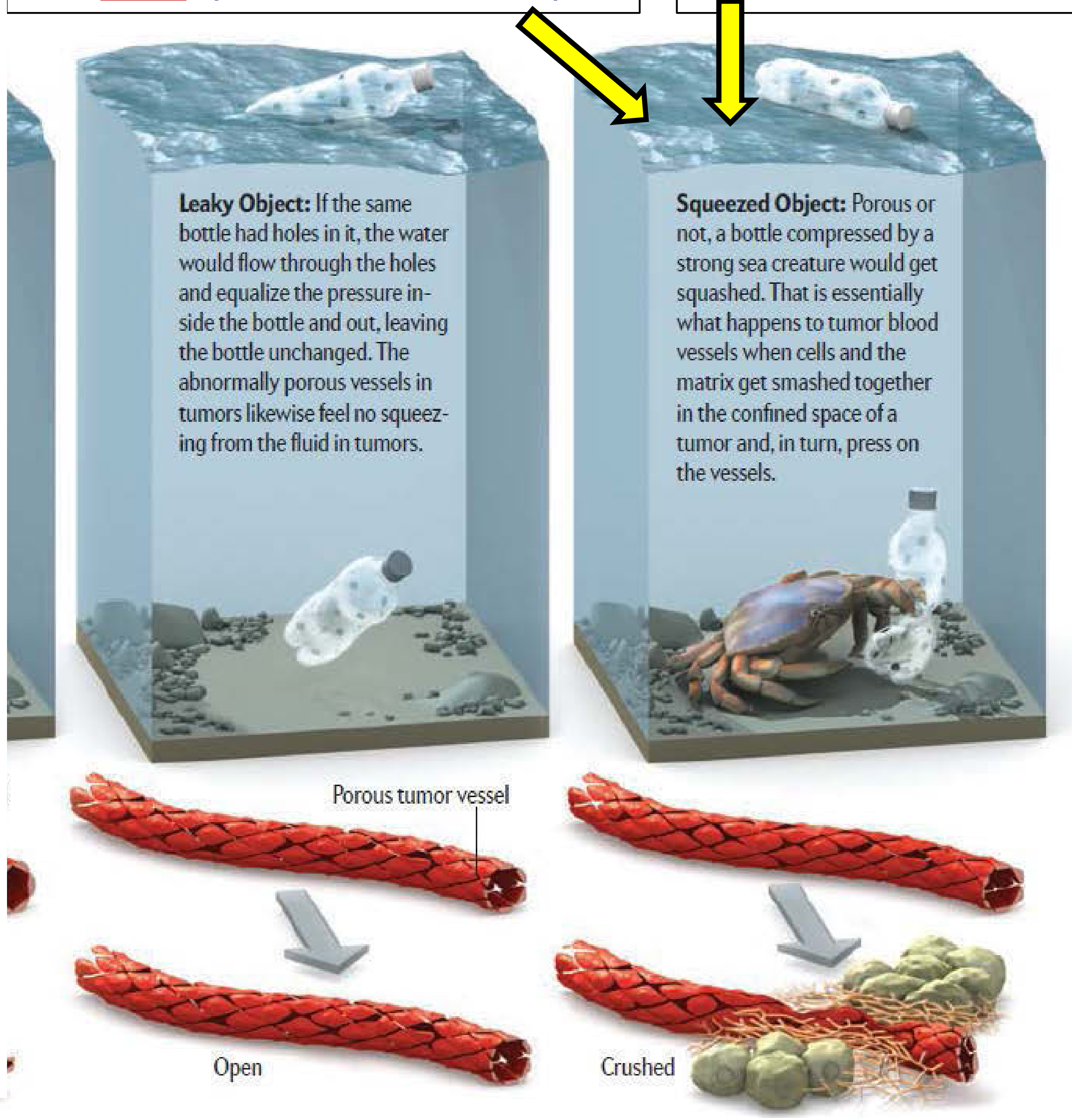
"Diffusion is driven by concentration gradients between intravascular and interstitial compartments; …Convection is driven by pressure gradients composed of hydrostatic and oncotic components"

Source of profoundly elevated fluid pressures and vascular collapse in PDA**??** :

HA organizes a hydrogel containing "immobilized fluid" ….that also has an **elastic modulus**….a property of solids; Gel has "fluid pressure, colloid osmotic pressure caused by **Donnan equilibrium**….."

Provenzano/Hingorani: **Donnan** also increases **IFP** (porous or not) **!!**

Jain: **Solid Stress** squeezes vessels shut, porous or not!



Source: Jain, Rakesh K. "An indirect way to tame cancer." Scientific American 310, no. 2 (2014): 46-53.

Examining Starling's principles of fluid flux reveals that IFP need not be limited by microvascular pressure. The Staverman-Kedem-Katchalsky solute transport equation formally defines fluid flux (J_F) based on Starling's principles as:

$$J_F = L_\rho \, . \, A \left[(p_v - p_i) - \sigma (\pi_v - \pi_i) \right]$$

in which L_ρ is the hydraulic conductivity; A is the surface area of the vascular bed; p_v and p_i are the hydrostatic pressures in the vessels and interstitium, respectively; π_v and π_i are the oncotic pressures in the same respective spaces; and σ is the reflection coefficient of the endothelium (Kedem and Katchalsky, 1961).

Jain and colleagues assume π_i is always lower than π_v....

From Letter-Reply DelGiorno et al., Cancer Cell 2014

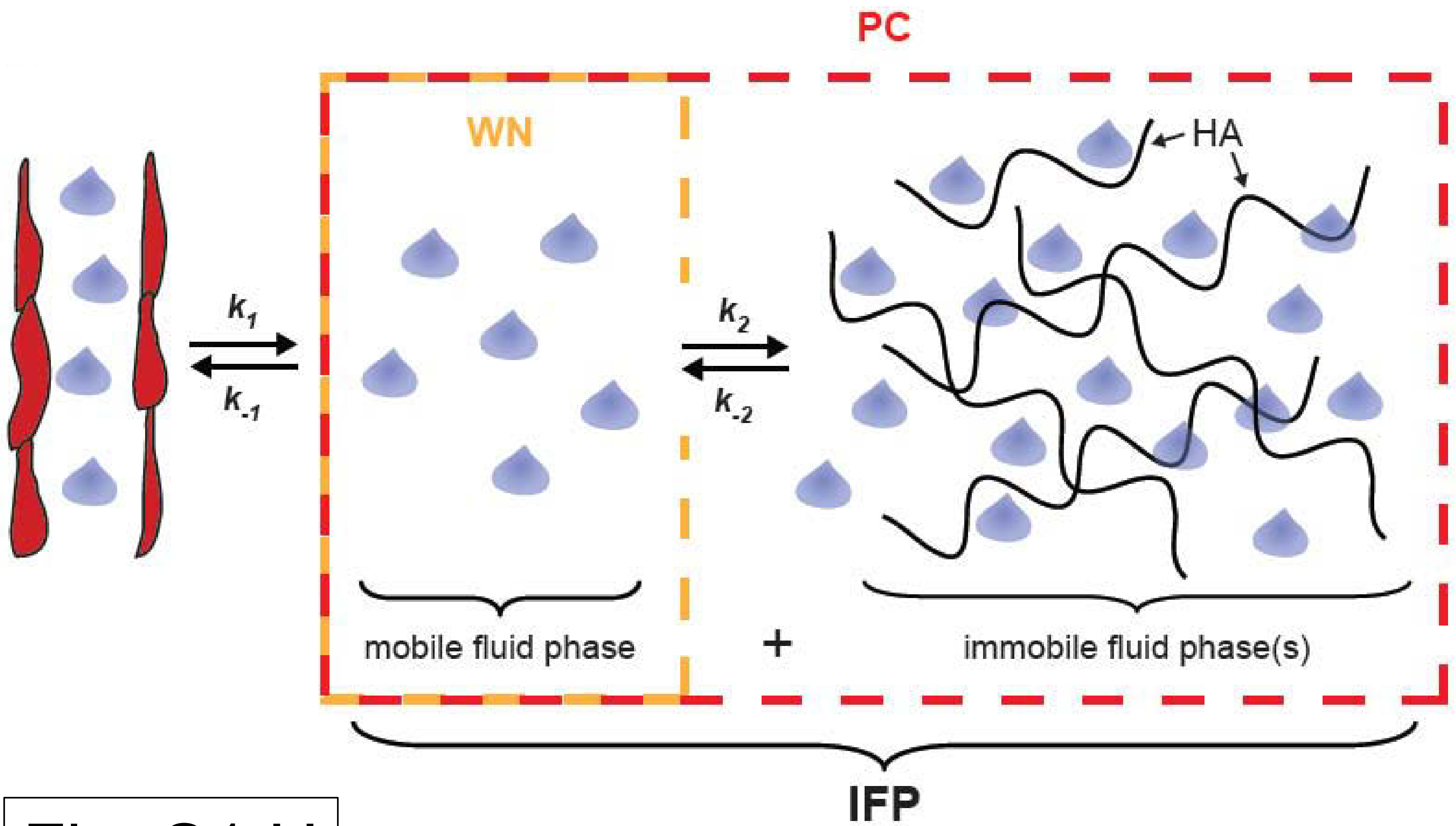


Fig. S1 H

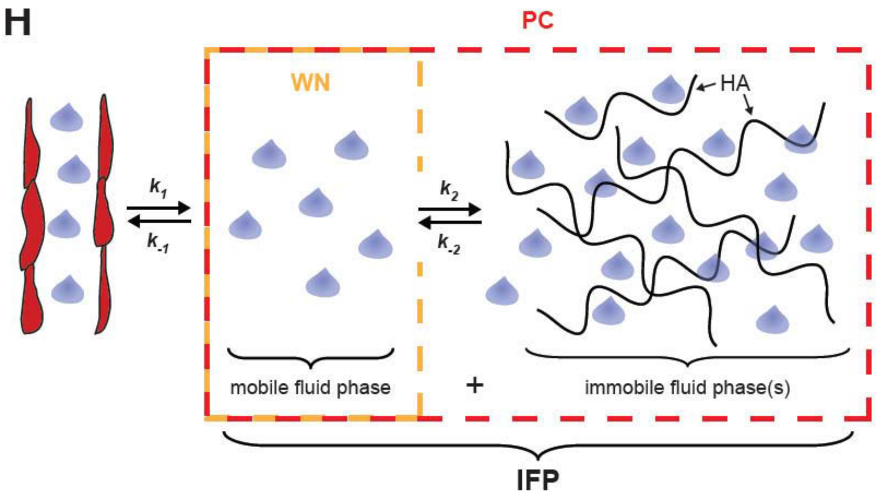
Source: DelGiorno, Kathleen E. et al. "Interstitial pressure and vascular collapse in pancreas cancer: fluids and solids, measurement and meaning." Cancer Cell 26, no. 1 (2014): 16.

Calculating total interstitial fluid pressure (IFPtotal)

The majority of interstitial fluid is **immobile(?)**. Hyaluronan, or **hyaluronic acid, HA,** a naturally occurring, megadalton polyelectrolyte **avidly binds water (?)** and contributes extensively to both hydrostatic and oncotic fluid pressures in the interstitium (Guyton et al., 1971; Ogston, 1966; Tanford, 1961).

Building on Starling's insights, a more complete formula for interstitial fluid pressure that takes into account both the freely mobile and less mobile fluid phases is:

$$\mathrm{IFP}_{total} = p_v - \sigma(\pi_v - \pi_i) + \underbrace{\{P_{immobile} + \Pi_{immobile}\}}_{?}$$



?

Source: DelGiorno, Kathleen E. et al. "Interstitial pressure and vascular collapse in pancreas cancer: fluids and solids, measurement and meaning." Cancer Cell 26, no. 1 (2014): 16.

$$\mathrm{IFP}_{total} = p_v - \sigma(\pi_v - \pi_i) + P_{immobile} + \Pi_{immobile}$$

- P_{imm} is comprised of an elastic recoil component from structural elements in the interstitium, together with the electrostatic repulsion of negative charges on HA that contributes to its tendency to expand or swell. This term includes pressure exerted from the collagen network in tension as it resists the expansion of hydrated HA.
- Π_{imm} is defined largely by the Donnan potential, the unequal distribution of diffusible cations as a result of non-diffusible negative charges, plus a minor component from van't Hoff forces. Together, these terms represent a complete depiction of interstitial fluid pressures (Figure S1H)

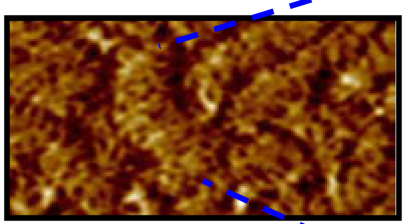
Del Giorno et al., Cancer Cell, July 2014

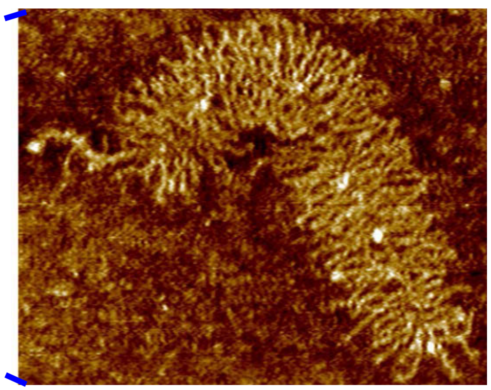
- Thus, it is important to remember that **in addition to resisting compression, HA also expands** and this **swelling pressure stresses the collagen fibrils**, which are loaded in response through cell contraction in an effort to maintain tensional homeostasis.

- We propose, therefore, that **fibrillar collagen** represents an **additional target** to decrease transmitted fluid pressure, and hypothesize further that its removal or inhibition would cause an incremental or stepwise drop in pressure rather than complete normalization.

Astronauts gain 1-2 inches in height during space flight: swelling of the intervertebral discs under 0-gravity:

"swelling pressure" of highly charged ECM: aggrecan GAG chains





"Double Layer repulsion: Nano "DLVO Theory"

(Chapter 4)

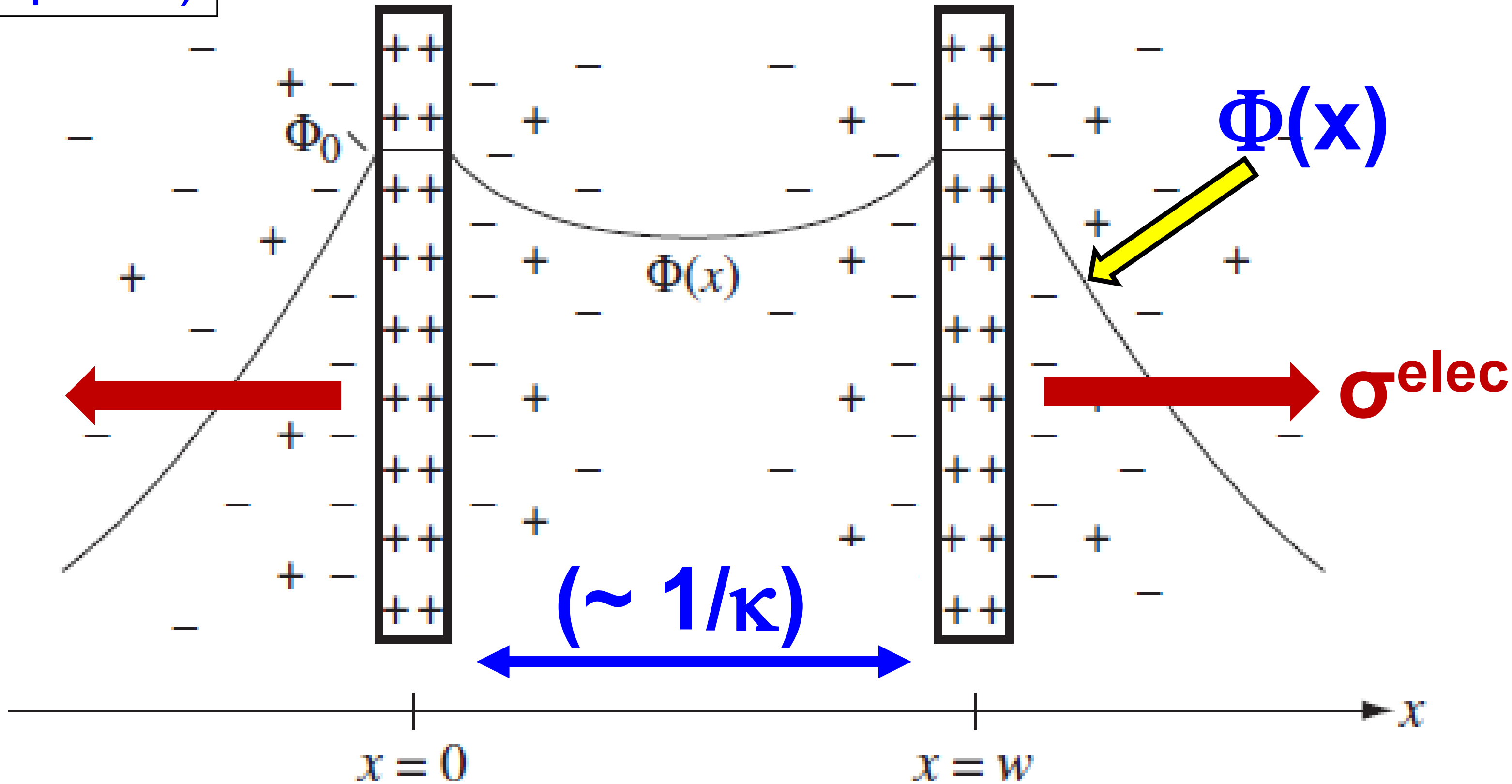
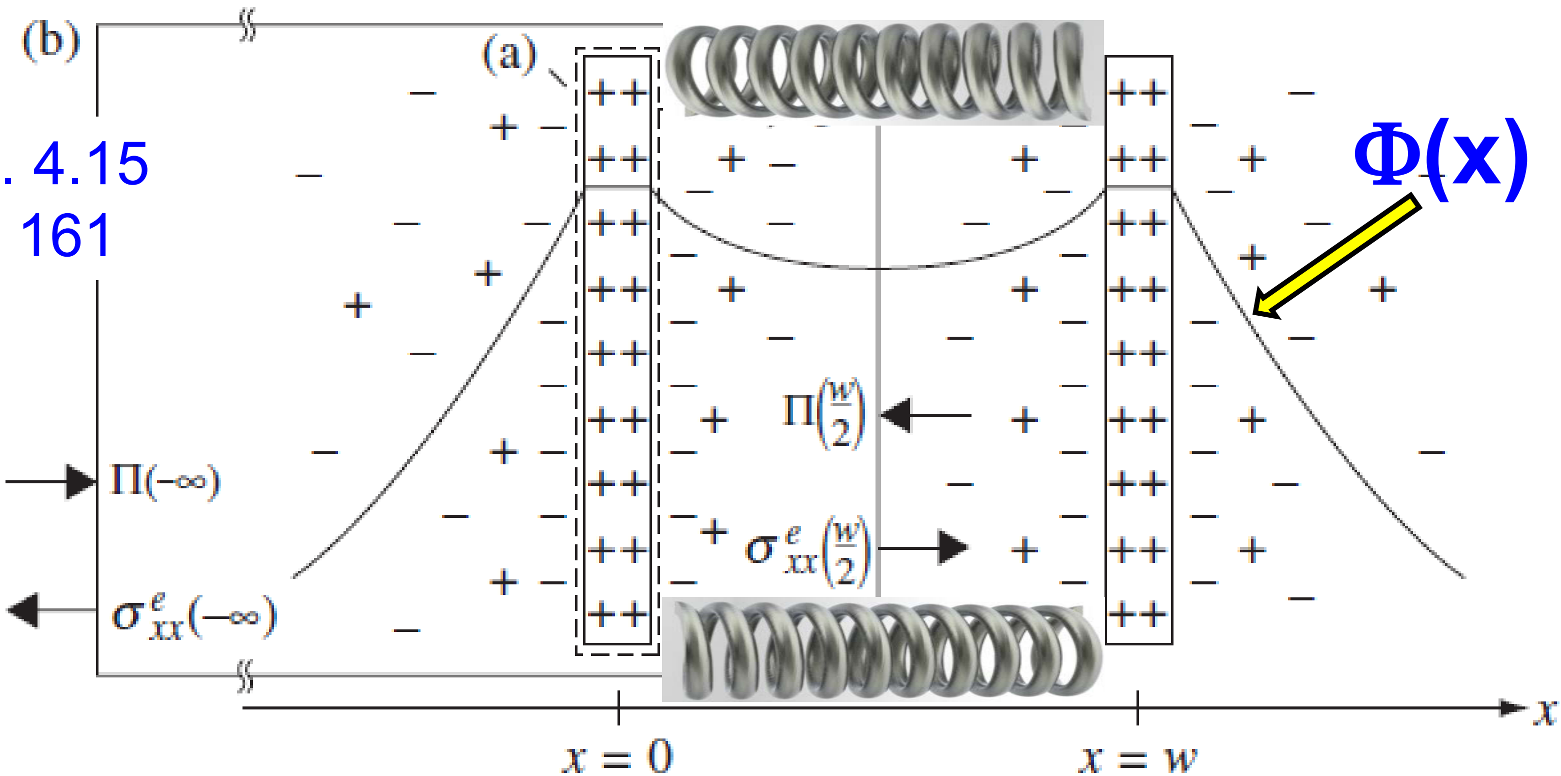


Figure 4.14 The potential profile for plane parallel interacting double layers. Local $E_x = -(d\Phi/dx) \rightarrow$ "double layer **repulsion**"

electrical stress: $\sigma^{elec} \sim (\sigma_d E) \propto E(x)^2$

In Equilibrium, the SUM of the [electrical + osmotic + "elastic recoil"] stresses = 0

Fig. 4.15
p. 161



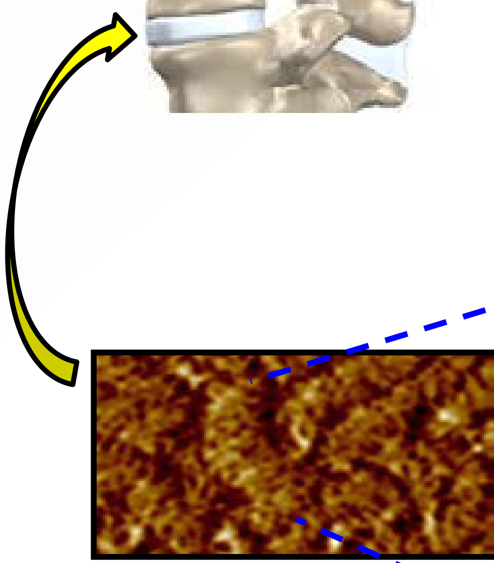
x-linked
HA "Hydrogel"

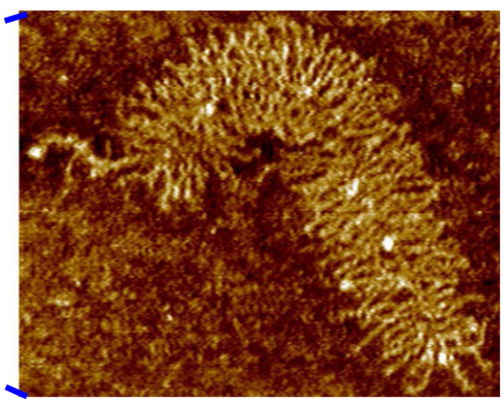
van't Hoff (local/molecular):

$$\pi^{os} = RT\left(\sum c_i\,(x) - \sum c_{io}^{bath}\right)$$

Astronauts gain 1-2 inches in <u>height</u> during space flight: <u>swelling of the intervertebral discs under 0-gravity</u>:

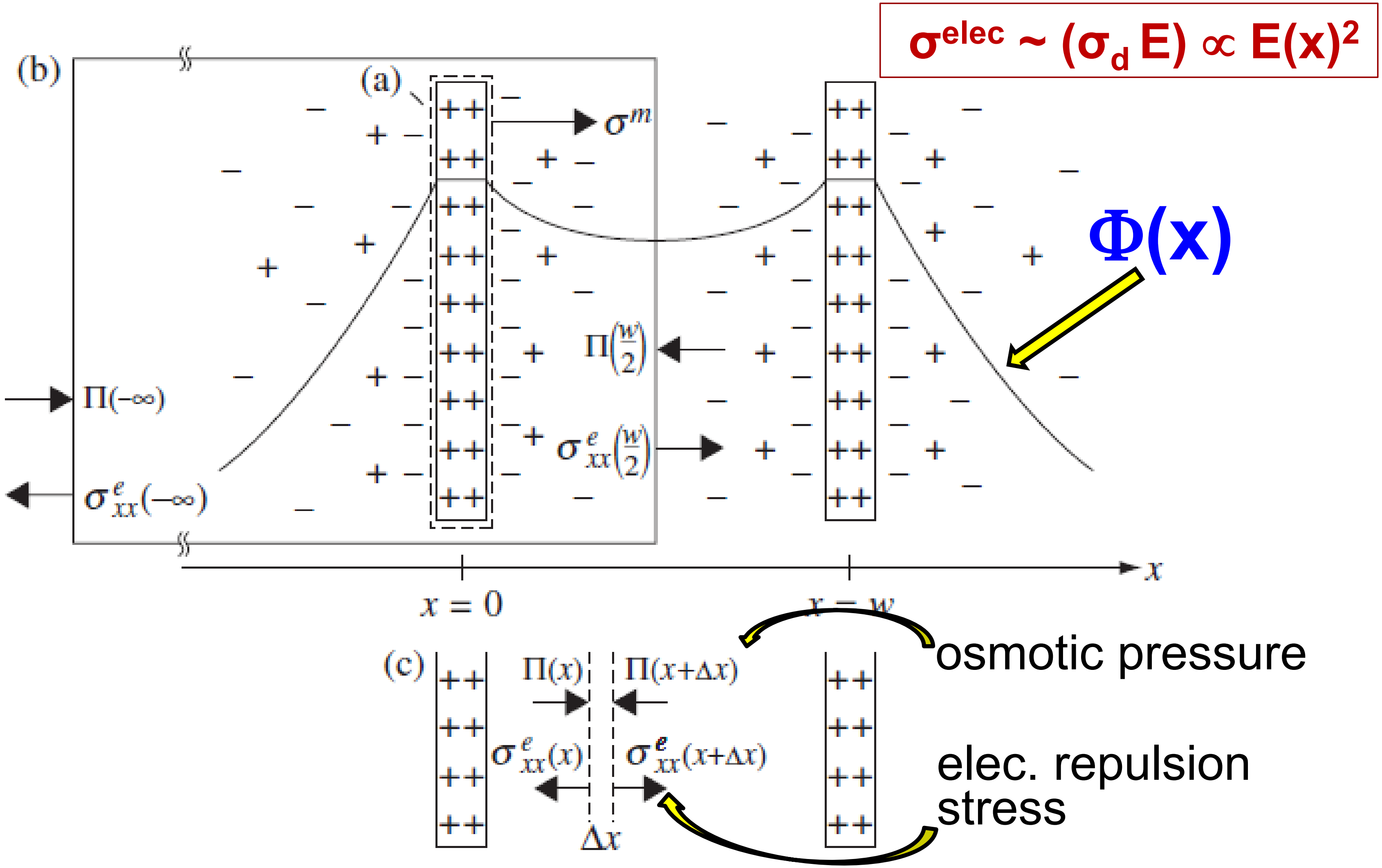
"swelling pressure" of highly charged ECM: aggrecan GAG chains





repulsive stresses

mech Elec. osmotic



Midterm: Find $\bar{c}_i$

Macro Donnan Model

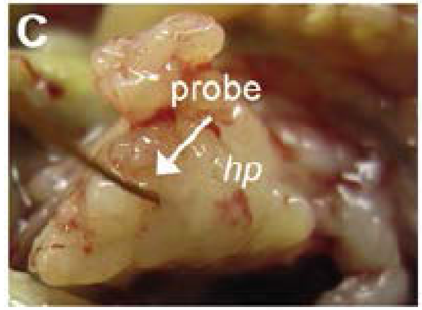
Boltzmann (for all species)

$$\left(\frac{\bar{c}_i}{c_i^{bath}}\right)^{\frac{1}{z_i}} = \textbf{const} = \left(\frac{\bar{c}_{Na}}{c_{Na}^{bath}}\right) = \left(\frac{\bar{c}_{H}}{c_{H}^{bath}}\right) = \left(\frac{c_{Cl}^{bath}}{\bar{c}_{Cl}}\right) = \left(\frac{c_{OH}^{bath}}{\bar{c}_{OH}}\right)$$

Electroneutrality (with approximations):

$$\bar{\rho}_m + F(\bar{c}_{Na} - \bar{c}_{Cl}) = 0$$

Term Paper: π^{os} in PDA



Source: Provenzano, Paolo P. et al. "Enzymatic targeting of the stroma ablates physical barriers to treatment of pancreatic ductal adenocarcinoma." Cancer Cell 21, no. 3 (2012): 418-429.

van't Hoff:

$$\pi^{os} = RT\left(\sum \overline{c}_i - \sum c_i^{bath}\right)$$

Boltzmann (for all species)

$$\left(\frac{\overline{}}{^{bath}}\right) = \textbf{const} = \left(\frac{\overline{}}{}\right) = \left(\frac{\overline{}}{}\right) = \left(\frac{^{bath}}{\overline{}}\right) = \left(\frac{C_{OH}^{bath}}{\overline{}}\right)$$

Electroneutrality (with approximations):

$$\overline{\rho}_m + F(\overline{C}_{Na} - \overline{C}_{Cl}) = 0$$

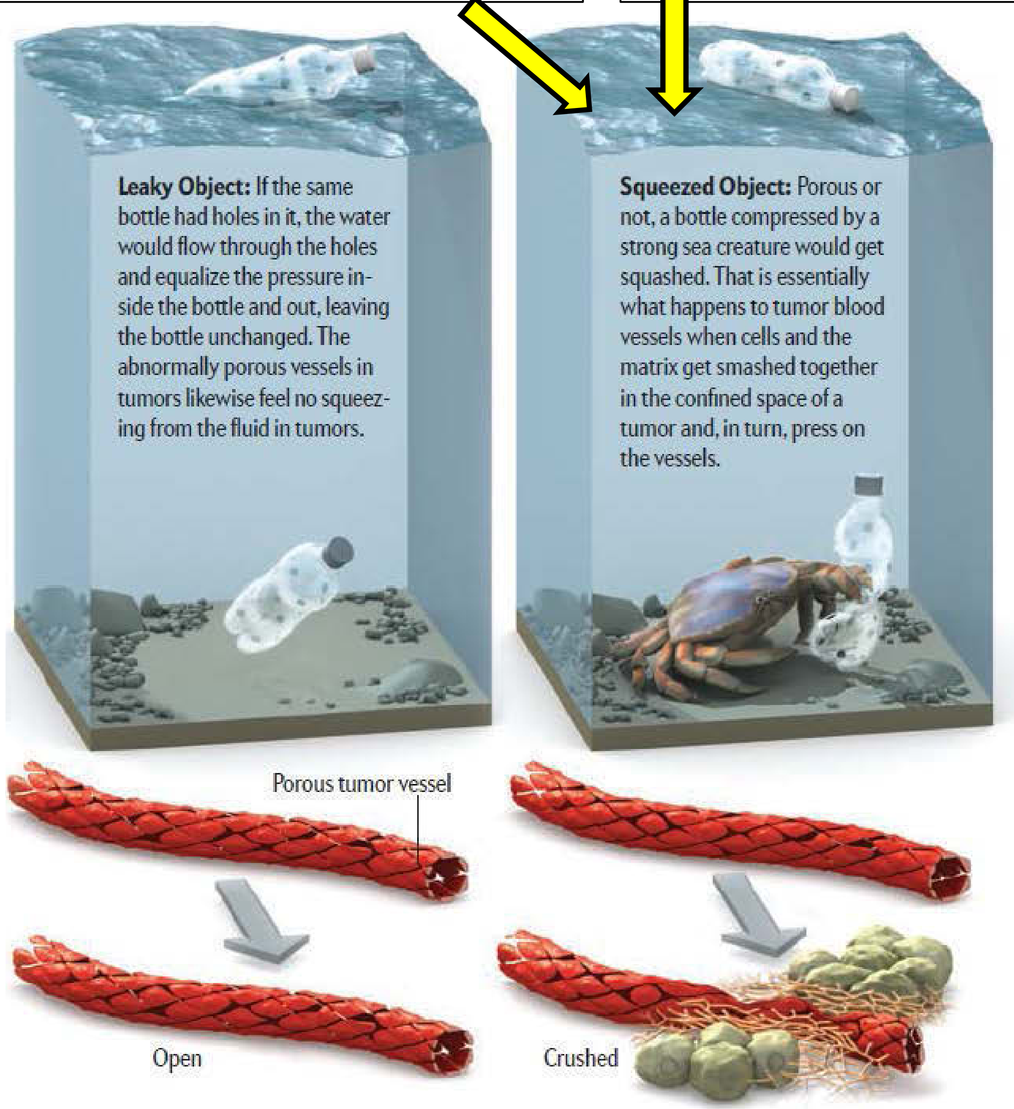
Provenzano/Hingorani: **Donnan** also increases **IFP** (porous or not) **!!**

Jain: **Solid Stress** squeezes vessels shut, porous or not!

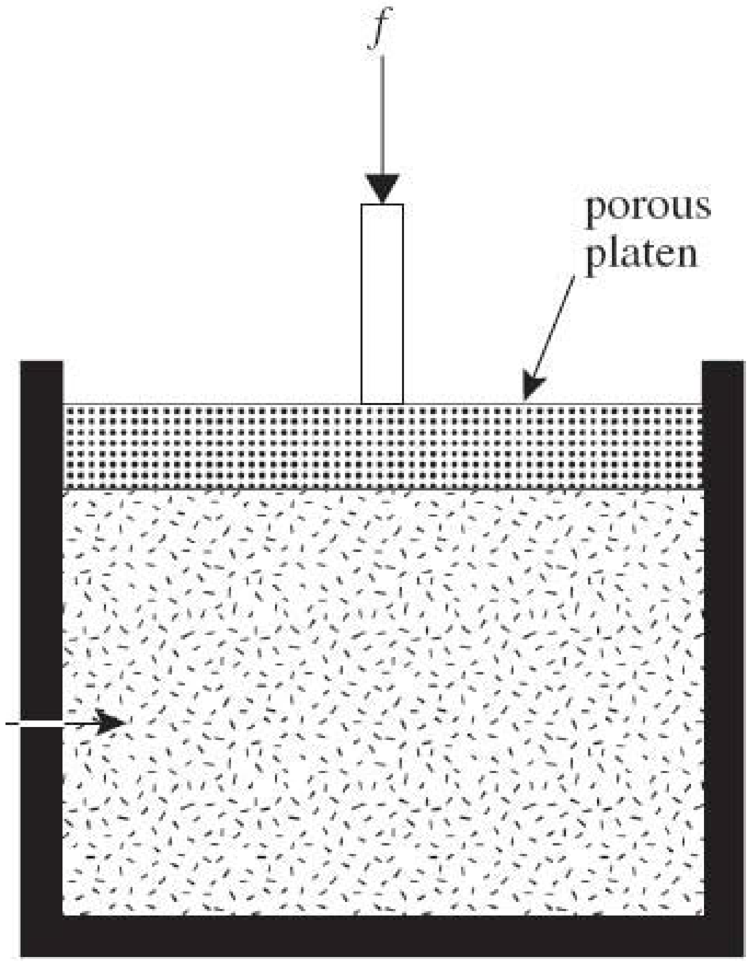
…Convection is driven by pressure gradients composed of hydrostatic and oncotic components"

HA organizes a hydrogel containing "immobilized fluid" ….that also has an **elastic modulus**….a property of solids;

…and gel has "fluid pressure"; **gradients in pressure drive convective fluid flow**



Source: Jain, Rakesh K. "An indirect way to tame cancer." Scientific American 310, no. 2 (2014): 46-53.



Gels are also Poroelastic

☺

Cancer Research 2000

Role of Extracellular Matrix Assembly in Interstitial Transport in Solid Tumors[1]

Paolo A. Netti,[2] David A. Berk,[3] Melody A. Swartz,[4] Alan J. Grodzinsky, and Rakesh K. Jain[5]

Steele Laboratory for Tumor Biology, Department of Radiation Oncology, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts 02114 [D. A. B., M. A. S., R. K. J.], and Department of Electrical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139 [A. J. G.]

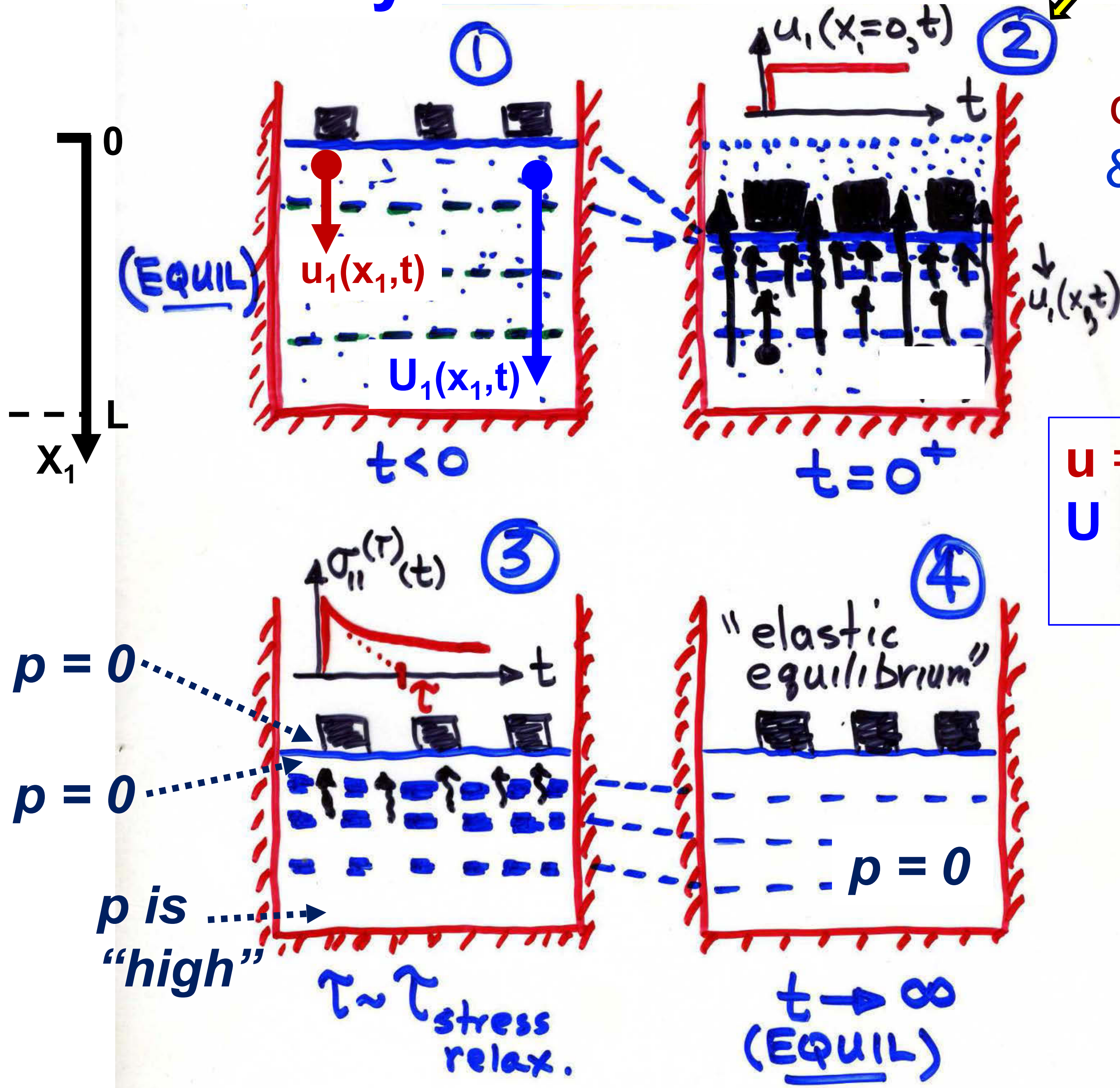
ABSTRACT

- The extracellular matrix may contribute to the drug resistance of a solid tumor by preventing the penetration of therapeutic agents. We measured differences in interstitial resistance to macromolecule (IgG) transport in 4 tumor types and **found an unexpected correspondence between transport resistance and the mechanical stiffness.**

- **The interstitial diffusion coefficient of IgG was measured *in situ* by FRAP. Tissue elastic modulus and hydraulic permeability were measured by confined compression of excised tissue (*using poroelastic theory*).**

- **Conclusions: collagen influences tissue resistance to macromolecule transport, possibly by binding and stabilizing the GAG component of ECM …findings suggest a new method to screen tumors for resistance to macromolecule-based therapy…. Moreover, collagen & collagen-proteoglycan bonds are ….. potential targets of treatment to improve delivery.**

Poroelasticity: Stress relaxation



Apply a "step" in compressive displacement u_1

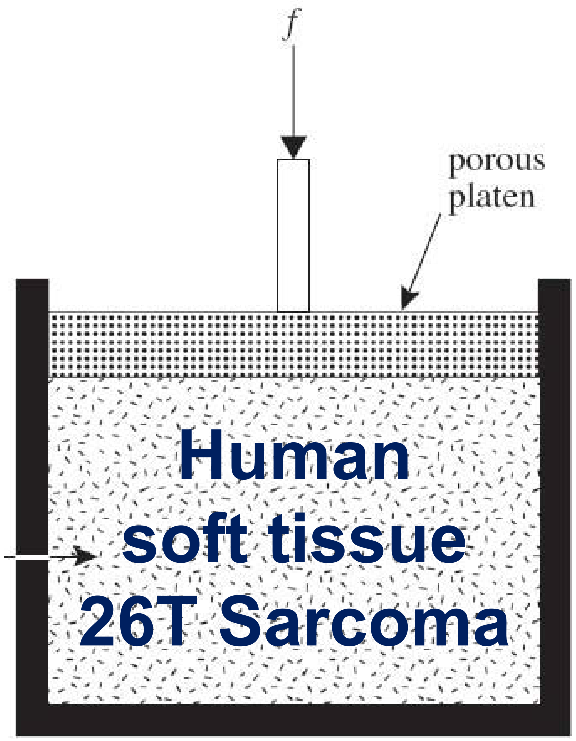
& hold thickness constant

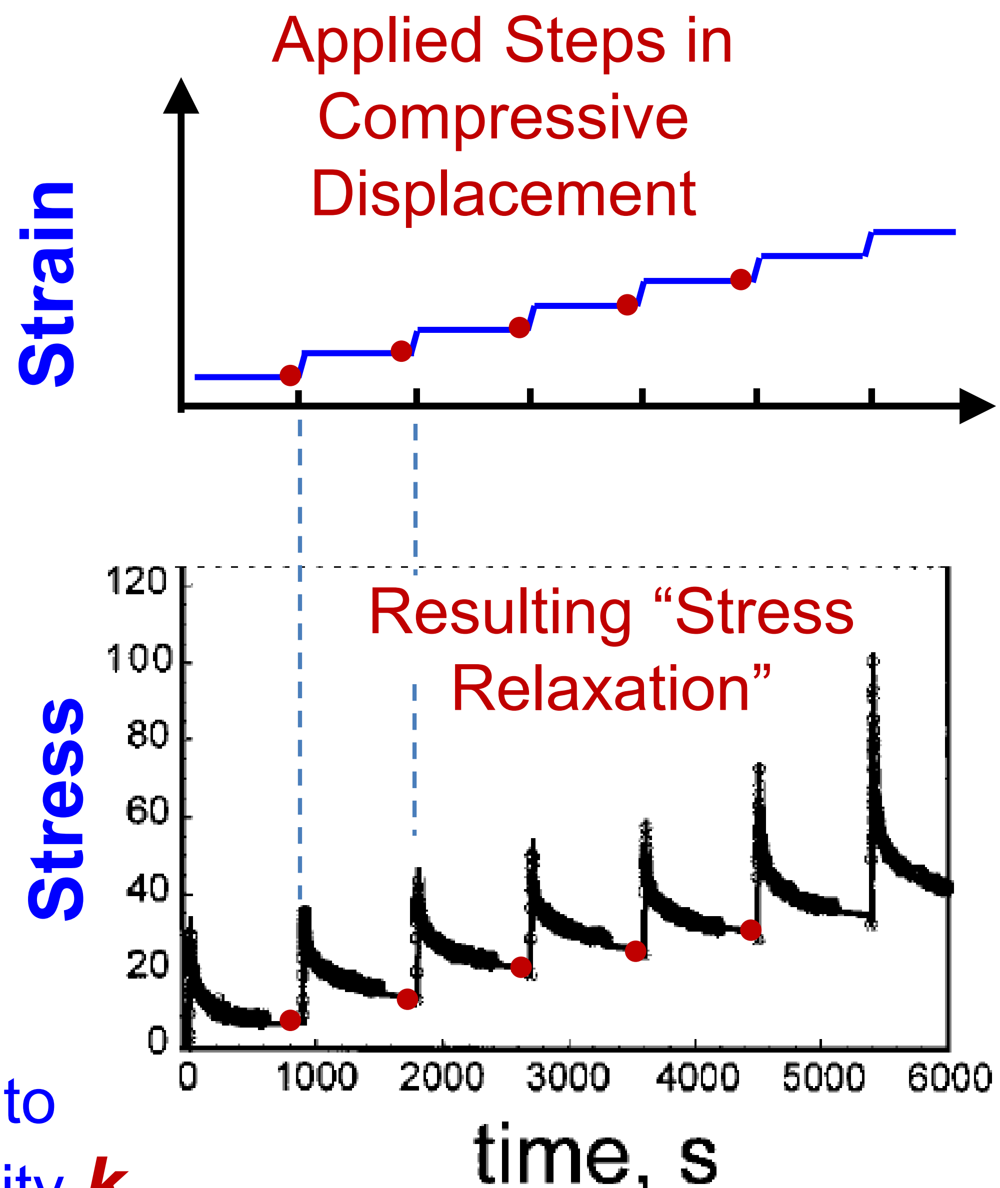
u = displacement

U = "macro" fluid velocity

$$\tau_{\text{gel}} = \frac{L^2}{\pi^2 D_{\text{gel}}}$$

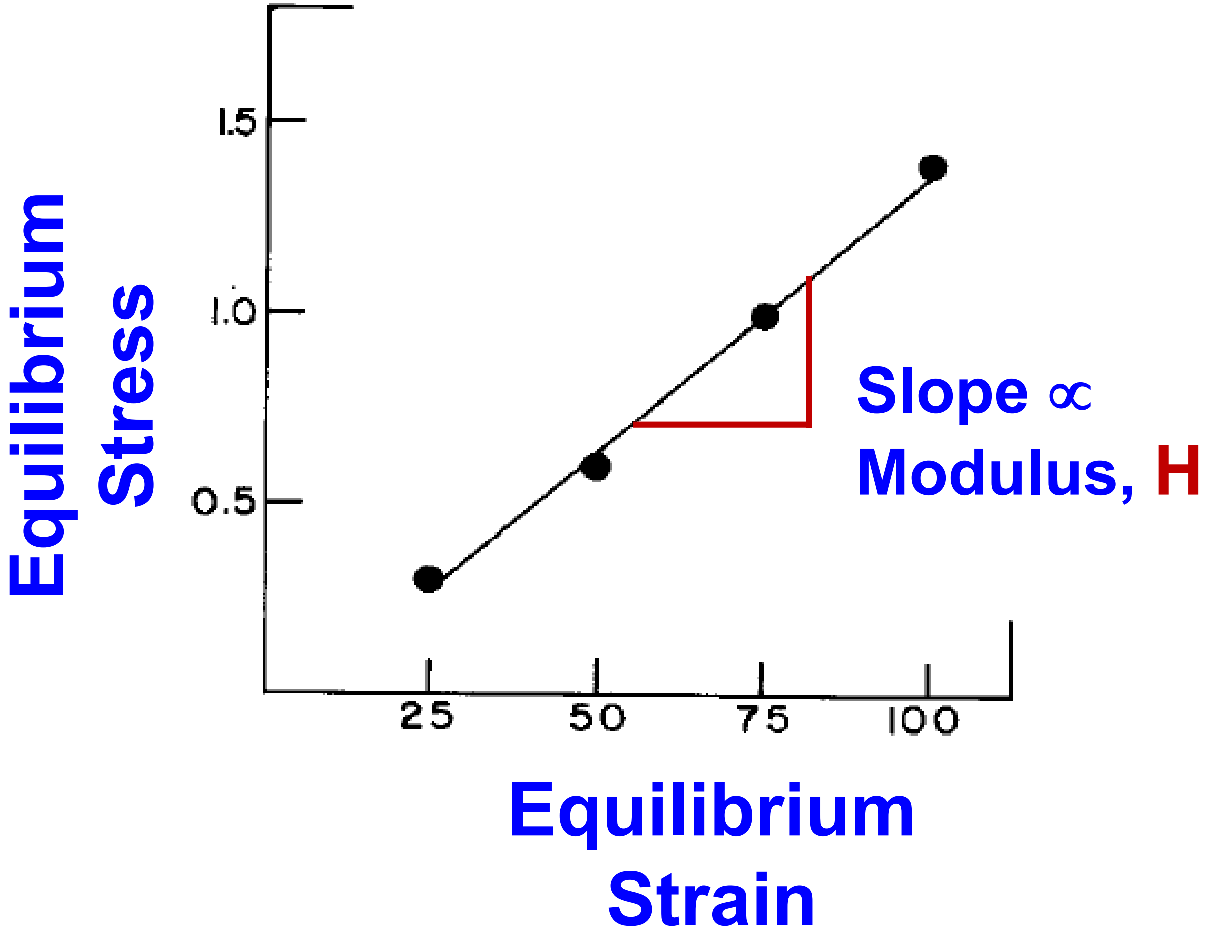
$$D_{gel} = H \cdot k$$





$$\tau_n = \frac{L^2}{n^2 \pi^2 H k}$$

n = 1 exponential curve fit to estimate hydraulic permeability ***k***


Equilibrium
Stress
1.5
1.0
0.5
25
50
75
100
Slope ∝
Modulus, H
Equilibrium
Strain

Kinetics of swelling of gels

Toyoichi Tanaka and David J. Fillmore

(J Chem Phys, 1979)

Gel displacement *u* satisfies a poroelastic diffusion equation!

$$\frac{\partial u}{\partial t} = D_{\mathrm{gel}} \frac{\partial}{\partial r}\left[\frac{1}{r^2}\left(\frac{\partial}{\partial r}(r^2 u)\right)\right]$$

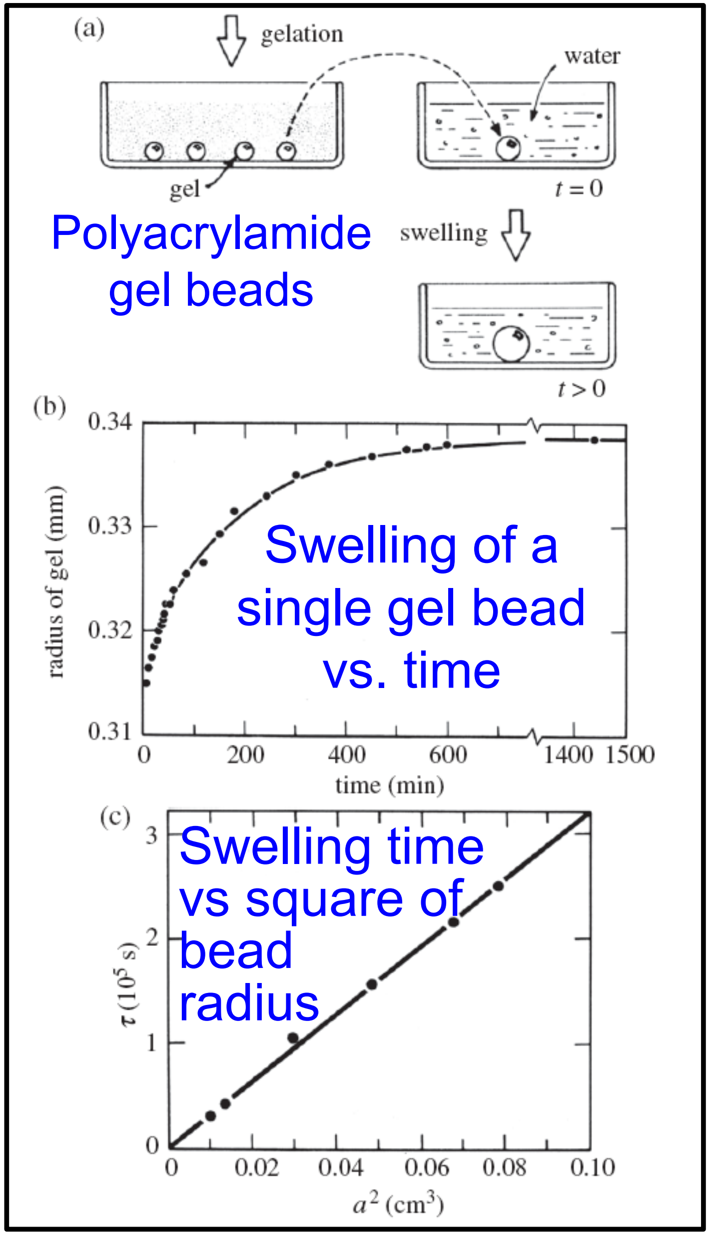
$$\tau_{\mathrm{gel}} = \frac{L^2}{\pi^2 D_{\mathrm{gel}}}$$

“D_{gel}” = “H” • “k”

H = (2G + λ) gel elasticity

k = gel hydraulic permeability

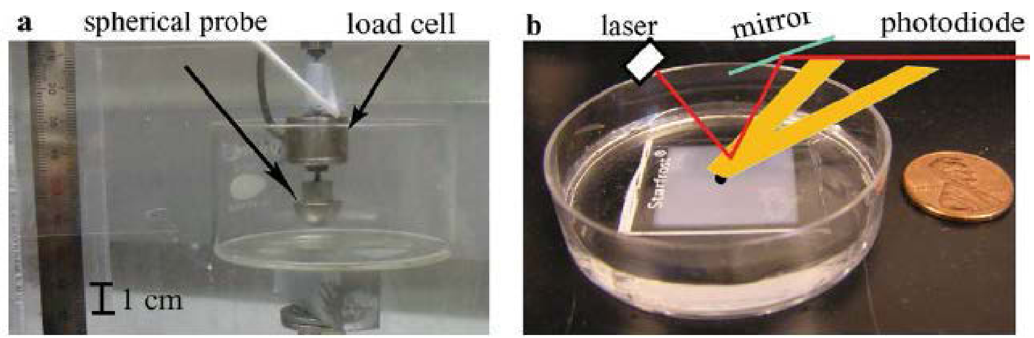
(Text, page 260-261)



From macro- to microscale poroelastic characterization of polymeric hydrogels *via* indentation†

Soft Matter 2012

Z. Ilke Kalcioglu,‡[a] Roza Mahmoodian,‡[a] Yuhang Hu,[b] Zhigang Suo[b] and Krystyn J. Van Vliet*[ac]



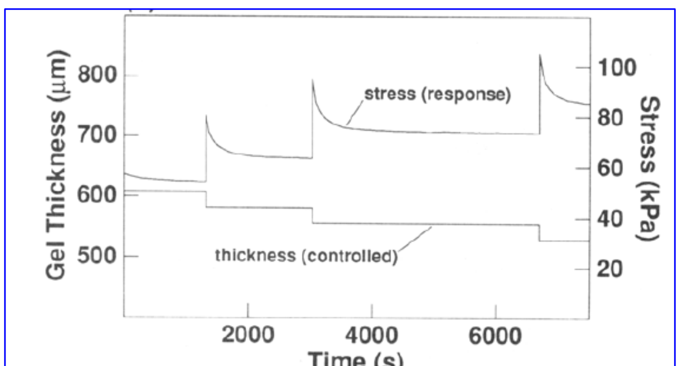
Source: Kalcioglu, Z. Ilke, Roza Mahmoodian, Yuhang Hu, Zhigang Suo, and Krystyn J. Van Vliet. "From macro-to microscale poroelastic characterization of polymeric hydrogels via indentation." Soft Matter 8, no. 12 (2012): 3393-3398.

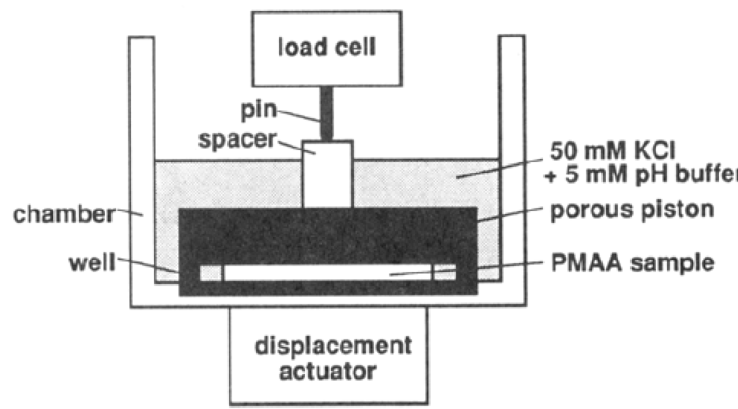
H k

Longitudinal Modulus and Hydraulic Permeability of Poly(methacrylic acid) Gels: Effects of Charge Density and Solvent Content

Macromolecules, 1993

Thomas M. Quinn and Alan J. Grodzinsky*



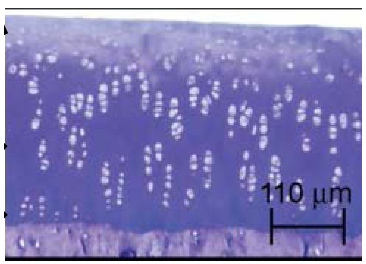


Source: Quinn, Thomas M., and Alan J. Grodzinsky. "Longitudinal modulus and hydraulic permeability of poly (methacrylic acid) gels: effects of charge density and solvent content." Macromolecules 26, no. 16 (1993): 4332-4338.

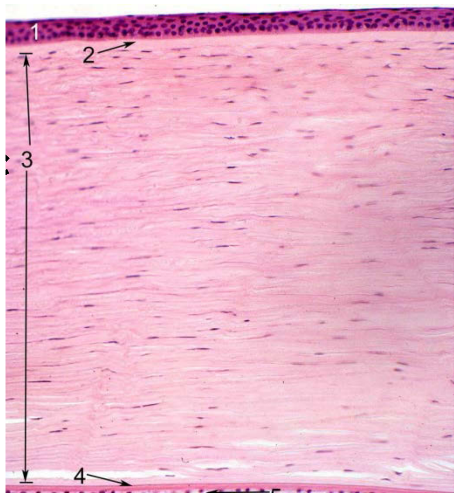
"1-D Confined Compression" measurements:

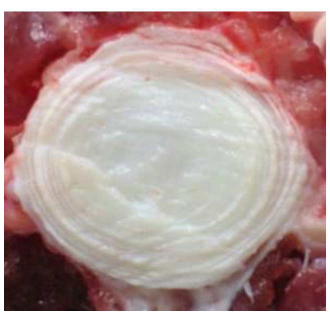
- **Poroelastic: material behavior "H" and "k"**
- **Tissue Swelling and Swelling Pressure**

Cartilage



Human Cornea





Human intervertebral disc