Ingénierie tissulaire

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UEL 340
Des biomatériaux à l'ingénierie tissulaire
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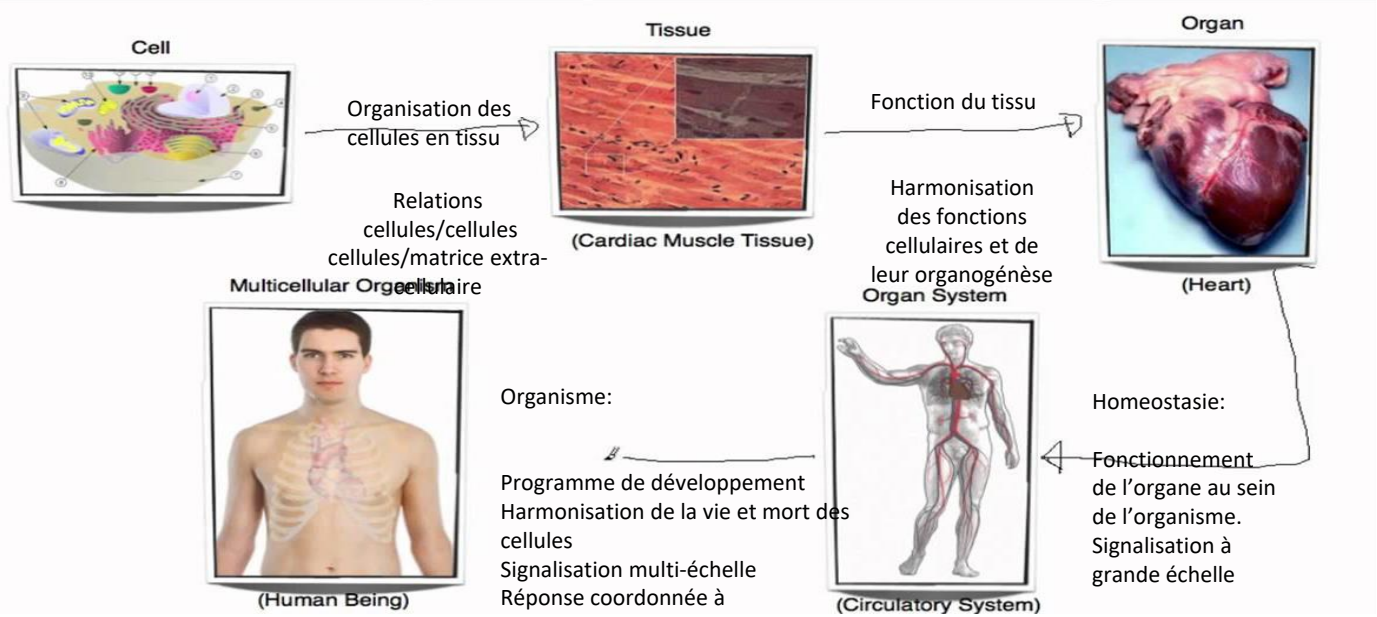
Définitions et plan de cours

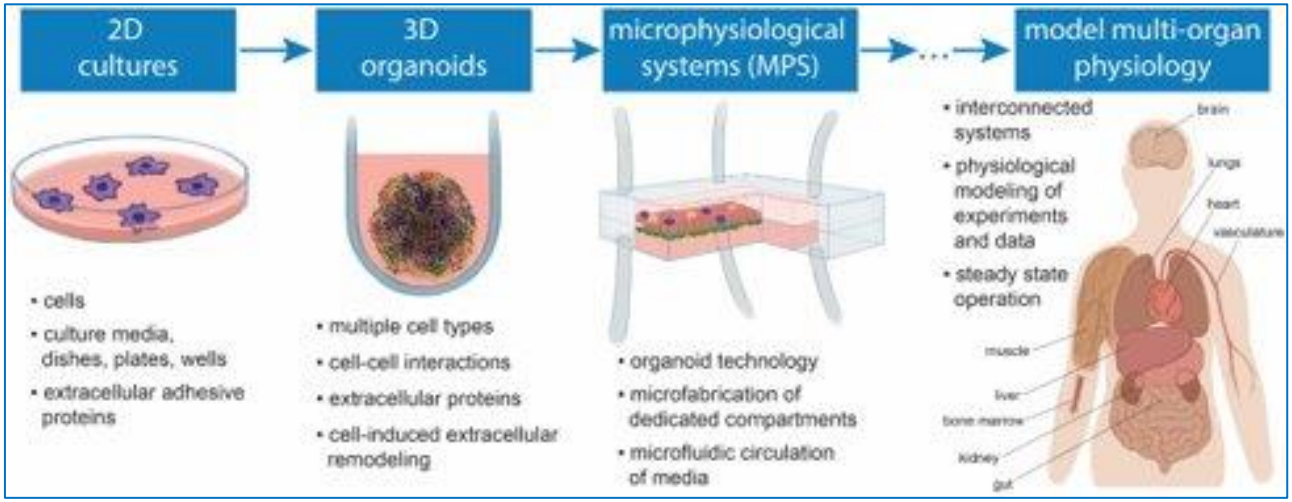
- L'**ingénierie tissulaire** ou **génie tissulaire** (en anglais, *tissue engineering*) est l'ensemble des techniques faisant appel aux principes et aux méthodes de l'ingénierie, de la culture cellulaire, des sciences de la vie, des sciences des matériaux pour comprendre les relations entre les structures et les fonctions des tissus normaux et pathologiques des mammifères, afin de développer des substituts biologiques pouvant restaurer, maintenir, comprendre ou améliorer les fonctions des tissus.

- Elle implique notamment d'identifier et maitriser les facteurs biochimiques et physico-chimiques de la croissance tissulaire maitrisée. Elle est souvent basée sur la construction ou l'utilisation d'un « échafaudage » qui servira de support à la croissance de nouveaux tissus viables, généralement à des fins médicales.

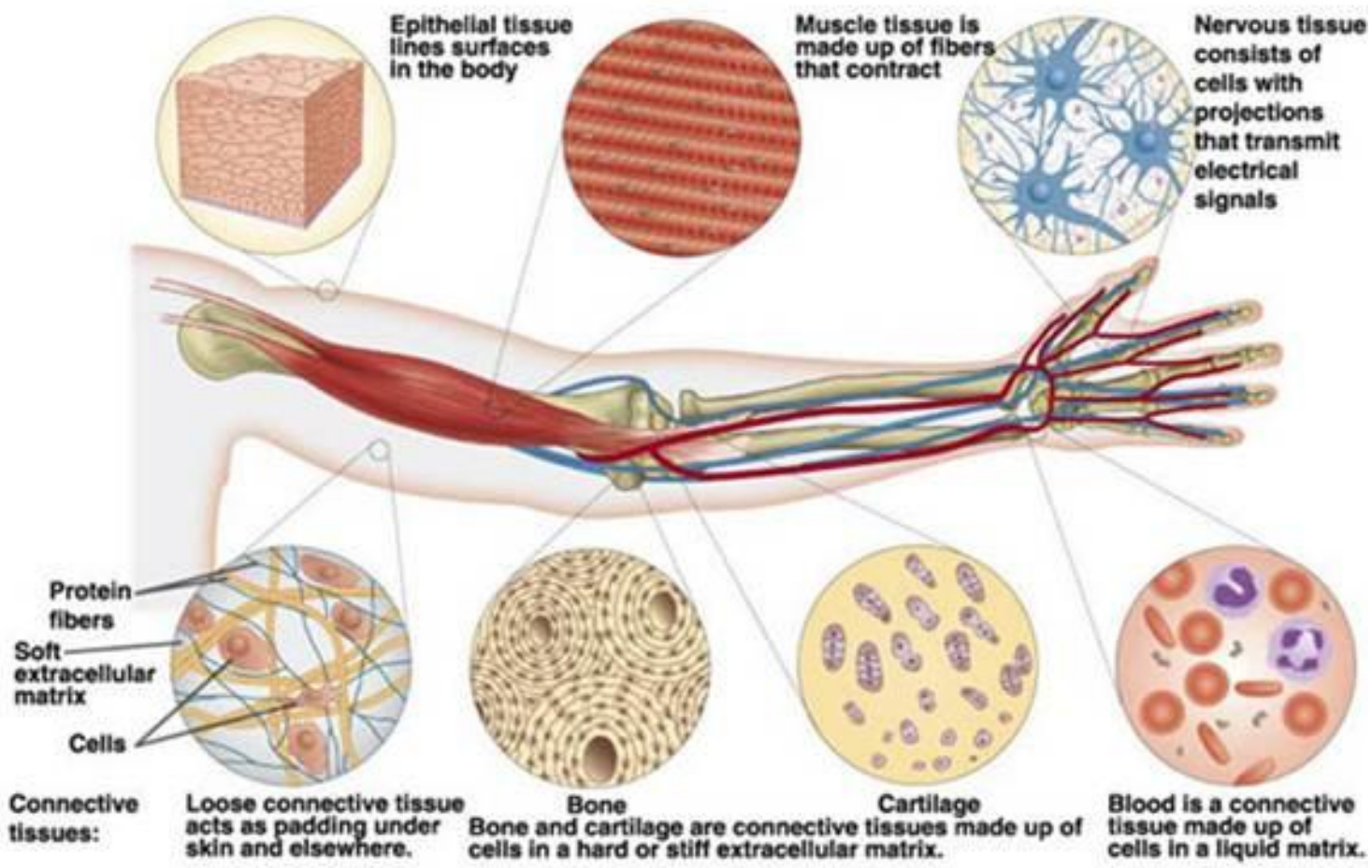
- Les définitions de l'ingénierie tissulaire couvrent une large gamme d'applications ; recherche fondamentale, drug testing, médecine personnalisée, médecine régénérative...

Organisation du vivant: de la cellule à l'organisme

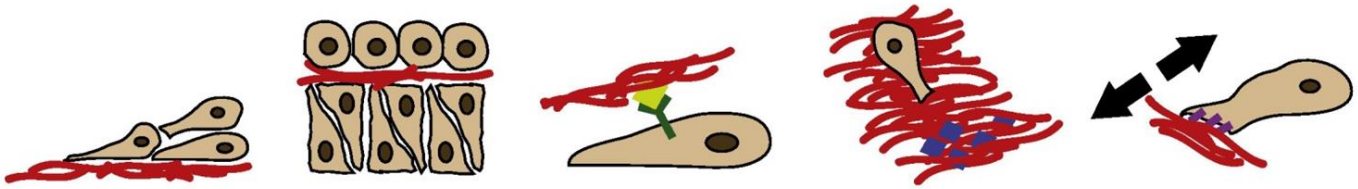




Les différents tissus de l'organisme sont composés de cellules et de matrices extra-cellulaires spécialisées



La matrice extra-cellulaire fournit aux cellules une niche chimiquement et mécaniquement contrôlée



Functions as adhesive substrate

- *tracks to direct migratory cells*
- *concentration gradients for haptotactic migration*

Provides structure

- *defines tissue boundaries*
- *provides integrity and elasticity to developing organs*
- *degraded by invasive cells during development and disease*

Presents growth factors to their receptors

- *controls spatial distribution of ECM-bound surface molecules*
- *facilitates crosstalk between growth factor receptors and ECM receptors*

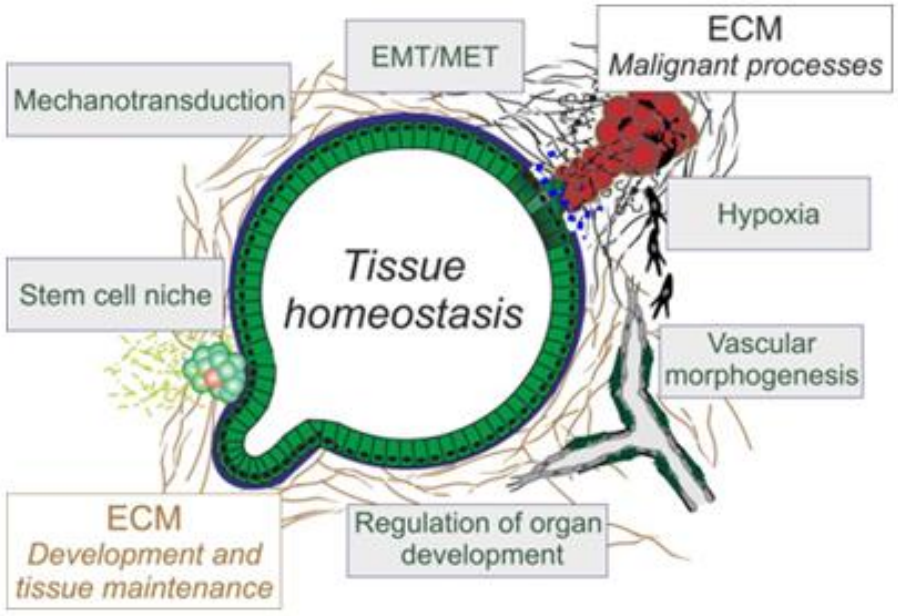
Sequesters and stores growth factors

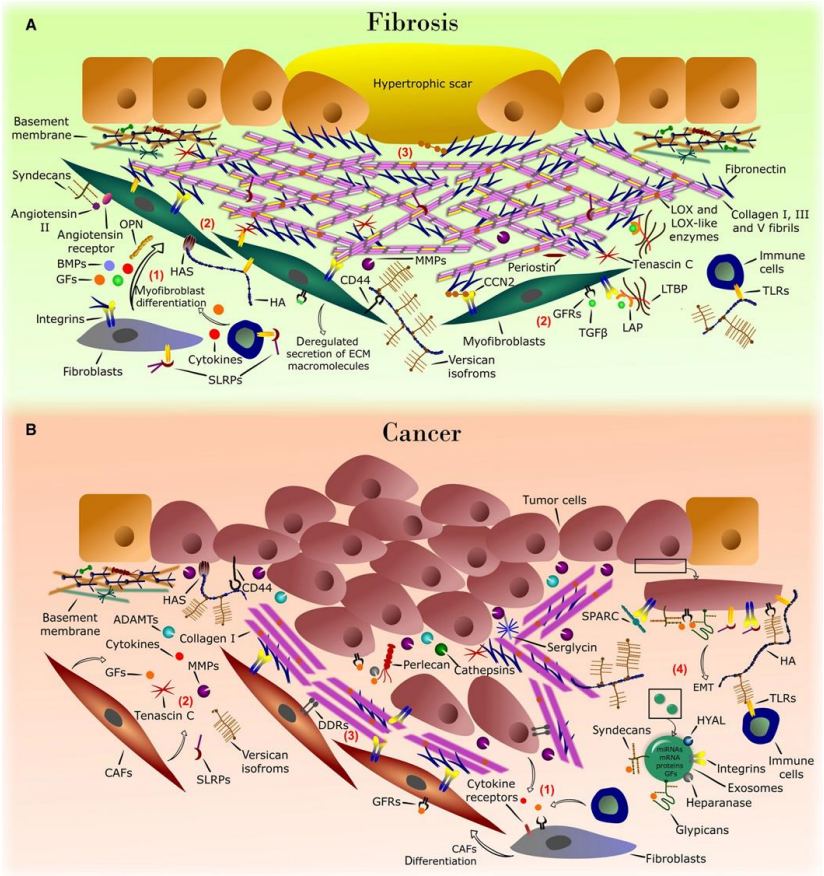
- *allows for spatio-temporal regulation of factor release*
- *organizes morphogen gradients*
- *mediates release of factors in the presence of appropriate cell-mediated forces or proteolytic degradation*

Senses and transduces mechanical signals

- *defines mechanical properties permissive/ instructive to cell differentiation*
- *activates intracellular signaling through interaction with cell-surface receptors*
- *engages cytoskeletal machinery and synergizes with growth factor signaling*

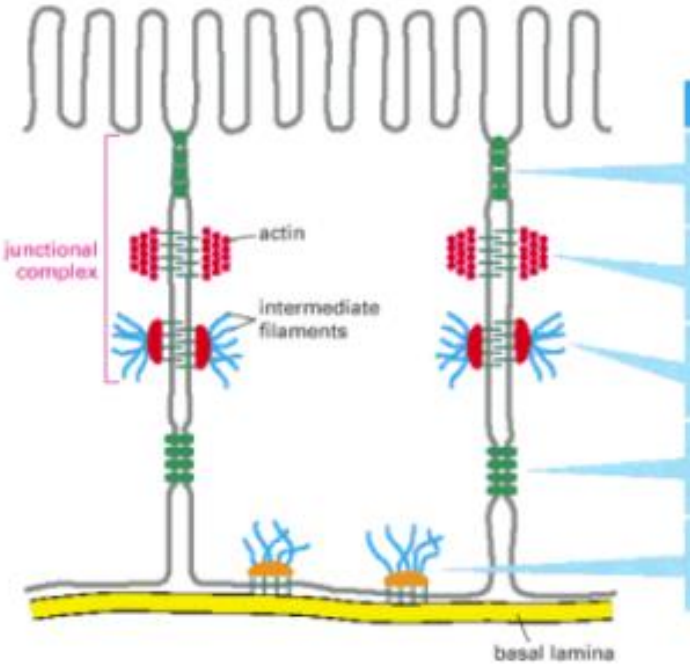
La différenciation et son maintien répondent à des stimuli biochimiques et mécaniques spécifiques





Conséquences pathologiques d'une perte de l'homéostasie épithéliale

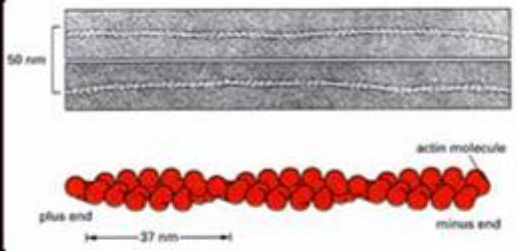
Relations cellules/cellules et cellules/matrice extra-cellulaire



name	function
tight junction	seals neighboring cells together in an epithelial sheet to prevent leakage of molecules between them
adherens junction	joins an actin bundle in one cell to a similar bundle in a neighboring cell
desmosome	joins the intermediate filaments in one cell to those in a neighbor
gap junction	allows the passage of small water-soluble ions and molecules
hemidesmosome	anchors intermediate filaments in a cell to the basal lamina

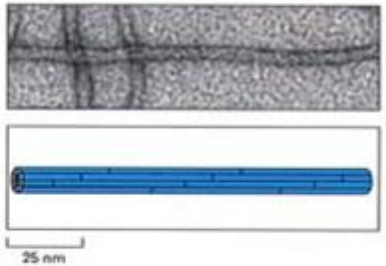
The Three Types of Cytoskeletal Filaments

Persistence length



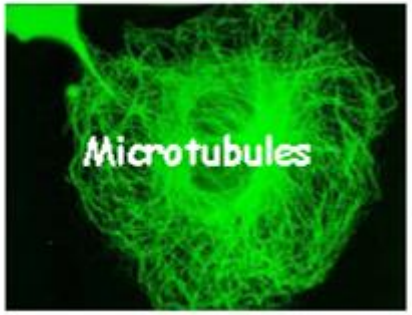
- Ø 5-9 nm
- polar filaments (+/- end)
- actin monomers
- ATP/ADP nucleotide binding

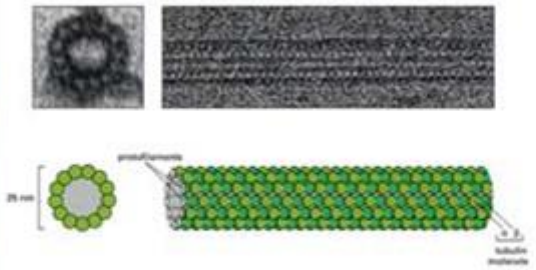
15 µm



- Ø 10 nm
- non-polar filaments
- tetramers
- no nucleotide binding

0,5 µm





- Ø 25 nm
- polar filaments (+/- end)
- α/β tubulin hetero-dimers
- GTP/GDP nucleotide binding

5000 µm

•All 3 are polymers that control cell's shape and mechanics, are organized into networks that resist deformation and can reorganize in response to external forces.
•Pol/depol of MTs and actin generate directed forces
•Architecture of networks based on: nucleation factors, capping proteins, depolymerizing and severing factors, and cross-linkers

Le micro-environnement (ECM) détermine l'organisation du cytosquelette et de la physiologie cellulaire

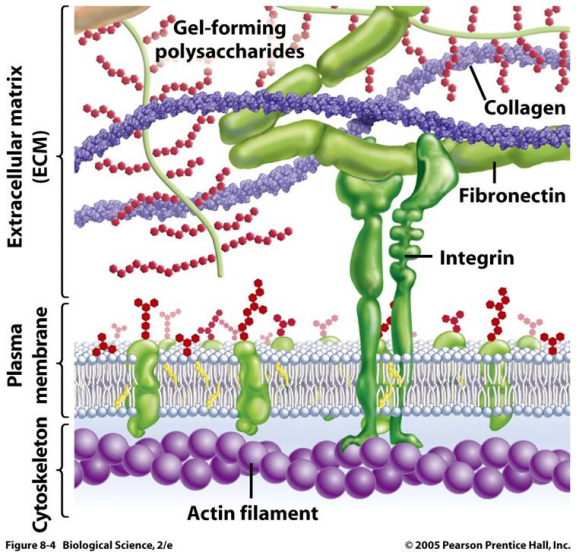
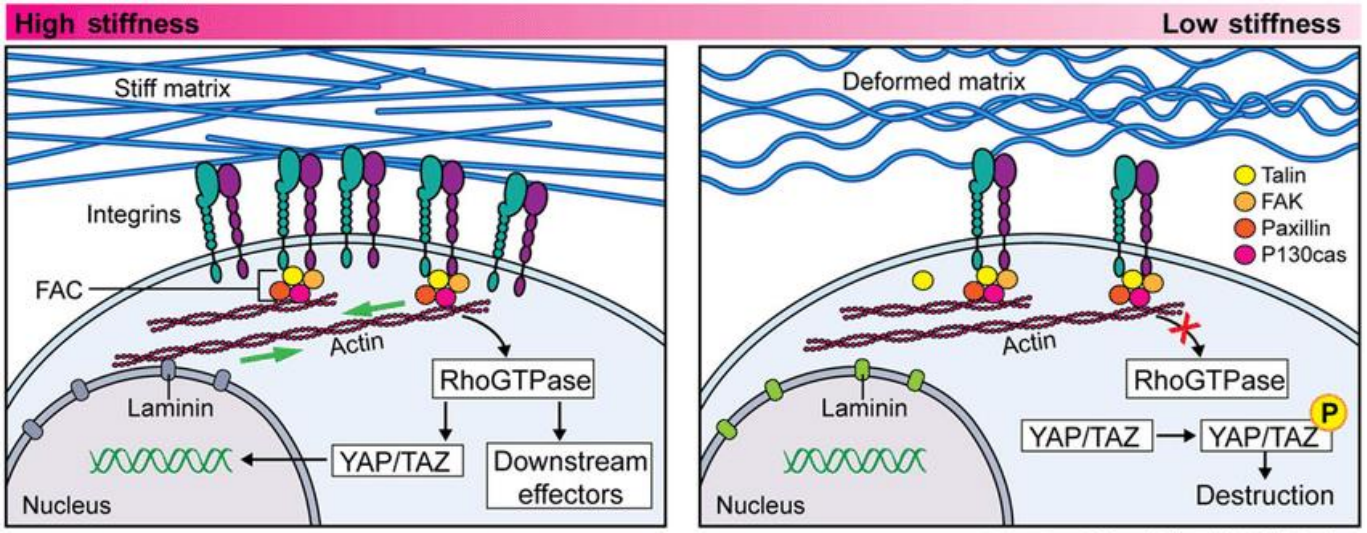


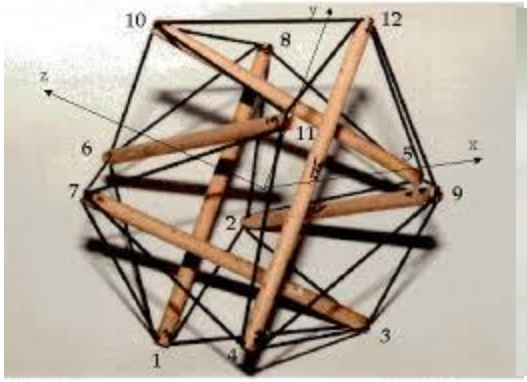
Figure 8-4 Biological Science, 2/e

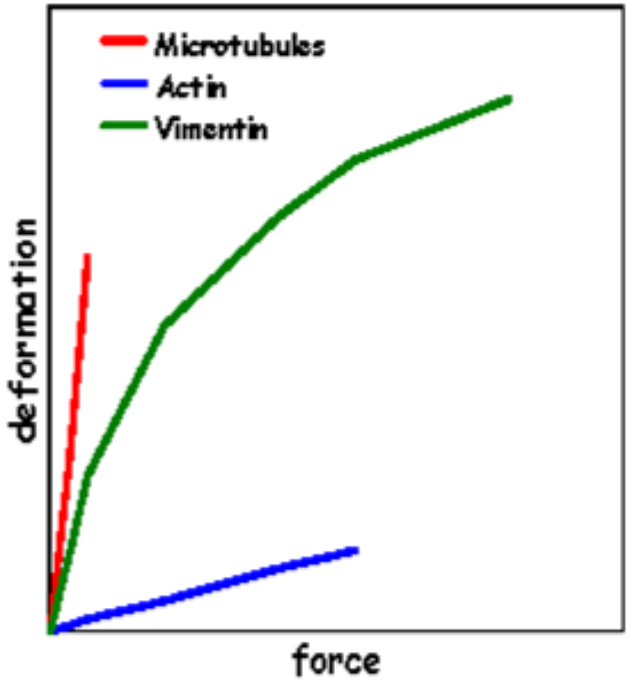


Biomechanical Differences Between Filaments

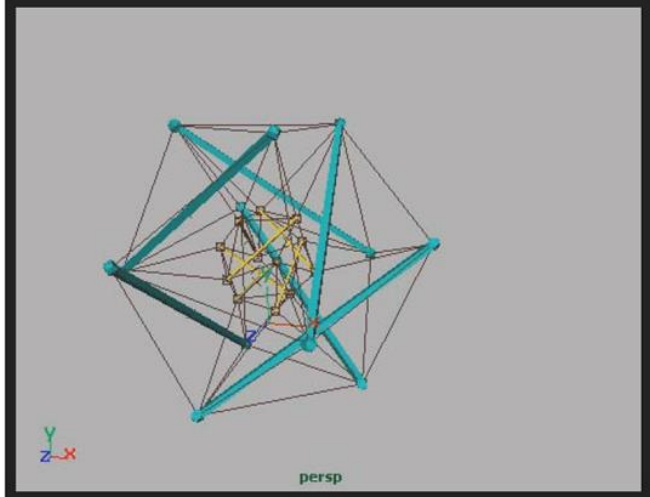
Pioneer studies (Janmey, 1991) investigating the mechanical properties of cytoskeletal filaments used viscometry on polymerized networks of different filament types at similar concentration:

- **Microtubule** networks are easily deformed but rupture upon deformation >150%
- **Actin** filament networks are rather stiff and also rupture easily
- **Intermediate filament** networks (vimentin) are easily deformed and resist large-scale deformation





Cellular Tensegrity Model



(Ingber et al., *PNAS* 78:3901-5, 1981; Ingber & Jamieson,1985;
Wang et al. *Science* 1993, *PNAS* 2001; Ingber *J. Cell Sci* 1993, 2003)

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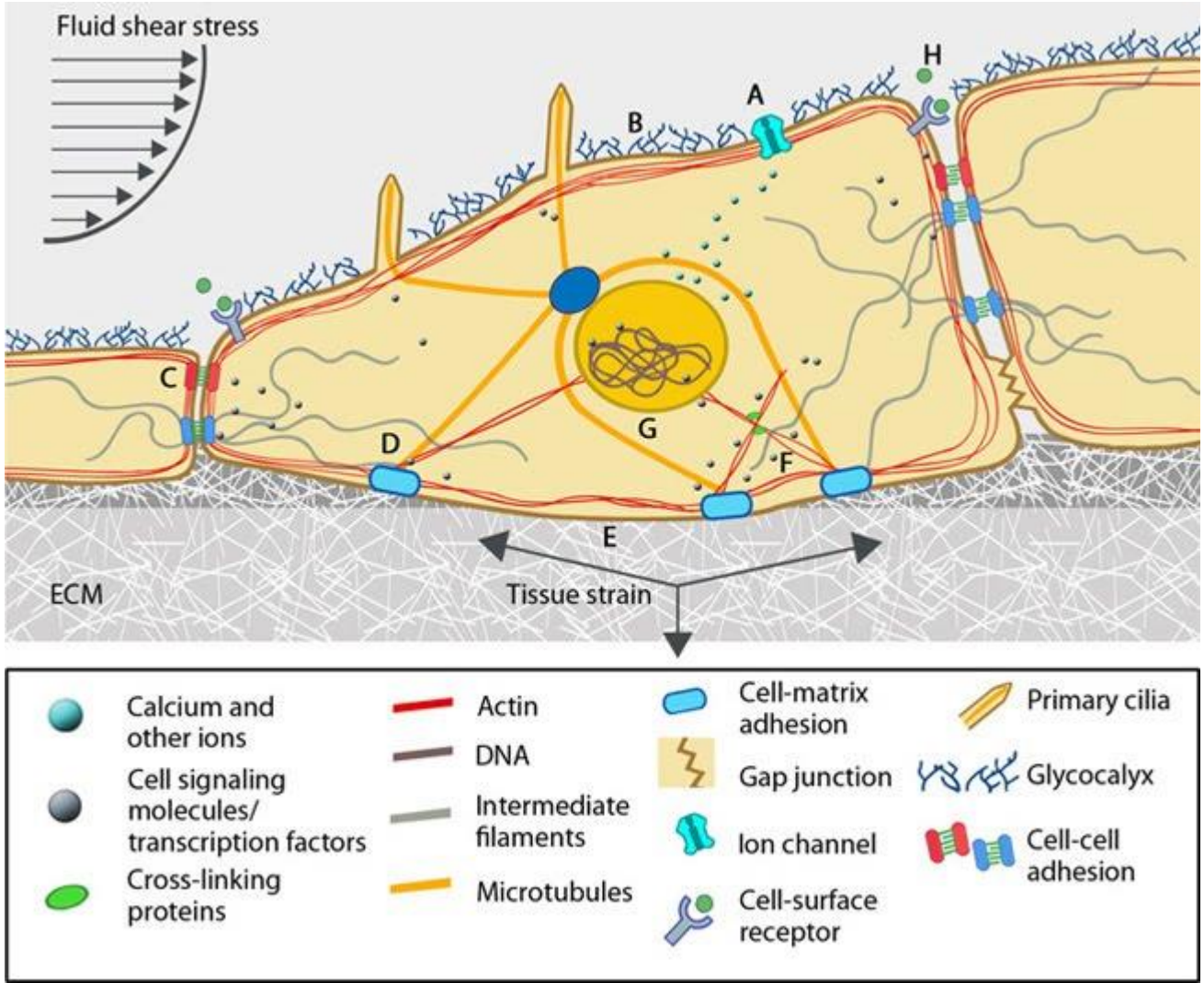
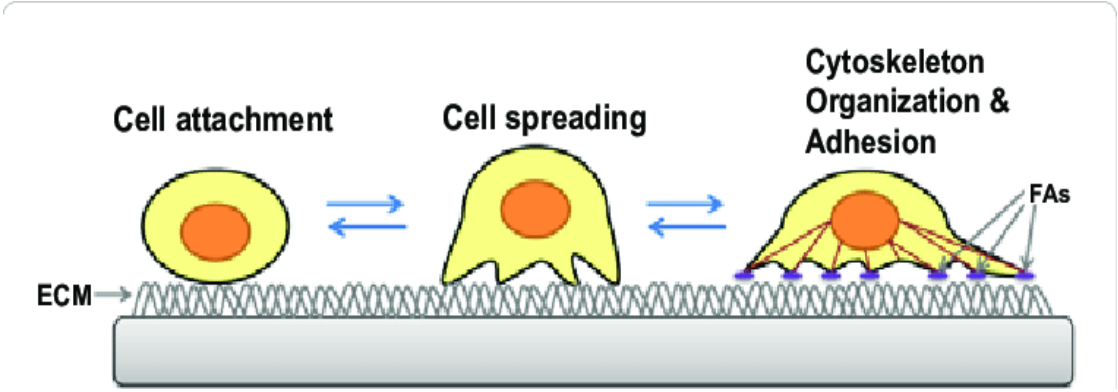
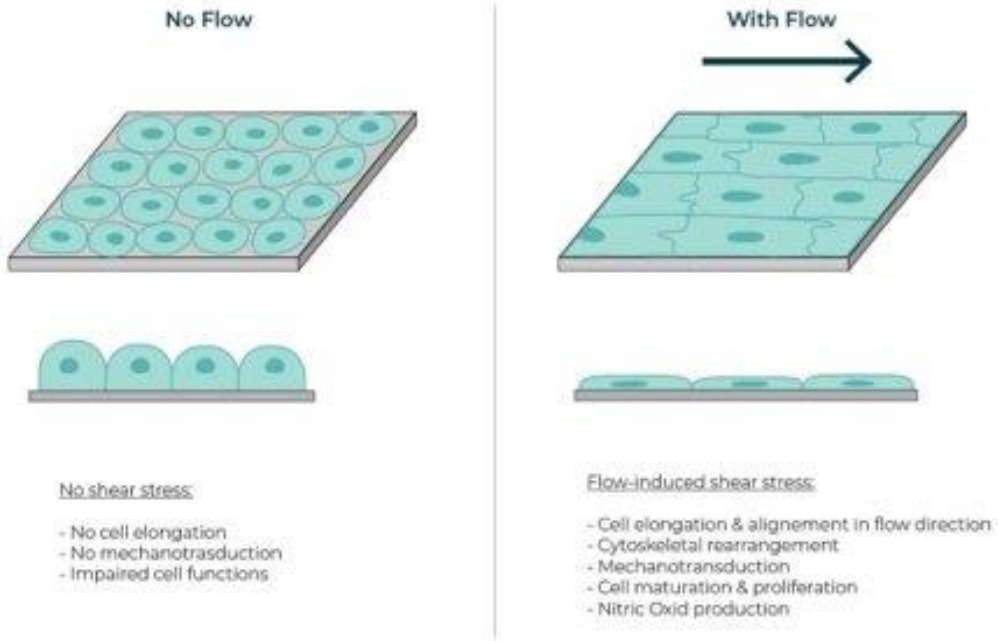
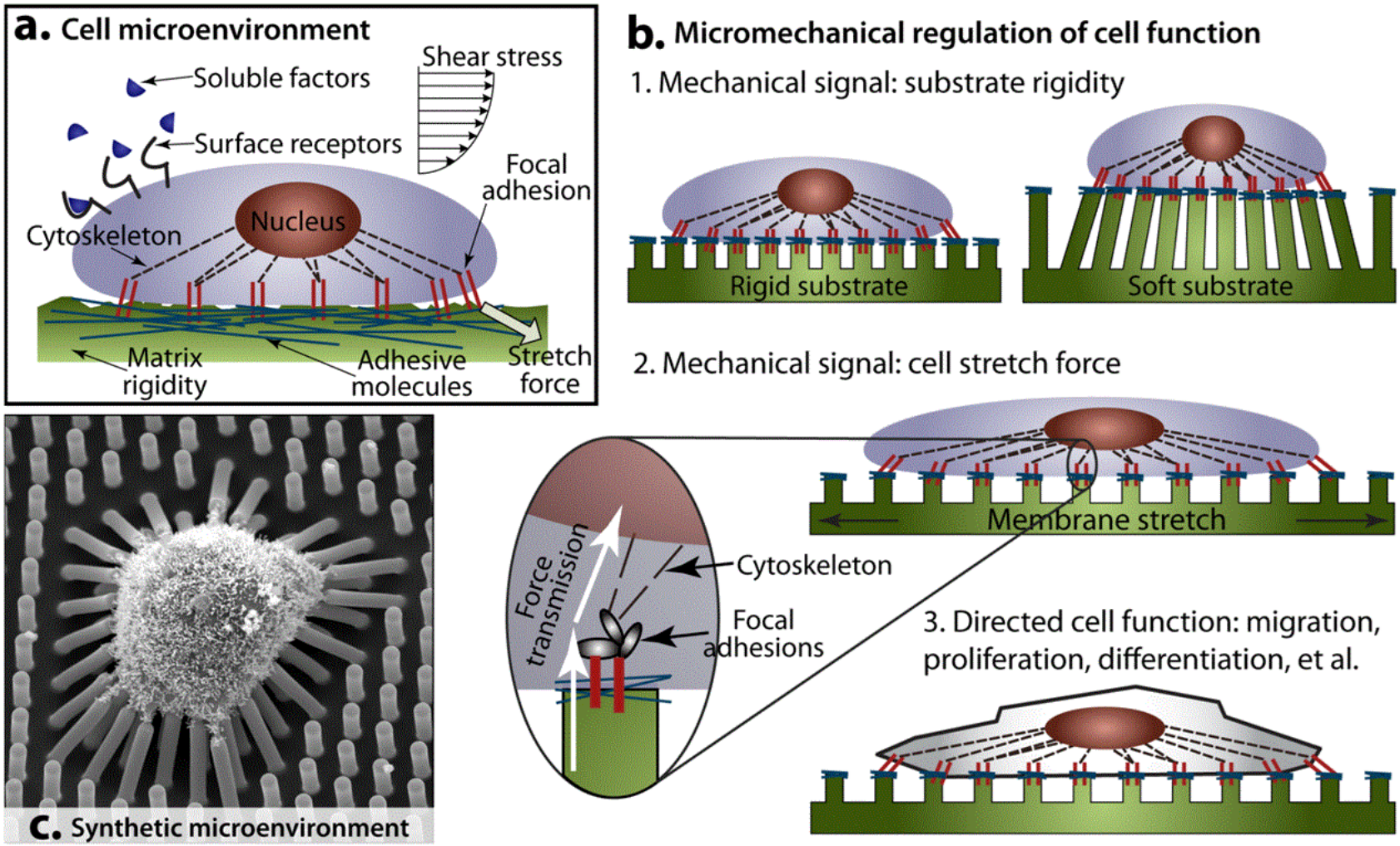

Fluid shear stress
A
B
C
D
E
F
G
H
ECM
Tissue strain
Calcium and other ions
Cell signaling molecules/ transcription factors
Cross-linking proteins
Actin
DNA
Intermediate filaments
Microtubules
Cell-matrix adhesion
Gap junction
Ion channel
Cell-surface receptor
Primary cilia
Glycocalyx
Cell-cell adhesion

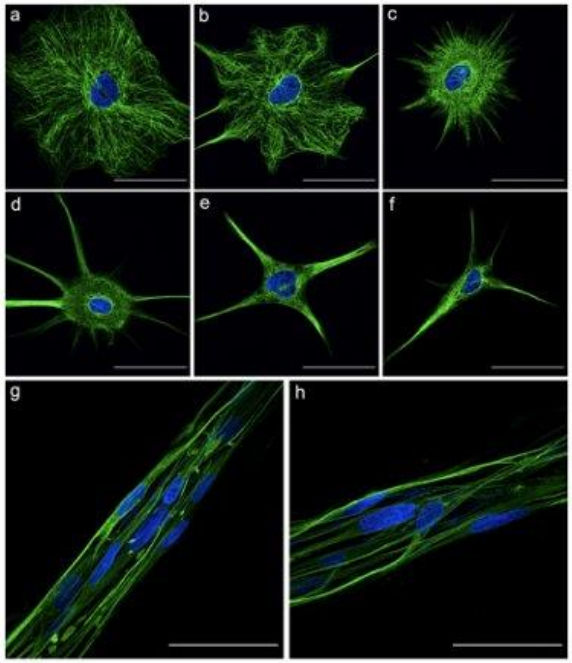
Relation adhésion/cytosquelette



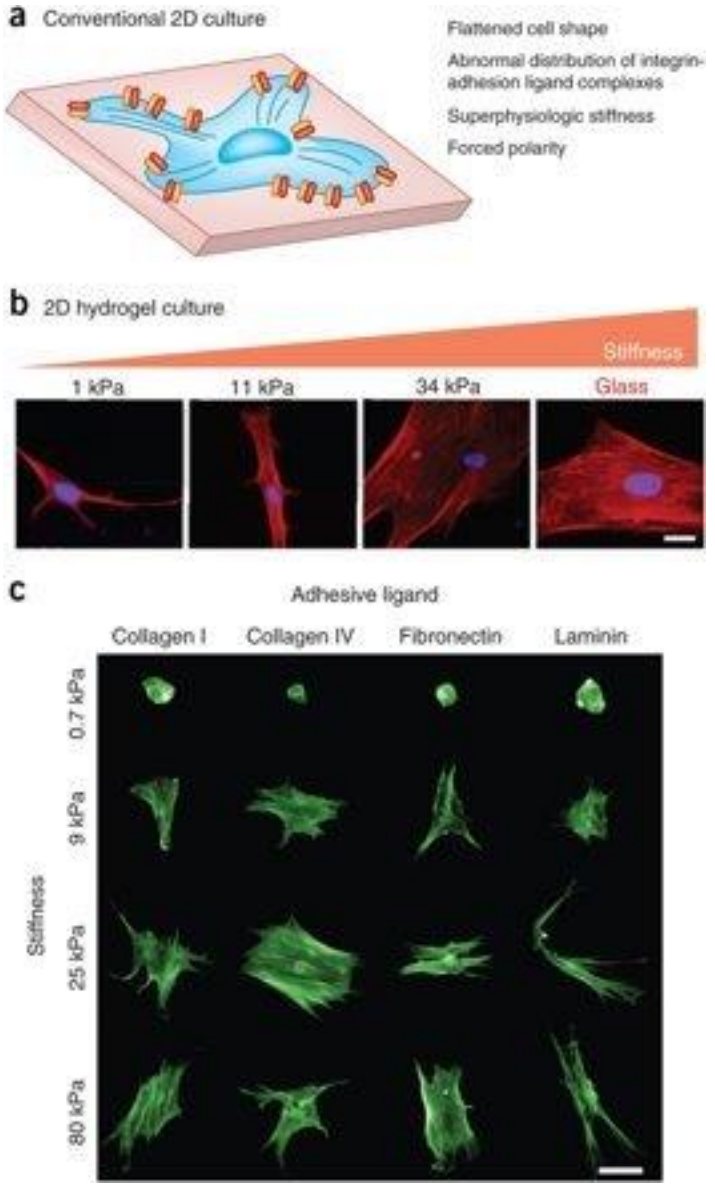



a. Cell microenvironment
Soluble factors
Surface receptors
Shear stress
Focal adhesion
Nucleus
Cytoskeleton
Matrix rigidity
Adhesive molecules
Stretch force
b. Micromechanical regulation of cell function
1. Mechanical signal: substrate rigidity
Rigid substrate
Soft substrate
2. Mechanical signal: cell stretch force
Membrane stretch
Force transmission
Cytoskeleton
Focal adhesions
3. Directed cell function: migration, proliferation, differentiation, et al.
c. Synthetic microenvironment

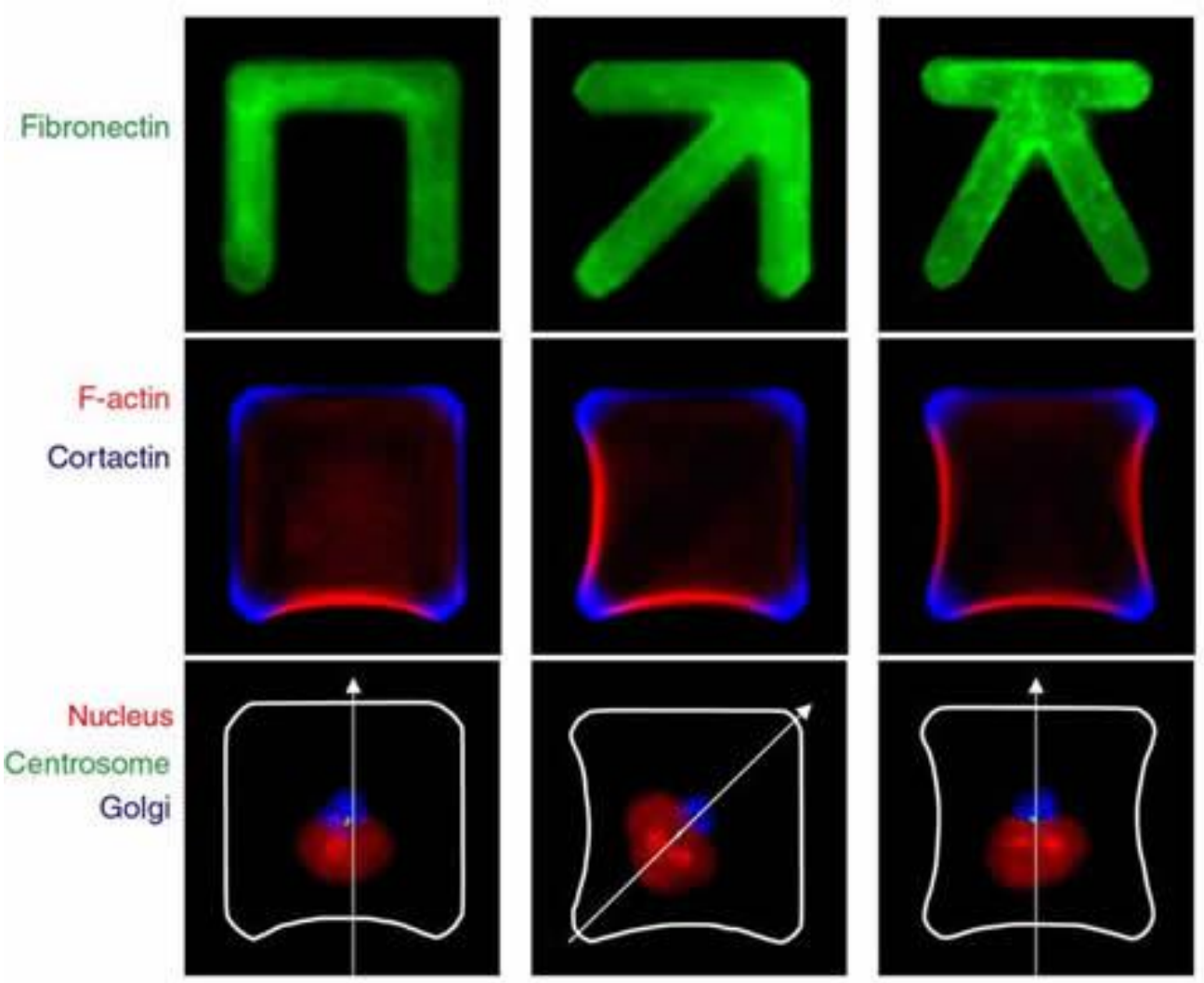
La surface d'adhésion conditionne la morphologie cellulaire



Astrocytes; différentes ECM



Ingénierie cellulaire: le micropatterning



Biophysical Journal Volume 106, Issue 11, 3 June 2014, Pages 2340-2352

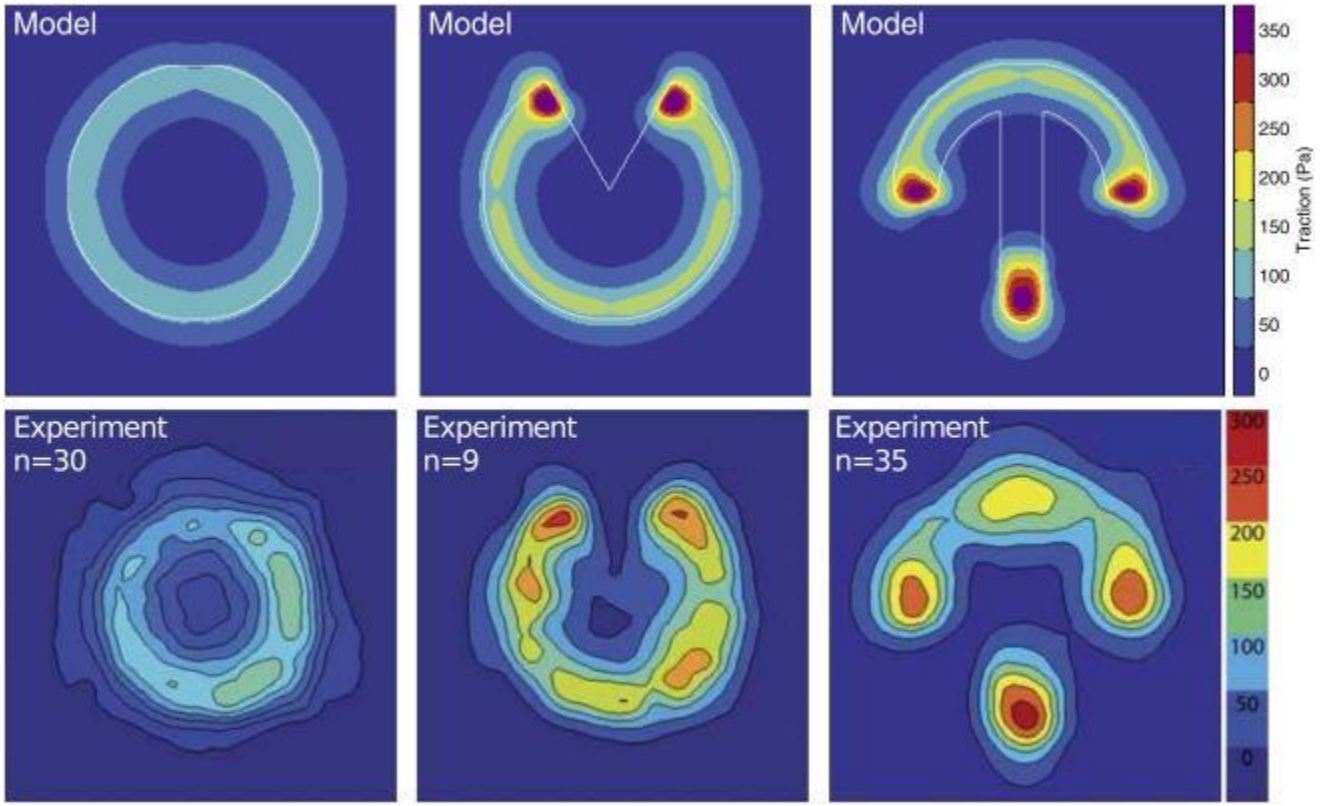
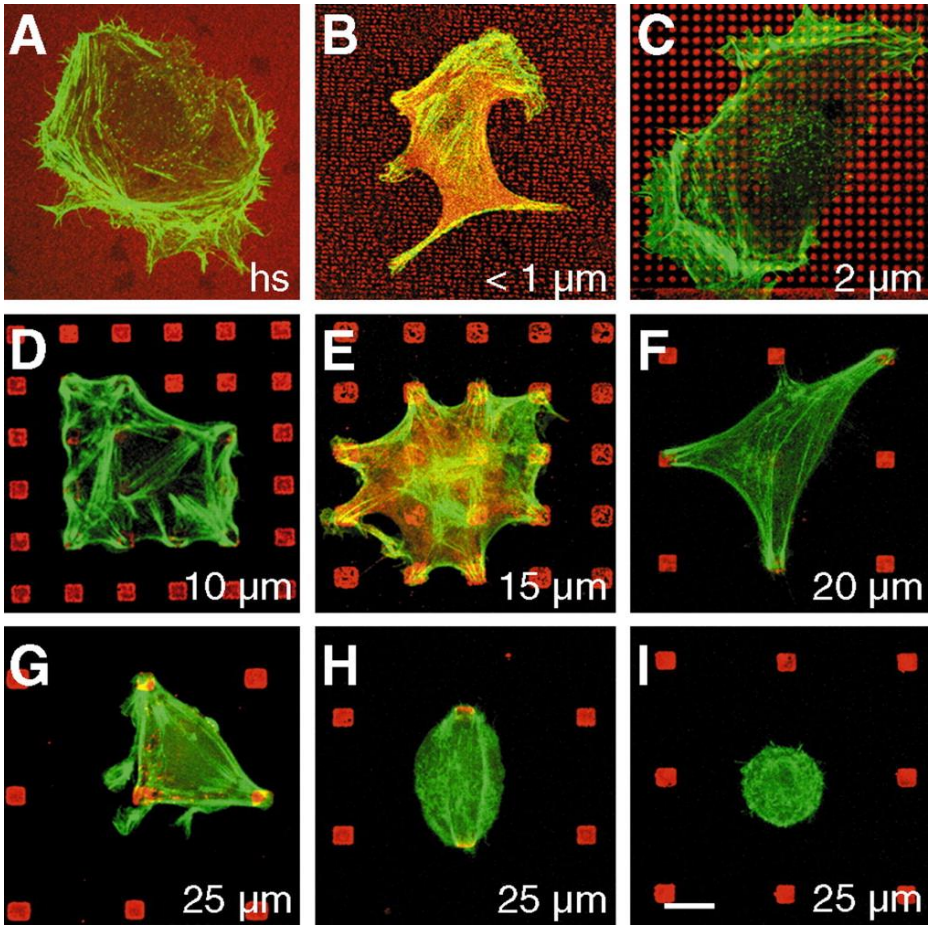


Figure 5. (*Upper row*) Reconstructed traction forces for the TEM with the best-fit parameters (λ_s = 2.30 nN, σ = 0.83 nN/μm, and EA = 40 nN). (*Lower row*) Traction forces reconstructed from experimental data for MCF10A-cells on fibronectin patterns on a polyacrylamide substrate.

Cell spreading in relation to substratum geometry.

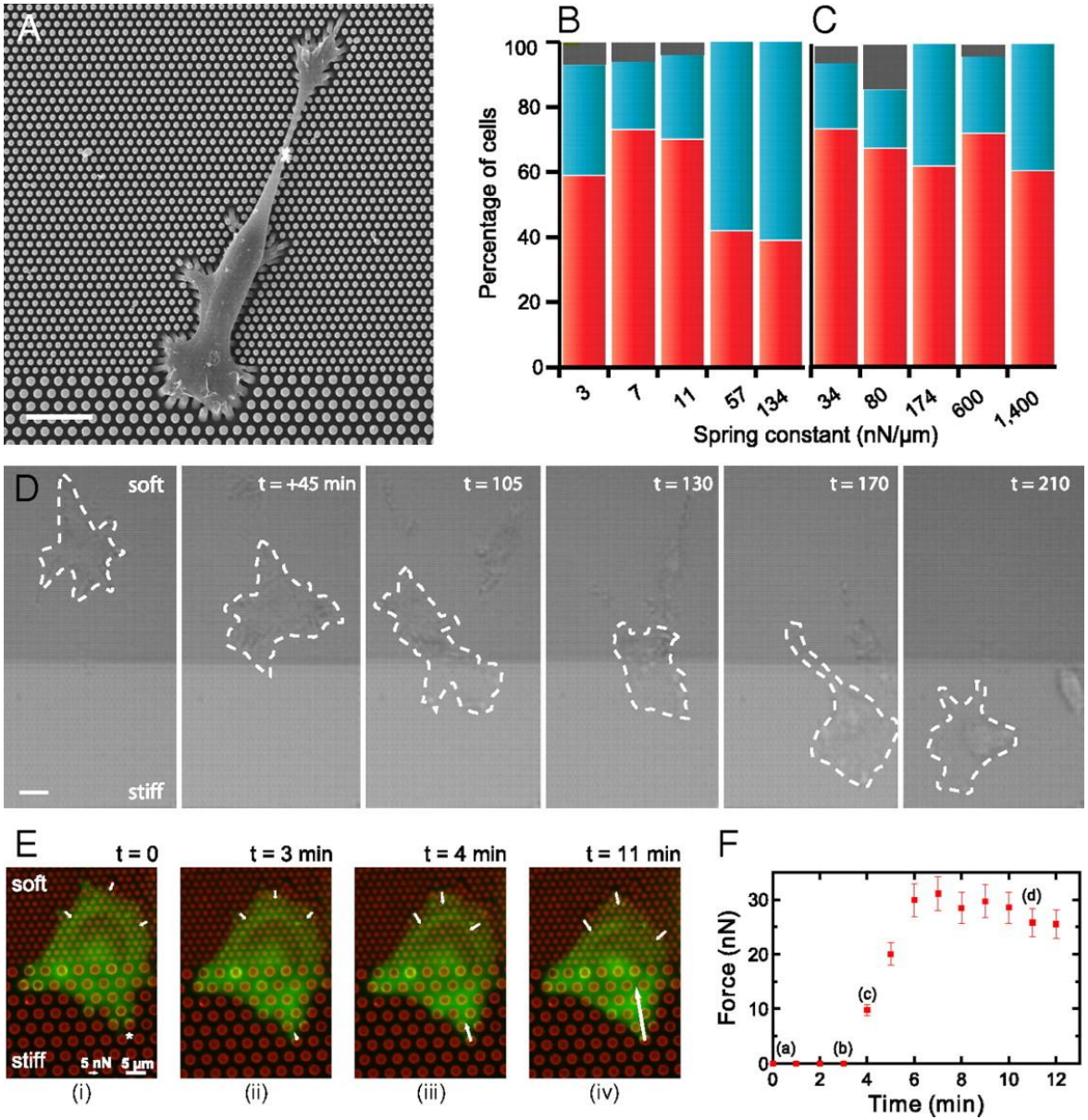


Cell spreading in relation to substratum geometry. B16 cells were cultured on fibronectin substrata prepared with µCP and labelled for fibronectin (red) and actin (green). (A) On a homogeneous substratum (hs), actin filaments are distributed throughout the cell periphery. (B,C) If the space between dots is ≤2 µm (B: 0.1 µm2 squares 1 µm apart, C: 1 µm2 squares 2 µm apart) cells spread as on a homogeneous substratum. (D-I) Cell growth on patterned substrata of 9 µm2 dots with spacing as indicated in the right-hand corner. (D-F) With distances of 5-20 µm between dots, cells spread and the actin cytoskeleton formed stress fibres between adjacent dots. (G-I) At a distance of 25 µm, spreading was limited and cells became triangular, ellipsoid or round. Scale bar: 10 µm.

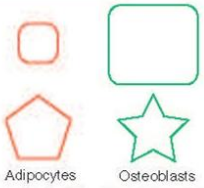
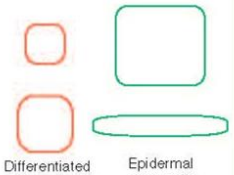
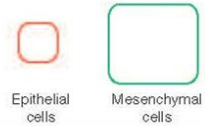
Dirk Lehnert et al. J Cell Sci 2004;117:41-52

Journal of Cell Science

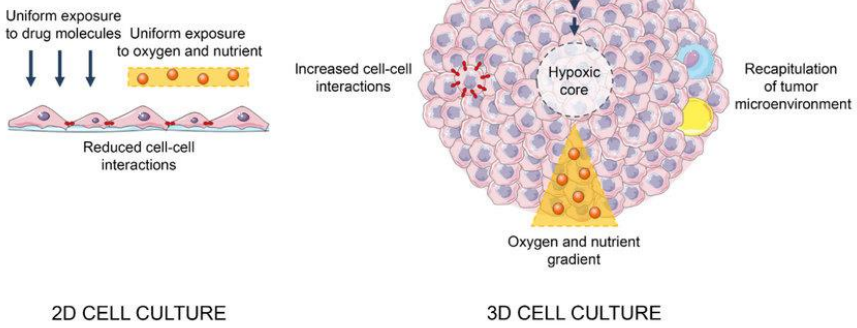
Un exemple de comportement cellulaire méchano-dépendant: la durotaxie

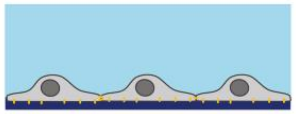


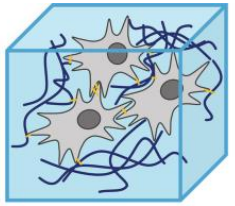
L'ingénierie cellulaire révèle les effets de l'adhésion sur la contractilité et la différenciation cellulaires

Cell type	Conditions		Cell fate	References
	ECM micropattern (LOW contractility / HIGH contractility)	Biochemical cues		
Mesenchymal stem cells		**Mixed medium** inducing adipogenic and osteogenic differentiation	 Adipocytes Osteoblasts	(McBeath et al., 2004) (Kilian et al., 2010)
		TGF-β	 Chondrocytes Myocytes	(Gao et al., 2010)
Epidermal stem cells		Growth factors	 Differentiated epidermal cells Epidermal stem cells	(Connelly et al., 2010)
Epithelial cells		Matrix metalloproteinase-3 Low concentration of TGF-β	 Epithelial cells Mesenchymal cells	(Nelson et al., 2008) (Gomez et al., 2010)

De la culture 2D à la 3D

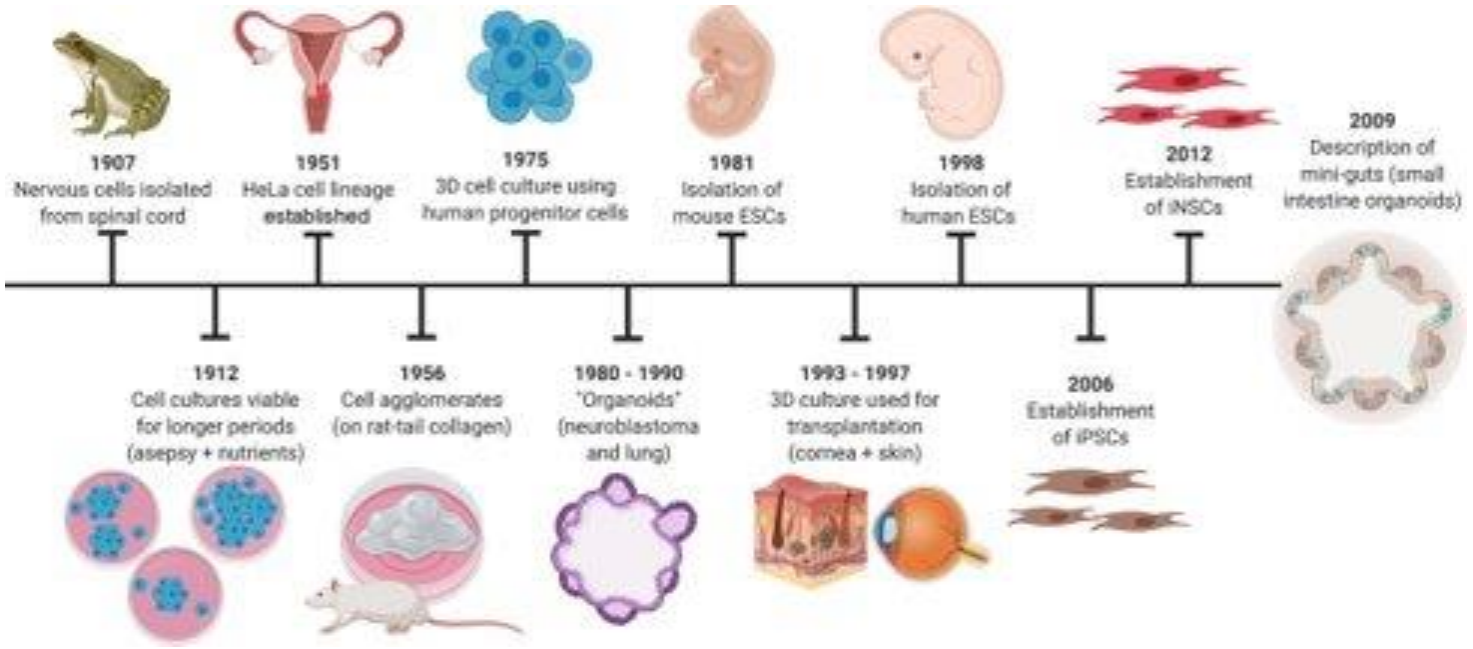






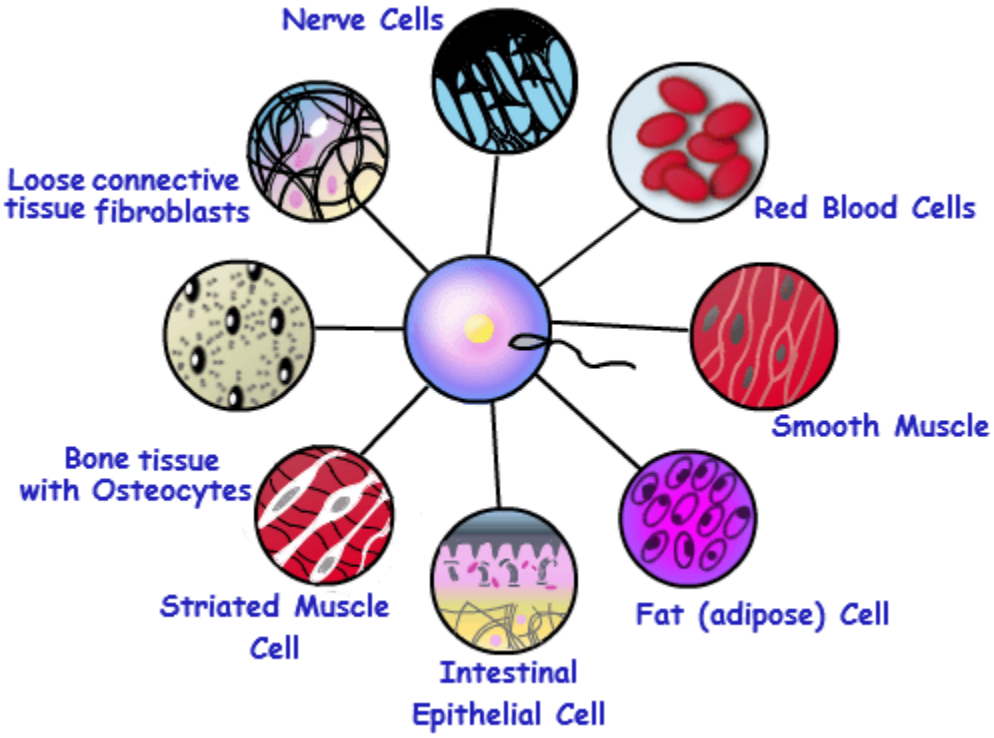
	2D	3D
Substrate	• high stiffness • continuous flat surface • unhindered spreading	• lower stiffness, tunable • porosity and roughness of surface helps adhesion • degree of porosity hinders cell movement
Oxygen & Nutrient Supply	• high abundance/oversupply of nutrients and oxygen	• gradients of nutrients and oxygen (diffusion)
Shape and Geometry	• forced apical basal polarity • high substrate interaction • low number of cell to cell contacts	• non-polar shape • higher number of cell-to-cell contacts • self-organisation in 3D structures

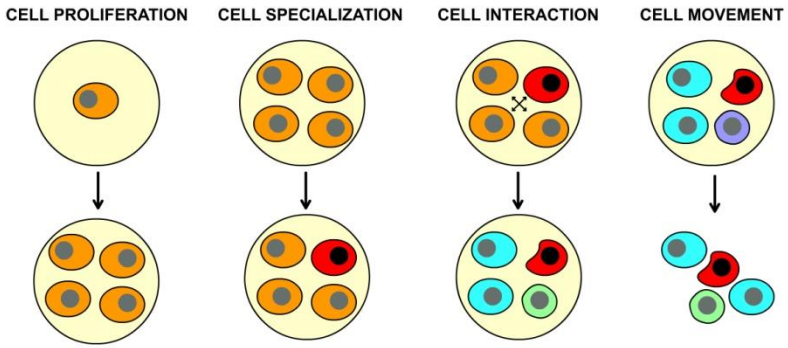
Une histoire de la culture cellulaire



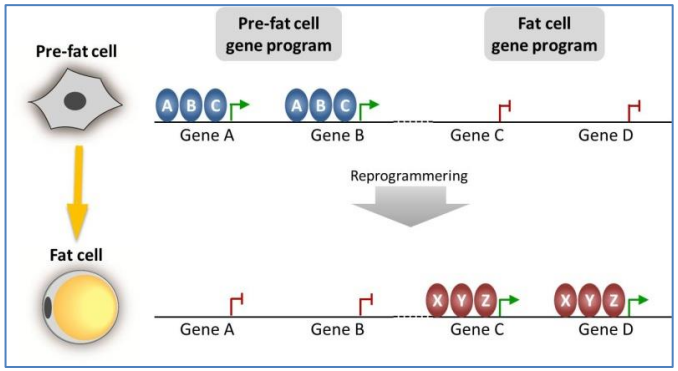
Différenciation cellulaire

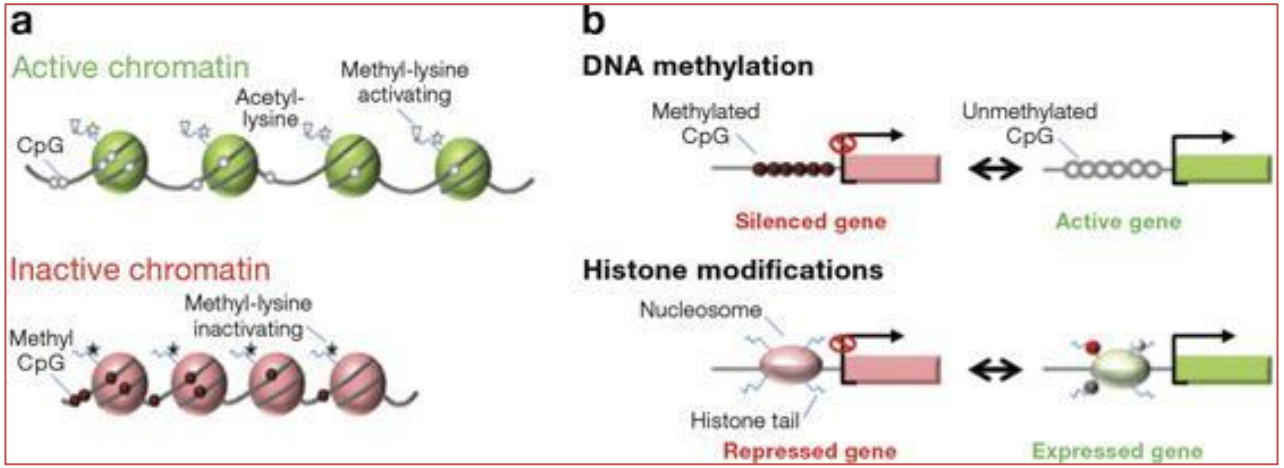
La différenciation cellulaire





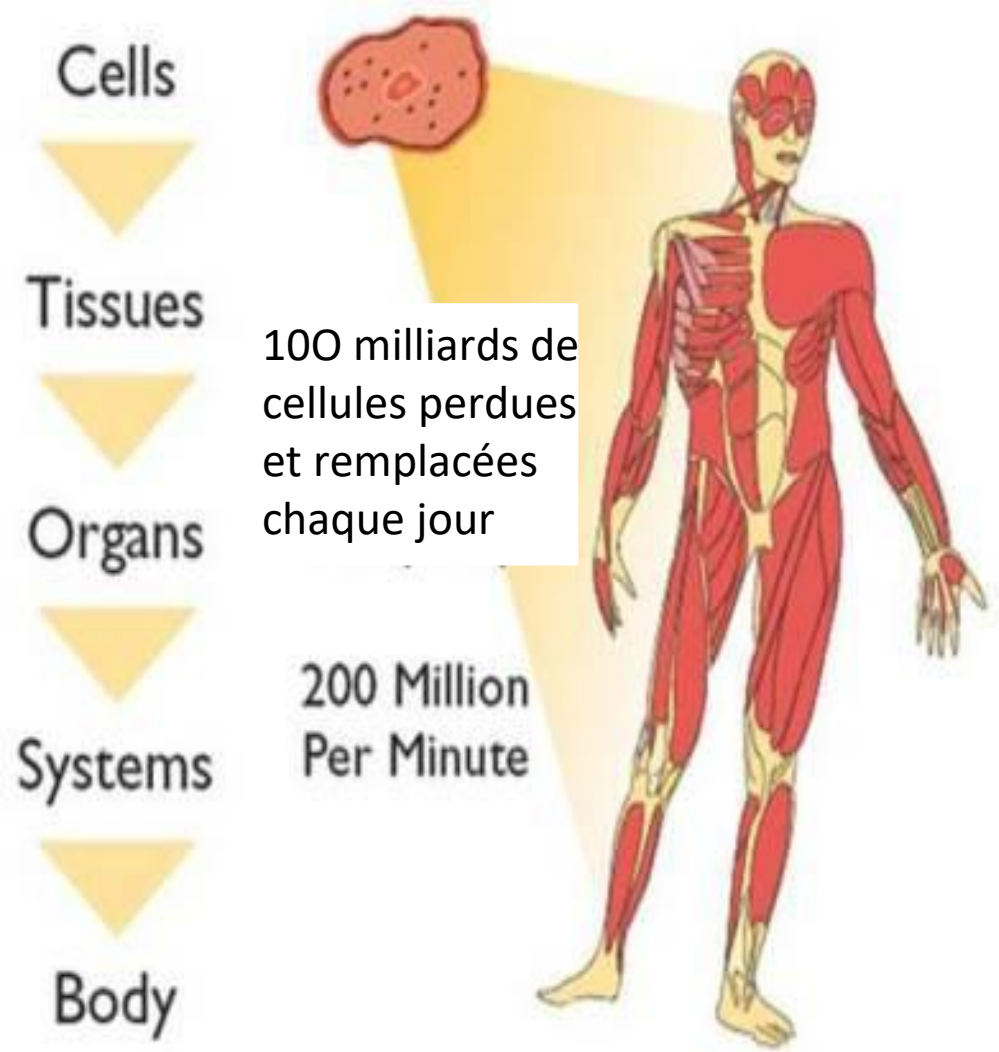
La différenciation cellulaire résulte de modifications épigénétiques qui modifient la structure de la chromatine





DNA methylation and histone modifications. DNA methylation and histone modifications are two of the best-defined epigenetic mechanisms of gene expression, Chromosomal DNA is packaged around histone cores to form nucleosomes. Nucleosome spacing (panel **a**, top) in active chromatin is an open structure that is accessible to nuclear factors and is maintained in part by posttranslational modification of histone tails, including lysine acetylation and specific lysine methylation. Cytosine–guanine (CpG) dinucleotides are distributed unequally throughout chromosomal DNA and may be concentrated in regions called CpG islands that typically overlap with gene promoters. Methylation of cytosine in CpG dinucleotides is associated with inactive, condensed states of the chromosome (panel **a**, bottom). This inactive state is also maintained by specific histone lysine modifications. Histone modifications occur posttranslationally, and different combination changes (phosphorylation, ubiquitination, acetylation, and methylation) may regulate chromatin structure and transcriptional status (Adapted from Handy *et al.* (2011) and Zaidi *et al.* (2011).

Division et mort cellulaires: renouvellement de l'organisme



10O milliards de cellules perdues et remplacées chaque jour

10 000 milliards de cellules

250 types cellulaires différents

En une année, chacun d'entre nous produit et élimine une masse équivalente au poids de son corps.

Sauf certaines cellules du cerveau et du cœur, nos cellules sont plus jeunes que nous!

Cellules souches

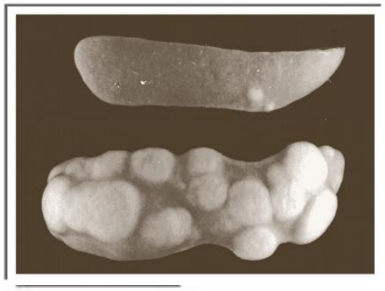
Concept de cellules souches

CELLULES SOUCHES HEMATOPOIETIQUES
TRANSFERT ADOPTIF=>GREFFE DE MOELLE

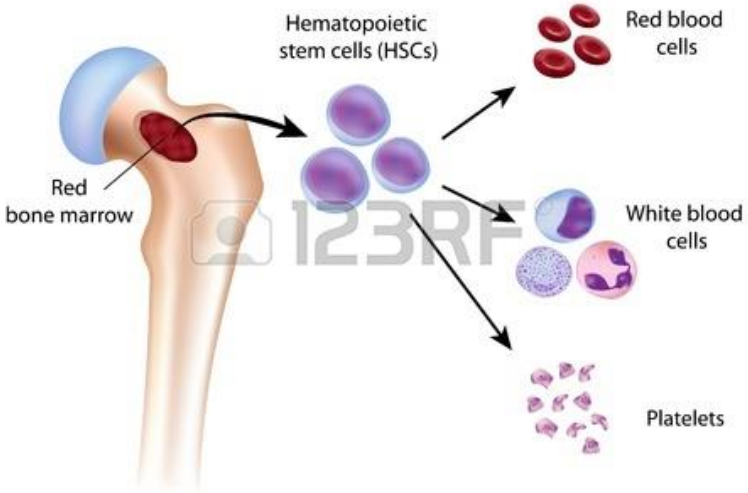
Expérience princeps: Till et Mc Culloch, 1961

Irradiation sub-léthale
1000Gy

+ Greffe de moelle (IV)



J10: colonies de cellules hématopoiétiques clonales, multi-linéales, dans la rate (CFU-S)



Qu'est-ce qu'une cellule souche?

Une cellule souche a la capacité unique de:

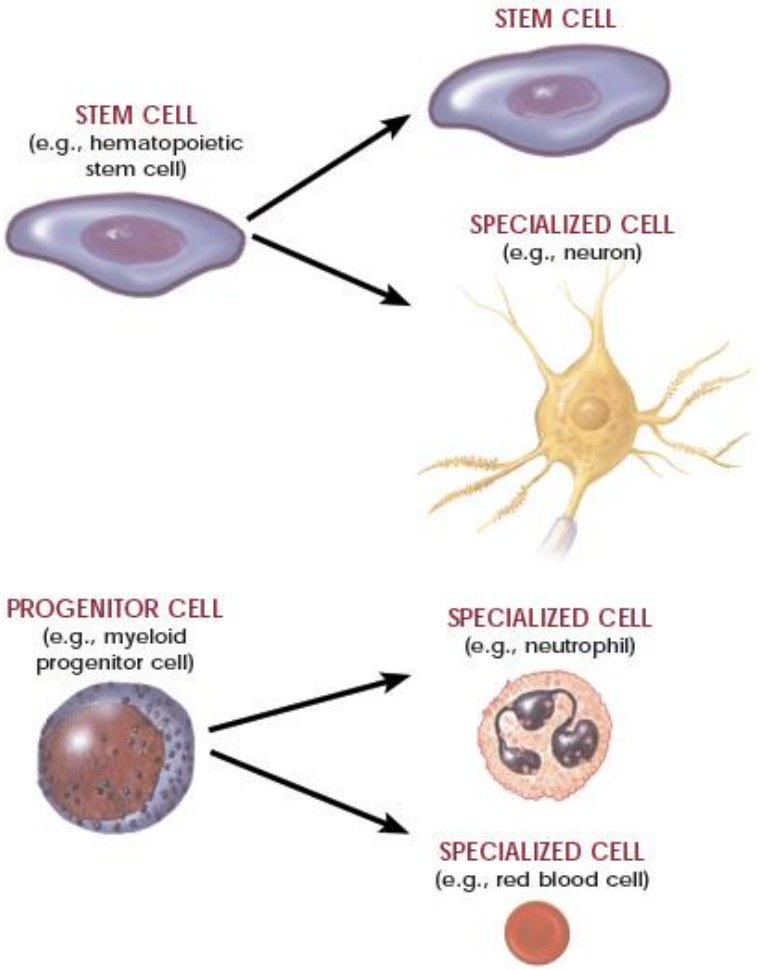
- ✓ s'auto-renouveler indéfiniment ou de manière prolongée

- ✓ produire différentes cellules spécialisées (différenciées)

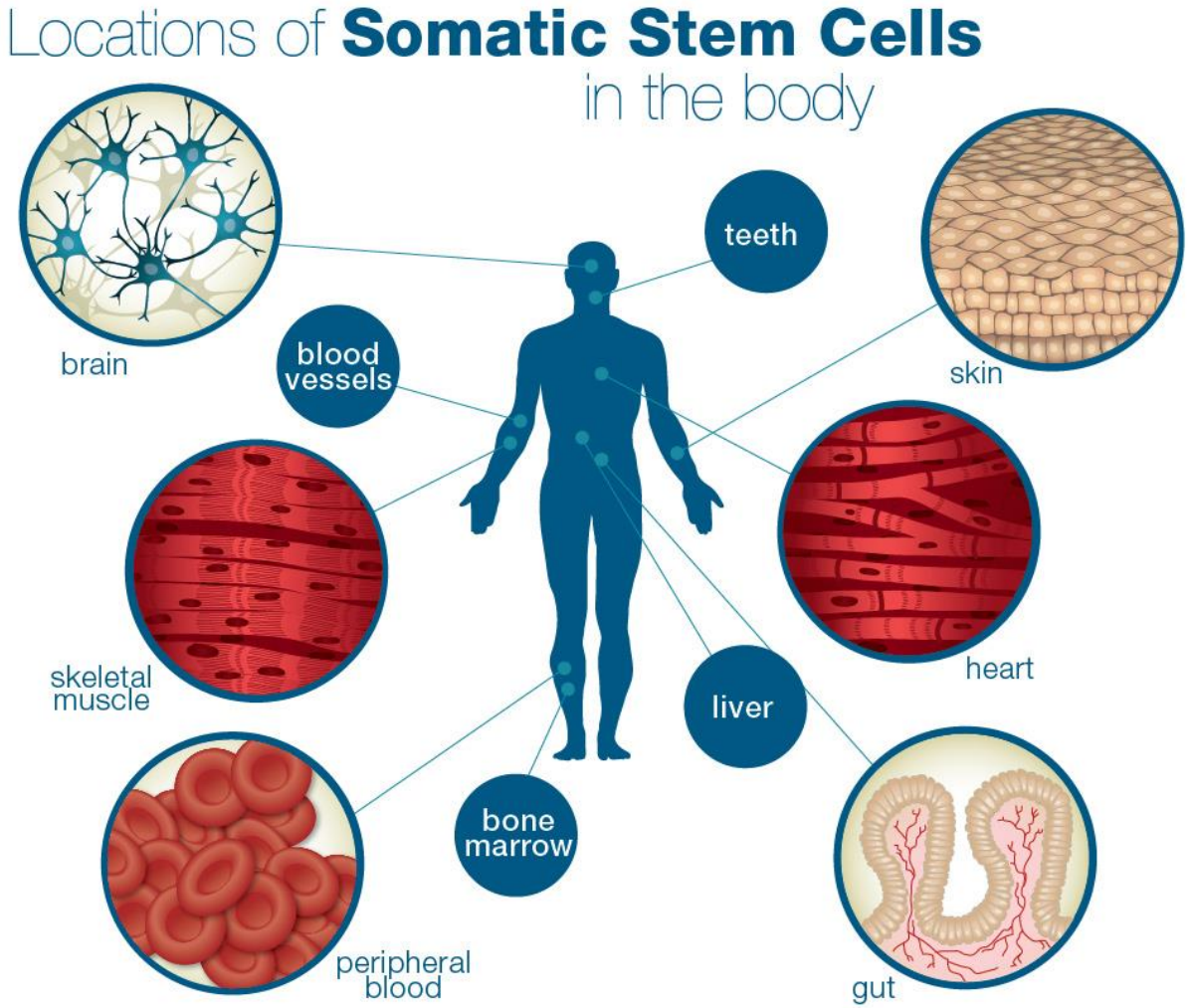
Qu’entend-on par auto-renouvellement?

- La division d’une cellule souche est asymétrique - les cellules filles ne sont pas identiques, et seule une des deux est identique à la cellule mère

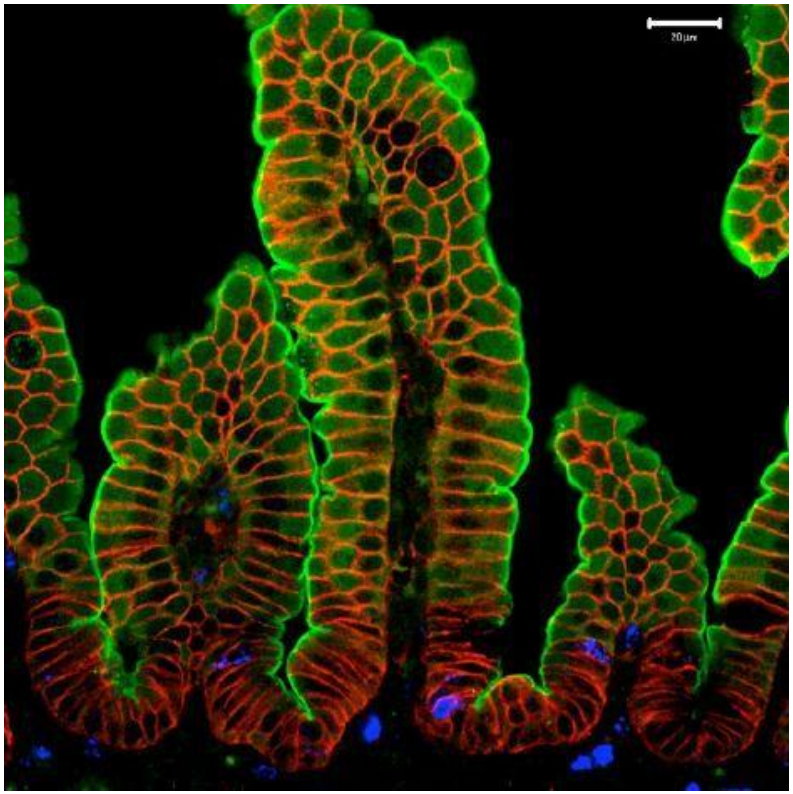
Divisions asymétriques



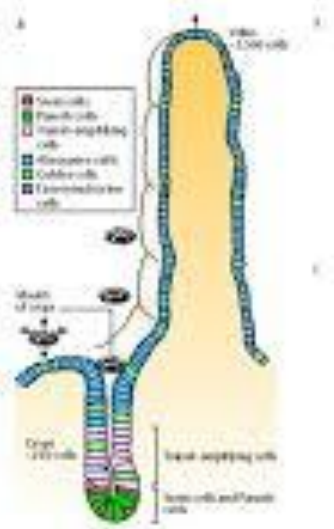
Les cellules souches adultes



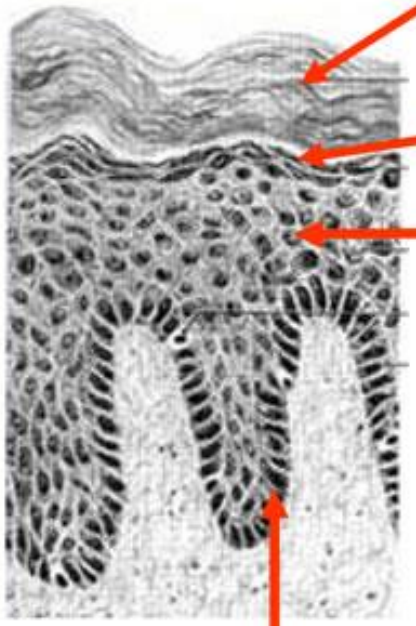
Notion de « niches »: exemples des cryptes intestinales



Les cellules souches sont localisées dans les cryptes



L'épiderme humain



Couche cornée

Couche granuleuse

Couche épineuse

Couche basale

Cornéocyte

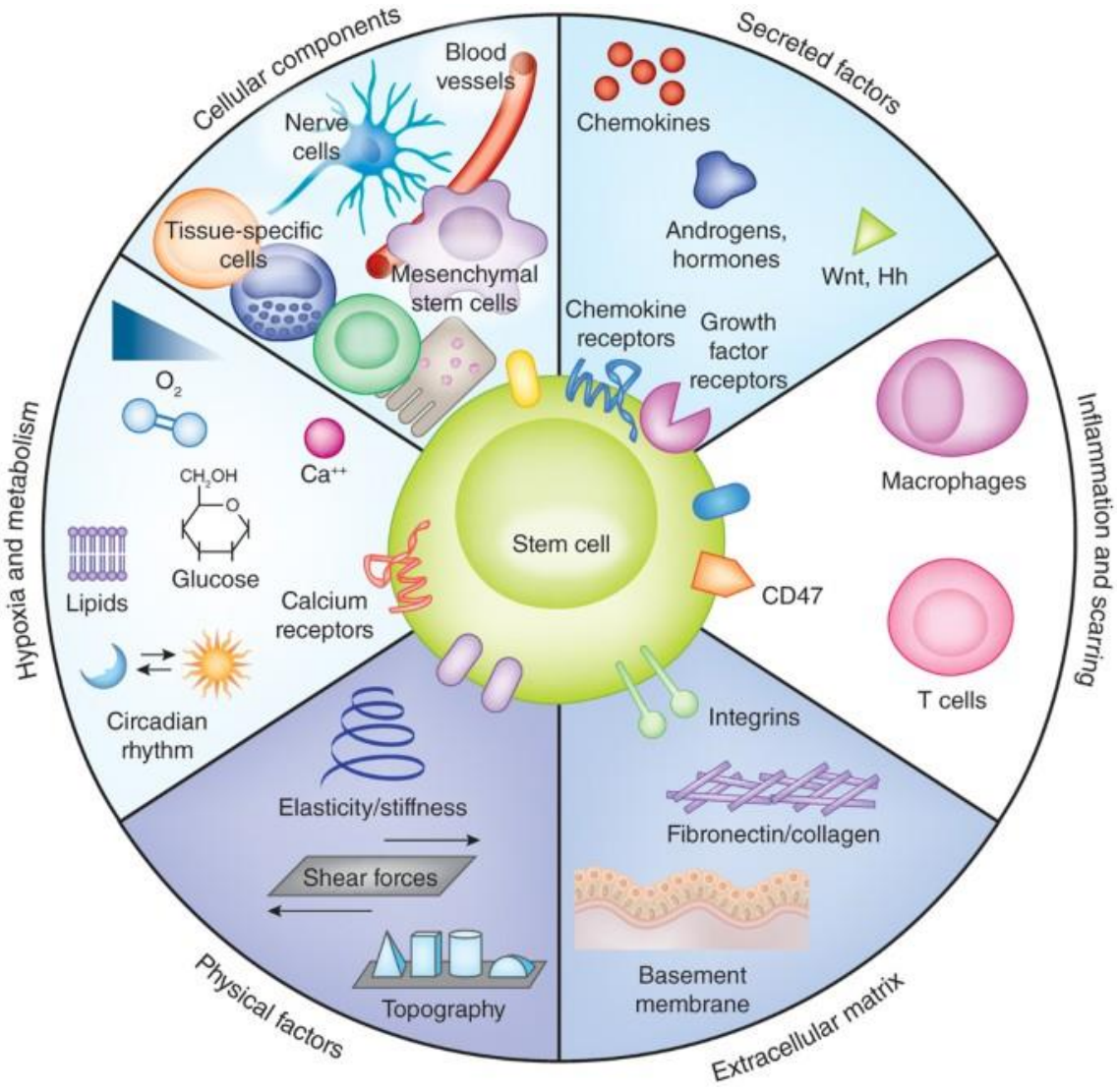
Kératinocyte granuleux

Kératinocyte épineux

Cellule fille souche

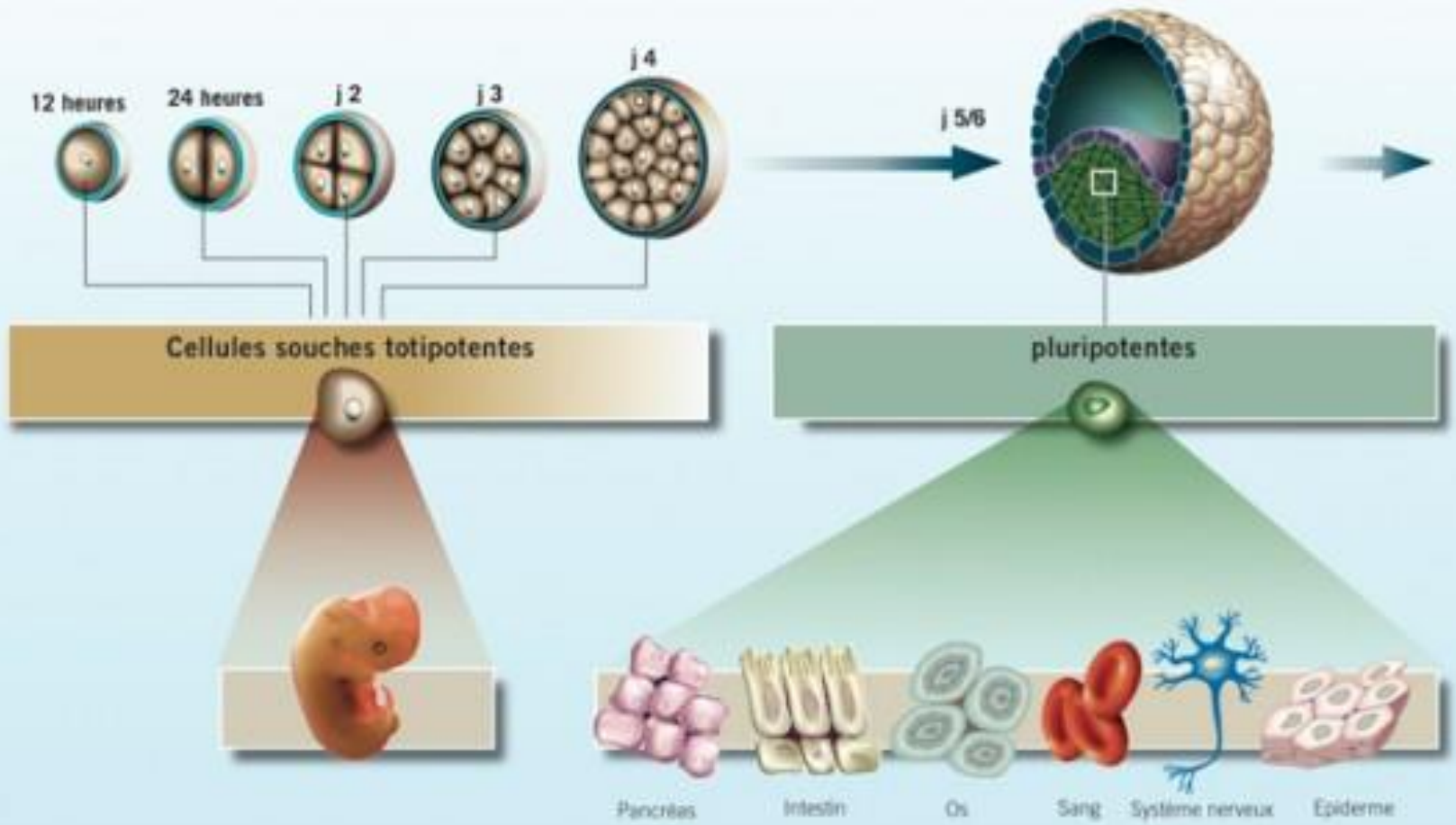
Kératinocyte souche

Cellules d'amplification transitoire proliférantes



Modulating the stem cell niche for tissue regeneration | Nature Biotechnology

Quatre types de cellules souches

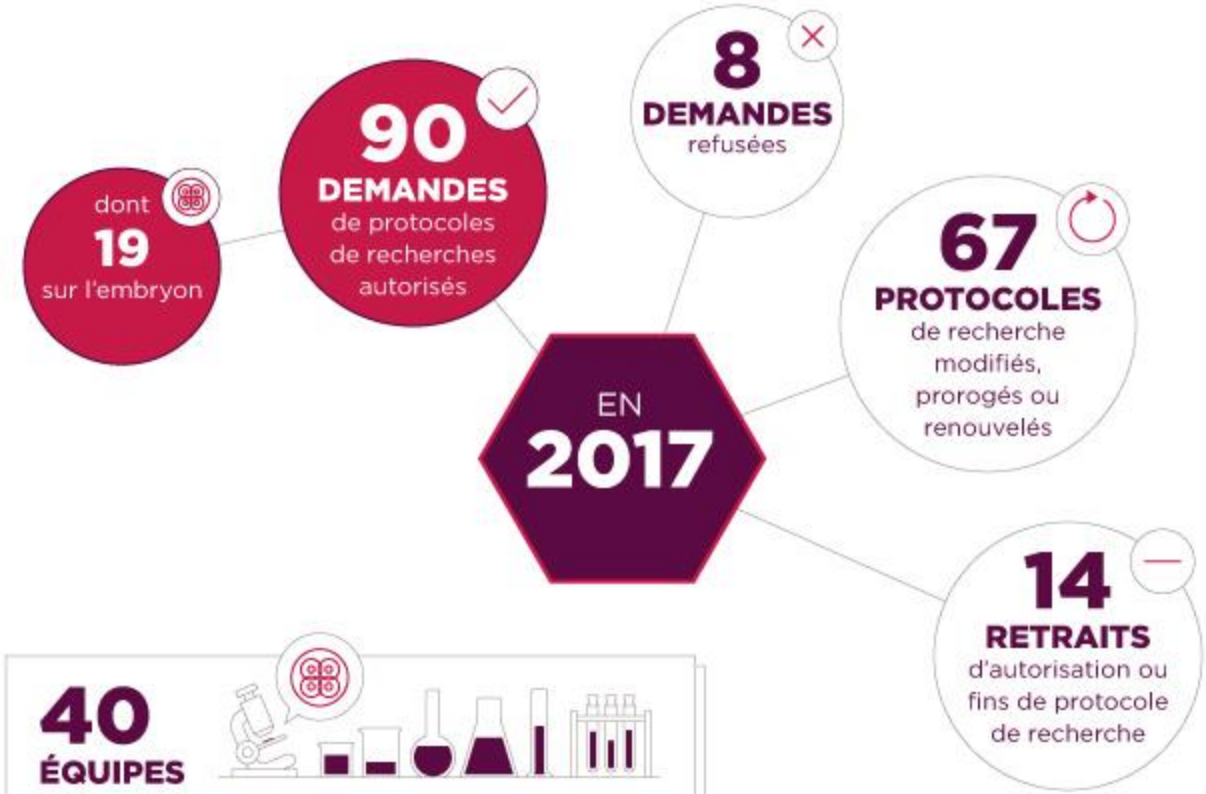


Issues des premières divisions de l'œuf fécondé, ces cellules sont indifférenciées et immortelles. Dites totipotentes, elles sont celles ayant la plus grande capacité de différenciation. Une seule d'entre elles – si elle était réimplantée dans un utérus – permettrait d'aboutir à un individu complet.

Présentes dans la masse interne du préembryon au stade de blastocyste, ces cellules pluripotentes sont immortelles et capables de se différencier en n'importe lequel des 200 types cellulaires. C'est sur ces fameuses « cellules souches embryonnaires humaines » (CSEh) que se concentre la recherche biomédicale actuelle.

ÉTAT DES LIEUX

DE LA RECHERCHE EN FRANCE SUR L'EMBRYON HUMAIN ET LES CELLULES SOUCHES EMBRYONNAIRES HUMAINES



Source : Agence de la biomédecine (2017)

Différences entre cellules souches adultes et cellules souches embryonnaires

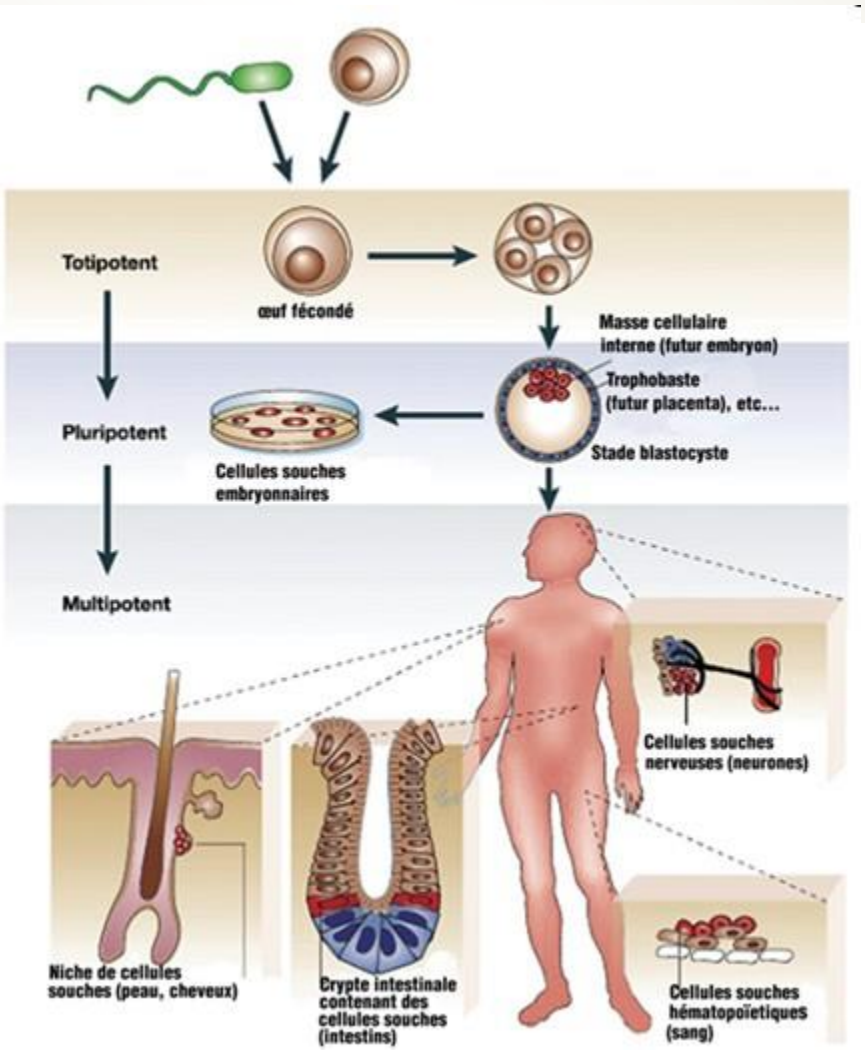
Adultes

Différenciation limitée : multipotent + plasticité

Rares dans les tissus matures

Difficiles à cultiver en culture

Plus “éthiques” que les cellules souches embryonnaires



Embryonnaires

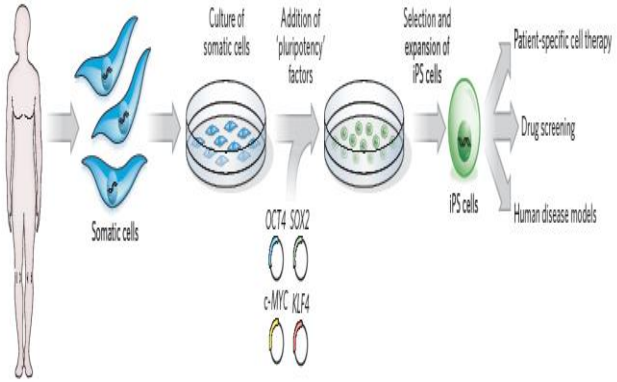
Différenciation illimitée: pluripotent

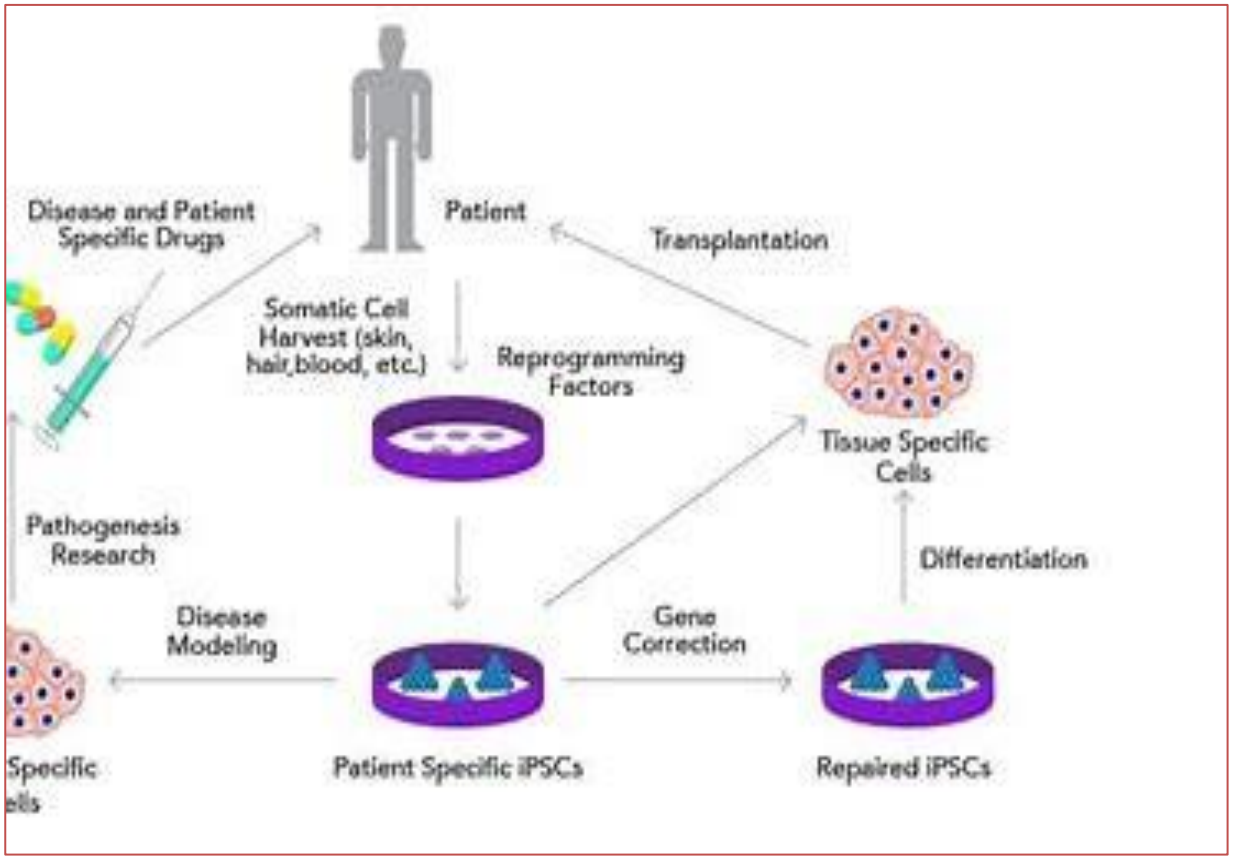
Présentes en grand nombre

Faciles à cultiver en culture

Prix Nobel de médecine 2012

Induced Pluripotent Stem Cells



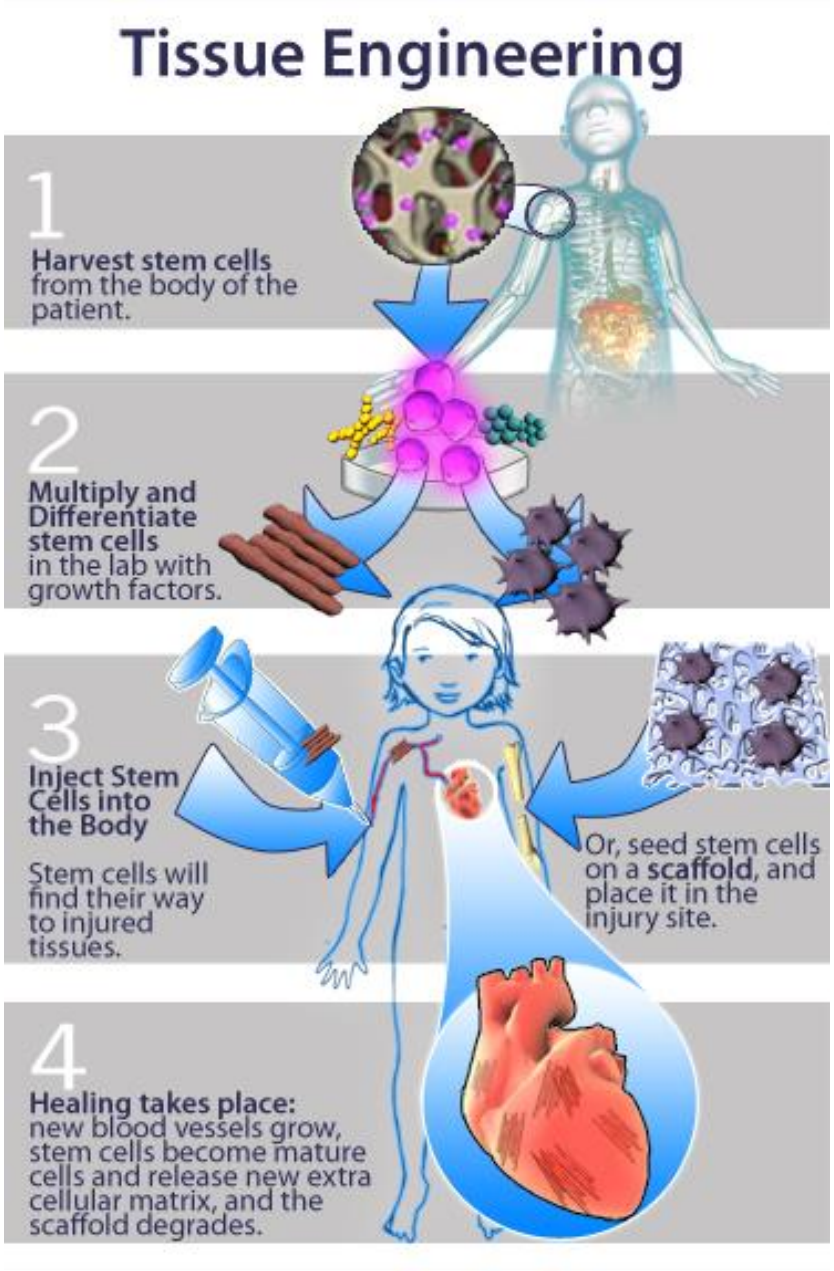


Nuclear reprogramming to a pluripotent state by three approaches
Shinya Yamanaka & Helen M. Blau
NATURE|Vol 465|10 June 2010|doi:10.1038/nature09229

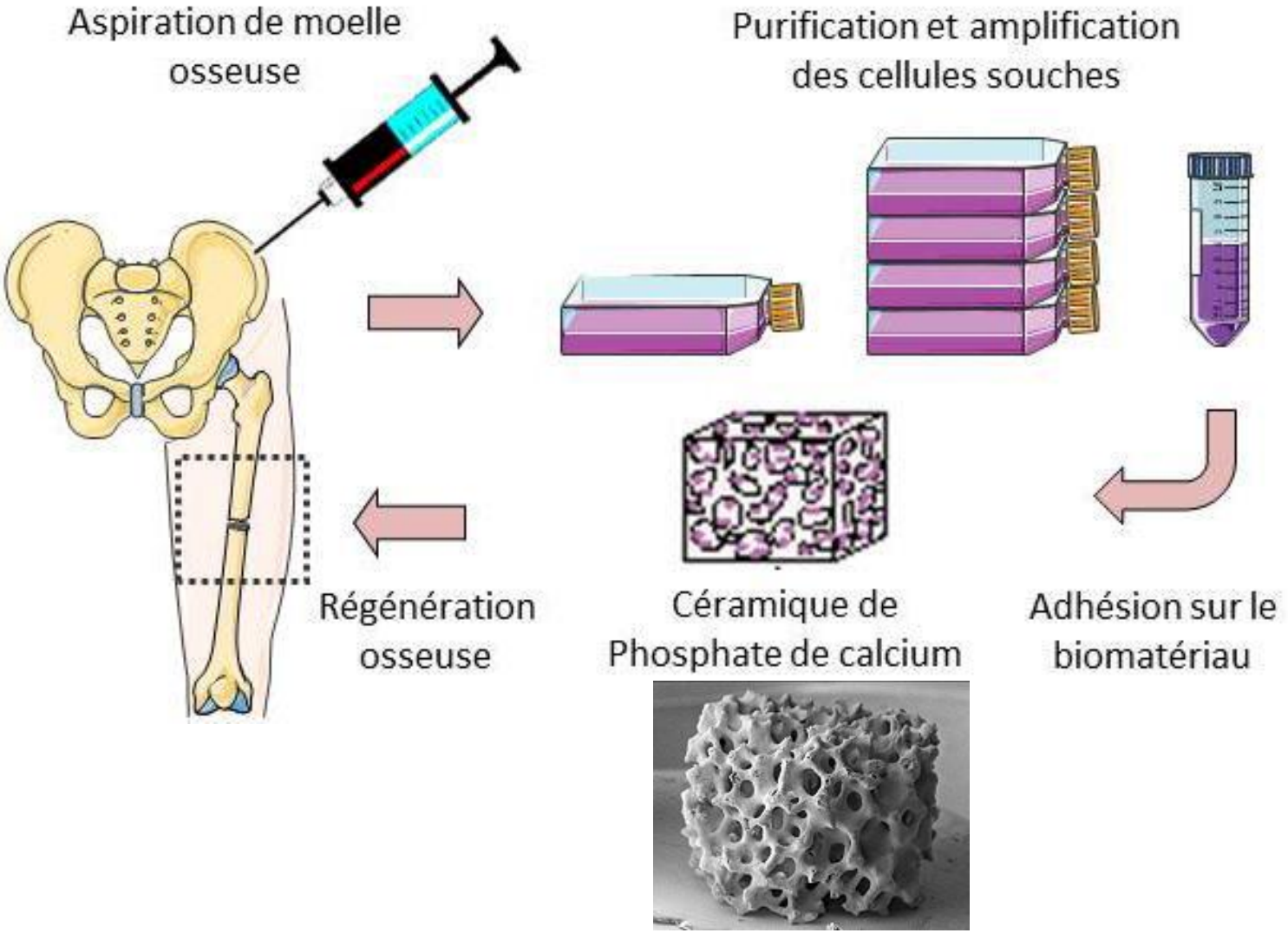
Buts de l'ingénierie tissulaire

- Créer des produits qui améliorent la fonction des tissus ou les guérissent.
- Remplacer les tissus malades ou endommagés
- Créer des modèles de tissus « malades »
- Créer des tissus modèles pour les tests pharmacologiques

Médecine régénérative: thérapie cellulaire



Réparation osseuse



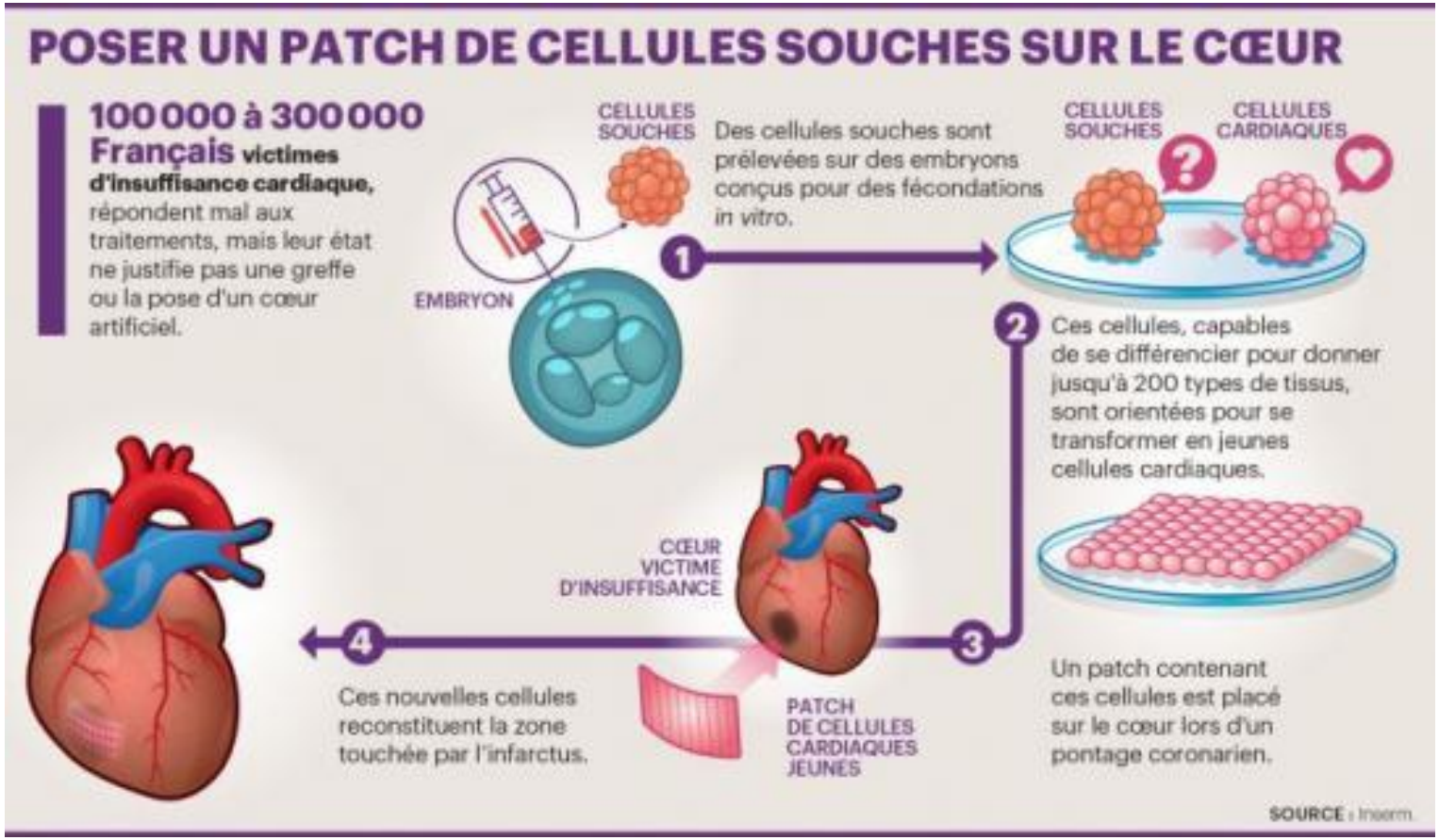
Première mondiale : des cellules au secours du cœur

LE MONDE | 16.01.2015 à 16h51 • Mis à jour le 16.01.2015 à 18h15 |

Par **Paul Benkimoun**

Le secret a été bien gardé, et le professeur Philippe Menasché, chirurgien cardiaque à l'Hôpital européen Georges-Pompidou (Paris), en collaboration avec l'équipe du professeur Jérôme Larghero (hôpital Saint-Louis, Paris), spécialisée dans les biothérapies, a réussi pour la première fois au monde une thérapie cellulaire chez une patiente en insuffisance cardiaque, qui se porte bien trois mois après l'intervention. Une étape qui couronne vingt ans de recherches.

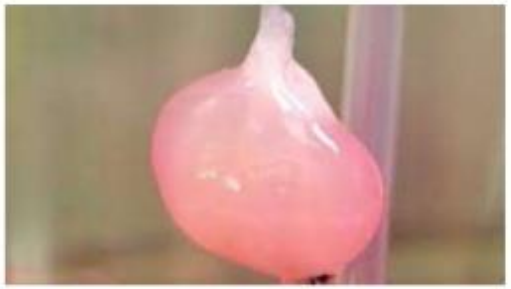
En savoir plus sur http://www.lemonde.fr/sante/article/2015/01/16/premiere-mondiale-des-cellules-au-secours-du-coeur



Les organes décellularisés

Challenge of Regenerative Medicine

Bioengineering of Organs

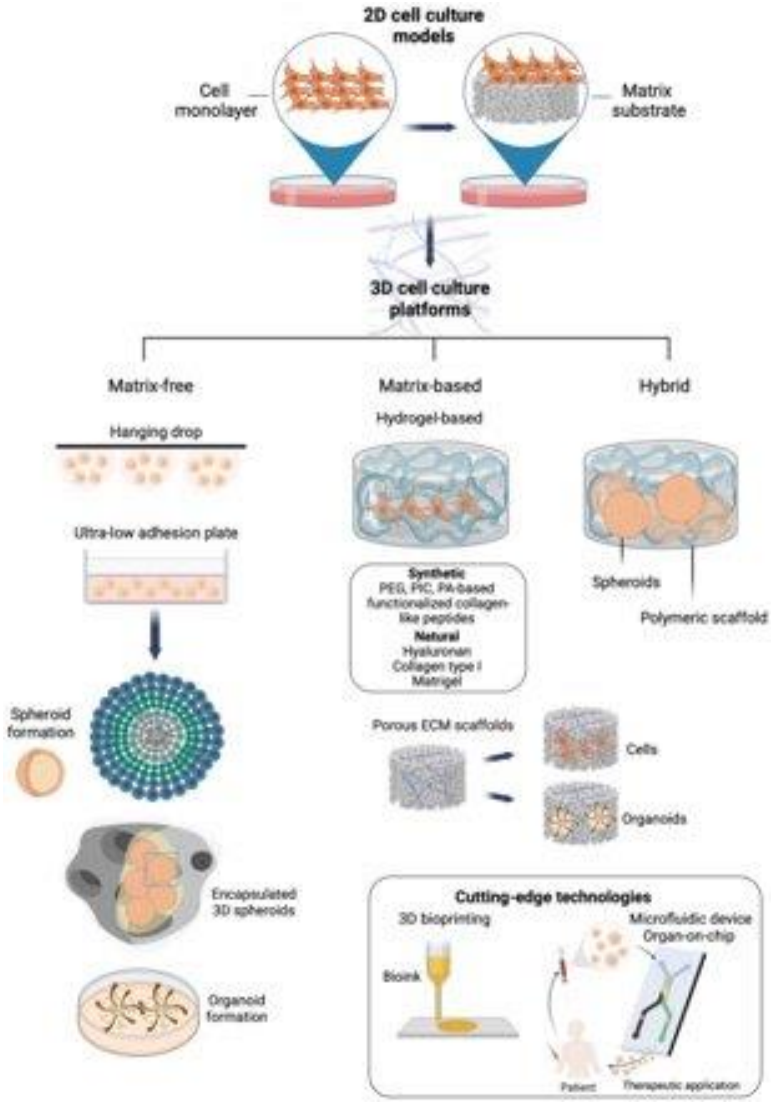


Ott HC et al., Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart, Nature Medicine, 2008

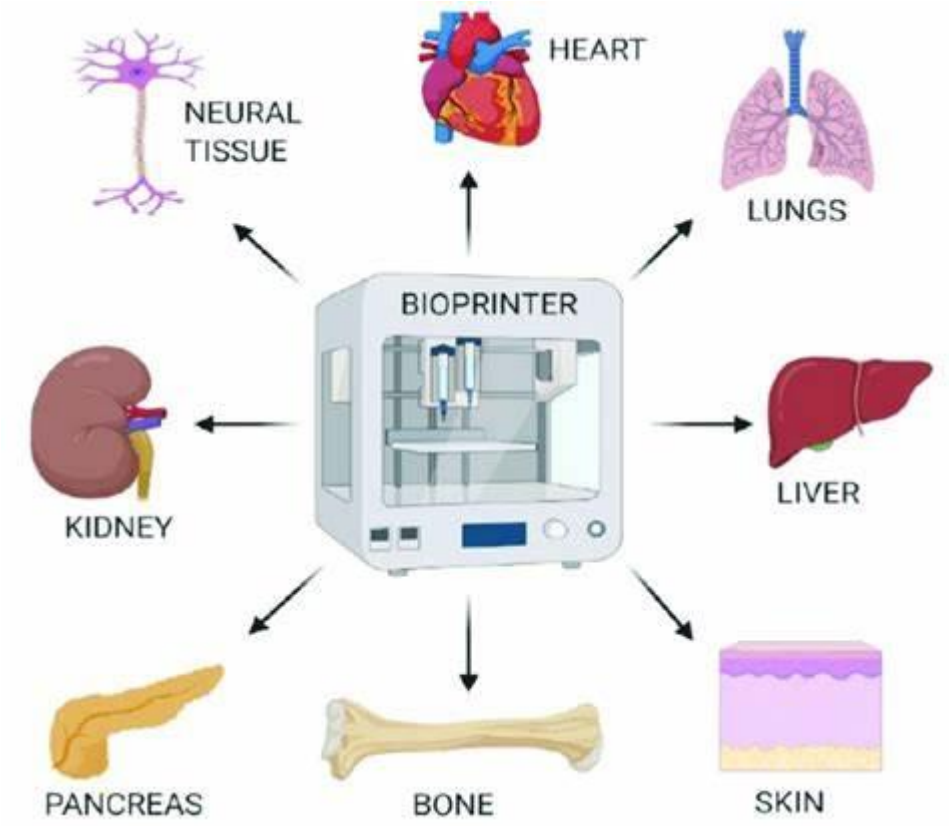
https://www.youtube.com/watch?v=YiKI3ppo0pM

Ingénierie tissulaire: fournir aux cellules le microenvironnement adéquat pour leur différenciation ou leur homéostasie fonctionnelle

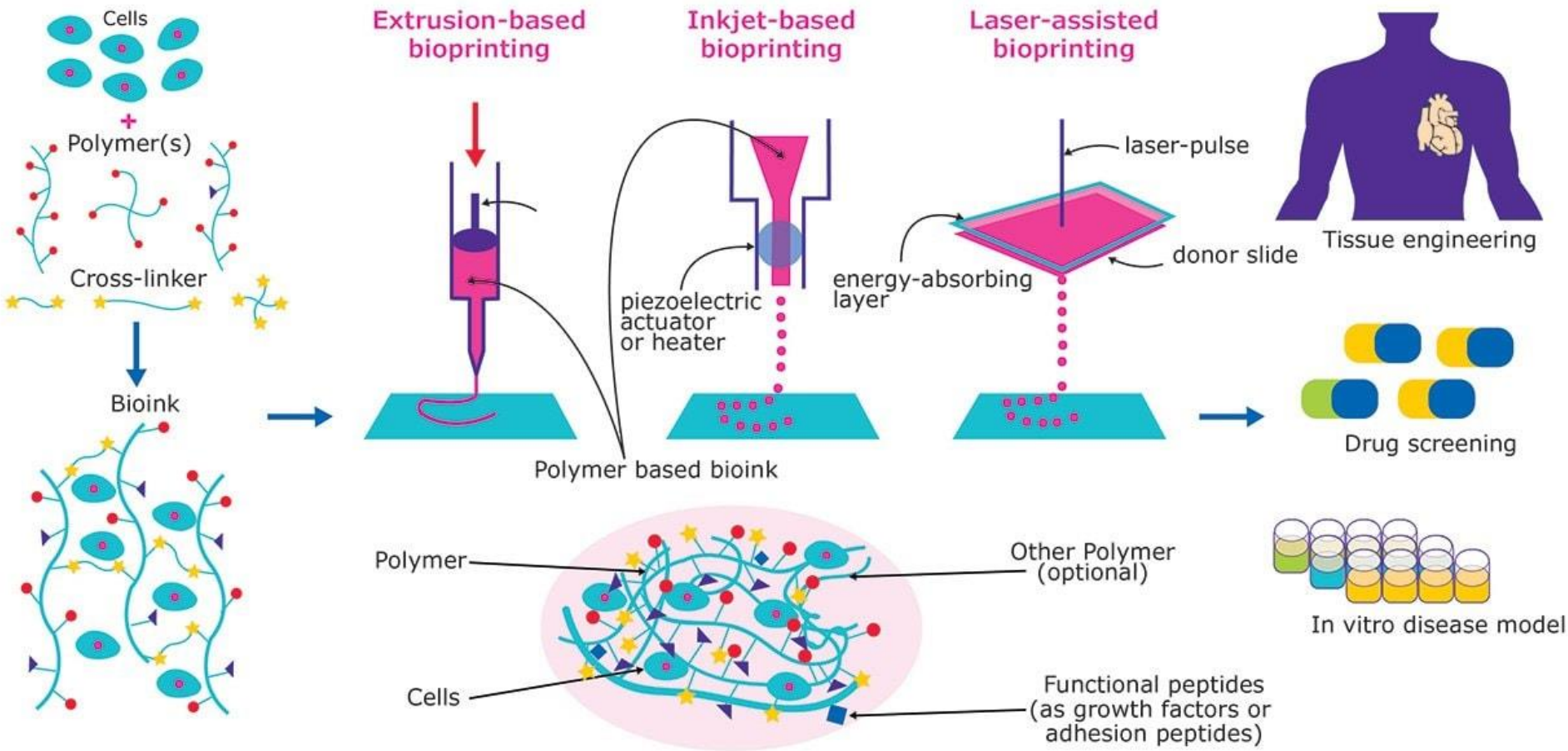
Thérapie cellulaire



Organes sur puces


NEURAL TISSUE
HEART
LUNGS
BIOPRINTER
KIDNEY
LIVER
PANCREAS
BONE
SKIN

Bioprinting



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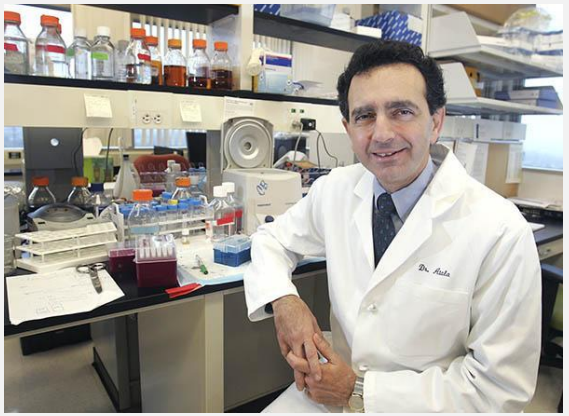
Wake Forest Physician Reports First Human Recipients of Laboratory-Grown Organs

J'aime 47 Tweet G+1 0

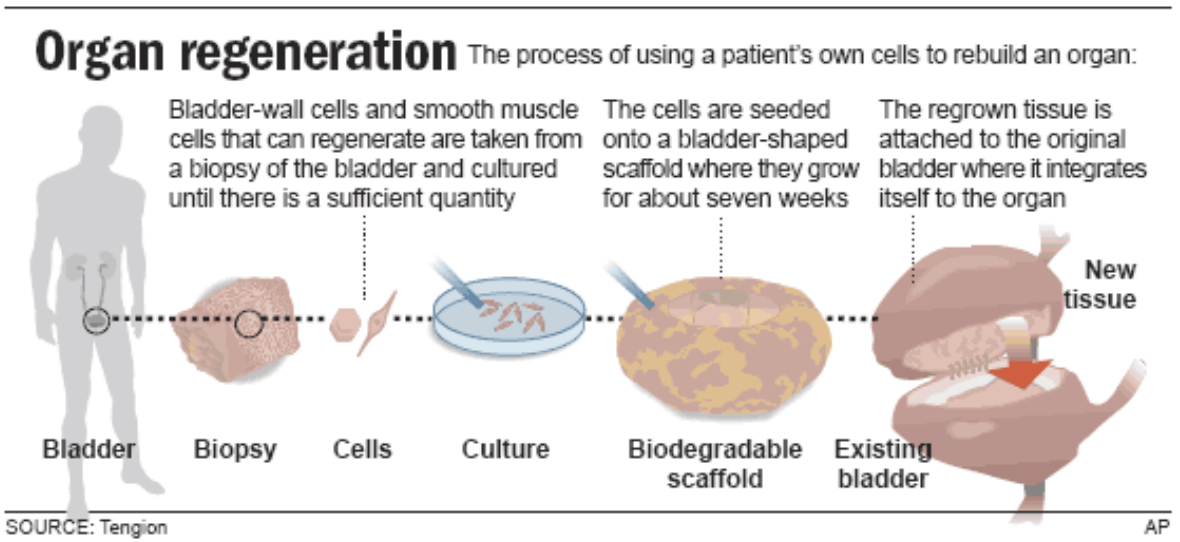
WINSTON-SALEM, N.C. -- May 2006 -- The first human recipients of laboratory-grown organs were reported today by Anthony Atala, M.D., director of the Institute for Regenerative Medicine at Wake Forest University School of Medicine. In The Lancet, Atala describes long-term success in children and teenagers who received bladders grown from their own cells.

"This is one small step in our ability to go forward in replacing damaged tissues and organs," said Atala, who is now working to grow 20 different tissues and organs, including blood vessels and hearts, in the laboratory.



In 1999, his team (Boston) reported the successful creation and transplantation of artificial urinary bladder in beagle dogs that showed excellent functional capabilities in terms of retaining urine and normal elastic properties

In 2006, his team (Wake forest) reported succesful transplantation of engineered bladder in humans without any major complications.

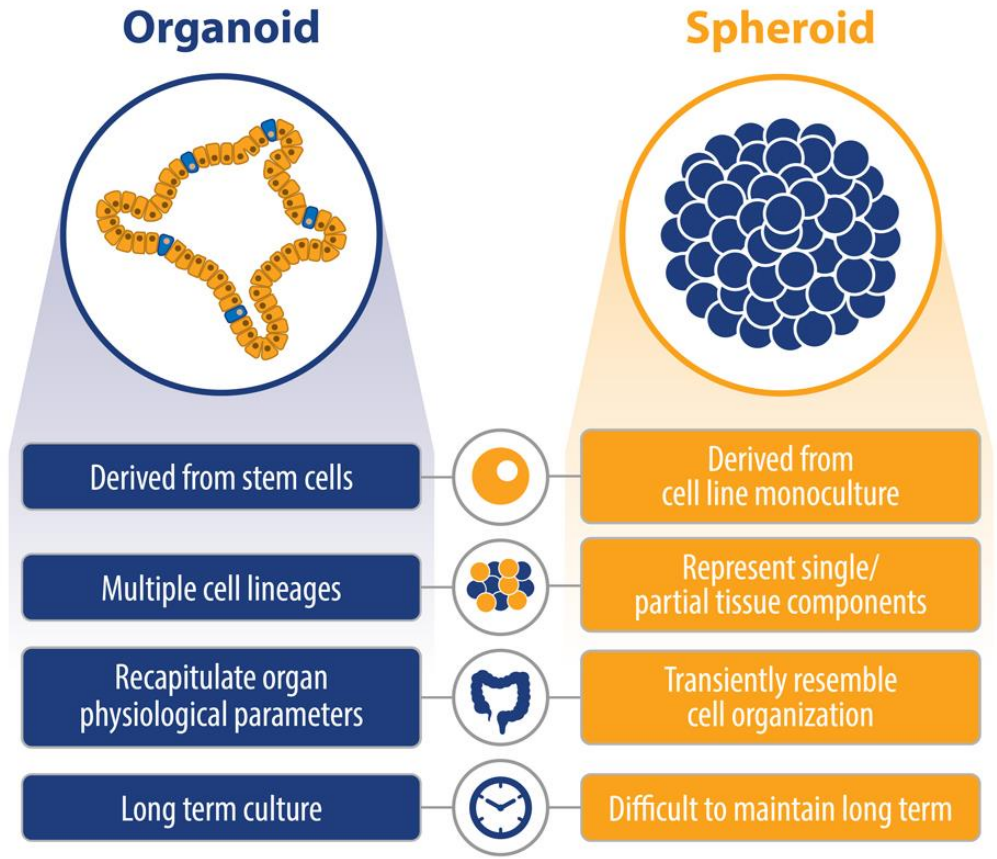


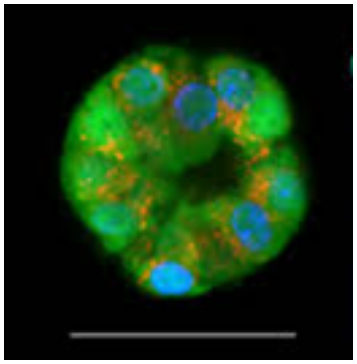
[(1) 3D bioprinting of organs - YouTube]

Organoid vs Spheroid 3D Culture

CrownBio
CONNECTING SCIENCE TO PATIENTS

Lung organoid

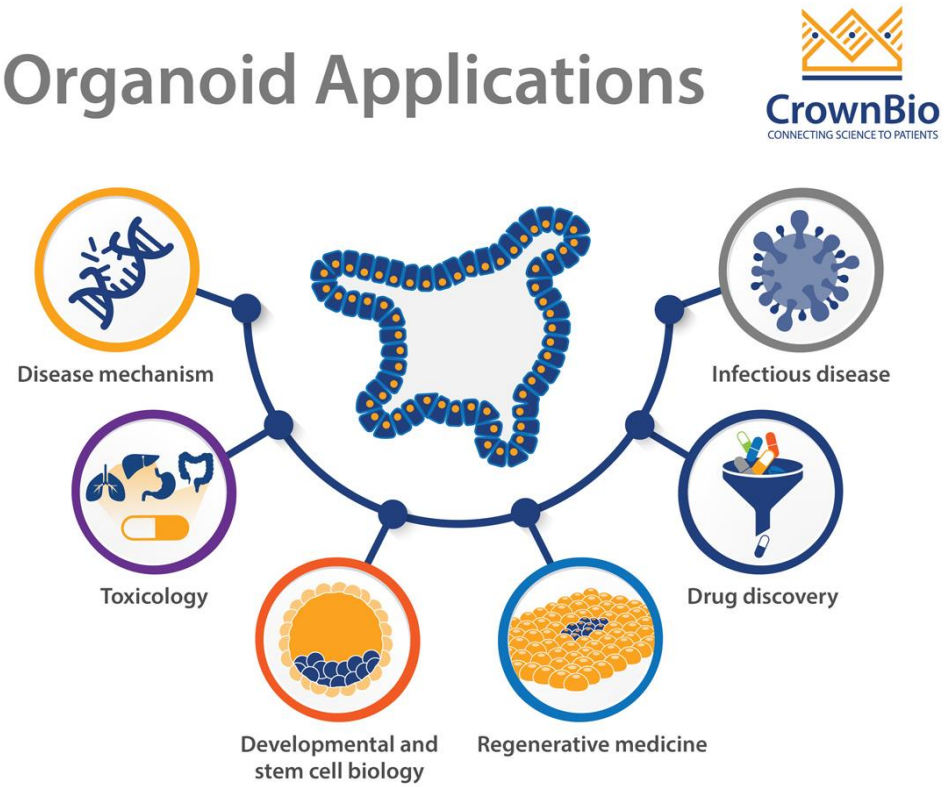




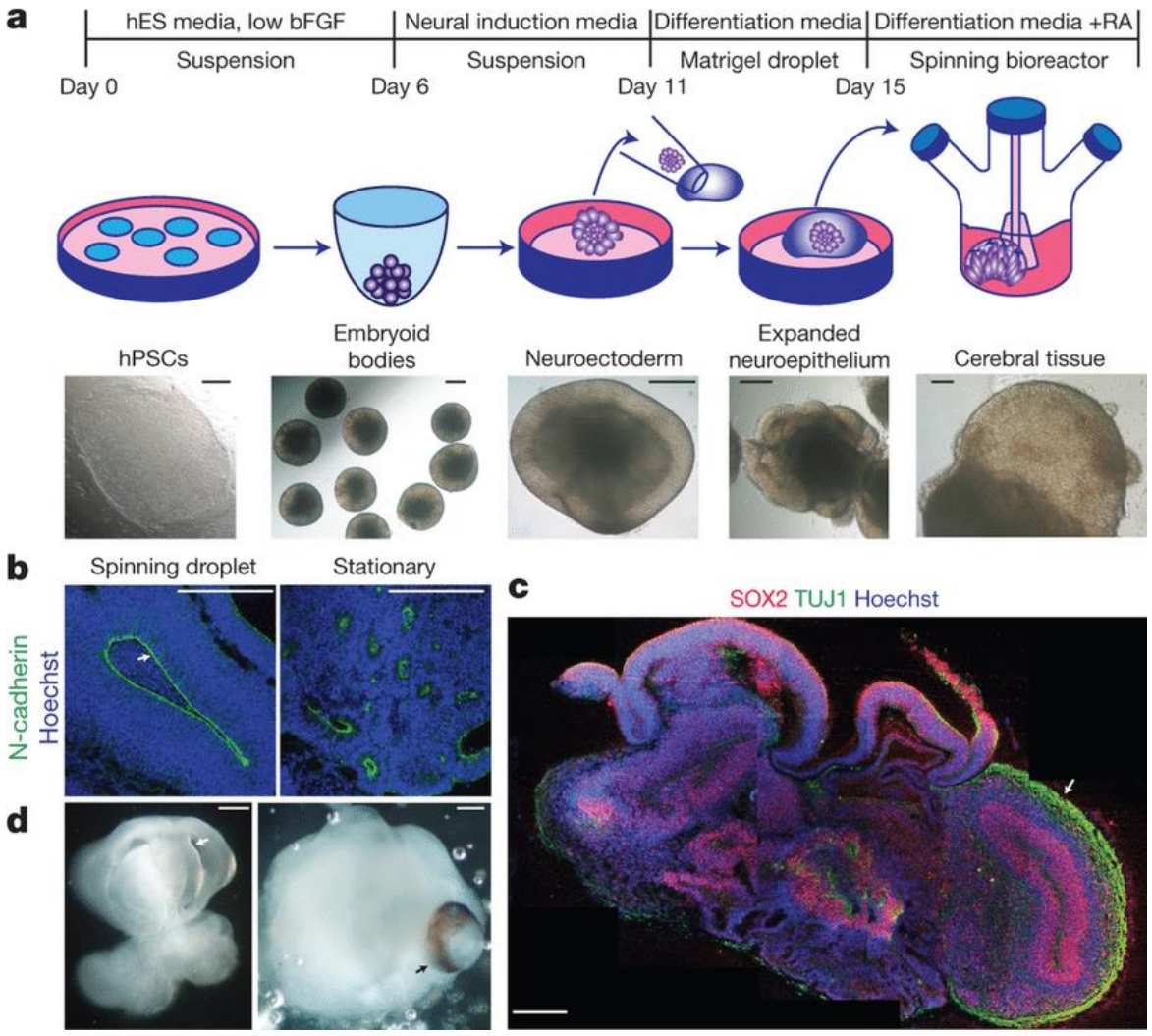
Lung speroid

Les organoides: exploiter les propriétés d'auto-organisation des cellules

- https://www.youtube.com/watch?v=2SG5ivm6jkw

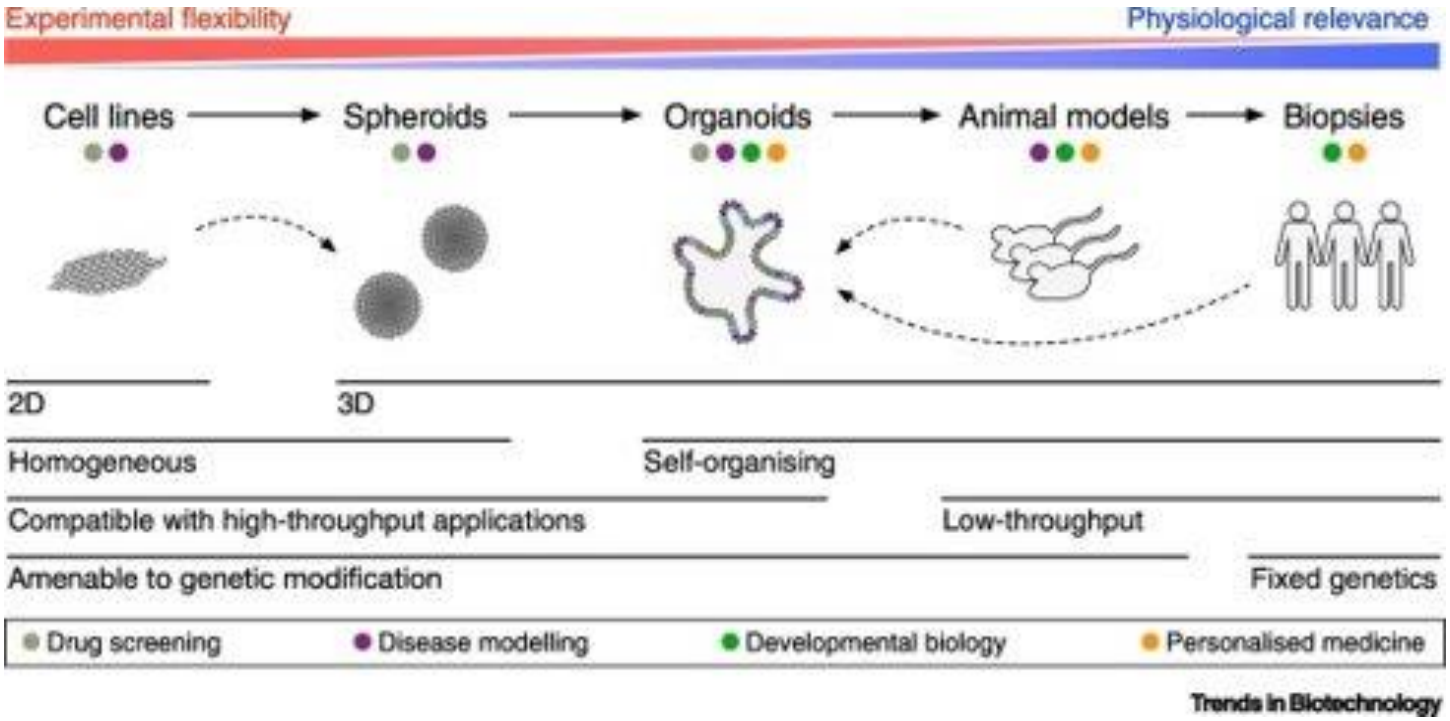


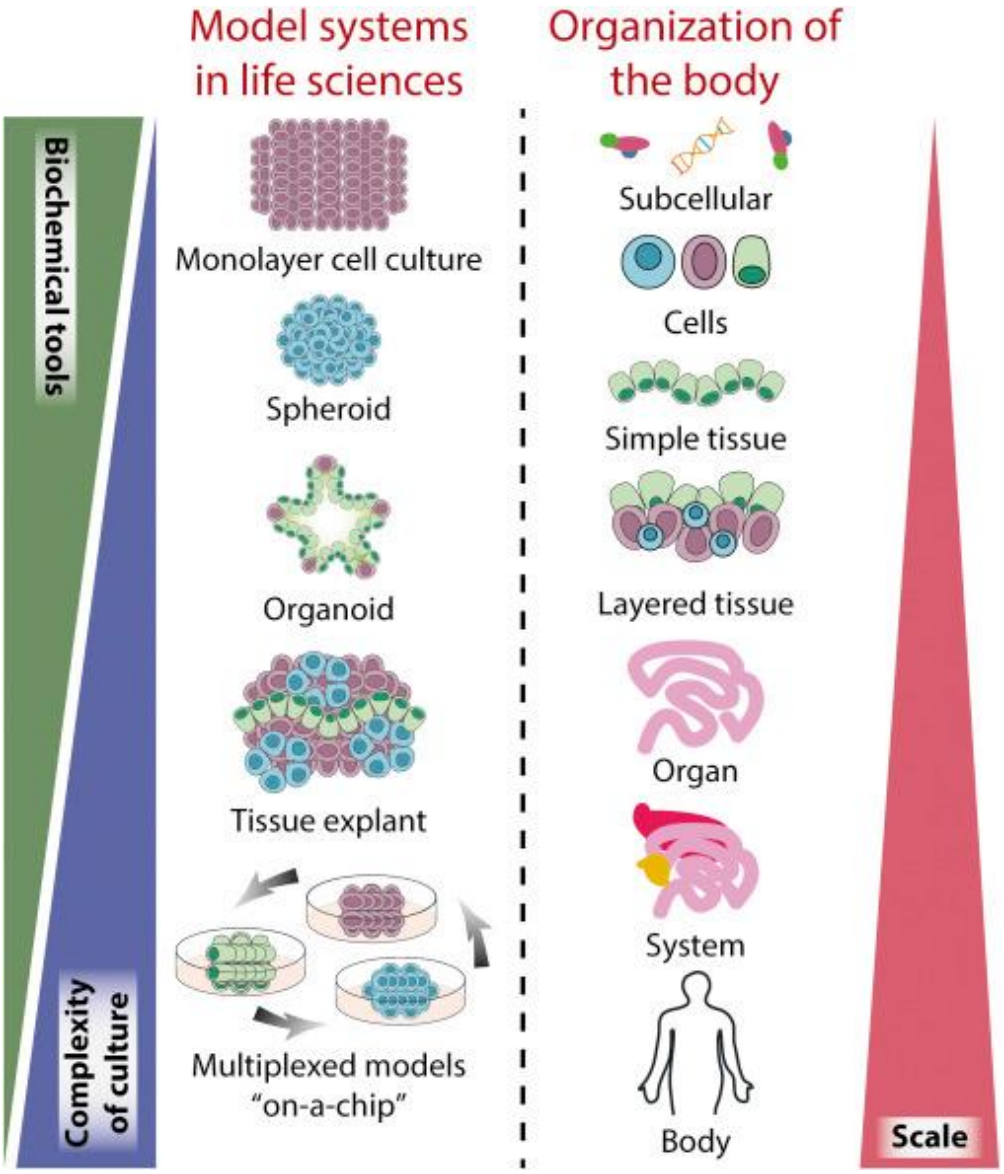
An **organoid** is a miniaturized and simplified version of an organ produced in vitro in three dimensions that shows realistic micro-anatomy. They are derived from one or a few cells from a tissue, embryonic stem cells or induced pluripotent stem cells, which can self-organize in three-dimensional culture owing to their self-renewal and differentiation capacities. The technique for growing organoids has rapidly improved since the early 2010s, and it was named by *The Scientist* as one of the biggest scientific advancements of 2013.



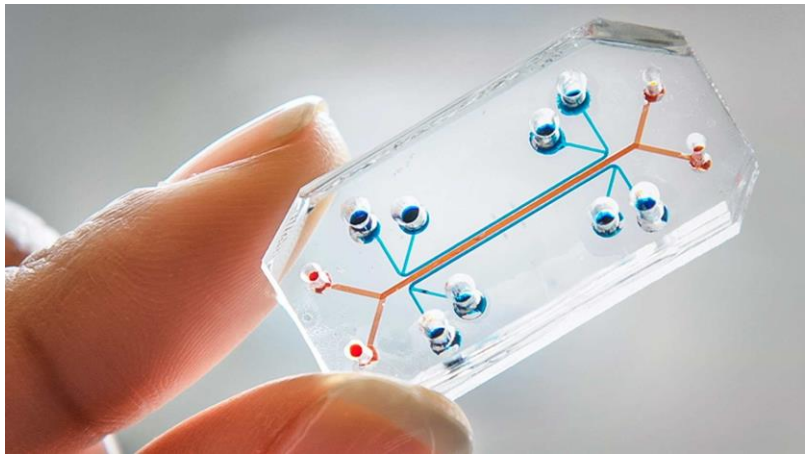
Lancaster, M et al. Nature, 2013

Modèles cellulaires et animaux

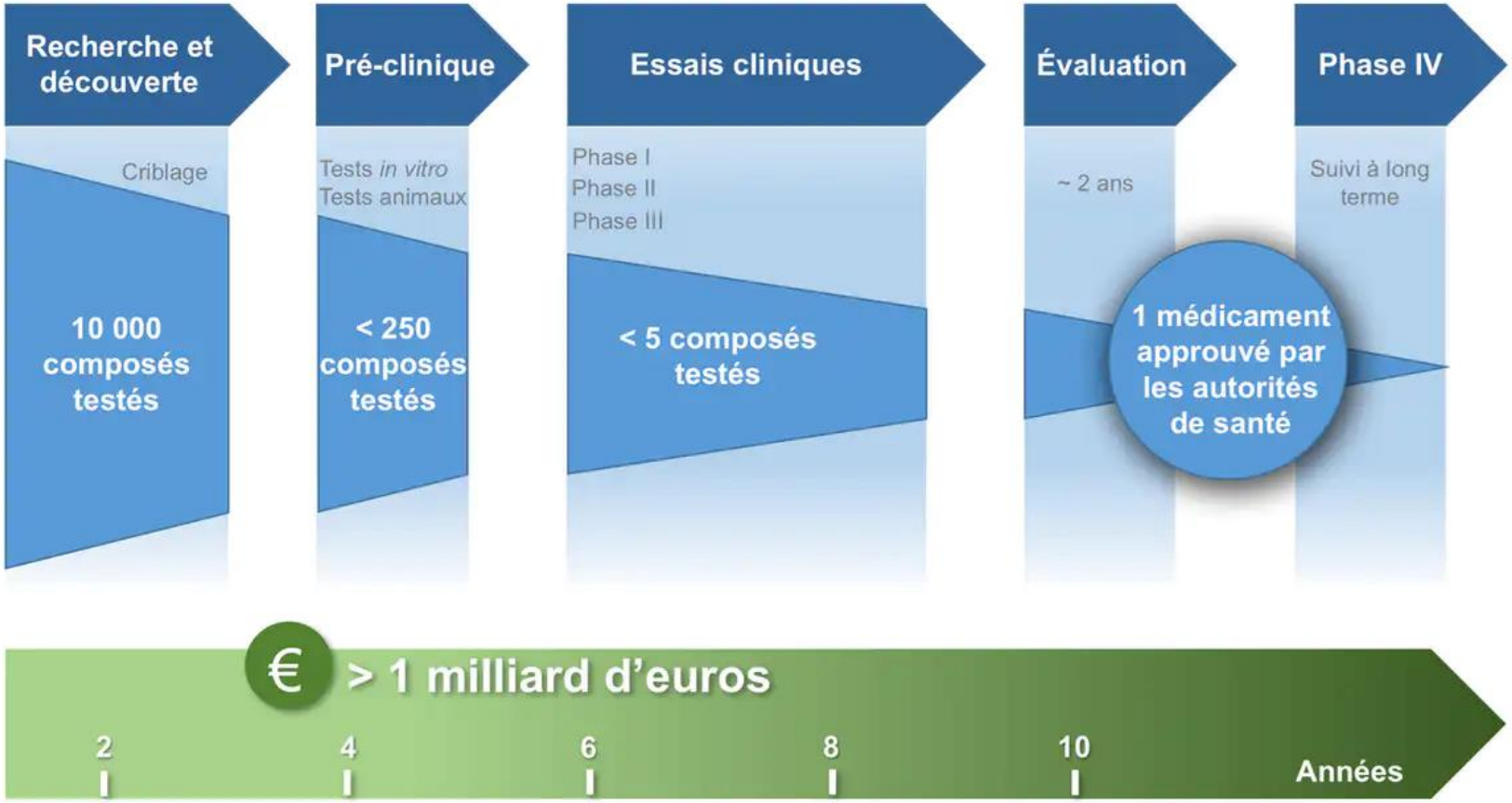



Model systems in life sciences
Organization of the body
Biochemical tools
Complexity of culture
Monolayer cell culture
Spheroid
Organoid
Tissue explant
Multiplexed models "on-a-chip"
Subcellular
Cells
Simple tissue
Layered tissue
Organ
System
Body
Scale

Les organes sur puces

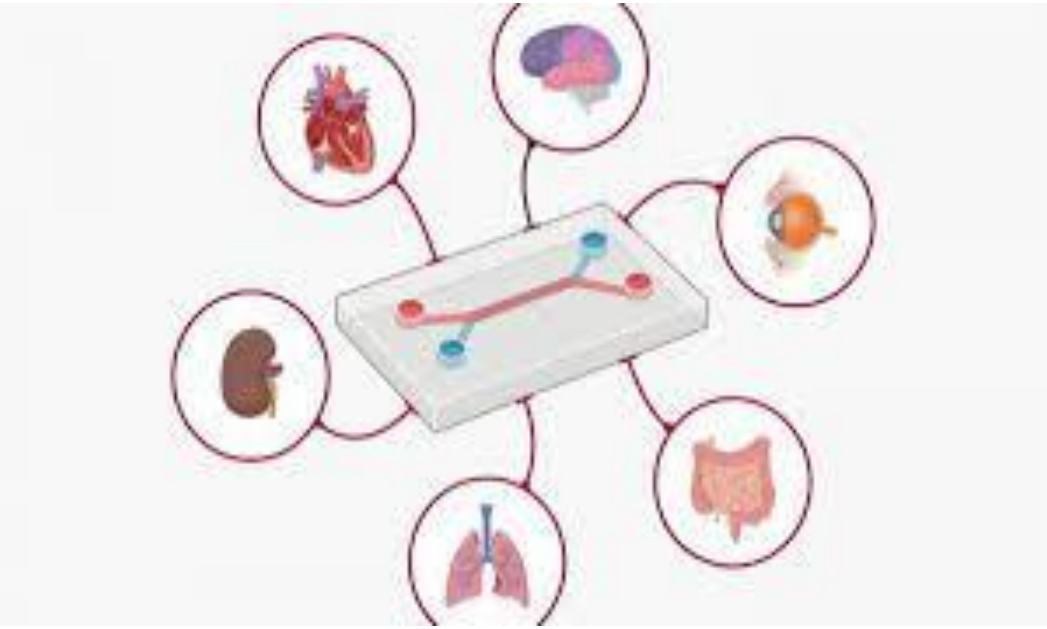


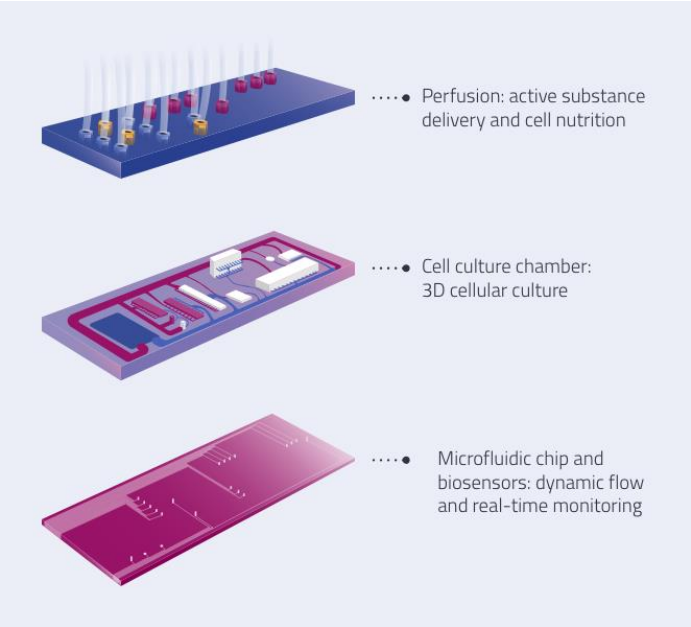
Nous sommes actuellement confrontés à un défi mondial pour la santé : la façon dont nous découvrons et développons de nouveaux médicaments est trop coûteuse et trop longue



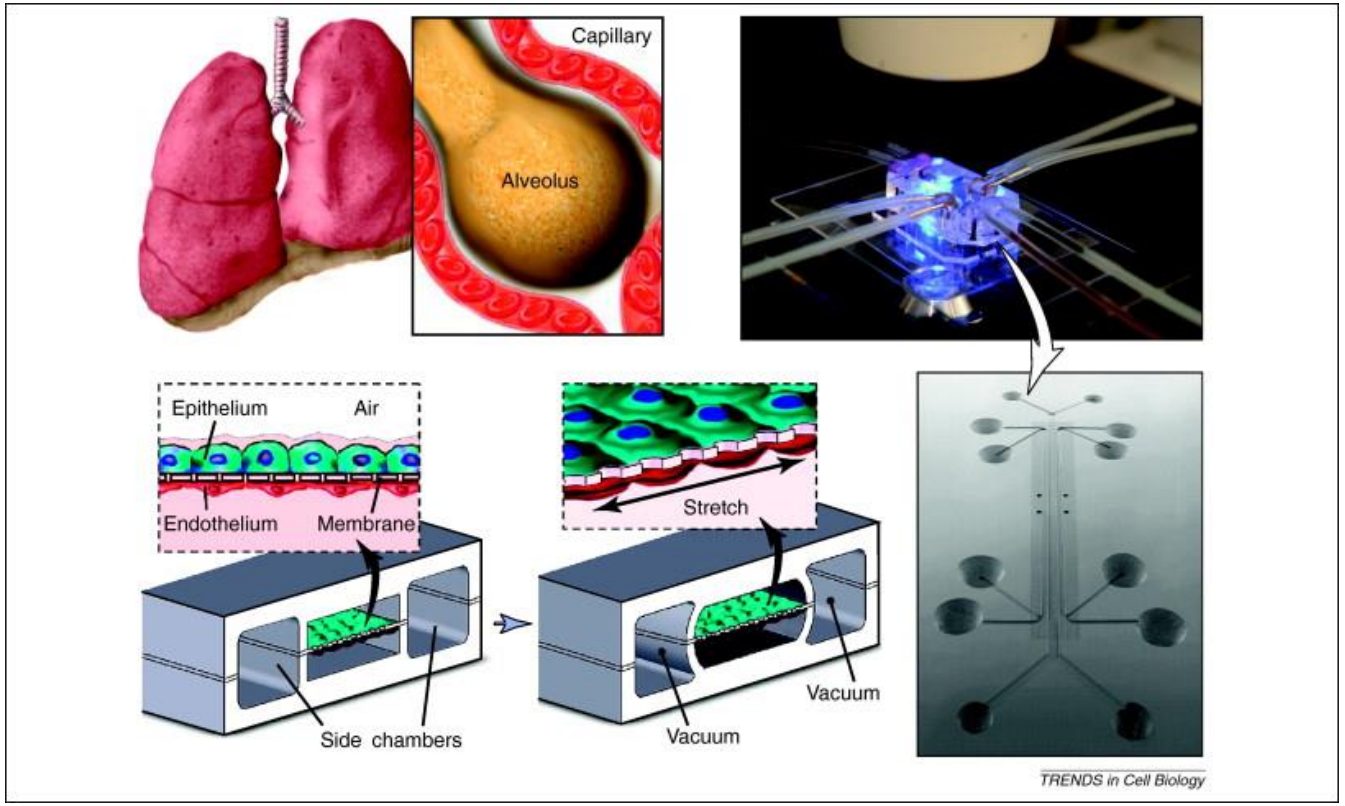
. Les organes sur puce pourraient permettre de répondre à ces difficultés et pourraient à l'avenir ouvrir la possibilité de faire des tests personnalisés pour chaque individu.

Les avantages des puces: mieux mimer les conditions physiologiques





Poumon sur puce



A human breathing lung-on-a-chip. A microengineered model of the alveolar–capillary interface within a clear flexible microfluidic chip approximately the size of a computer memory stick (top right visualized on the microscope under fluorescence illumination; bottom right shows scanning electron micrographic view) that reconstitutes the cellular, biochemical and mechanical functions of the living human lung. The crucial tissue–tissue interface of the alveolus (top left) is replicated in this bioinspired microdevice by co-culturing human alveolar epithelial cells and pulmonary capillary endothelial cells on the opposite sides of a thin, flexible, porous, ECM-coated PDMS membrane (bottom left). To accomplish mechanical actuation that mimics physiological breathing movements, air pressure in two hollow side chambers microfabricated within the device is decreased and increased in a cyclic manner by using a small vacuum pump; this causes the membrane and attached human cell layers to cyclically stretch and relax under physiological mechanical strain. The lung epithelial cells are cultured at an air–liquid interface, and culture medium is pumped through the lower microchannel containing the capillary cell layer to mimic blood flow through lung microvasculature. This system effectively mimics the entire human inflammatory response when pathogens or inflammatory cytokines are placed in the air channel and human neutrophils are introduced into the capillary channel; the device also can be used to study absorption and toxic effects of airborne particles, chemicals or drugs.

Our Work
- Enabling Technology Platforms
- Emerging Science

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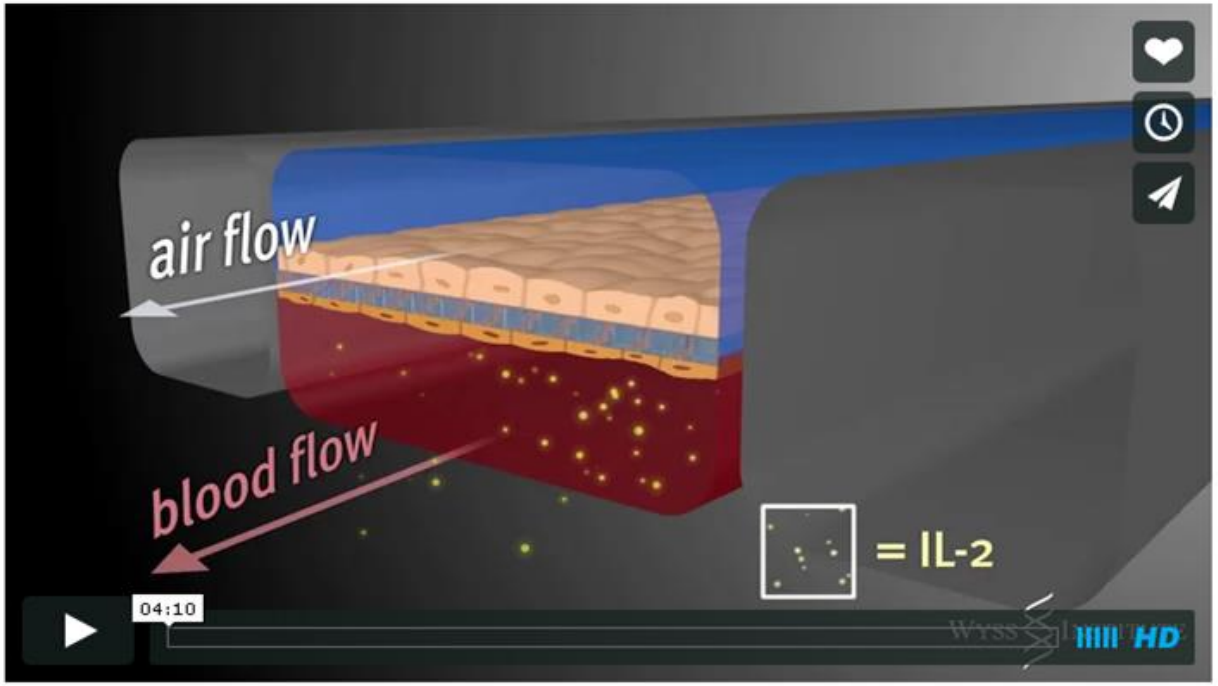
Lung-on-a-chip

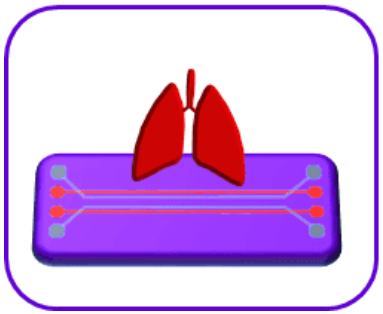
Combining microfabrication techniques with modern tissue engineering, lung-on-a-chip offers a new in vitro approach to drug screening by mimicking the complicated mechanical and biochemical behaviors of a human lung. This version of the video (updated January 29, 2013) includes our findings when we mimicked pulmonary edema on the chip. Watch this video to see how it works.



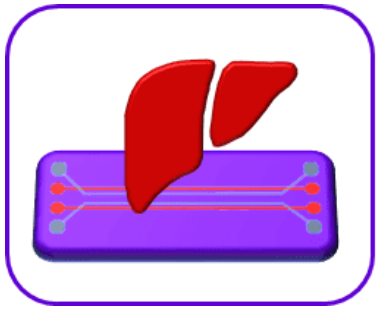
Related video: Organs-on-a-Chip

Extended interview: The Discovery Channel's *Curiosity Project* discusses organs-on-a-chip with Wyss

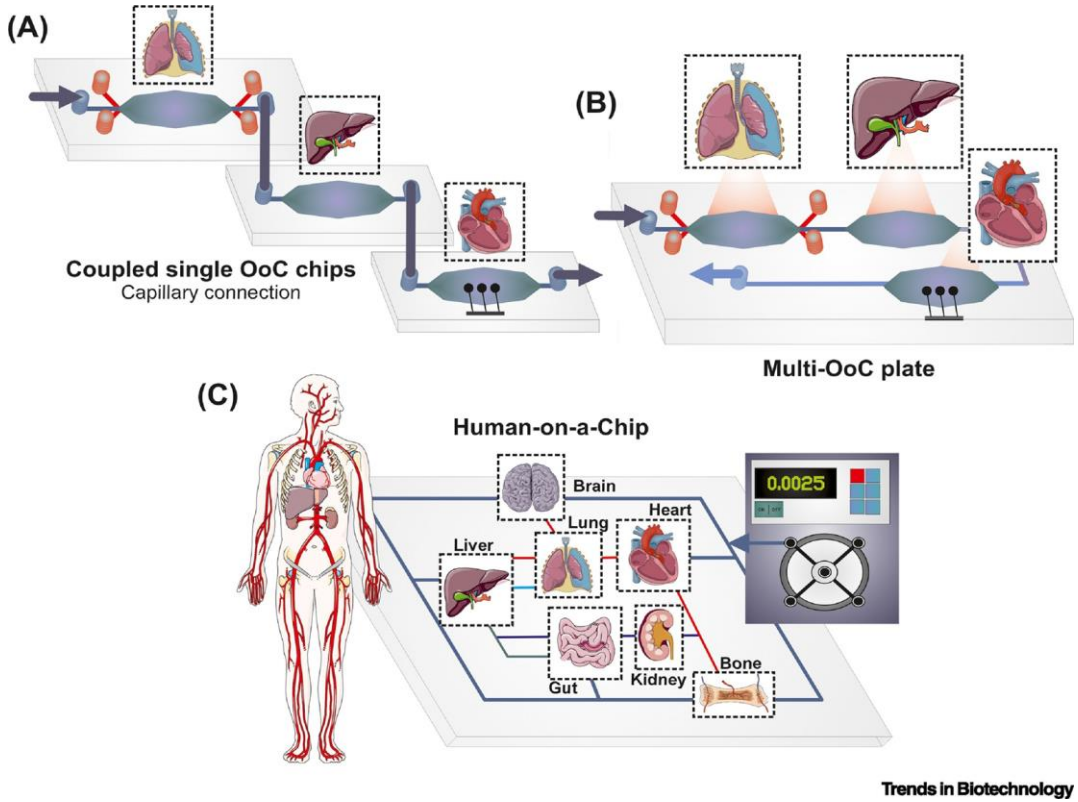
From organ to multi organs on chip

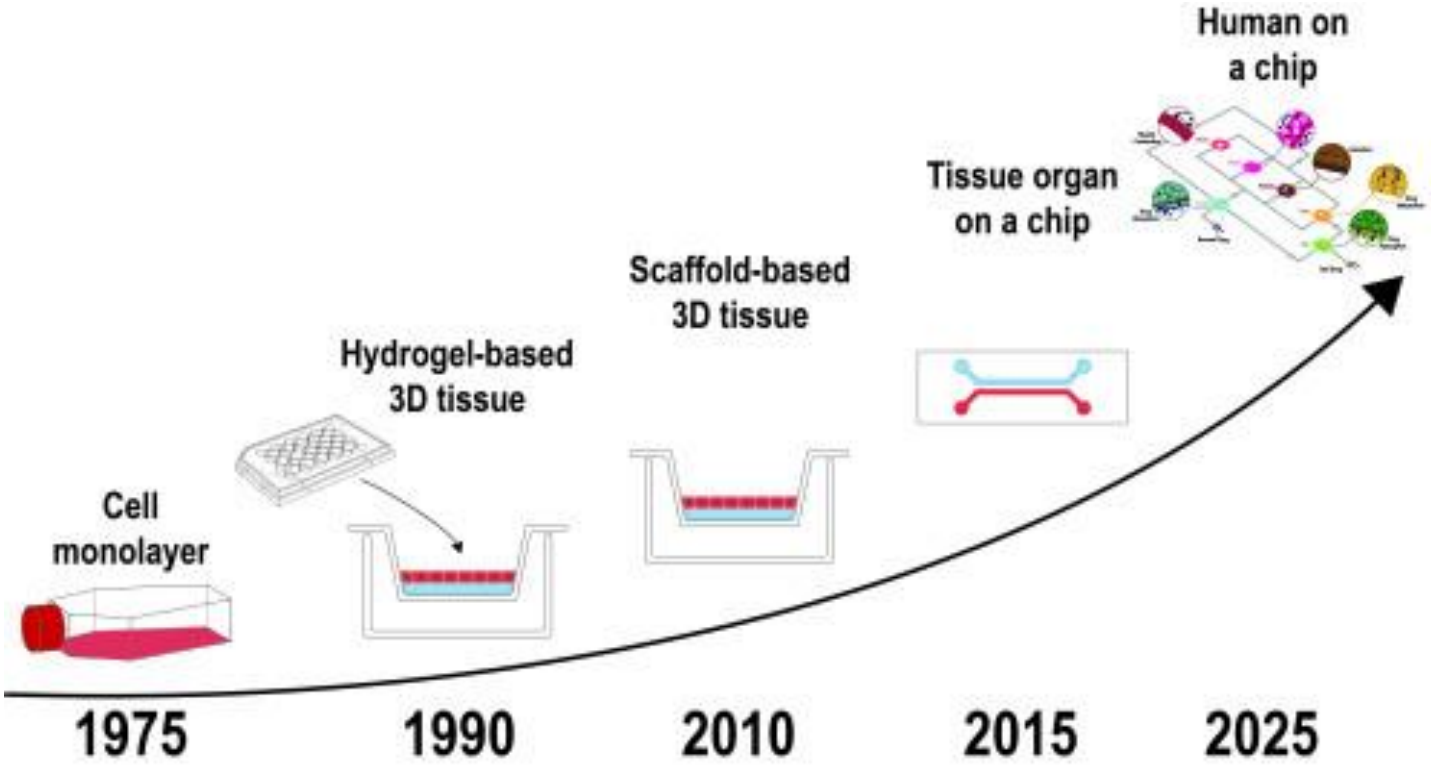


First Lung-on-Chip
Huh et al., 2010



Liver-on-Chip
Liver Acinus with oxygen zonation
Li et al., 2018





The human-on-a-chip concept. Biomimetic microsystems representing different organs can be *integrated into a single microdevice and linked by a microfluidic circulatory system in a physiologically relevant manner to model a complex, dynamic process of drug absorption, distribution, metabolism and excretion, and to more reliably evaluate drug efficacy and toxicity. As shown in this example, an integrated system of microengineered organ mimics (lung, heart, gut, liver, kidney and bone) can be used to study the absorption of inhaled aerosol drugs (red) from the lung to microcirculation, as well as to measure their cardiotoxicity (e.g. changes in heart contractility or conduction), transport and clearance in the kidney, metabolism in the liver, and immune-cell* contributions to these responses. Drug substances (blue) also can be introduced into the gut compartment to investigate interplay between orally administered drugs and molecular transporters and metabolizing enzymes expressed in the various organs.

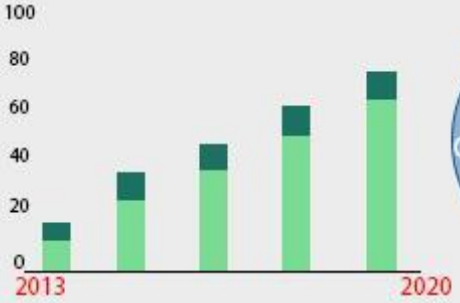
Global Regenerative Medicines Market

Size & Forcast, (2013-2020)

For More Details See Table Of Contents

Global Regenerative Medicines Market

Expected to Reach $67.5 Billion by 2020



Growing At a CAGR of 23.2% (2014-2020)

Global Regenerative Medicines Market by Technology

Gene Therapy

Small molecules and Biologics

Cell Therapy

Global Regenerative Medicines Market by Geography

Asia Pacific, Europe, LAMEA

North America
Highest Revenue Generating Region
$33.5 Billion
By 2020

Global Regenerative Medicines Market by Application

Dermatology

Cardiovascular

CNS

Dental

Others - Autoimmune disorders
Muscle regeneration
Ocular diseases

Orthopedic

Global Regenerative Medicines Market Dynamics

Drivers

- Advancements in stem cell technology
- Potential of nanotechnology
- Increasing incidences of chronic Diseases and trauma injuries

Restraints

- Stringent regulatory policies and ethical issues
- Expensive treatment