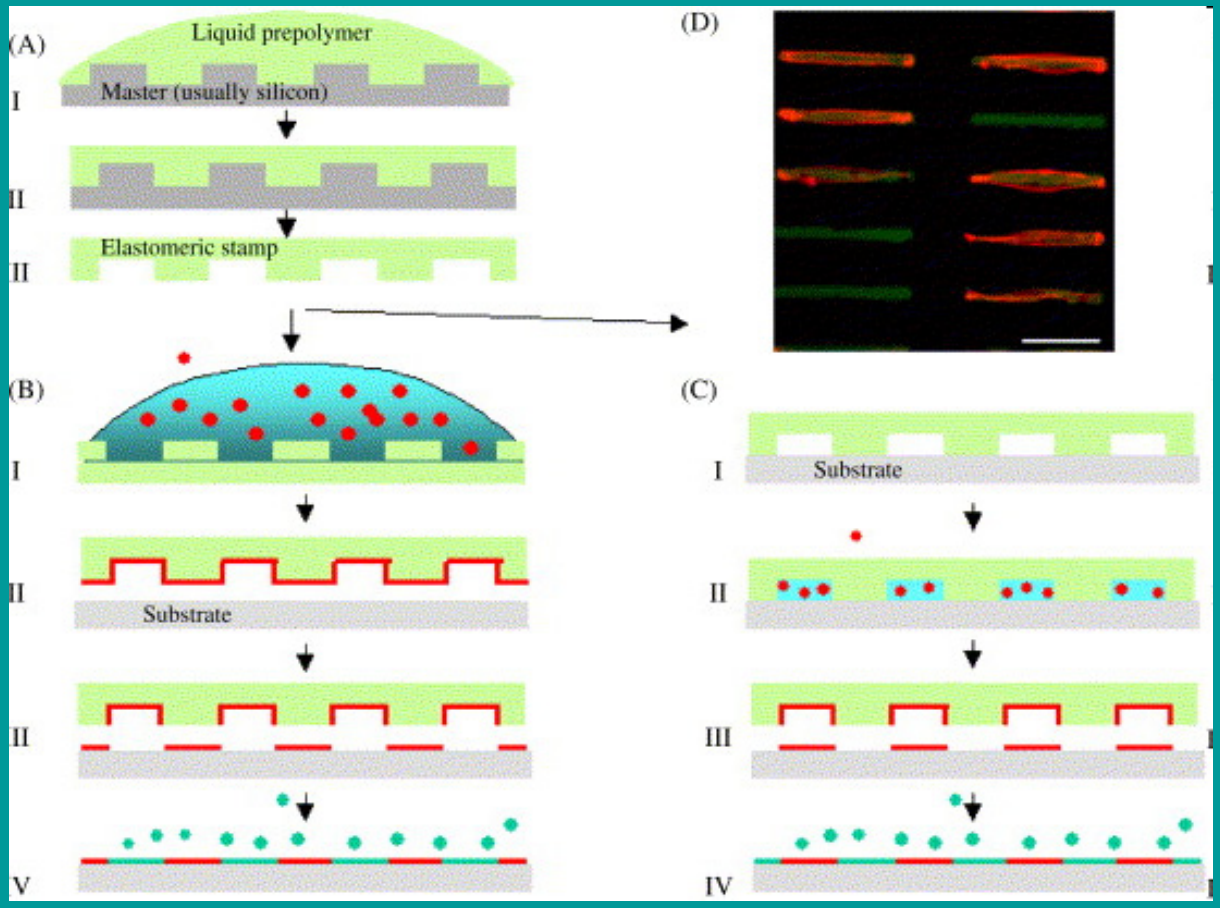
Techniques for Surface Patterning

- Photolithography
- Printing Techniques
  - Nanoprinting
  - Microcontact Printing
  - Direct Writing
- Patterning with Block Copolymers
- Instability-Induced Polymer Patterning

Nie, Z, Nature Materials 7, 2008, 277

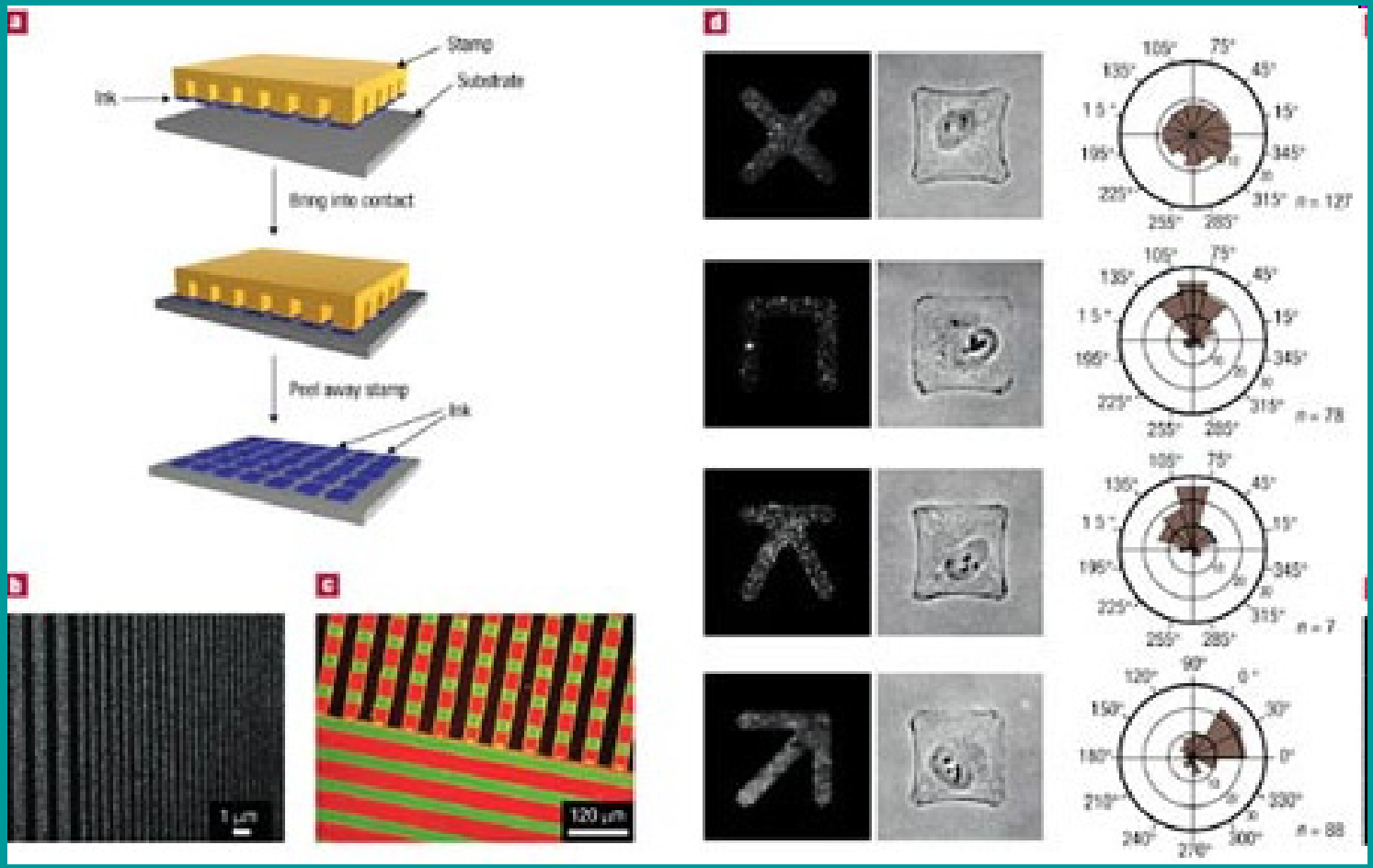
Microcontact Printing and Microfluidic Patterning



Red: Biomolecules

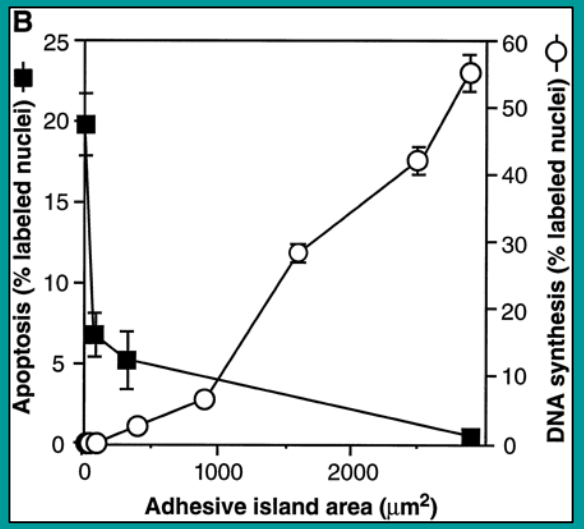
Falconnet, D., Biomaterials 27 (2006), 3044

Microcontact printing

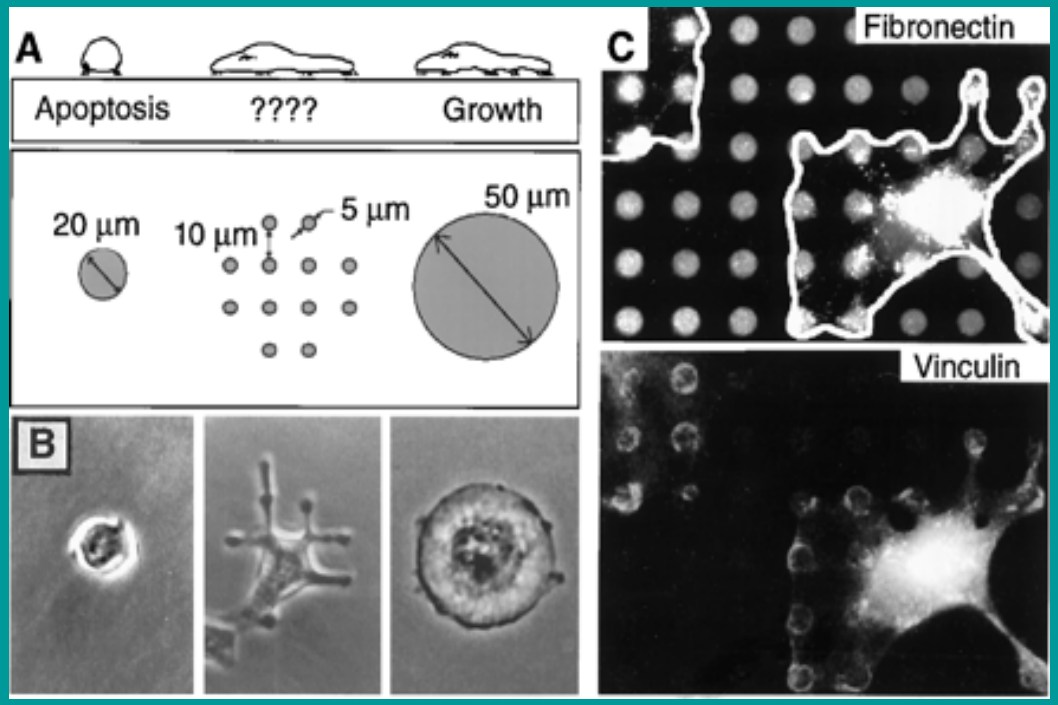


Cell shape controls cell physiology

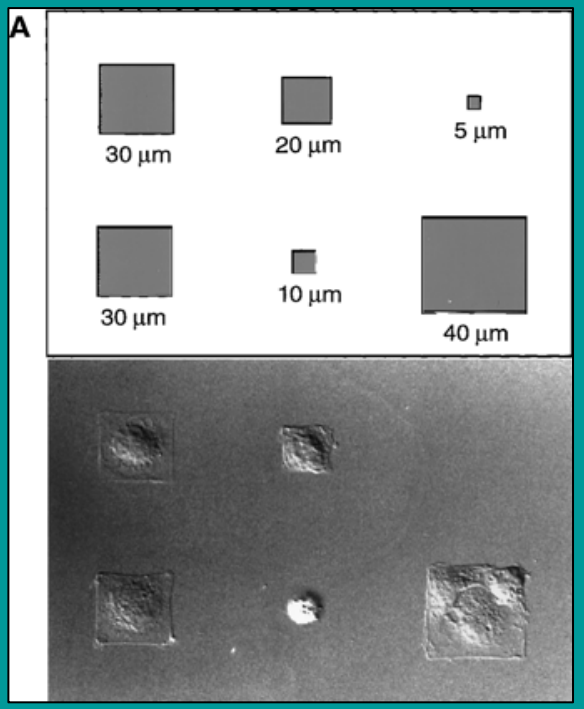
Round endothelial cells apoptote, while spread cells replicate



Cell shape control whithout changing the adhesion area

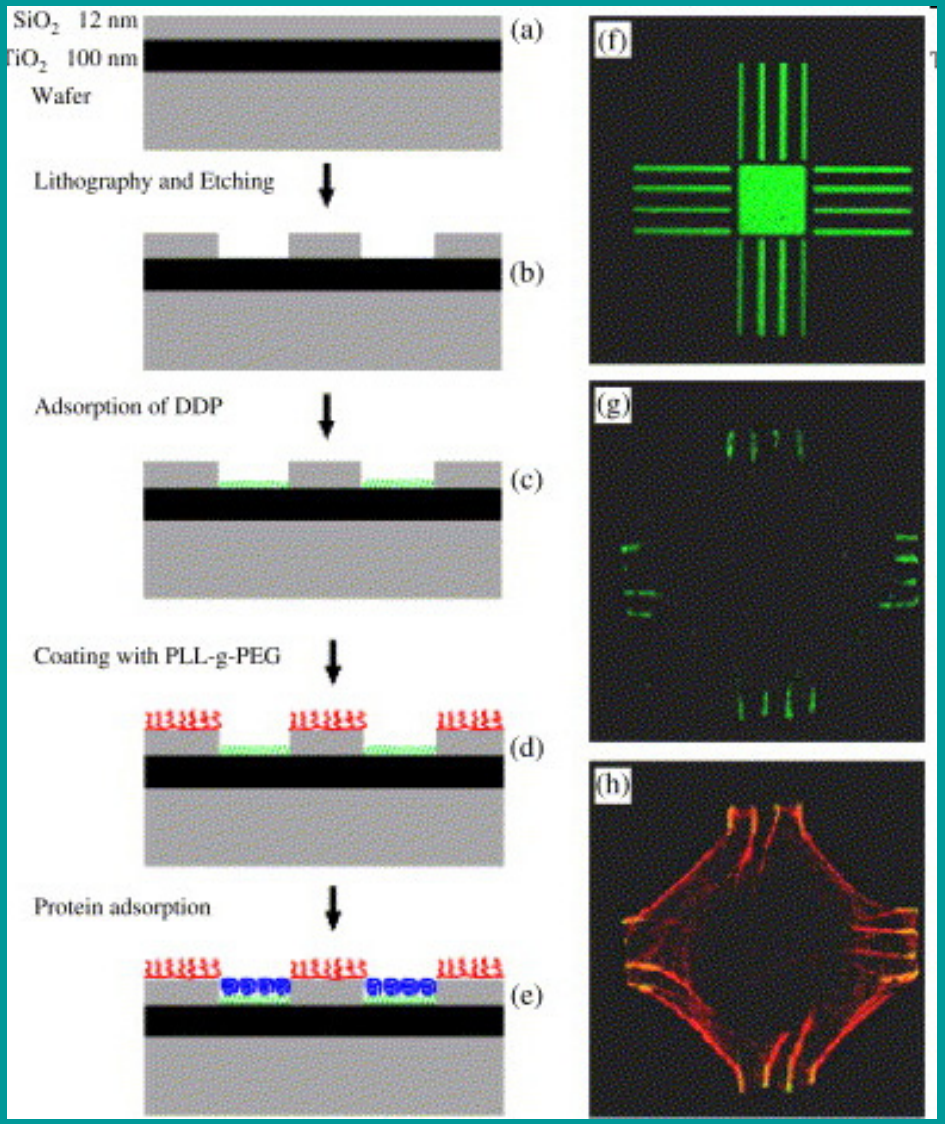


Fabrication of cell geometry



Chen et al., Science 276 (1997)

Defined Structural Organisation of the Aktincytoskeleton



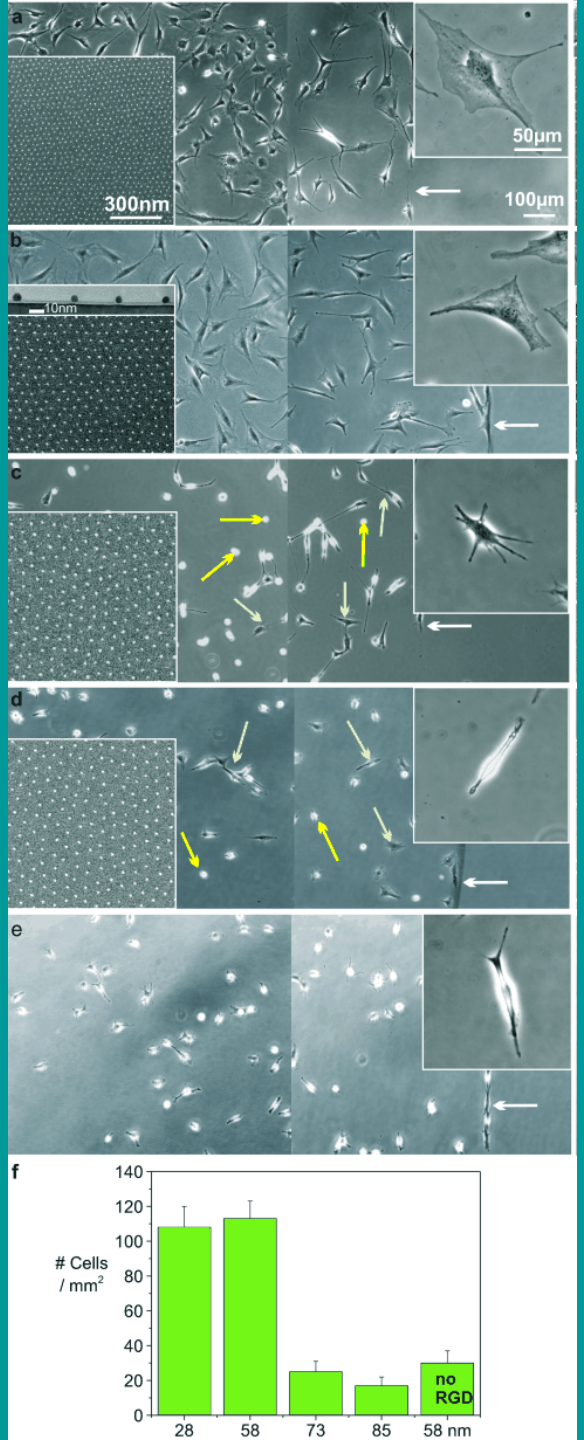
Falconnet, D., Biomaterials 27 (2006), p. 3044

28 nm

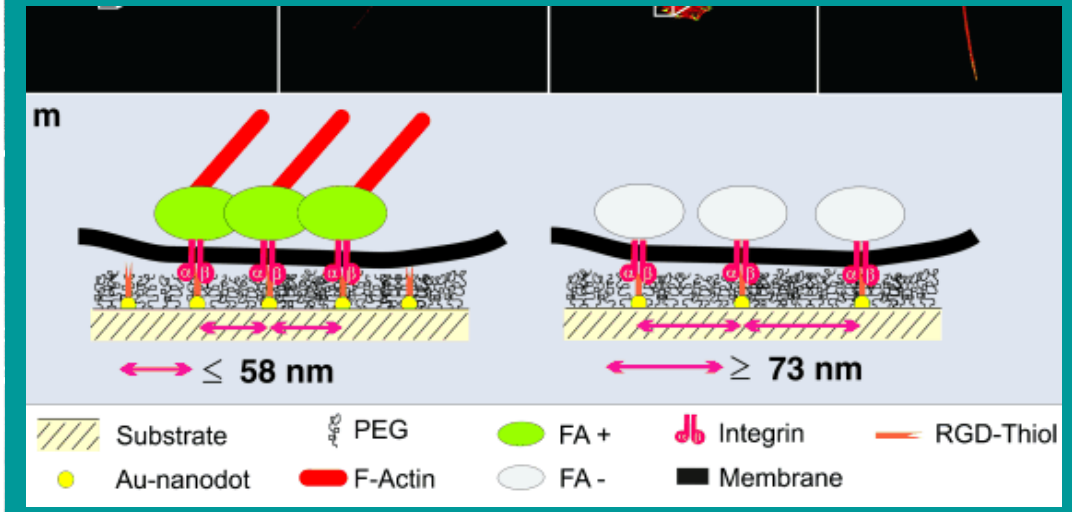
58 nm

73 nm

85 nm

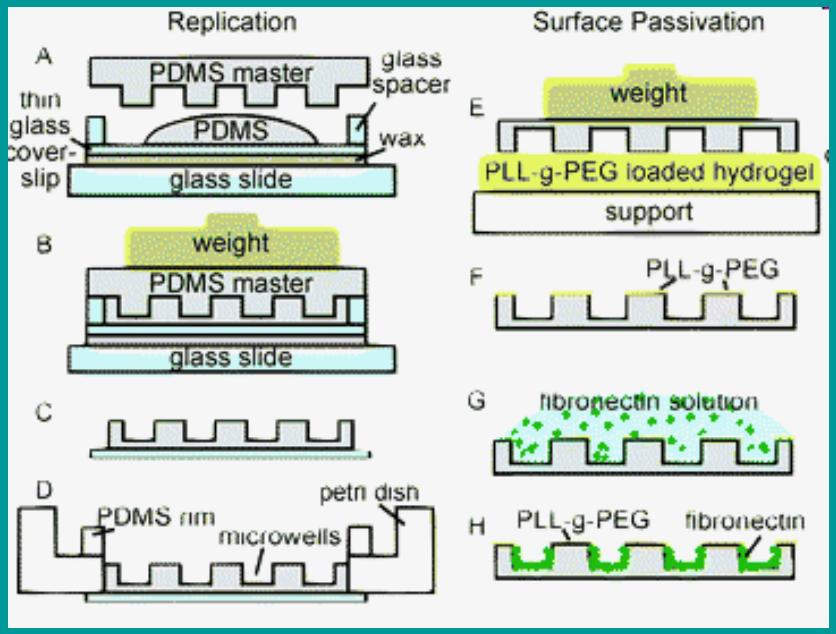


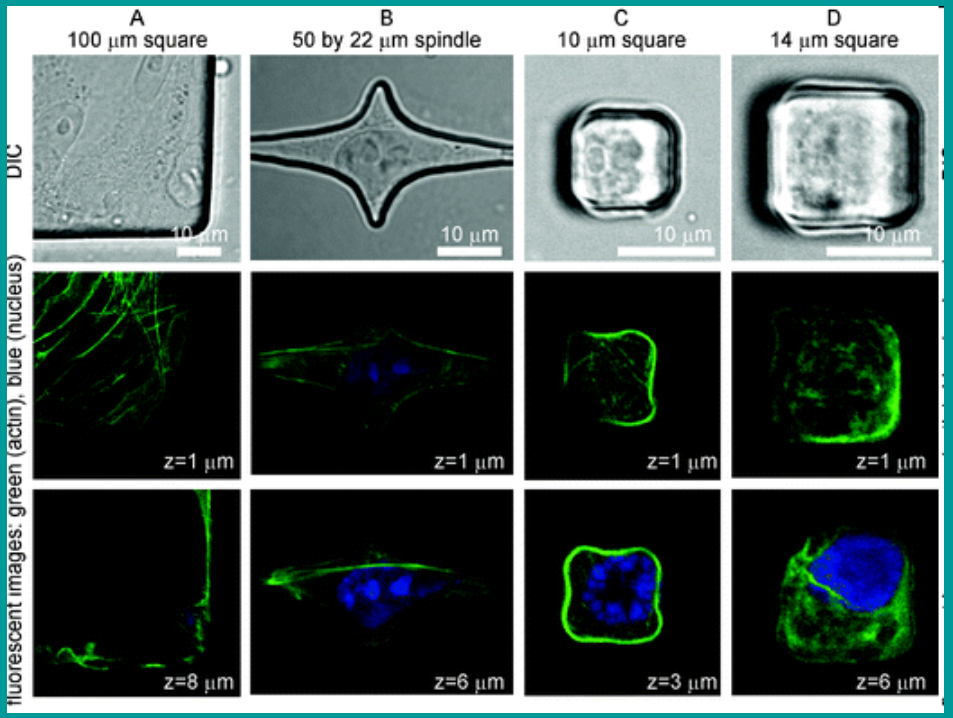
Cell spreading requires a minimal distance of the ligand



Arnold, M., et al. ChemPhysChem, 5 (2004)

3-D shape control for single cells





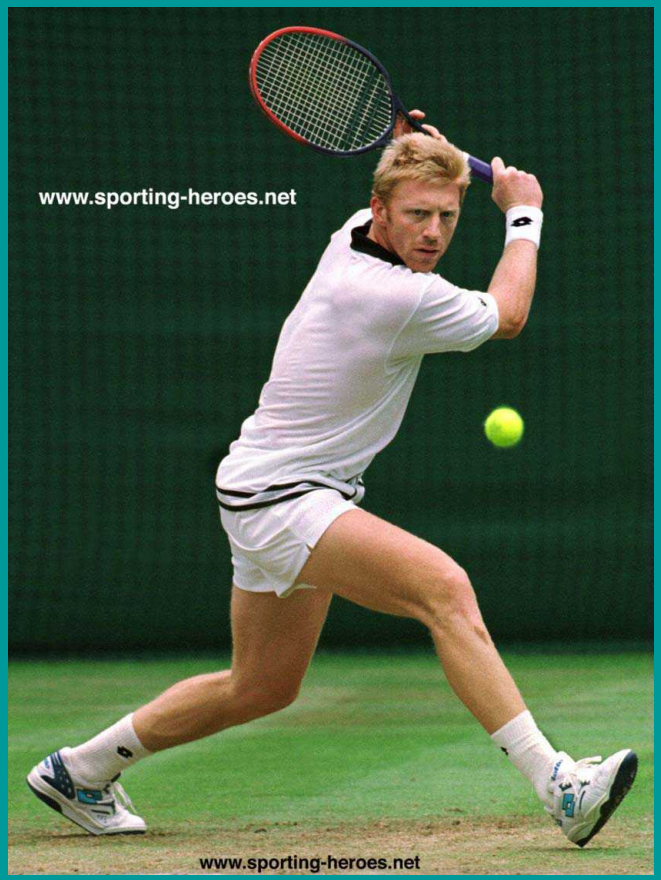
PDMS – polydimethyl-siloxane
Poly-L-lysine-graft-poly(ethylen)glycol

Ochsner,M. Lab Chip 7 (2007)

Mechanical Properties of Biomaterials

Julius Wolff

- **"Remodeling of bone ... occurs in response to physical stresses - or to the lack of them - in that bone is deposited in sites subjected to stress and is resorbed from sites where there is little stress"**



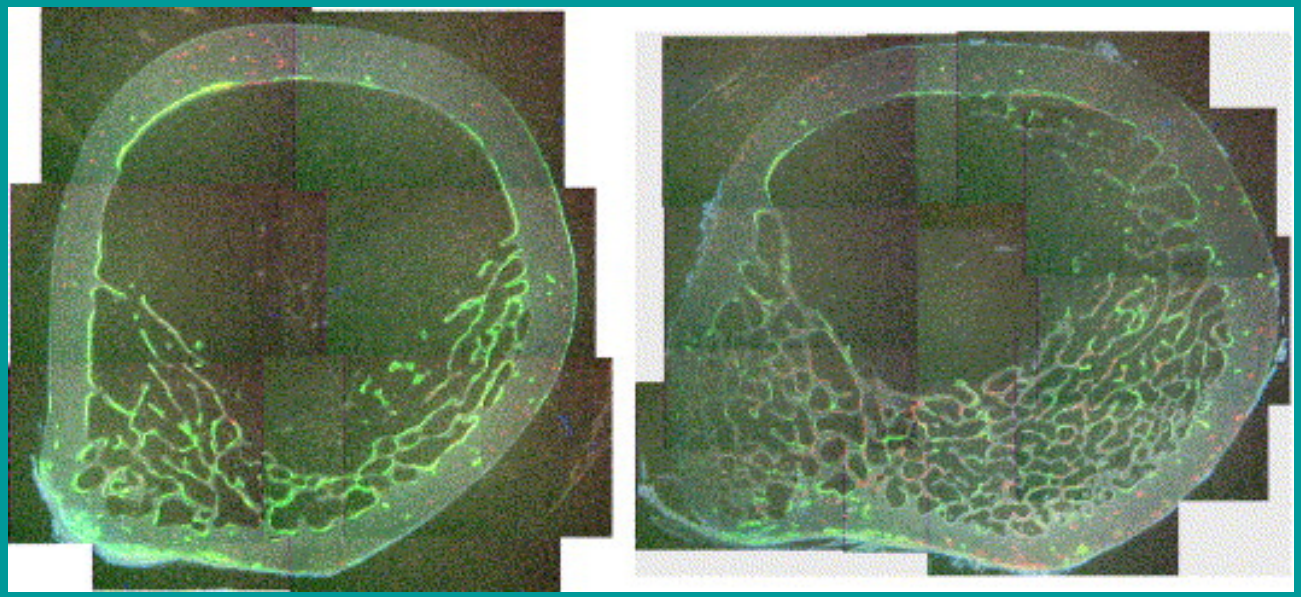
Tennis Player May Have 30% More Bone in the Playing Arm

Astronauts lose 1- 2% of Bone Mass per Month

„Good Vibrations“

Clinton Rubin, University Stony Brook, N.Y.

Vibration Therapy

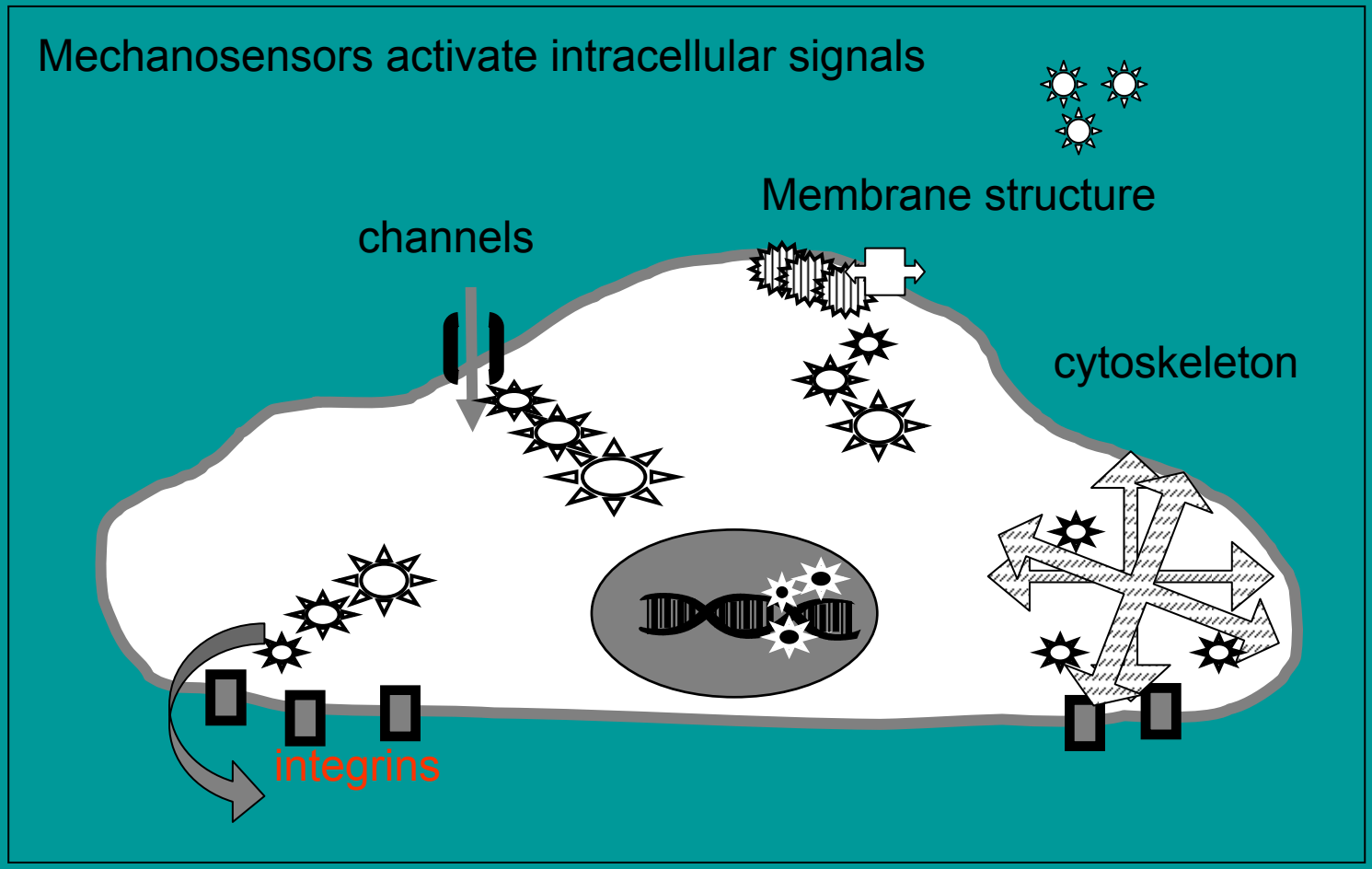


34% More Spongiosa in Sheep

After 1 Year Daily 20 min Mechanical Load, 0,3 g, 30 Hz

Rubin, Nature 2001

Cells Sense Mechanical Signals

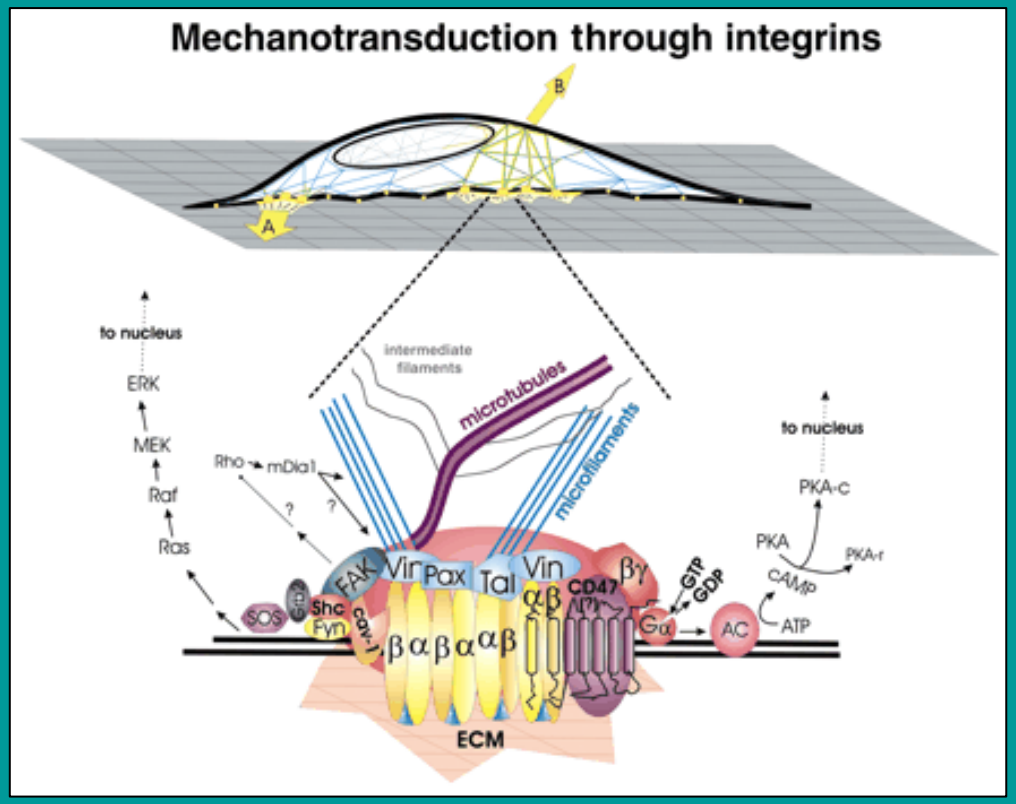


Mechanical properties of the biomaterial

Mechanical forces control cell physiology:

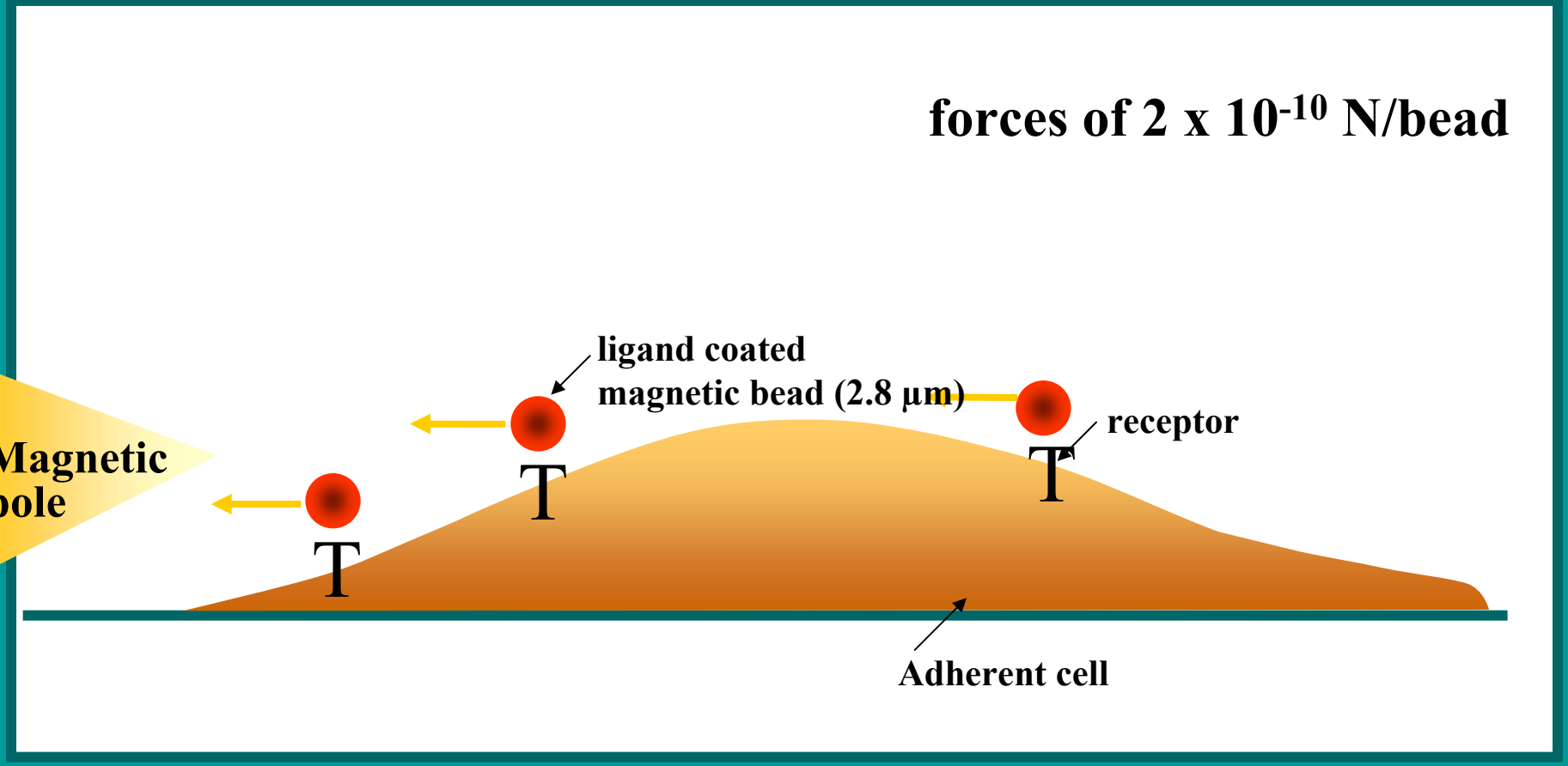
1. From outside the cell mediated by the material and extracellular matrix

2. From inside the cell to the substrate



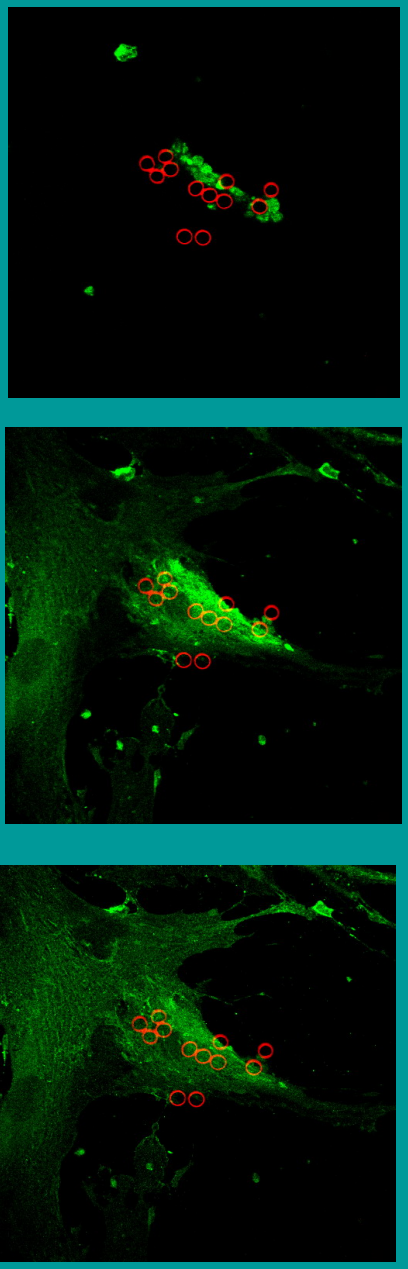
Mechanical stress to integrins induces signal tranduction

Design for application of mechanical stress to cell receptors



Pommerenke et al., EJCB 70 (1996) 157
Schmidt et al., JCB 273 (1998) 5081
Pommerenke et al., JBMR 17 (2002) 603

Clustering of focal adhesion proteins due to mechanical stress to the integrin receptor



1

2

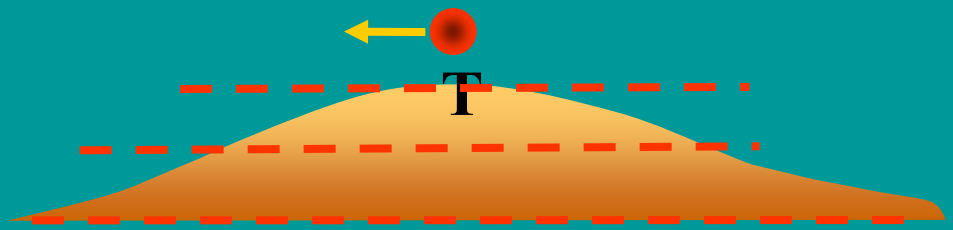
3

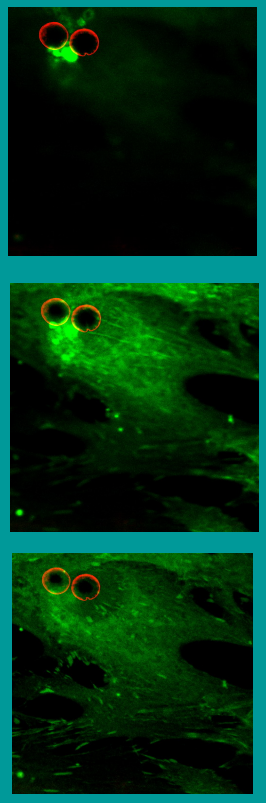
talin

1

2

3





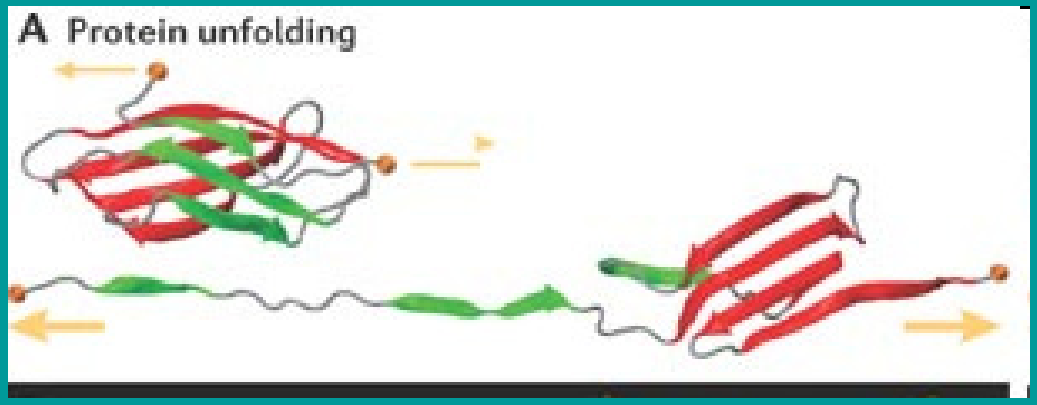
1

2

3

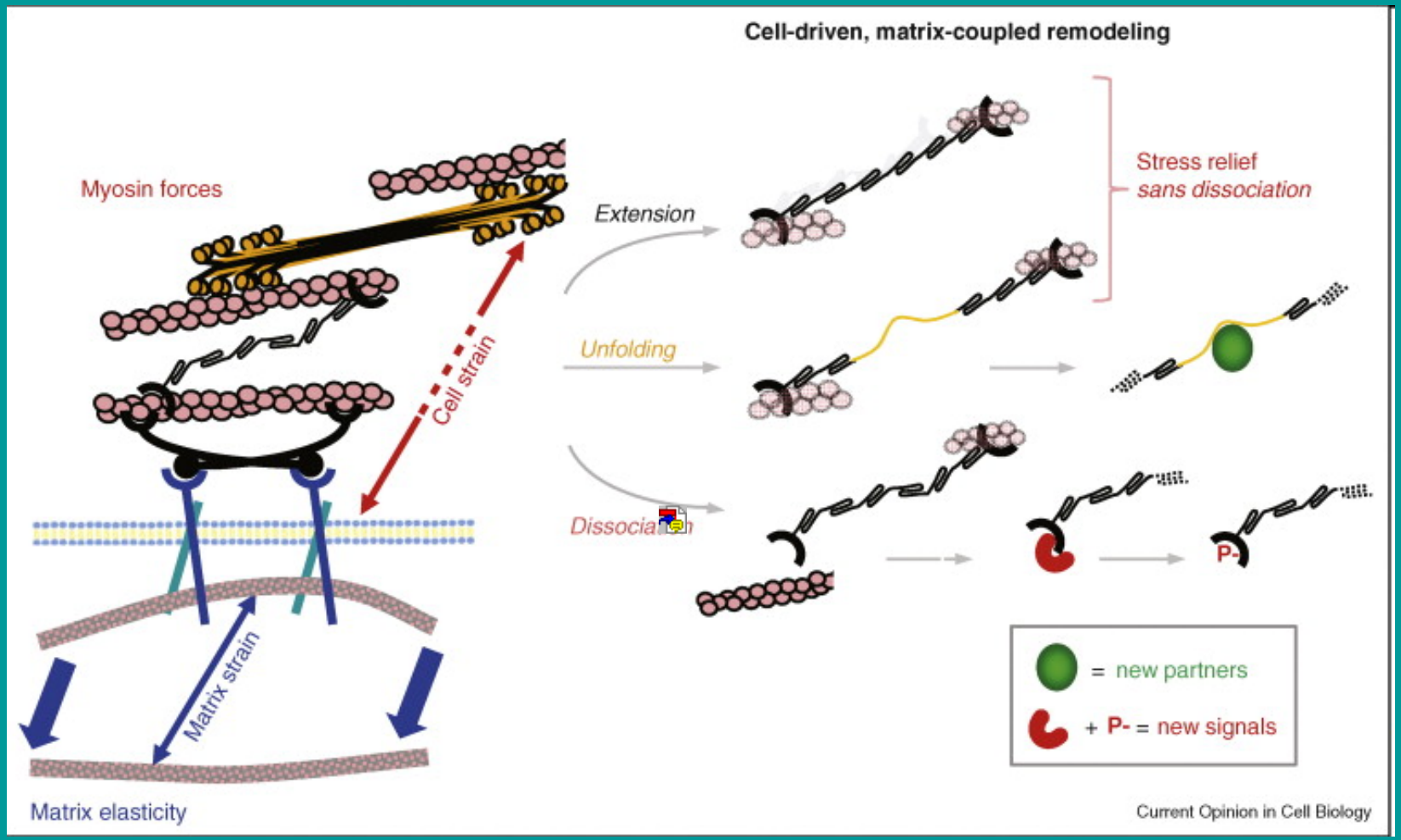
vinculin

Conversion of force into biochemical signals by protein unfolding



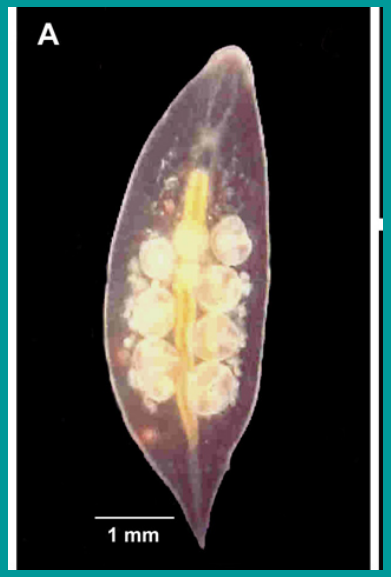
fibronectin

Vogel, V., Sheetz, M,:Nature Reviews, Molecular Cell Biology,7, 2006


Cell-driven, matrix-coupled remodeling
Myosin forces
Cell strain
Matrix strain
Matrix elasticity
Extension
Unfolding
Dissociation
Stress relief
sans dissociation
P-
= new partners
+ P- = new signals
Current Opinion in Cell Biology

Stem Cells and Biomaterials

Ability of Animals to Regenerate Tissue Depends on the Stage of Development



Flatworm

Earthworm

Salamander

Invertebrates

Vertebrates

Which tissue can regenerate?

Connective Tissue

- **Bone** — **Yes**
- **Articular cartilage, ligament, Intervertebral disc** — **No**

Epithelia (e. g. Epidermis) — Yes

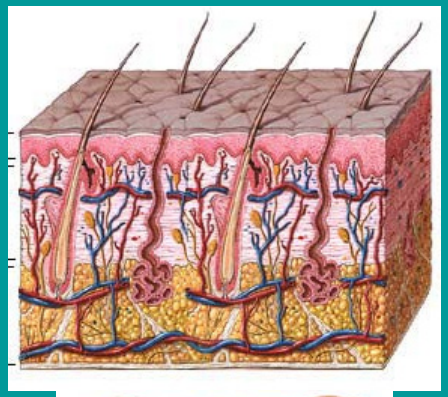
Muscle

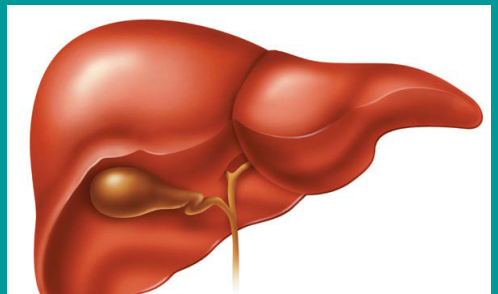
- **Cardiac, Skeletal** — **No**
- **Smooth** — **Yes**

Nerve — No

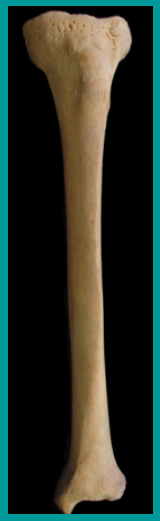
Regeneration in Humans

High

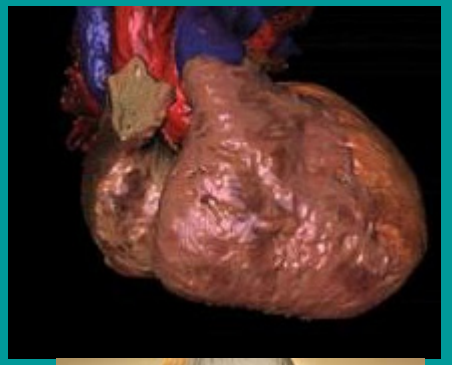


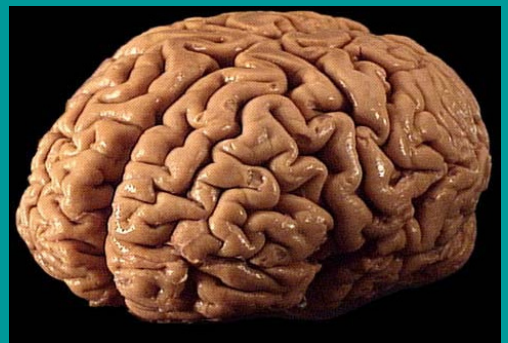


Moderate

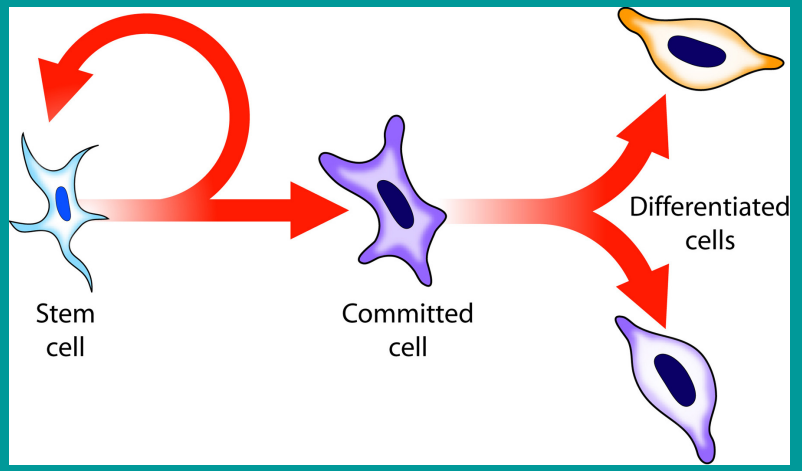


Low





Stem cells



Definition:

1. Cells which are undifferentiated and are able of self-renewal for a longer time.

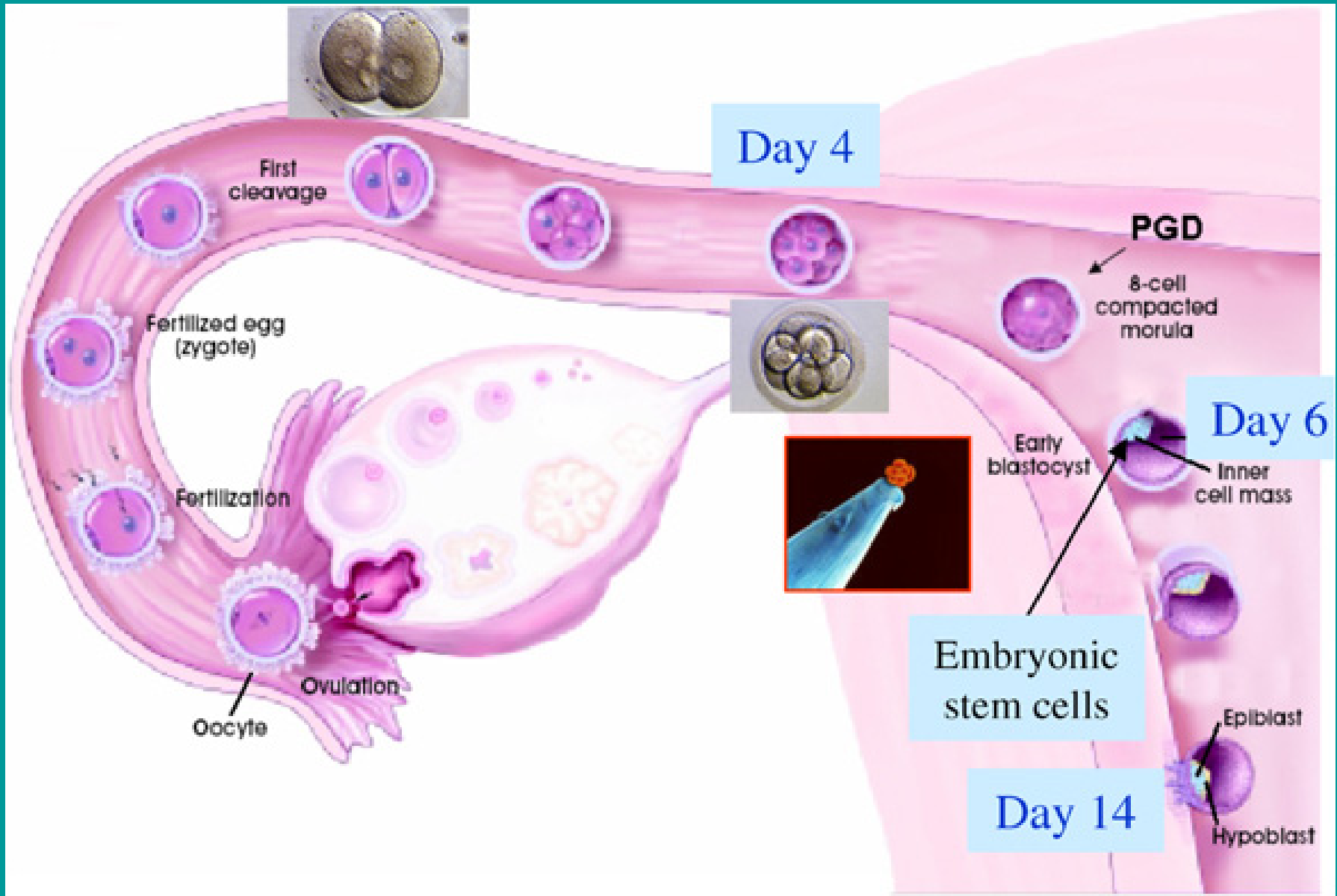
2. Under specific physiological or experimental conditions they have the ability to differentiate to different cell types with specific functions.

Stammzellen

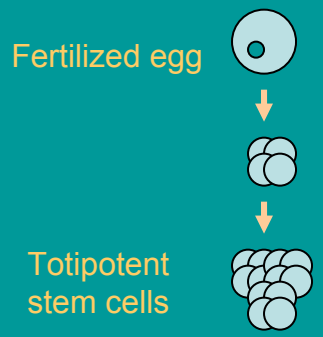
Embryonale
Stammzellen

Adulte
Stammzellen

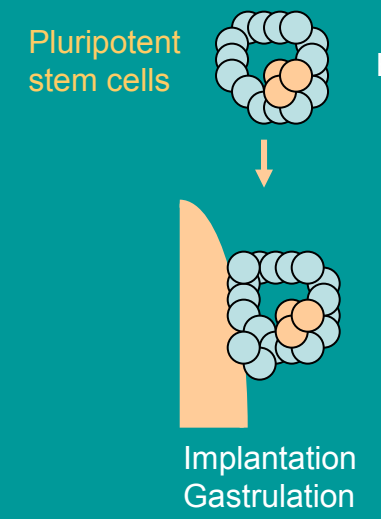
Development of a Blastocyst Containing Embryonic Stem Cells



Early Development



Fate Decision



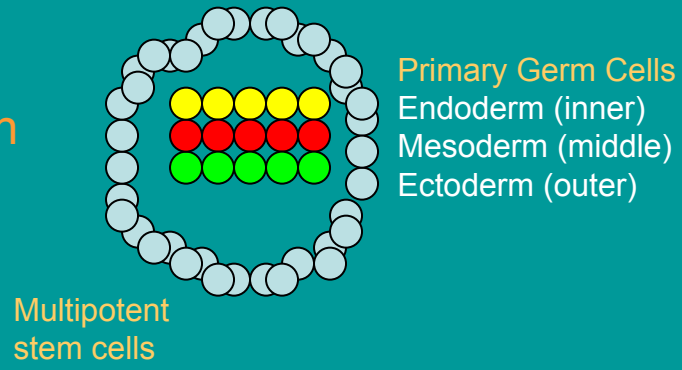
Embryonic stem cells come from inner cell mass of blastocyst.

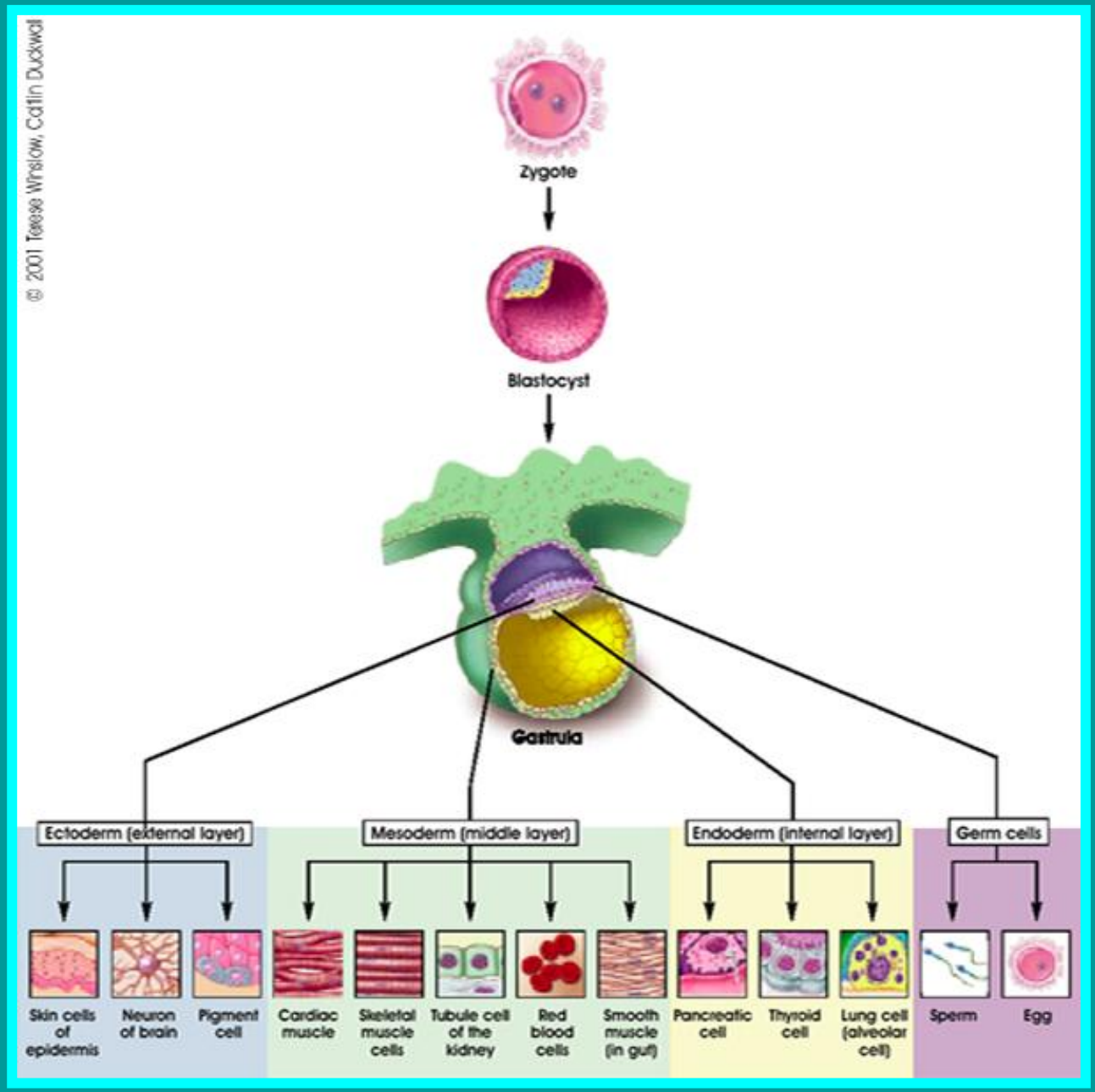
Totipotent: Can become any cell in body or placenta

Pluripotent: Can become any cell in body

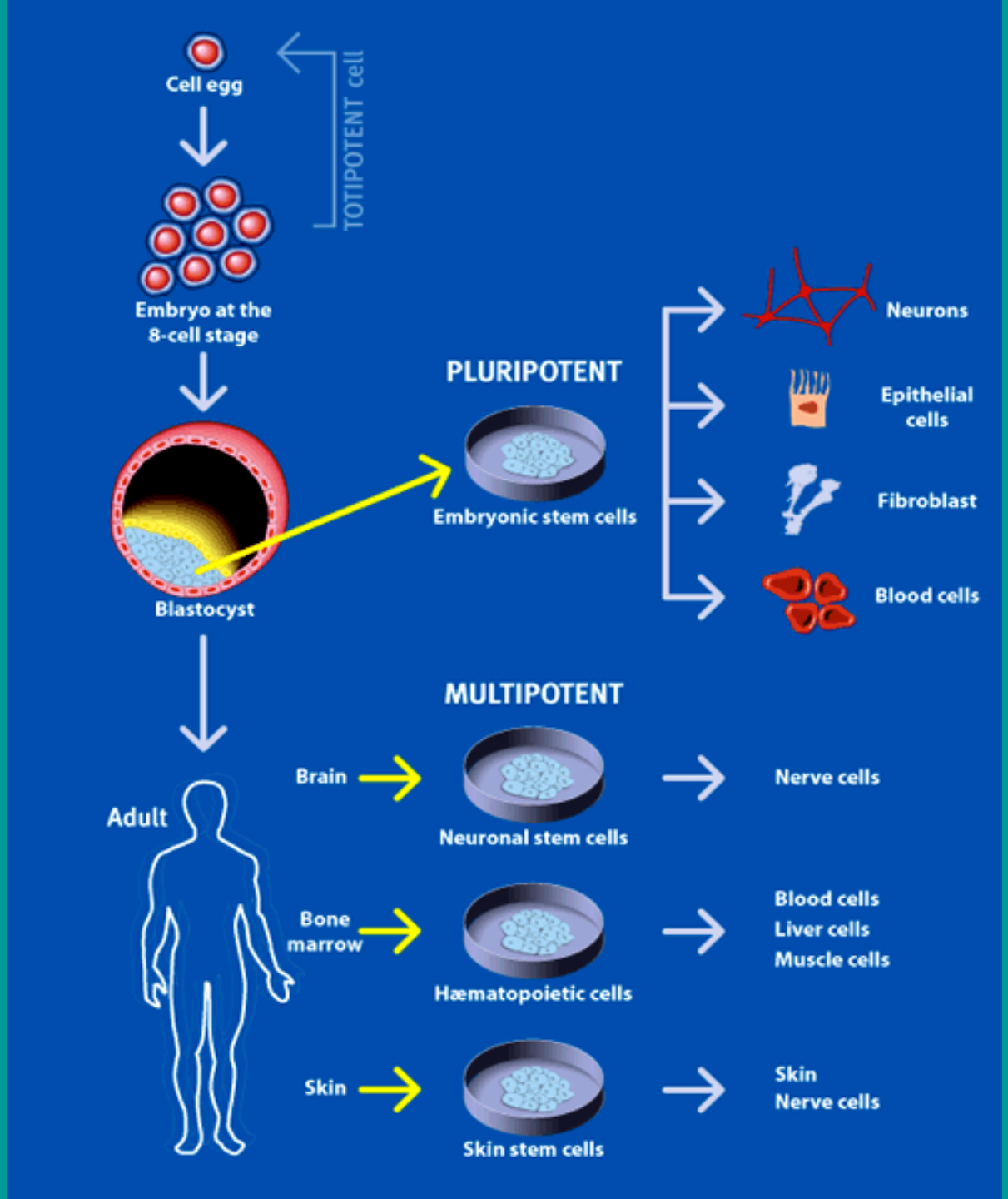
Multipotent: Can become any cell within a specific germ layer or cell lineage

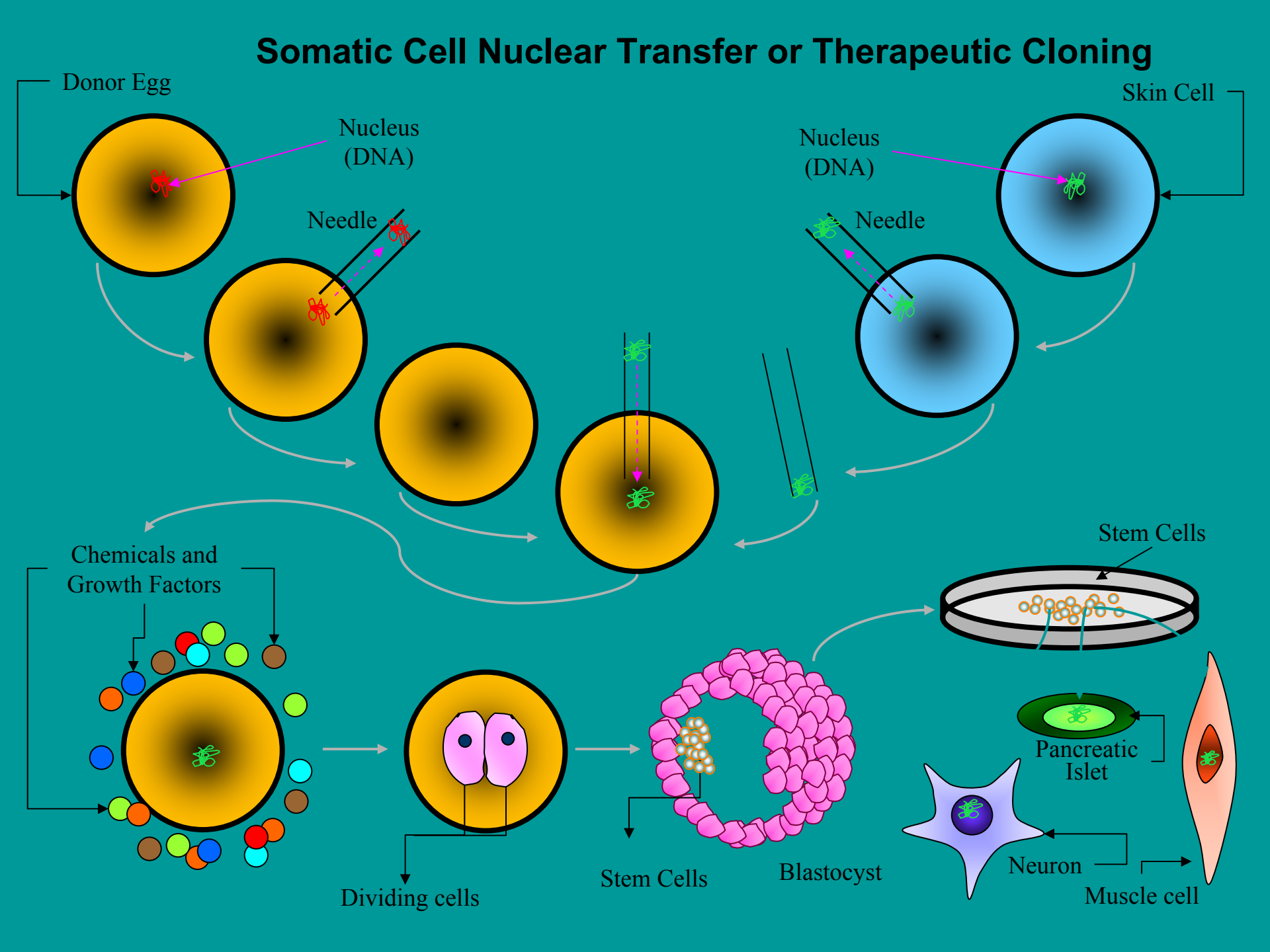
Fate Decision




© 2001 Terese Winslow, Catlin Duckwall
Zygote
Blastocyst
Gastrula
Ectoderm (external layer)
Mesoderm (middle layer)
Endoderm (internal layer)
Germ cells
Skin cells of epidermis
Neuron of brain
Pigment cell
Cardiac muscle
Skeletal muscle cells
Tubule cell of the kidney
Red blood cells
Smooth muscle (in gut)
Pancreatic cell
Thyroid cell
Lung cell (alveolar cell)
Sperm
Egg

Stem Cells


Cell egg
TOTIPOTENT cell
Embryo at the 8-cell stage
PLURIPOTENT
Embryonic stem cells
Neurons
Epithelial cells
Fibroblast
Blood cells
Blastocyst
MULTIPOTENT
Adult
Brain
Neuronal stem cells
Nerve cells
Bone marrow
Hæmatopoietic cells
Blood cells
Liver cells
Muscle cells
Skin
Skin stem cells
Skin
Nerve cells


Somatic Cell Nuclear Transfer or Therapeutic Cloning
Donor Egg
Nucleus (DNA)
Needle
Skin Cell
Nucleus (DNA)
Needle
Chemicals and Growth Factors
Stem Cells
Dividing cells
Stem Cells
Blastocyst
Pancreatic Islet
Neuron
Muscle cell

Embryonic Stem Cells

Strengths

Unlimited self-renewal

- Enhanced telomerase activity

Highest level of pluripotency

- All somatic cell types

Markers:

Oct-4, Nanog, SSEA-3/4

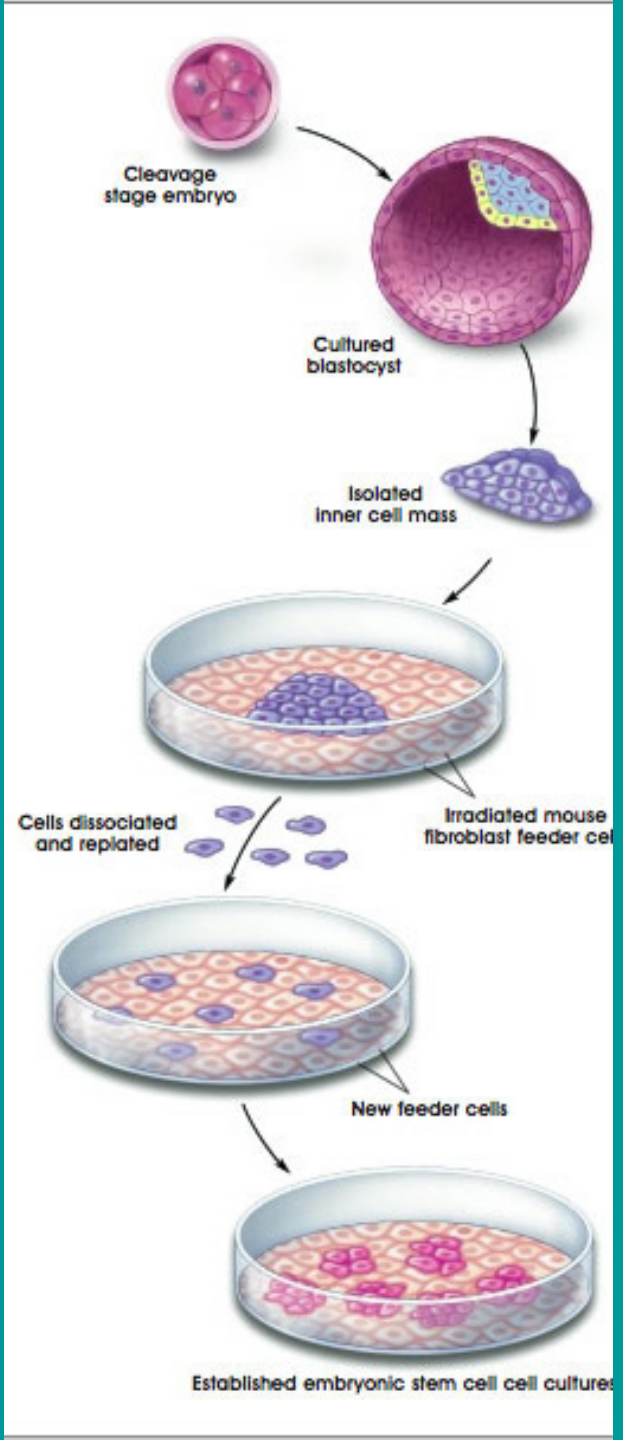
Limitations

Teratoma Formation

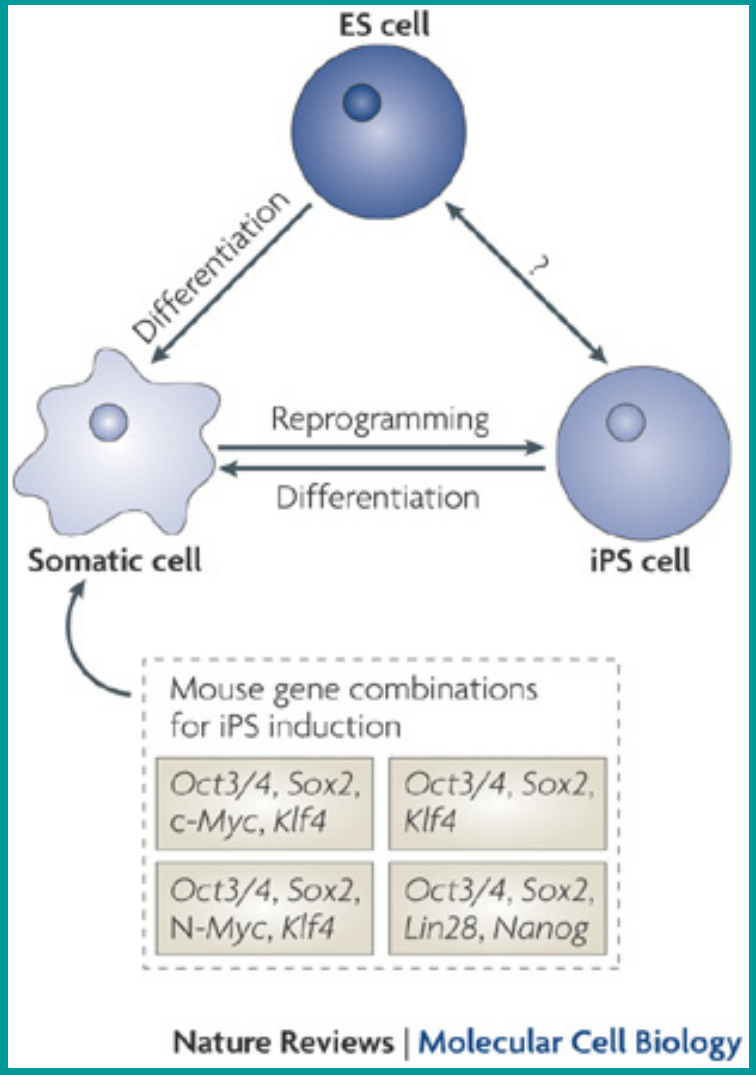
Animal pathogens

Immune Response

Ethics



Induced Pluripotent Stem Cells (iPS)



Adult Stem Cells

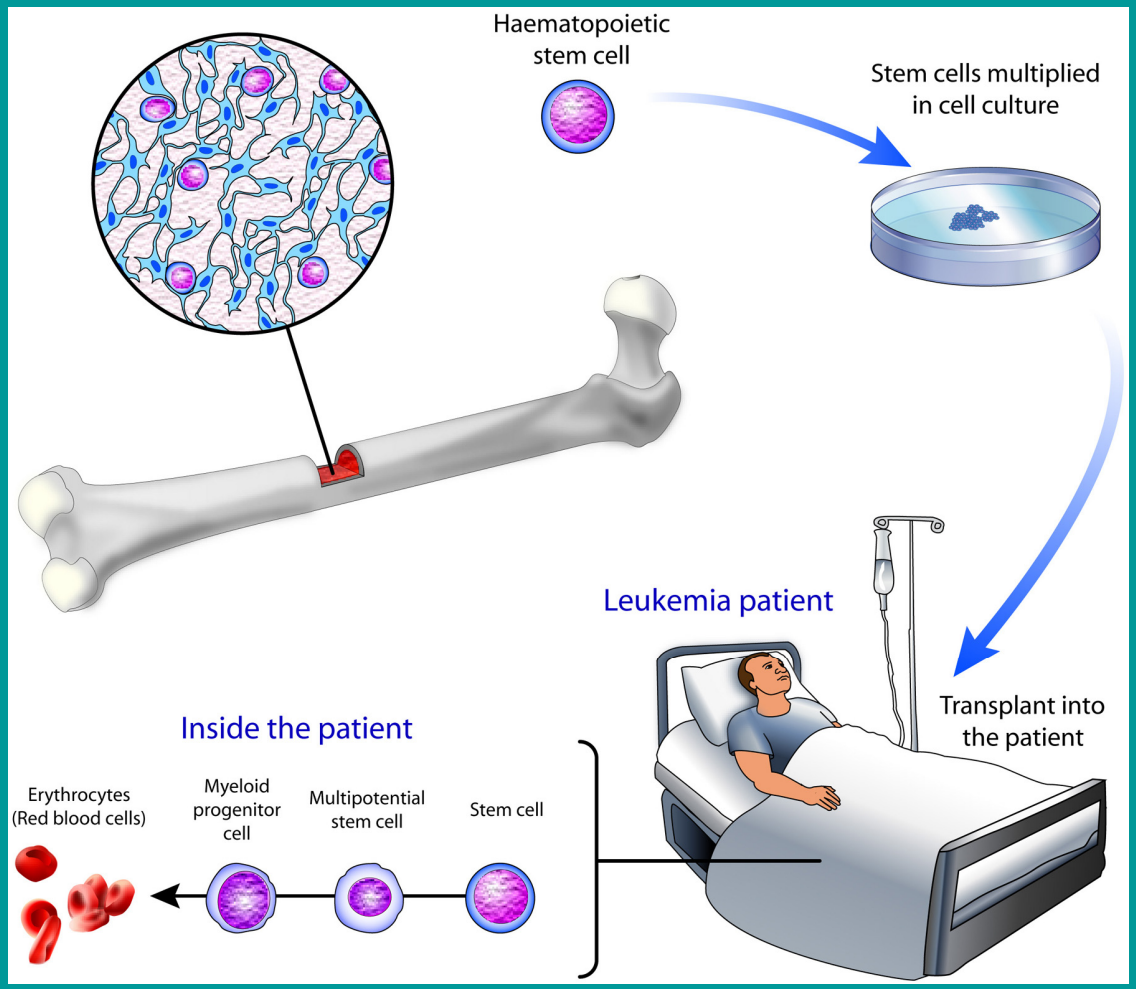
Strengths

- Ethics, not controversial
- Immune-privileged
  - Allogenic, xenogenic transplantation
- Many sources
  - Most somatic tissues

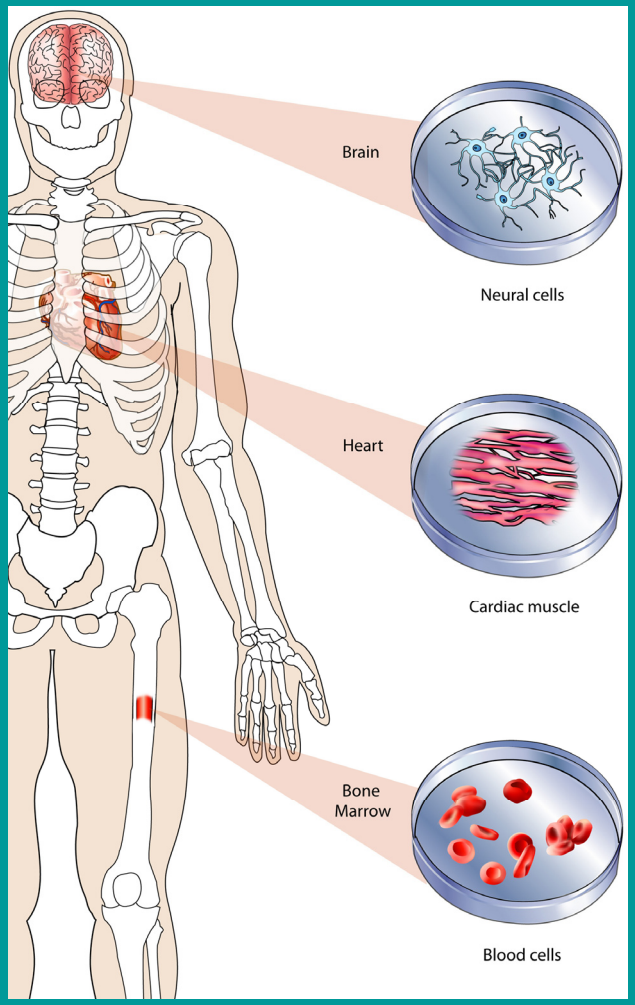
Limitations

- Differentiation Capacity?
- Self-renewal?
- Rarity among somatic cells

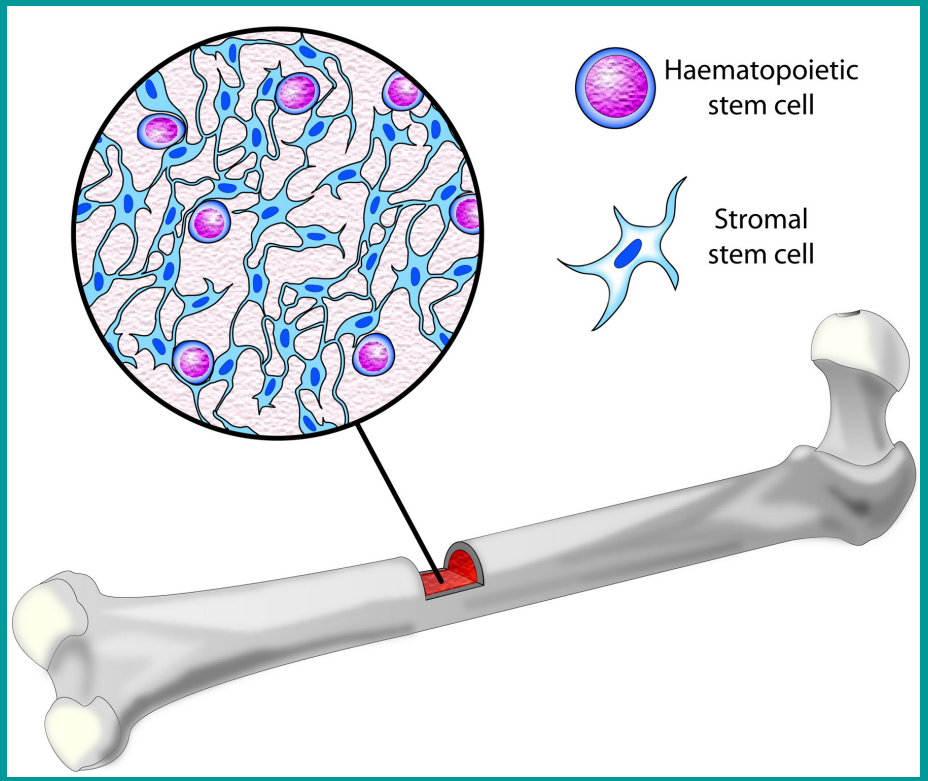
What Is a Treatment Example?



What Are the Sources of Adult Stem Cells?



Adult Body Tissues



Mesenchymal Stem Cells from Adipose Tissue

Photo: Liu Jin / Getty

What Are the Sources of Mature Stem Cells?

Umbilical Cord & Placenta

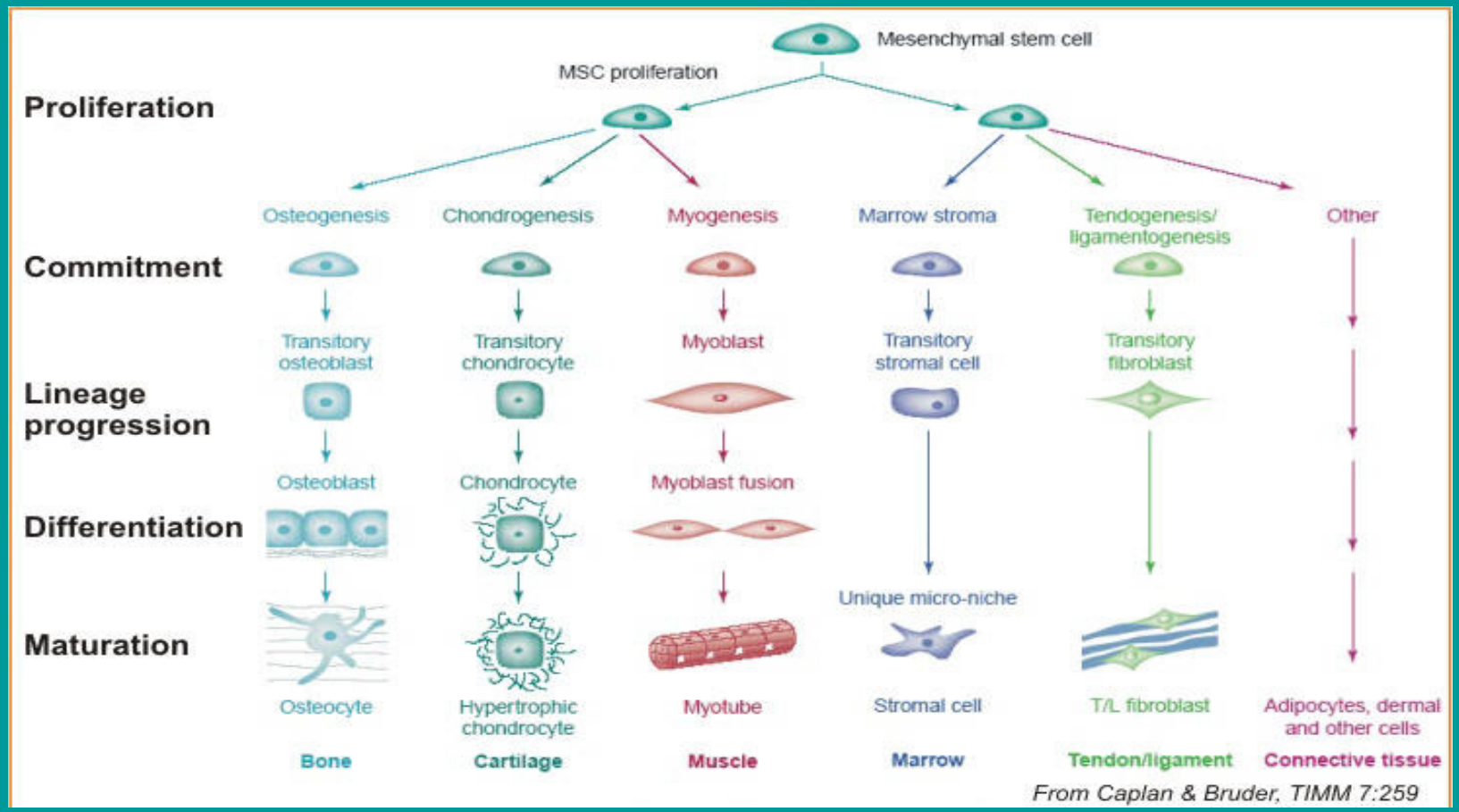
- Wharton's Jelly showing promise as a source
- Some pluripotent characteristics
- Isolated immediately following birth
- Research is limited but growing

Sources for Mesenchymal Stem Cells

- Bone Marrow
- Adipose Tissue
- Placenta
- Umbilical Cord
- etc.

Mesenchymal Stem Cells

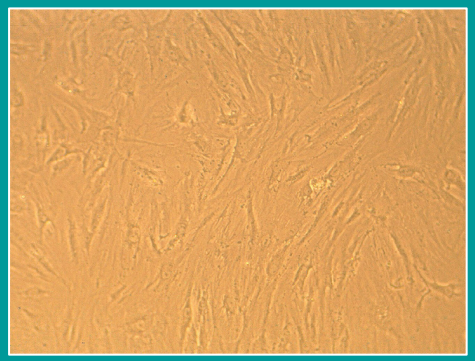
- Easy isolation, high expansion,



Humane mesenchymale Stammzellen aus dem Knochenmark

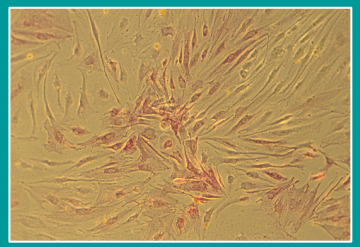
Differenzierung in Osteoblasten oder Fettzellen:

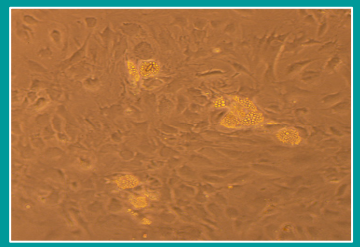
ODM:
Glycerophosphat
Askorbinsäure
Dexamethason



ADM:
Isobutyl-methylxanthin
Indomathazin
Dexamethason

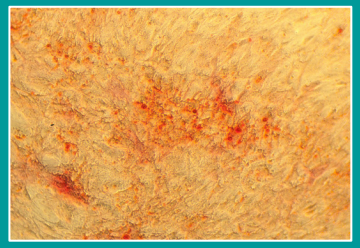
ALP Aktivität

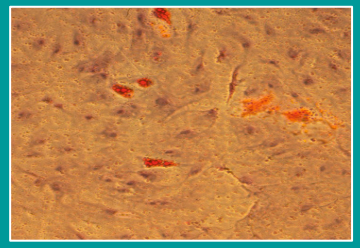




Fetttropfen

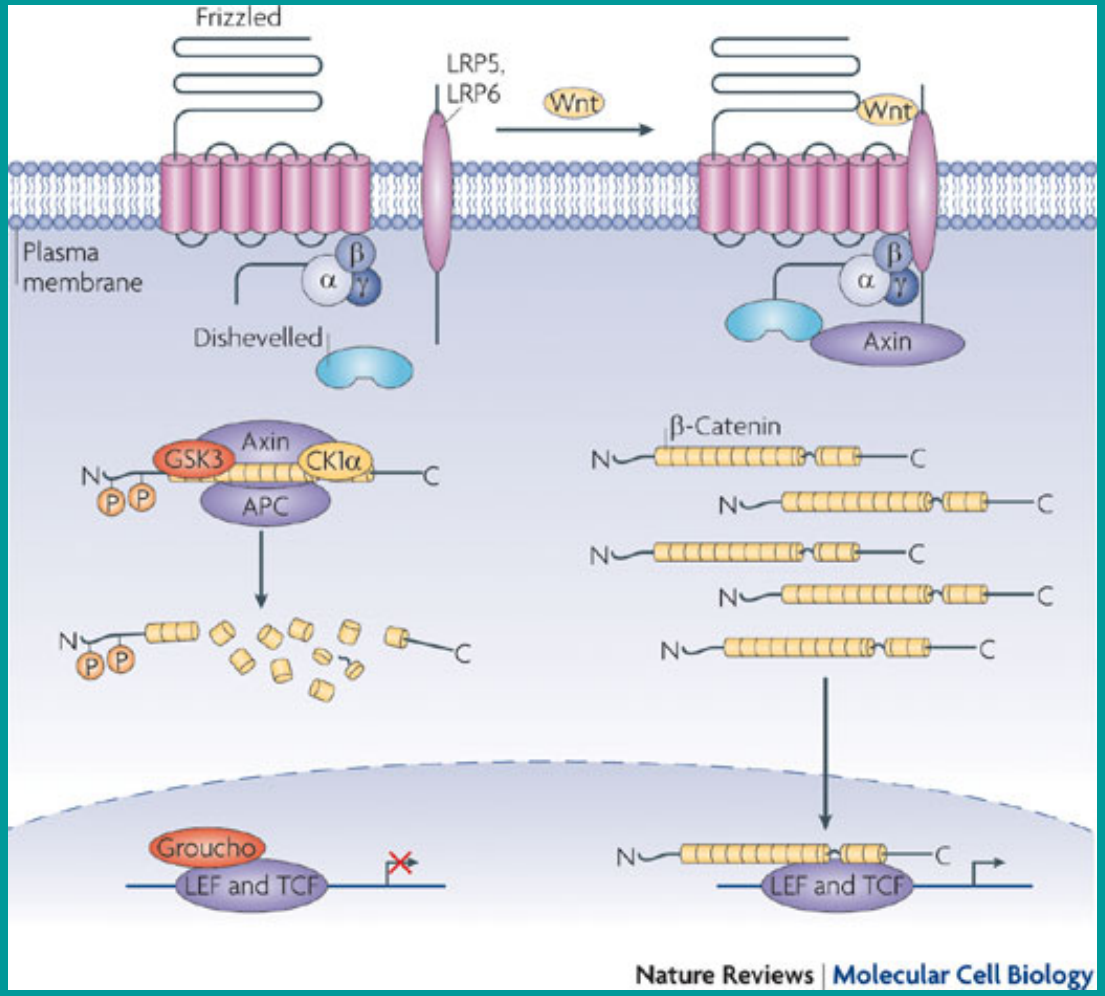
Mineral.

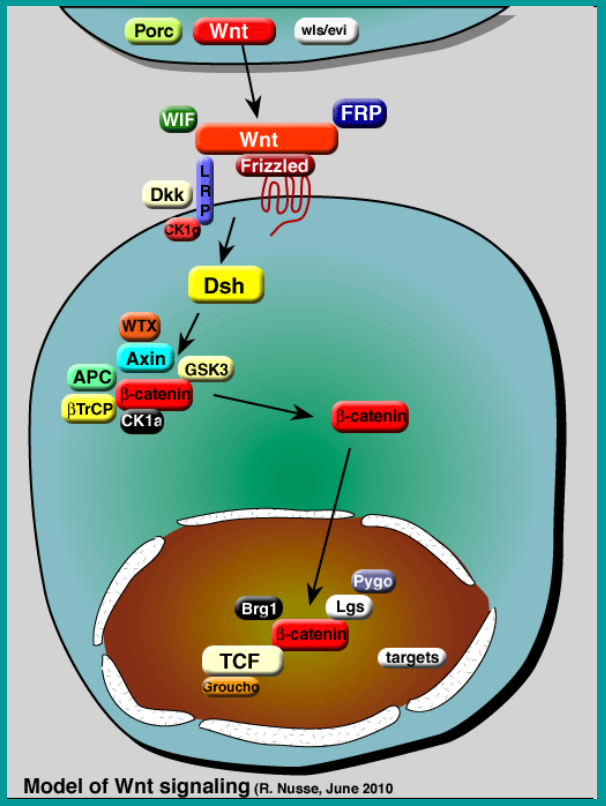




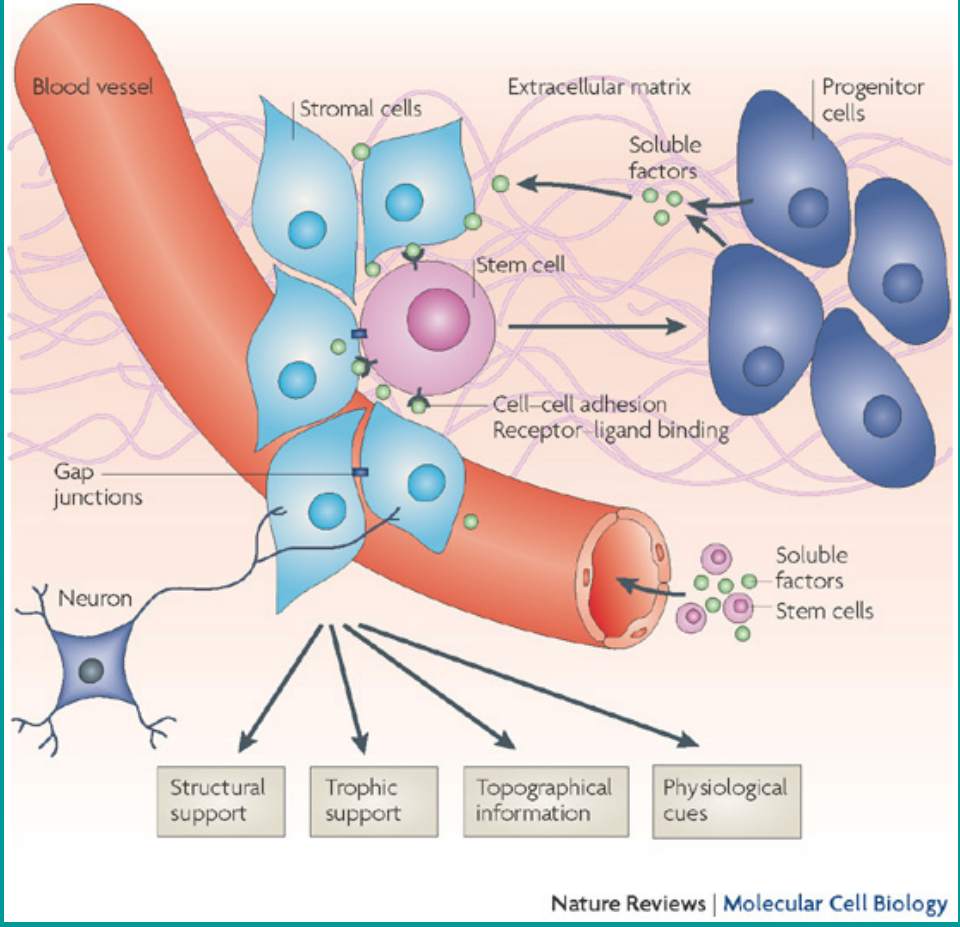
Oil Red Färbung

Wnt-Signalweg

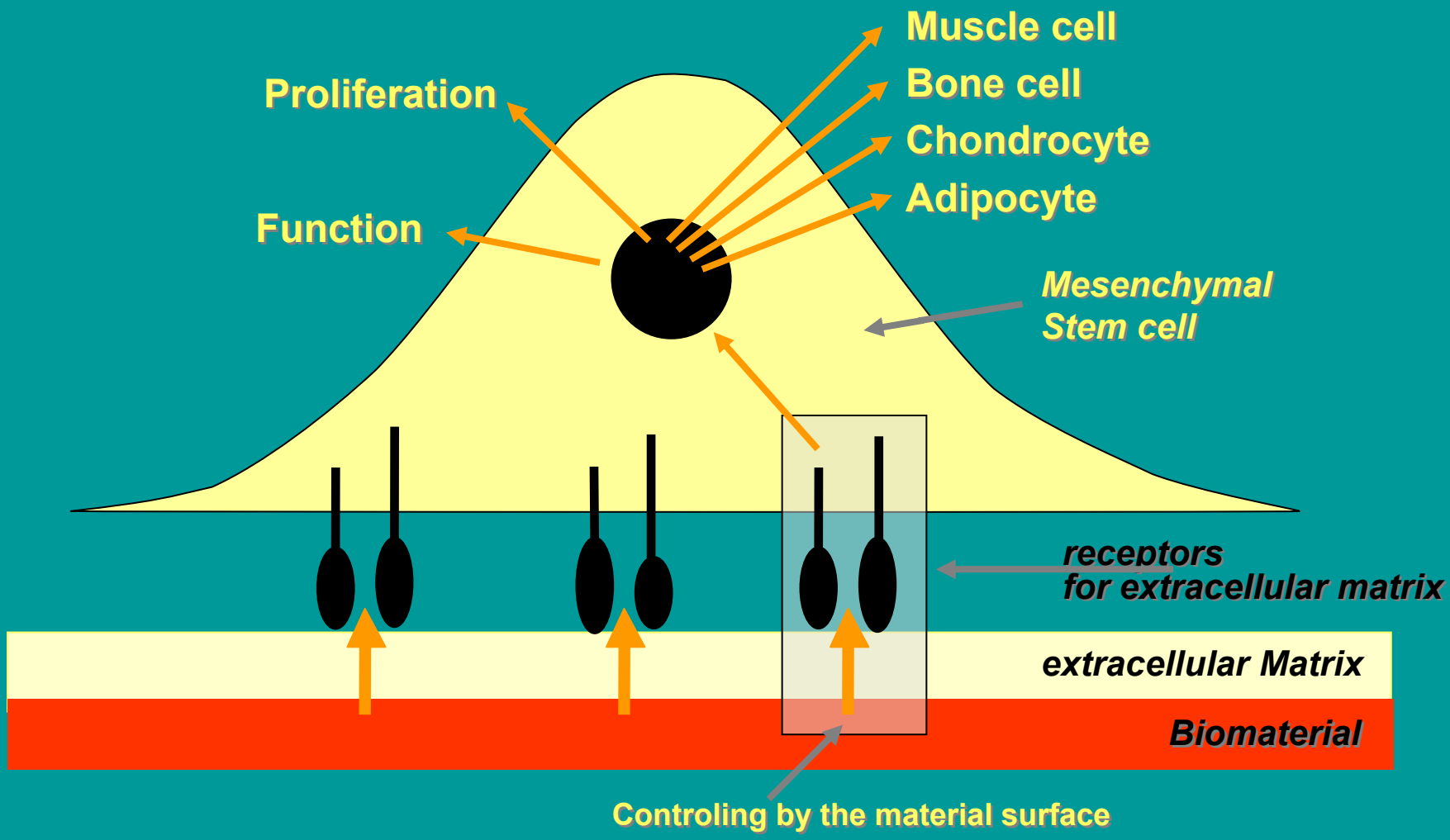




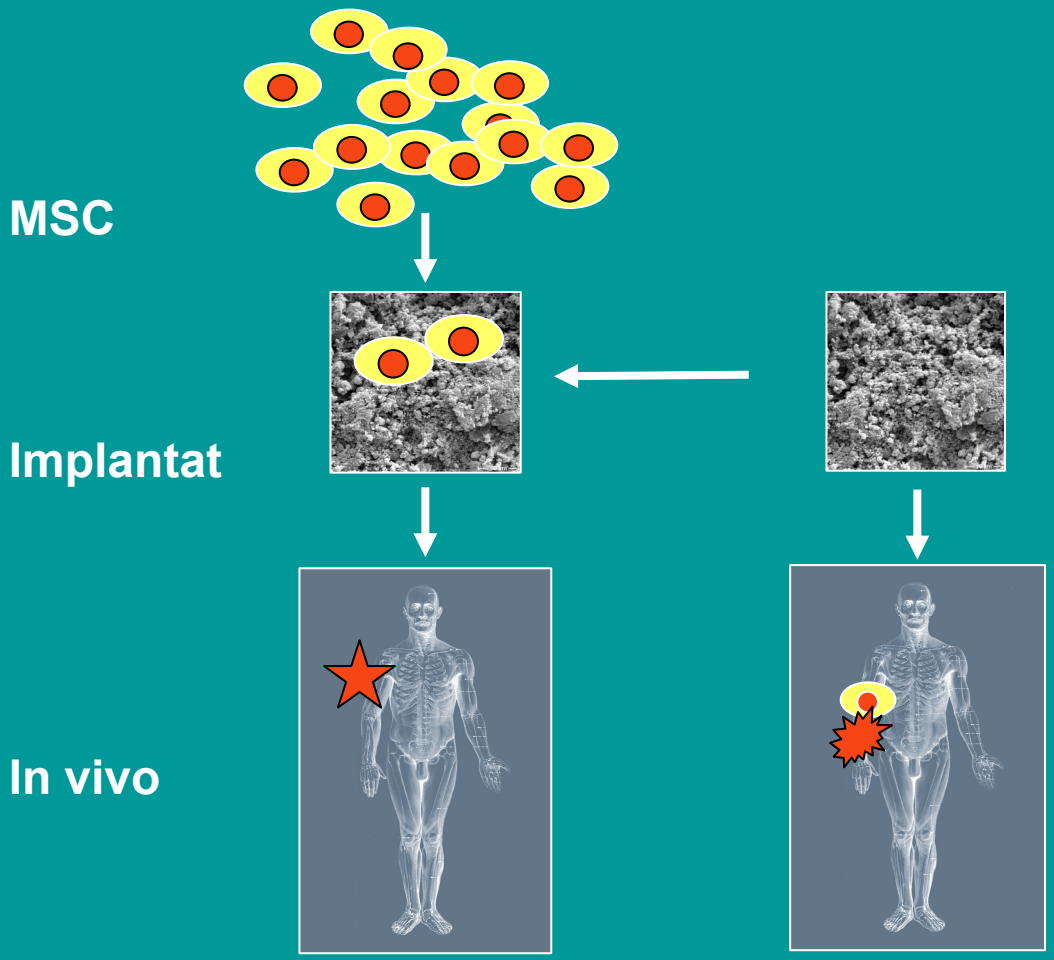
Stem Cell Niche



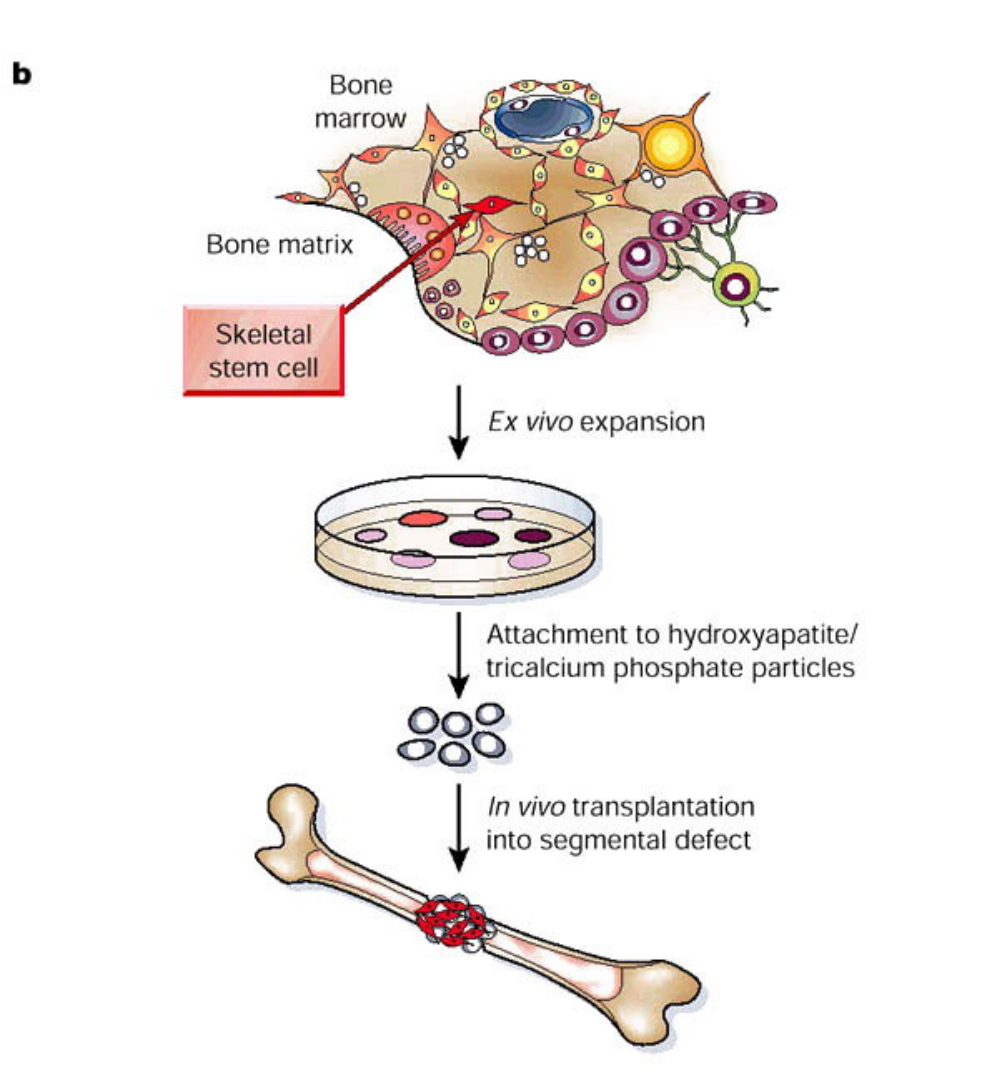
Signals of the Extracellular Matrix Regulate the Fate of Mesenchymal Stem Cells



Regeneration of Bone by Mesenchymal Stem Cells

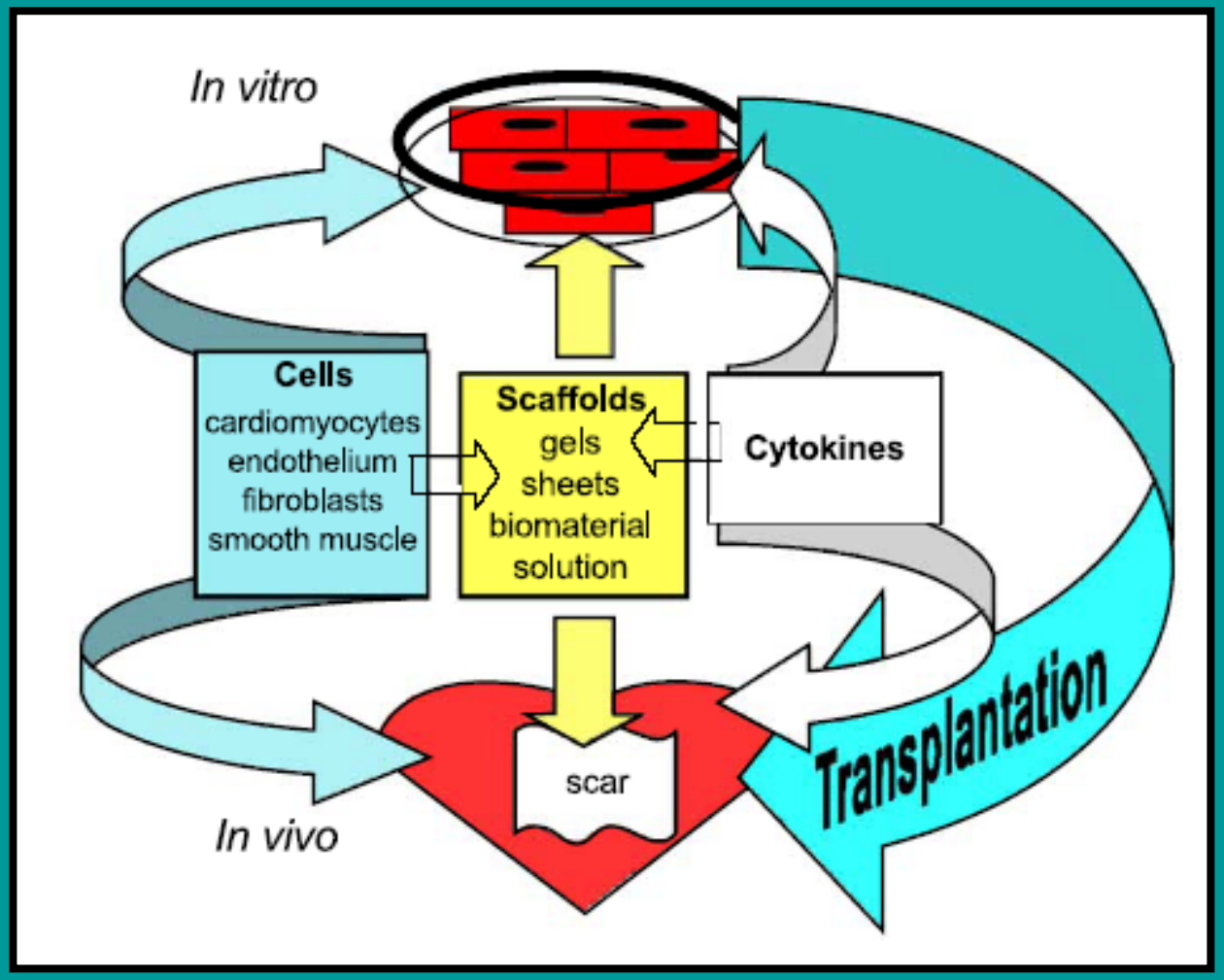


Tissue Engineering of Bone



Bianco et al., Nature 2001

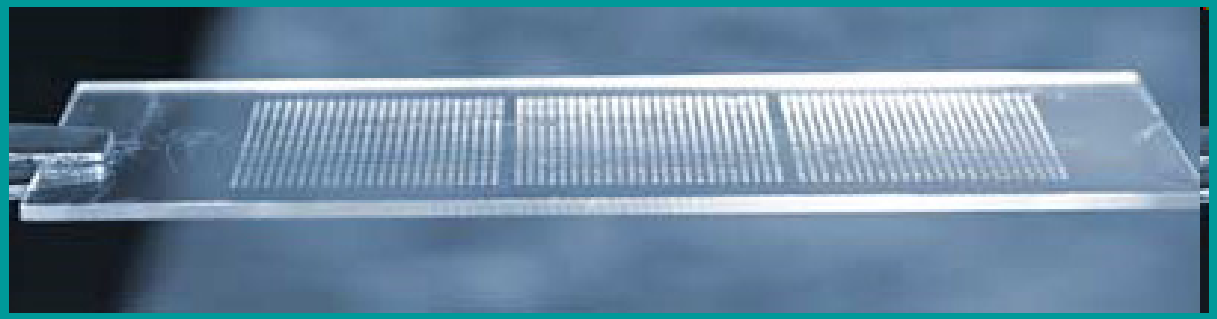
Strategies in Tissue Engineering

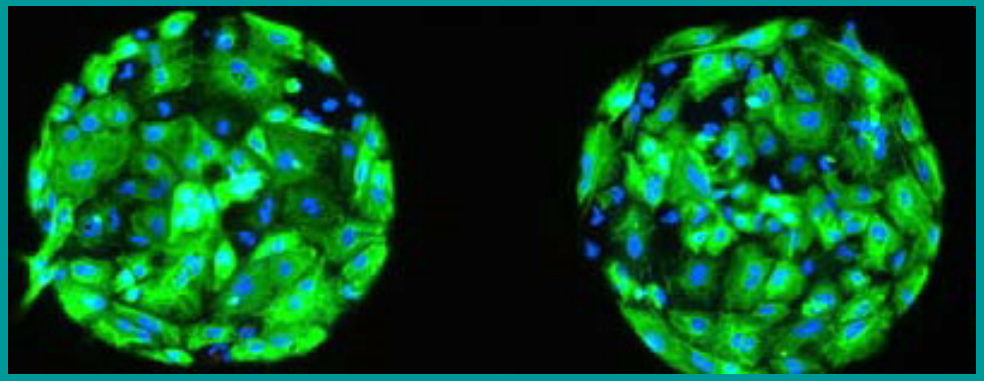


Control of Stem Cells

by Chemical and Physical Characteristics of a Biomaterial

Screening of 500 polymers for epithelial differentiation of embryonic stem cells

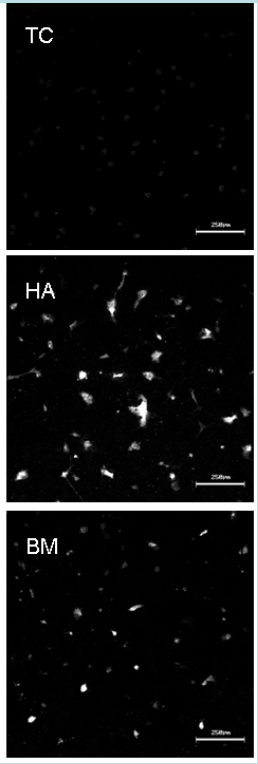




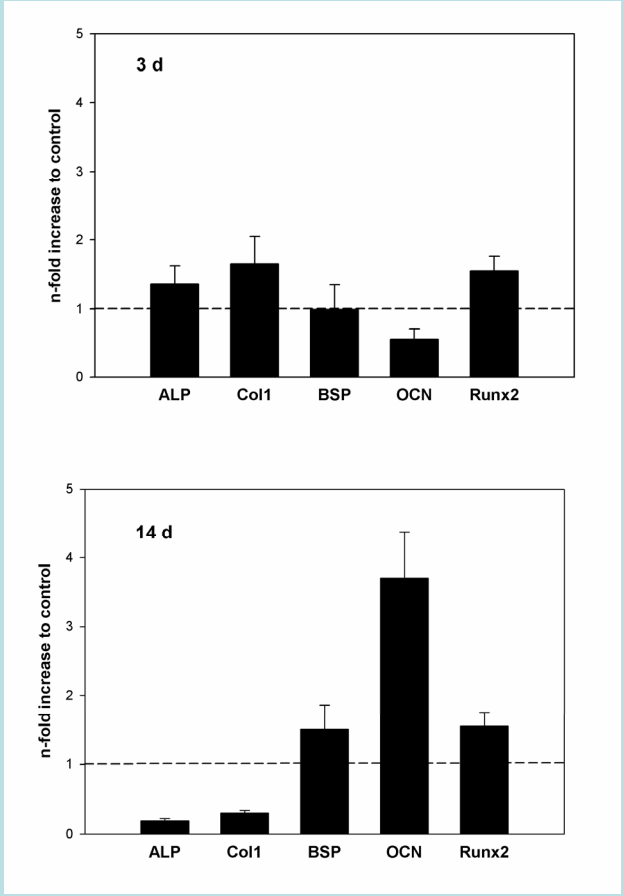
Cytokeratin staining as a marker for epithelial cells

Anderson et al., Nature Biotechn. 2004

Increased osteogenic differentiation of mesenchymal stem cells on calcium phosphate surfaces



Increased activity of alkaline phosphatase in cells after 14 days on calcium phosphate surfaces without additional soluble differentiation factors

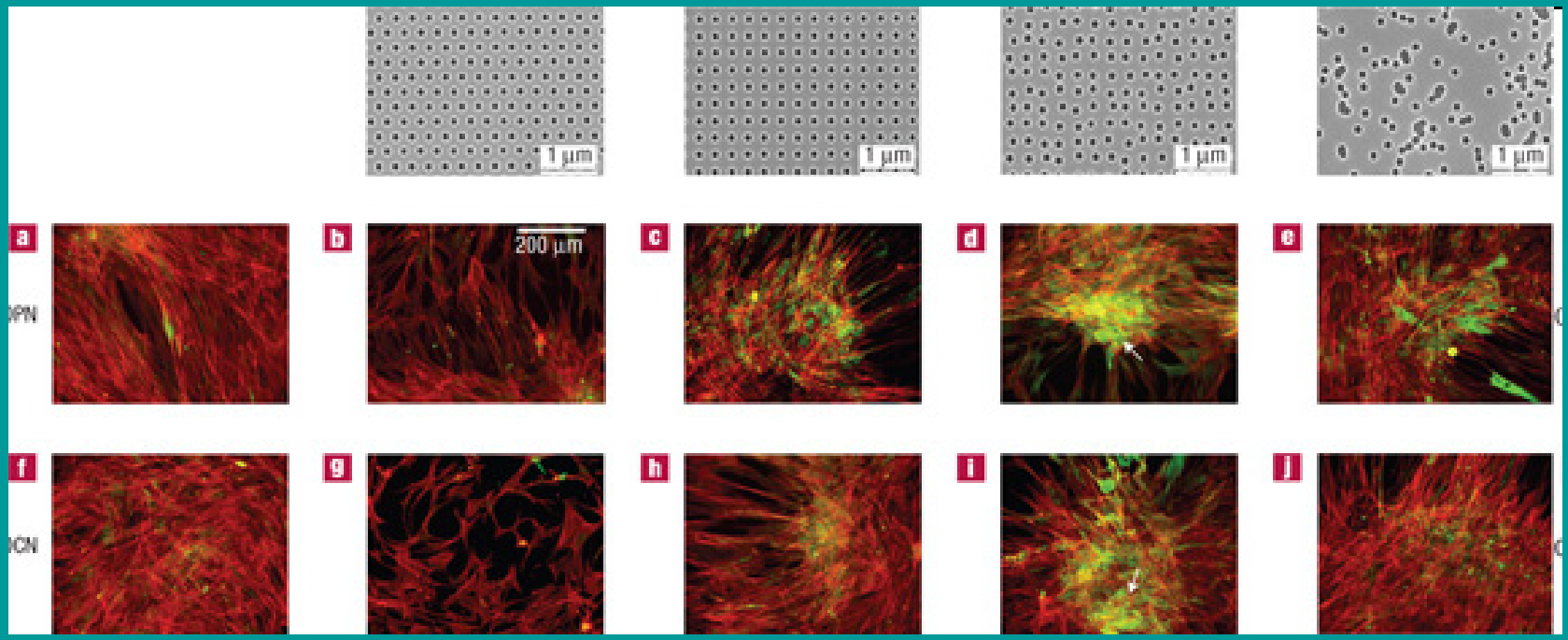


Increased expression of some osteogenic markers on calcium phosphate without additional soluble factors

Disordered Pattern of Nanotopographies Stimulate Osteogenic Protein Expression in Mesenchymal Stem Cells

OPN

OCN

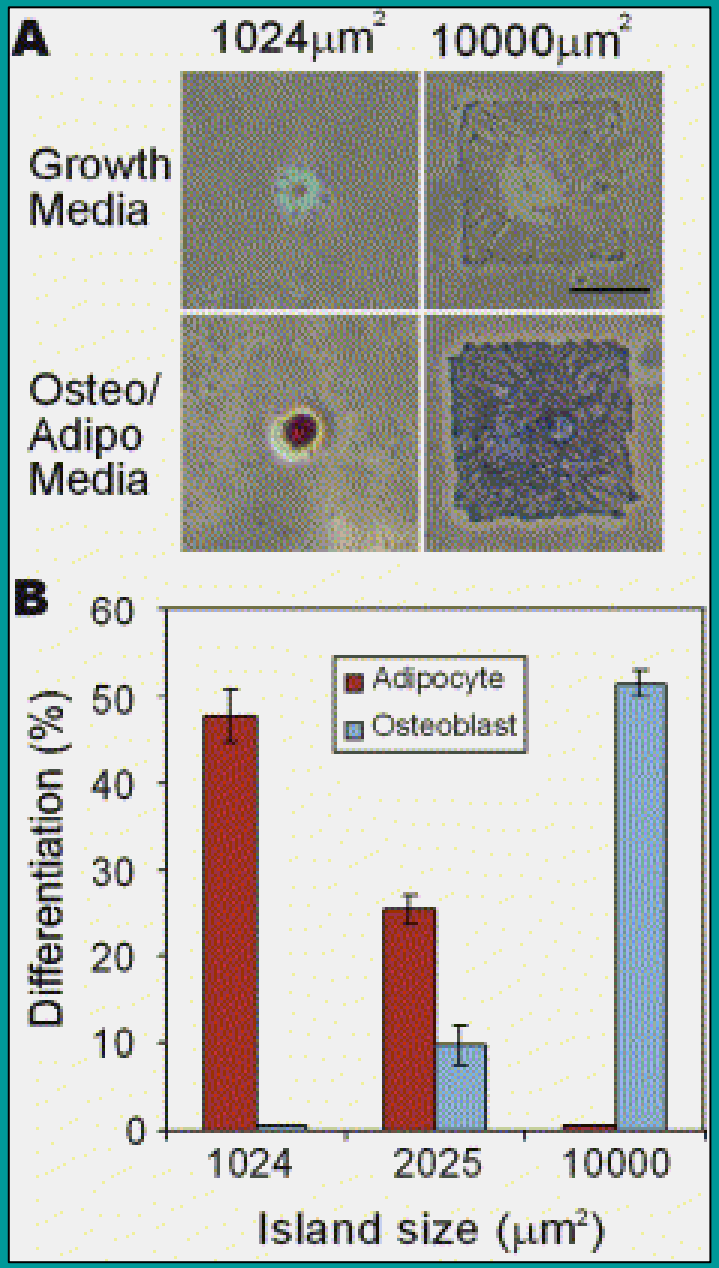


Dalby et al., Nature Mat., 2007

Cell shape regulate stem cell lineage commitment

Mesenchymal stem cell differentiation:

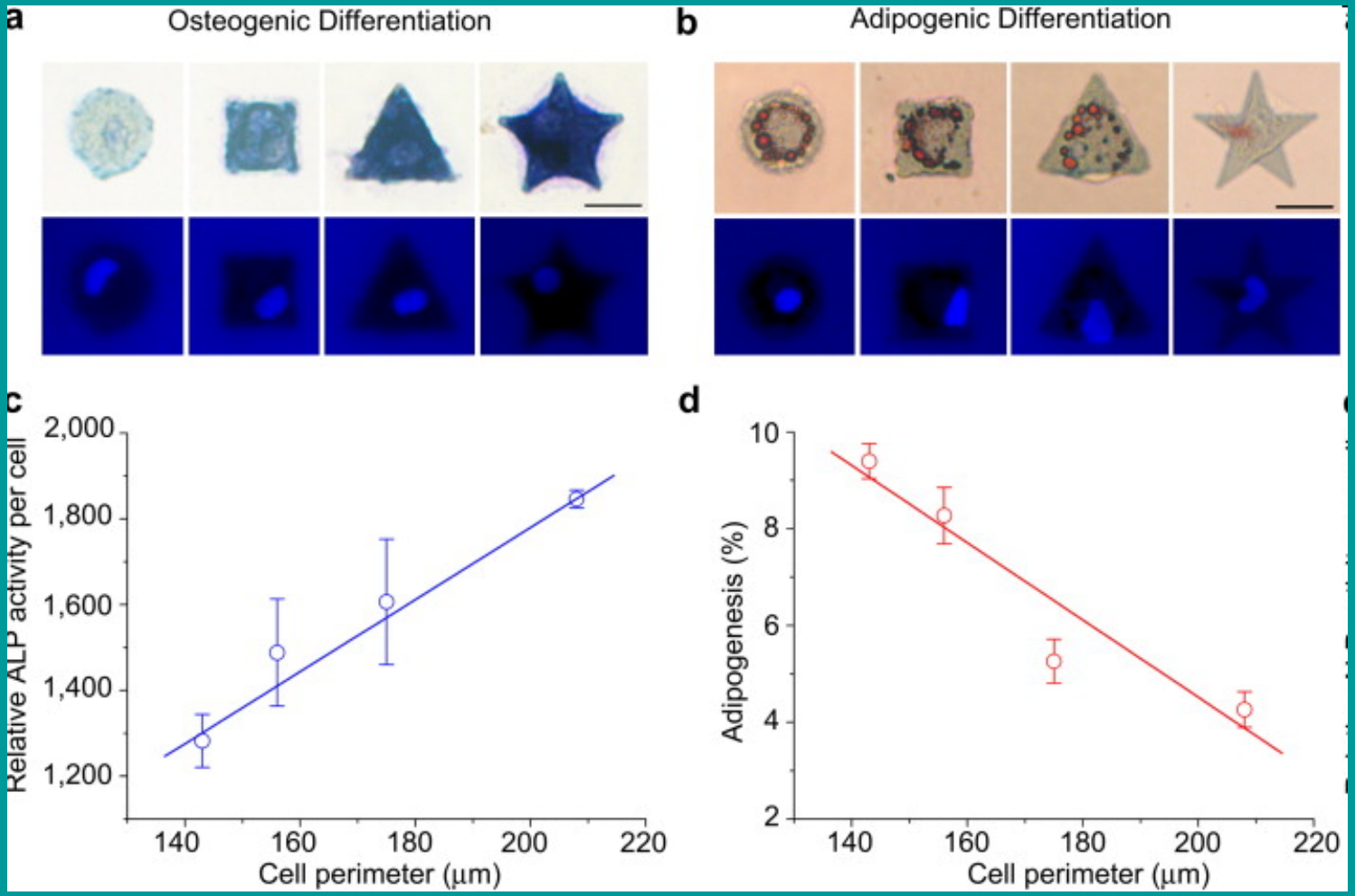
- ***Round cells become adipocytes***
- ***Flat cells become osteoblasts***



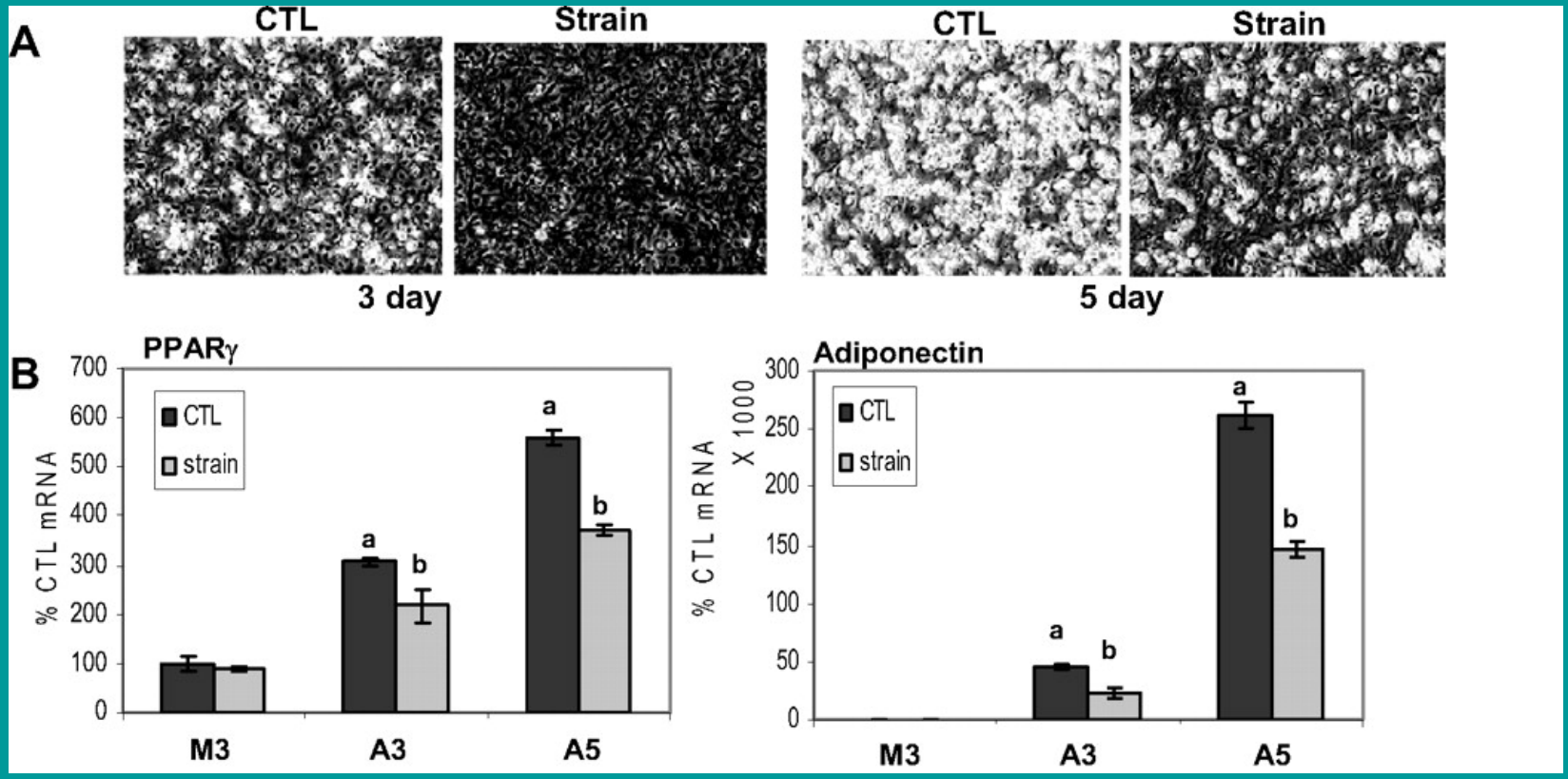
McBeath et al., Dev. Cell 6 (2004)

Differentiation of Mesenchymal Stem Cells

Depends on Cell shape



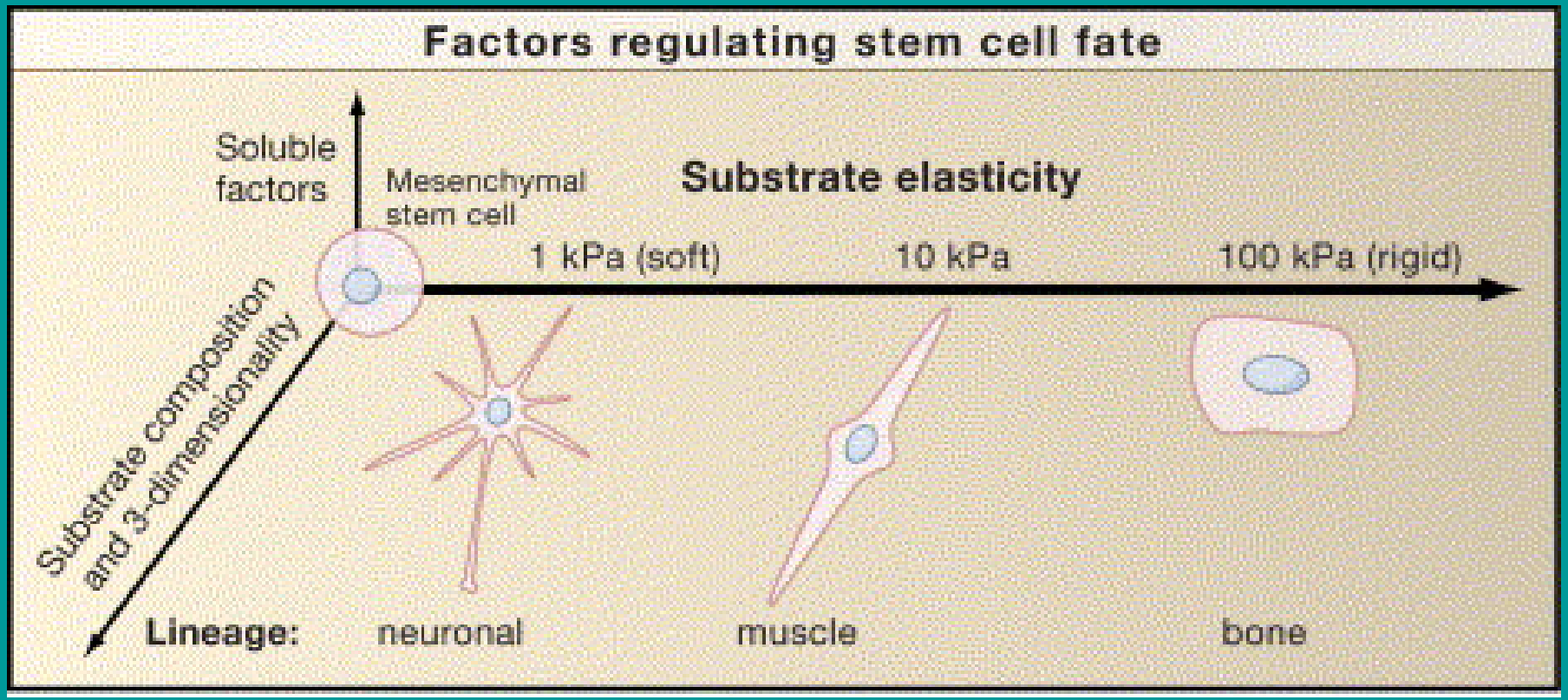
Mechanical loading inhibits adipogenesis of mesenchymal stem cells



Sen, B. et al. Endocrinology 2008;149:6065-6075

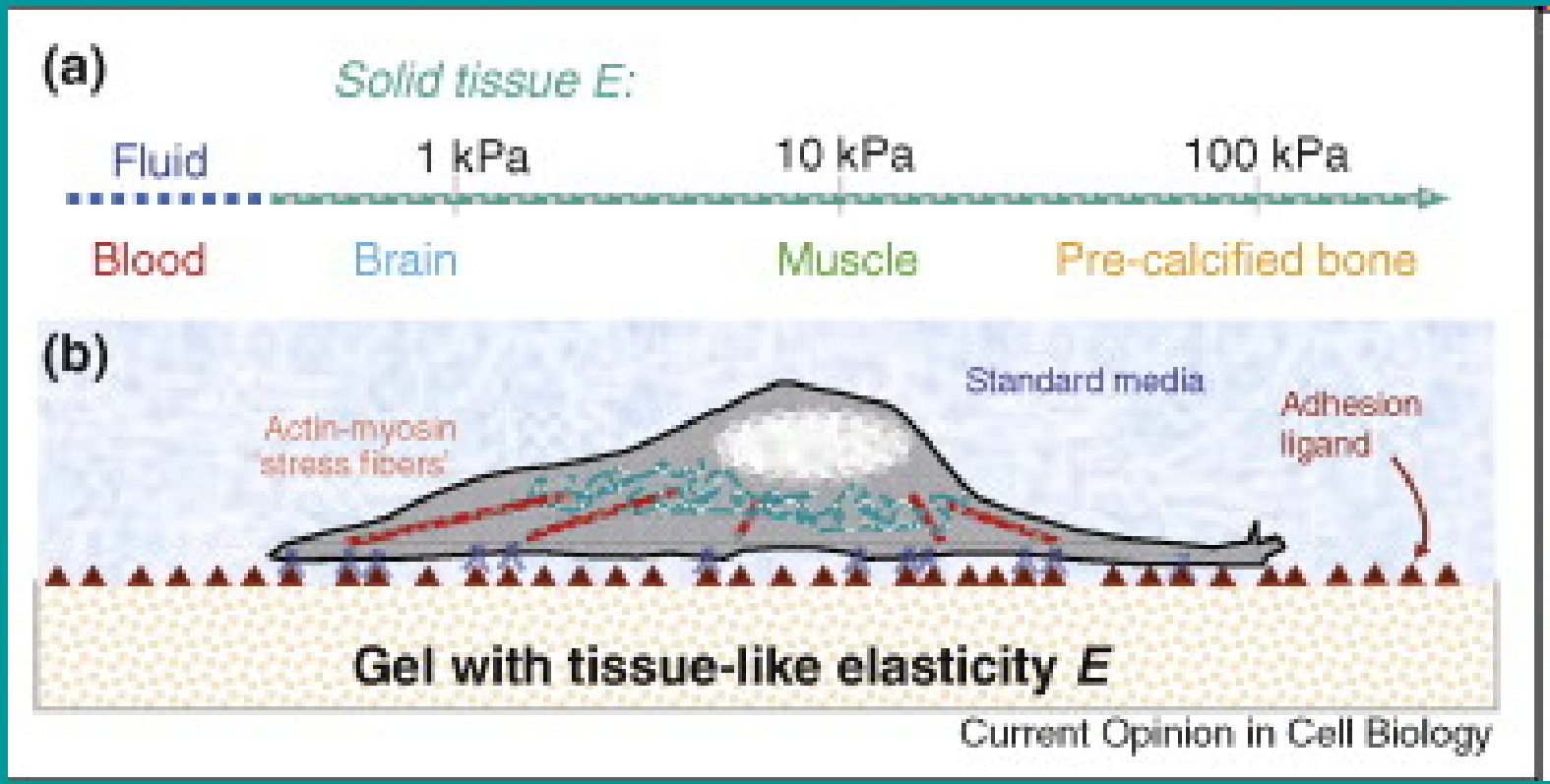
Endocrinology

Mechanical Interaction with the Substrate Determines Stem Cell Fate

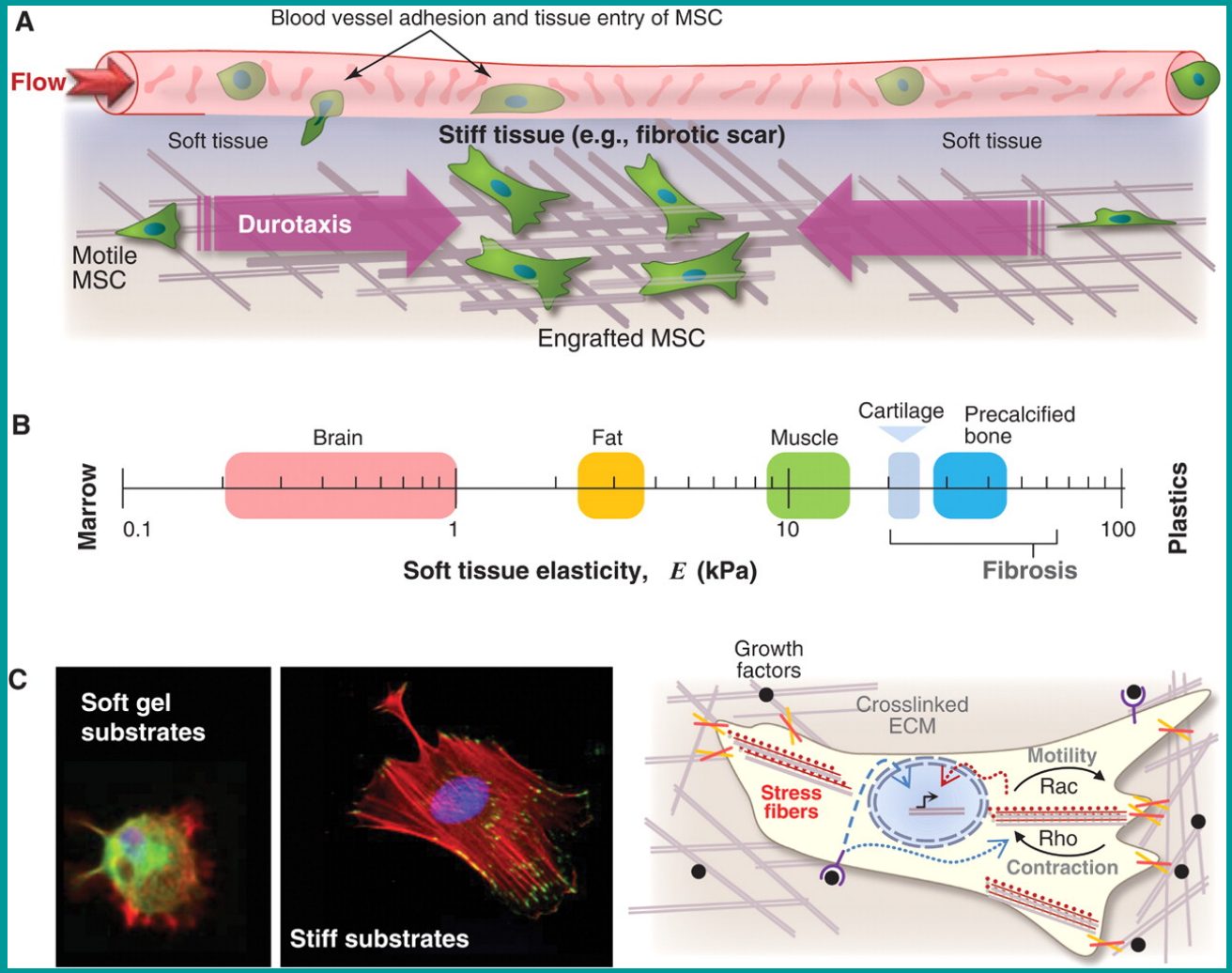


S. Even-Ram, Cell 126, 2006, p. 645

A. J. Engler, Cell 126, 2006, p. 677


(a)
Solid tissue E:
Fluid
1 kPa
10 kPa
100 kPa
Blood
Brain
Muscle
Pre-calcified bone
(b)
Standard media
Actin-myosin 'stress fibers'
Adhesion ligand
Gel with tissue-like elasticity E
Current Opinion in Cell Biology

Forces and ECM in stem cell trafficking



D. E. Discher et al., Science 324, 1673 -1677 (2009)

Published by AAAS

Science

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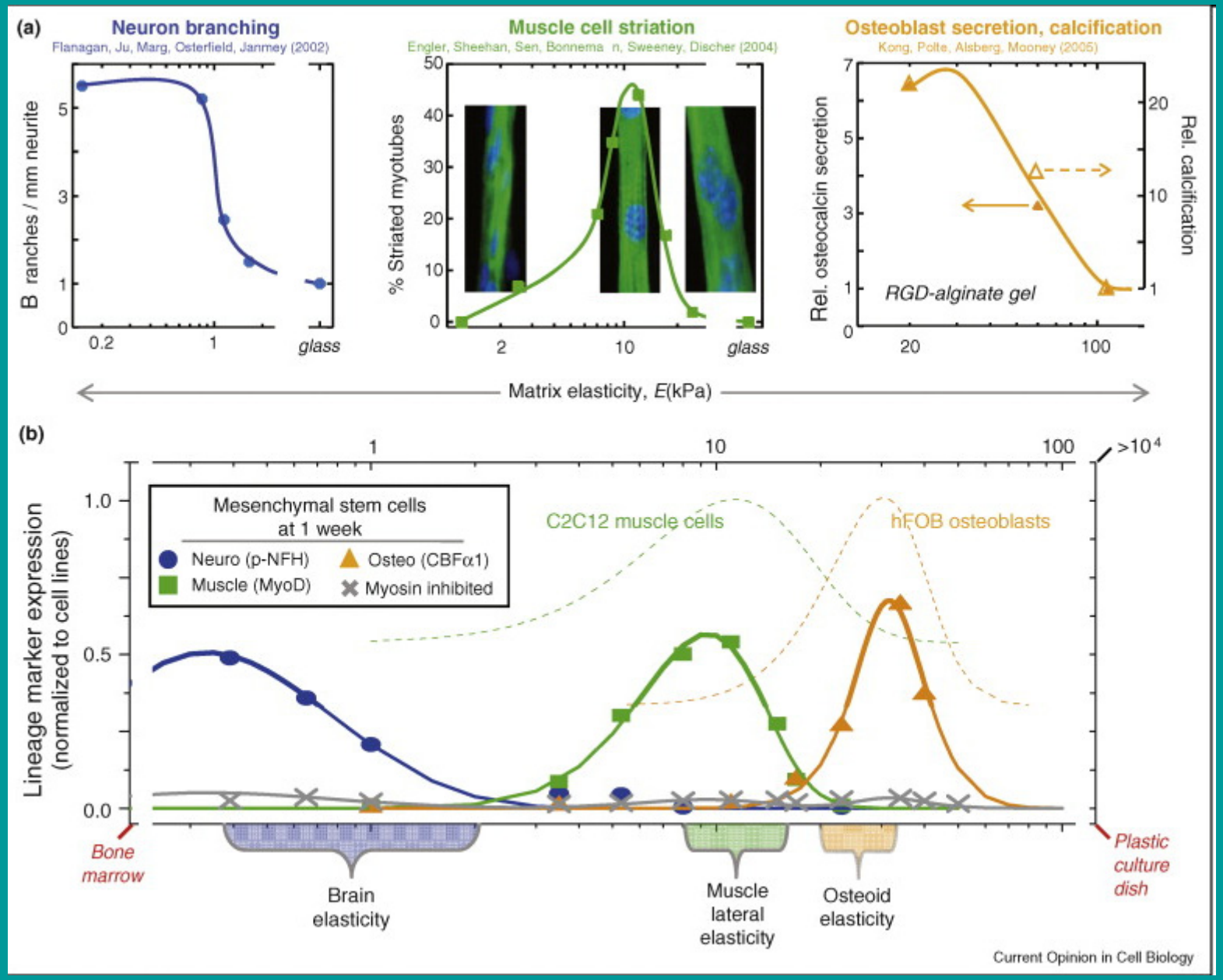
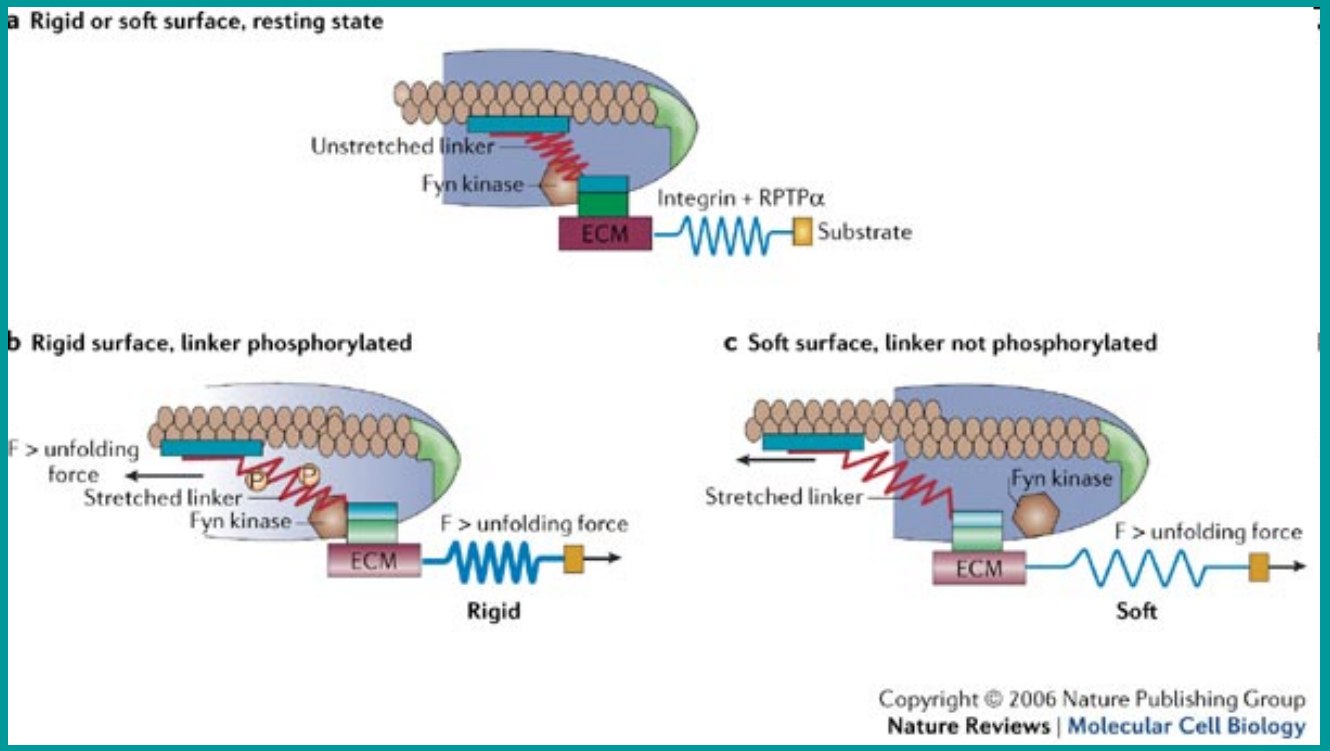

(a)
Neuron branching
Flanagan, Ju, Marg, Osterfield, Janmey (2002)
B ranches / mm neurite
0
1
3
5
0.2
1
glass
Muscle cell striation
Engler, Sheehan, Sen, Bonnema n, Sweeney, Discher (2004)
% Striated myotubes
0
10
20
30
40
50
2
10
glass
Osteoblast secretion, calcification
Kong, Polte, Alsberg, Mooney (2005)
Rel. osteocalcin secretion
Rel. calcification
RGD-alginate gel
0
1
3
5
7
1
10
20
20
100
Matrix elasticity, E(kPa)
(b)
1
10
100
>10^4
Lineage marker expression
(normalized to cell lines)
0.0
0.5
1.0
Mesenchymal stem cells
at 1 week
Neuro (p-NFH)
Osteo (CBFα1)
Muscle (MyoD)
Myosin inhibited
C2C12 muscle cells
hFOB osteoblasts
Bone
marrow
Brain
elasticity
Muscle
lateral
elasticity
Osteoid
elasticity
Plastic
culture
dish
Current Opinion in Cell Biology



Position dependent mechanism of rigidity sensing

Vogel, V., Sheetz, M,:Nature Reviews, Molecular Cell Biology,7, 2006