Microenvironnement tumoral: approches physique II

Mecanique et Mecanotransduction de la progression tumorale in vitro

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Rencontres scientifiques des Grands Causses, 30 sept. 2015

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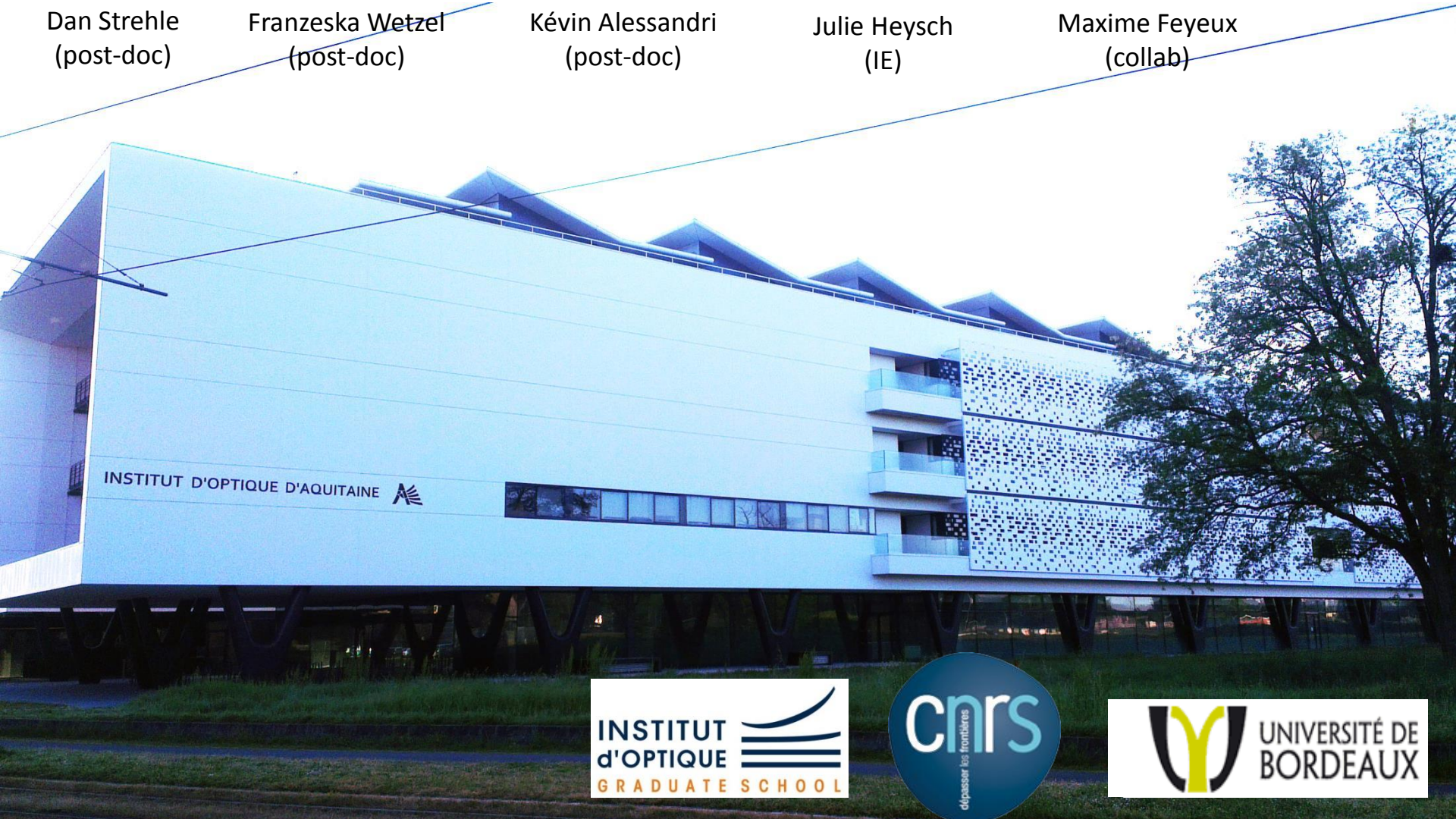

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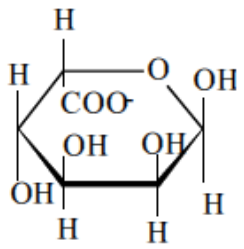
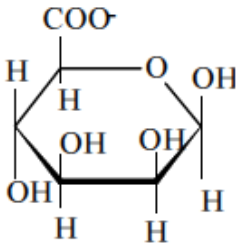
Activities in the lab (1)

(Thierry Marx)



G

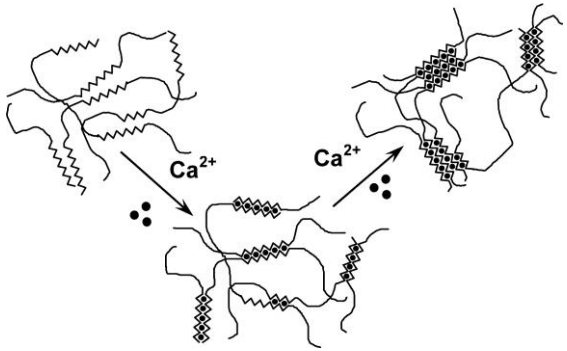


M

Alginate

Biocompatible polysaccharide

Wine pearls

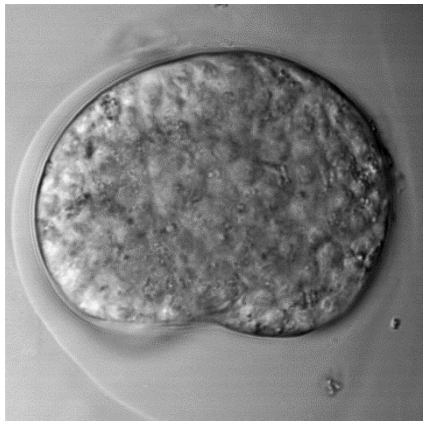


Alginate gel

Transparent, permeable (pore size, 15 nm)

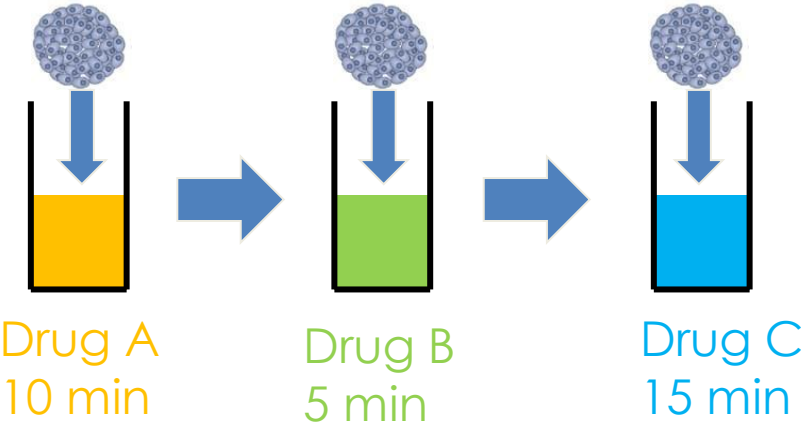
Activities in the lab (2)

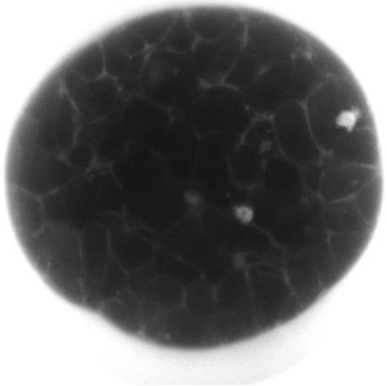
Hepato-toxicity assays (for cosmetic industry)



Liver pearls

… for high throughput screening of « drugs »

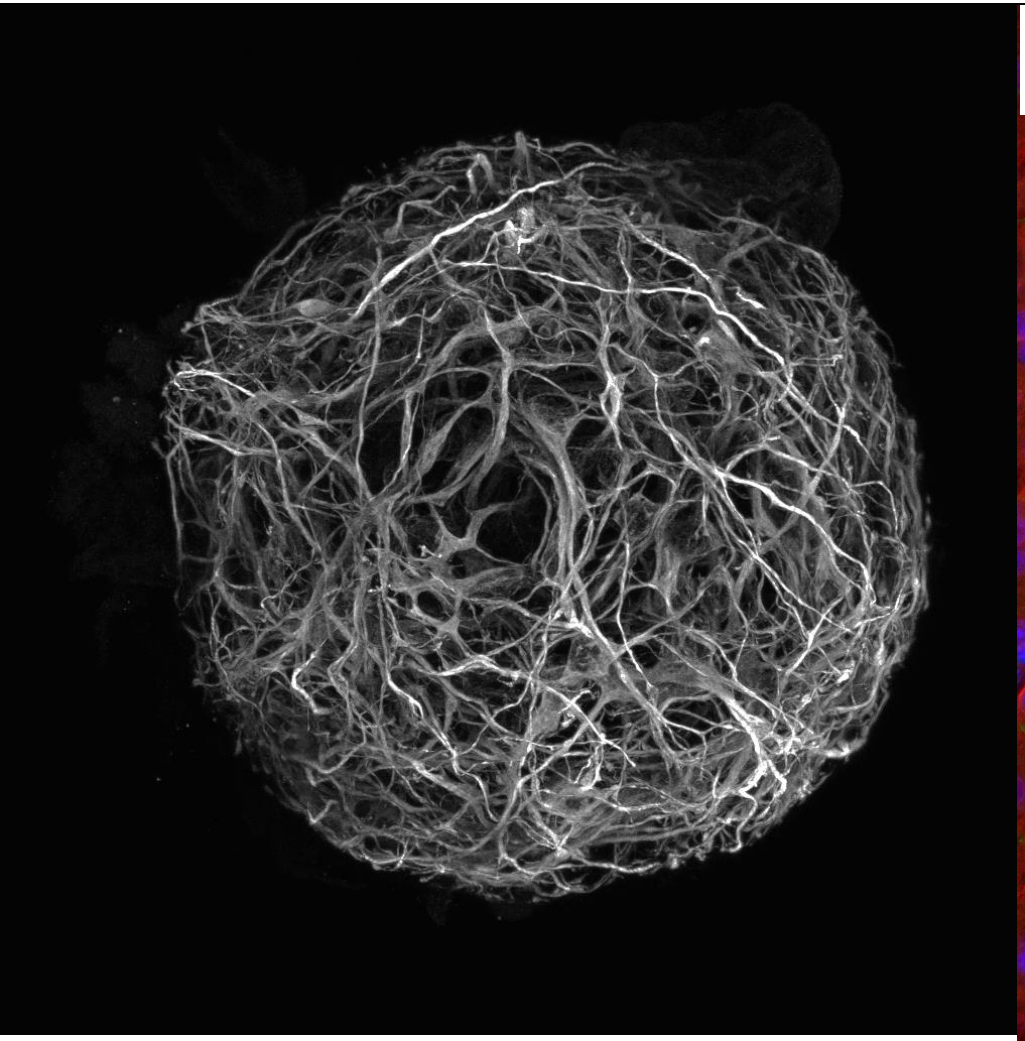




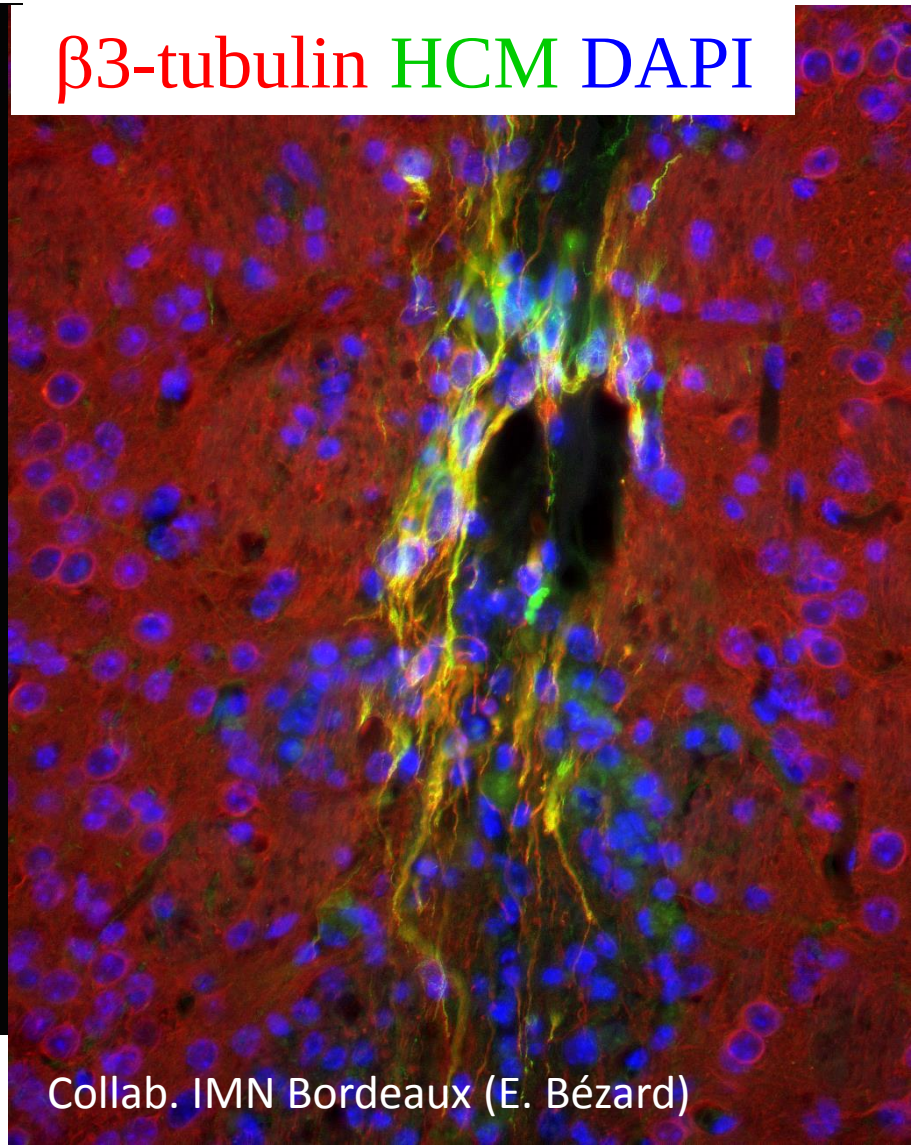
CFDA accumulation in active canaliculi from equatorial plane

Activities in the lab (3)

Cell therapy for Parkinson disease



Collab: Univ. Genève (A. Roux)



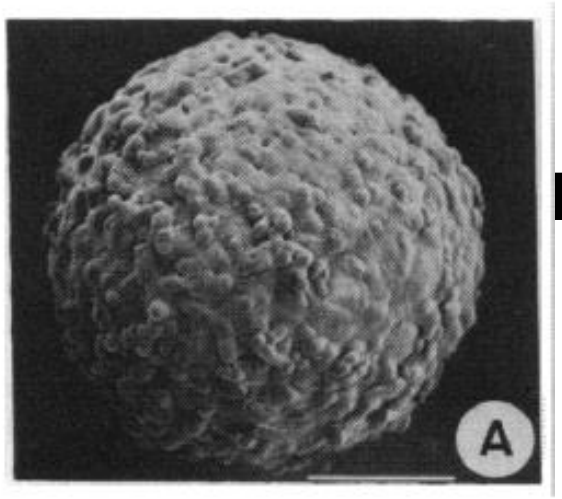
Collab. IMN Bordeaux (E. Bézard)

Activities in the lab (4)

Oncological physics

The Multicell Spheroid Model

ROBERT M. SUTHERLAND 8 APRIL 1988



Pétri dish(2D)

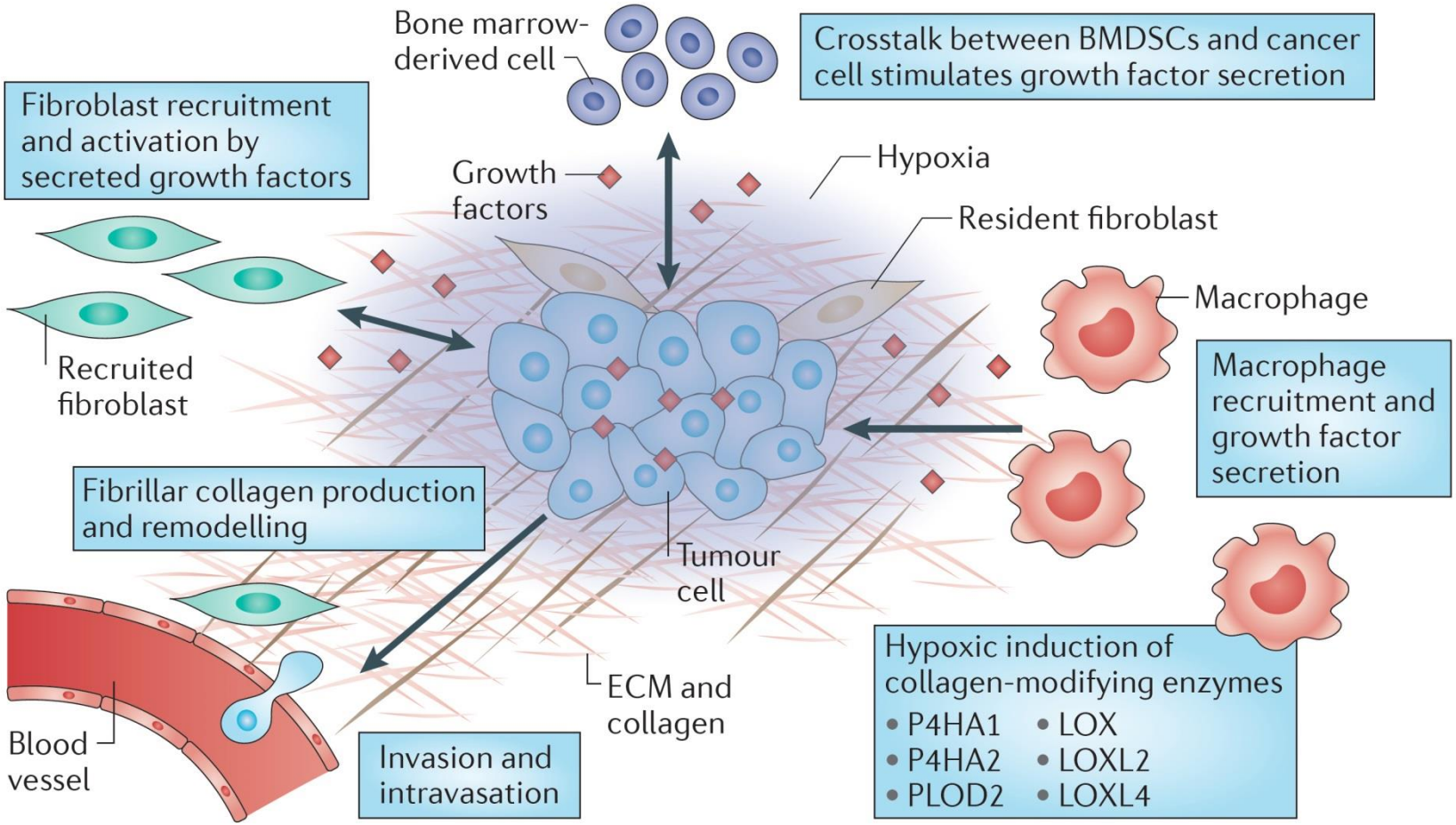
Animal testing (3D)

BMC Cancer

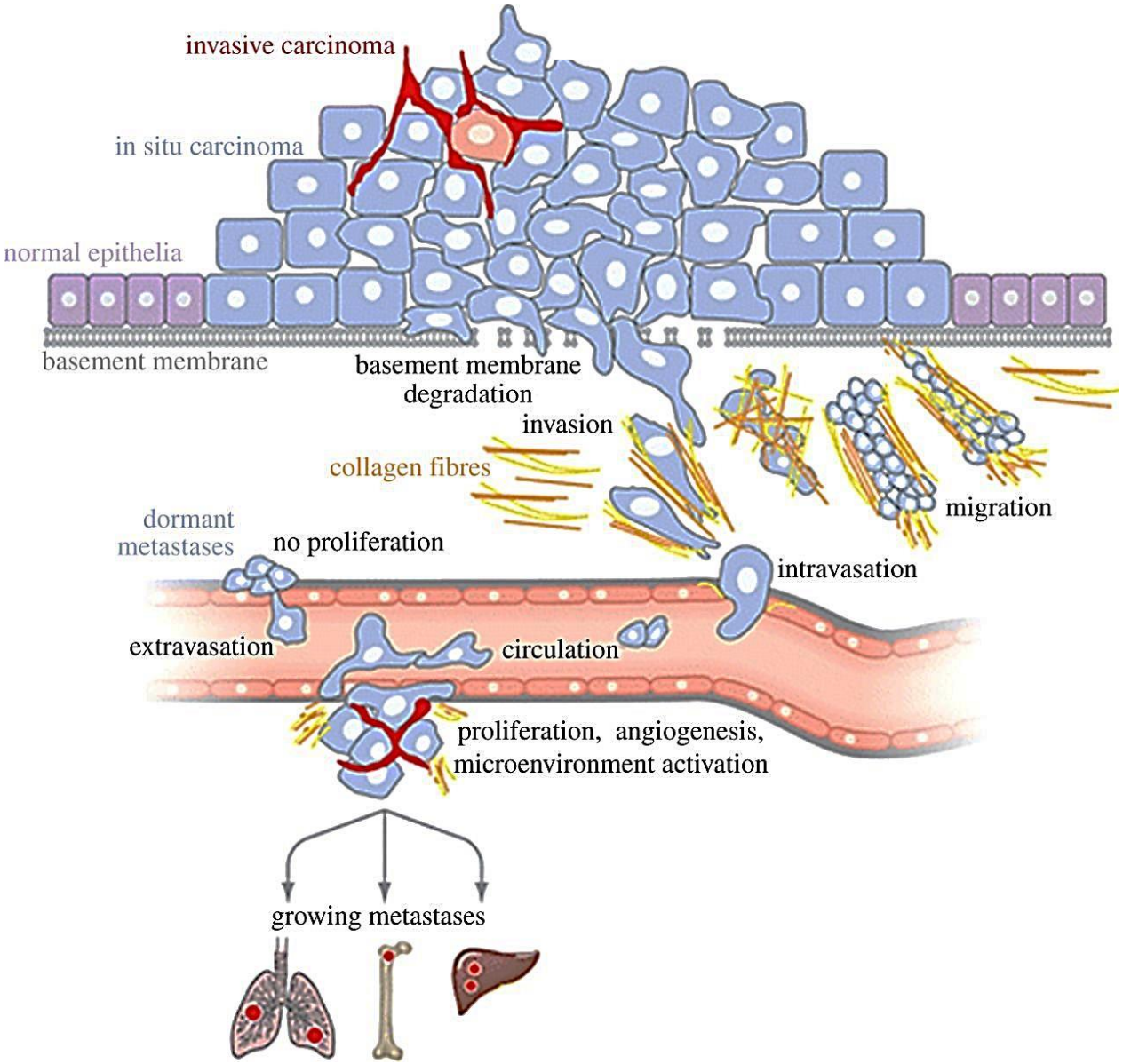
3D pancreatic carcinoma spheroids induce a matrix-rich, chemoresistant phenotype offering a better model for drug testing

Longati *et al. BMC Cancer* 2013, **13**:95

Tumor microenvironment



Metastasis

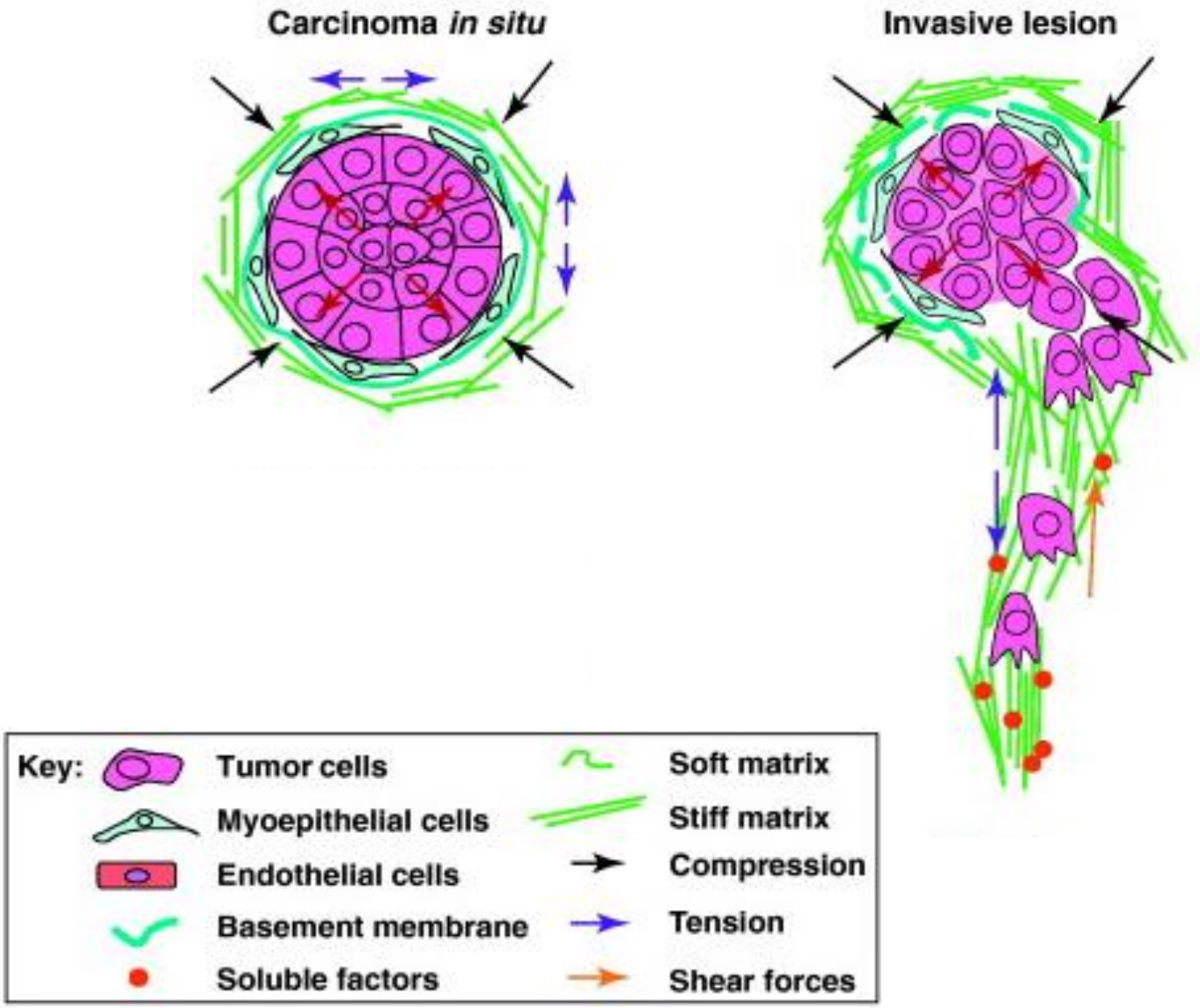

invasive carcinoma
in situ carcinoma
normal epithelia
basement membrane
basement membrane degradation
invasion
collagen fibres
migration
dormant metastases
no proliferation
intravasation
extravasation
circulation
proliferation, angiogenesis, microenvironment activation
growing metastases

Tumor Microenvironment: A New Treatment Target for Cancer

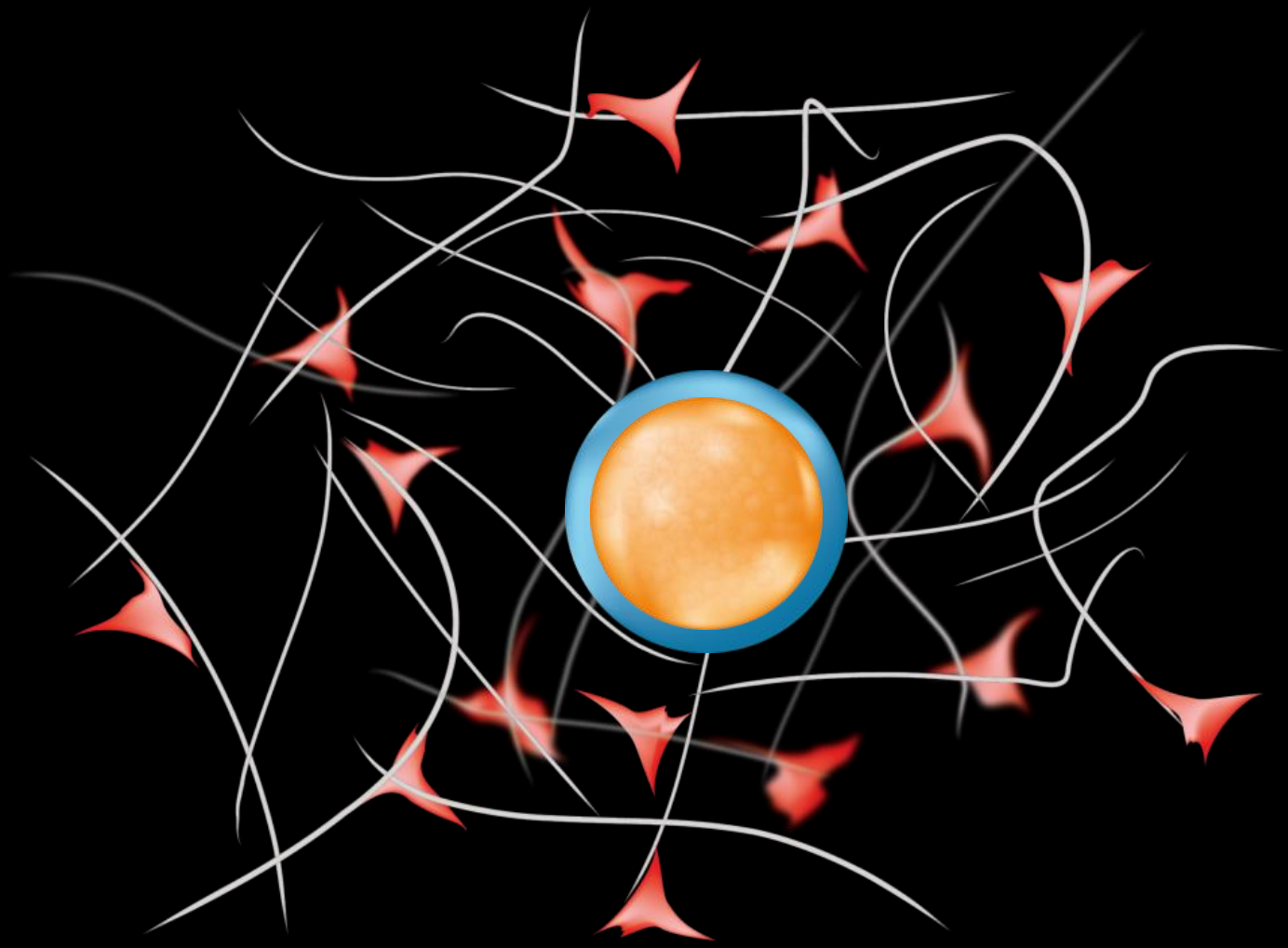
Molecule	Target	Molecule type	Company	Status (reference)
ECM/fibroblasts				
Marimastat	MMP — broad spectrum	Small molecule	British Biotech	Phase III negative for NSCLC, SCLC and breast cancer (NCT00002911, NCT00003010, NCT00003011)
Prinomastat	MMP 2, 3, 9, 13 and 14	Small molecule	Agouron/Pfizer	Phase III negative for NSCLC and prostate cancer (NCT00004199, NCT00003343)
Tanomastat	MMP 2, 3 and 9	Small molecule	Bayer	Phase III terminated (NCIC-CTG trial 0V12)
Neovastat	VEGFR2, MMP 2, 9, 12	Small molecule	AEterna Laboratories	Phase III negative for NSCLC (NCT00005838)
Rebimastat	MMP 1, 2, 8, 9 and 14	Small molecule	Bristol-Myers Squibb	Phase III negative for NSCLC (NCT00006229)
Vismodegib	SMO	Small molecule	Genentech/Roche	Phase II negative for CRC and ovarian cancer and phase II for PDAC (NCT00636610, NCT00739661, NCT01064622)
Saridegib	SMO	Small molecule	Infinity Pharmaceuticals	Phase II terminated for PDAC (NCT01130142, NCT01310816)
Sonidegib	SMO	Small molecule	Novartis	Phase III (NCT01708174)
Vasculature				
Bevacizumab	VEGFA	Antibody	Genentech/Roche	FDA-approved ((BLA) 125085)
Vandetanib	VEGFRs, PDGFRs, EGFR	Small molecule	AstraZeneca	FDA-approved ((NDA) 022405)
Sunitinib	VEGFRs, PDGFRs, FLT3, CSF1R	Small molecule	Pfizer	FDA-approved ((NDA) 021938)
Axitinib	VEGFRs, PDGFRs, KIT	Small molecule	Pfizer	FDA-approved ((NDA) 202324)
Sorafenib	VEGFRs, RAF, PDGFRs, KIT	Small molecule	Bayer	FDA-approved ((NDA) 021923)
Pazopanib	VEGFRs, PDGFRs, KIT	Small molecule	GlaxoSmithKline	FDA-approved ((NDA) 022465)
Cabozantinib	VEGFR2, RET, MET	Small molecule	Exelixis	FDA-approved ((NDA) 203756)
Ziv-aflibercept	VEGFA, VEGFB, PlGF	Receptor–Fc fusion	Regeneron	FDA-approved ((BLA) 125418)
Cilengitide	Integrins $\alpha_v\beta_3$, $\alpha_v\beta_5$ and $\alpha_5\beta_1$	Small molecule	Merck Serono	Phase III negative for GBM (NCT00689221)
AMG386	ANG2	RP–Fc fusion protein	Amgen	Phase III (NCT01281254)
Parsatuzumab	EGFL-7	Antibody	Genentech/Roche	Phase II (NCT01399684, NCT01366131)
Enoticumab	DLL4	Antibody	Regeneron	Phase I (NCT00871559)
Demcizumab	DLL4	Antibody	OncoMed	Phase I (NCT00744562, NCT01189968, NCT01189942, NCT01189929)
Nesvacumab	ANG2	Antibody	Regeneron	Phase I (NCT01688960, NCT01271972)
Immune				
Ipilimumab	CTLA-4	Antibody	Bristol-Myers Squibb	FDA-approved ((BLA) 125377)
Sipuleucel-T	PAP	DC vaccine	Dendreon	FDA-approved ((BLA) 125197)
Aldesleukin	IL-2	RP	Prometheus	FDA-approved ((BLA) 103293)
IFN α-2b	IFN-α receptor	RP	Merck	FDA-approved ((BLA) 103132)
MK-3475	PD1	Antibody	Merck	Phase III (NCT01866319)
Nivolumab	PD1	Antibody	Bristol-Myers Squibb	Phase III (NCT01668784, NCT01673867, NCT01642004, NCT01721772, NCT01721746, NCT01844505)
Nivolumab	OX40	Antibody	Bristol-Myers Squibb and PPMC	Phase III (NCT01668784, NCT01642004, NCT01673867, NCT01721772, NCT01721746, NCT01844505)
MPDL3280A	PDL1	Antibody	Genentech/Roche	Phase II (NCT01846416)
PLX3397	KIT, CSF1R, FLT3	Small molecule	Plexxikon	Phase II (NCT01349036)
BMS-663513	CD137 (4-1BB)	Antibody	Bristol-Myers Squibb	Phase II (NCT00612664)
Blinatumomab	CD3 and CD19	Bi-specific scFv	Amgen	Phase II (NCT01741792, NCT01466179, NCT01207388, NCT01471782, NCT00560794, NCT01209286)
AMG 820	CSF1R	Antibody	Amgen	Phase I (NCT01444404)
AMP-224	PD1	Antibody	GlaxoSmithKline	Phase I (NCT01352884)
TRX-518	GITR	Antibody	GITR, Inc.	Phase I (NCT01239134)
IMC-CS4	CSF1R	Antibody	ImClone/Eli Lilly	Phase I (NCT01346358)
CP-870,893	CD40	Antibody	Pfizer	Phase I (NCT00711191, NCT01008527, NCT00607048, NCT01456585, NCT01103635)

References listed pertain to the molecule as a stromal-modifying agent, either the FDA application, where approved, or the national clinical trial identification of the oncology trial in the latest phase is listed (note that in some cases the drug may also be tested or approved for an indication for which it acts directly on the tumour cell compartment, which will not be referenced here). ANG2, angiopoietin 2; BLA, biological license application; CRC, colorectal cancer; CSF1R, colony stimulating factor 1 receptor; CTLA-4, cytotoxic T-lymphocyte-associated antigen 4; DC, dendritic cell; DLL4, delta-like 4; ECM, extracellular matrix; EGFL-7, epidermal growth factor like 7; EGFR, epidermal growth factor receptor; FDA, Food and Drug Administration; FLT3, Fms-like tyrosine kinase 3; GBM, glioblastoma multiforme; GITR, glucocorticoid-induced TNFR-related; IFN, interferon; IL-2, interleukin 2; MMP, matrixmetalloproteinase; NCT, national clinical trial; NDA, new drug application; NSCLC, non-small cell lung cancer; PAP, prostatic acid phosphatase; PD-1, programmed death-1; PDAC, pancreatic ductal adenocarcinoma; PDGFR, Platelet-derived growth factor receptor; PDL1, programmed death ligand 1; PLGF, placental growth factor; PPMC, Portland Providence Medical Center; RP, recombinant peptide; scFv, single-chain Fv; SCLC, small-cell lung cancer; SMO, smoothened; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

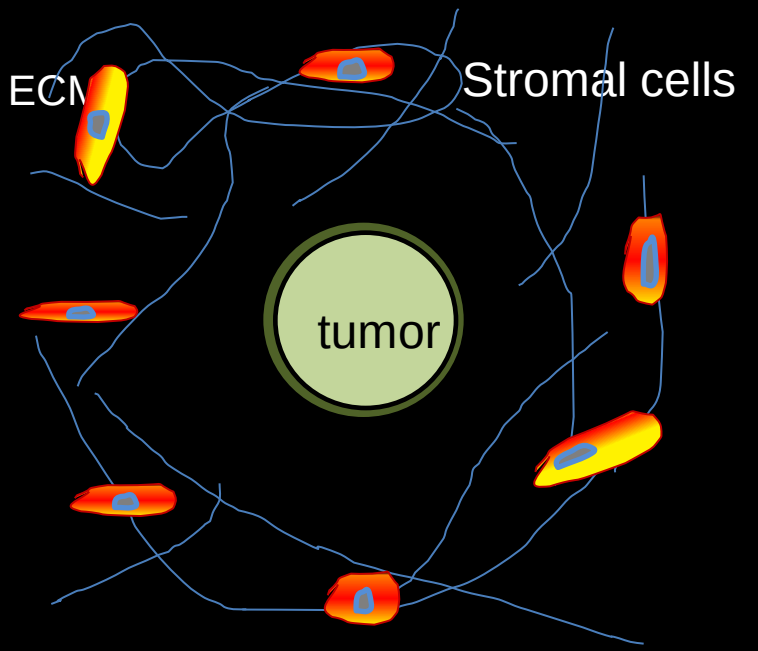
Chemical and physical interactions between stromal and cancer cells



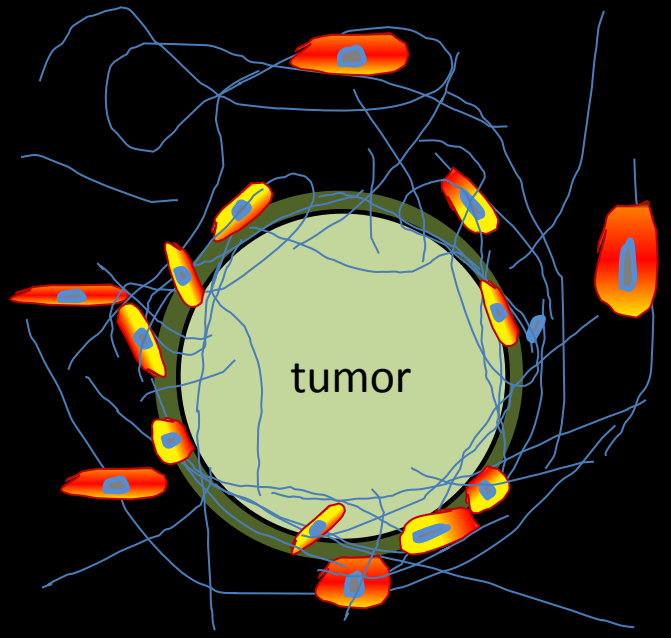
Realistic cellular model of tumor and micro-environment



Naive intuition

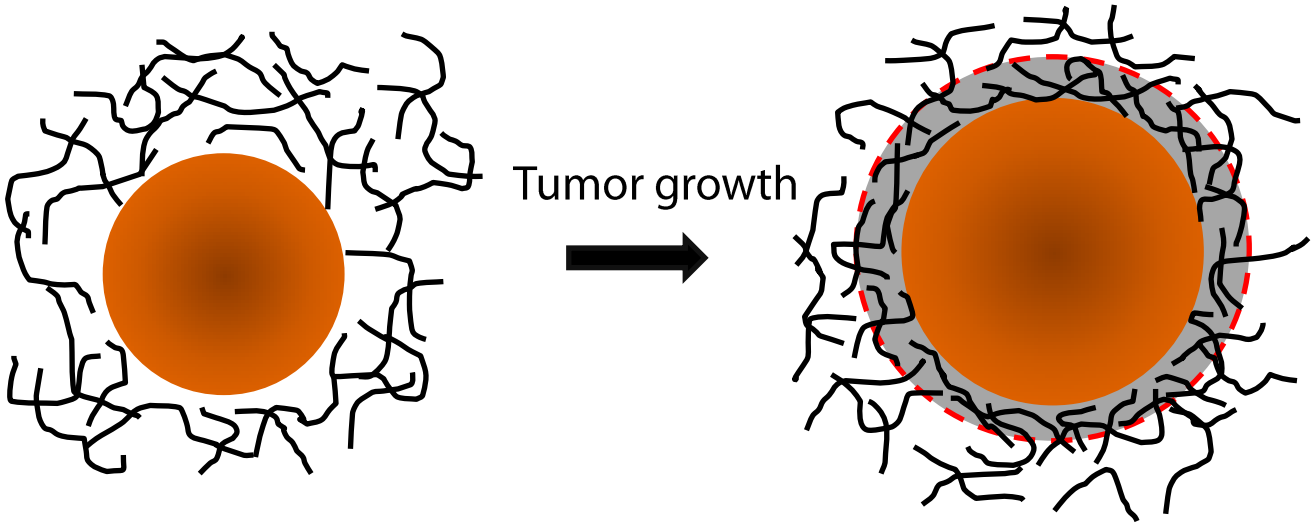


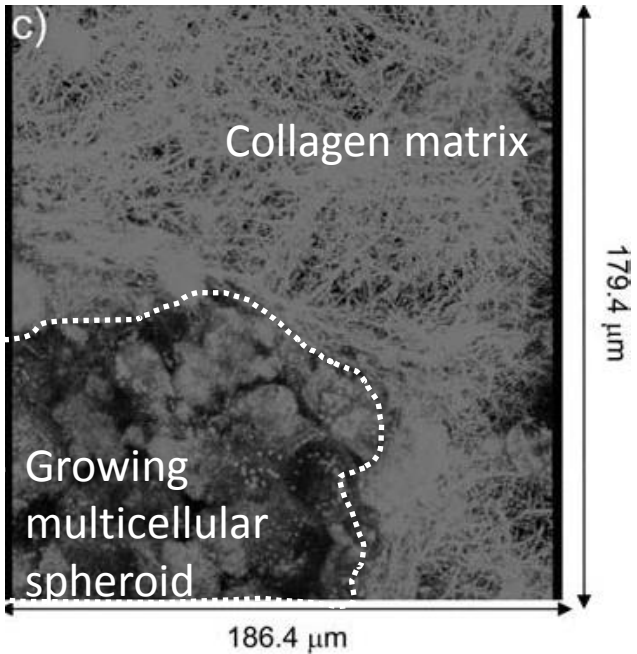
“weak” stroma = low pressure
Tumor grow



“strong” stroma = high pressure
Tumor shrink

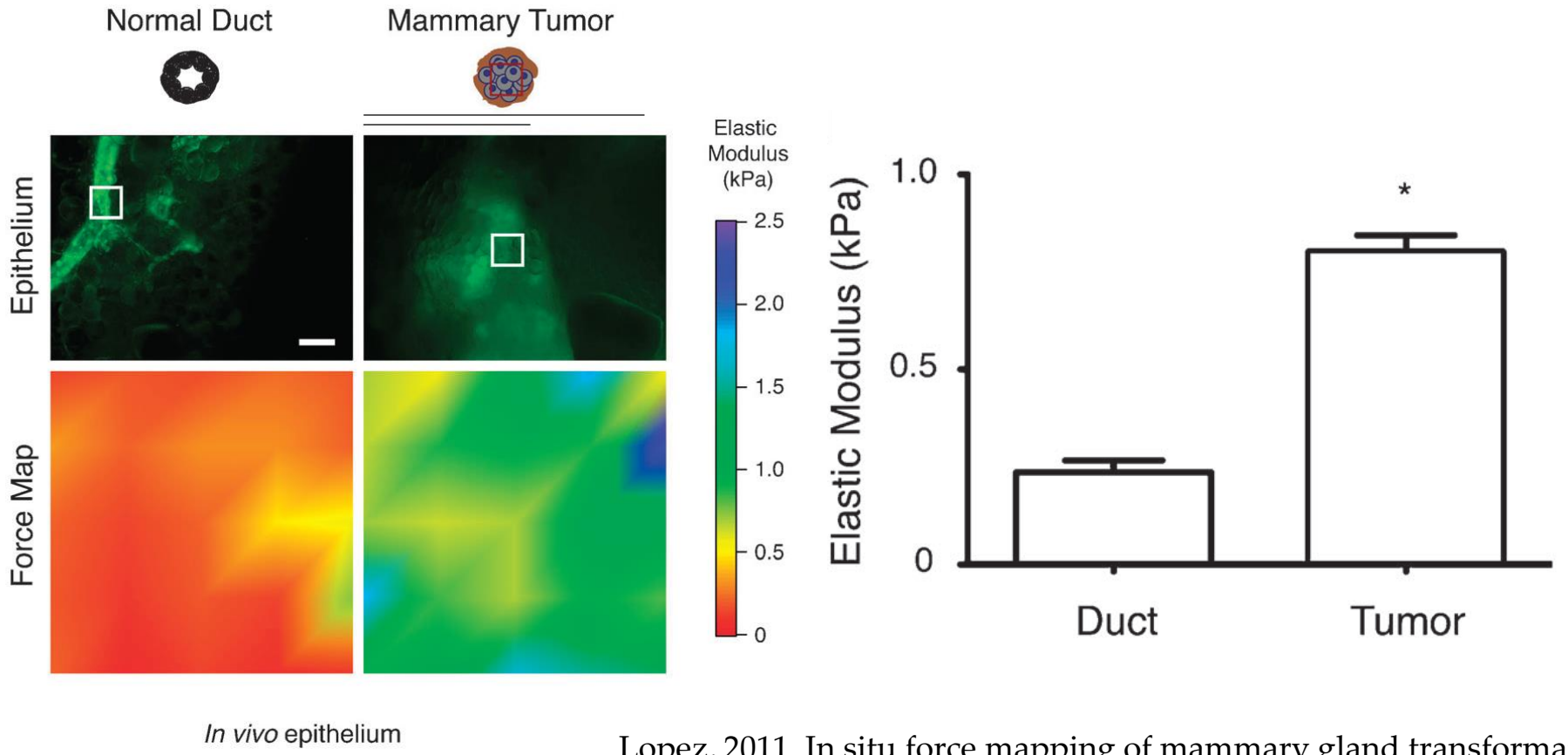
ECM stiffening *(in the vicinity of the tumor)*





Kaufman et al. BJ 2005

ONCOGENESIS



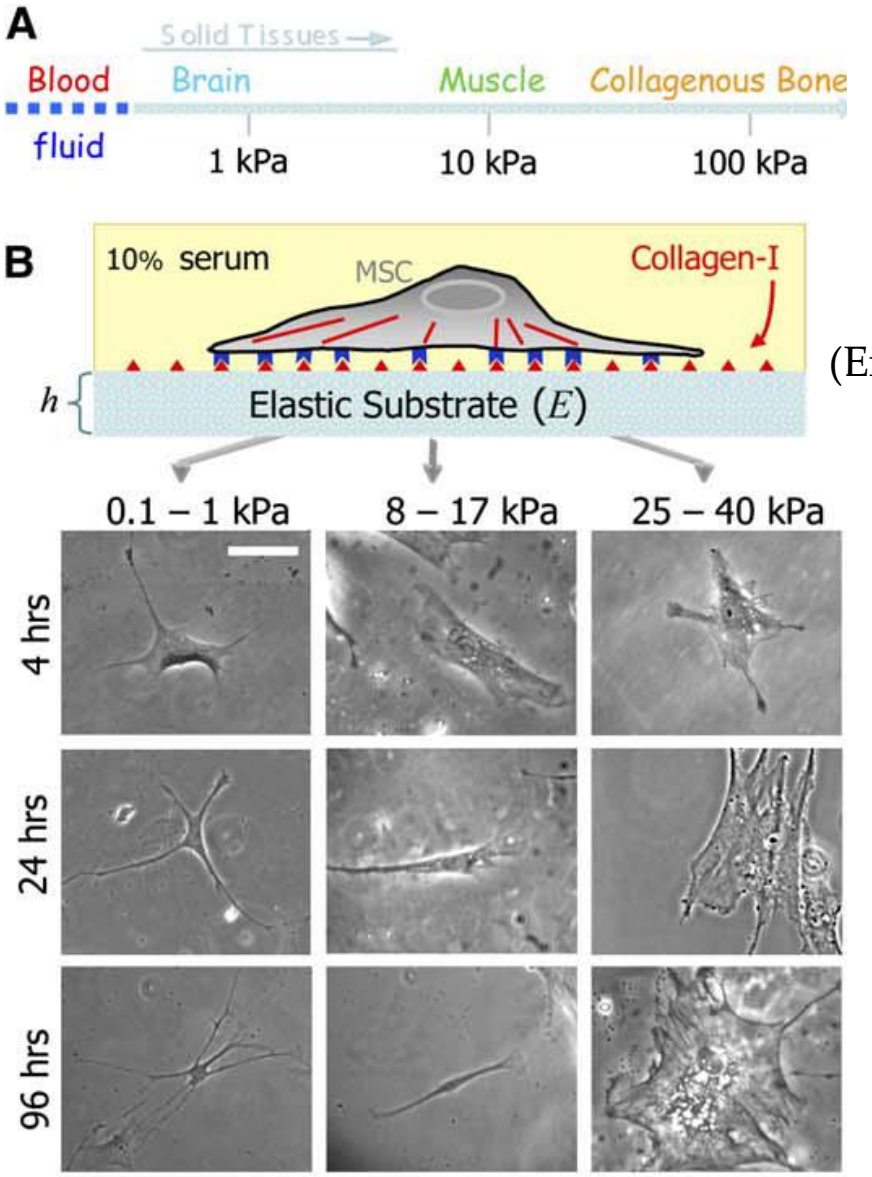
In vivo epithelium

Lopez, 2011. In situ force mapping of mammary gland transformation

Mammary **tumor** epithelia, tumor-associated **vasculature** and **ECM** all contribute to mammary gland stiffening as it transitions from normal to invasive carcinoma.

Physical interactions and **mechanical forces**

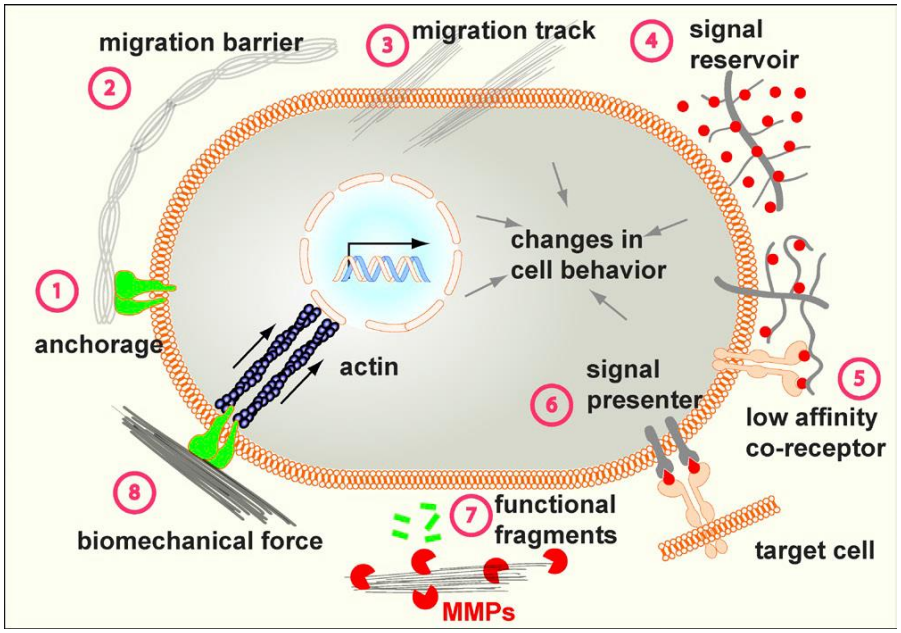
… are key regulators of cell differentiation



(Engler, 2006)

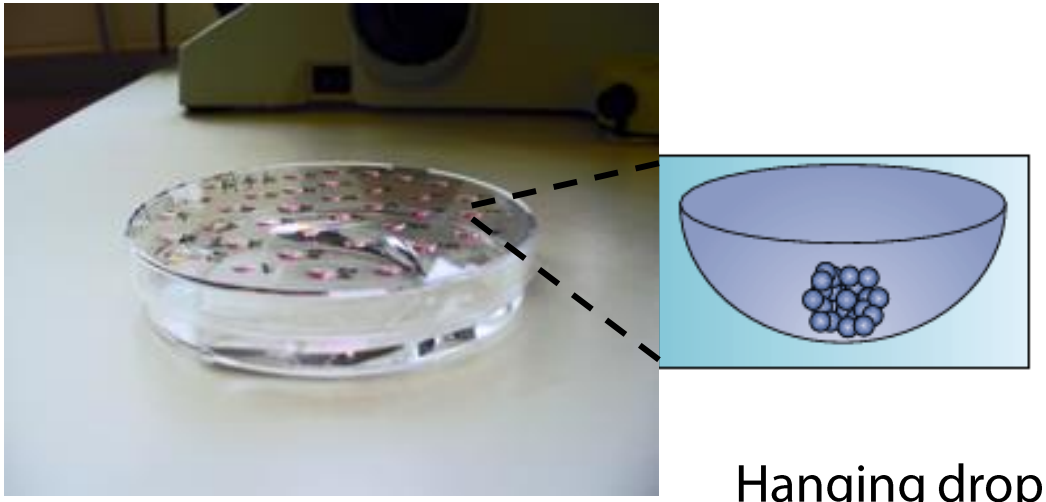
Summary:

1. *Tumorogenesis is a complex process!*
2. *There are mutual interactions between cancer cells, stromal cell and ECM*

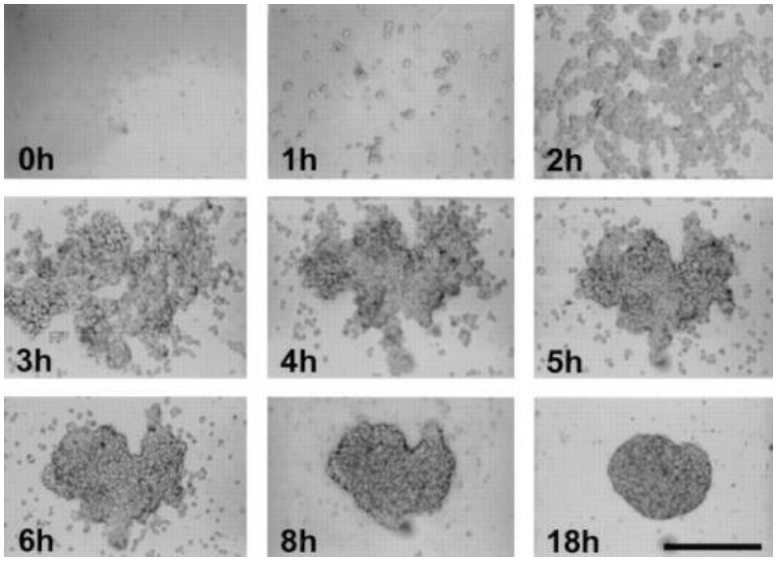


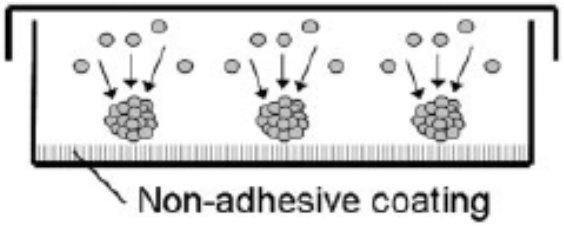
***Question*: Can we design a minimal *in vitro* system in which different cues are decoupled in order to investigate or mimic the sole mechanical influence of ECM on tumor cells, tumor growth and cell invasion?**

Formation of multicellular spheroids (current techniques)



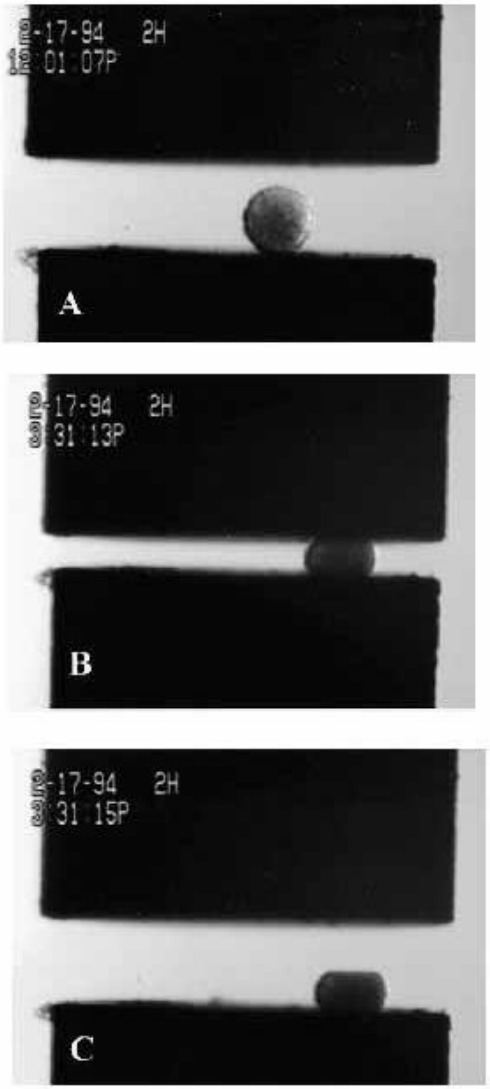
Hanging drop



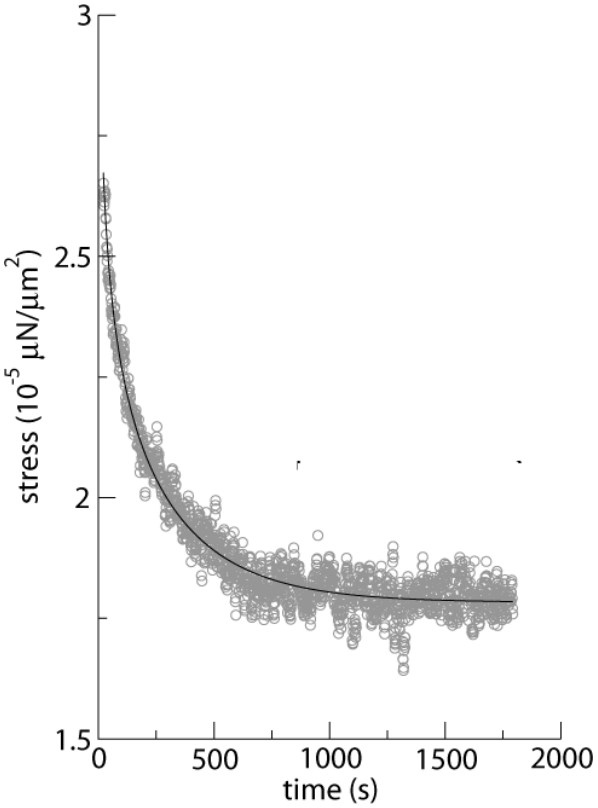


Agarose « cushion »

Compression of a non-growing multicellular spheroid



(Foty, Dev. 1996)



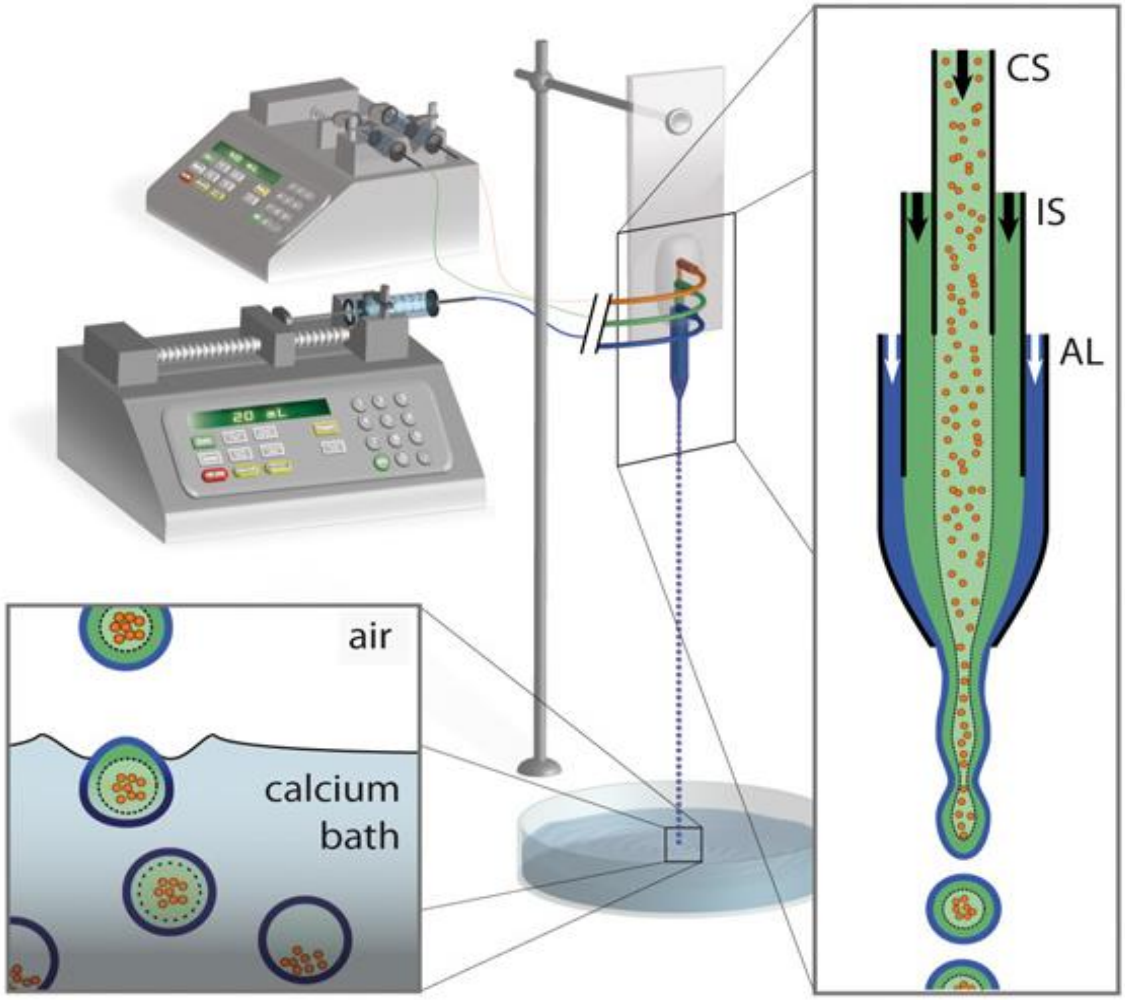
(Marmottant, PNAS 2009)

A spheroid is a visco-elastico-plastic material

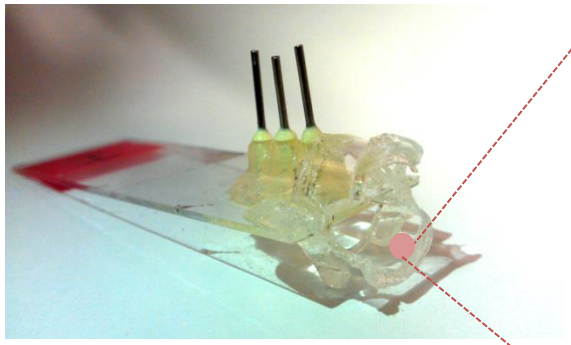
Note: time scale <1 hour

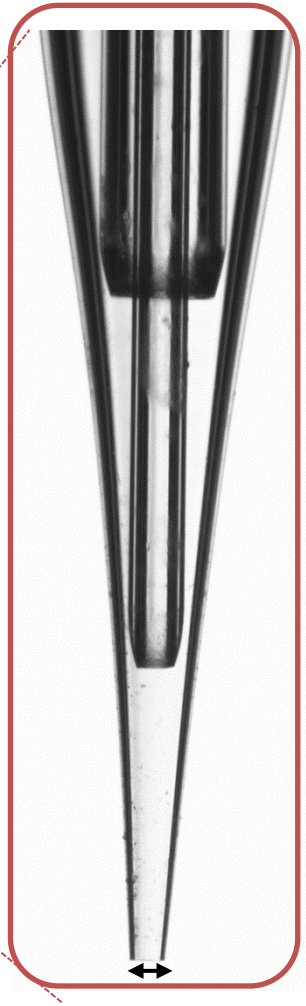
Cellular Capsules Technology

Microfluidics-assisted production of sub-millimetric aqueous core capsules made of an alginate permeable and elastic membrane

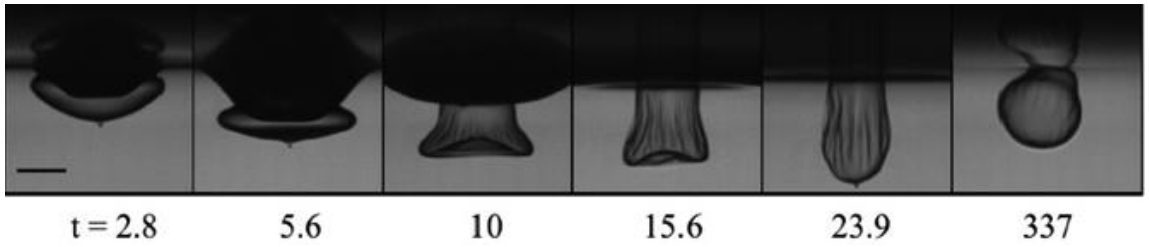


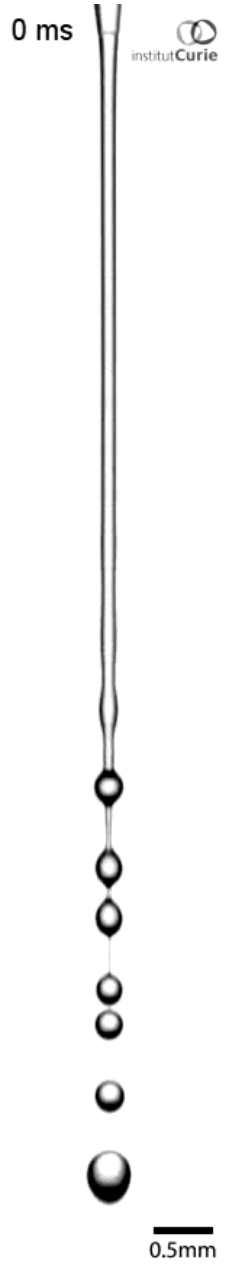
Microfluidic Capsule Production



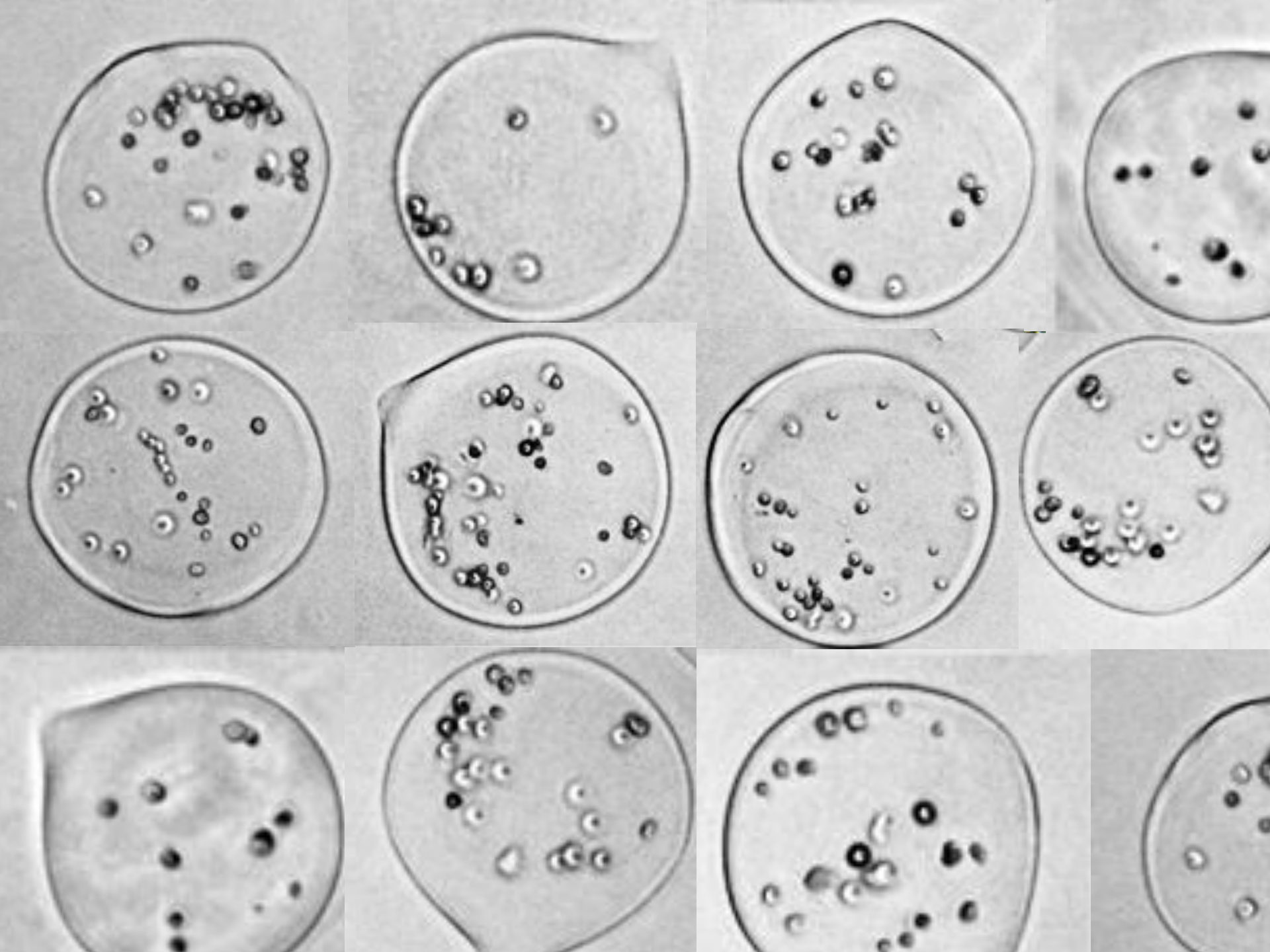


100 µm

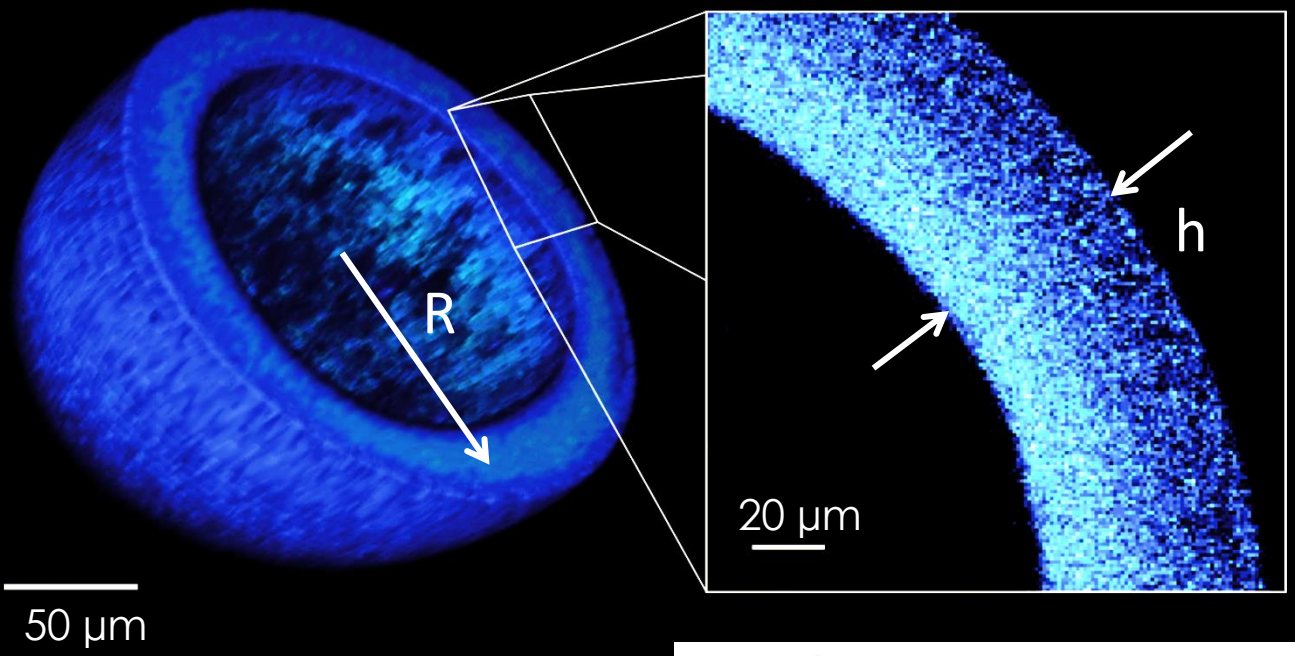




To make micro-capsules, operate in a *jetting* regime

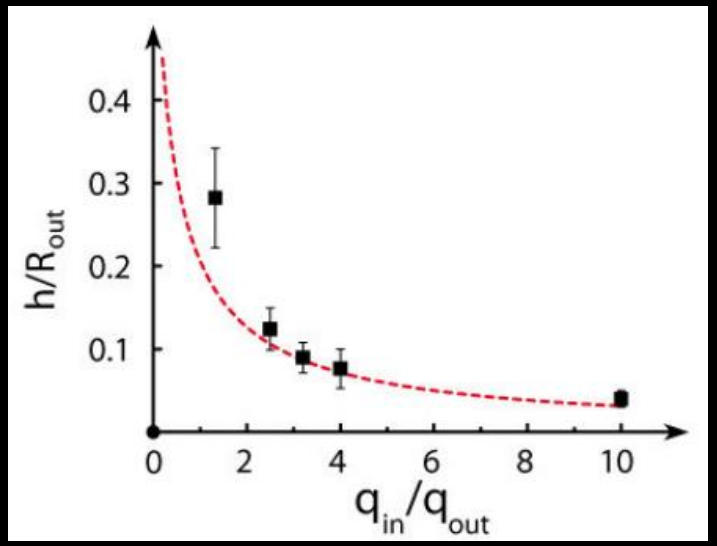


Capsule structure

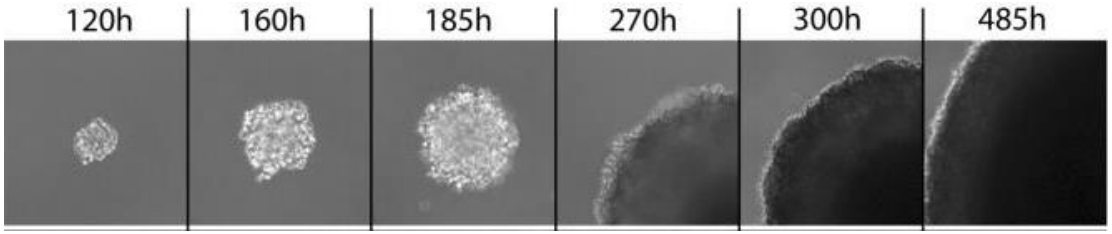


Capsule radius is fixed:
R~ 100 μm

Shell thickness can be tuned:
5 μm<h<35 μm



Control experiment: **Growth kinetics of free spheroids**



Short times : bulk growth

$$R \cong \exp(t / 3\tau_d)$$

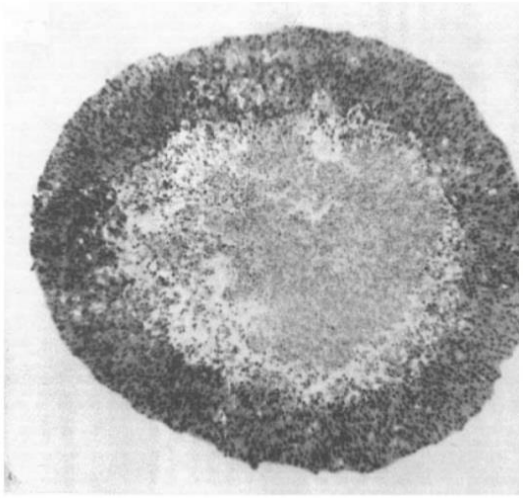
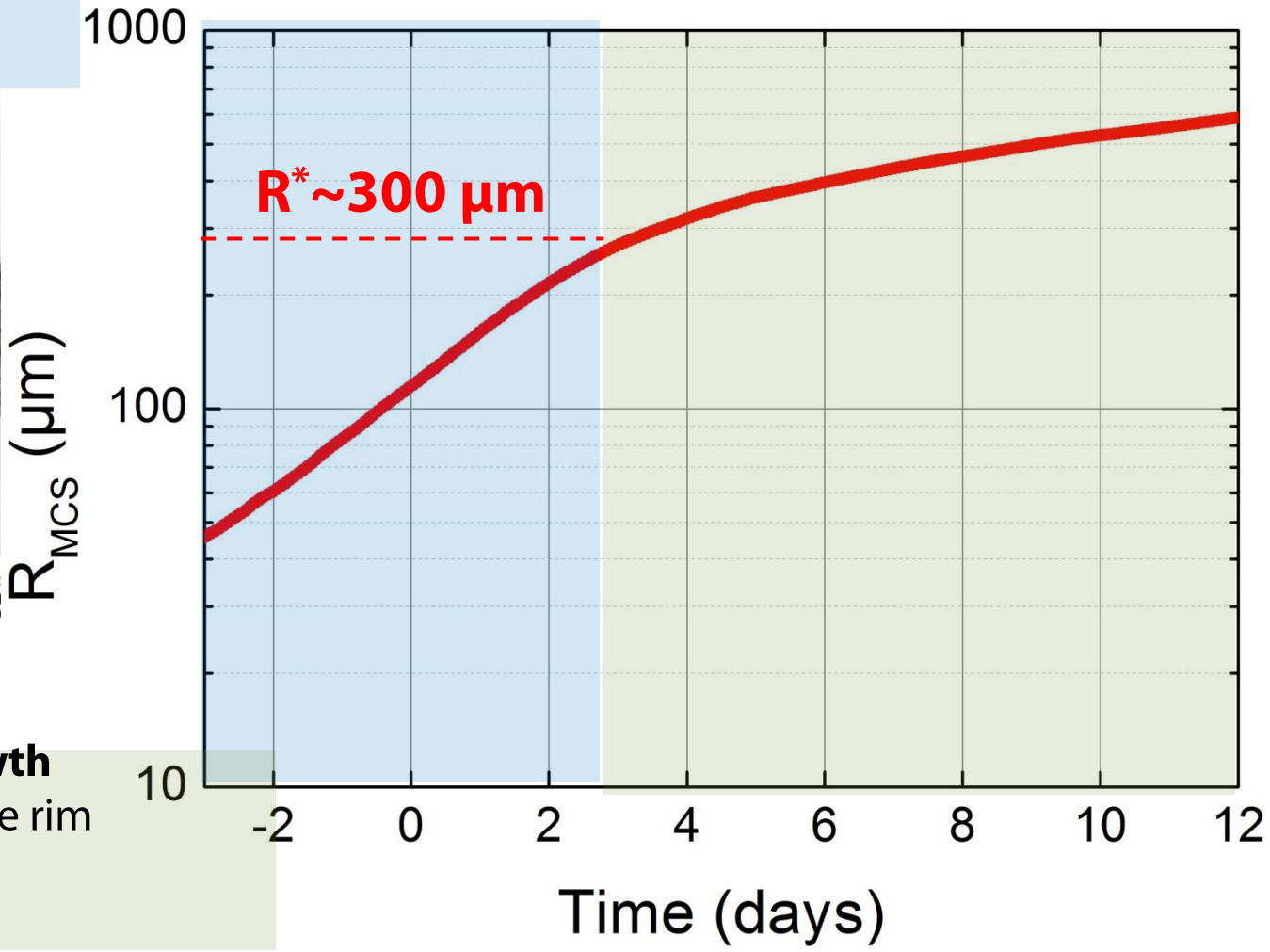


Fig. 1. An illustration of the structure of a multicellular tumour spheroid, with an o of proliferating cells and an inner necrotic core; these are separated by a layer of q cells. The spheroid diameter is 1.4 mm. [Reproduced from Sutherland et al. (Can 46, 5320–5329, 1986) by courtesy of the publisher and the authors].

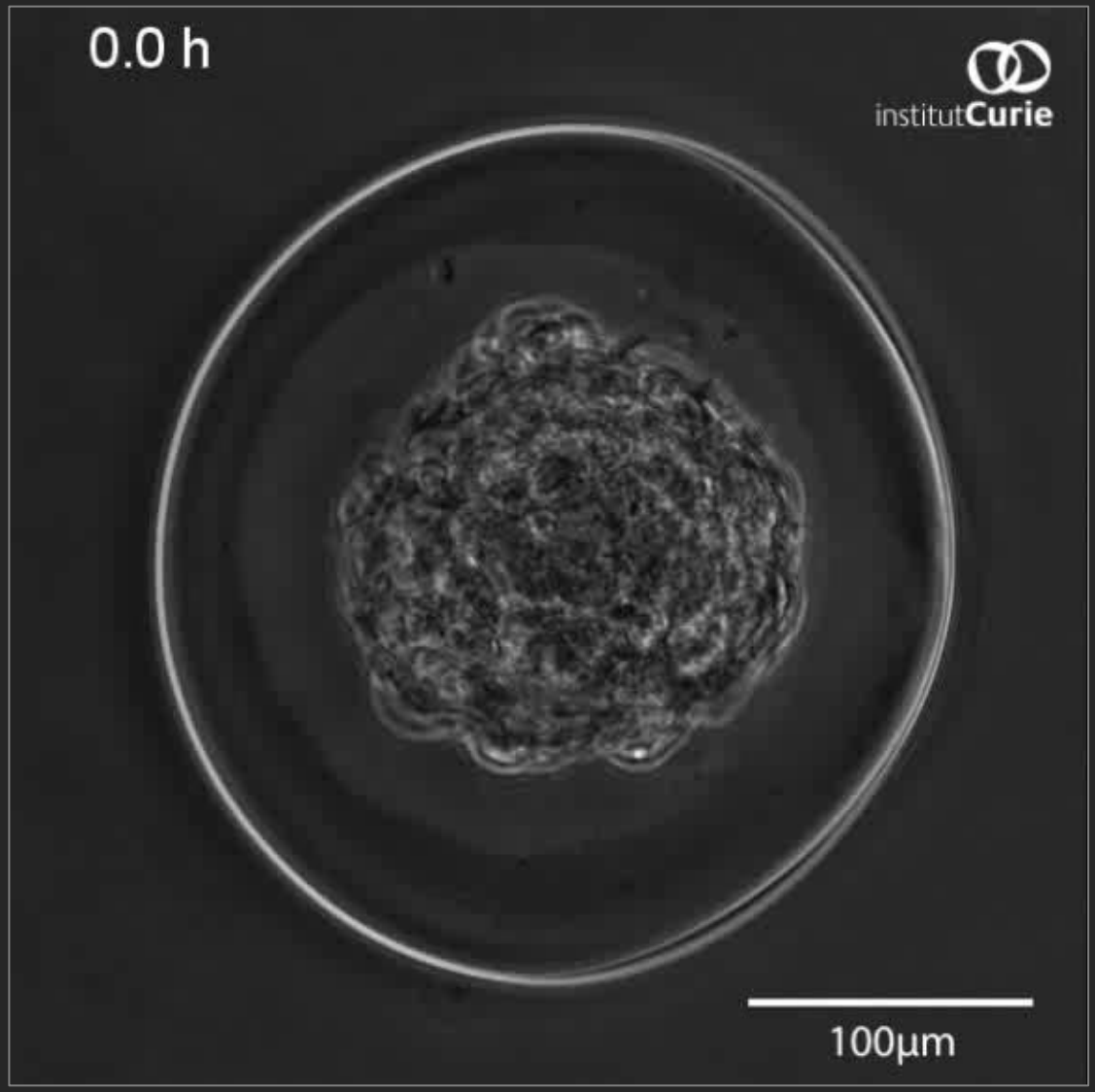


Long times : surface growth

Necrotic core + proliferative rim

$$R \cong v \cdot t$$

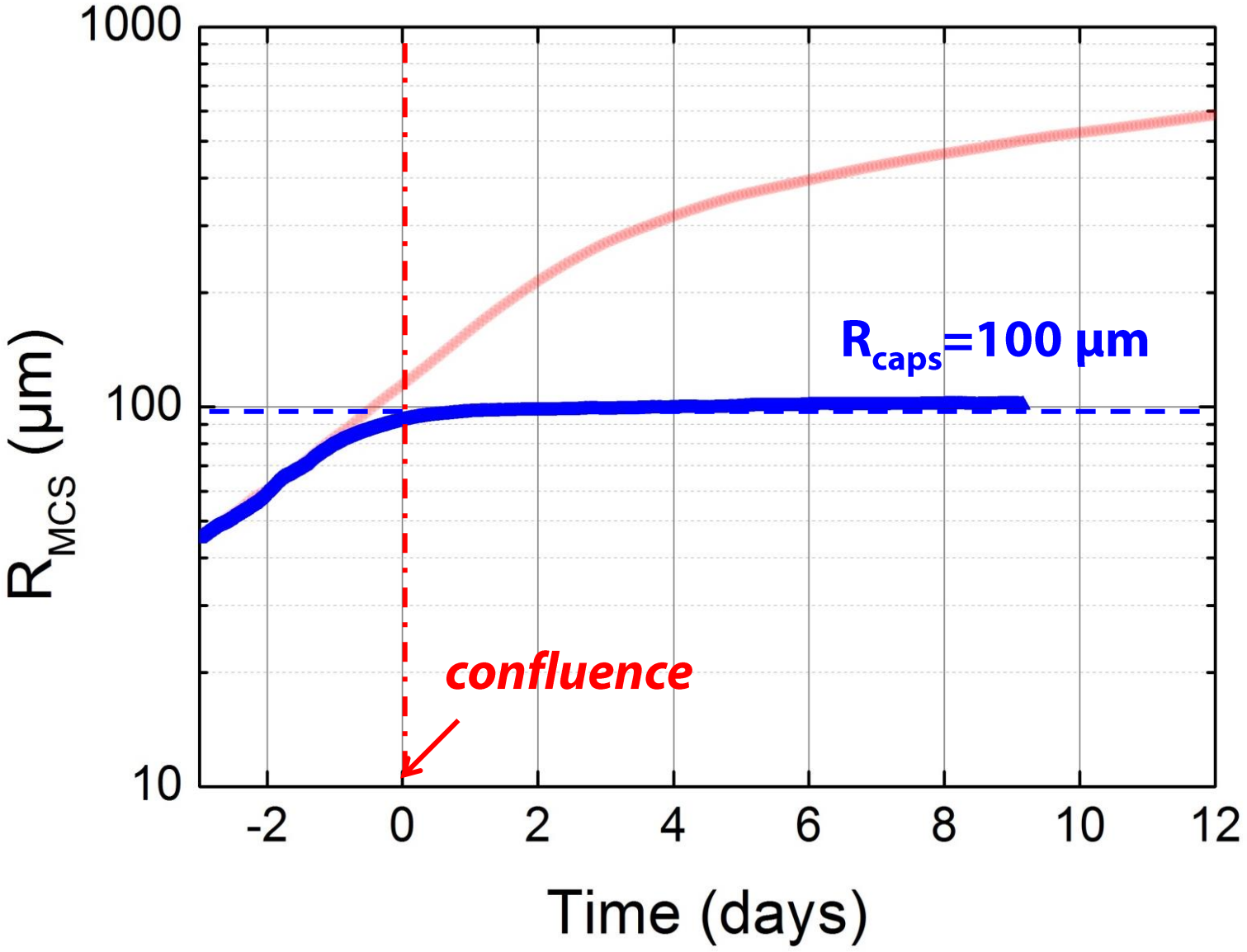
In capsulo cell growth - Formation of confined spheroids



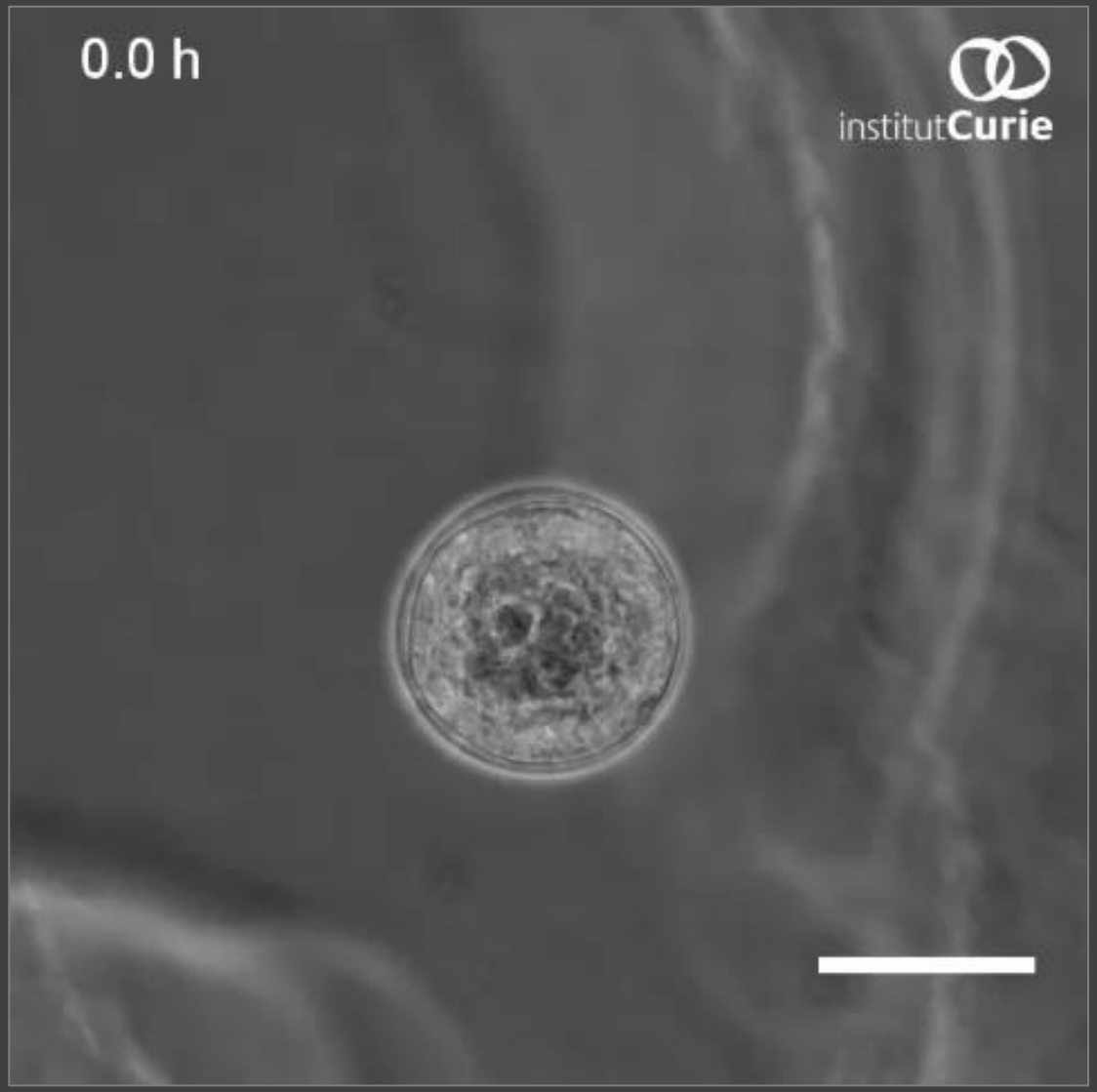
CT26 mouse colon carcinoma cells

« Thick » capsule h~35 µm

Growth kinetics of confined spheroids



Post-confluent spheroid evolution

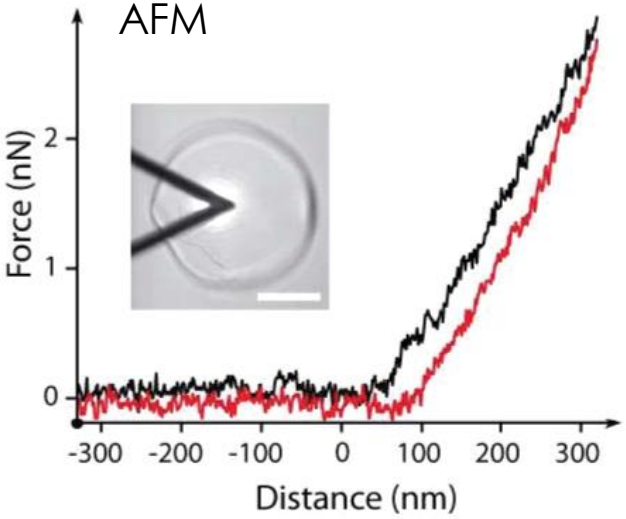


[Same size,
larger field of view]

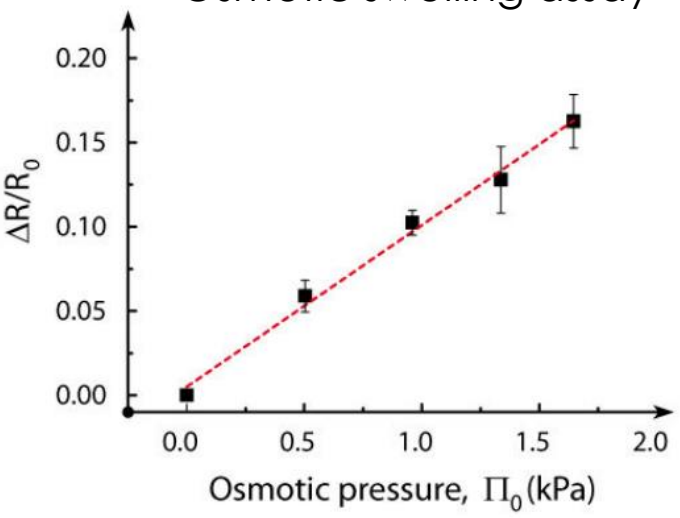
« Thin » capsule h~7 µm

Alginate gels are elastic and stiff

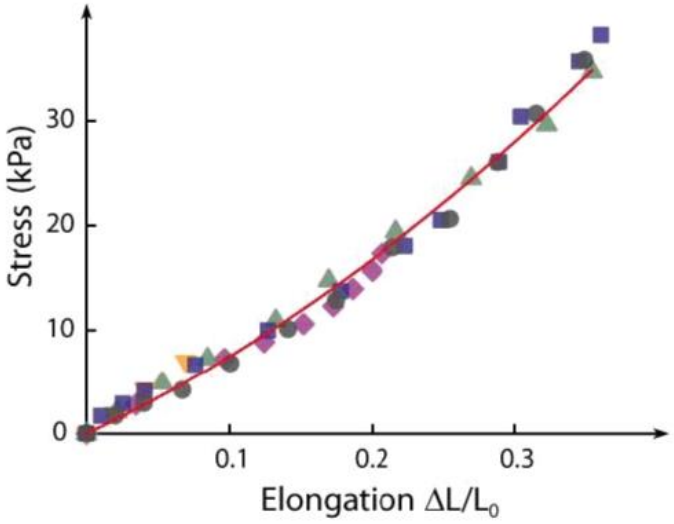
AFM

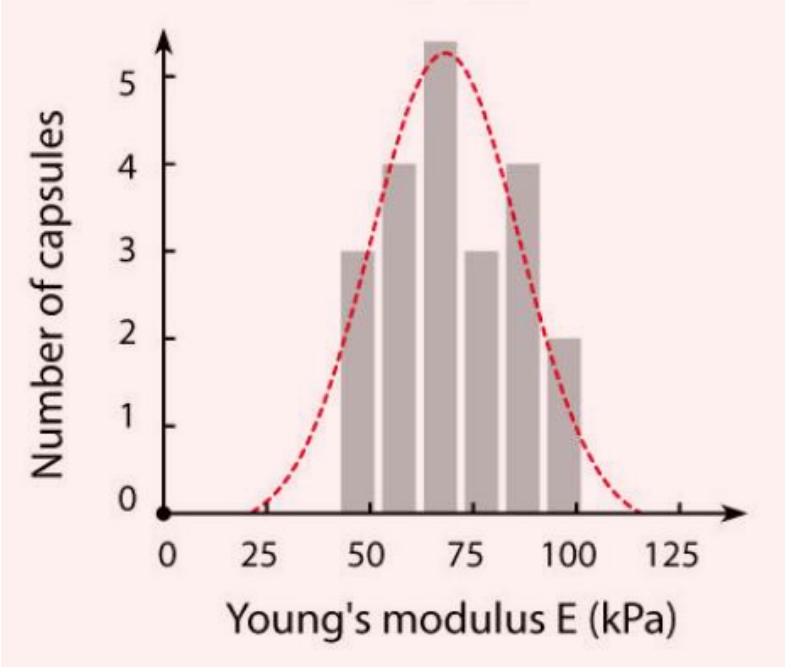


Osmotic swelling assay

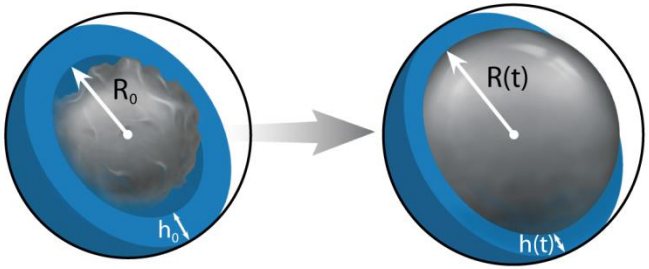


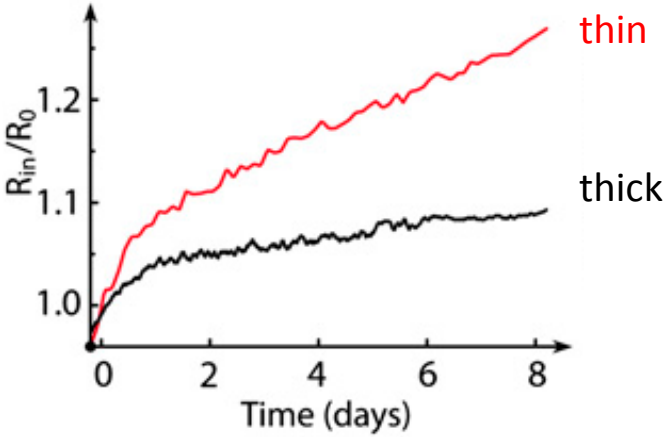
Stretching of alginate threads

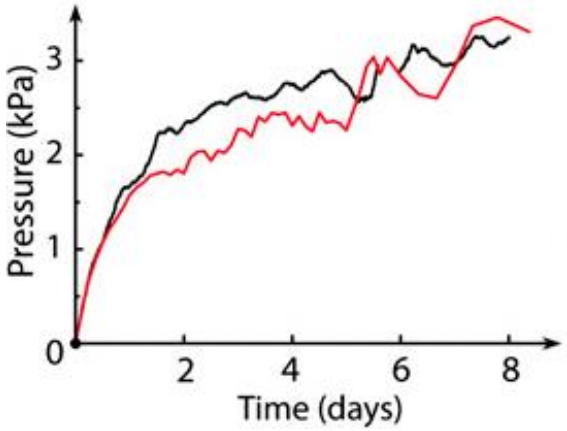


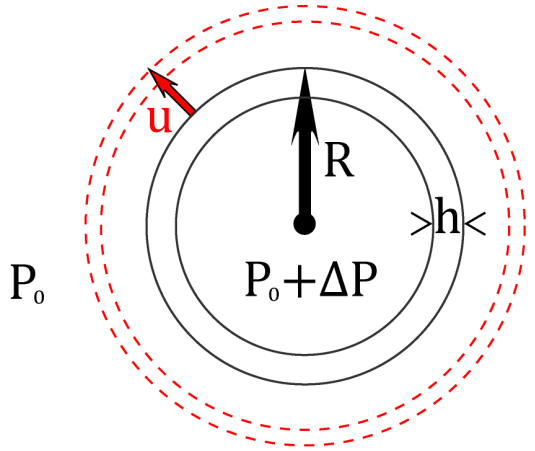


Capsules can be soft and used as pressure sensors





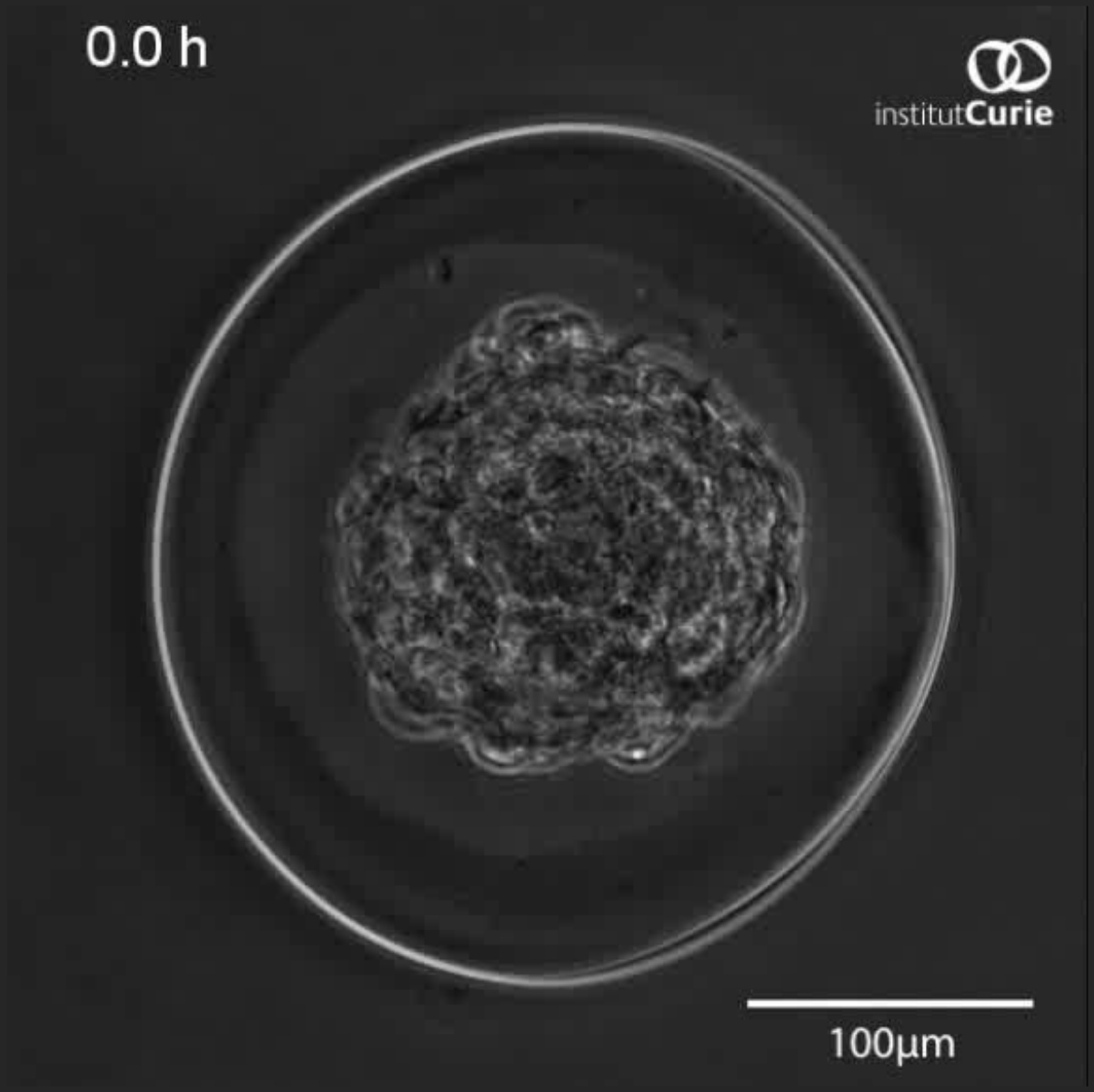




Dilation of a pressurized balloon

$$\Delta P = \frac{2E}{(1-\sigma)} \frac{h}{R} \frac{u}{R}$$

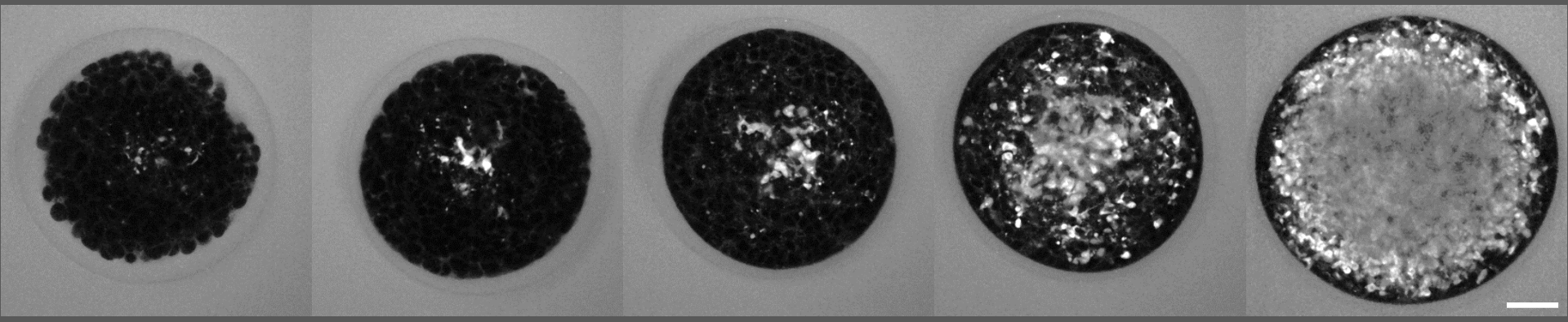
Post-confluent spheroid growth



Influence of confinement on the internal cellular organization of spheroids

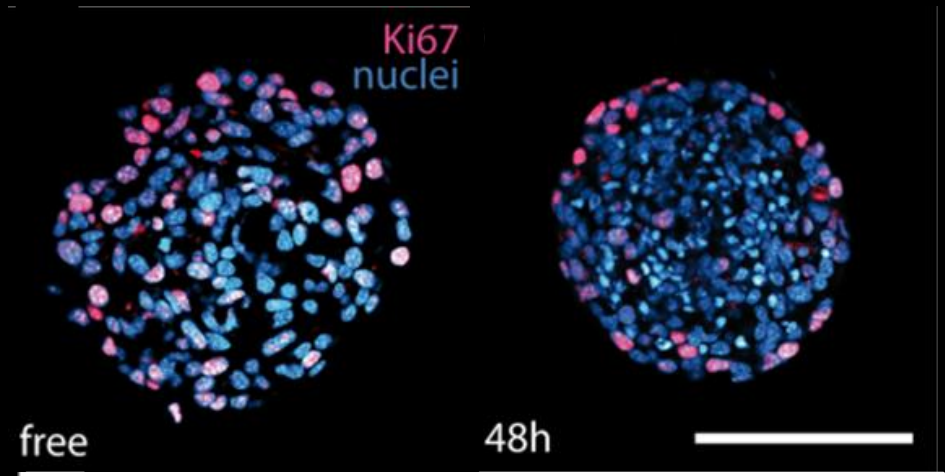
2-photon microscopy

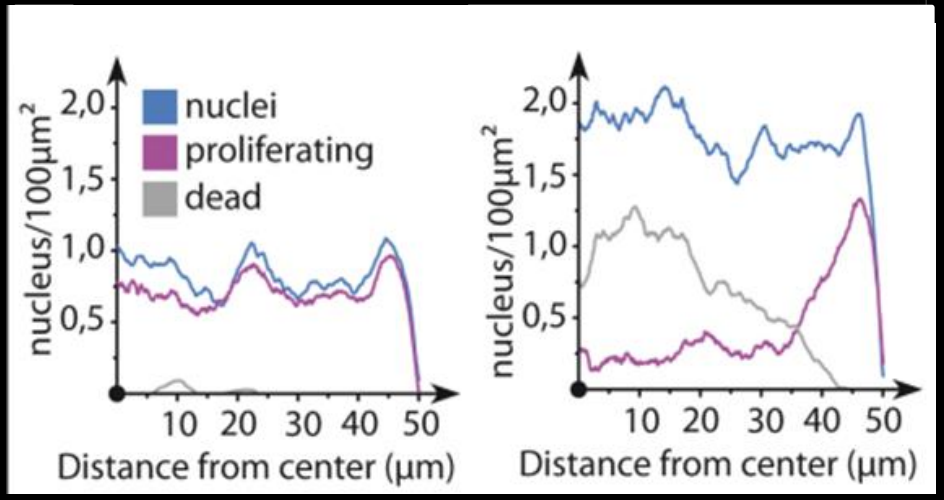
Sulforhodamin B : *labels dead cells and extracellular proteins*



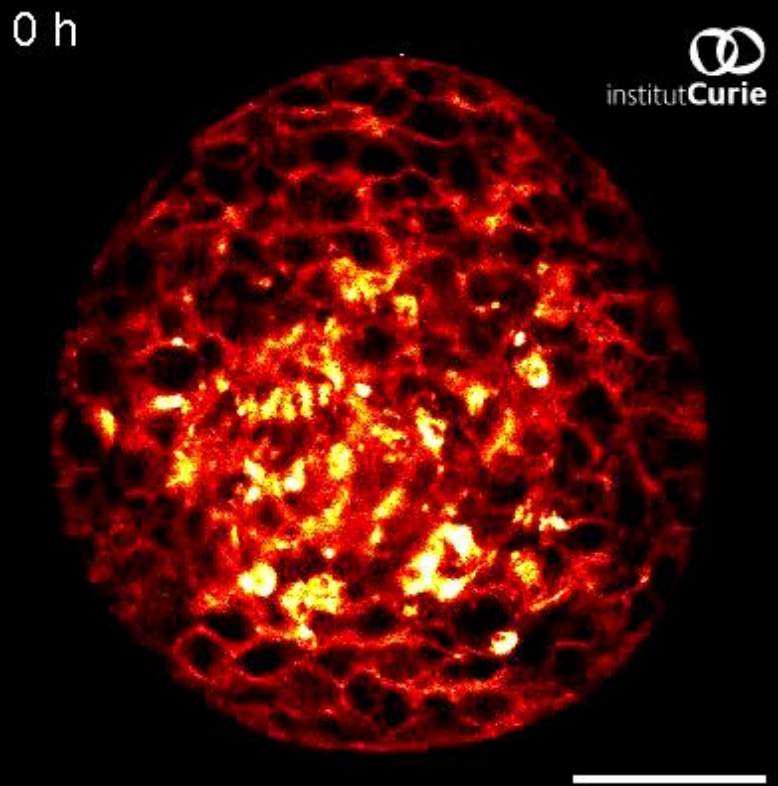
0h 8h 15h 26h 144h

Influence of confinement on the internal cellular organization of spheroids





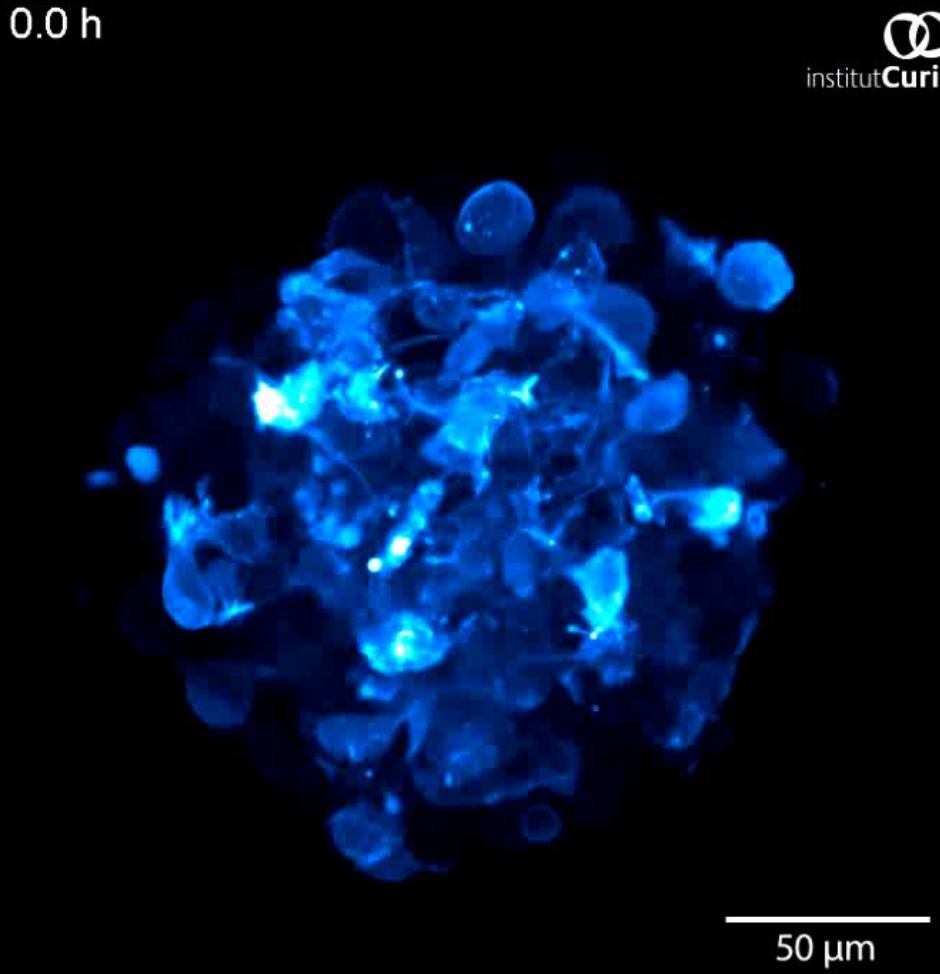
Compaction

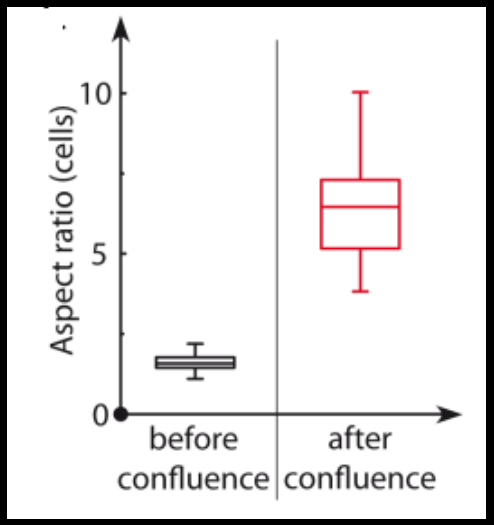


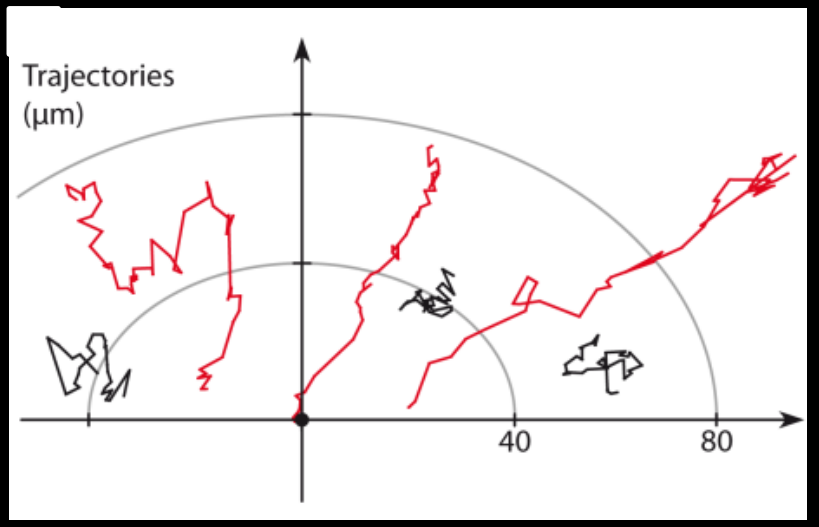
Core necrosis

FM4-64 labelling
Cell membranes Necrotic cells

Peripheral cells exhibit long protrusions and acquire enhanced motility upon confinement



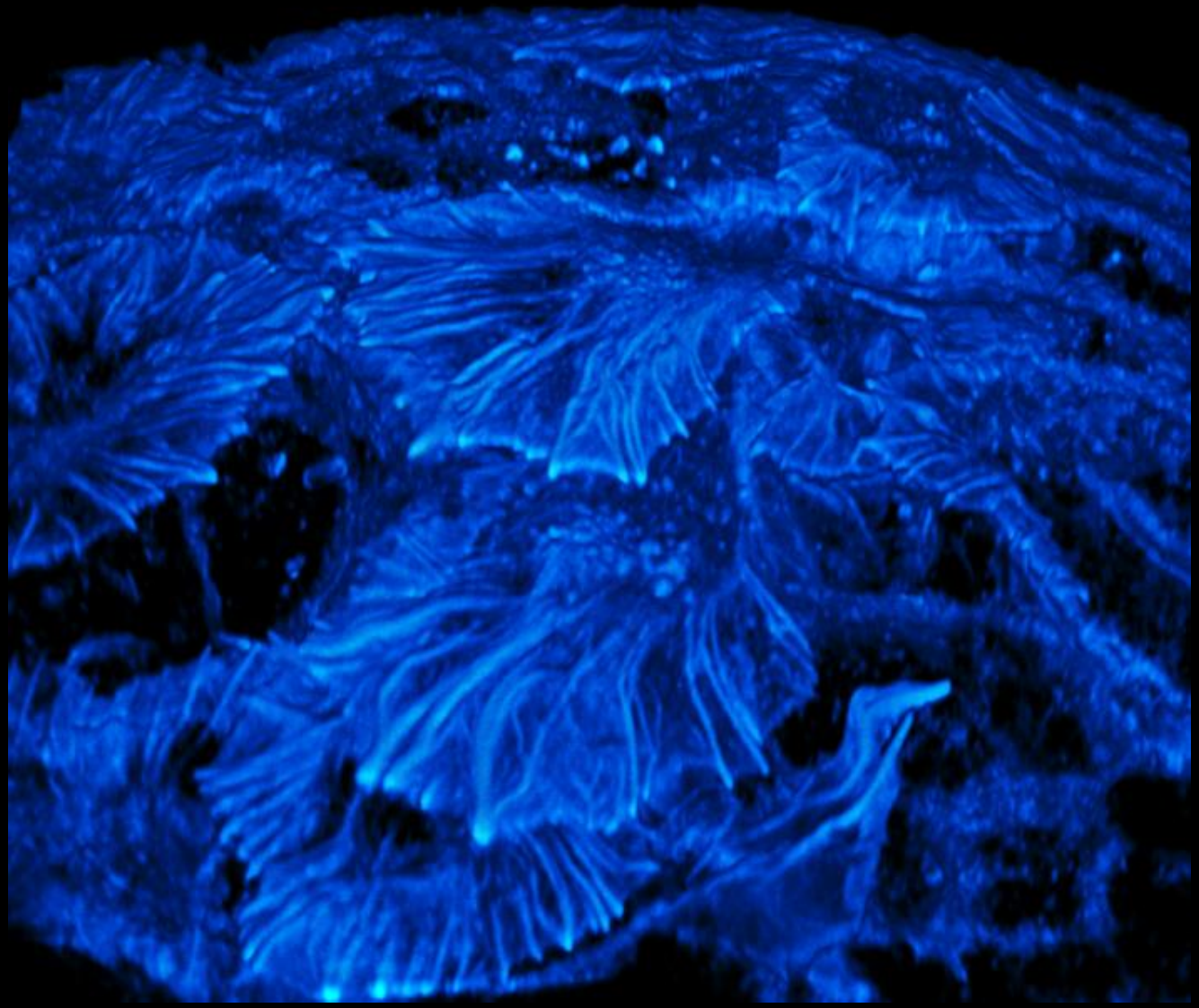




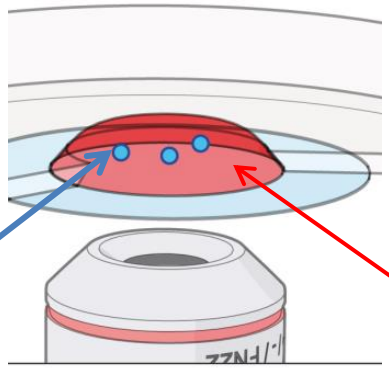
1photon confocal microscopy
LifeAct-mCherry
CT26 cells

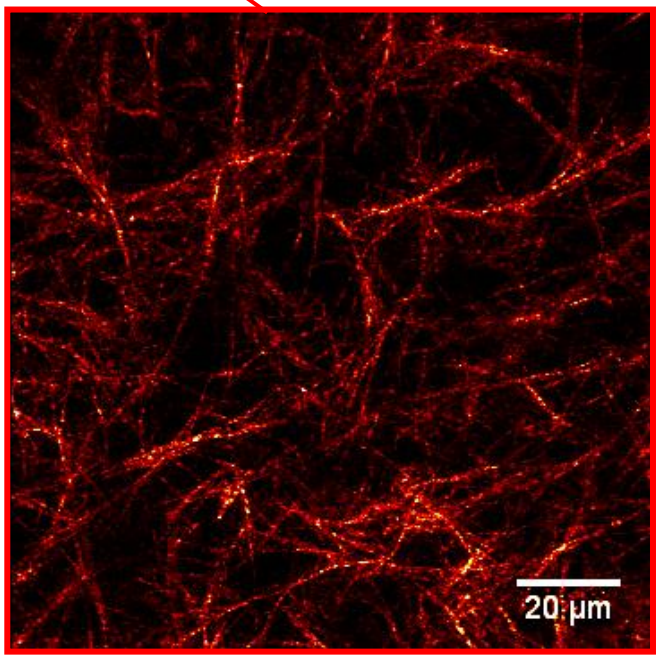
Fixed spheroid

Phalloidin Alexa488
(Actin bundles)



Invasion assay

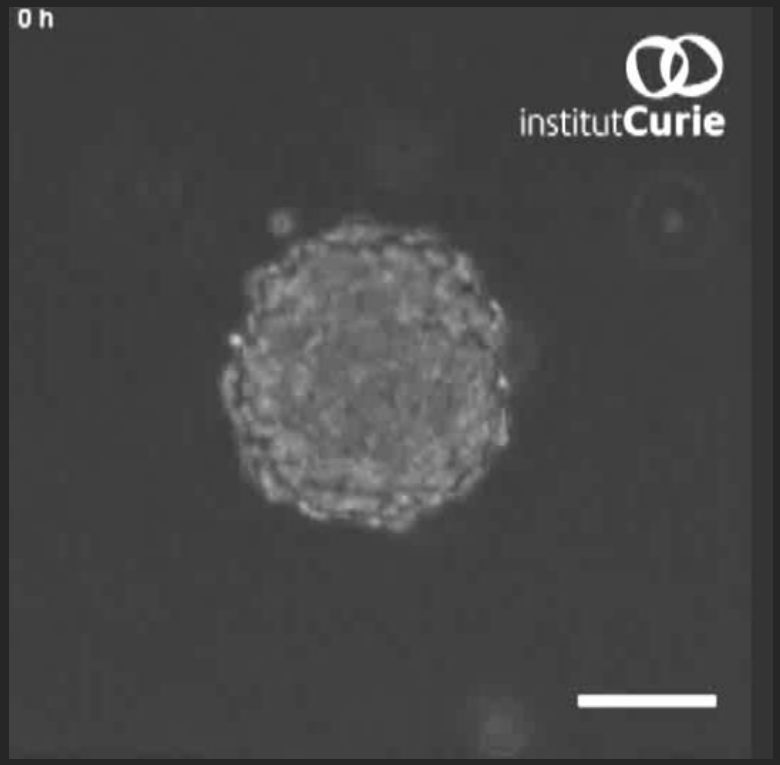




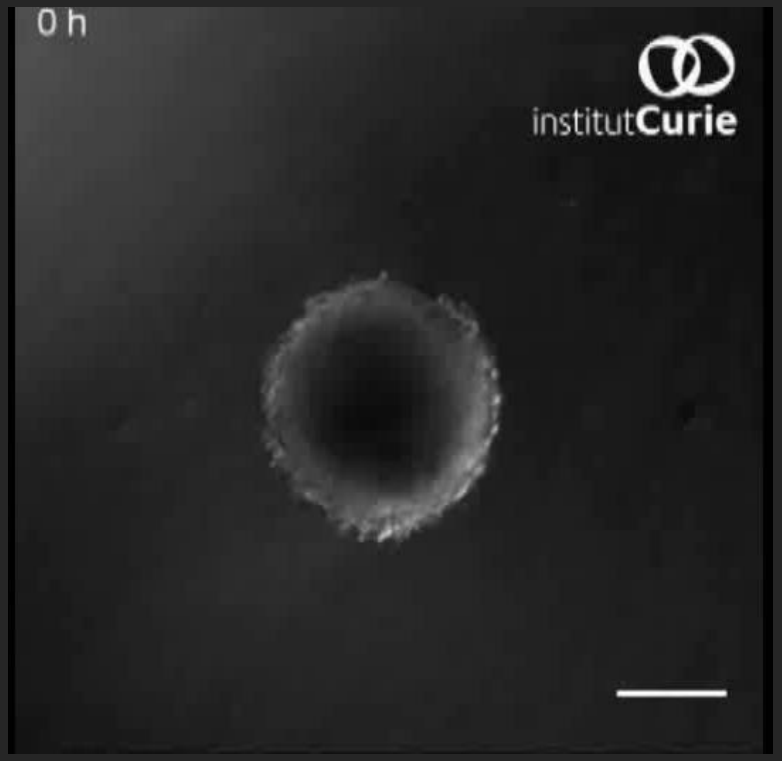
Bare spheroid
(after incubation for 5 min in PBS)

Collagen I matrix (2mg/ml)

Invasion assay

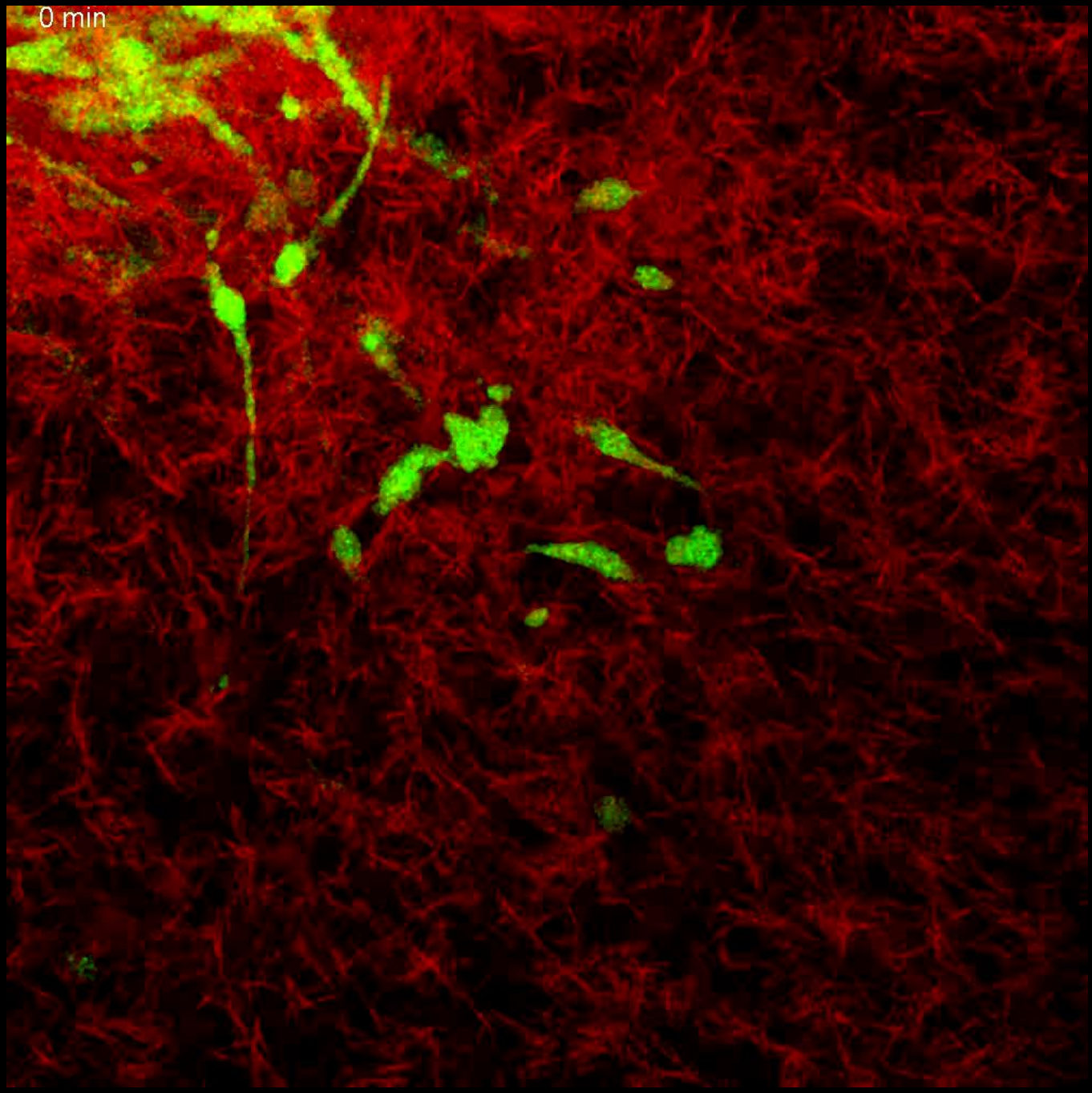


Freely grown spheroid



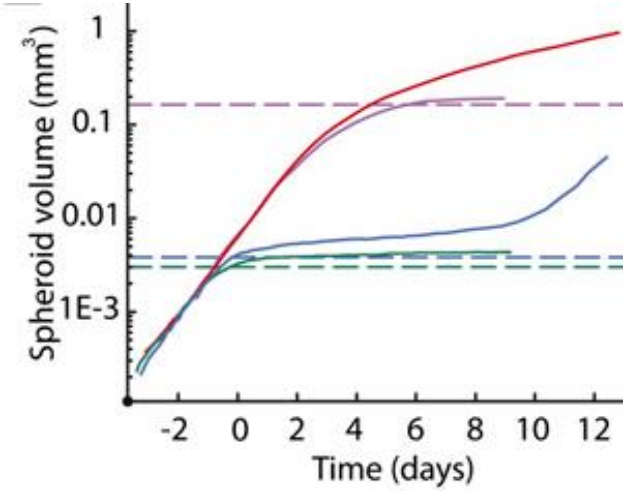
Pre-compressed spheroid

Invasion assay

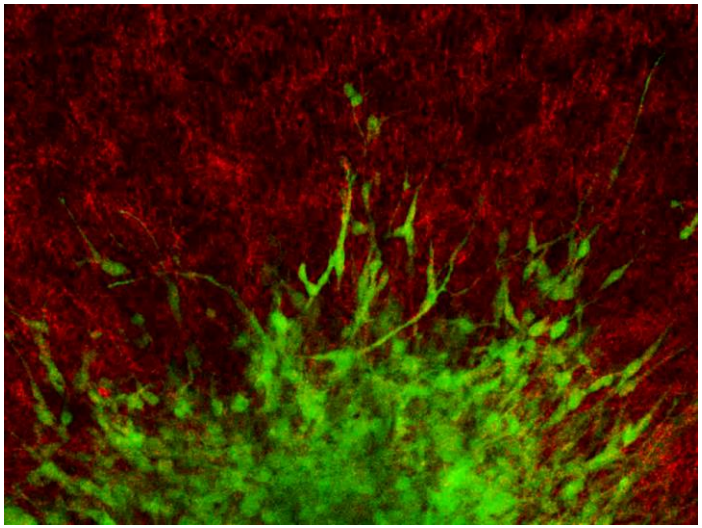


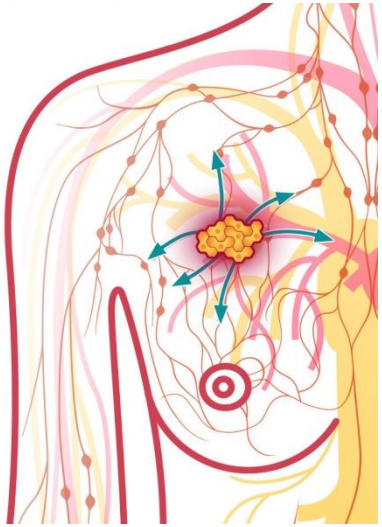
Take-home messages for cancer biologists

1. Confinement (or compressive stress) slows down tumor progression

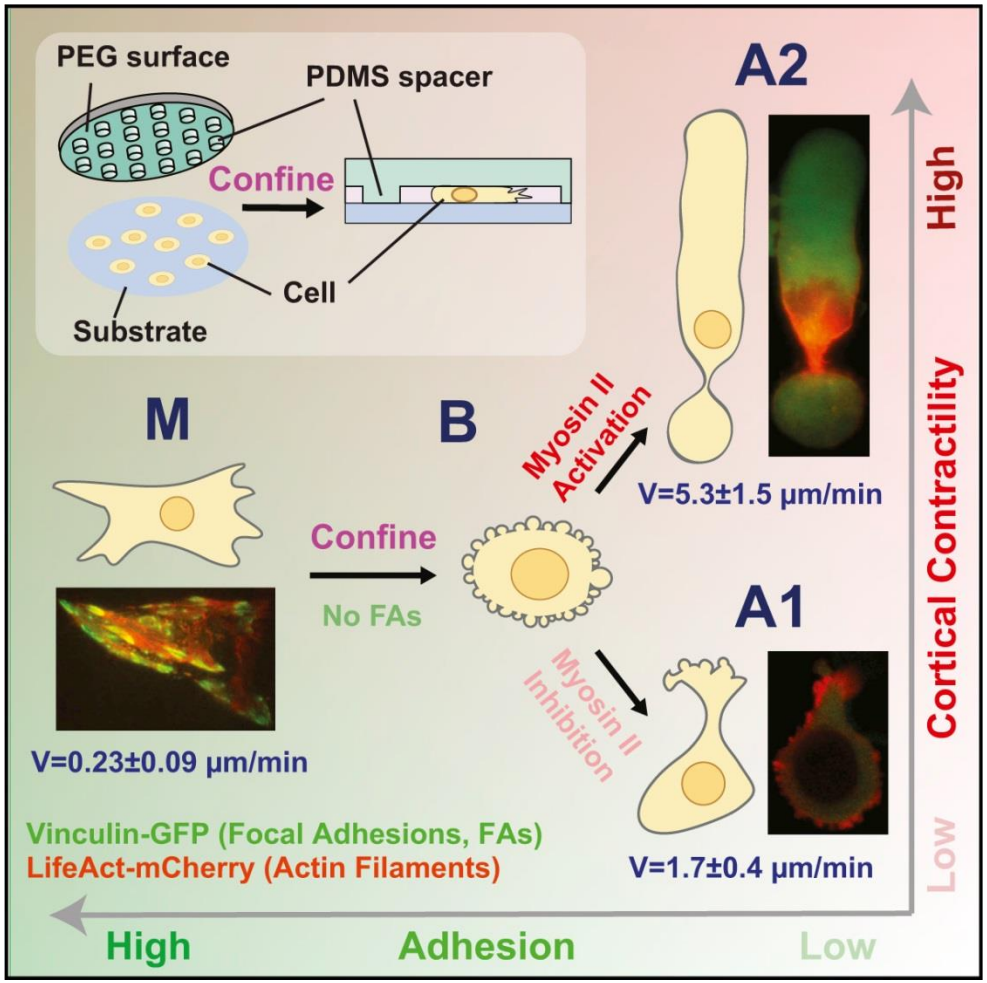


2. Elastic confinement (or compressive stress) may trigger cell escape (metastasis)





Can mechanical stress enhance cell motility?



A large range of slow mesenchymal cells can switch to fast amoeboid-like migration under conditions of low adhesion and strong confinement, suggesting that tumor cells may spontaneously escape primary tumors and invade tissues without any specific genetic alteration.

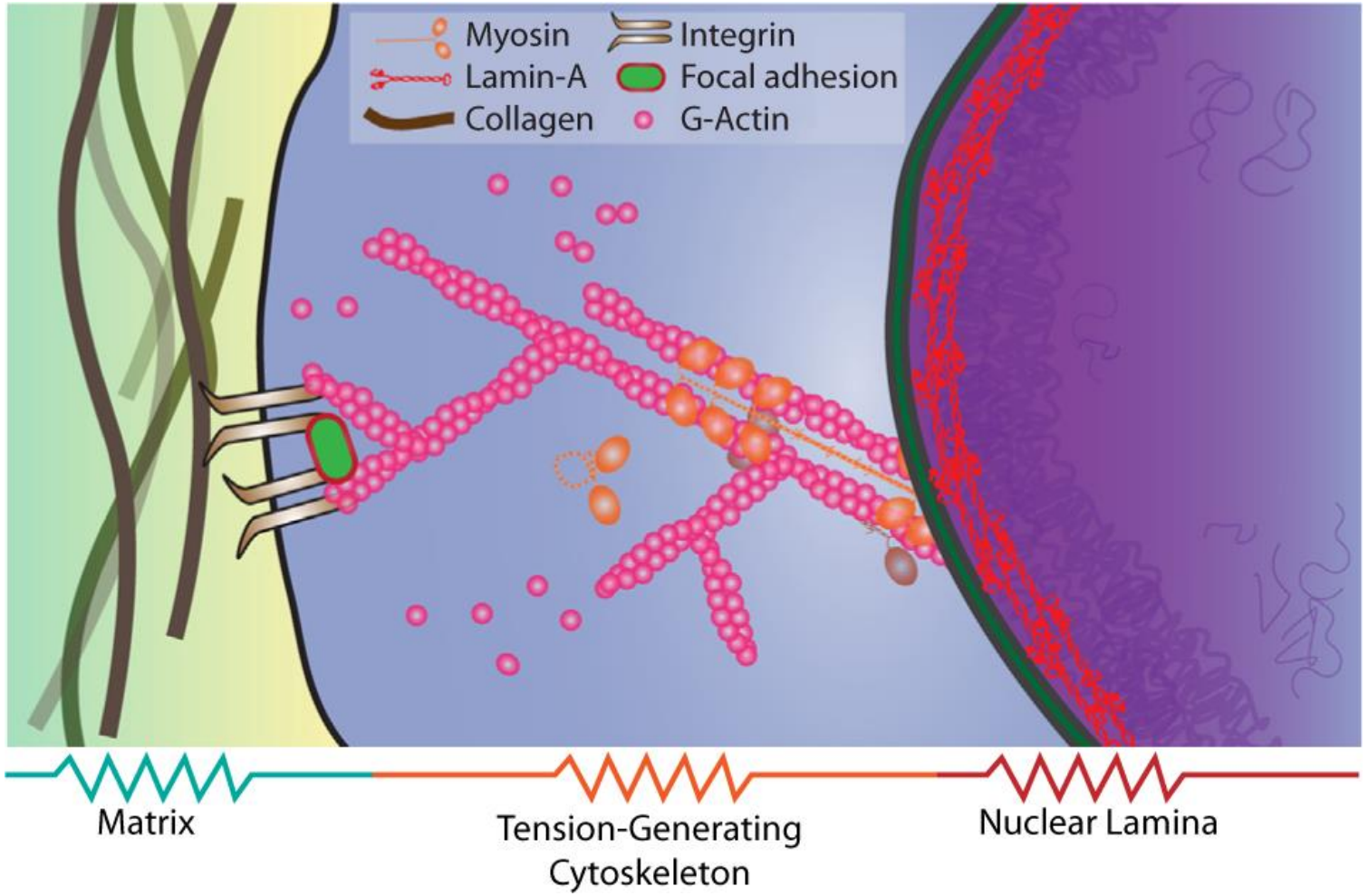
Confinement and Low Adhesion Induce Fast Amoeboid Migration of Slow Mesenchymal Cells

Authors

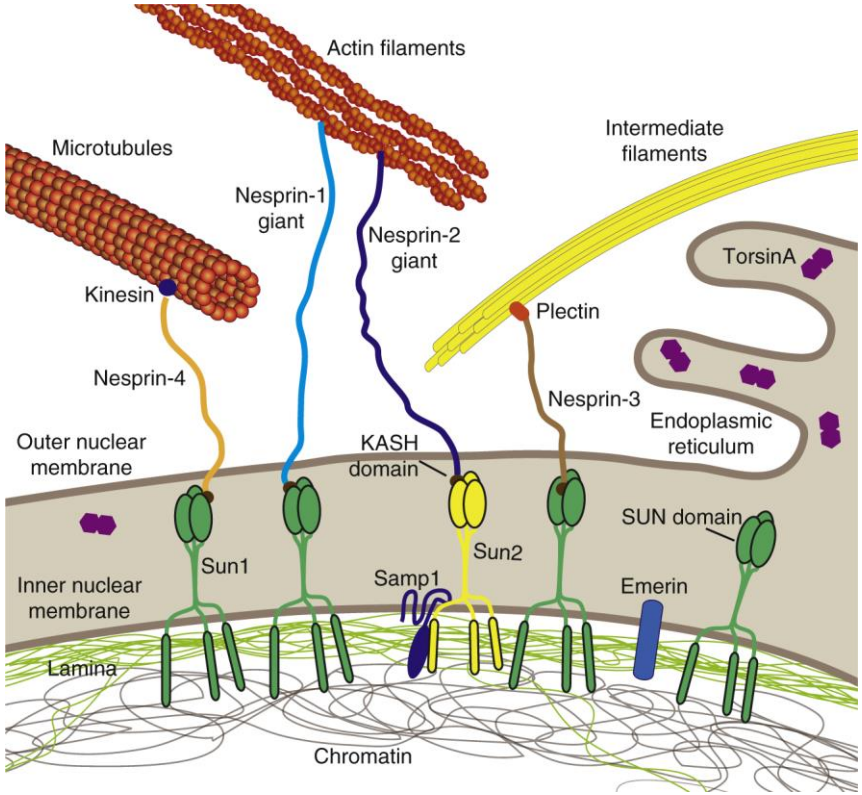
Yan-Jun Liu, Maël Le Berre, ..., Raphaël Voituriez, Matthieu Piel

Cell 2015

Connection between cytoskeleton and nucleus



Molecular details

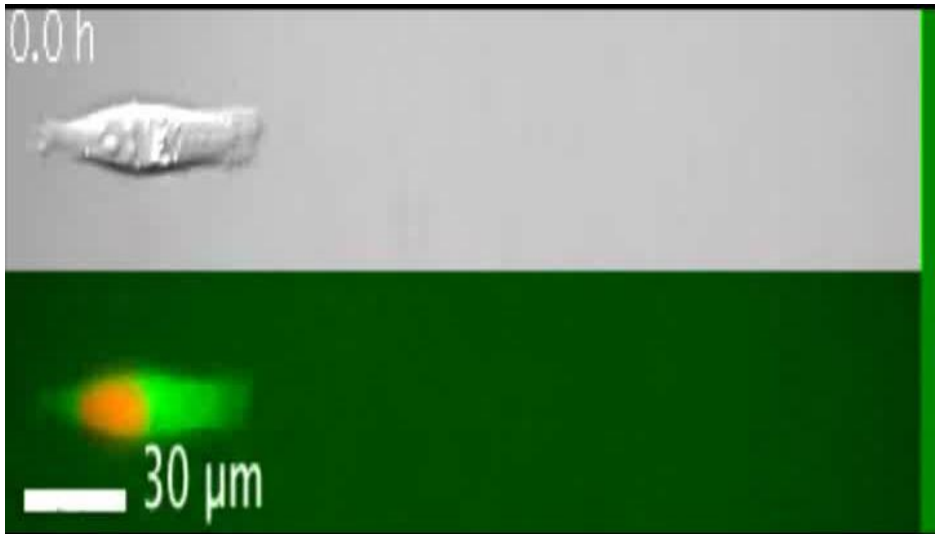


Jan Lammerding's lab

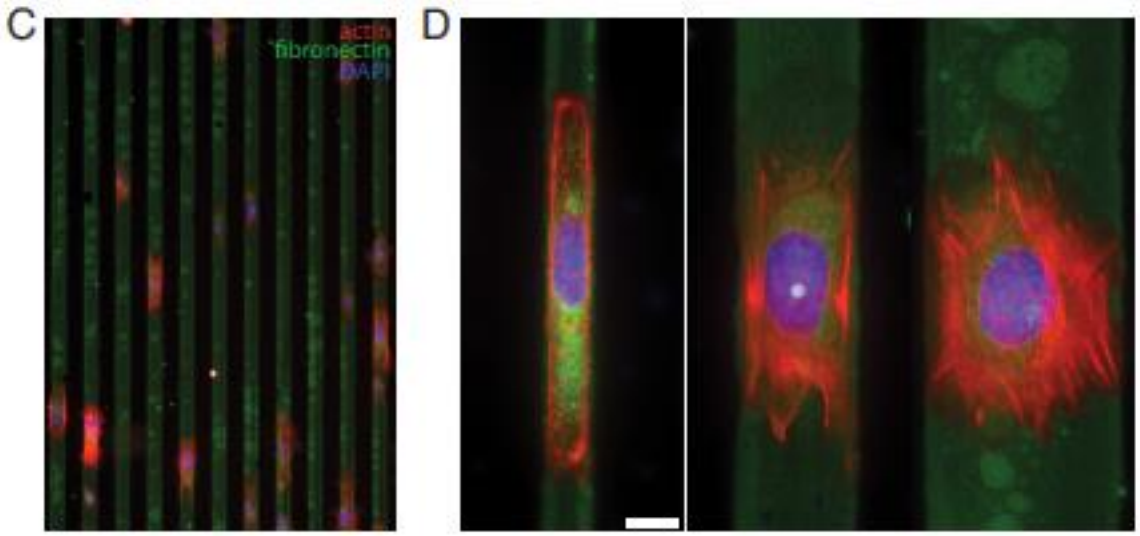
Figure 1. Schematic overview of LINC complex proteins and their connections to the cytoskeleton and nuclear interior.

SUN proteins at the inner nuclear membrane bind to the nuclear lamina and other nucleoplasmic proteins while interacting with KASH domain proteins at the outer nuclear membrane. KASH domain proteins directly or indirectly interact with cytoskeletal filaments, thereby forming a physical connection between the nuclear interior and cytoskeleton. Please note that SUN and KASH domain proteins can exist in multiple isoforms encoded by several genes. In human somatic cells, the most predominant KASH domain proteins are nesprin-1, -2, and -3 and their various isoforms, and Sun1 and Sun2 are the predominant SUN proteins [16]. Illustrated are only the largest isoforms for nesprin-1–4; cells express many additional shorter nesprin isoforms, including some lacking the KASH domain. Smaller nesprin isoforms may also be located on the inner nuclear membrane. Note that nesprin-1, -2, -4 and KASH5 can also interact with kinesin and/or dynein. Samp1 and torsinA are involved in the regulation of the LINC complex. Not depicted are KASH5 and the SUN protein isoforms Sun3–5, as their expression is restricted to germ cells. The nuclear lamina comprises A-type and B-type lamins. Note that torsinA can be localized in the endoplasmic reticulum and the perinuclear space, with the distribution varying depending on expression levels.

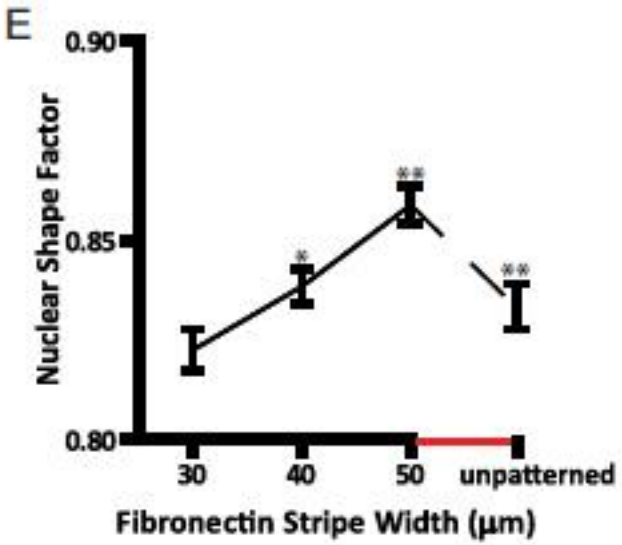
Evidence from cell guidance

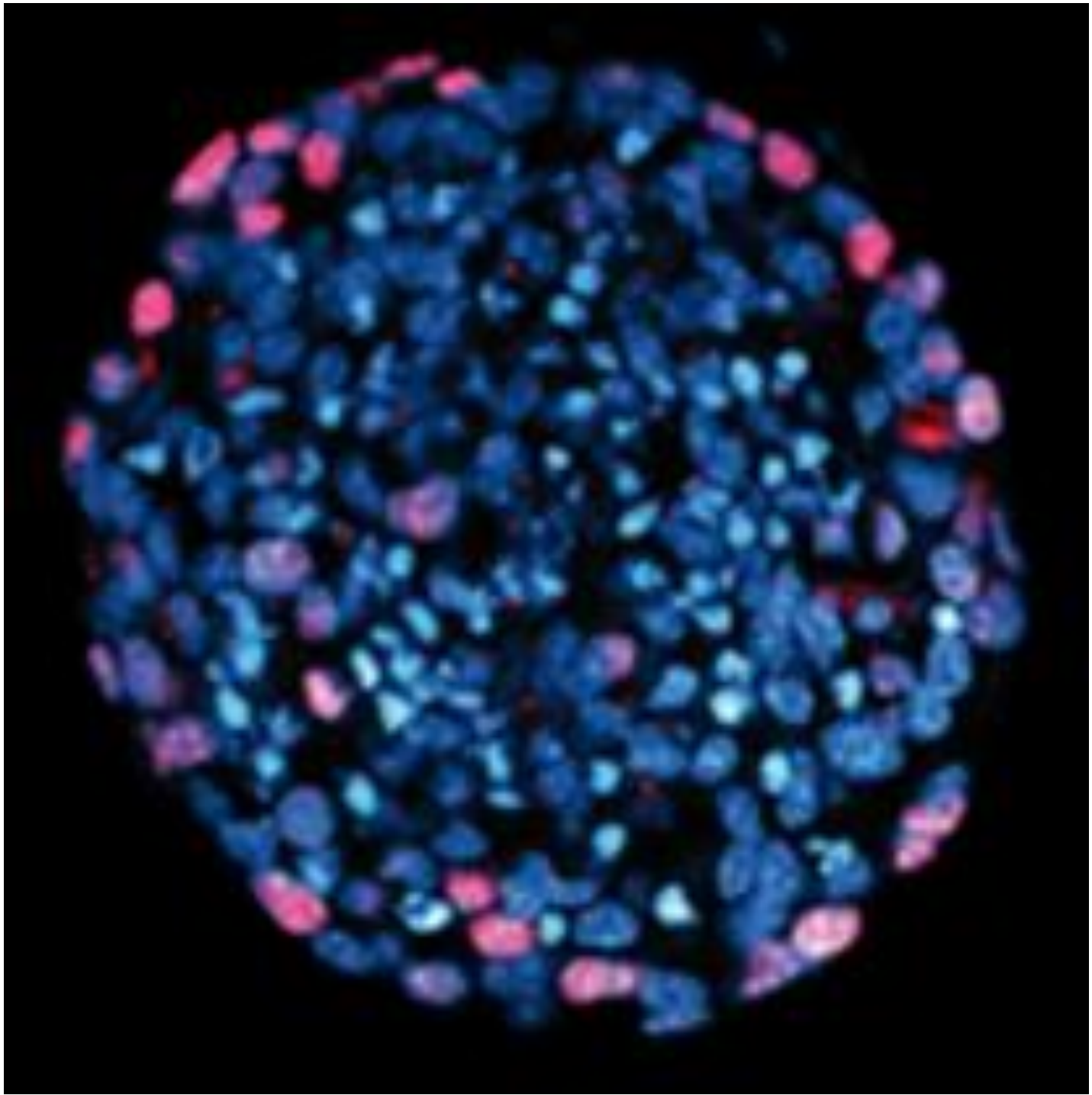


Manuel Théry – Matthieu Piel

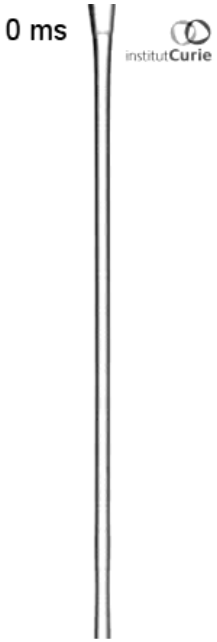


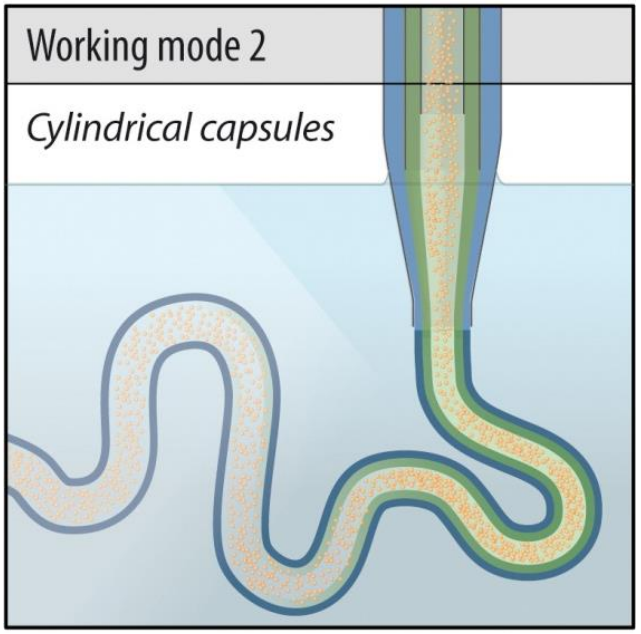
Denis Wirtz, USA

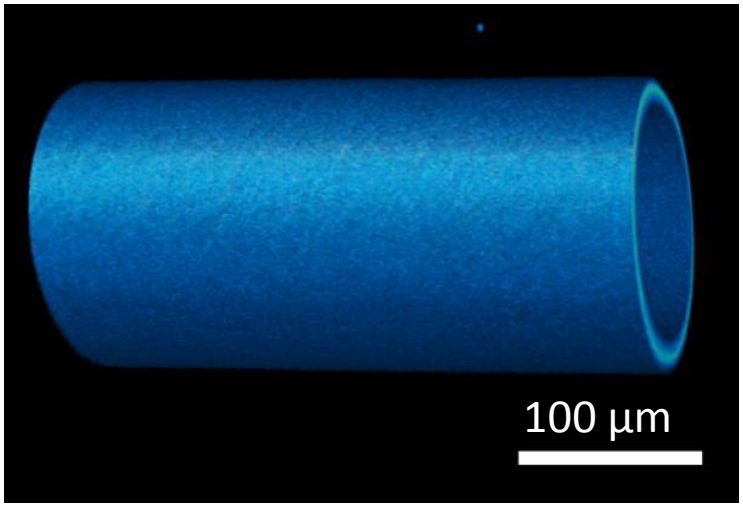


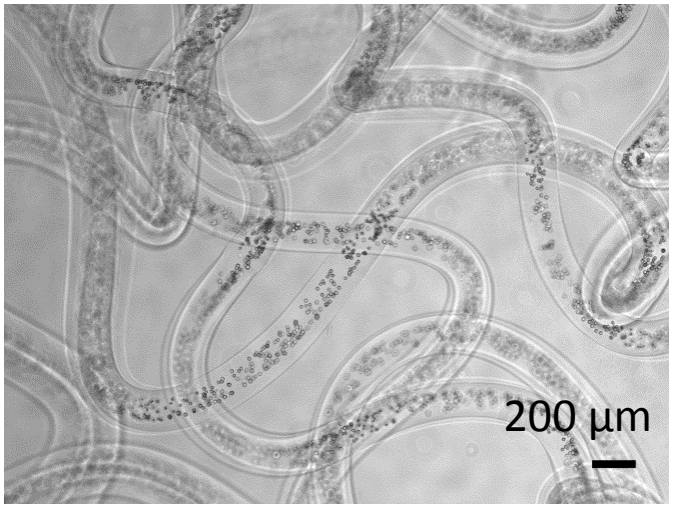


Long micro-tubes, … Sausages, micro-canelloni, cylindroids



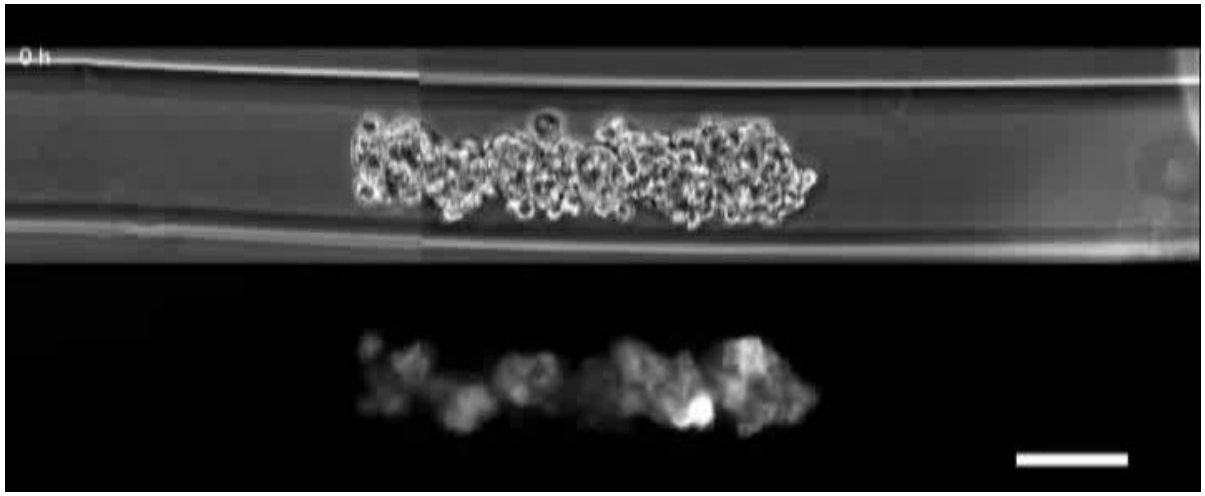






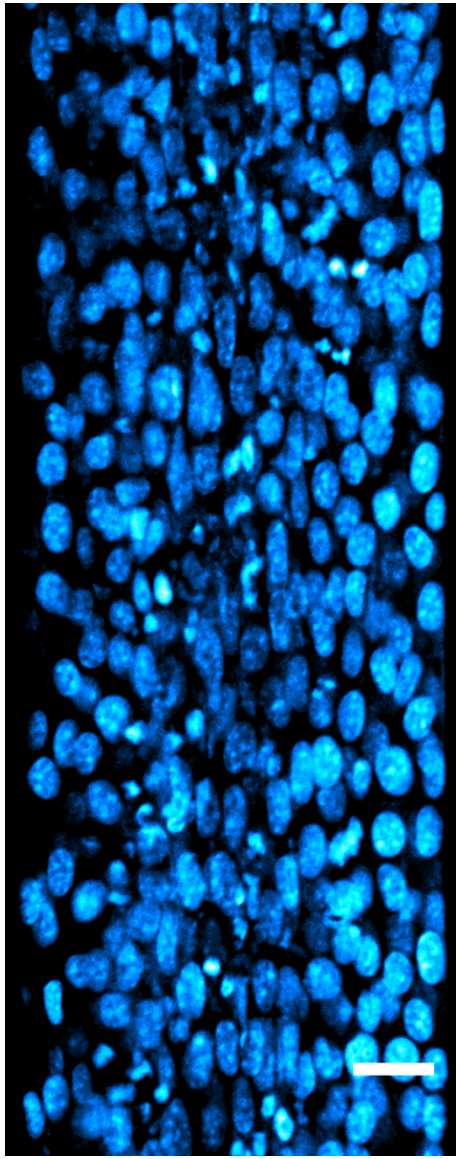
Insight from « 1D » growth of cylindroids?

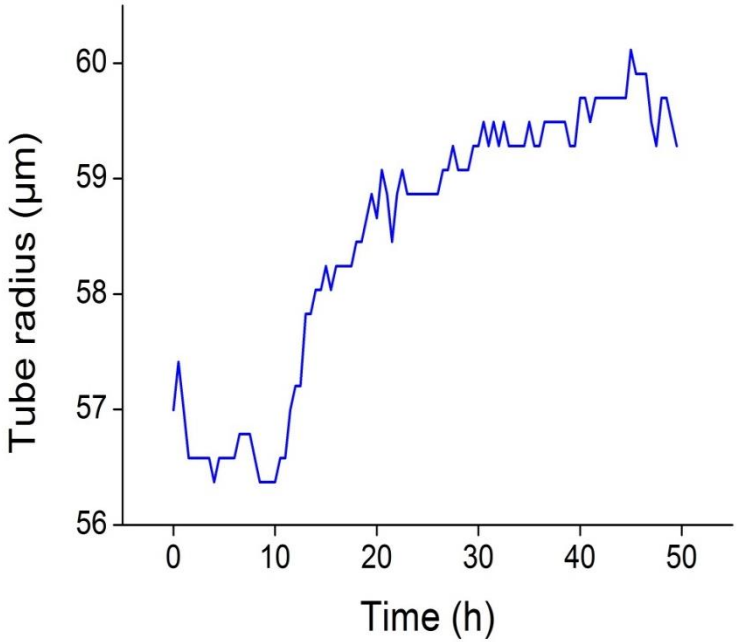
Single cylindroid



Coalescence of cylindroids

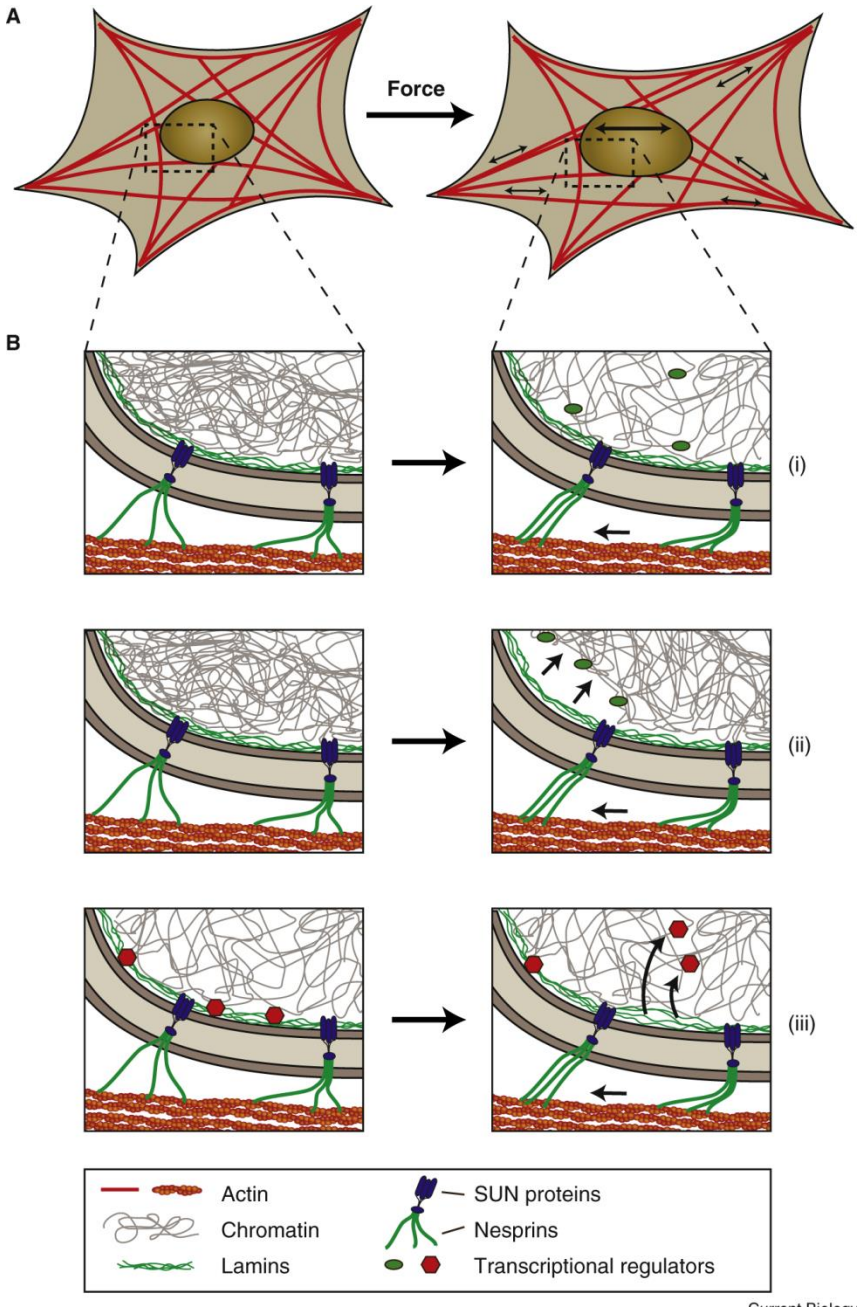
Pressure builds up in open tubes



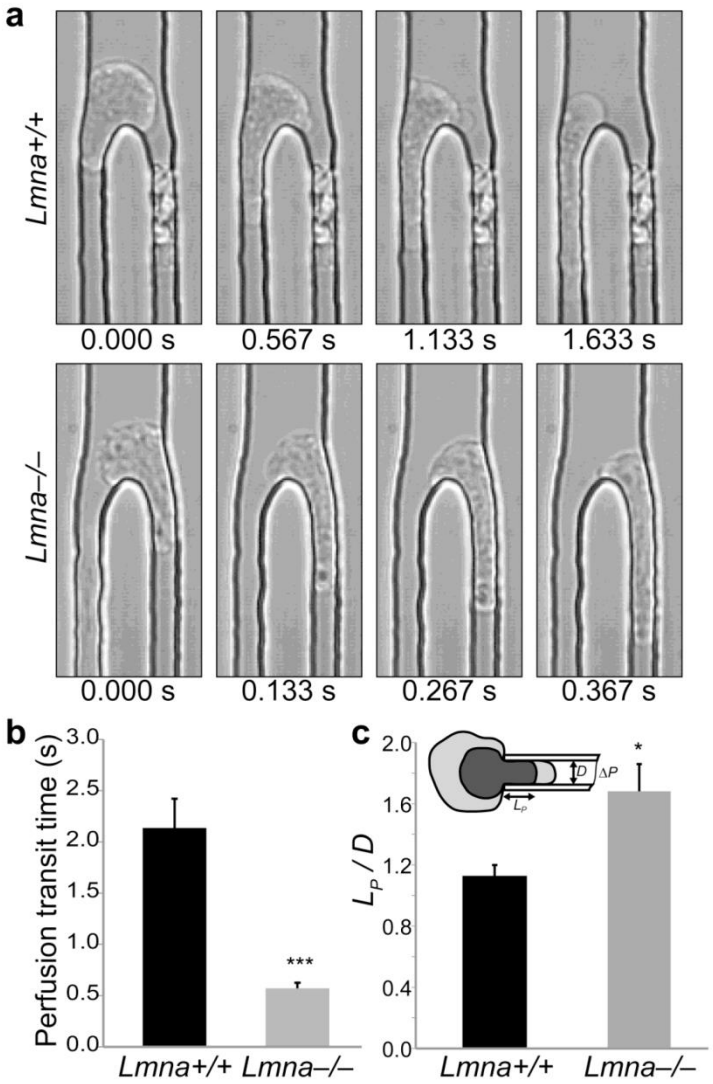


DAPI staining

Potential mechanisms of nuclear mechanosensing



Schematic illustration of how force-induced nuclear deformation could modulate expression of mechano-responsive genes. (A) A cell exposed to a uniaxial stretch, resulting in nuclear deformation by forces transmitted from focal adhesions through the (actin) cytoskeleton to the nucleus. (B) Potential molecular mechanisms for nuclear mechanosensing. (i) Opening of chromatin structures under force, enabling access of transcriptional regulators to the chromatin. (ii) Chromatin detachment from the lamina, freeing genes from the often transcriptionally repressive nuclear periphery; this process could also result in further changes in chromatin structure, promoting access to transcriptional regulators. (iii) Stretching the lamina could result in conformational changes or partial unfolding of lamins, altering their interaction with transcriptional regulators. Shown here is the release of transcription factors, which can then interact with their target genes. Phosphorylation and other post-translational modifications of nuclear envelope proteins could further contribute to nuclear mechanosensing.



Lamin A/C-deficient cells have more deformable nuclei

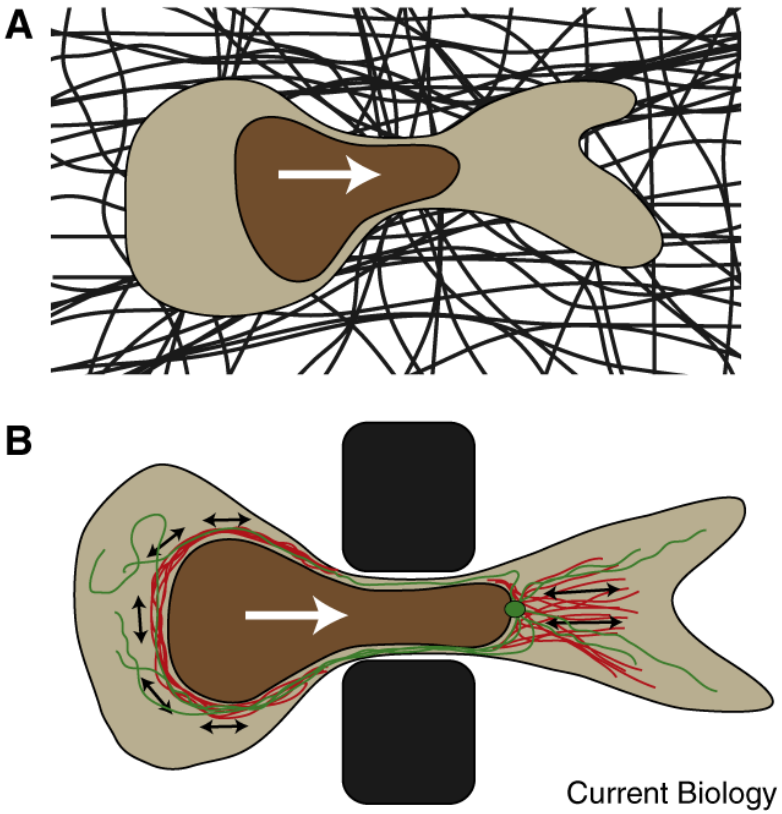
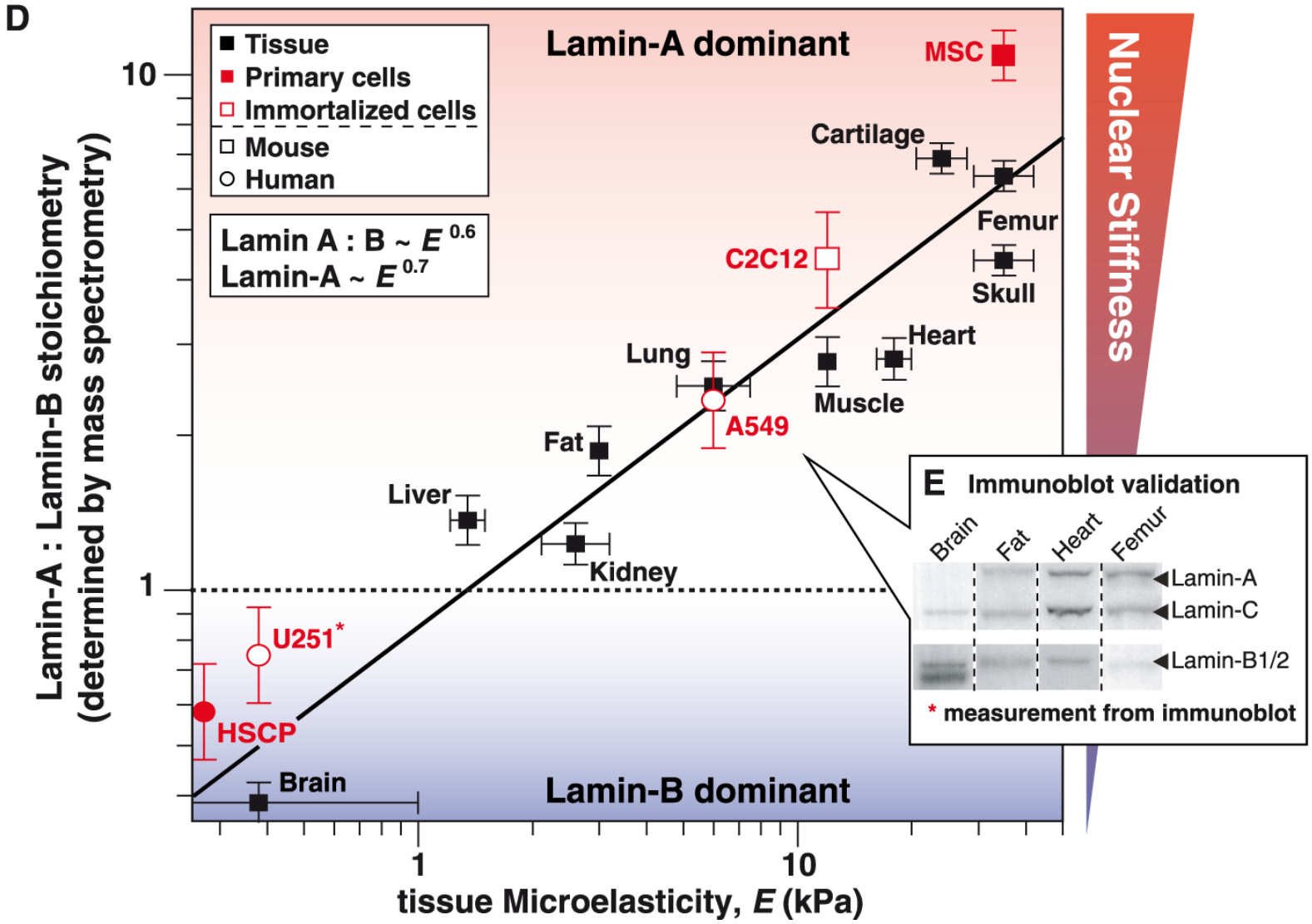
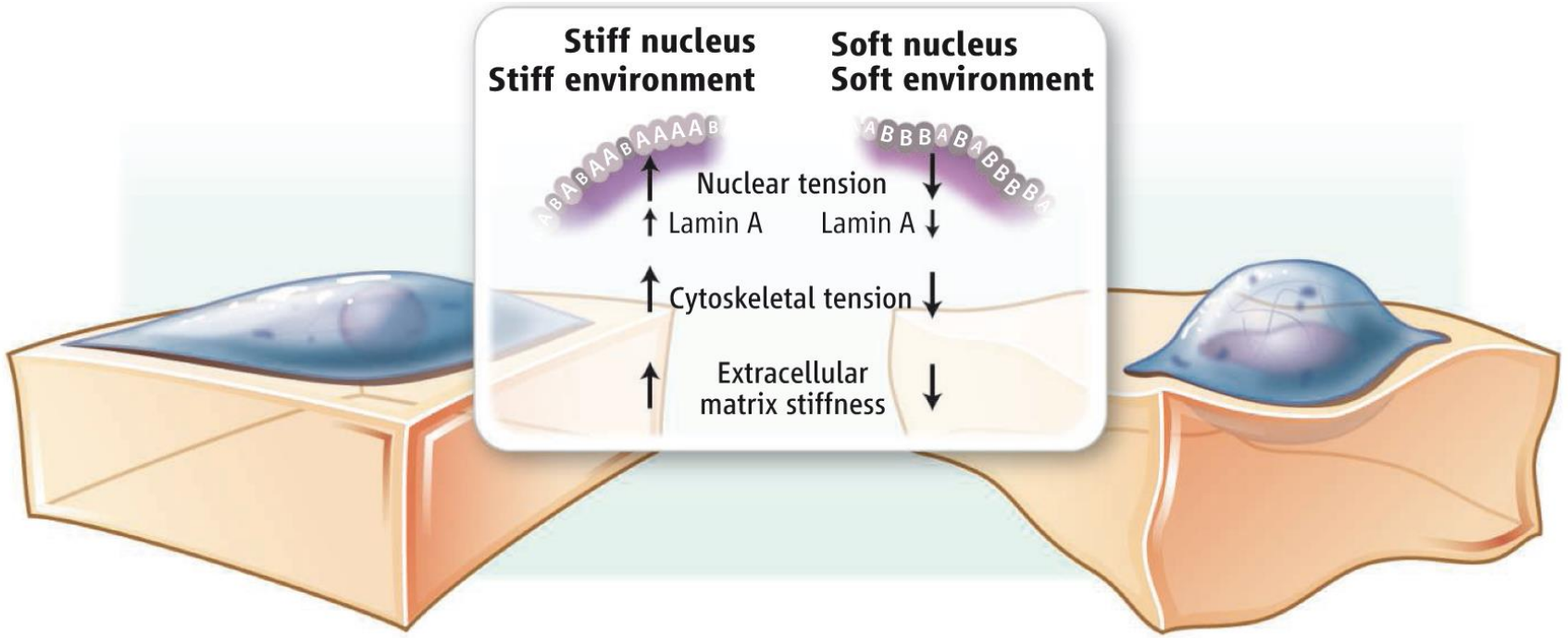


Figure 4. Nuclear deformation during cell migration through tight constrictions.

(A) Schematic depiction of a cross-section of a cell migrating through a constriction in the dense extracellular matrix (dark fibers) that is smaller than the nuclear diameter. The white arrow denotes the direction of cell migration. The nucleus is depicted in brown. (B) Side view of a cell migrating through a polycarbonate filter or microfabricated device used to study nuclear deformation during cell migration through precisely defined pores. Illustrated in red are actin–myosin networks, applying contractile forces (black arrows) to the nucleus, either posterior to the nucleus, resulting in a pushing force, or anterior, pulling on the nucleus. Molecular motors on the microtubule network (green, with centrosome) may apply additional forces to the nucleus, particularly during neuronal migration. White arrow indicates the direction of migration.


D
Tissue
Primary cells
Immortalized cells
Mouse
Human
Lamin A : B ~ E 0.6
Lamin-A ~ E 0.7
Lamin-A dominant
Lamin-B dominant
MSC
Cartilage
Femur
Skull
C2C12
Heart
Muscle
Lung
A549
Fat
Liver
Kidney
U251*
HSCP
Brain
Nuclear Stiffness
E Immunoblot validation
Brain
Fat
Heart
Femur
Lamin-A
Lamin-C
Lamin-B1/2
* measurement from immunoblot
Lamin-A : Lamin-B stoichiometry
(determined by mass spectrometry)
10
1
1
10
tissue Microelasticity, E (kPa)



Mechano-response. Tension from the extracellular matrix affects cytoskeletal tension on the nucleus. This affects the turnover of lamin A in the nuclear envelope, expression of *LMNA,* and stiffness of the nucleus.

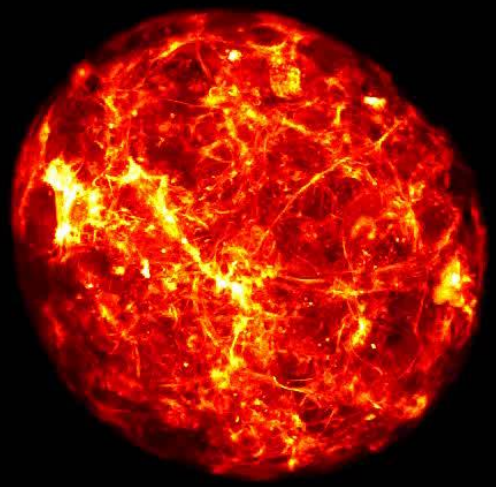
Mechanical tension on a cell from its environment alters the expression of a protein that changes the physical properties of the nucleus.

Dennis Discher's lab

Physics & Biology

Kevin Alessandri

Vasily Gurchenkov



Institut Curie - Biology

Sara Geraldo

Fabien Bertillot

Anthony Simon

« D. »

Institut Curie – Physics

Bibhu Ranjan Sarangi

ANR Agence Nationale de la Recherche
Institut National du Cancer
Inserm
Institut national de la santé et de la recherche médicale