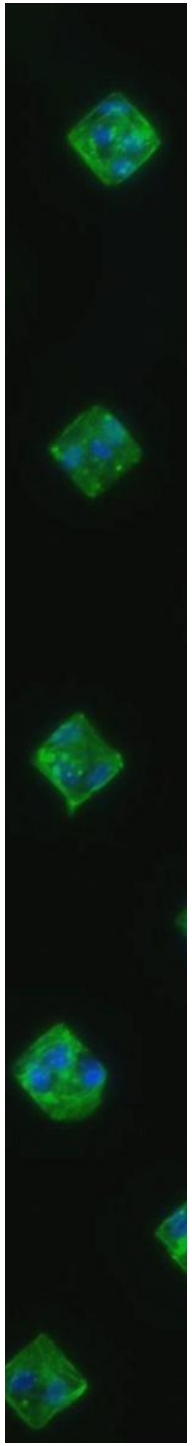


Materials Science & Technology

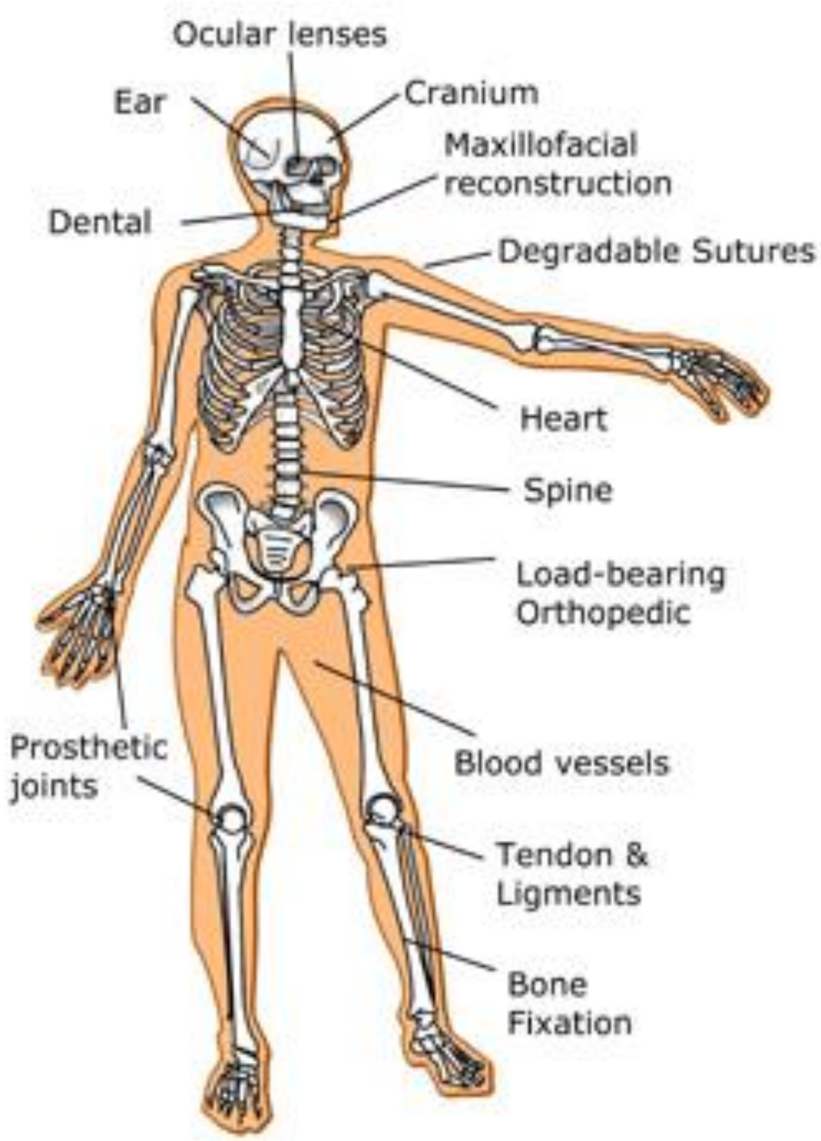
# Steering cellular function by physical cues- potential for medical applications

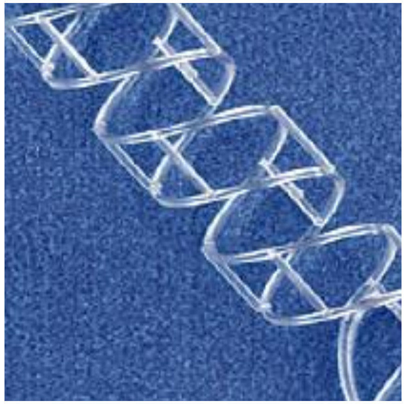
Katharina Maniura

Lab for Materials-Biology Interactions
Empa
Swiss Federal Laboratories for Materials Testing and Research
Department: Materials Meet Life
St. Gallen, CH

Materials Valley e.V., Hanau- January 21, 2010

# Replacement on demand



# Two examples for “success”

# History of artificial heart valves

**Marmorkugel in Käfig (1960)**

# History of artificial heart valves

**Drehscheibe (1977)**

# History of artificial heart valves

**Saloon Doors (1970ies)**

# History of artificial heart valves

**Herzklappe vom Schwein (fixiert und immunokompatibel, 1965)**

# History of artificial heart valves

**Herzklappe vom Rind – in Kombination mit Dacrontextil (1980s)**

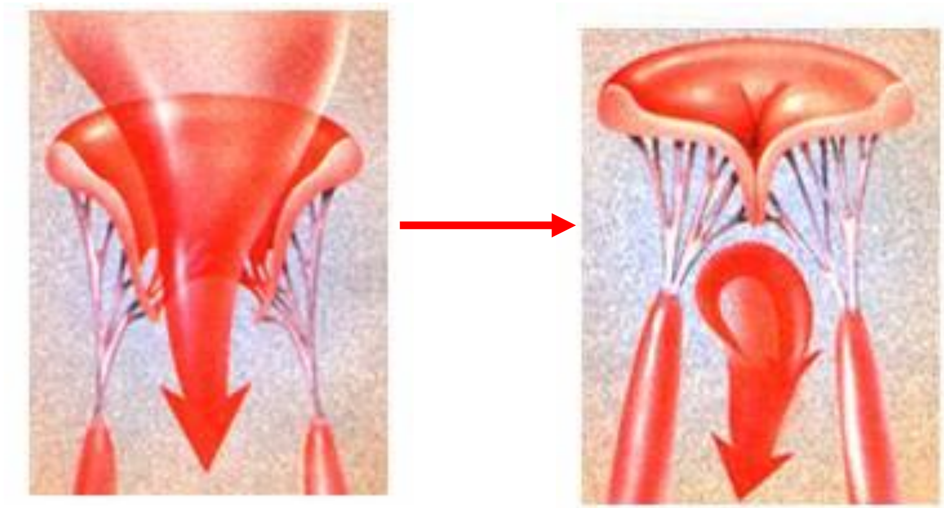
# History of artificial heart valves

**Reparatur der Klappe**

# History of artificial heart valves

**Kollabierbare Klappe: durch Beingefäss einführbar + expandierbar durch Balon (2007)**

# Natural heart valves



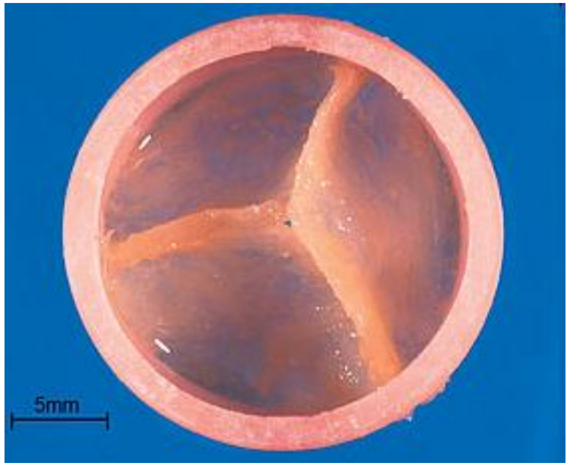
Valve opening:
blood flow

Valve closing:
backflow of blood
stopped

**Movie:**

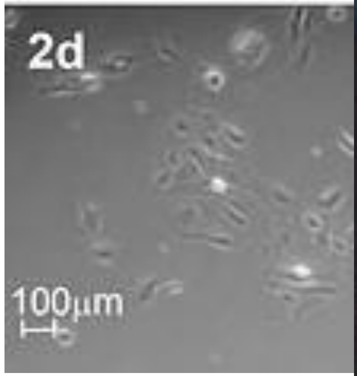
**http://www.youtube.com/watch?v=WXwYYsi6z7Q**

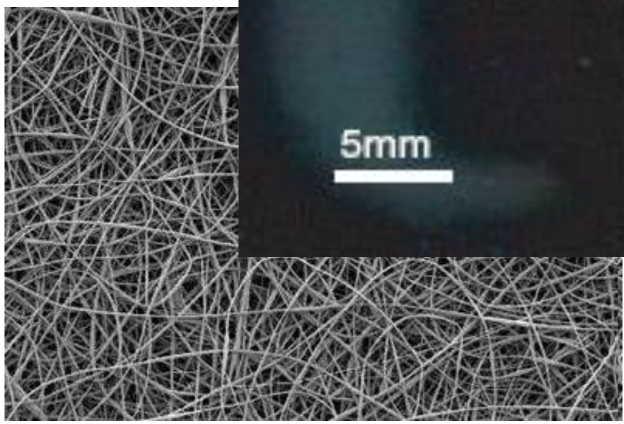
# Dream? Tissue Engineering

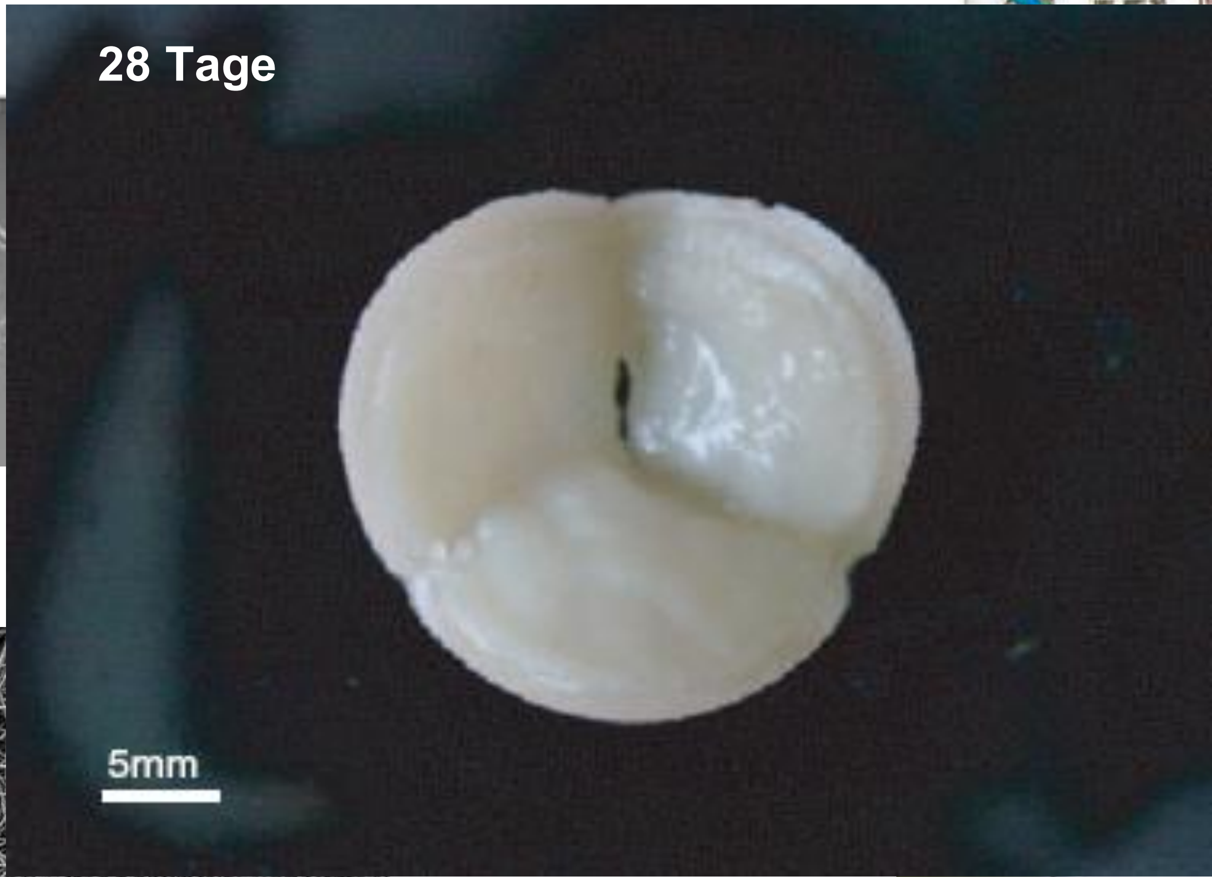


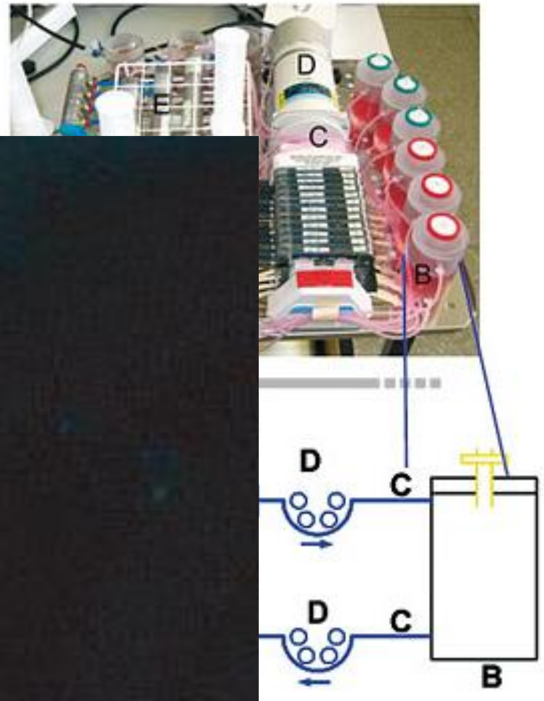

**Prof. Dr. Simon P. Hörstrup**
**Head Cardiovascular Research, Universität Zürich**

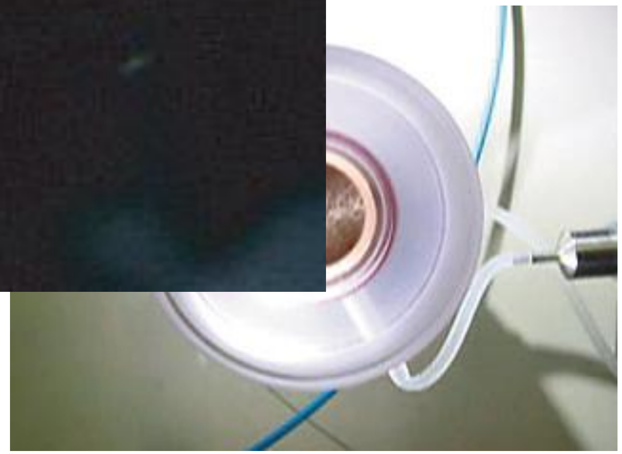
# Tissue Engineering- concept?





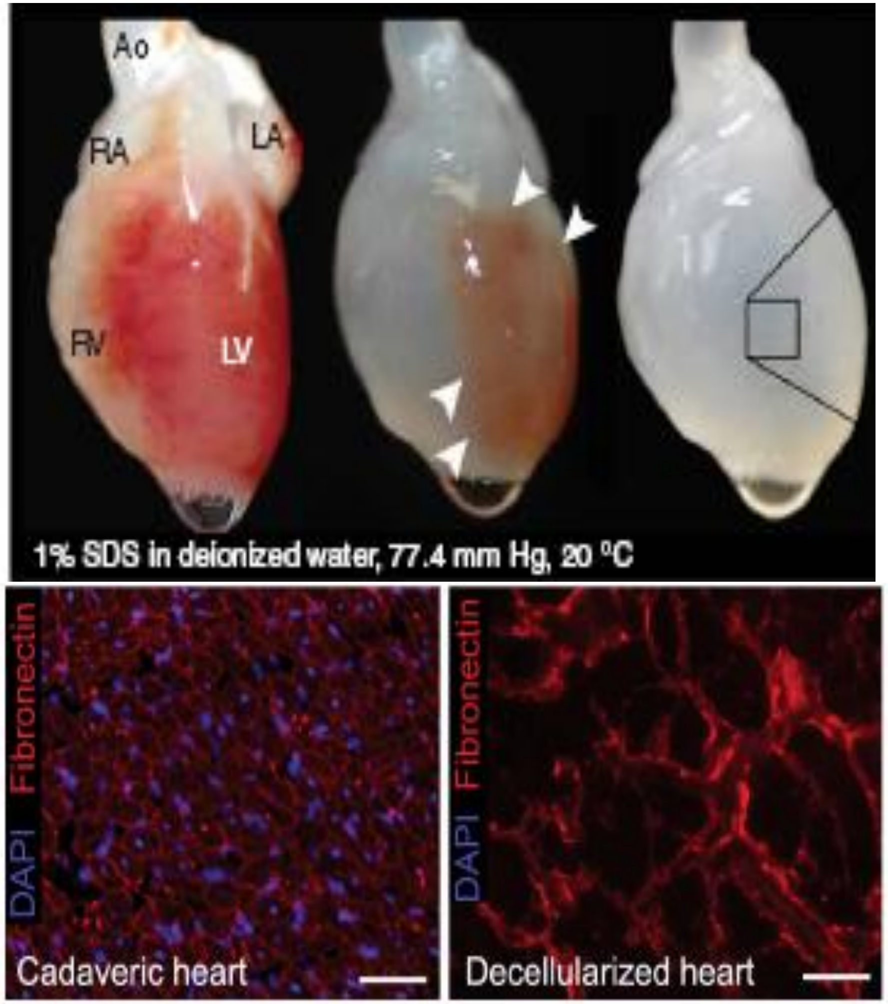



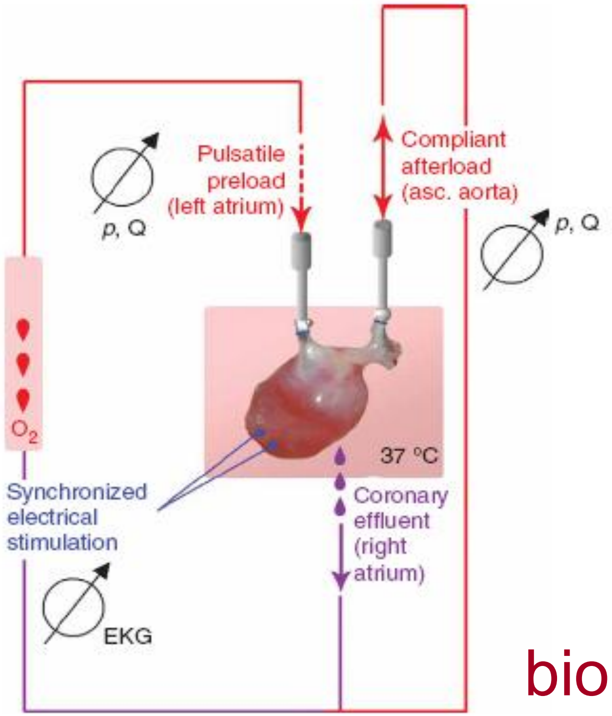
Prof. Dr. Simon P. Hörstrup
Head Cardiovascular Research, Universität Zürich

# „Bioartificial“ heart

## washing-out of cells

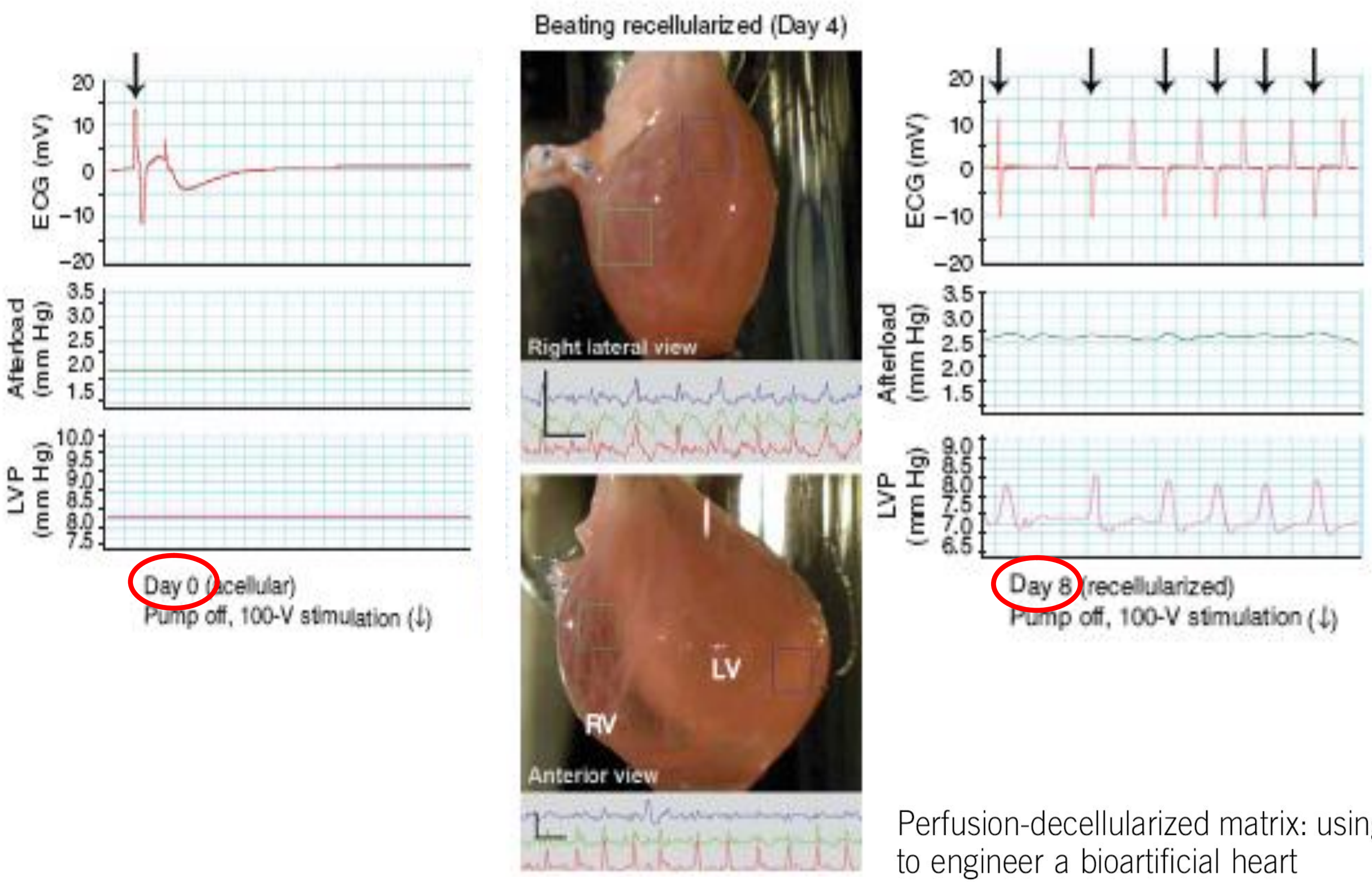



## addition of cultivated cells




## bioreactor

# „Bioartificial“ heart




Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart


Harald C Ott[1], Thomas S Matthiesen[2], Saik-Kia Goh[2], Lauren D Black[3], Stefan M Kren[2], Theoden I Netoff[3] & Doris A Taylor[2,4]

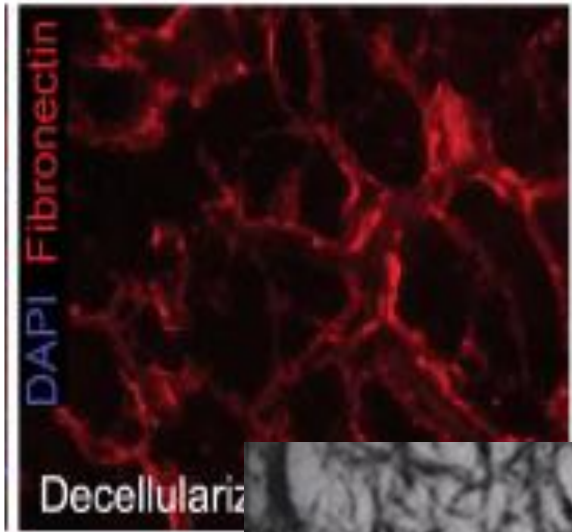


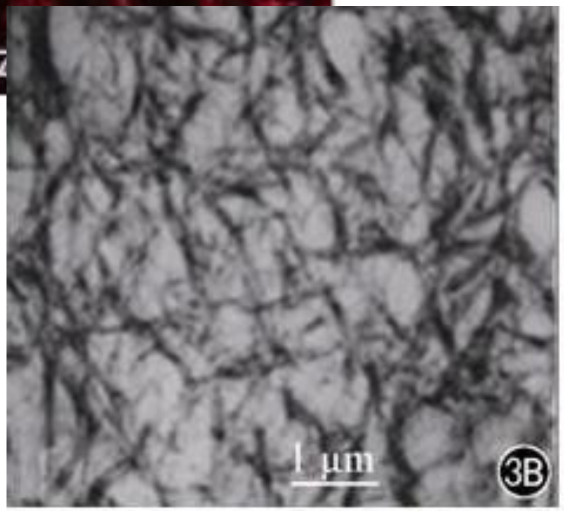
NATURE MEDICINE VOLUME 14 | NUMBER 2 | FEBRUARY 2008


**Harvard Medical School**

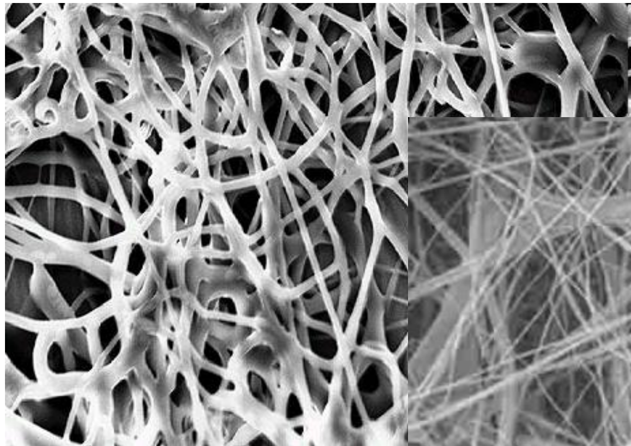
# Non-woven nano material: proteins

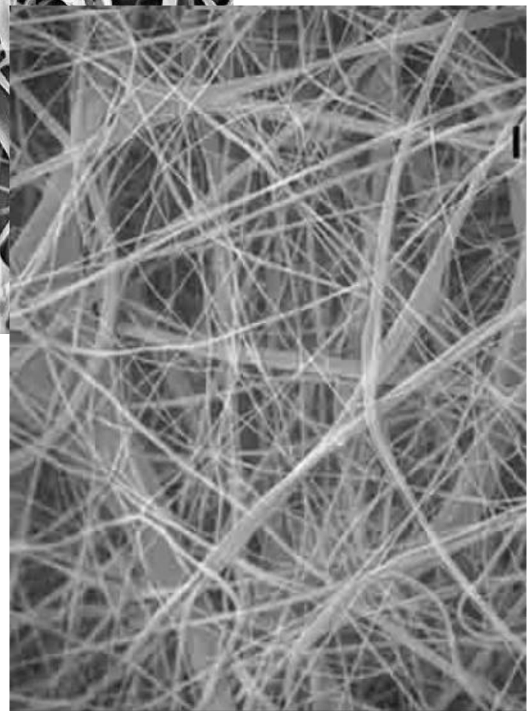
*ex vivo*




from the lab



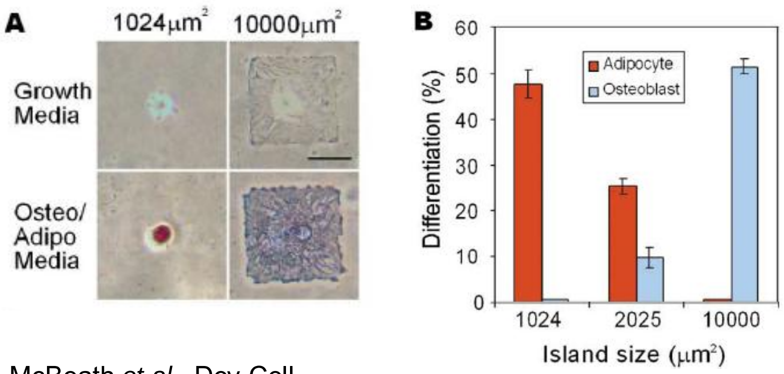


# Physical cues in the two examples:

| | |
|---|---|
| **structural** | geometry of substrate<br>- orientation<br>- dimensionality |
| **mechanical** | - different stiffness substrates<br>- mechanical stimulation (pressure, shear, …) |
| **biochemical** | proteins (e.g. fibronectin)<br>- protein interaction: niches, binding to other factors, …<br>- conformation ... |
| **physicochemical** | …. |

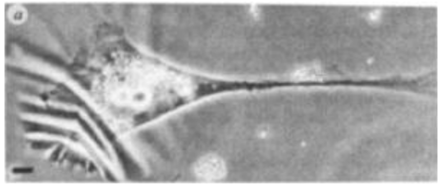
# Cell fate and materials

## 2D micro patterning

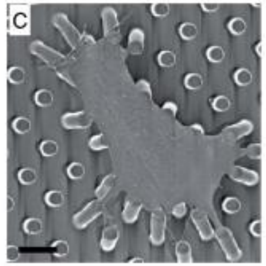



McBeath *et al*., Dev Cell, **6**, 483-495 (2004)

## force

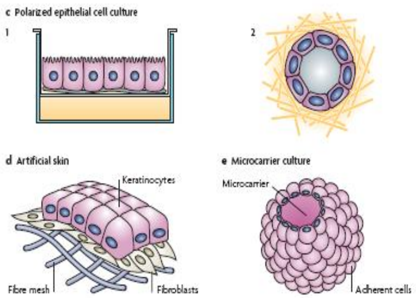


Harris *et al*., Science, **204**, 177-179 (1980)



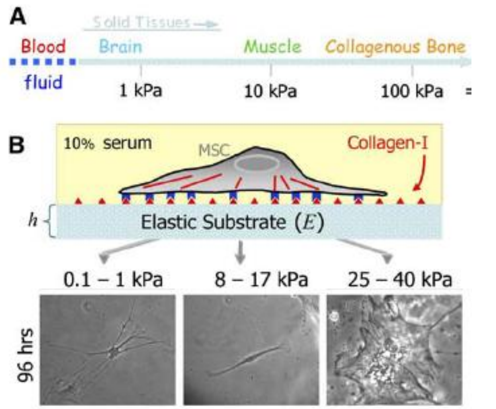

Sniadecki & Chen, Methods Cell Biol., **83**, 313-328 (2007)

## 3D




Pampaloni *et al*., Nat Rev Mol Cell Biol , **8**, 839-845 (2007)

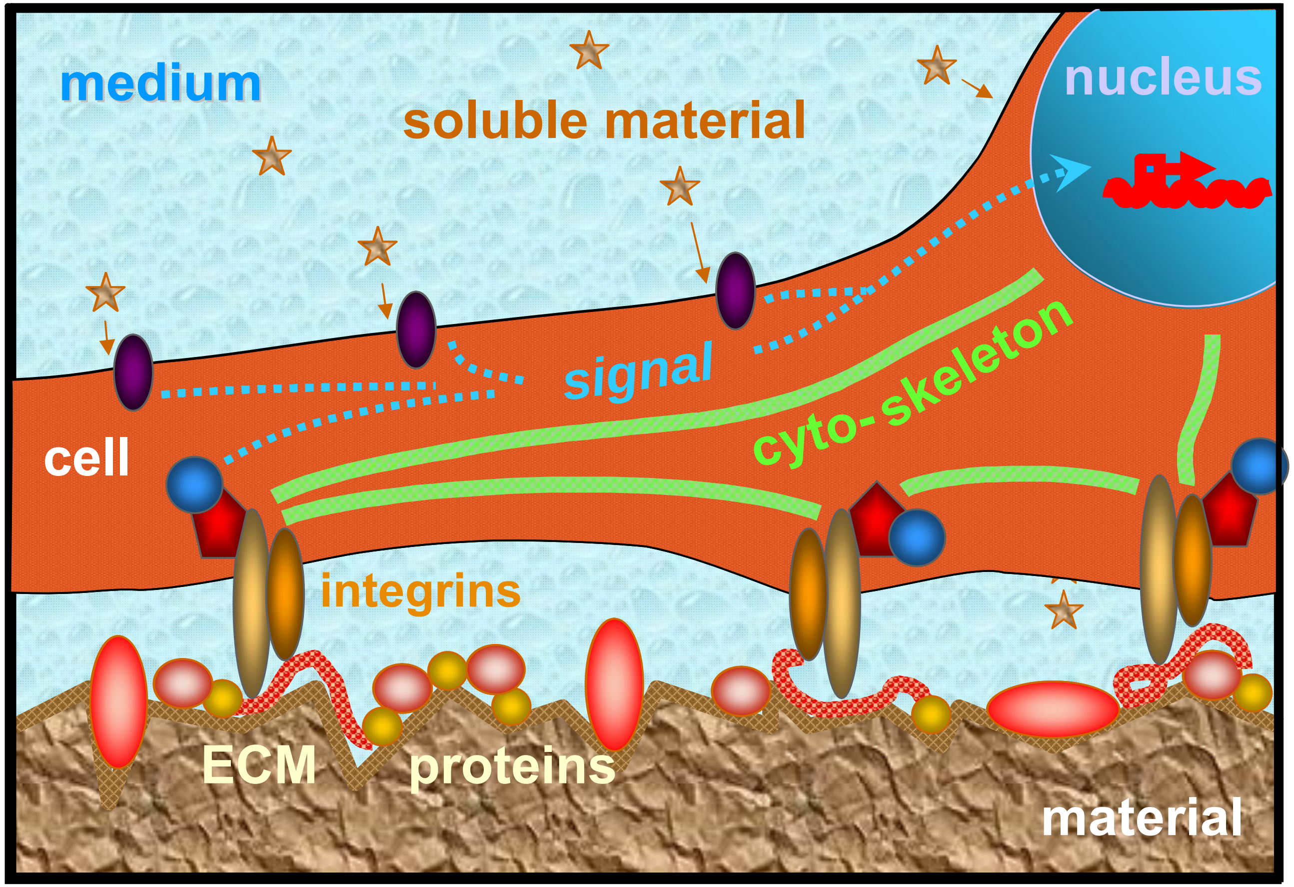
## mechanical properties



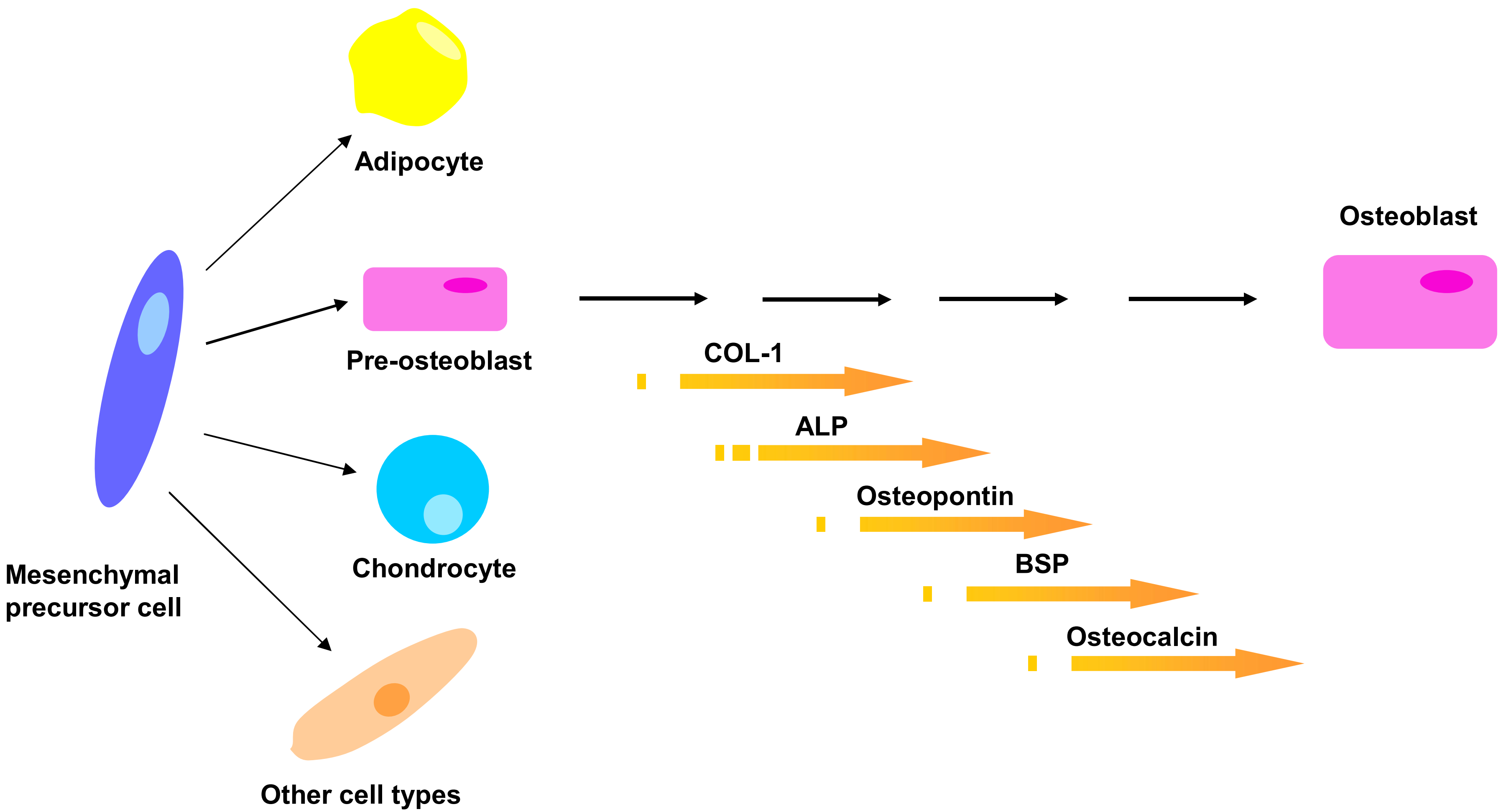

Engler *et al*., Cell, **126**, 677-689 (2006)

**mesenchymal stem cells → neuronal muscle bone cells**

# Cell – material interactions



# Cell model: mesenchymal stem cells

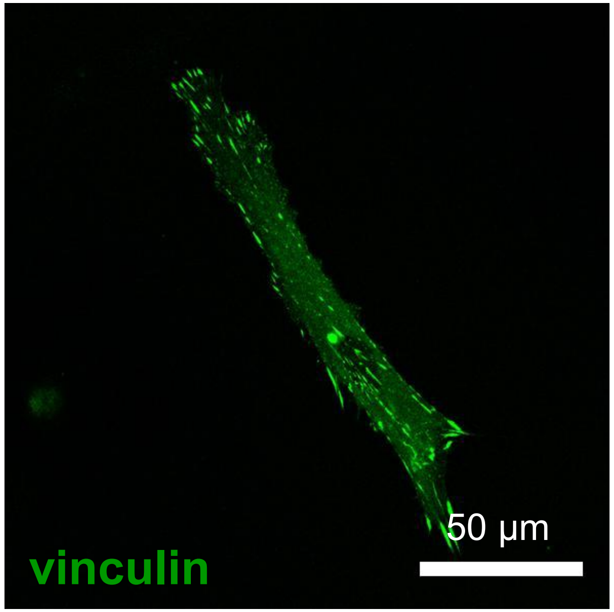



→ osteogenic differentiation of MSC is characterised by the expression of marker proteins

# Differentiation of MSCs

osteogenic differentiation
is accompanied by
changes in:

undifferentiated

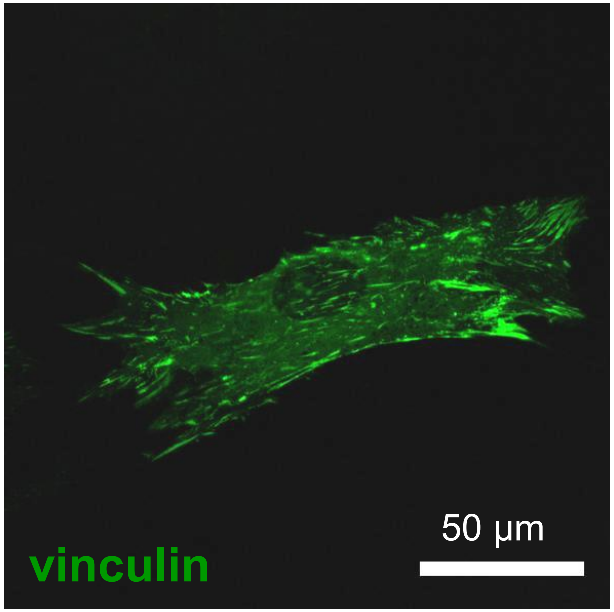



cell morphology

cell adhesion

expression of specific
marker proteins

differentiated




changes in cellular forces during osteogenic differentiation

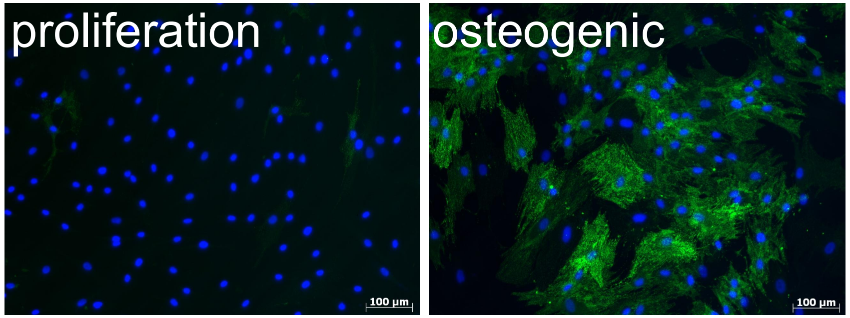
→ online monitoring of osteogenic differentiation and cell adhesion

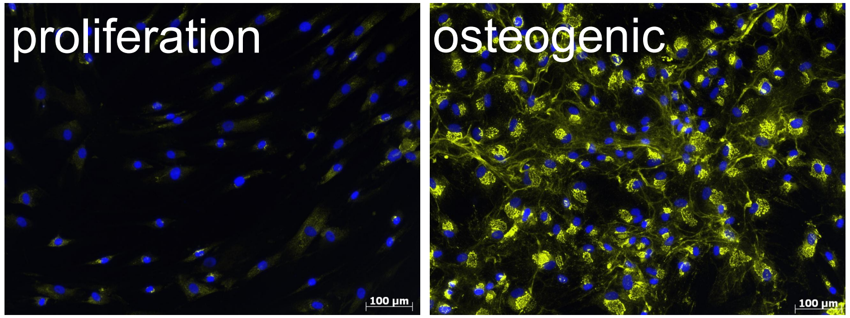
# Markers for osteogenesis

## immunohistochemistry

MSC day 7, bALP

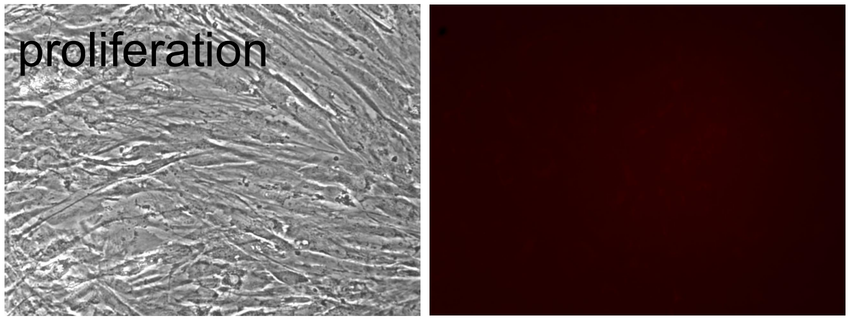
MSC day 14, col I

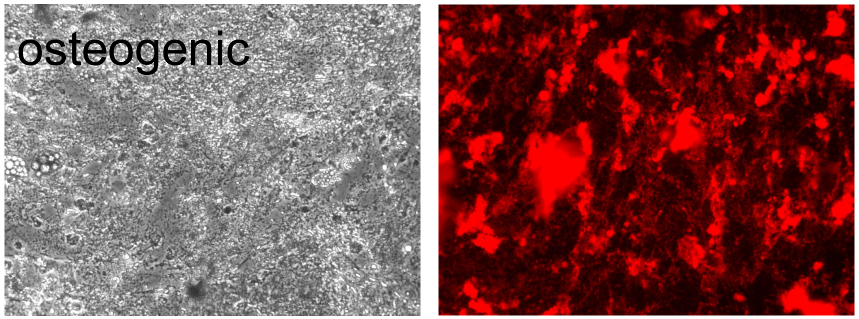



## mineralization

MSC day 21 (xylenol orange)

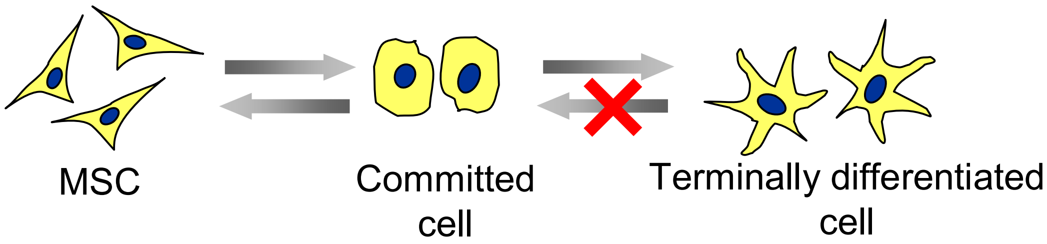


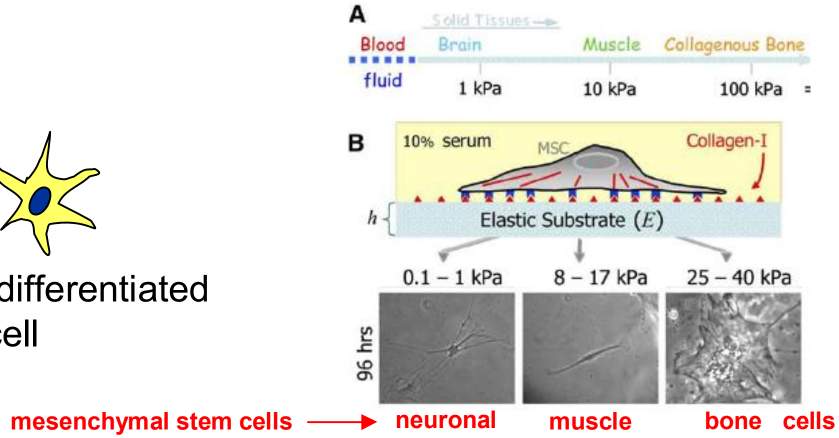



# Stem cell plasticity

**substrate induced - without adding chemical stimuli**

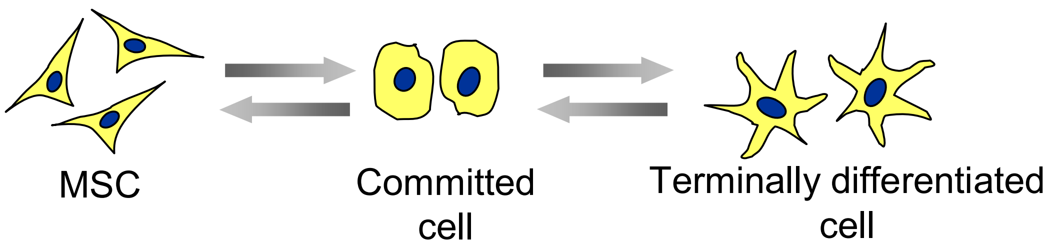
- first week - transdifferentiation
- three weeks - cells committed




Engler, A.J. et al. *Cell* **126, 677-689 (2006)**

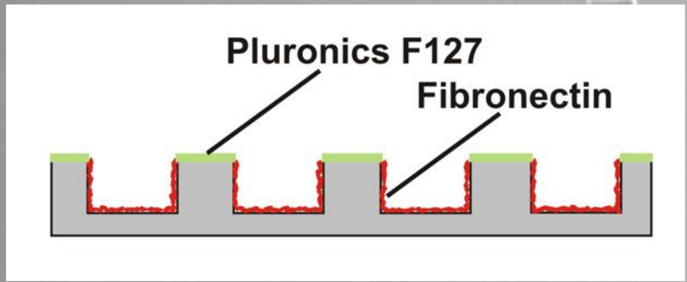
- **chemically induced**
  - four weeks - dedifferentiation and transdifferentiation

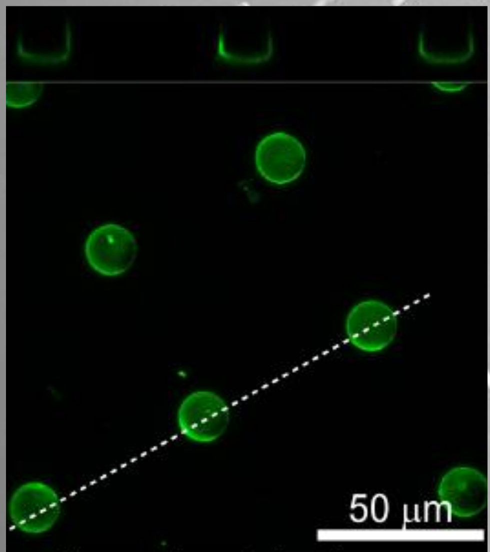



Song, L. & Tuan, R.S. *FASEB J.*, **03-1100fje (2004)**
Song, L. et al. *Stem Cells* 24, **1707-1718 (2006)**

# Stem cell fate in 2.5D microwells

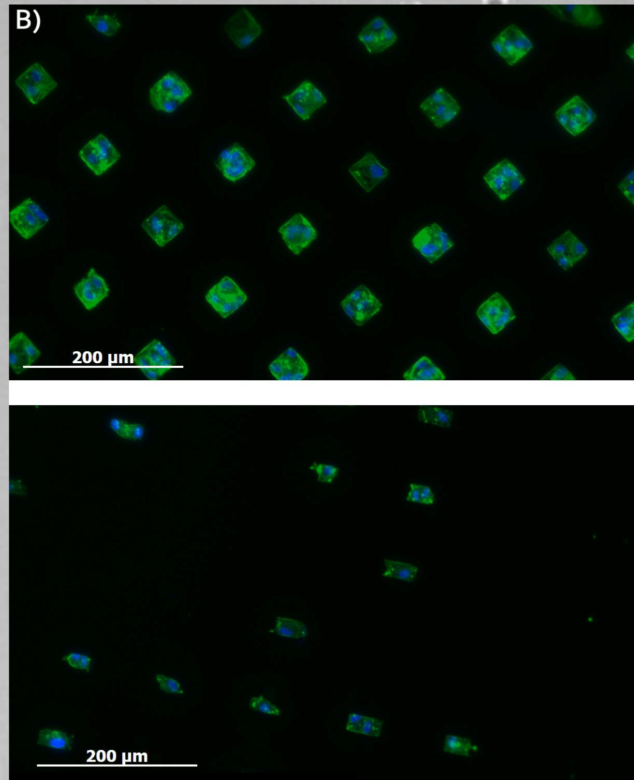
the wells




***fibronectin coated wells***
***(CLSM image)***

fibroblasts seeded in wells

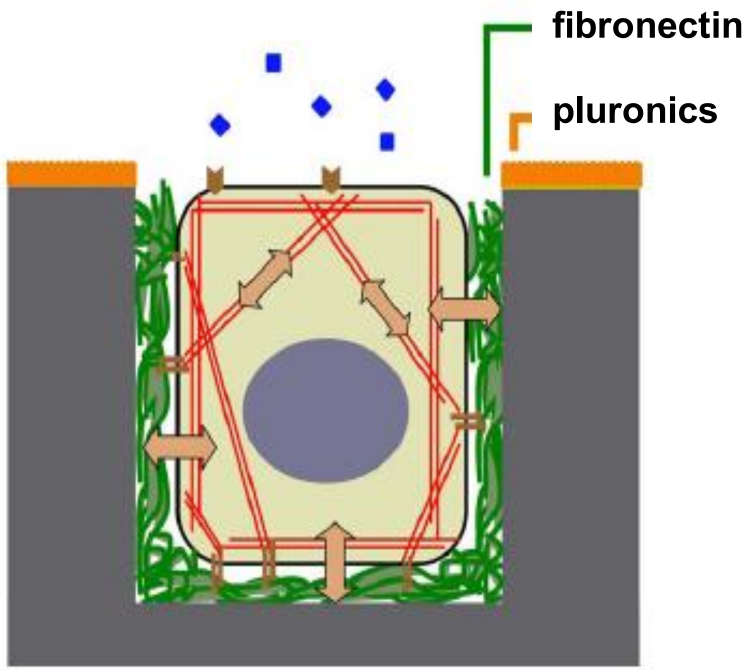



actin
nuclei

200 µm

collaboration with M. Textor, ETHZ; V. Vogel, ETHZ; M. Alini, AO Davos

# Microenvironment




**structural** geometry of well

**mechanical** different stiffness of PDMS

→ 10 kPa – 1 MPa

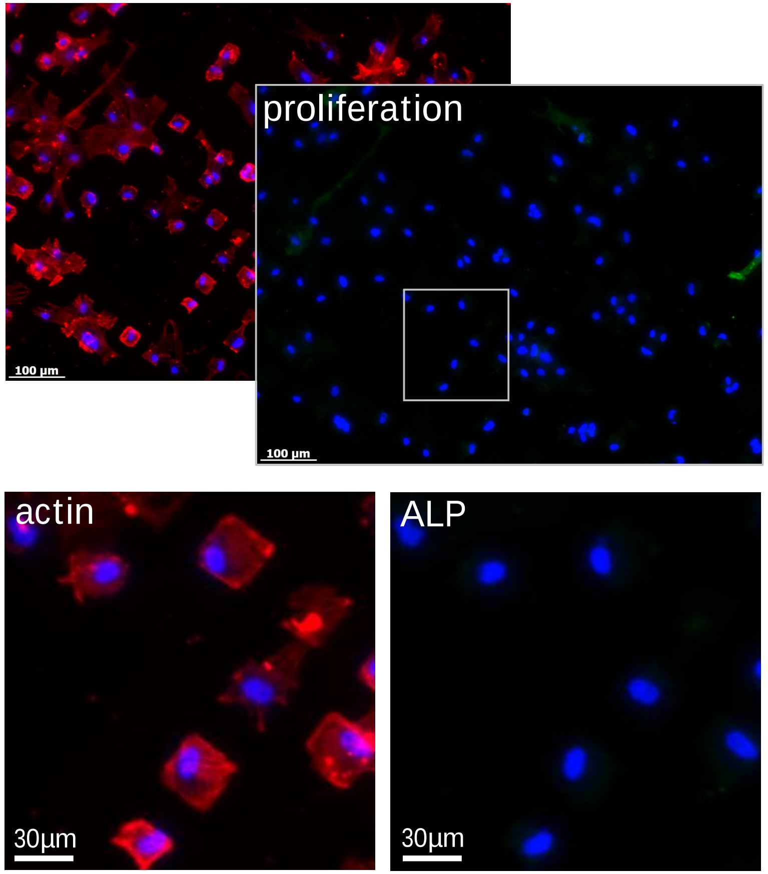
**chemical** coating of well

- fibronectin
- functionalised phospholipid bilayer

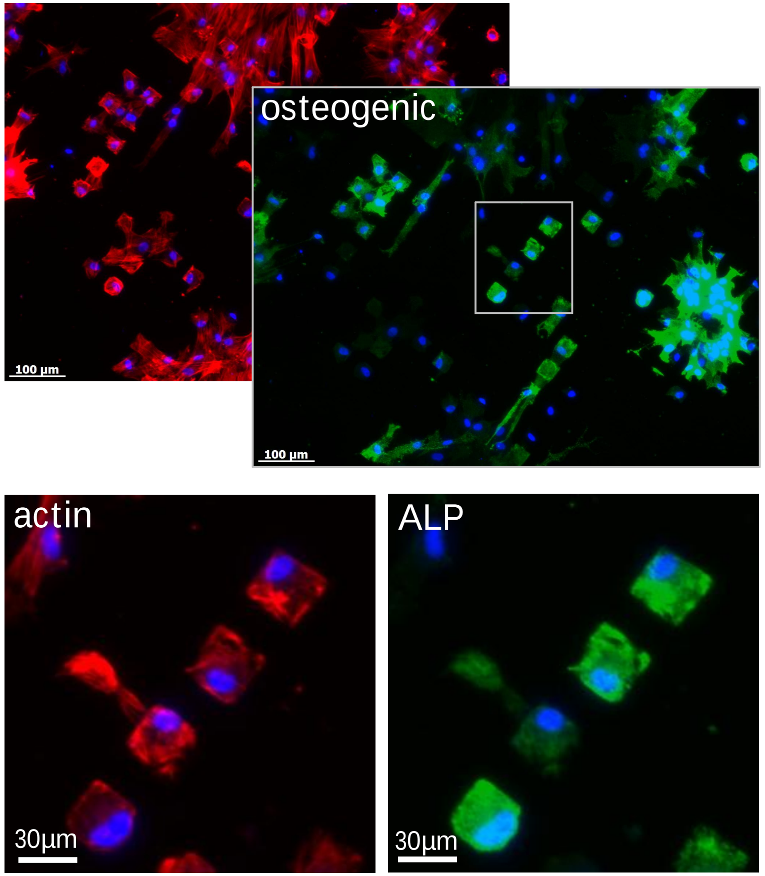
→ well controlled microenvironment of every single cell

# First results - osteogenesis

- Osteogenic differentiation of MSC in microwells **(d7)**




- 10 single cells (not bridging)
- 0/10 positive for ALP



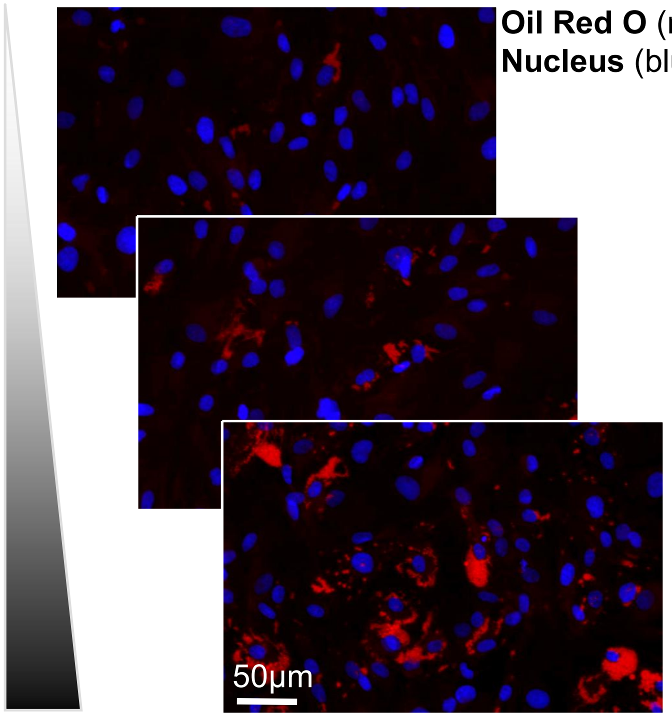

- 21 single cells (not bridging)
- 9/21 positive for ALP

# Adipogenic differentiation of MSC

- Adipogenesis requires high starting cell density on TCP

McBeath et al., *Dev Cell*, **6, 483-495 (2004)**

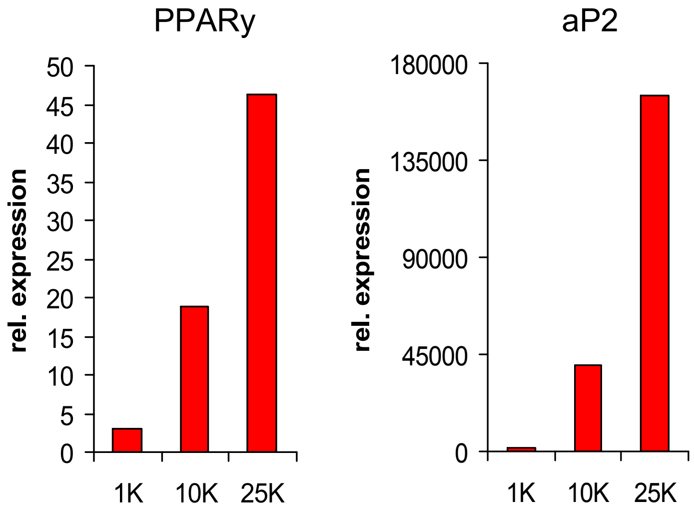
5K



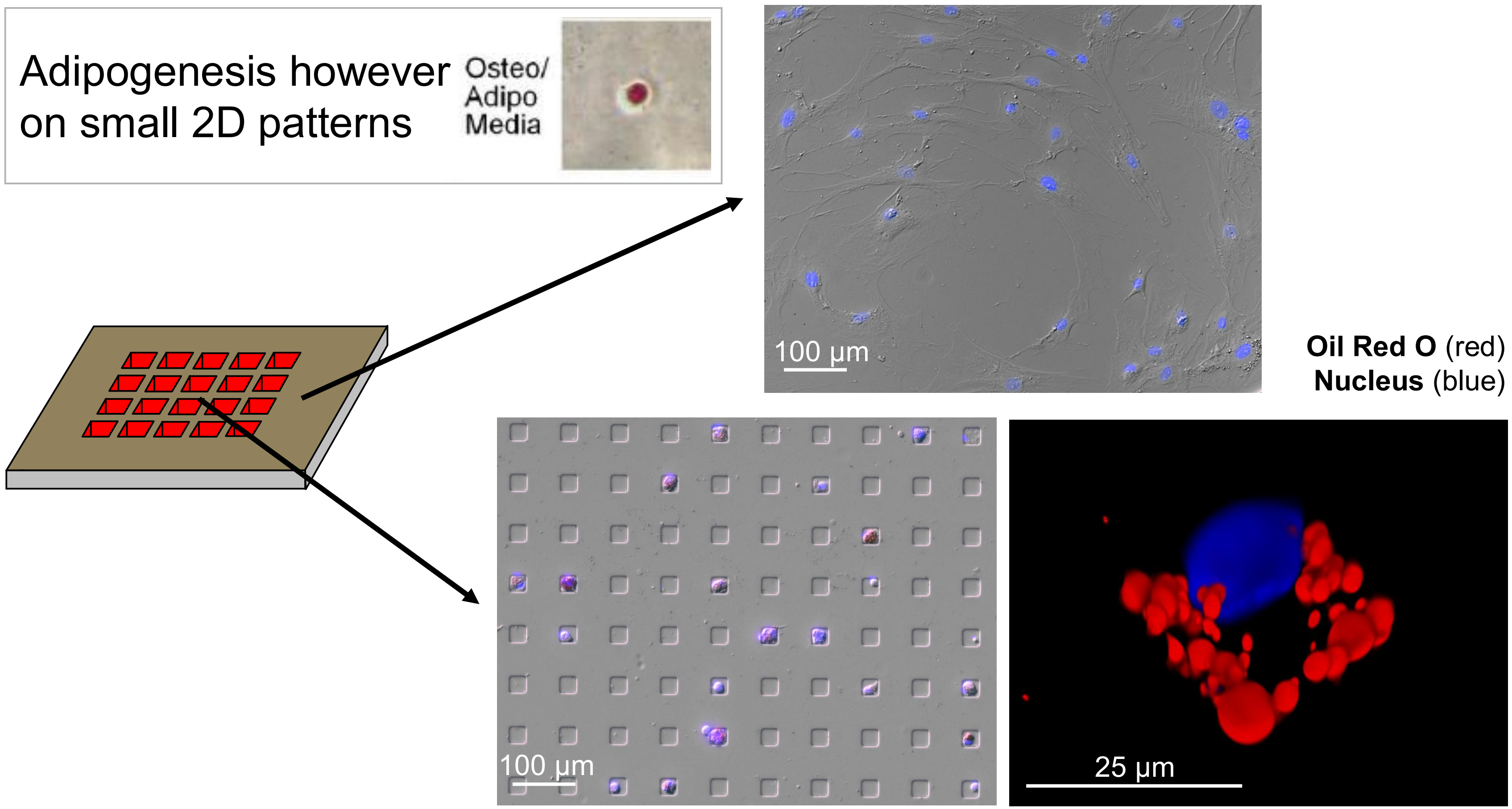

**Oil Red O** (red)
**Nucleus** (blue)

25K
Seeding density
cells/cm$^2$

qRT-PCR (n=3)



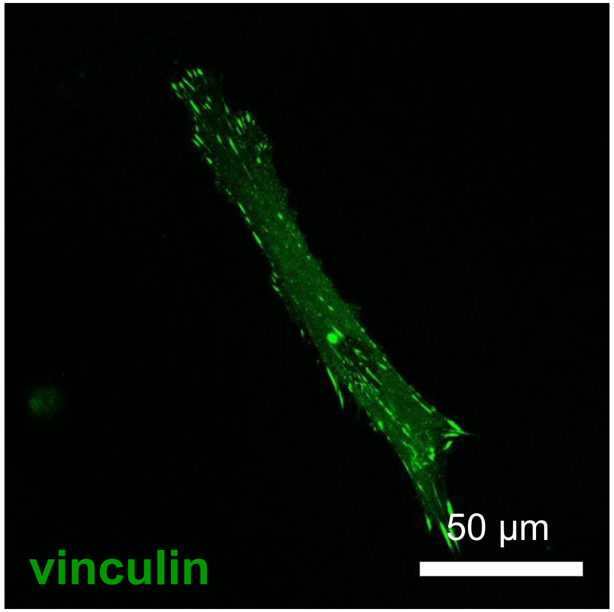
# Adipogenic differentiation of MSC in microwells **(d7)**



# Osteogenesis: changes in cell architecture

osteogenic differentiation
is accompanied by
changes in:

undifferentiated

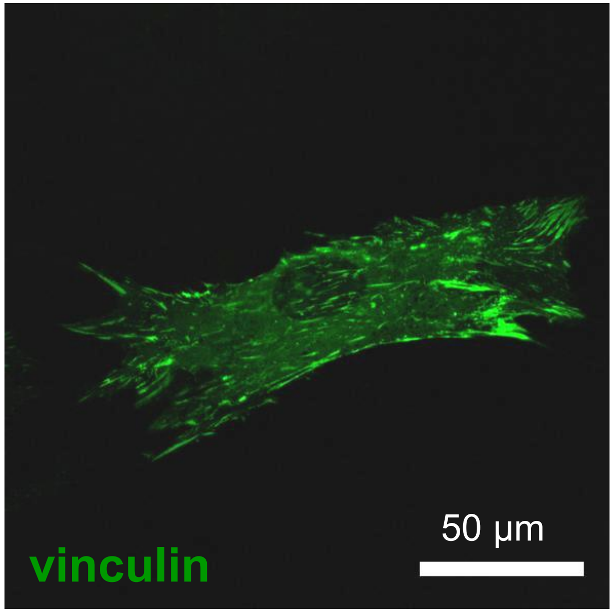



cell morphology

cell adhesion

expression of specific
marker proteins

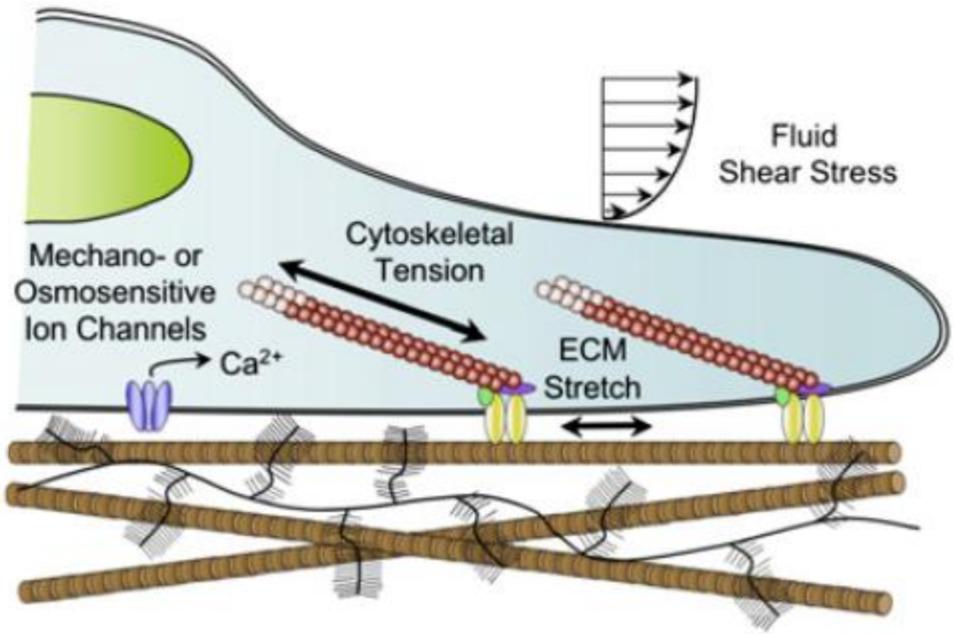
differentiated




## changes in cellular forces during osteogenic differentiation

→ online monitoring of osteogenic differentiation and focal adhesion dynamics

# Mechanical factors and the regulation of stem cell fate



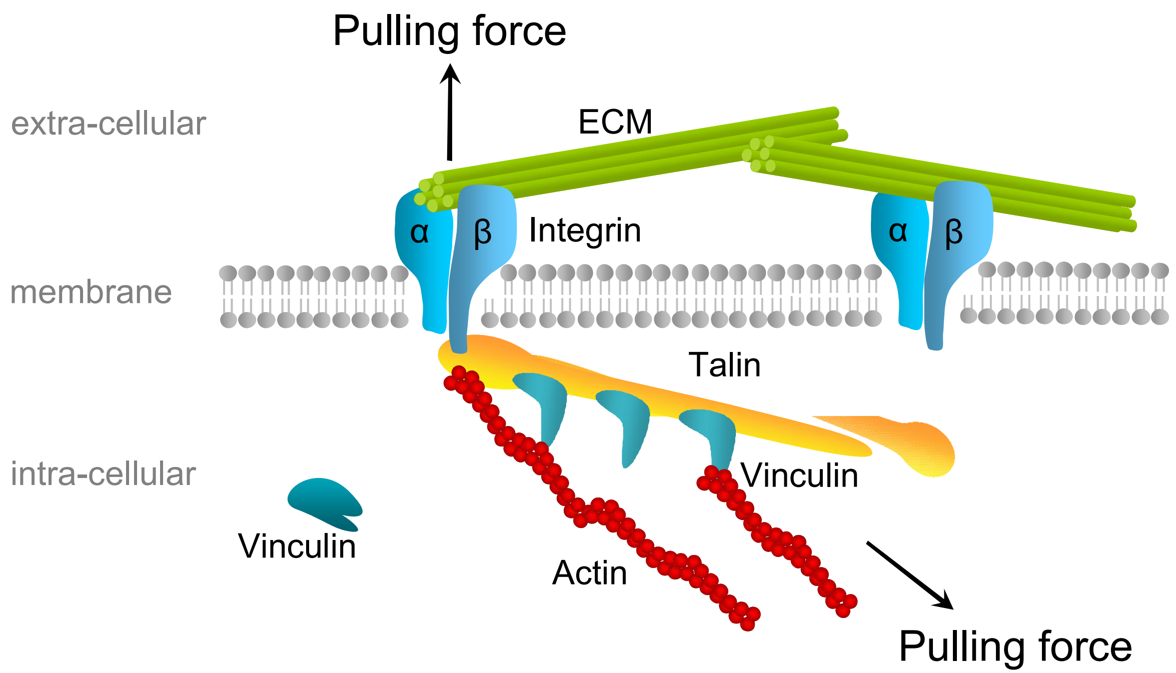

physical signals:

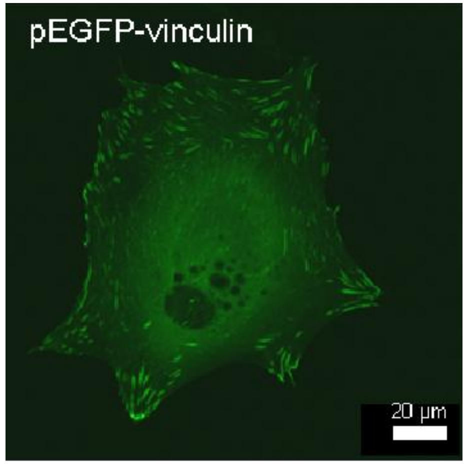
tensile
compressive
shear
osmotic
fluid stresses

often secondarily to biomechanical interactions with their ECM

Guilak et al., Cell Stem Cell, 2009

# Dynamics in focal adhesions



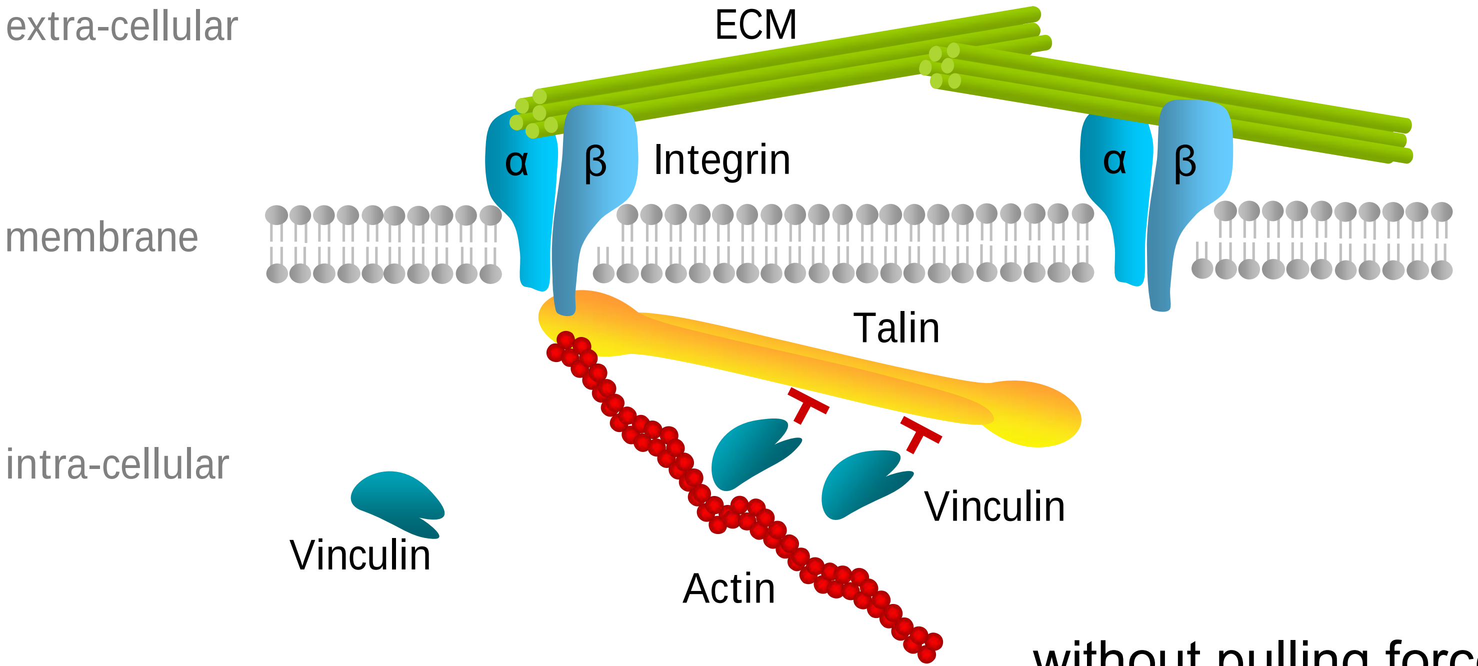




- → molecular interaction between vinculin and talin is force dependent
- → without forces → vinculin can not bind to talin
- → dynamic changes in cell adhesion during cellular processes can not be studied by conventional methods (e.g. stainings)

→ FRET between talin and vinculin

# Cell Adhesion

## Dynamics in focal adhesions

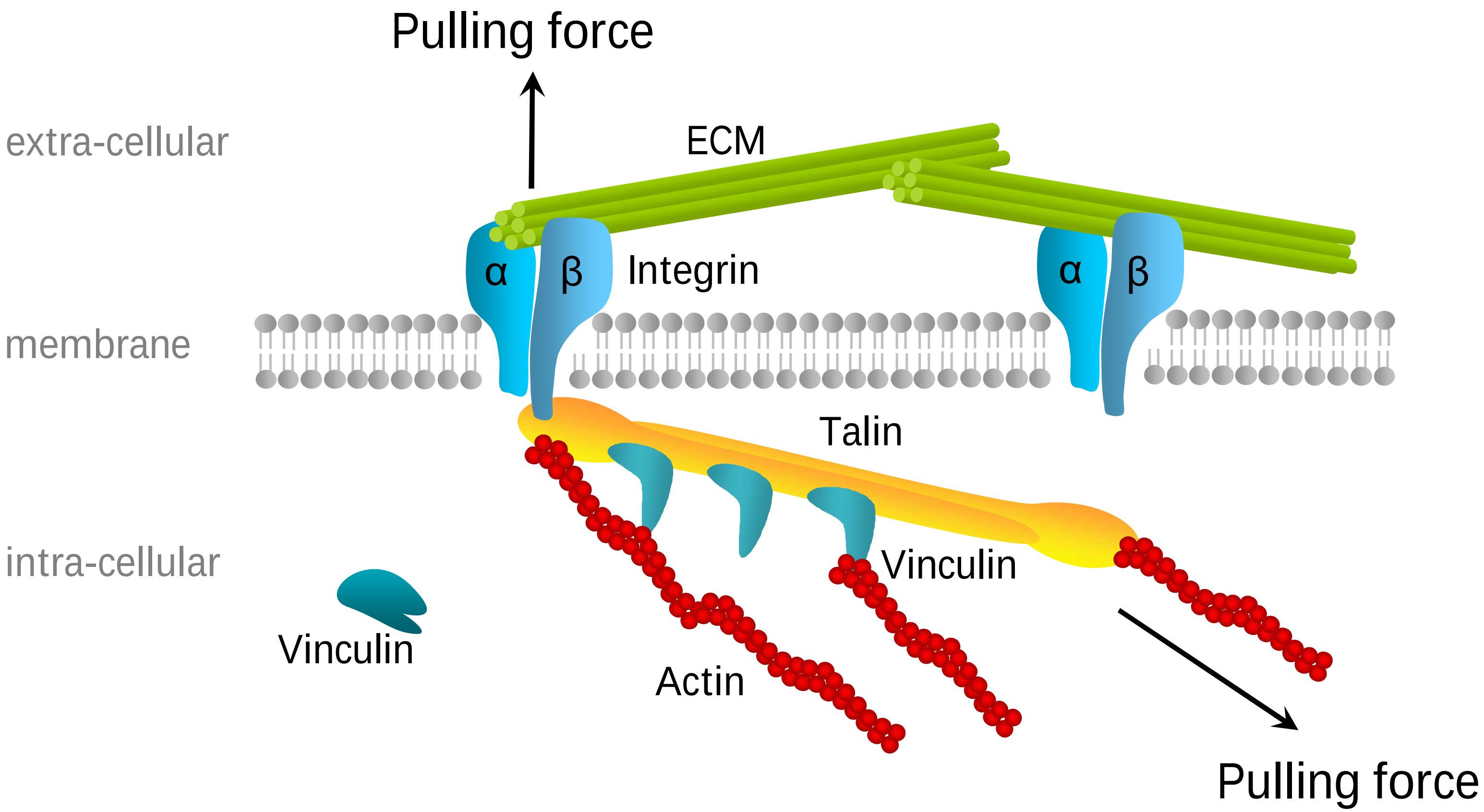



without pulling force

→ no vinculin binding to talin

# Cell Adhesion

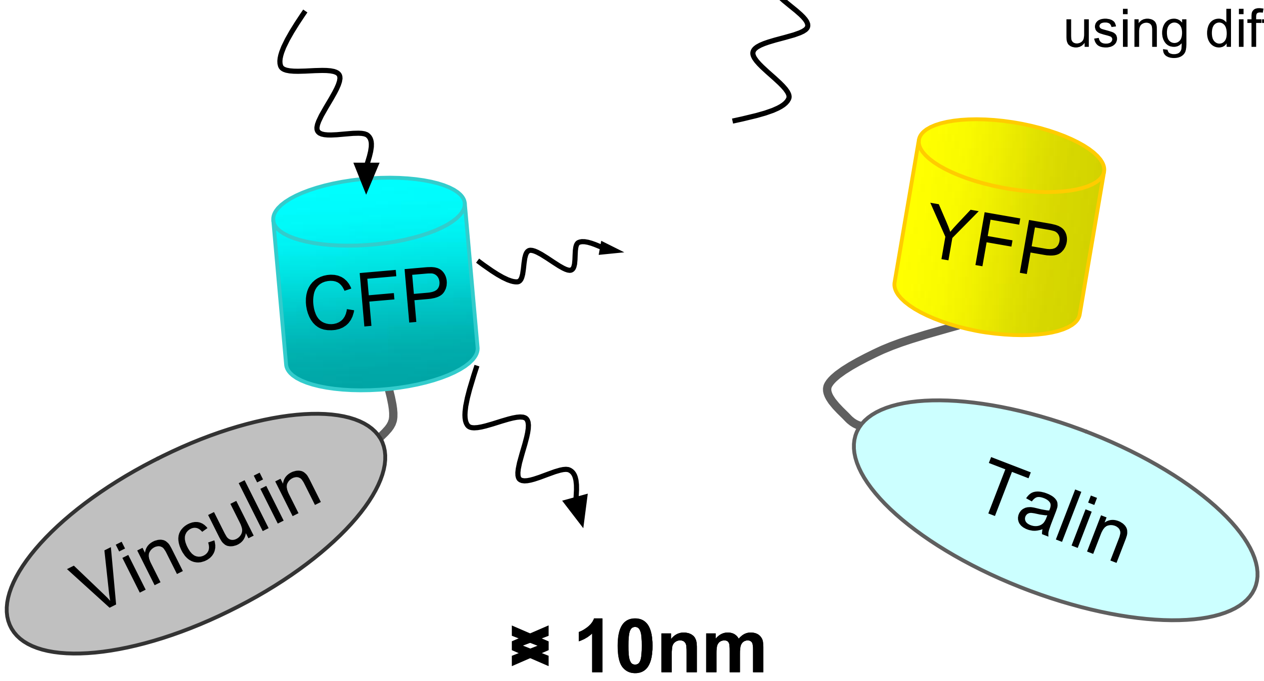
## Dynamics in focal adhesions

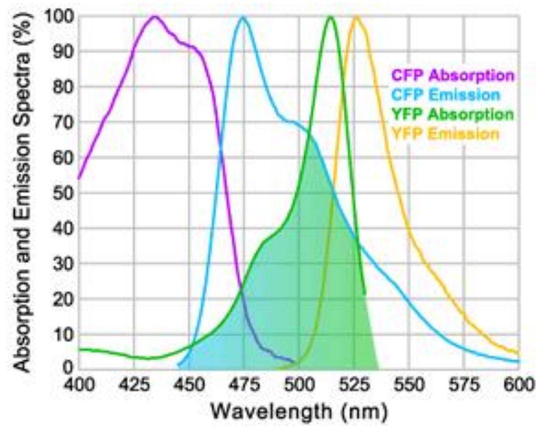


# FRET between vinculin and talin

- online monitoring of interaction between vinculin and talin
  - conformational changes of vinculin
- force dependent interaction by using different DNA constructs

no FRET signal

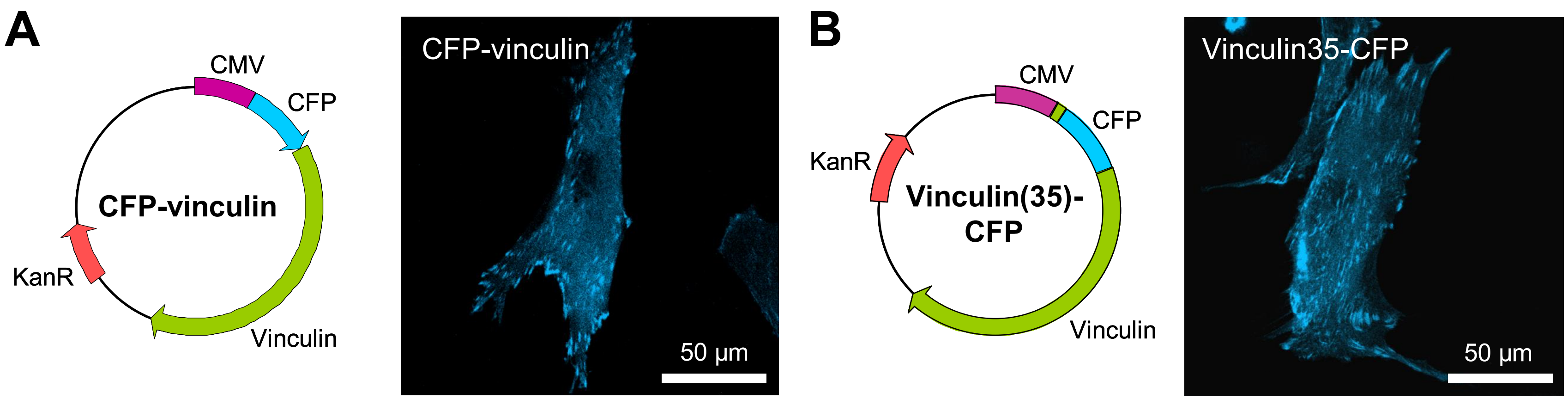


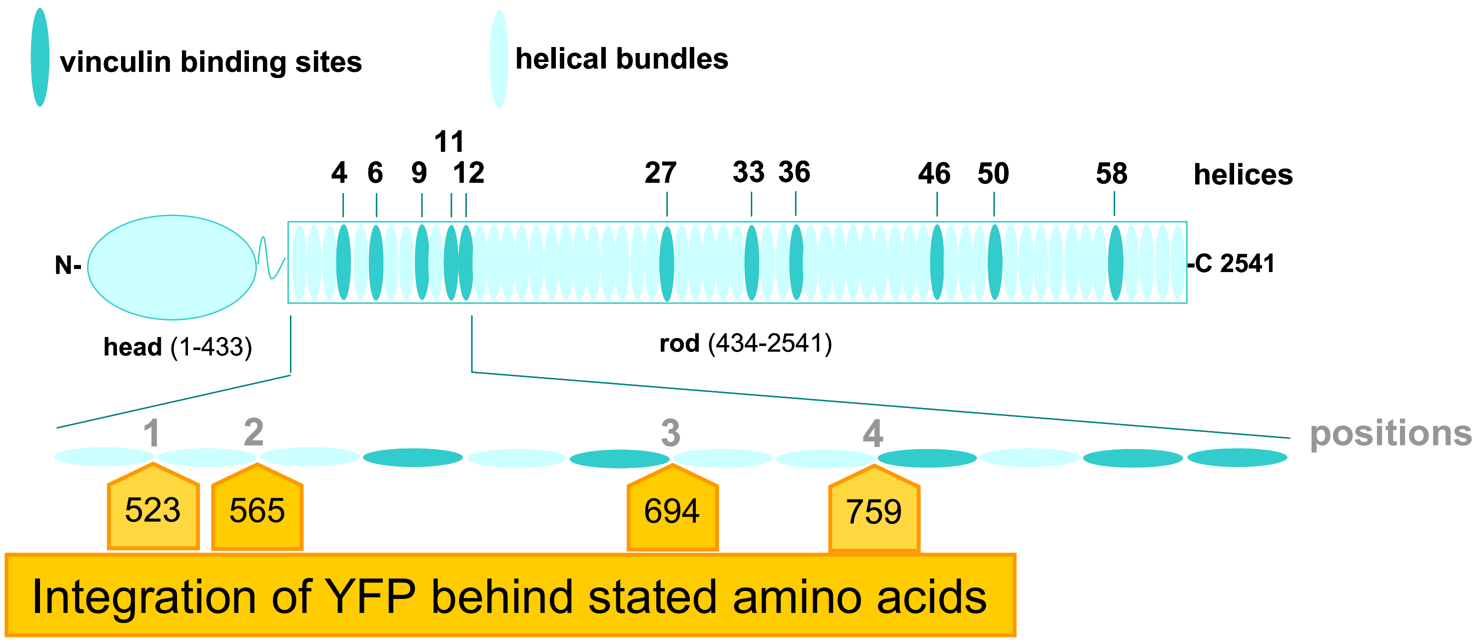

www.semrock.com

# Vinculin as FRET donor


- Vinculin was integrated in front of the cyan fluorescent protein sequence of pECFP-C1
- further, CFP was integrated within the vinculin sequence of plasmid pCMV-vinculin to create pVinculin(35)-CFP



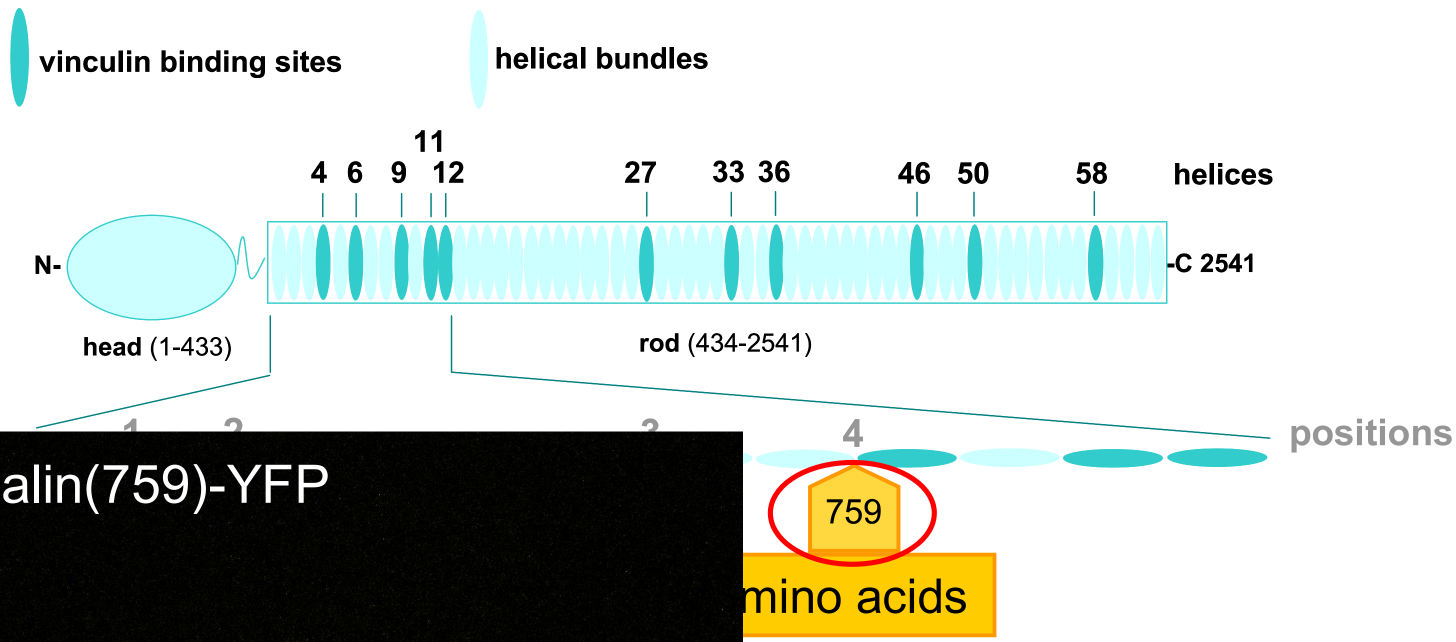
# Talin as FRET acceptor

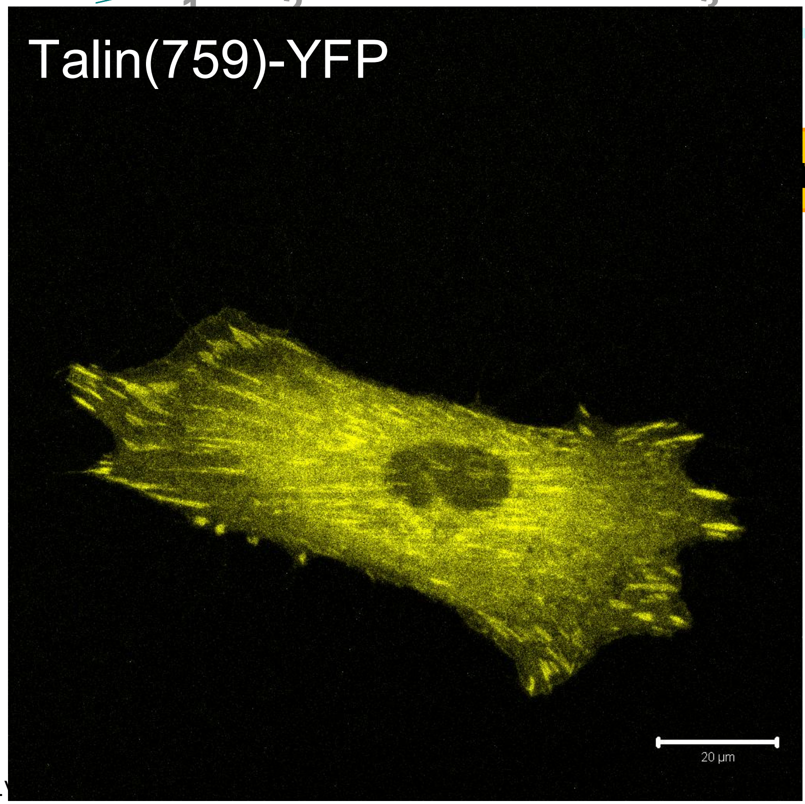



- different vinculin binding sites in talin become accessible under different tensile forces
- YFP integration sites were defined in collaboration with V. Vogel, ETHZ
- integration sites differ in their distance to vinculin binding sites

Patel et al., 2006; Hytönen & Vogel, 2008

# Talin as FRET acceptor



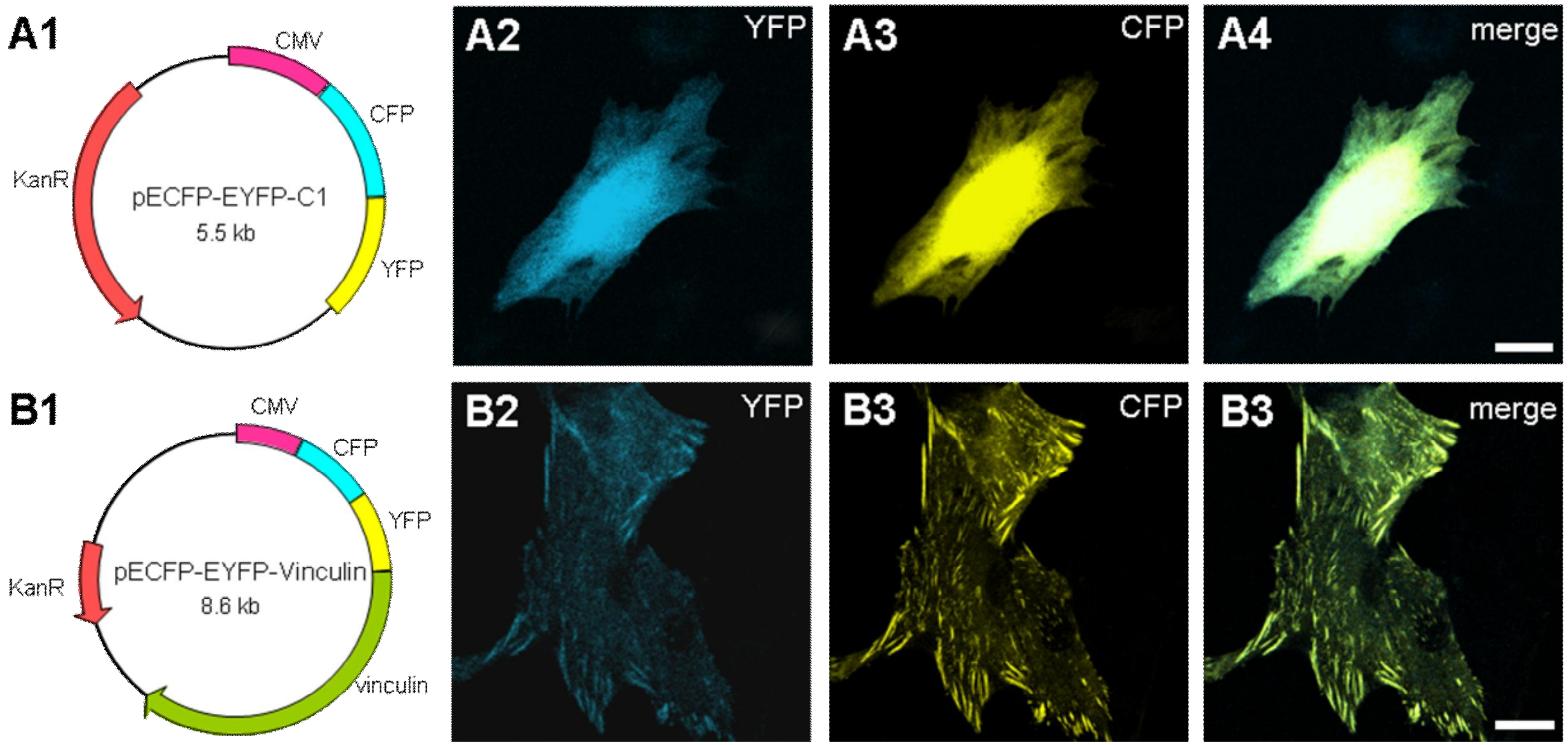




- YFP-tagged talin localises in focal adhesions
- integration of YFP does not alter talin function

# Negative and positive FRET controls

- negative control (1): transfection only with FRET donor → no FRET
- negative control (2): co-transfection of unlinked donor and acceptor → no FRET
- positive control: transfection with donor linked to acceptor → FRET

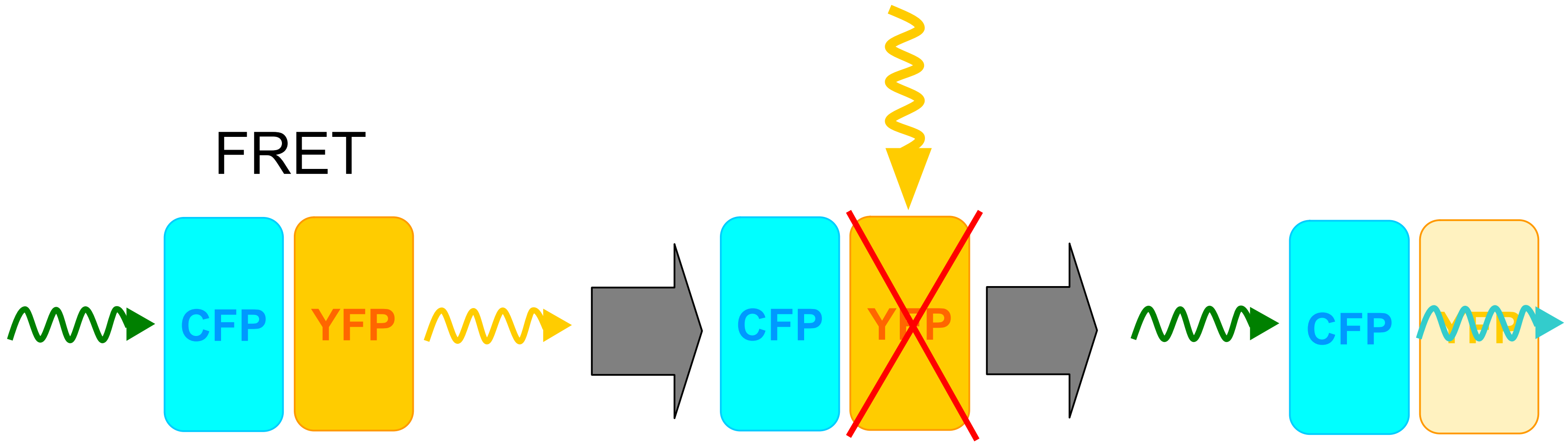
Positive FRET controls:




NHDF, 2 days post-transfection

# FRET analysis by acceptor photobleaching

FRET donor: CFP -vinculin FRET acceptor: YFP -talin




before photobleaching

BLEACHING

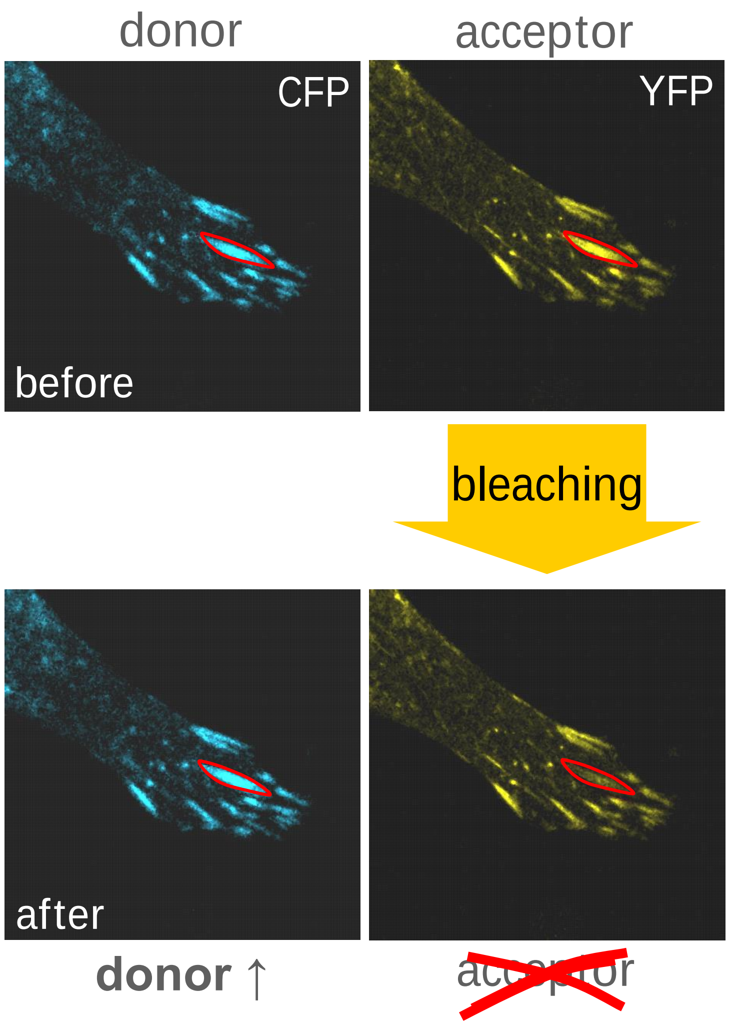
after photobleaching

→ before bleaching: donor ↓, acceptor ↑

→ after bleaching: donor ↑, acceptor ↓

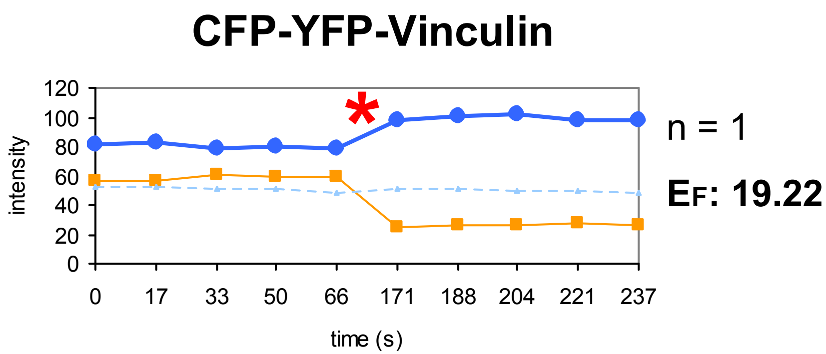
# FRET analysis by acceptor photobleaching

## CFP-YFP-Vinculin (NHDFs)




FRET energy transfer efficiency:

$E_F = (I_{after} - I_{before}) \times 100 / I_{after}$



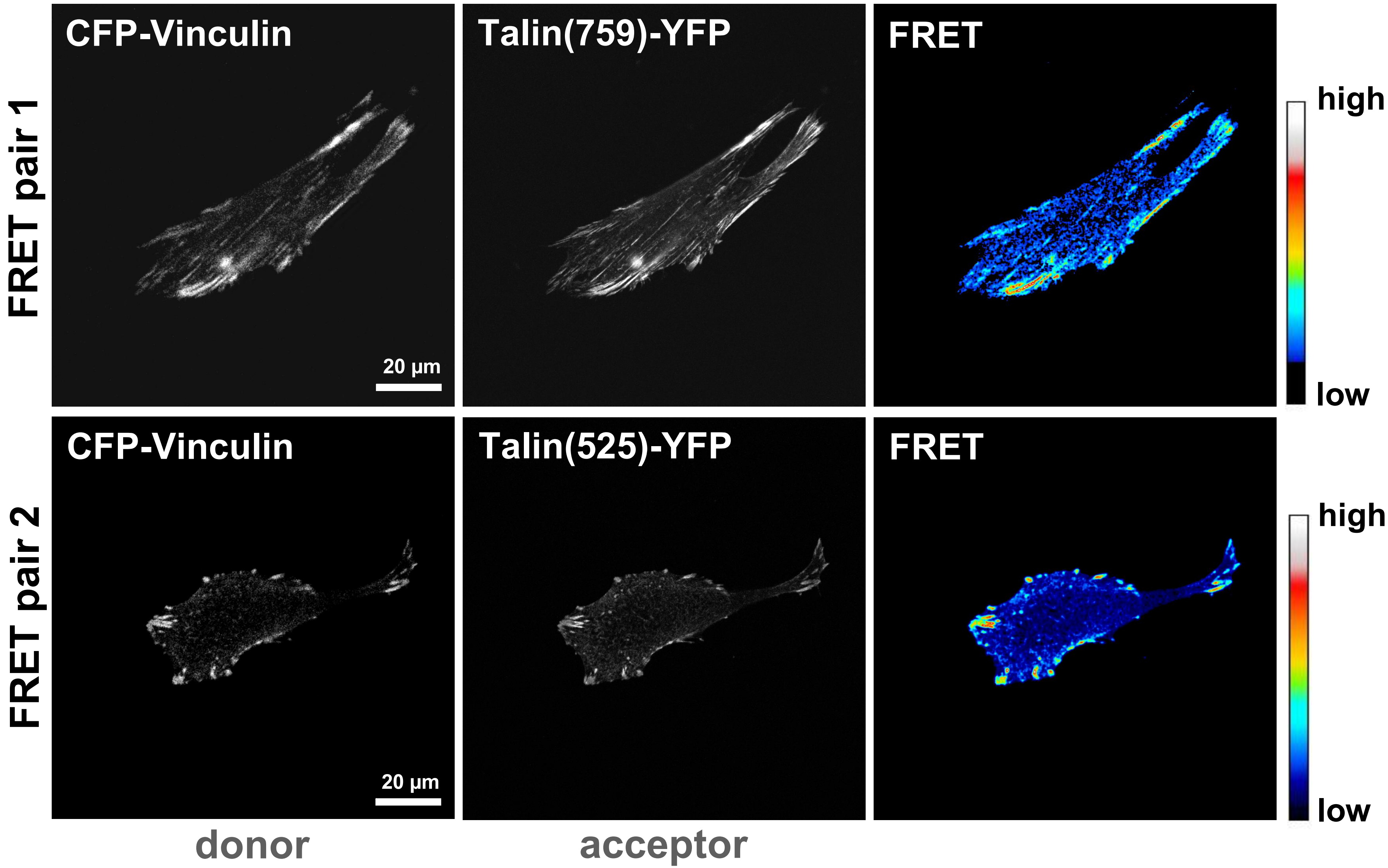

n = 31

**$E_F$: 14.19 ± 6.81**

$C_F$: 3.31 ± 4.24

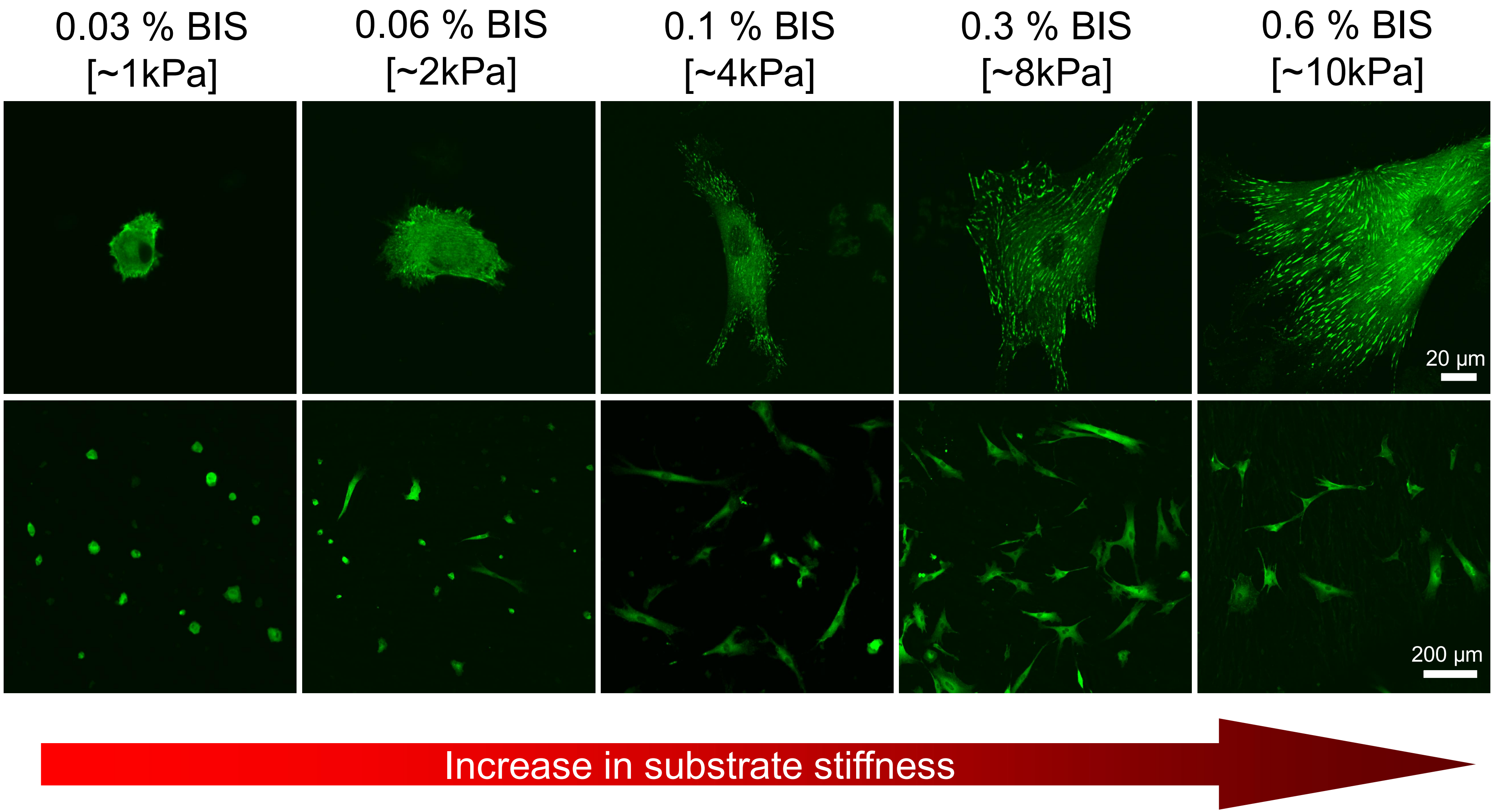
NHDFs 24h after transfection with pCFP-YFP-Vinculin

# Sensitized emission FRET



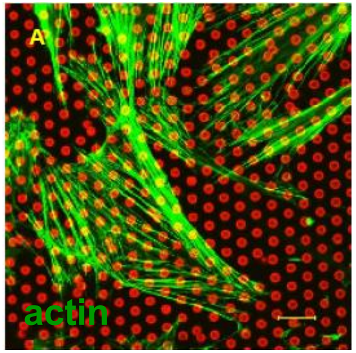
# Cell adhesion on polyacryl amide substrates

- AA conc. 5% fix, FN coated
- anti-vinculin

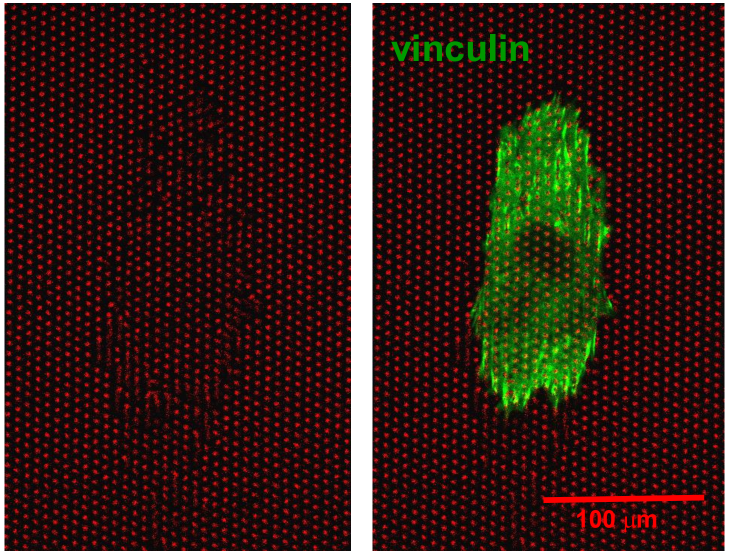


# Ongoing: FRET between vinculin and talin – correlation with forces

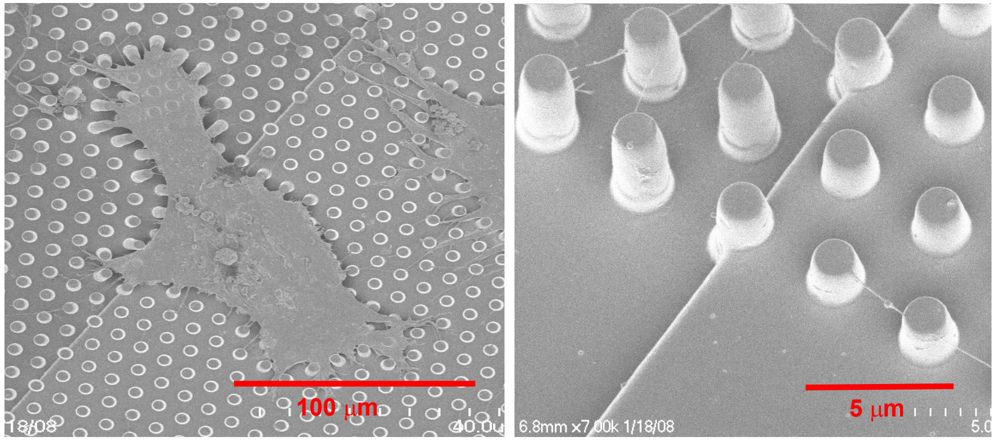
- fibroblasts on pillars

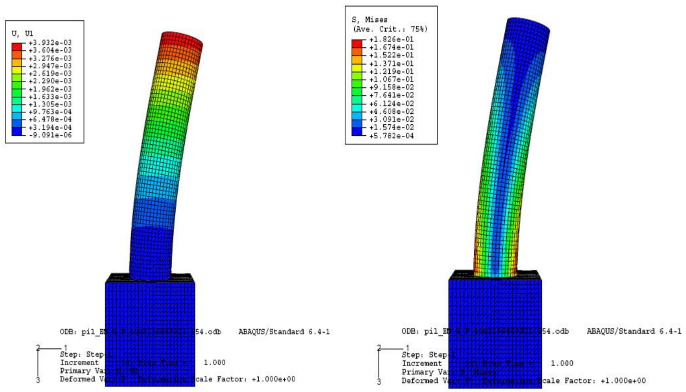



**fibronectin coated tops of pillars**

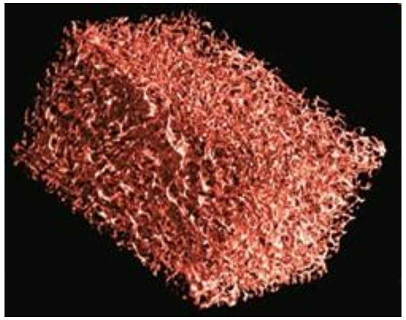



- measuring forces transduced by cells on pillars



# Conclusions for cell-based therapies



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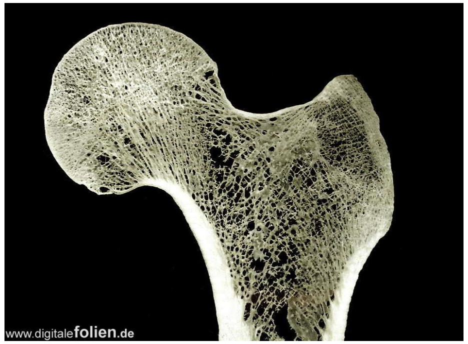
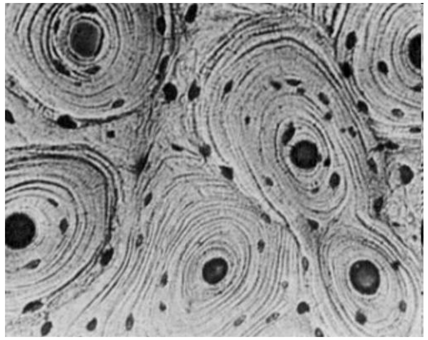
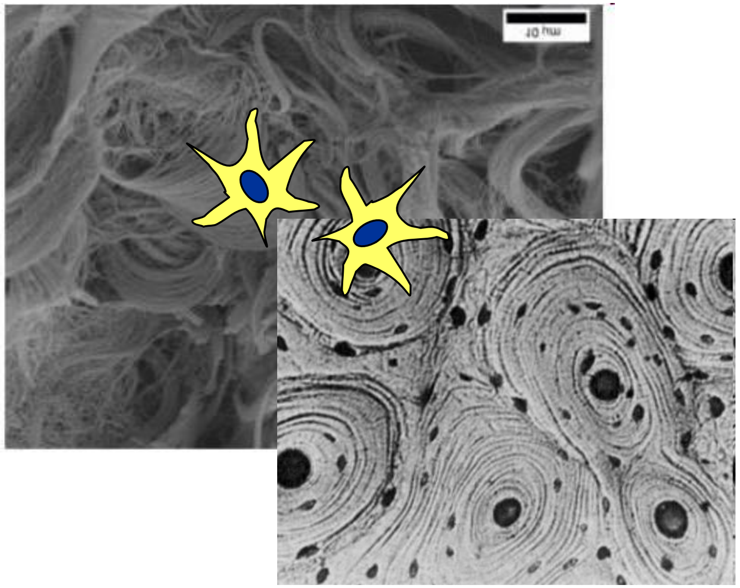
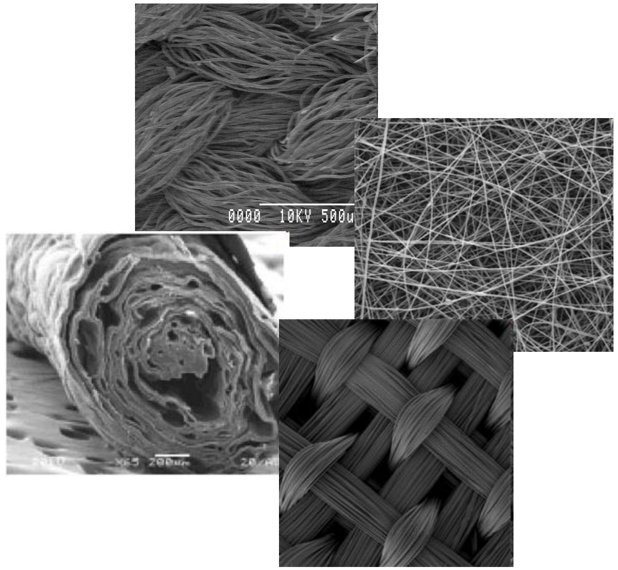




detailed understanding
of cellular processes
- tissue
- decellularised tissue
- engineered models

detailed planning of
tissue replacement
materials



# People + funding

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