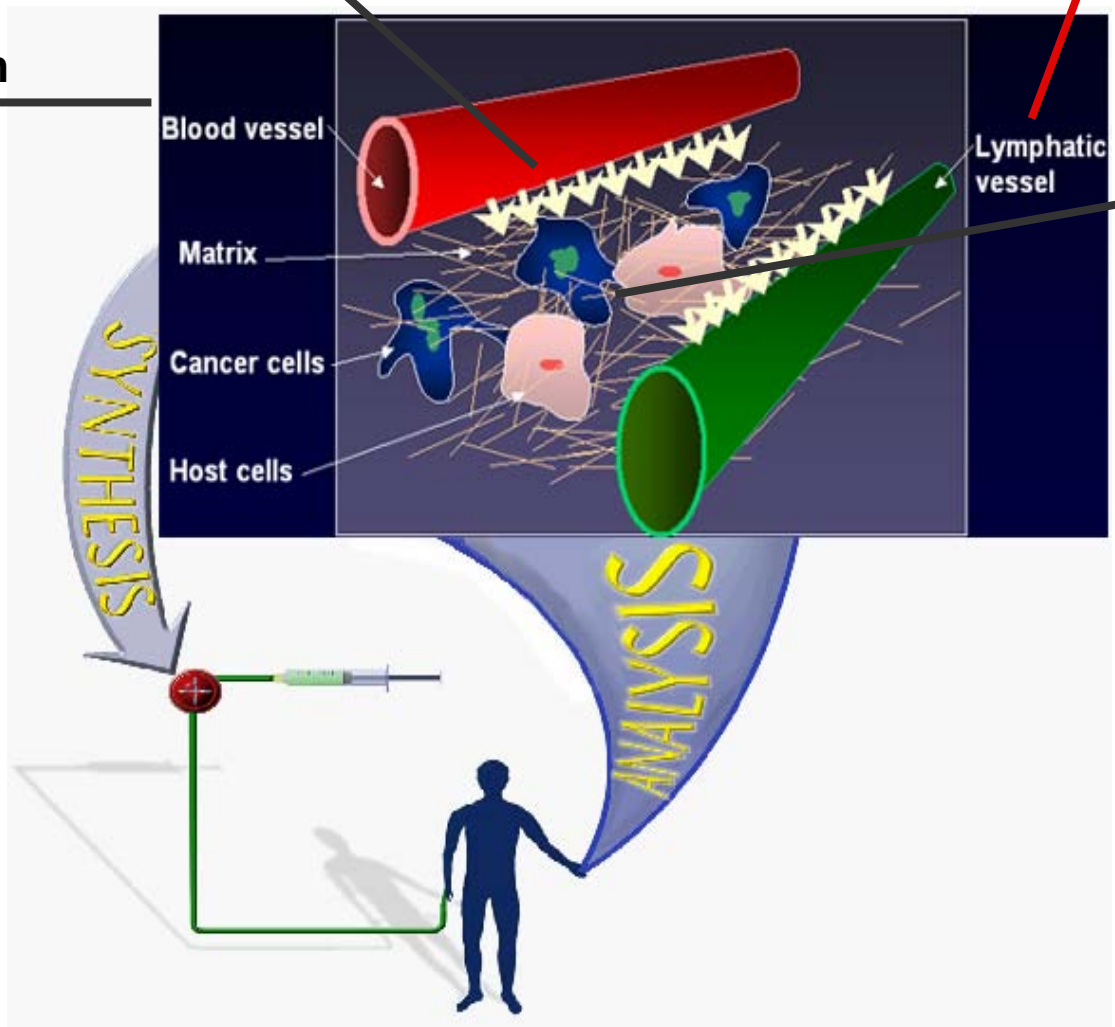
Delivery of Molecular and Cellular Medicine to Tumors

Vascular Transport (Lecture I)

Interstitial and Lymphatic Transport (Lecture II)

Angiogenesis & Microcirculation (Lecture I)

Vascular Normalization (Lecture II)



DELIVERY OF MOLECULAR MEDICINE TO TUMORS

Lecture II: Interstitial and Lymphatic Transport

OVERVIEW

R.K. Jain, “Transport of Macromolecules in the Tumor Interstitium: A Review,” Cancer Research, 3038- 47:3050 (1987).

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Interstitial Transport

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C. de L. Davies, D.A. Berk, A. Pluen, and R.K. Jain, “Comparison of IgG Diffusion and Extracellular Matrix Composition in Rhabdomyosarcomas Grown in Mice Versus In Vitro as Spheroids Reveals the Role of Host Stromal Cells,” BJC, 86:1639-1644 (2002)

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Lymphatic Transport

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A.J. Leu, D.A. Berk, A. Lymboussaki, K. Alitalo and R.K. Jain, “Absence of Functional Lymphatics within a Murine Sarcoma: a Molecular and Functional Evaluation,” Cancer Research, 60: 4324-4327 (2000).

M.A. Swartz, A. Kaipainen, P. Netti, C. Brekken, Y. Boucher, A.J.Grodzinsky and R.K.Jain, “Mechanics of Interstitial-Lymphatic Fluid Transport: Theoretical Foundation and Experimental Validation,” Journal of Biomechanics, 32: 1297-1303 (1999).

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C. Mouta Carreira, S.M. Nasse, E. diTomaso, T.P. Padera, Y. Boucher, S.I. Tomarev, R.K. Jain, “LYVE-1 is not Restricted to the Lymph Vessels: Expression in Normal Liver Blood Sinusoids and Down-regulation in Human Liver Cancer and Cirrhosis,” Cancer Research, 61:8079-8084 (2001).

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T.P. Padera, E. di Tomaso, A. Kadambi, C. Mouta Carreira, E.B. Brown, Y. Boucher, N.C. Choi, D. Mathisen, J. Wain, E.J. Mark, L.L. Munn, R.K. Jain, “Lymphatic Metastasis in the Absence of Functional Intratumor Lymphatics,” Science, 296:1883-1886 (2002)

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Interstitial Transport

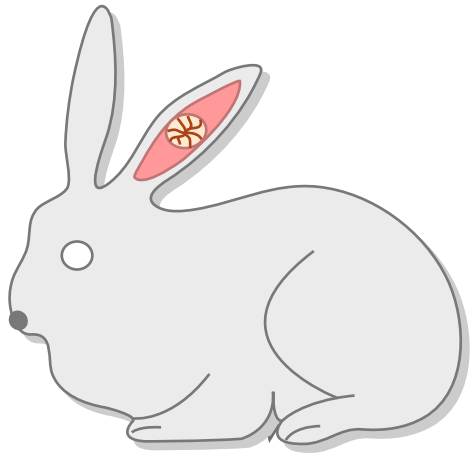
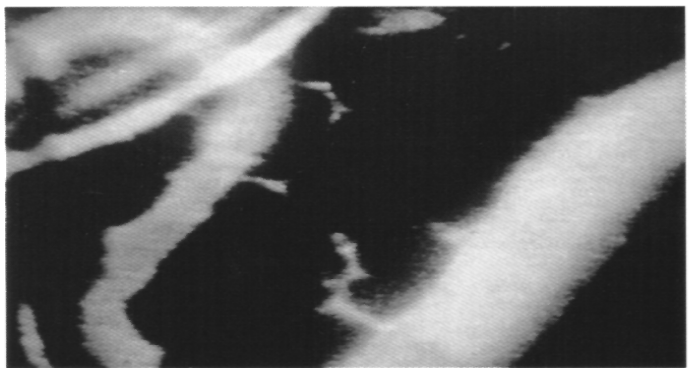
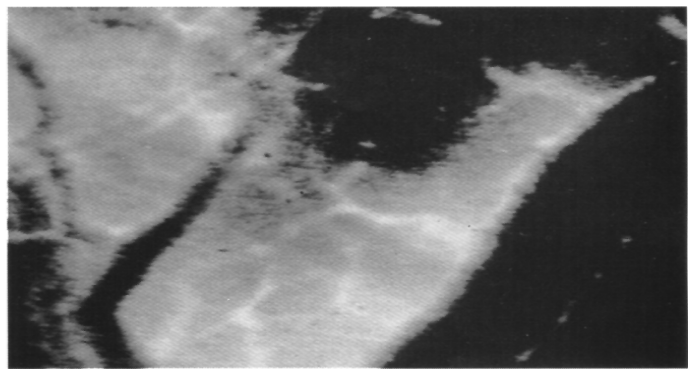
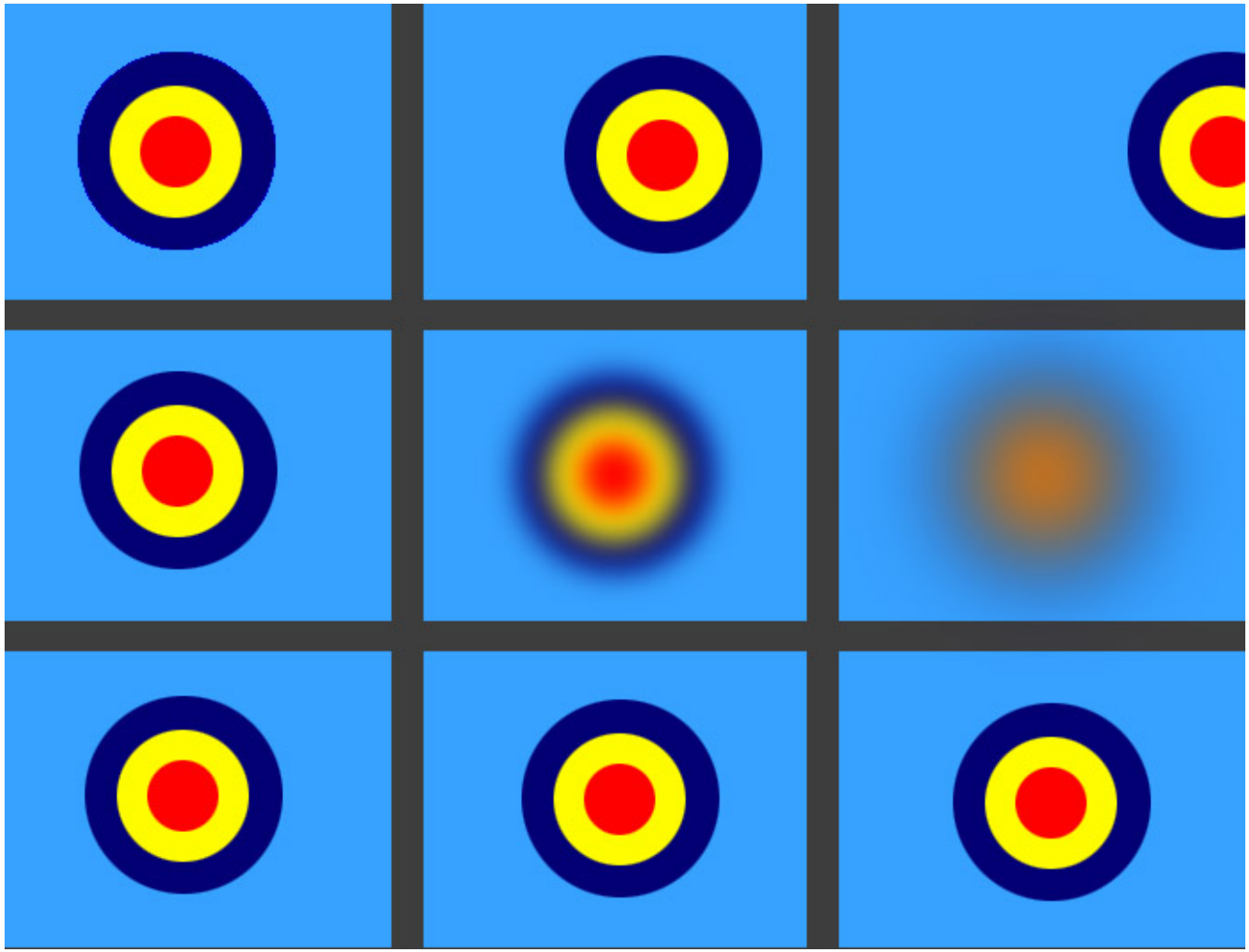


Figure by MIT OCW.

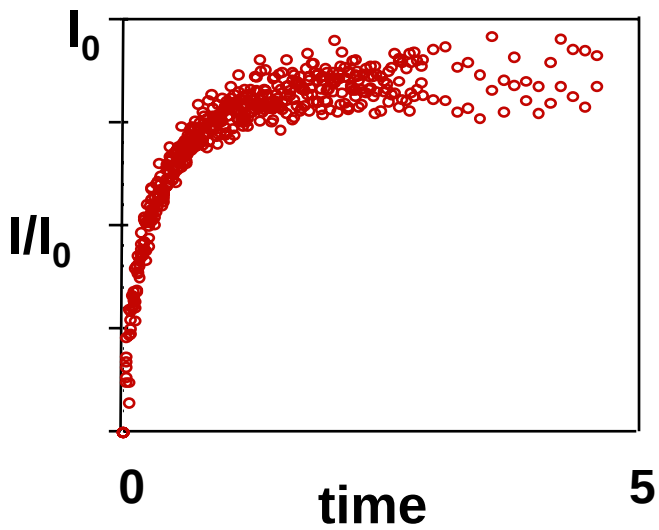




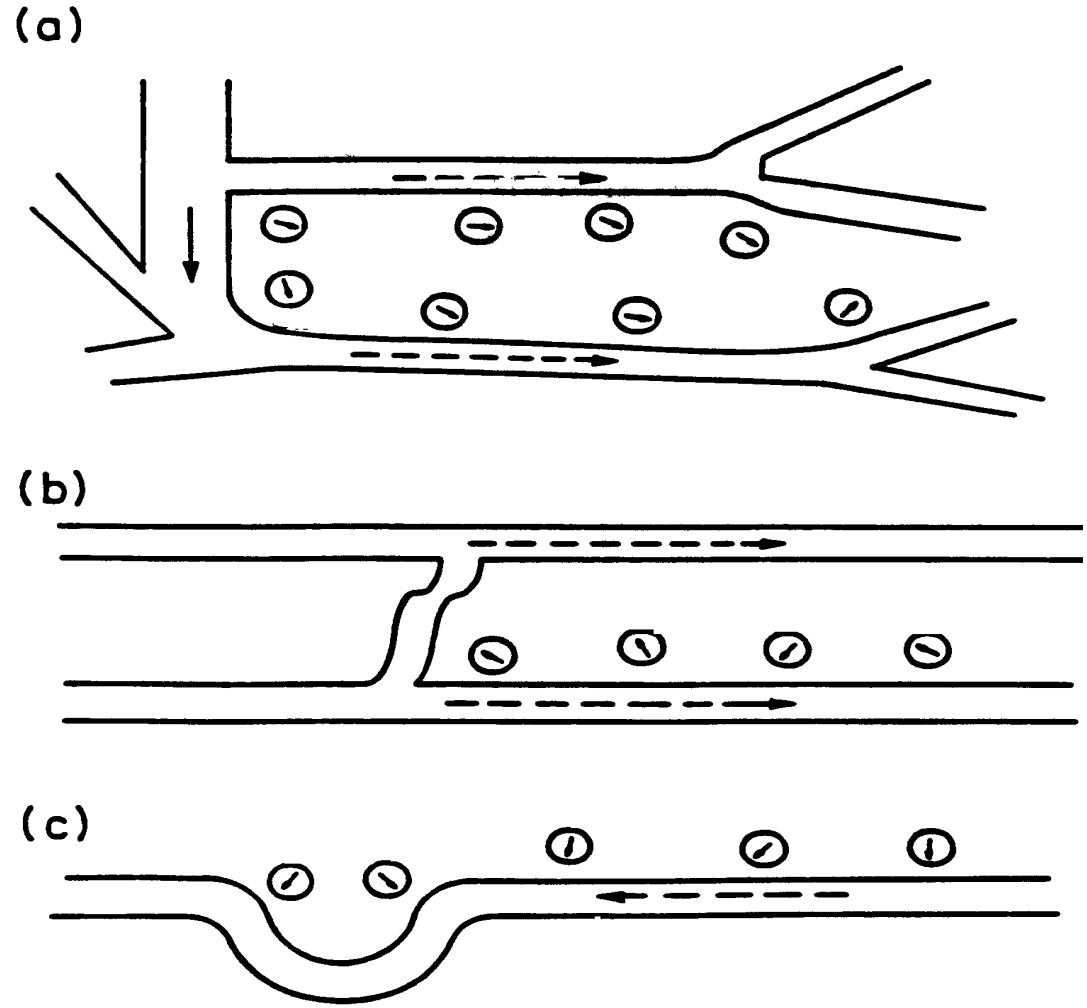
Measuring Convection, Diffusion and Binding



Fluorescence Recovery After Photobleaching

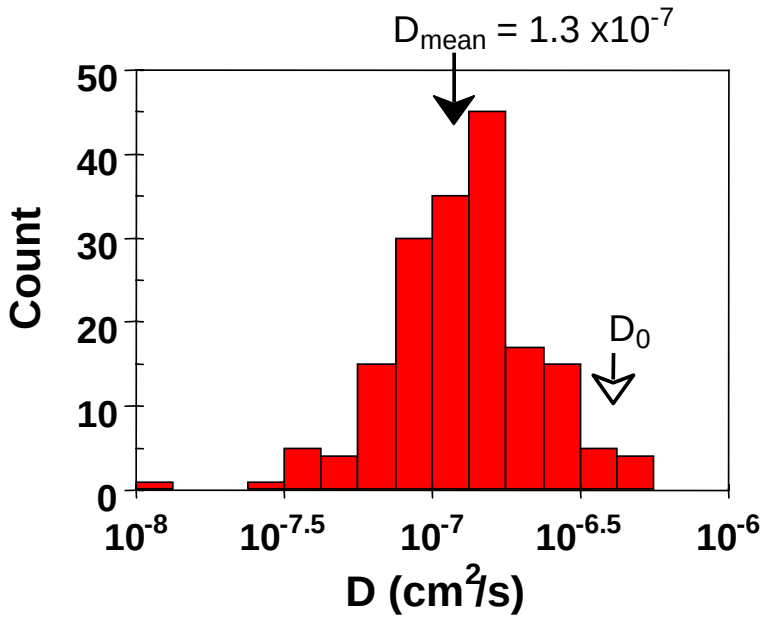


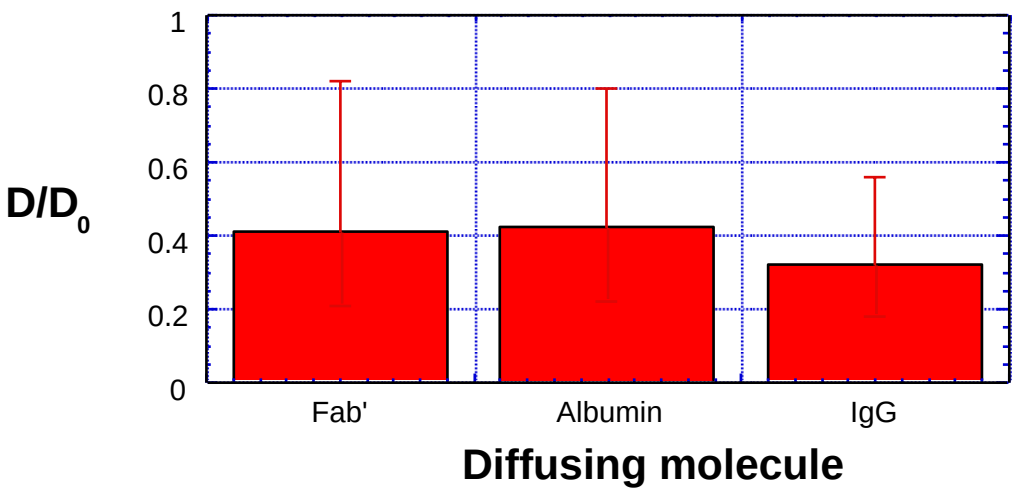
Typical Values of Interstitial Convective Velocities & Directions



Reference: *Chary & Jain, PNAS (1989)*

Interstitial Diffusion of IgG in LS174T Xenograft





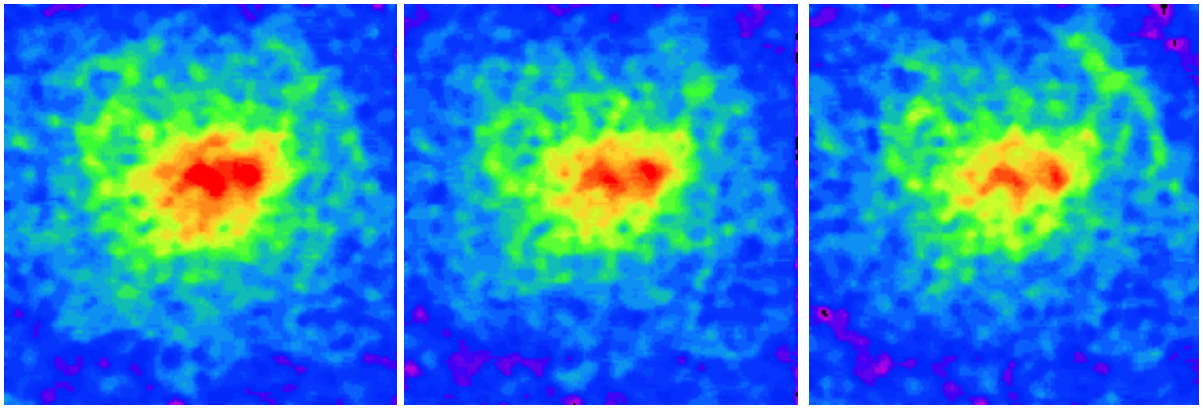
Reference: ***Berk et al. PNAS (1997)***

Time Constant for Diffusion

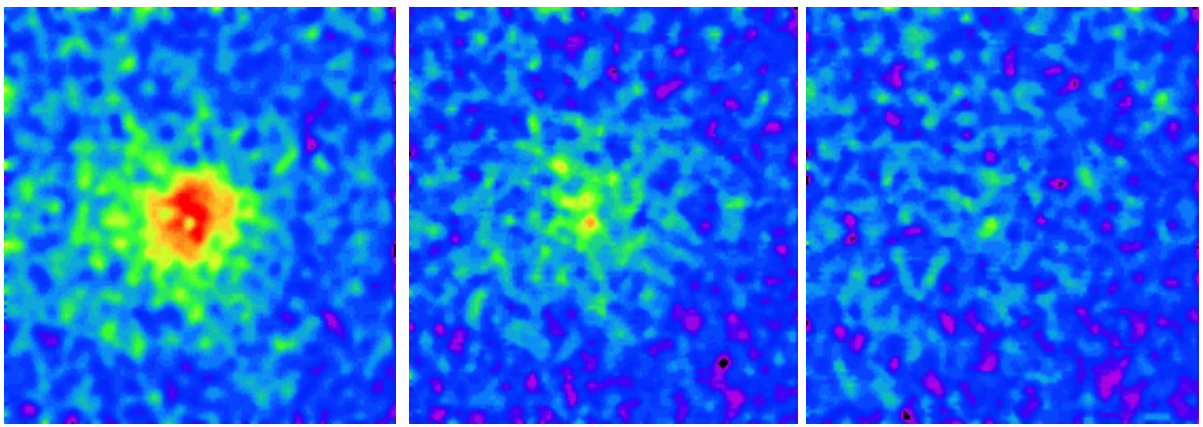
Distance	IgG	Fab
100 μm	~ 0.5 hours	~ 0.2 hours
1 mm	~ 2–3 days	~ 0.5–1 days
1 cm	~ 7 months	~ 2 months

Plasma Half-life	~ days	~ hours

Specific Antibody



1 sec → 10 sec → 100 sec

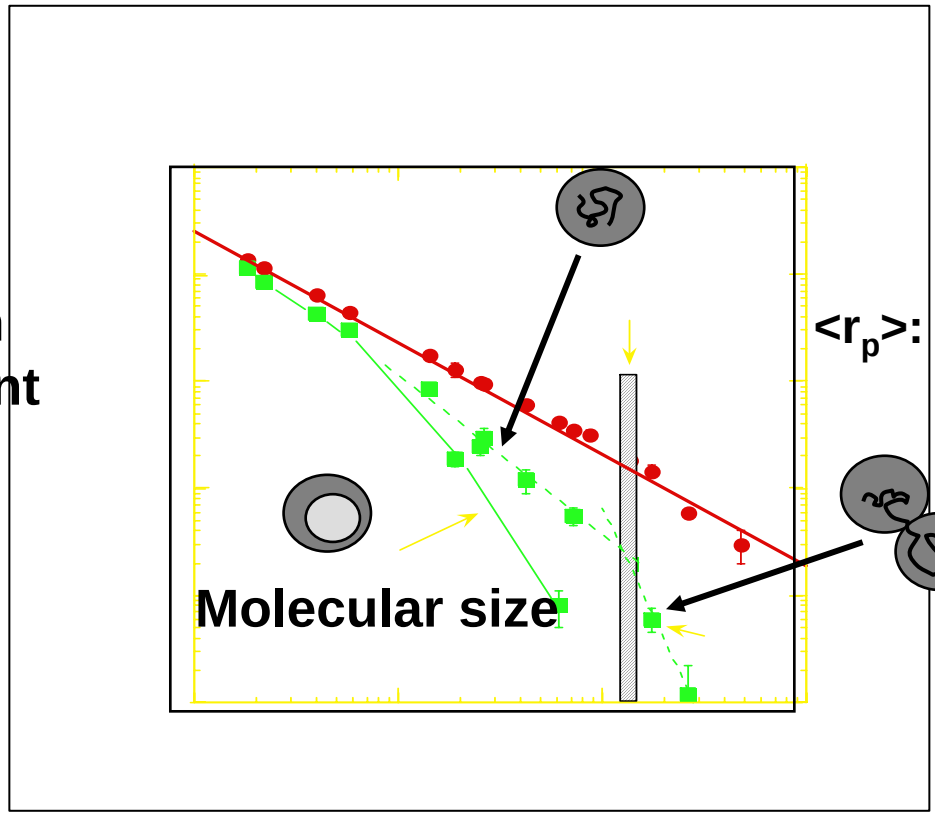


Non-Specific Antibody

Reference: *Berk et al. PNAS (1997)*

Effect of Molecular Configuration on Diffusion

Diffusion coefficient



$\langle r_p \rangle$: experimental pore radius

Reference: *Pluen et al. Biophysical Journal (1999)*

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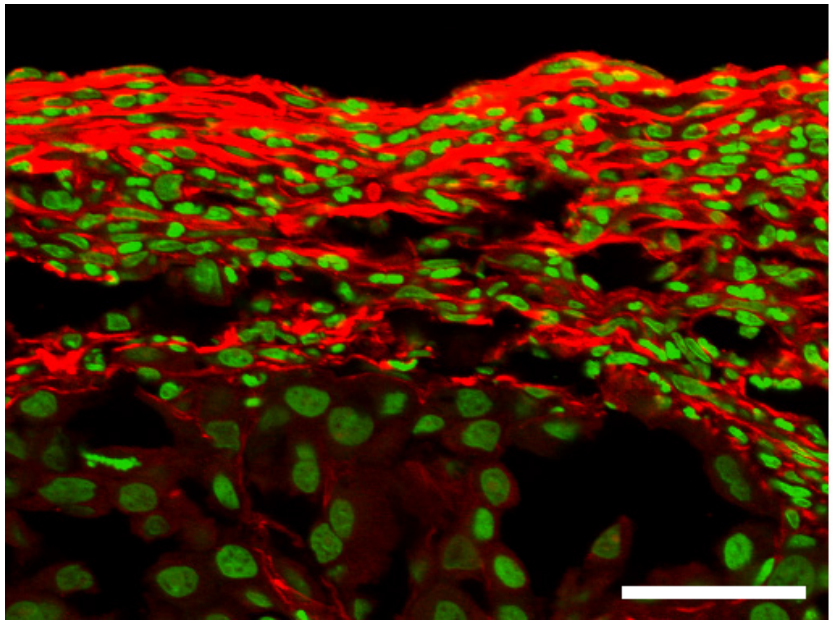
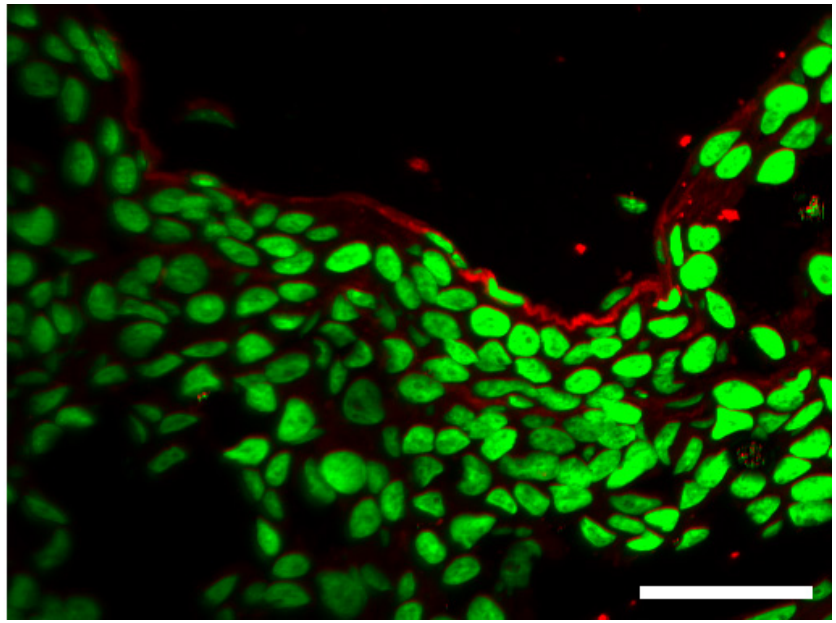
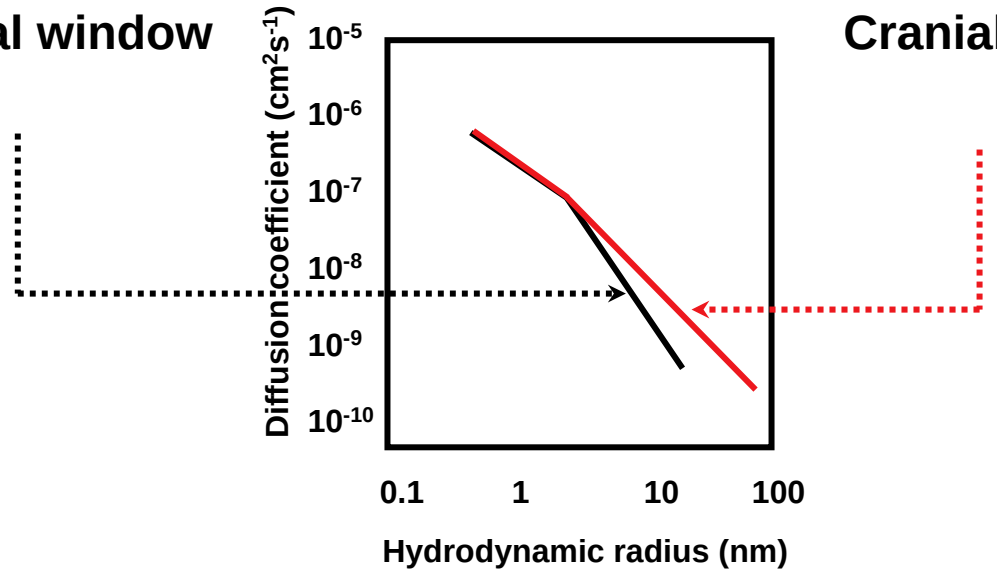
Interstitial diffusion varies with site of growth



Dorsal window



Cranial window



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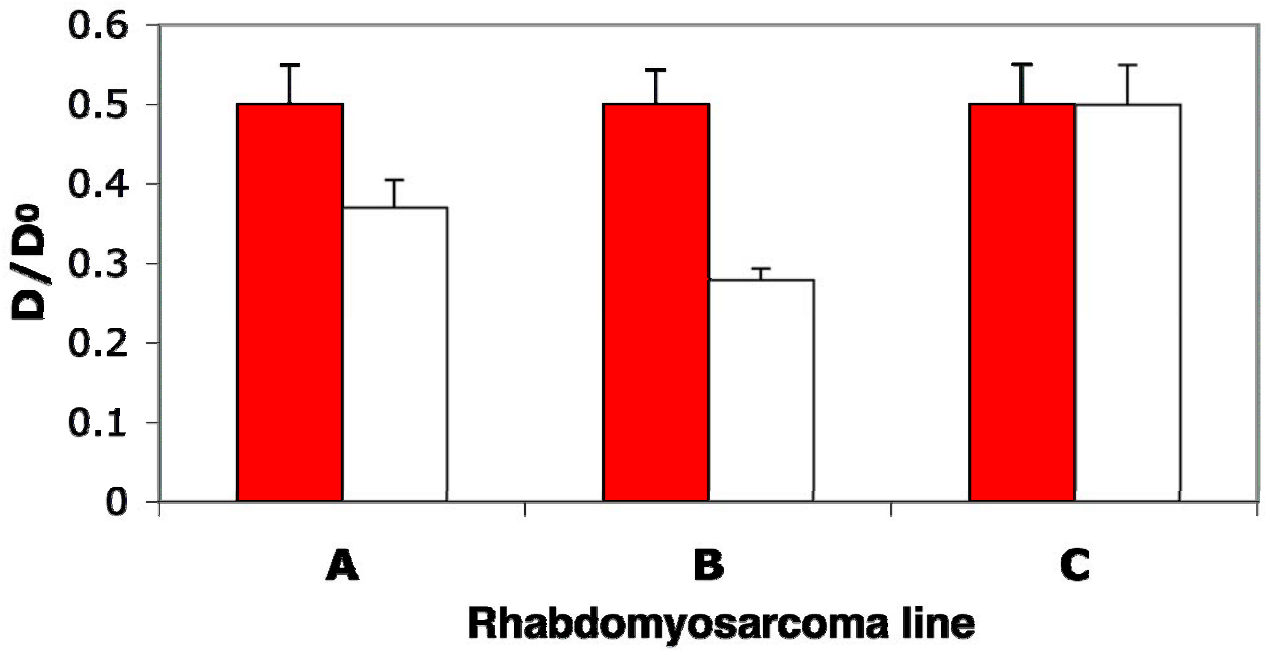
Tumor-Host Interaction Influences Drug Transport

Image removed for copyright reasons.

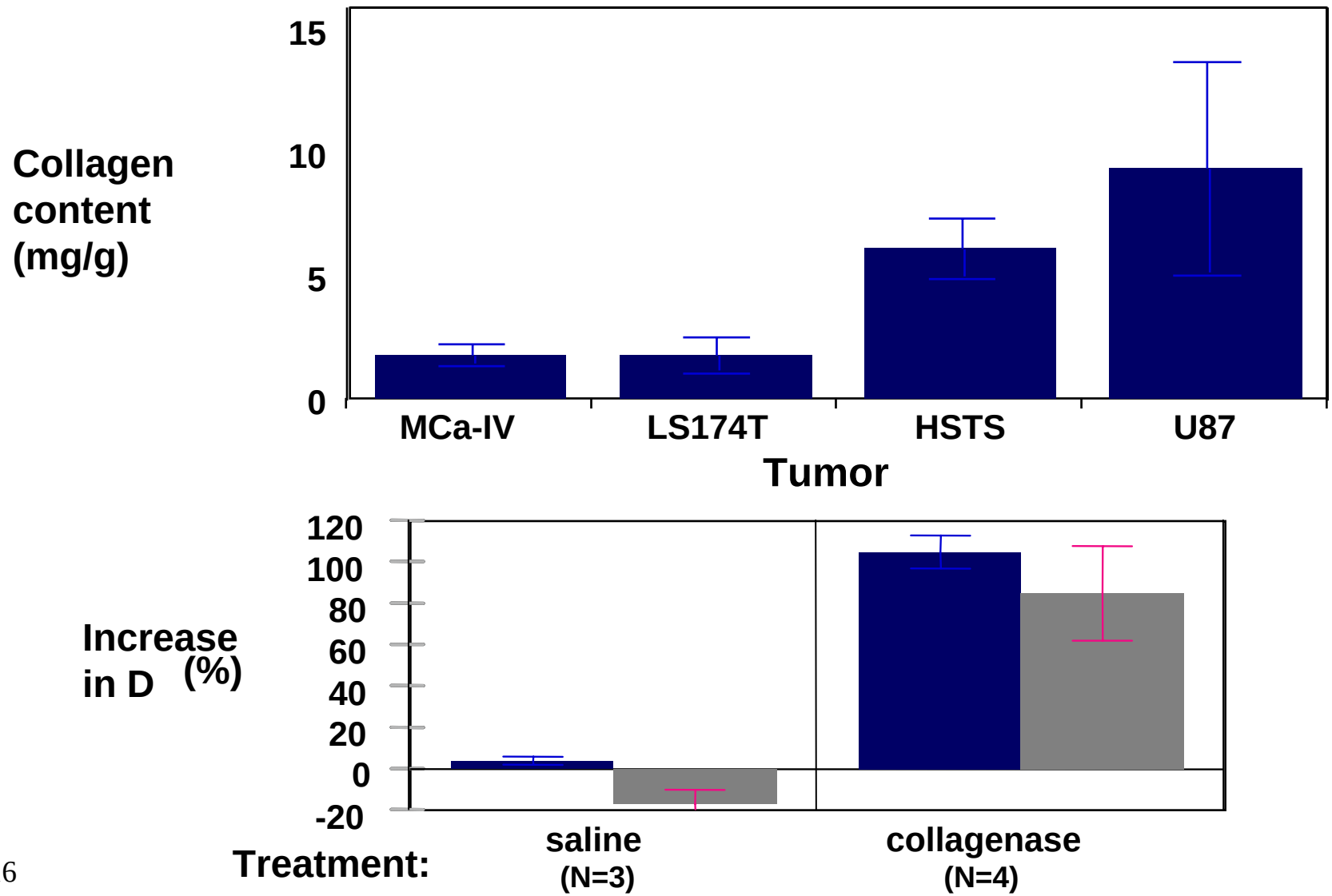
Multicell spheroid human squamous cell carcinoma.

See: *Science* 240, no. 4849 (April 8, 1988): cover page.

Figure by MIT OCW. After Jain.

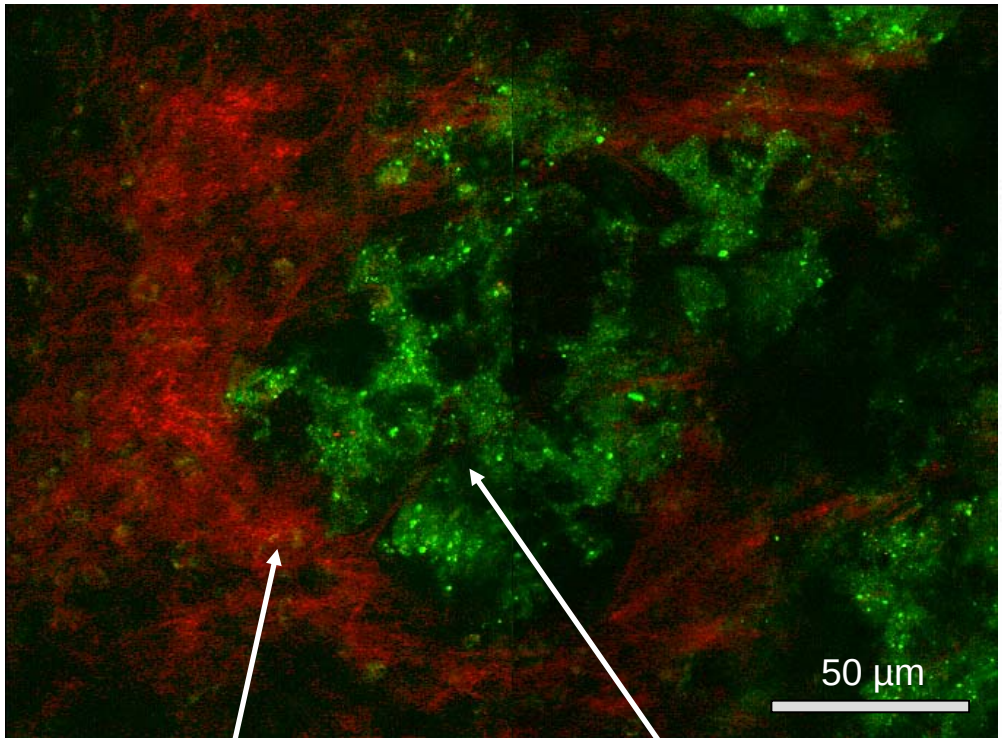


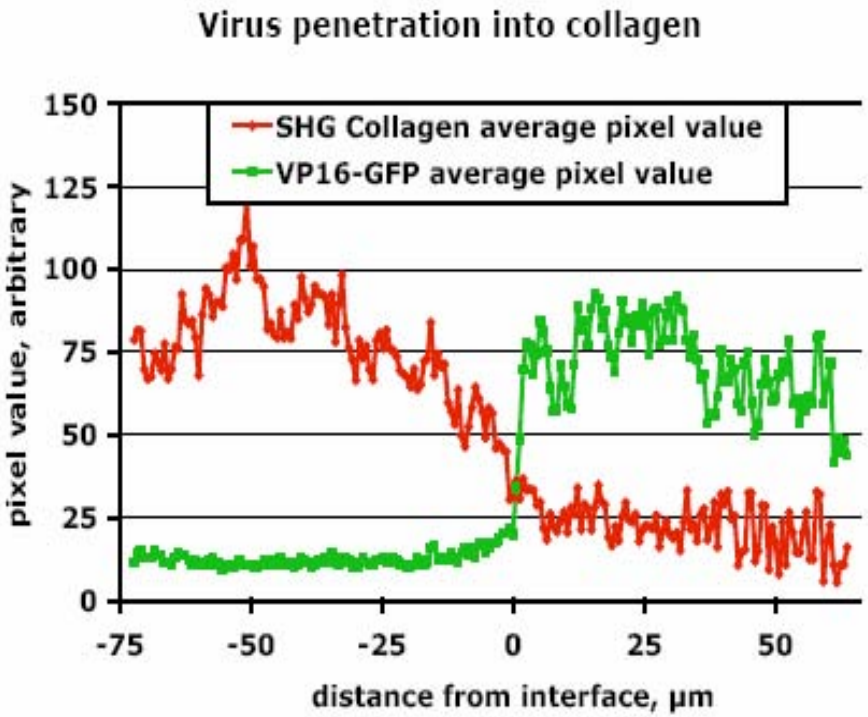
Role of Collagen in Interstitial Transport



Reference: *Netti et al. Cancer Research (2000)*

Collagen Restricts HSV Distribution

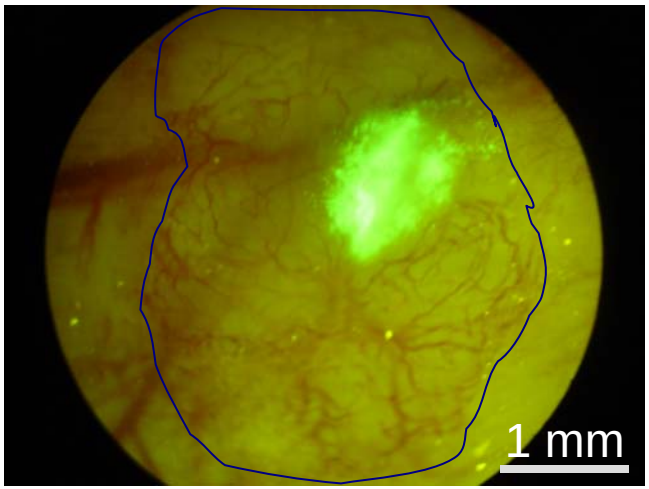




Fibrillar collagen HSV particles (150 nm)

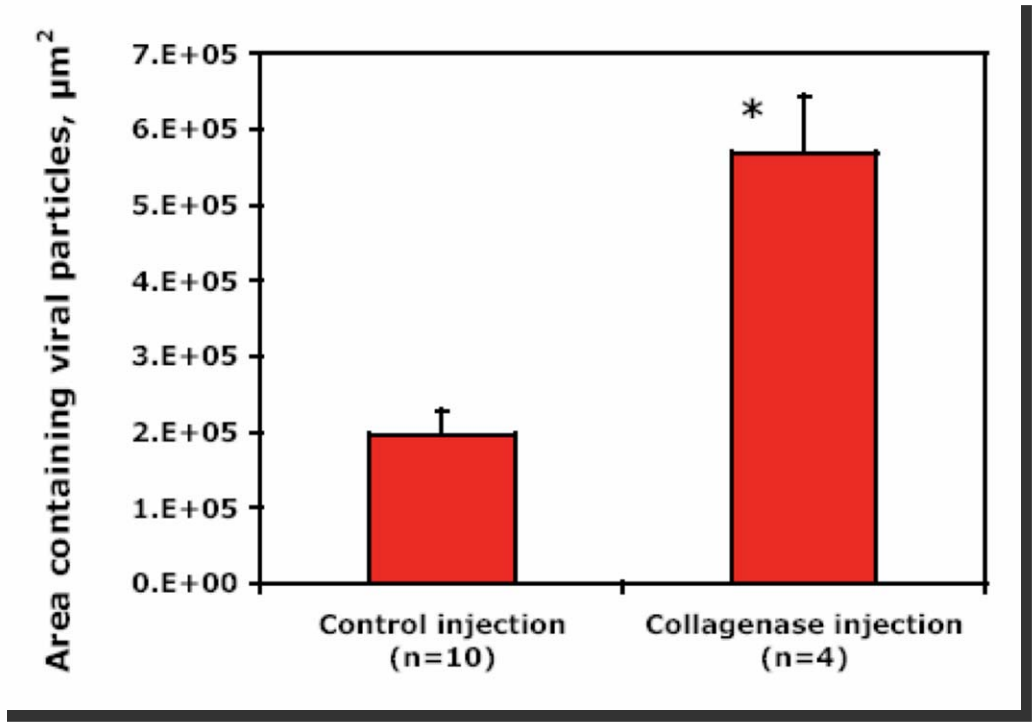
Reference: McKee, Grandi, Mok et al., submitted (2005)

Collagenase Co-Injection Improves Virus Distribution



Virus Alone

Collagenase Co-injection



Reference: McKee, Grandi, Mok et al., submitted (2005)

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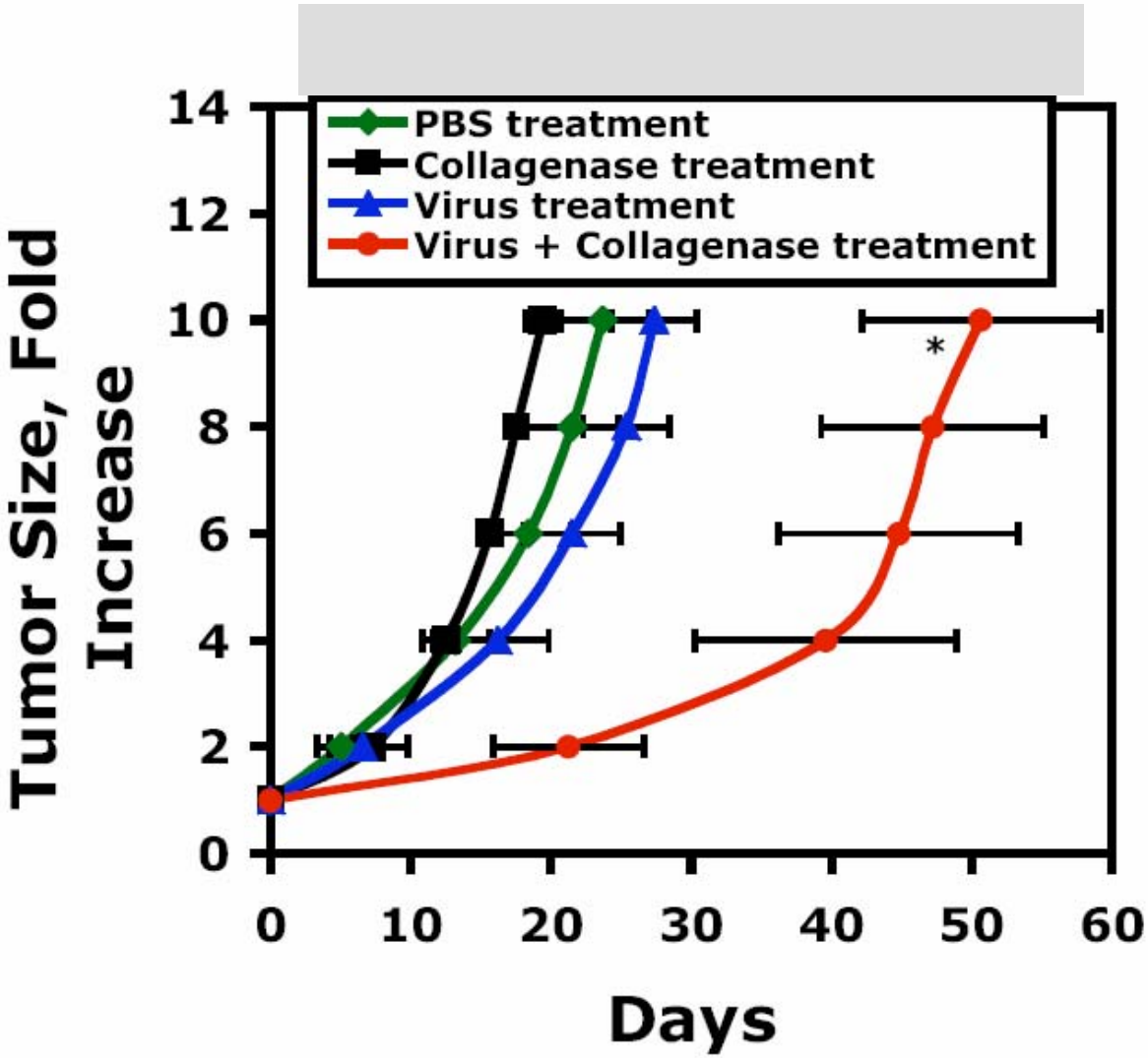
Collagenase Improves Efficacy of MGH2 Oncolytic Viral Therapy



Courtesy of the American Association for Cancer Research. Used with permission. From McKee, T. D., P. Grandi, W. Mok, G. Alexandrakis, N. Insin, J. P. Zimmer, M. G. Bawendi, Y. Boucher, X. O. Breakefield, and R. K. Jain. "Degradation of fibrillar collagen in a human melanoma xenograft improves the efficacy of an oncolytic herpes simplex virus vector." *Cancer Research* 66 (2006): 2509-2513.

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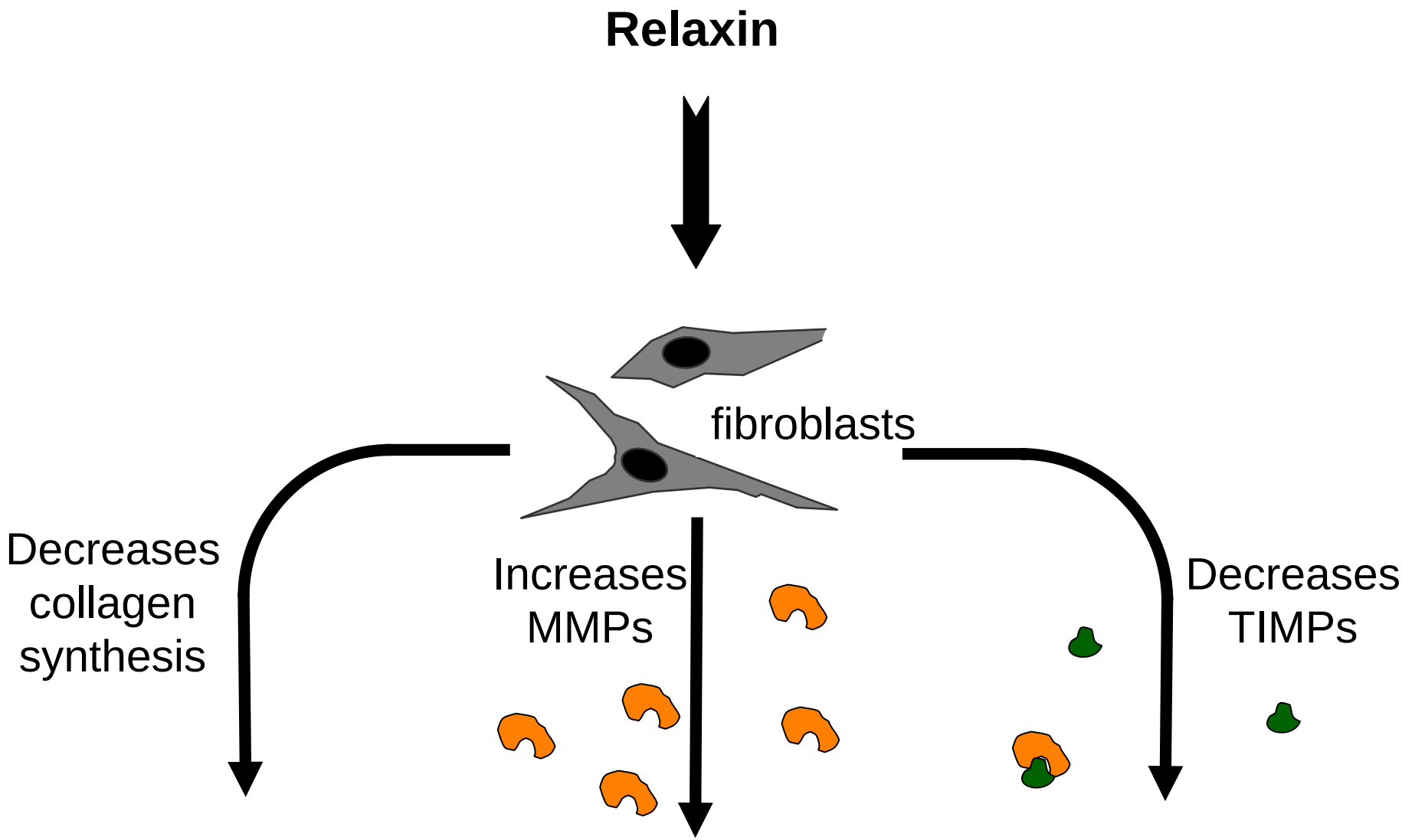
Relaxin
fibroblasts
Decreases collagen synthesis
Increases MMPs
Decreases TIMPs
Decreased collagen production
Increased collagen degradation

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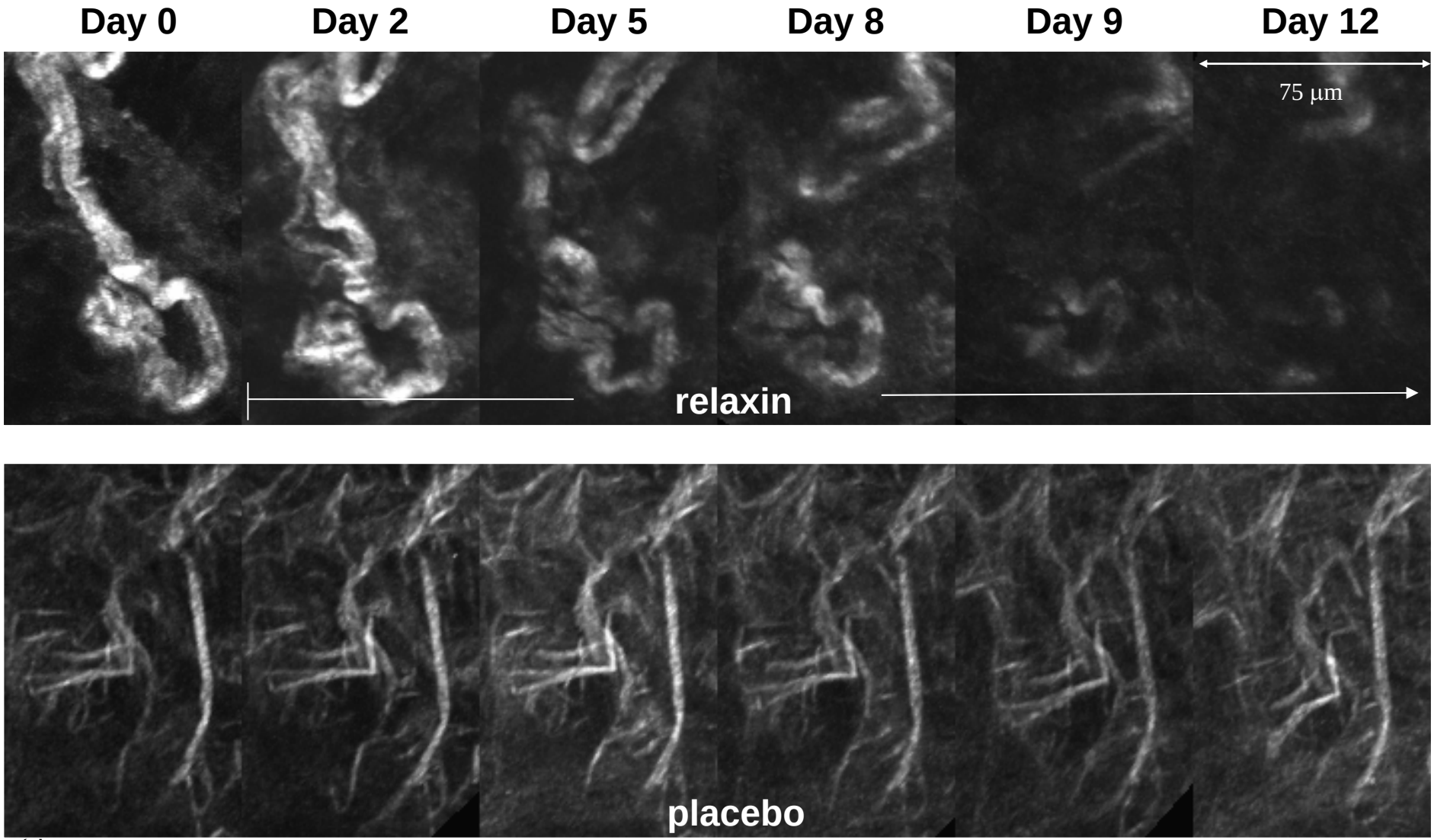
Relaxin treatment modifies collagen structure



Reference: Brown, McKee et al., Nature Medicine (2003)

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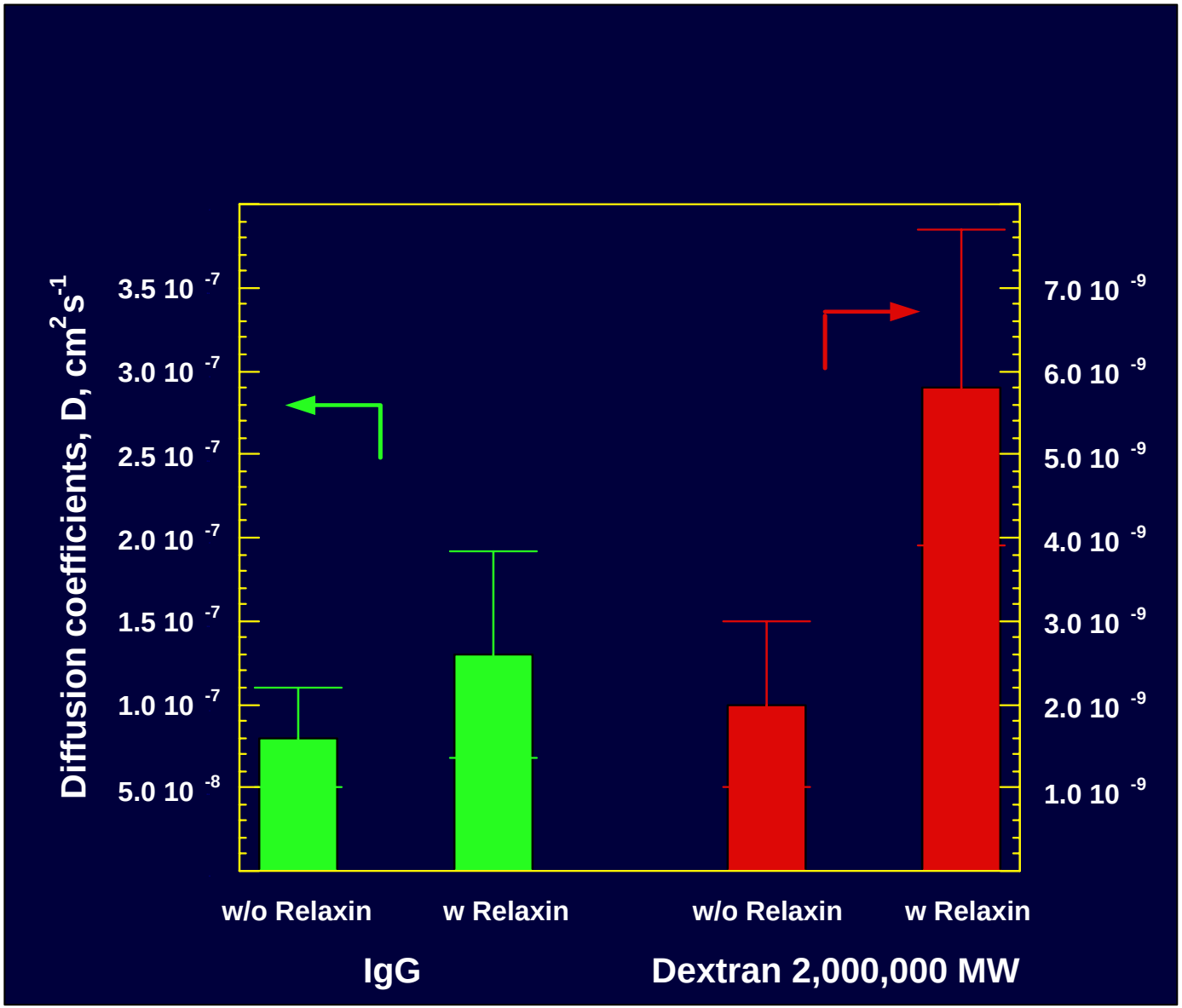
Relaxin treatment enhances macromolecular transport



Reference: *Brown, McKee et al. Nature Medicine (2003)*

Summary

- **FRAP can provide direct measurements of diffusion, convection and binding *in vivo***
- **Convection ~ 0.1 - μm/sec**
- **Diffusion ~ 1/3 of water**
- **Non-specific binding ~ 1/4 - 1/3**
- **Interfering with collagen synthesis and/or assembly may enhance interstitial transport.**
- **Interstitial diffusion depends on the host-tumor interaction.**
- **Fluorescence correlation microscopy can reveal the two-phase nature of transport in tumors.**

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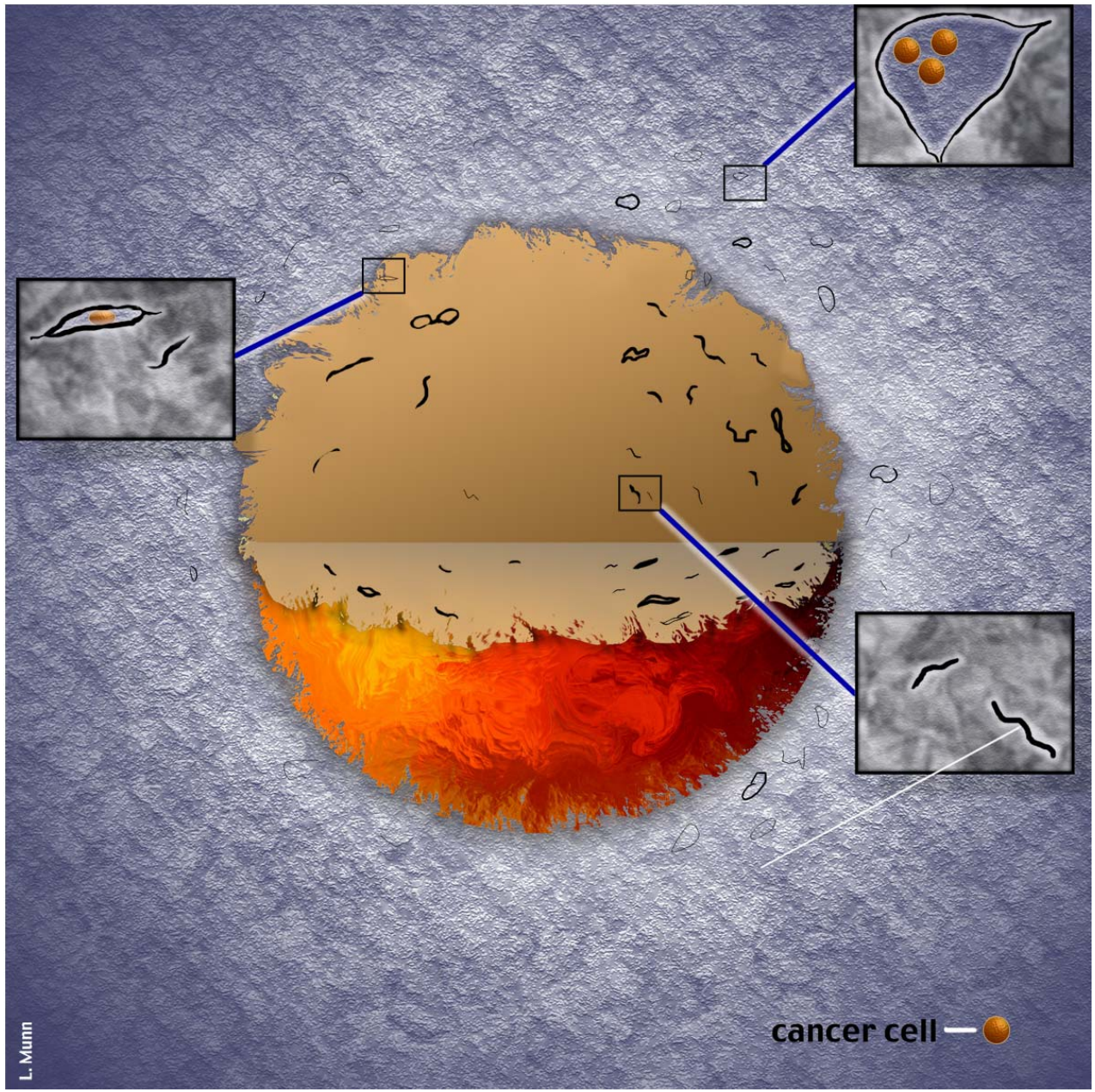
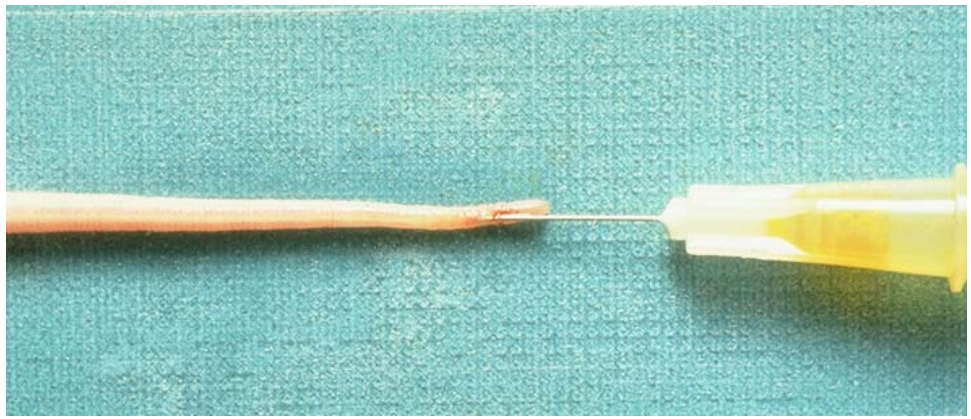


Courtesy of Lance Munn. Used with permission.

Intravital Lymphangiography



Reference: *Leu et al. Am J Physiol. (1994)*
Hagendoorn et al. (2004)

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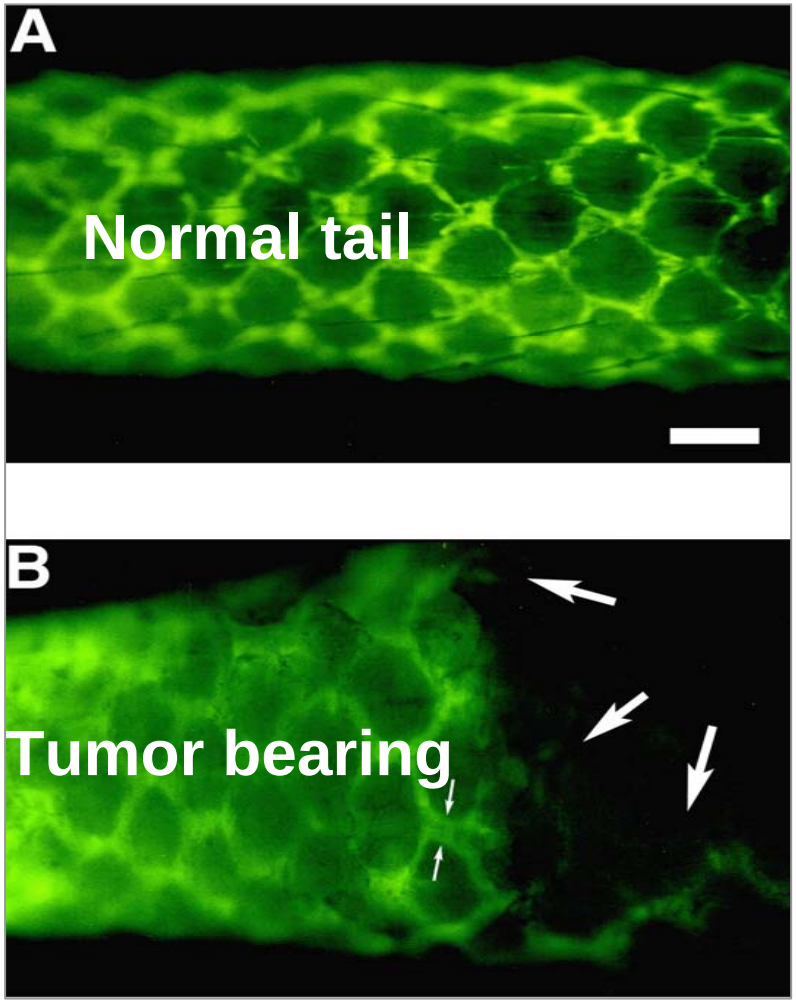
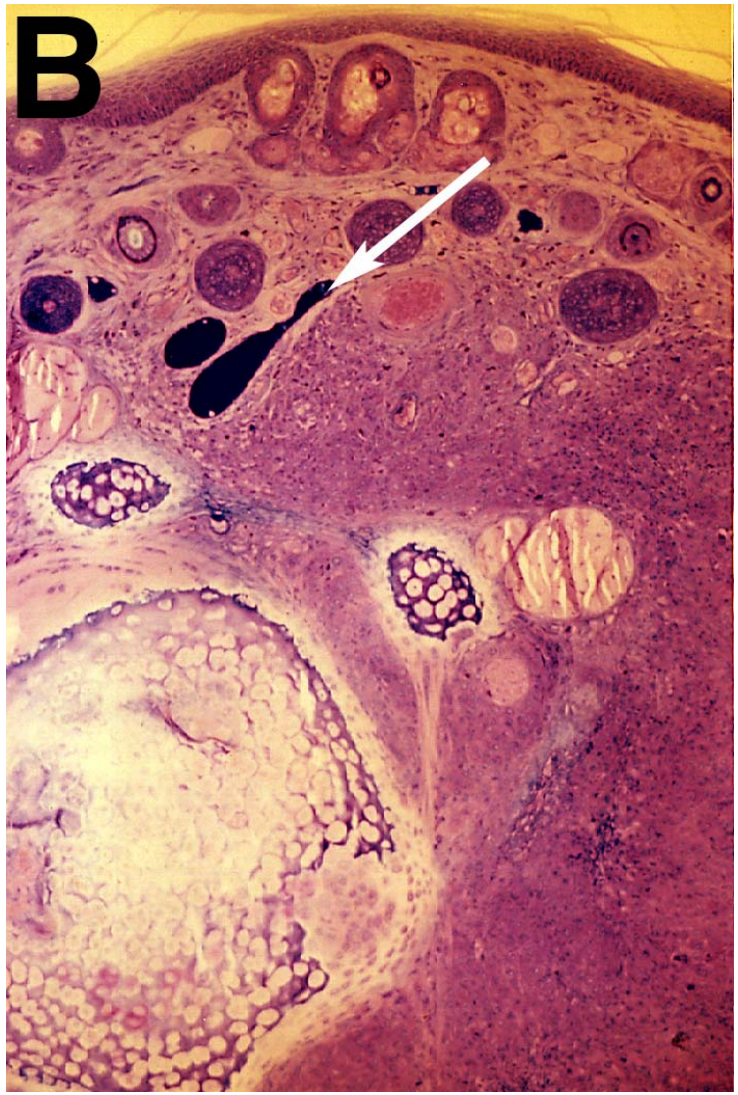
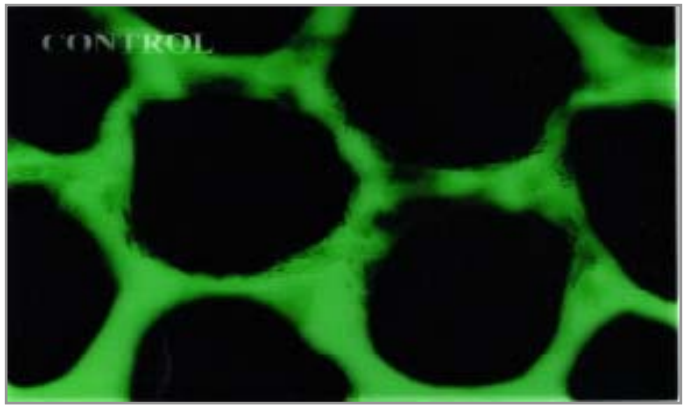
Lack of Functional Lymphatics in Tumors



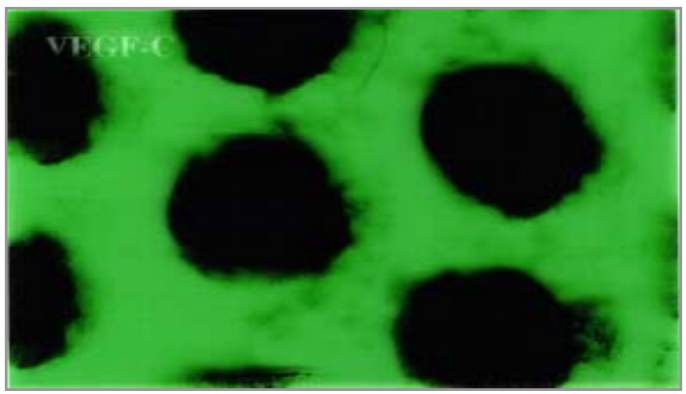


Courtesy of the American Association for Cancer Research. Used with permission. From Leu, A. J., D. A. Berk, A. Lymboussaki, K. Alitalo, and R. K. Jain. "Absence of functional lymphatics within a murine sarcoma: a molecular and functional evaluation." *Cancer Research* 60 (2000): 4324-4327.

VEGF-C Induces Lymphatic Hyperplasia



Control – Lymph Vessels



VEGF-C Induced Hyperplasia

Reference: Jeltsch et al. Science, (1997)

Effect of VEGF-C on Lymphatic Metastases

Property	Cell Line		P-value*
	B16F10-MT	B16F10-VEGF-C	
Lymphatic Metastasis	6/21	15/19	0.002
Local Invasion	3/21	12/18	0.001
Lung Metastasis†	5/22	2/19	N.S.
	T-241-MT	T-241-VEGF-C	
Lymphatic Metastasis	2/14	8/14	0.046
Local Invasion	4/14	5/14	N.S.
Lung Metastasis†	5/14	6/14	N.S.

Numerator is the number of mice that exhibited the property, denominator is the total number of mice.

**: P-value based on Fisher's Exact Test. N.S.: not significant*

†: Hematogenous metastasis was analyzed microscopically in 10-20 sections of lung per animal.

Reference: Padera et al., Science, (2002)

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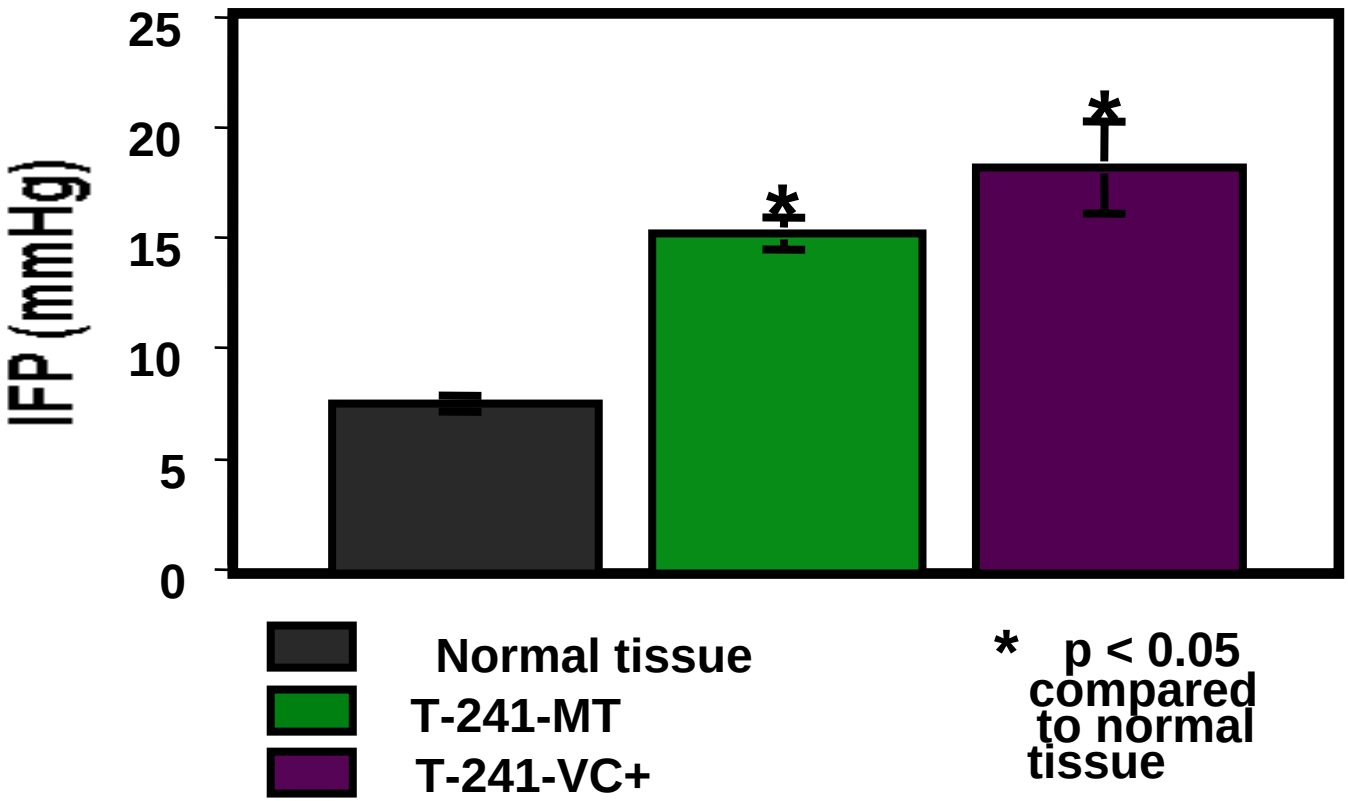
Lymphatic Function Studies

- Interstitial fluid pressure measurements
- Epifluorescence lymphangiography
- Multi-photon laser scanning microscopy
- Ferritin lymphangiography

Immunohistochemistry

- Lectin, CD31, LYVE-1, Prox 1

IFP is not changed by VEGF-C overexpression



Reference: Padera et al., Science, (2002)

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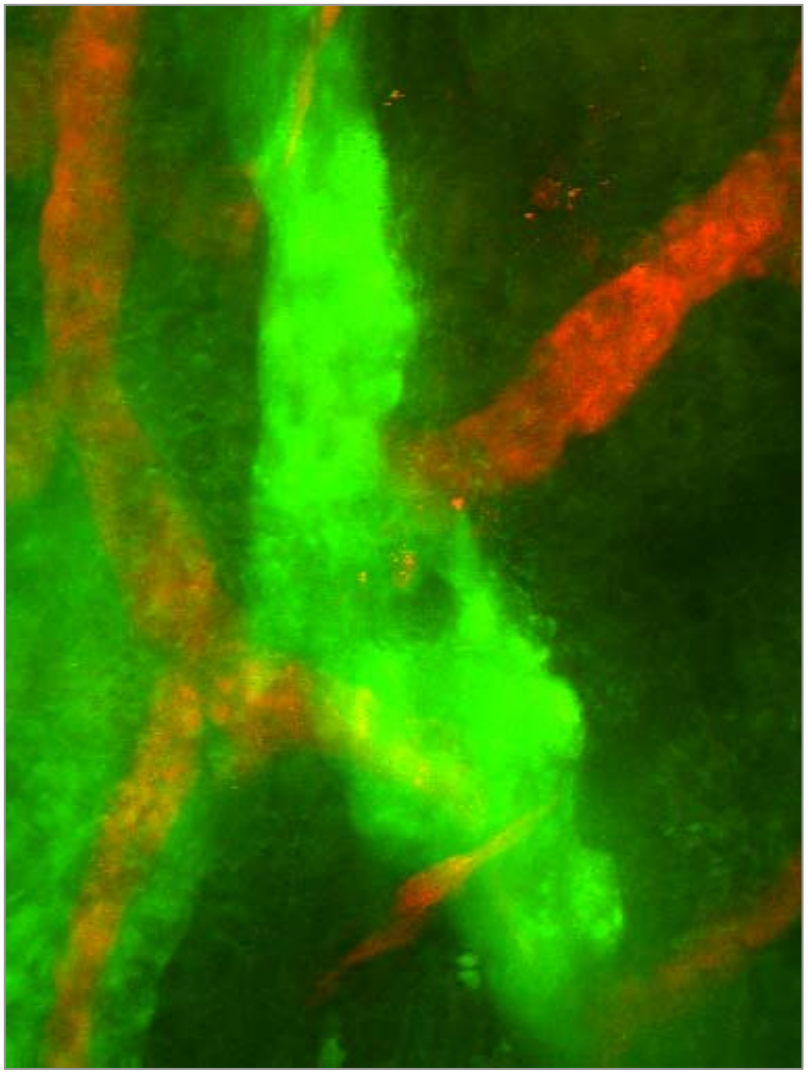
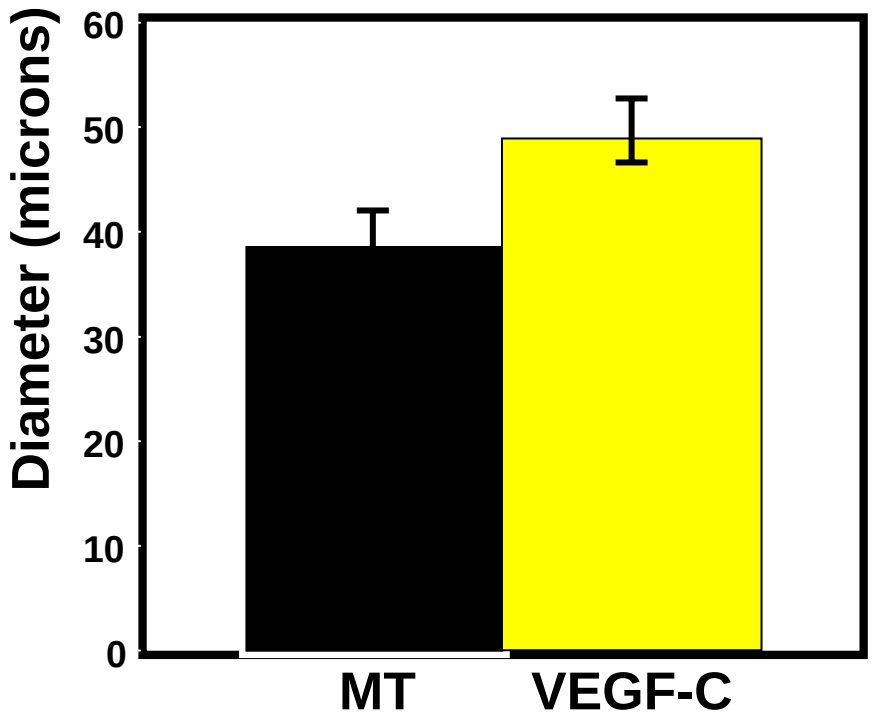
VEGF-C induced hyperplasia of peri-tumor lymphatics





Green: Lymphatic vessels
Red: Blood vessels

Reference: Padera et al., Science, (2002)

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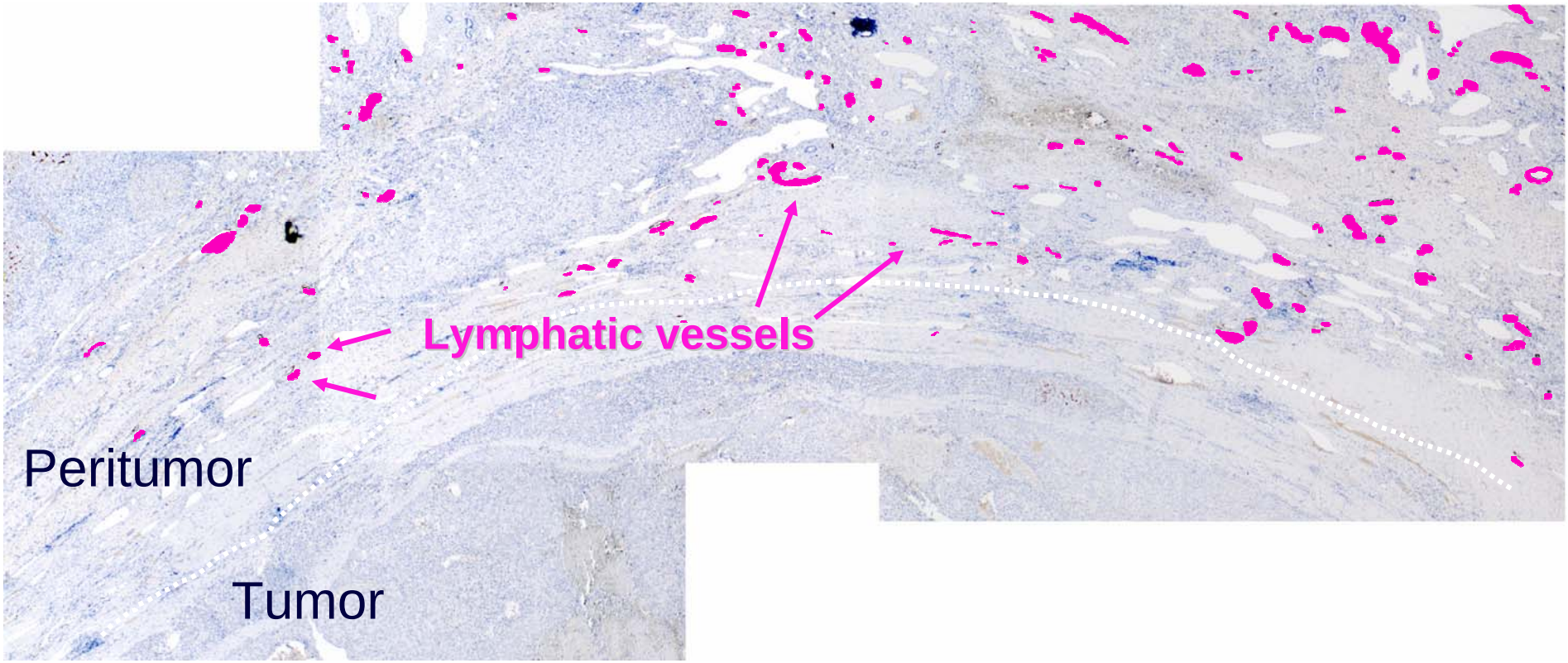
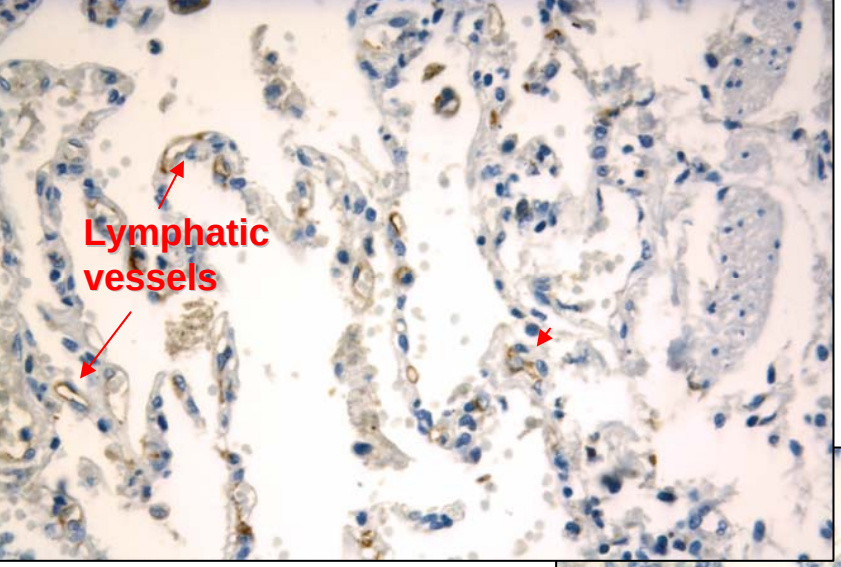
HCCs in Patients Lack Lymphatics



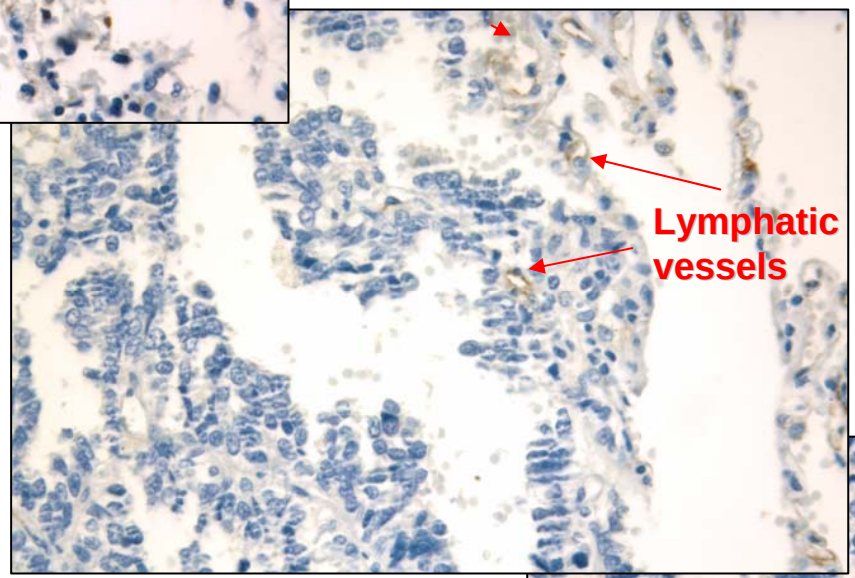
- **42 biopsies of liver tumor (HCC + metastases) examined**
- **No staining for Prox 1 + LYVE-1 was observed in the tumor area**

Courtesy of the American Association for Cancer Research. Used with permission. From C, Mouta Carreira, S. M. Nasser, E. di Tomaso, T. P. Padera, Y. Boucher, S. I. Tomarev, and R. K. Jain. "LYVE-1 is not restricted to the lymph vessels: expression in normal liver blood sinusoids and down-regulation in human liver cancer and cirrhosis." *Cancer Research* 61 (2001): 8079-8084.

Human Lung Tumors

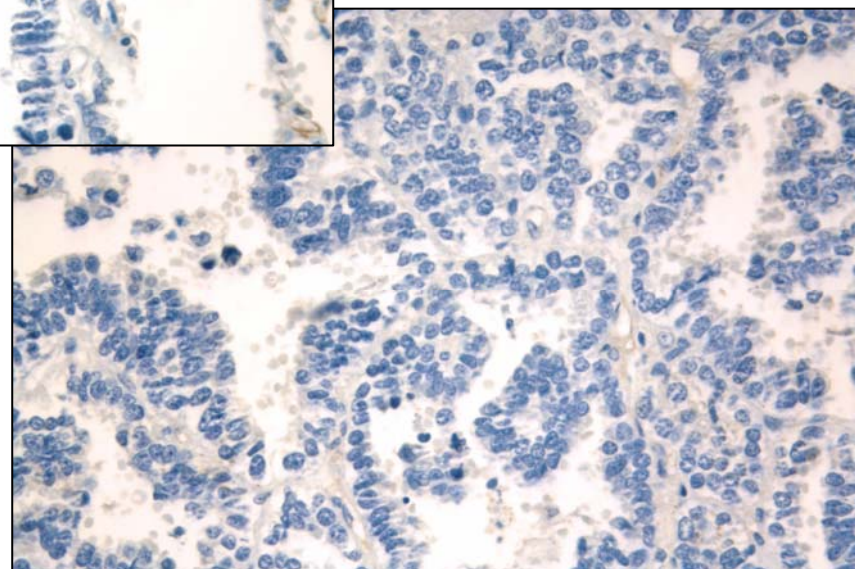


Tumor edge



200 microns

875 microns



IFP = 9.5 ± 1.5 mmHg
n = 27

Reference: *Padera et al., Science (2002)*

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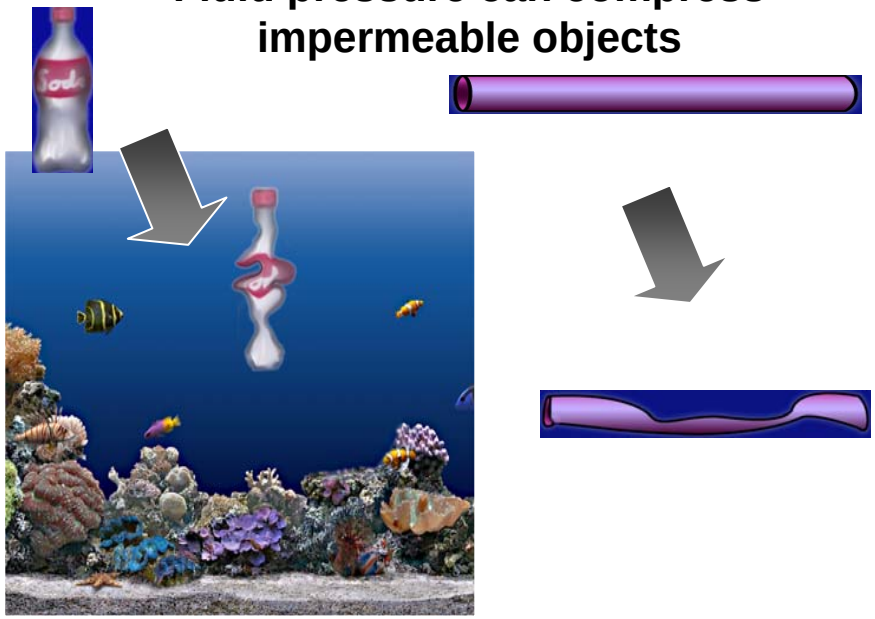
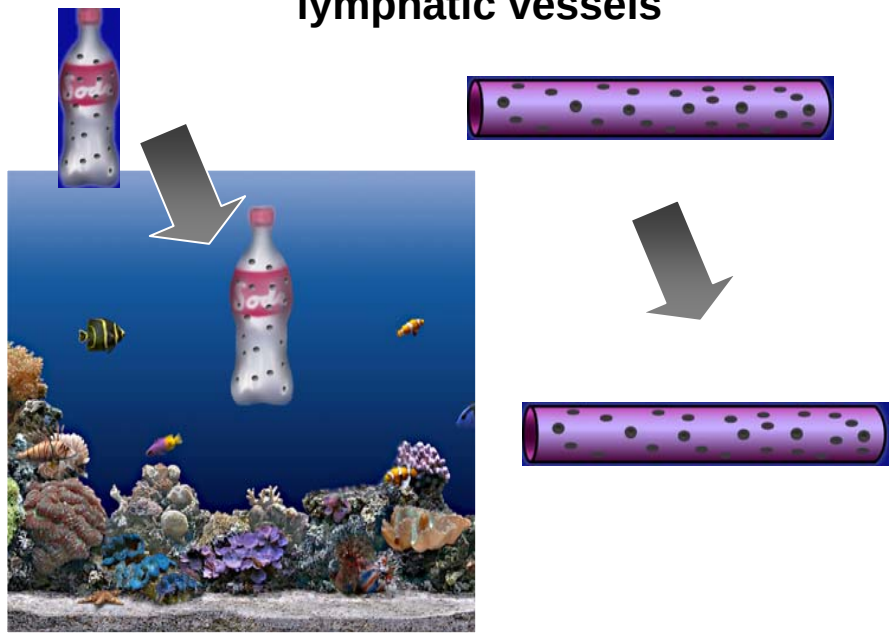
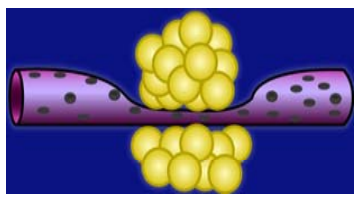
Fluid pressure can compress impermeable objects



Fluid pressure can NOT compress permeable vessels, such as blood and lymphatic vessels





Solid stress can compress permeable vessels, such as blood and lymphatic vessels

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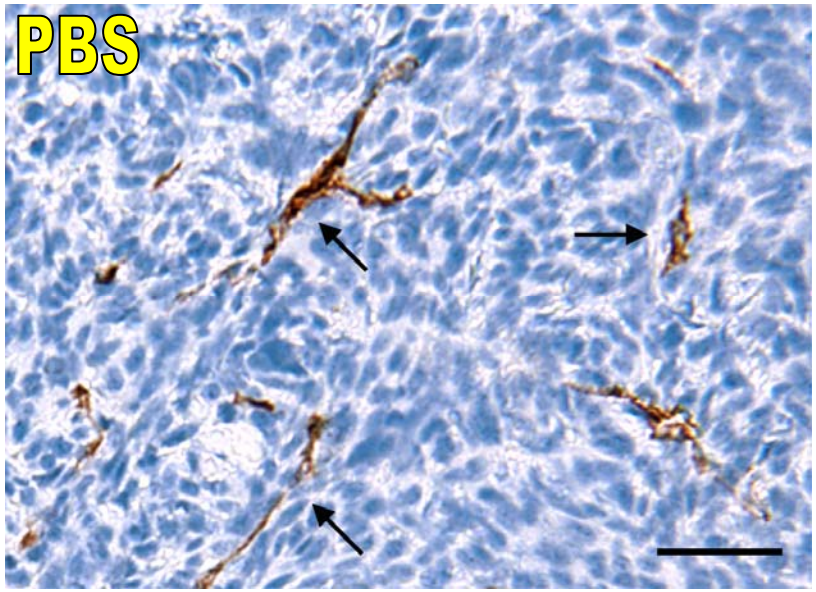
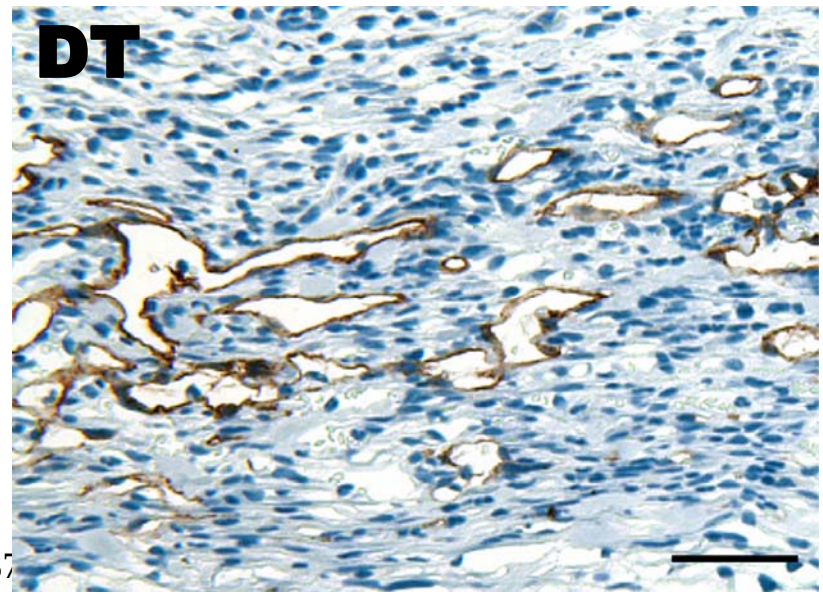
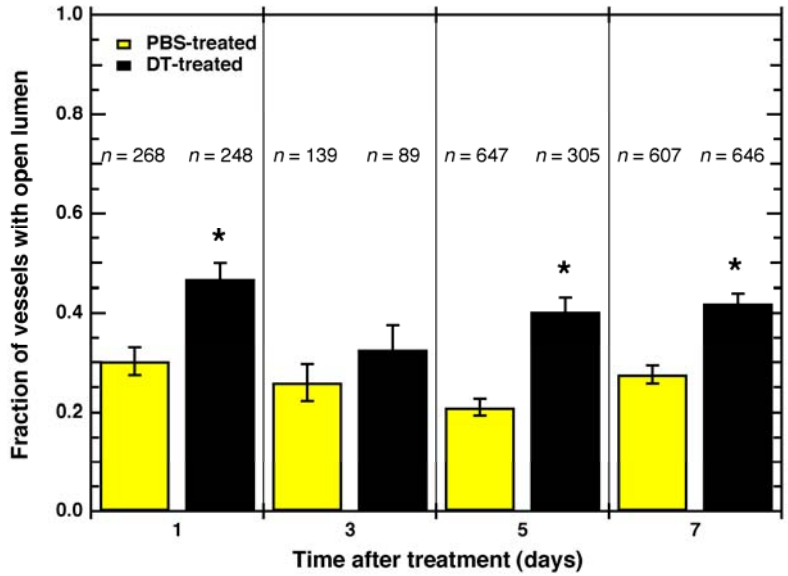
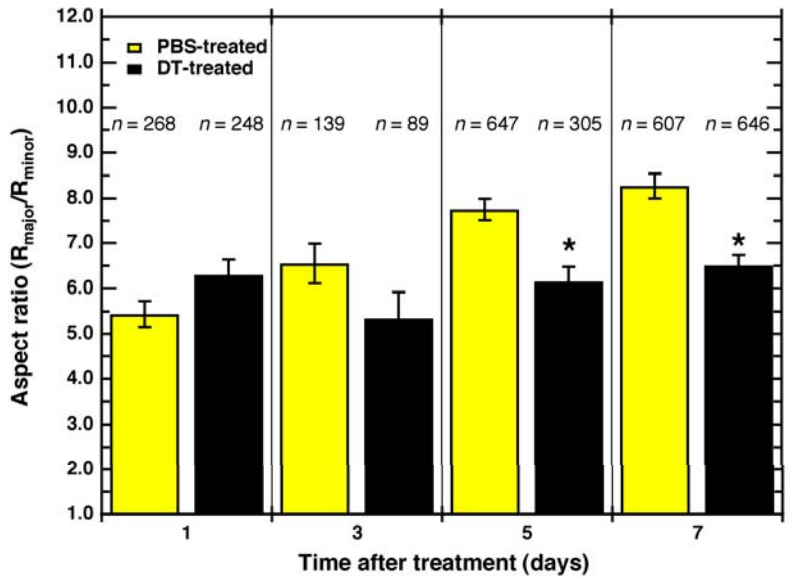
DT treatment opens lymphatic vessels









Reference: Padera, Stoll et al. Nature, (2004)

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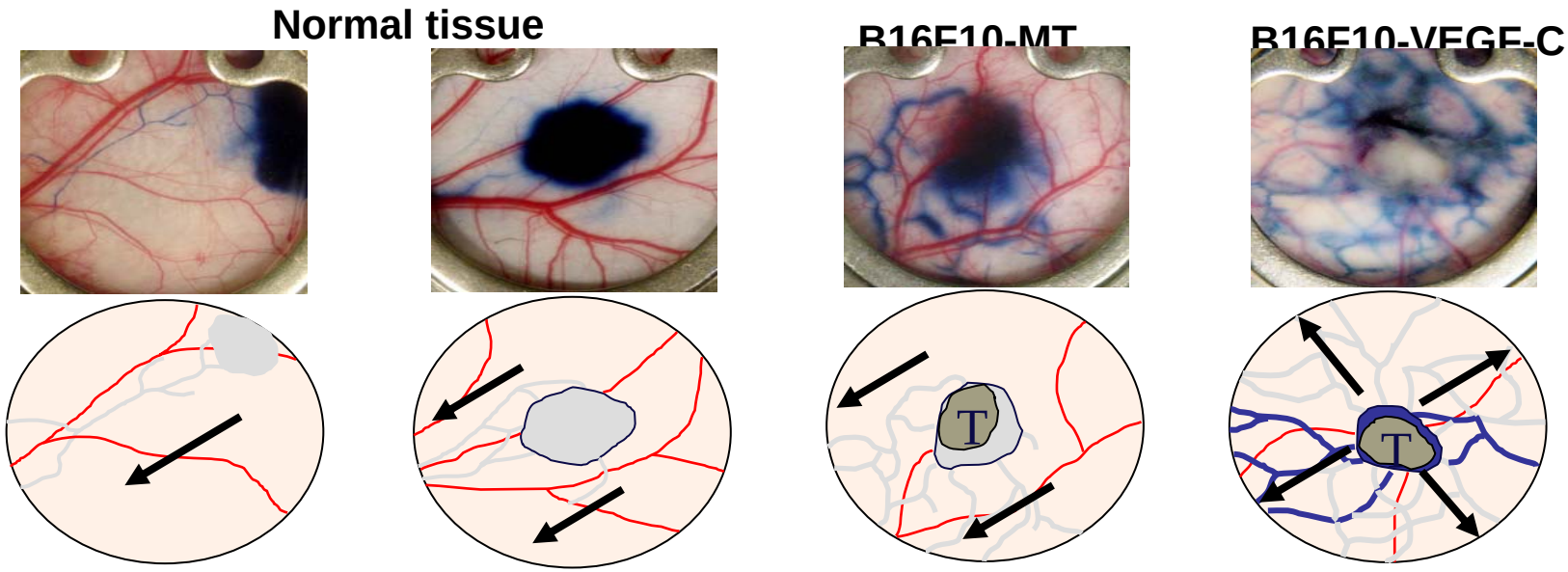
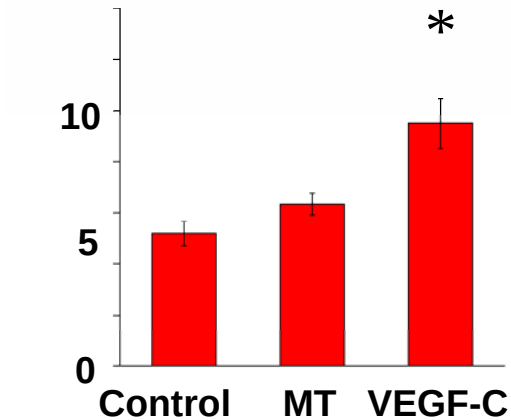
Peritumor lymphatics induced by VEGF-C exhibit abnormal function





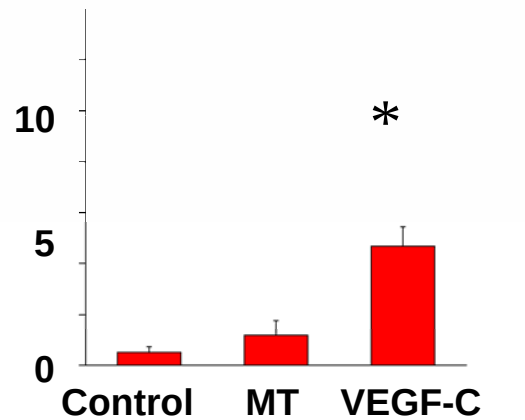


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Image removed for copyright reasons.

See: Jain, R.K., and B.T. Fenton. "Intra-Tumoral Lymphatic Vessels: A Case of Mistaken Identity or Malfunction?" *JNCI* 94 (2002): 417-421.

Summary

- **Despite the presence of VEGF-C and its receptor VEGFR3, functional lymphatics cannot be detected in tumors.**
- **VEGF-C overexpression increases diameter and number of peri-tumor lymphatics.**
- **Overexpression of VEGF-C and -D increases vascular angiogenesis.**
- **Levels of VEGF-C and -D correlate with increased lymphatic and hematogeneous metastasis.**
- **Blocking VEGF-C/-D can prevent lymphatic metastasis.**

Overall Summary

Mechanisms of Heterogeneous Distribution of Macromolecules in Tumors

- Heterogeneous blood flow
- Elevated interstitial pressure
- Fluid oozing from the periphery
- Slow interstitial diffusion
- Retardation by binding
- Heterogeneous expression of antigens

Strategies for Improved Delivery

- Make the vasculature the target for therapy
- Modify physiological parameters
  - Increase perfusion
  - Modulate interstitial pressure
  - Interfere with collagen synthesis (e.g., relaxin)
  - Use multi-step strategies
  - “Normalize” the abnormal tumor vasculature