Structure-Function of Interstitial Spaces: New Clues to Fluid-Balance Mechanisms

Department of Reconstructive and Plastic Surgery Research

Medical School of Wake-Forest University

The energy for fluid and solute transport is the work of the heart and concentration gradients of water and solutes across extravascular spaces.

The net force determining ***fluid exchange*** *is the resultant of hydrostatic and osmotic pressures (Starling Principle) across capillary membranes.*

~5 liters

~15 liters

(~6 – 10 liters in skin)

Plasma

Interstitial space

INTRACELLULAR
30 liters

P_c

COP_c

P_i ?

COP_i?

LYMPH FLOW

σ

(capillary osmotic reflection coefficient)

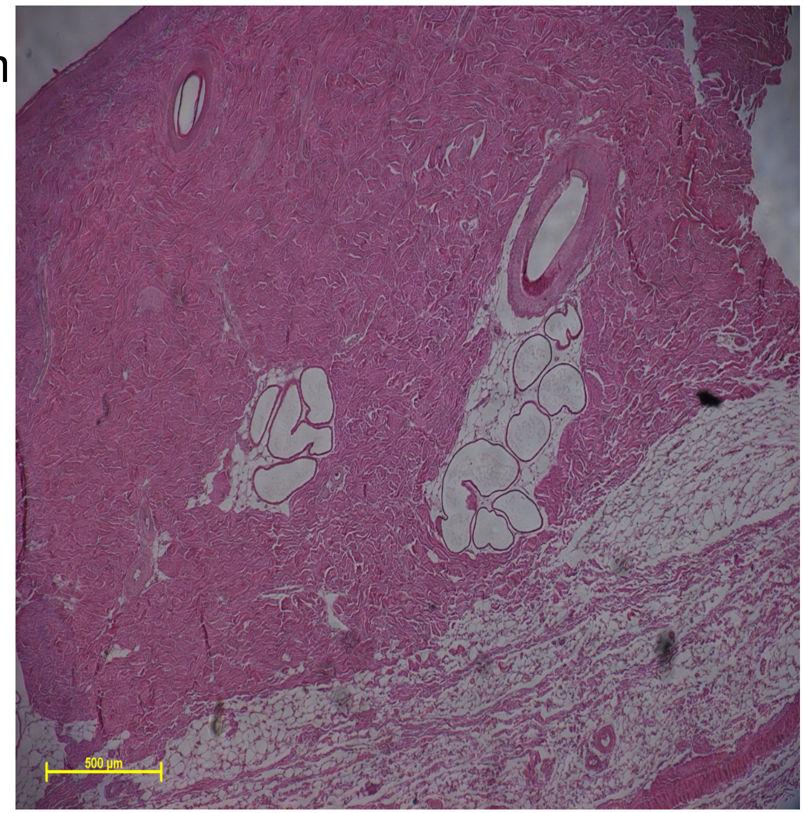
P(hydrostatic) /P(colloidosmotic) ---> filtration / adsorption

FORCES
GEOMETRY
PHYSICOCHEMICAL PROPERTIES

Porcine Skin

Layers ~11 mm:
- corneum
- epidermis
- dermis
- adipose
- cutaneous muscles

(corneum, epidermis, dermis) 2.5 mm = 2500 μm



P_c-P_i =(COP_c) –(COP_i)

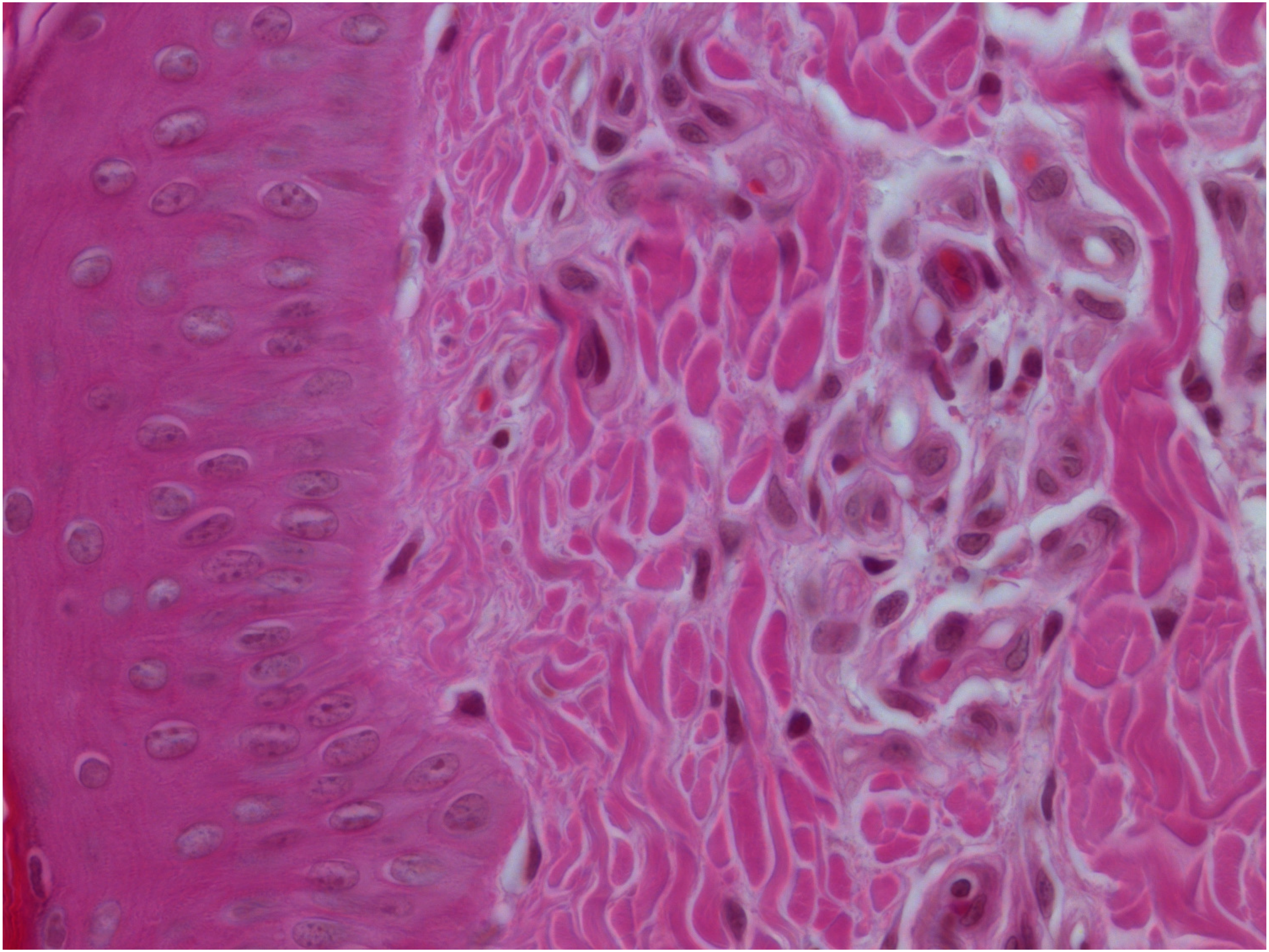
$\Delta IFV = k\int P_c - P_i - [(COP_c)-(COP_i)].dt + \int Fl.dt$

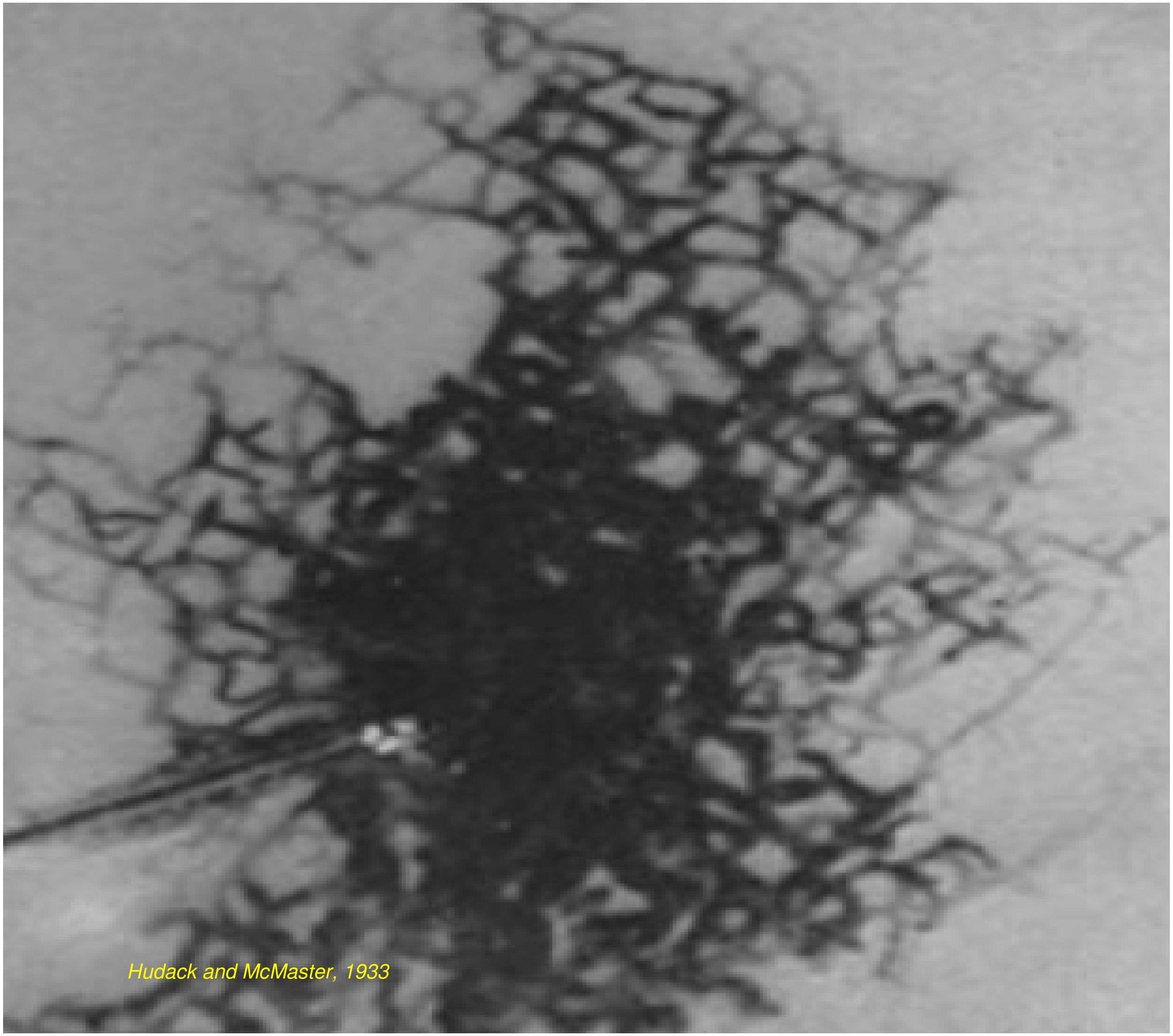
$\mu_j = \mu_0 + RT\ln a_j + PV + FE\, z_j + ghn_j$

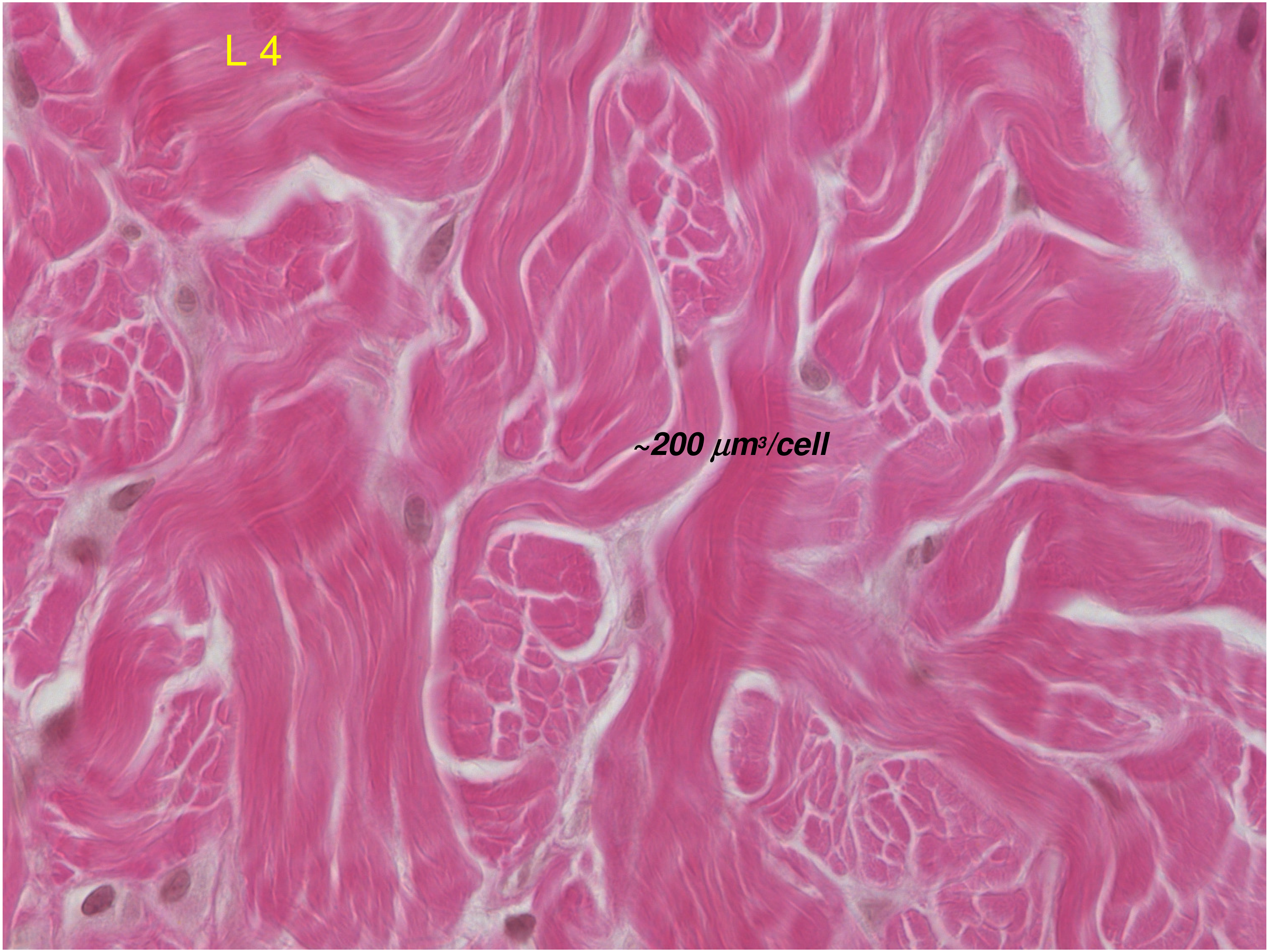
∂G/∂nj

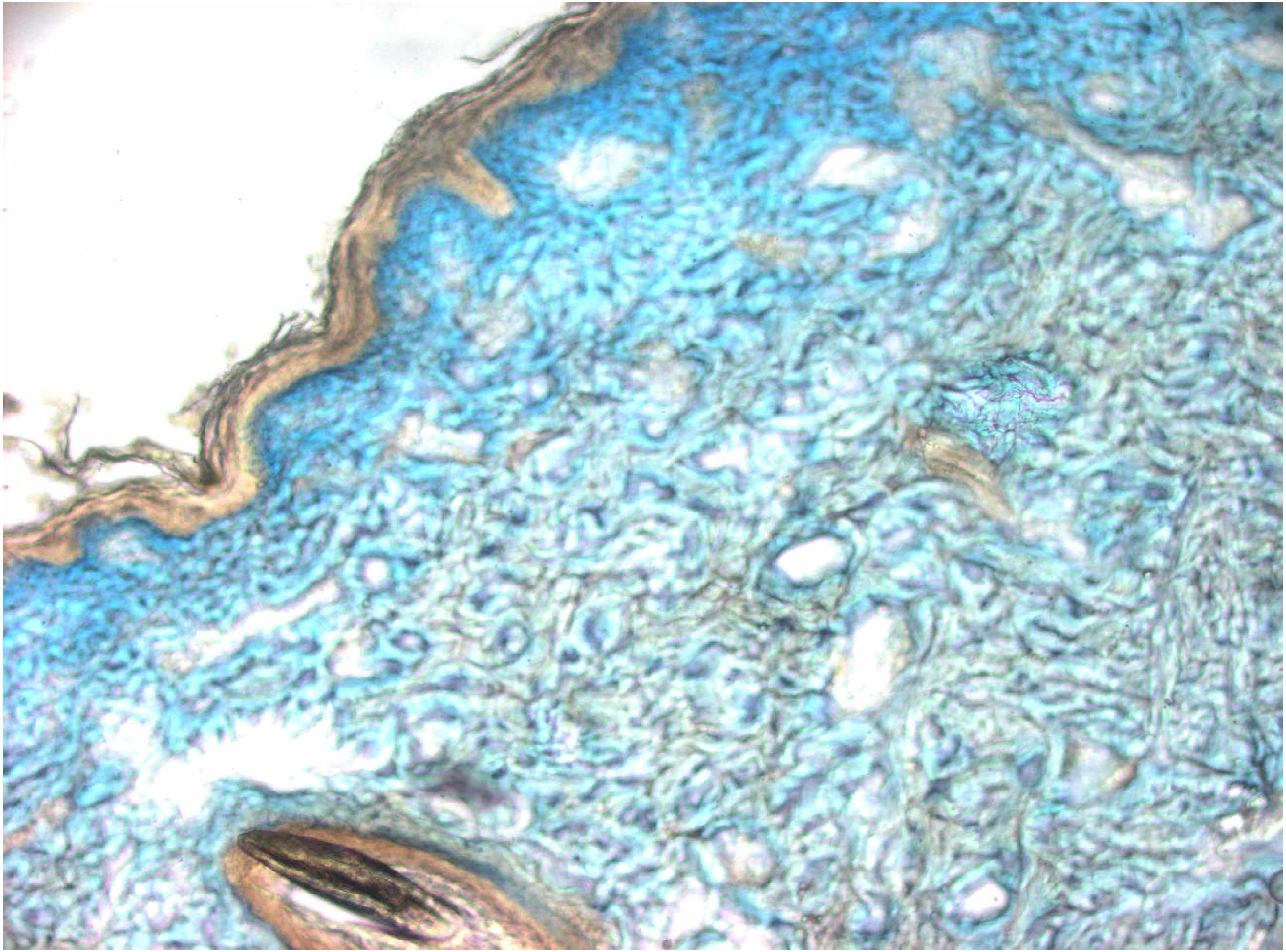
Chem. potential

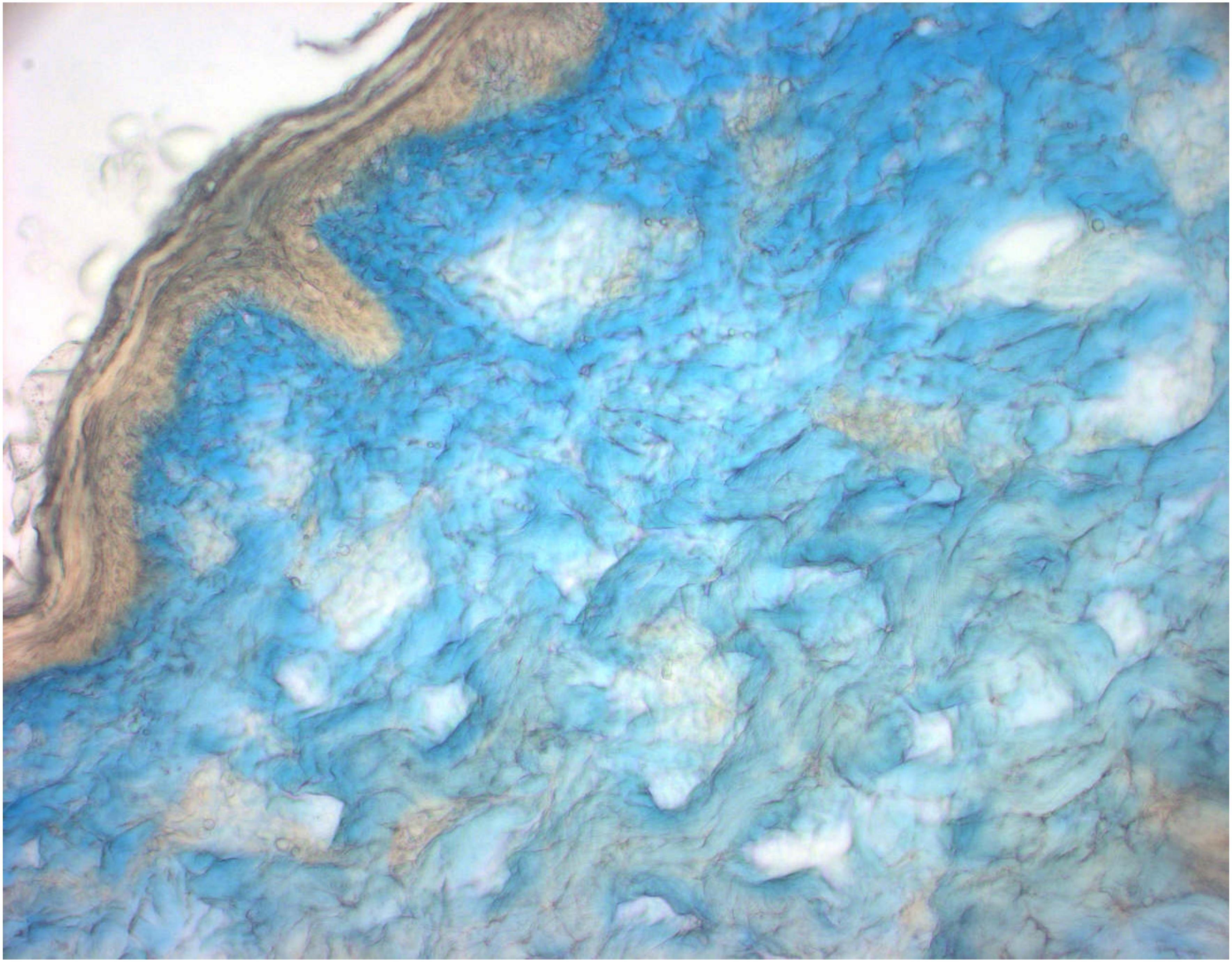
concentration + pressure vol. + charge +gravity

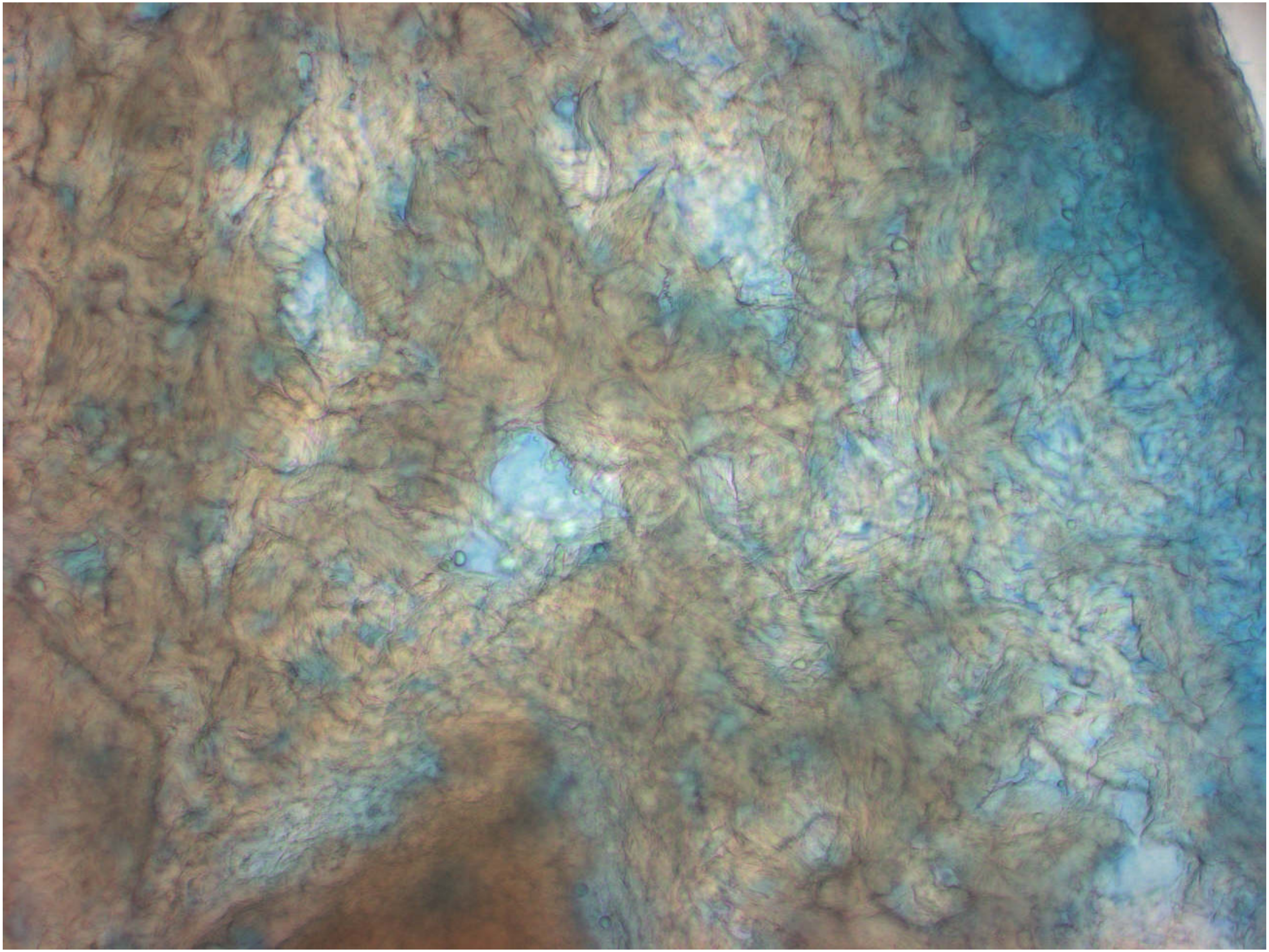


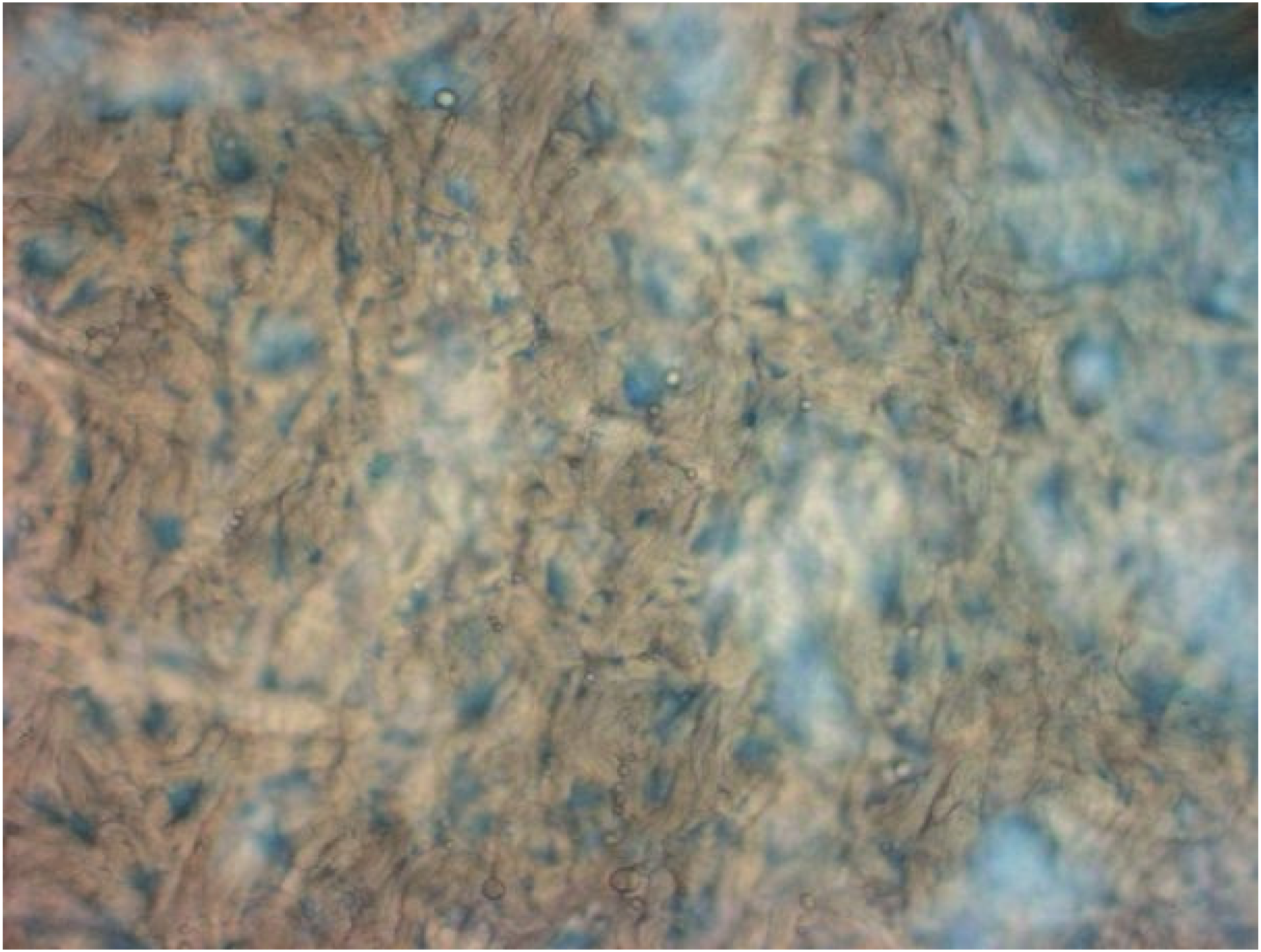

Hudack and McMaster, 1933


L 4
~200 μm3/cell



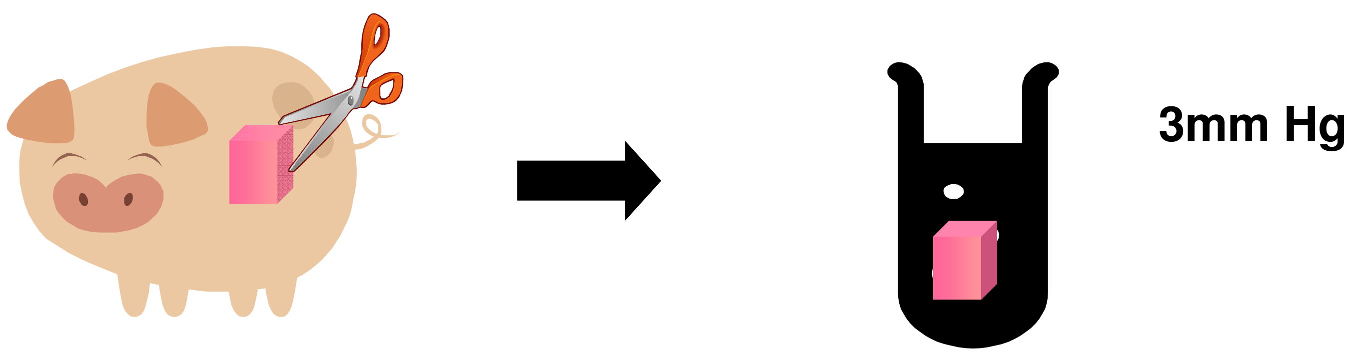




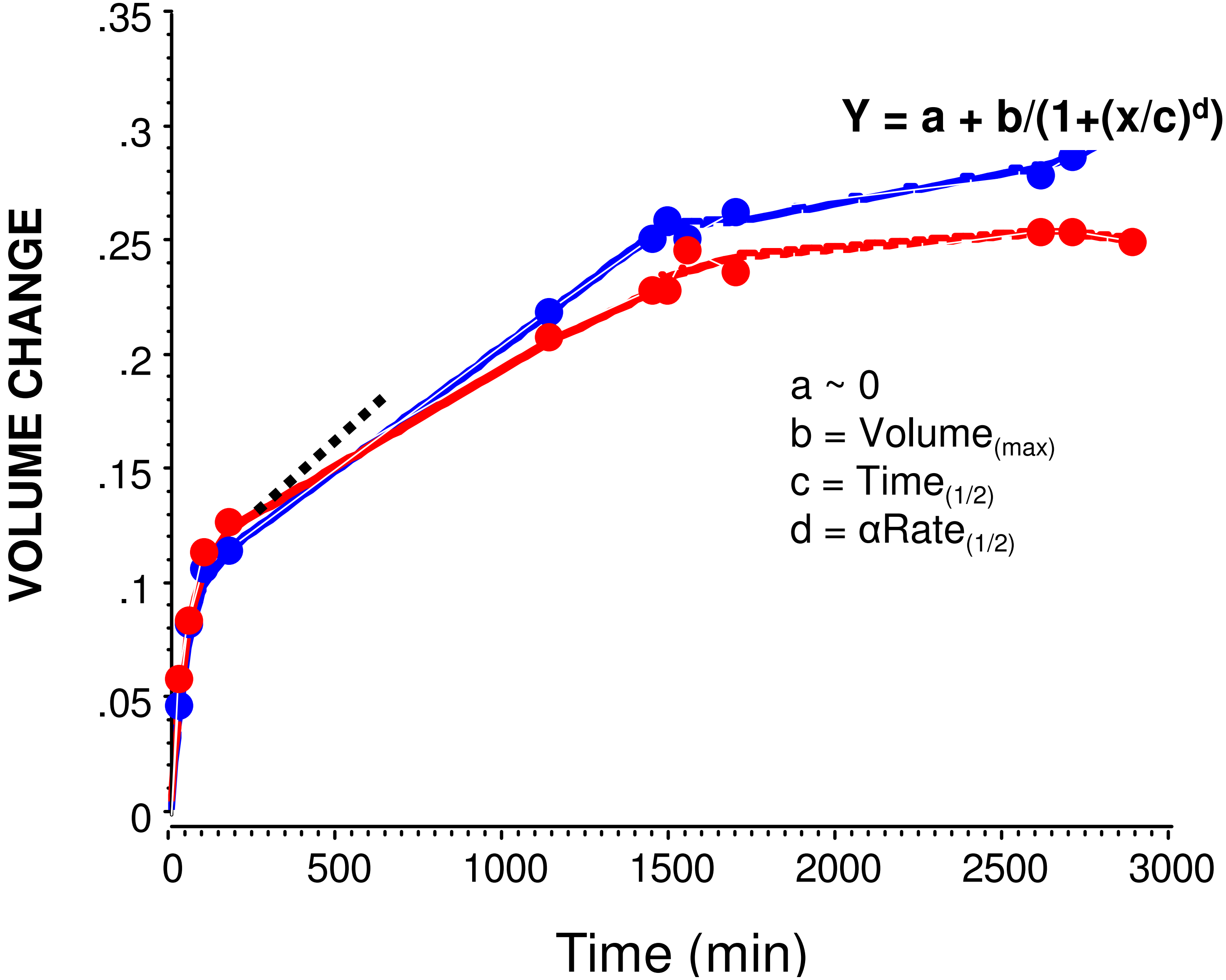


Swelling Kinetics of Dermal Explants

Time (min)	Volume Change (Mean ± SE; n = 6)	
	4º C	37º C
22	0.043 ± 0.002	0.047 ± 0.004
48	0.062 ± 0.005	0.066 ± 0.005
95	0.093 ± 0.008	0.087 ± 0.007
172	0.112 ± 0.006	0.101 ± 0.007
1128	0.214 ± 0.005	0.173 ± 0.010
1440	0.246 ± 0.004	0.186 ± 0.012



Swelling of Dermal Interstitium : Progress Curve at 4 and 37 ºC



Swelling of Dermal Explants
Kinetic Parameters

(Mean ± SE n=3)

	4 ºC	**37 ºC**
$Volume_{max}$	0.458 ± 0.068	0.225 ± 0.074
$Time_{1/2}$ (min)	1638 ± 688	426 ± 44
$\alpha Rate_{1/2}$	0.476 ± 0.078	0.555 ± 0.101

$$y = a + Volume/[1+(x/time)^{\alpha Rate}]$$

“EQUILIBRIUM” PRESSURE OF DERMAL INTERSTITIUM.
DESCRIPTIVE STATISTICS

Temperature	Mean ± SE	Max.	Min.	Difference	P-value	n
4 ºC	107.4 ± 22.3	211	50	60	0.012	7
37 ºC	47.3 ± 12.3	107	3	“	“	7

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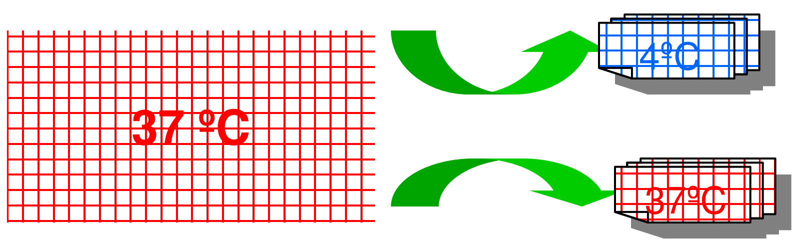
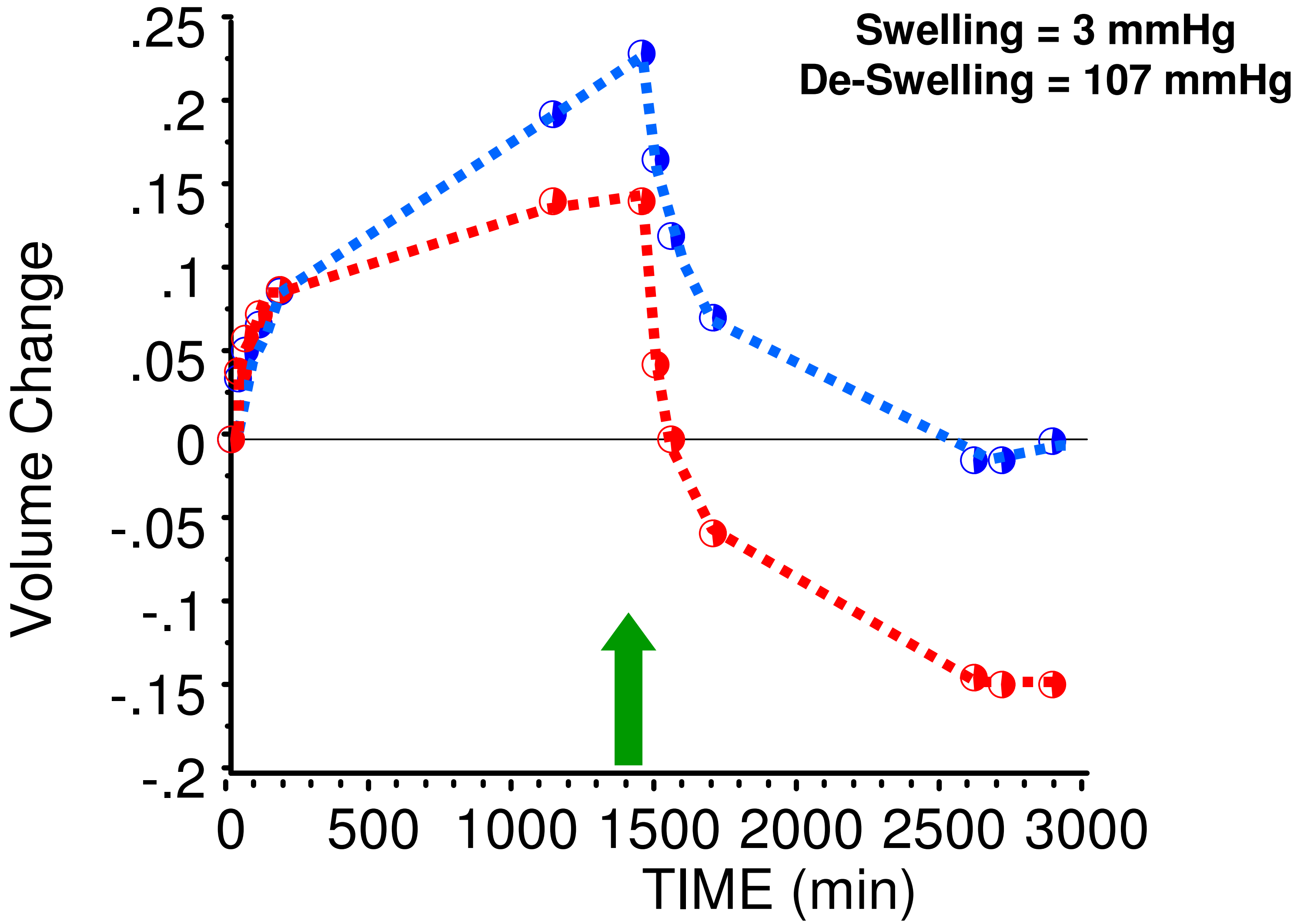


Replicate samples equilibrated in physiologic solutions of known colloidosmotic pressure within a **3 - 211 mmHg** range. Depending on pressure, explants swell, de-swell or do no change.

Swelling and De-Swelling of Dermal Interstitium



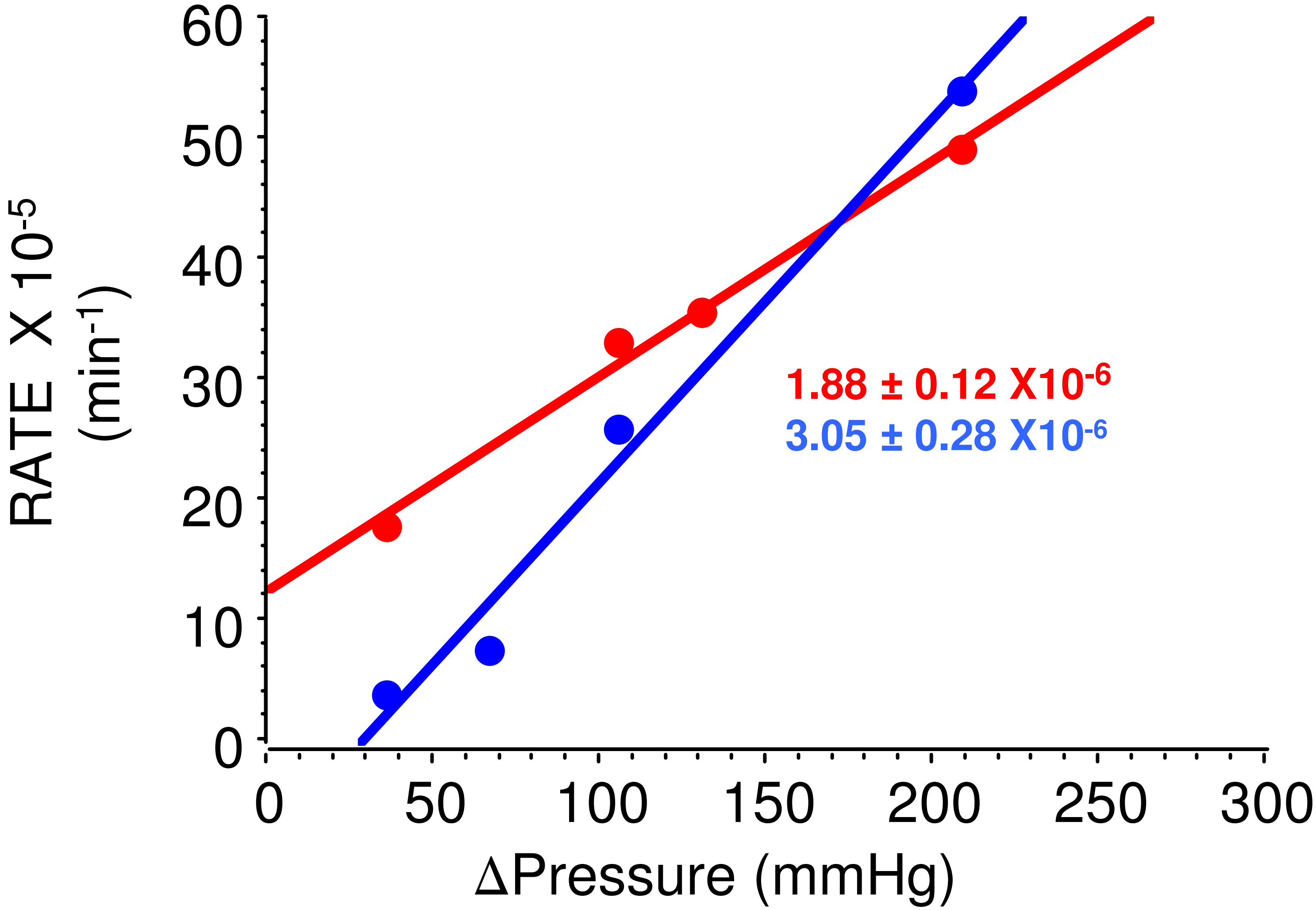
De-swelling of Dermal Explants. Kinetic Parameters

Parameter	4 ºC	37 ºC
Volume $_{(max)}$	0.408 ± 0.013	0.542 ± 0.021
Time $_{(1/2)}$	182 ± 17	192 ± 25
αRate $_{(1/2)}$	0.970 ± 0.075	0.698 ± 0.04

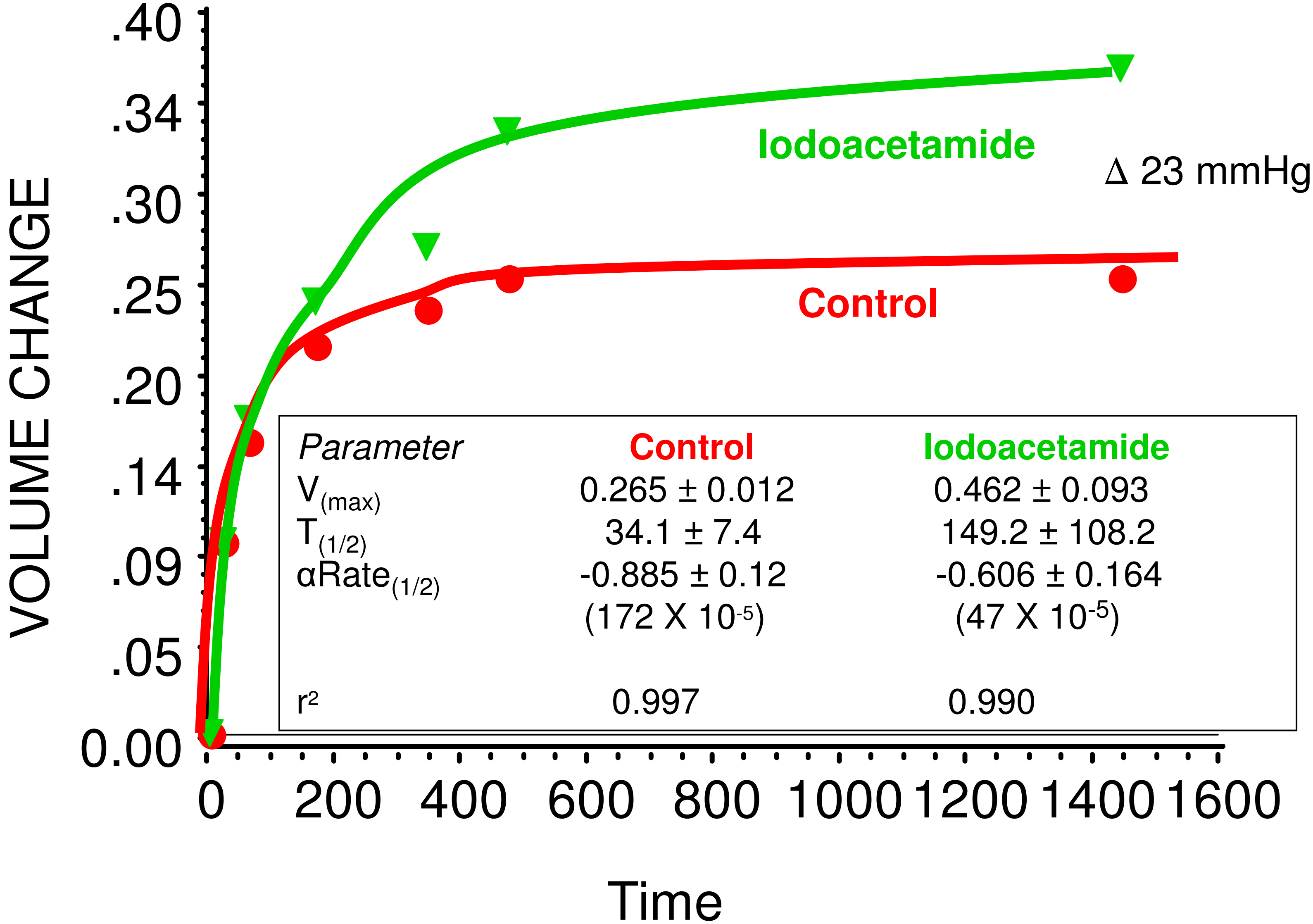
$$y = a + Volume/[1+(x/time)^{\alpha rate}]$$

r^2	0.9994	0.9997

VOLUME-CHANGE VELOCITY AS A FUNCTION OF PRESSURE



Swelling Parameters of Human Dermis after Inhibition of Anaerobic Glucose-Metabolism



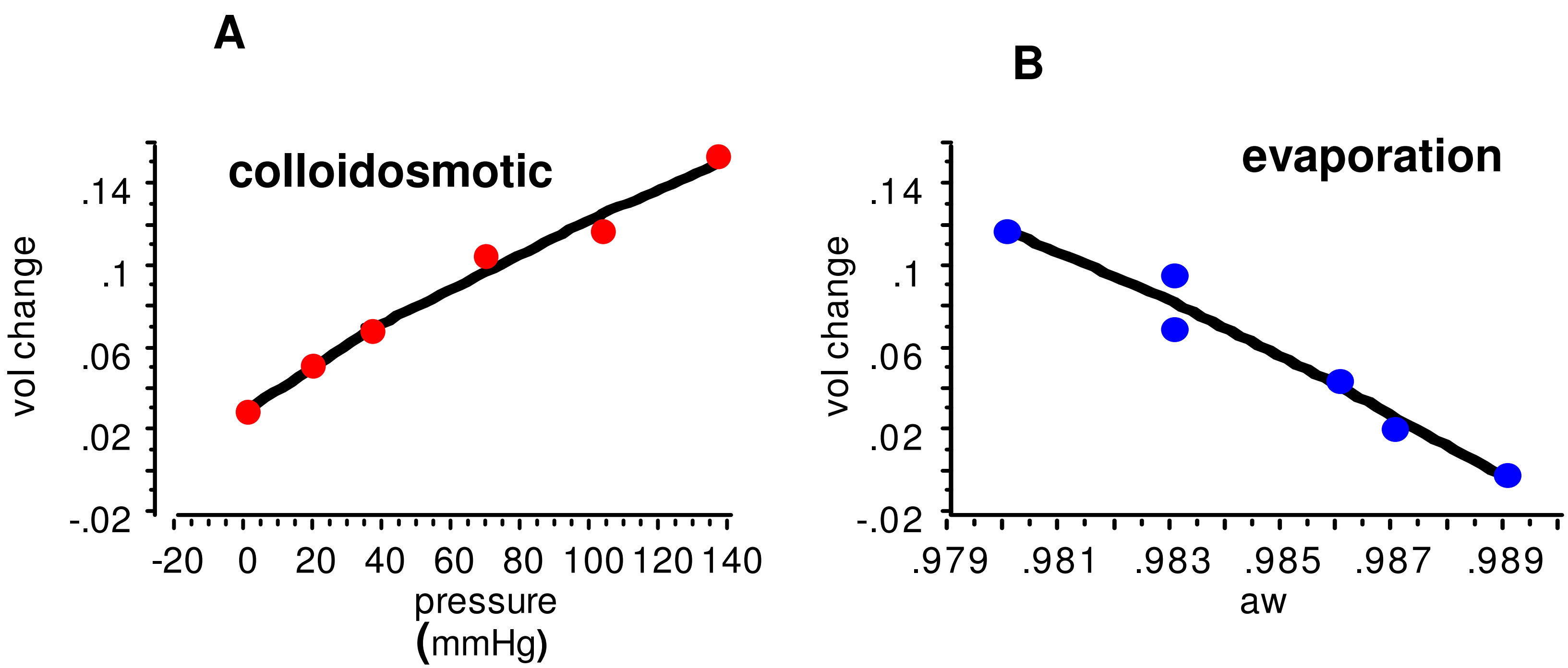
The Magnitude of Interstitial Pressure-Gradients is larger than previously considered.

The fluxes in/out of Interstitium are related linearly to the Pressure Gradients.

The Resultant Interstitial Pressure includes significant contribution from cell processes that require generation of energy from glucose metabolism.

The Geometry of Interstitial Fluid Pathways is …complex.

Interstitial-water transfer as a function of pressure and water activity gradients



A. Water transfer from interstitium to polymer solution.

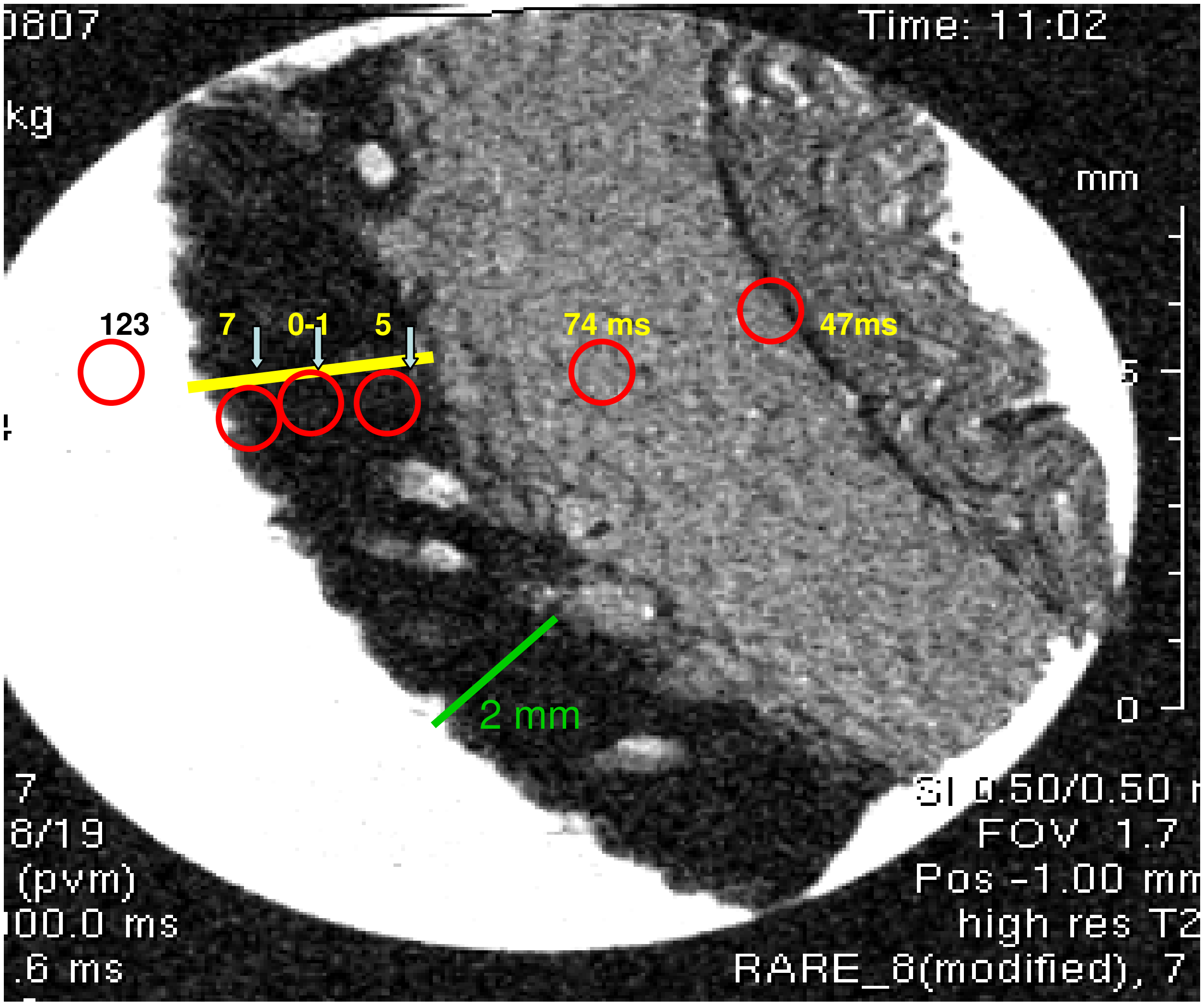
B. Water transfer from interstitium to air

$\Delta G - \Delta G° = RT \ln a$;

1atm . V_w = ~ 0.435 cal/mol

RT ~ 600 cal/mol

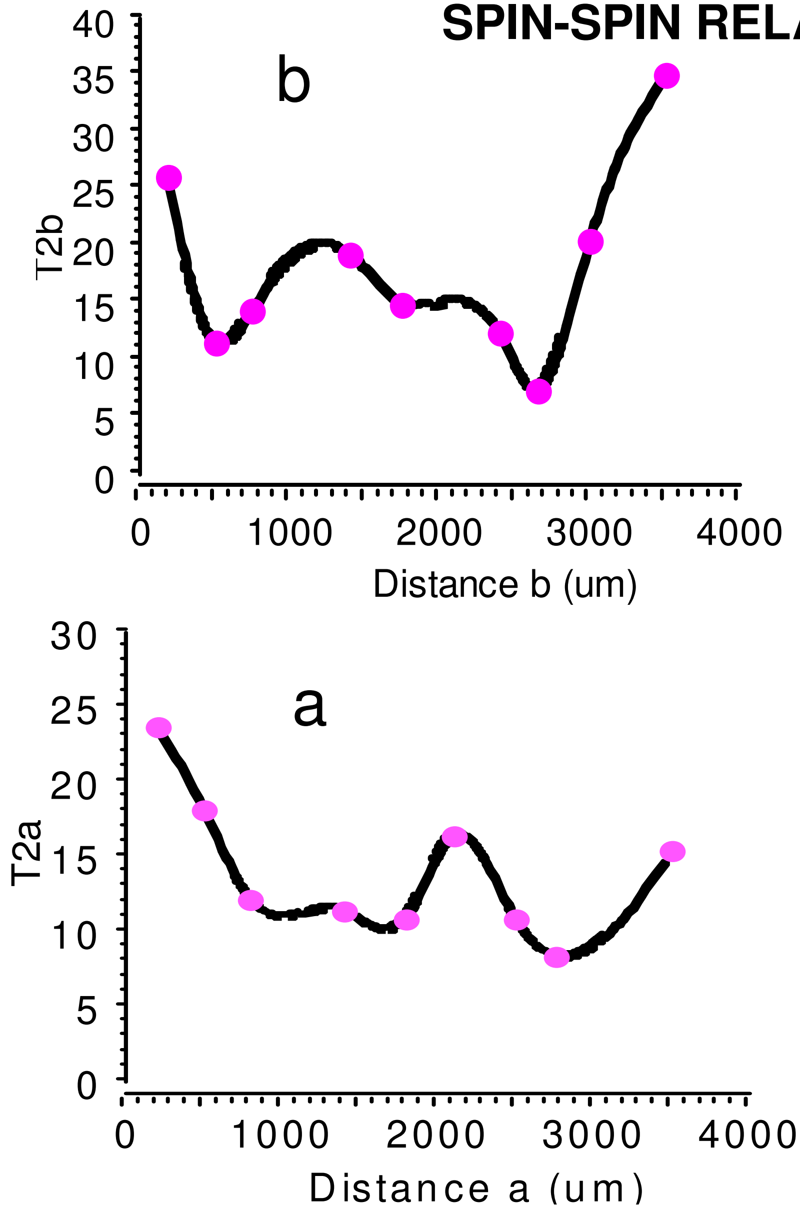
Magnetic Resonance Imaging: Transverse relaxation time

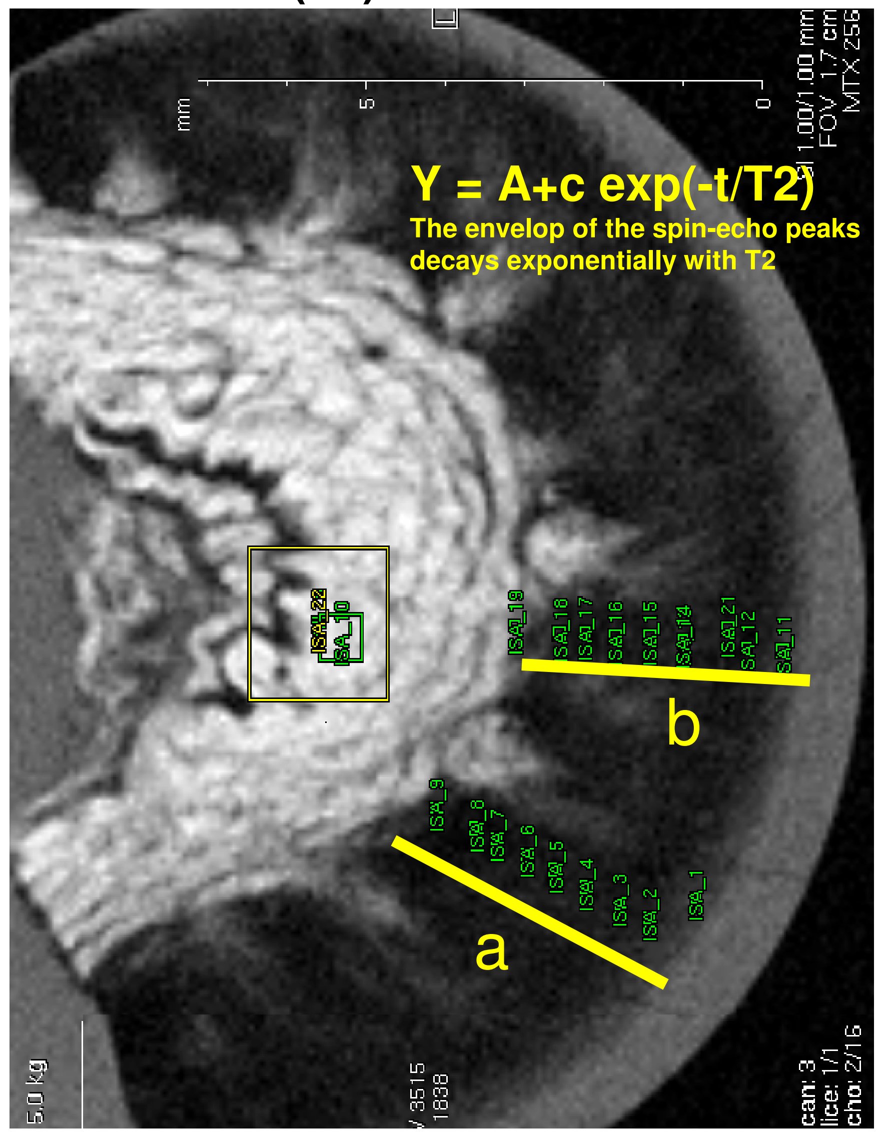


T_2 reflects water's freedom of motion

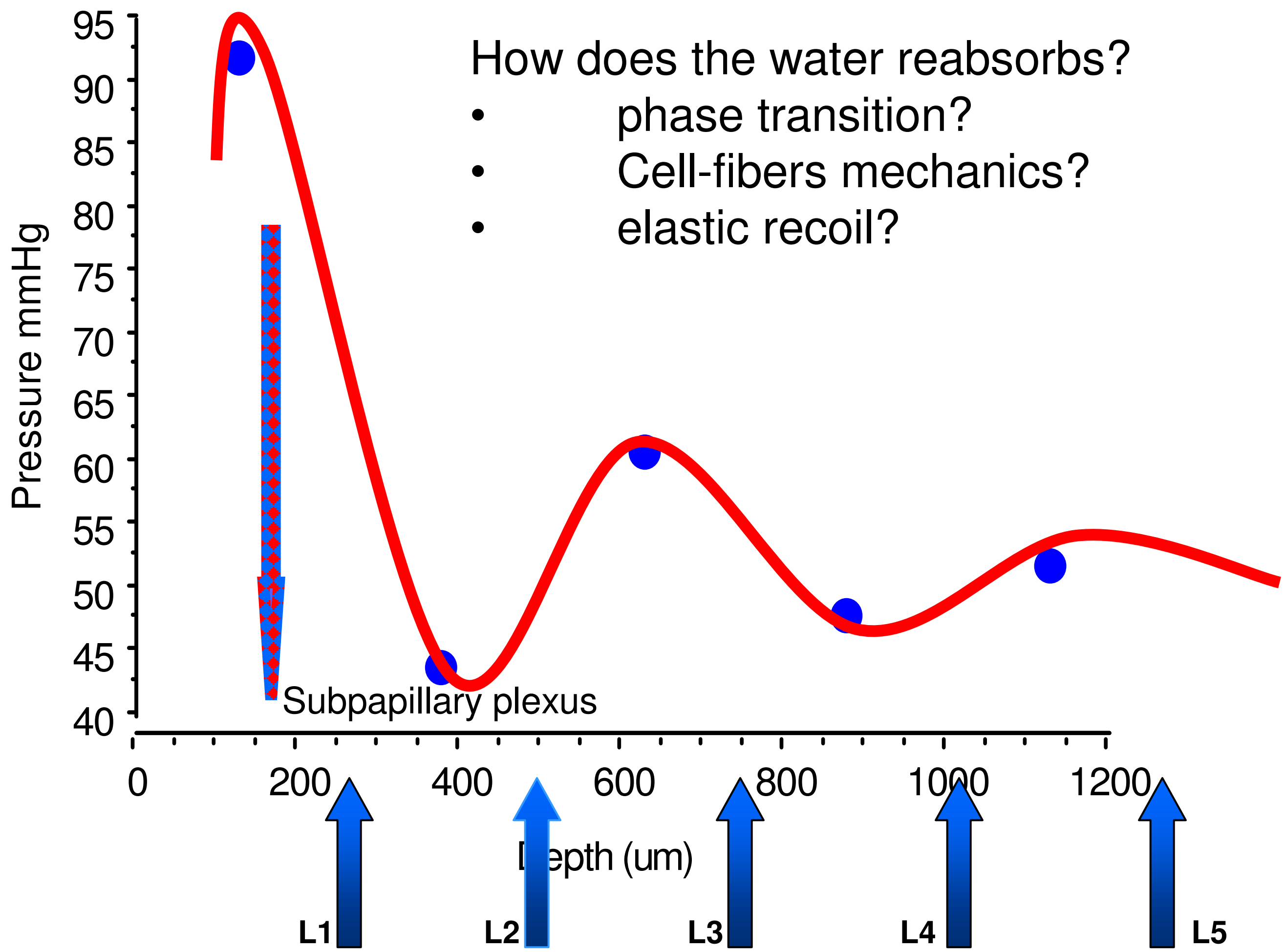
Correlates with a_w

Water activity gradients in skin interstitium.
SPIN-SPIN RELAXATION TIME (T2)

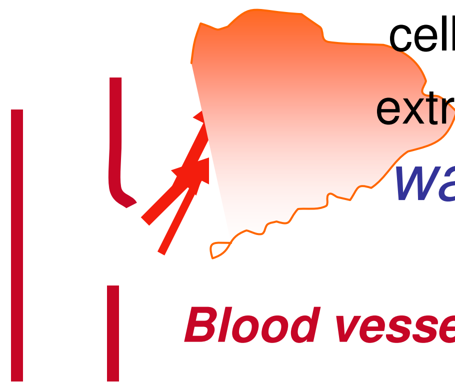




Osmotic equilibrium-pressure of pig skin layers



Local factors influence transport and distribution of reactants and their <u>microscopic rate coefficients</u> in extravascular spaces



cells→ Tissue factor = reactive sinks

extracellular matrix→ **Glycosaminoglycans**

water activity→ hydration/dehydration reactions

Blood vessel wall injury, inflammation

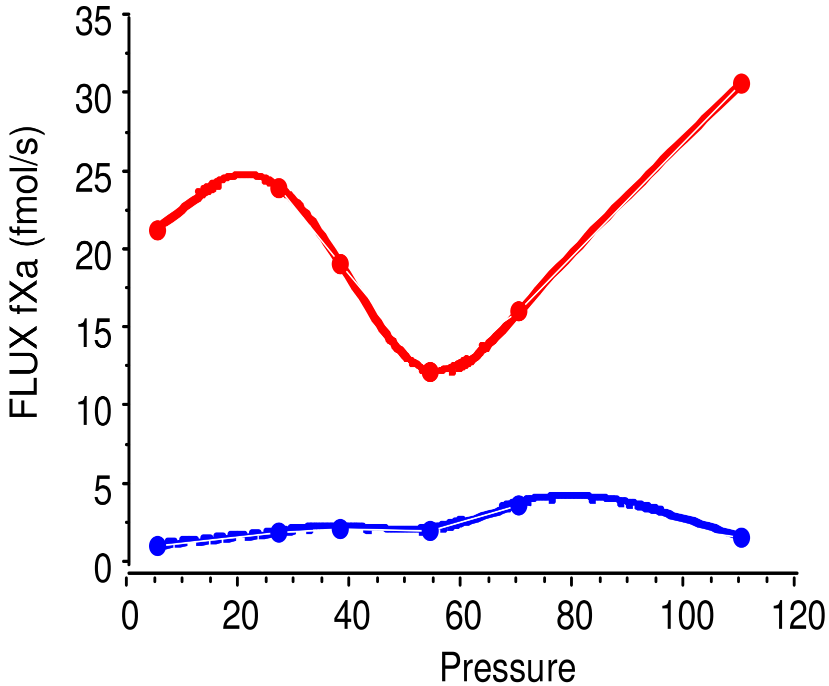
Vasodilatation→ extravasation of plasma proteins

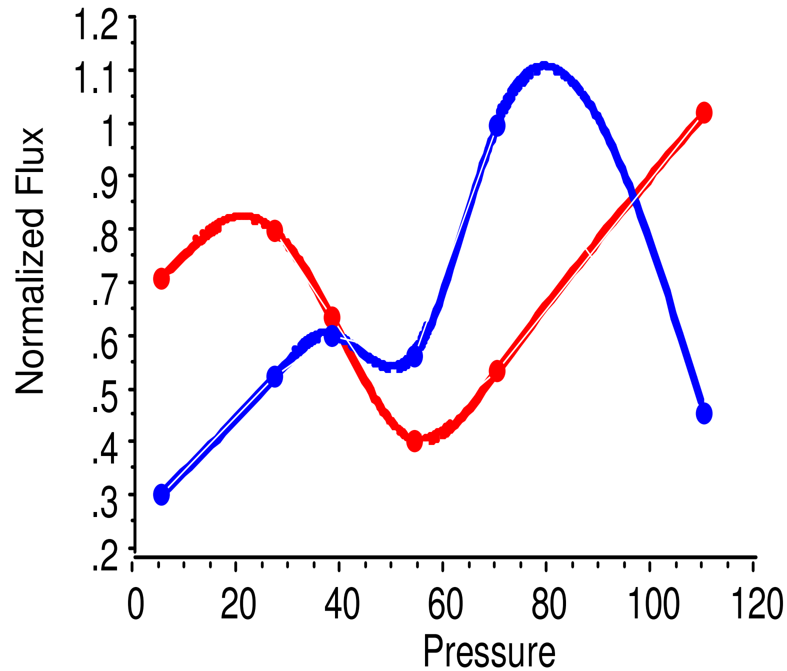
<u>Acceleration</u> of coagulation pathways-→ Fibrin and platelet clot →hemostasis

The transfer of blood to the extravascular space is stopped (or much slowed)

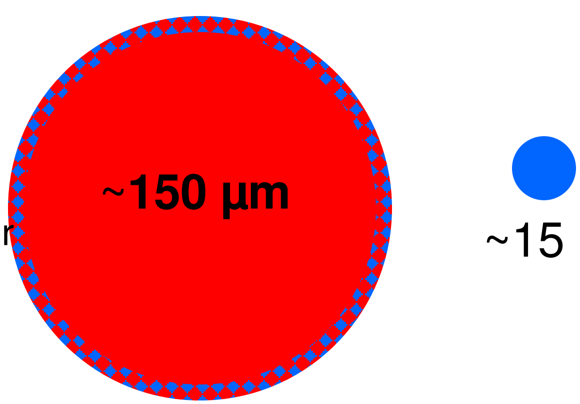
The clot is initial <u>scaffold</u> for tissue regeneration and repair and a source of signals for cell migration and differentiation

Surface-Mediated Diffusion-Limited Reactions.
Pressure Spectra and Source Intensity as a Function of Geometry





10^4 microcarriers
~200 cells/microcarrier



~15 µm

CELLS = ~ 2 X 10^6 /ml

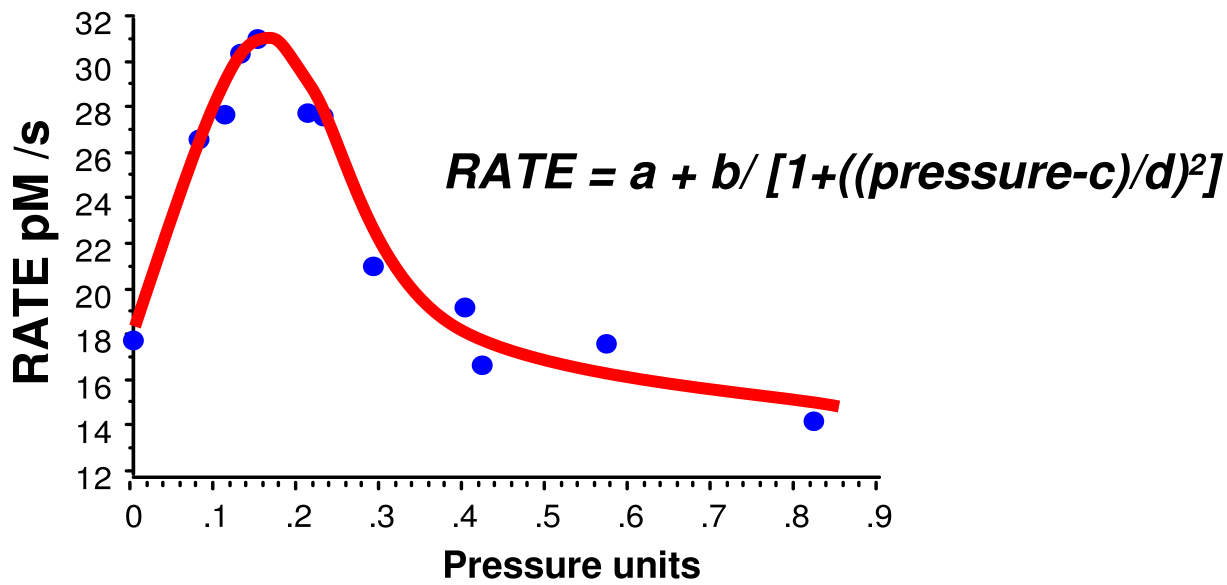
BLOOD COAGULATION PROTEOLYTIC PATHWAYS

ACCELERATE *(procoagulants)*	DECELERATE *(anticoagulants)*
Tissue factor (TF)*	Tissue factor pathway inhibitor*
Intrinsic loop**	Antithrombin**
Prothrombinase	Protein C pathway**
Thrombin activatable fibrinolysis inhibitor	Fibrinolytic pathway

* *Regulated by water activity*

* *Regulated by glycosaminoglycans*

FACTOR Xa GENERATION IN DILUTED PLASMA UNDER OSMOTIC STRESS



The rate of coagulation factor X activation is a function of the pressure (colloid osmotic)

TF + fVIIa ↔ TF/fVIIa + fX → fXa

GLYCOSAMINOGLYCANS

GALACTOSAMINOGLYCANS

Chondroitin sulfates
Dermatan sulfates

GLUCOSAMINOGLYCANS

Heparan sulfates
Keratan sulfates
Hyaluronan

Linear polysaccharides ; disaccharide units; variable sequences; variable distribution; variable density at nano-micro scales

The Skin interstitium is a transfer-media composed of gelled heterogeneous layers with fluctuating interfaces

Material properties of gels and of this gel at the appropriate scales for cellular and macromolecular rate process.

EVIDENCE FOR PRESURE AND CONCENTRATION GRADIENTS

- *Water activity gradients → magnetic resonance microscopy*
- *Responses to water activity changes →Osmotic Stress techniques*
- *Water desorption isotherms*
- *"Swelling pressure" ; ΔV/ΔP*

- "<u>Spontaneous" fluctuation in capillary blood flow with a frequency of 6-10 cycles/min ; 0.2 - 0.6 mm/s.</u>

- Irregular distribution of reactive sinks
- "Variable" lymphatic pressures (10 mmHg to -7mmHg)

<u>"Physiology is Physics"</u>

"We model to <u>organize</u> and to <u>understand</u> biological information"

- **Large Integrative Model (emergence of biological properties from complexity)**

- **Focused, Simplest Possible Model (abstracts key properties for analysis and hypothesis testing)**

COLLABORATIVE WORKING GROUPS MUST BE ESTABLISHED:

To incorporate broadening ranges of knowledge and technical expertise insuring that models are consistent with: Experimental Biological Observations, Mathematical Principles, Thermodynamic and Mechanical Laws, Computational Capabilities.