Il corso- Tecnologie per la Medicina Rigenerativa, 30 CFU 2019-2020

The course book: **Fondamenti di ingegneria dei tessuti per la medicina rigenerativa.** Author/s Mantero S, Remuzzi A, M.T. Raimondi, Ahluwalia A ISBN Code978-88-55-3039-2 Publisher :Patron: Number of pages212

- http://www.centropiaggio.unipi.it/~ahluwalia

(c’è un link al corso)

Portale biomedica

- www.biomedica.ing.unipi.it

Come si svolge l’esame

Orale

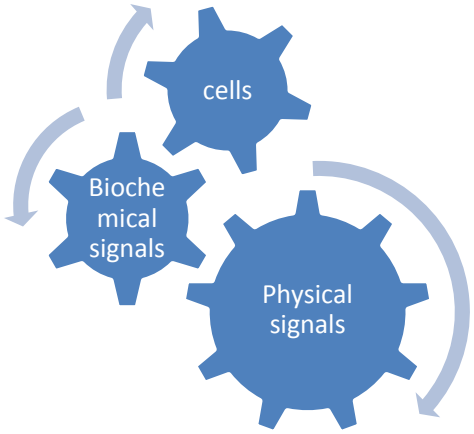
What is the course about?

Quantitive aspects of

- Tissues
- Development
- Nutrients in tissues

Why?

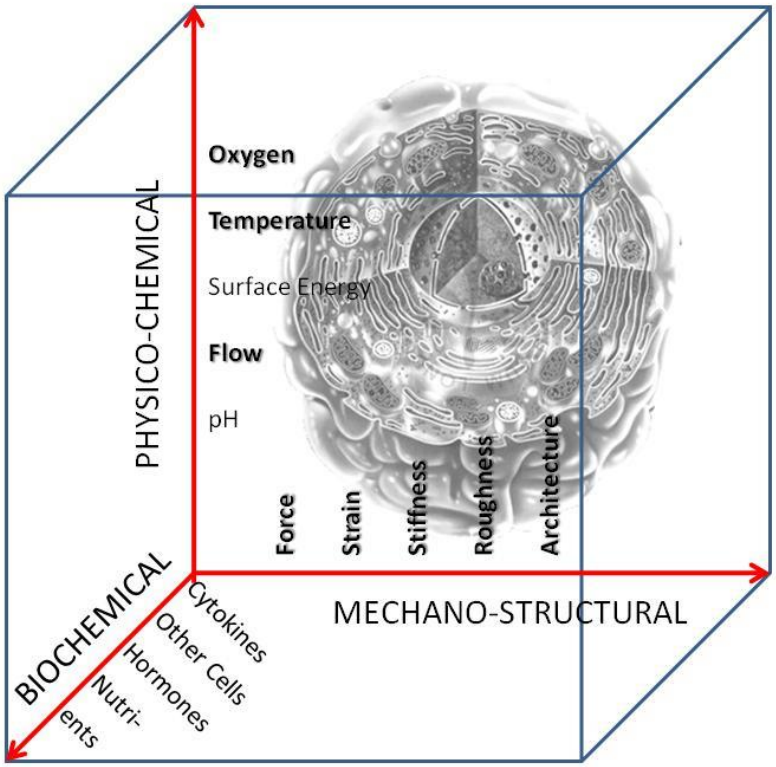
- Design downscaled biomimetic in-vitro systems for different applications
- Cell based engineering is the bioengineering of the future. ⟶ Biological engineering



Development: The cues of life

Stimuli

- Biochemical
- Physico-chemical
- Mechano-structural



Note even time has a role- thus a *dynamic* environment, is fundamental in all biological processes.

21 century tissue engineering (regenerative medicine)

Allopathy:a system of medical practice that aims to combat disease by use of remedies (as drugs or surgery) producing effects different from or incompatible with those produced by the disease being treated

New Regenerative medicine uses ATMP (advanced therapy medicinal products)

An ATMP is a medicinal product which is either:

- a gene therapy medicinal product
- a somatic cell therapy medicinal product (allogenic, autologus, or xenogenic)
- a tissue engineered product

They all involve a degree of manipulation in-vitro

Why do we need it?

(Lack of donor organs used to be the reason)

Allopathy cannot “cure” 21° century diseases

like :

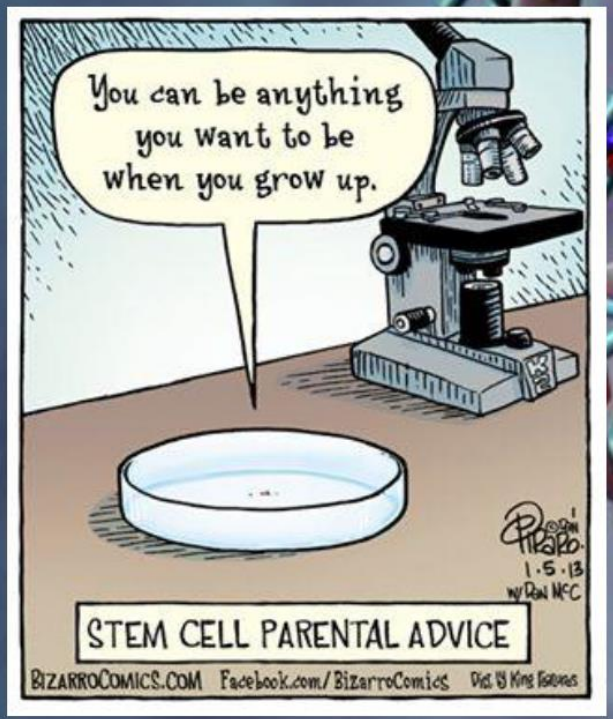
- Ageing & degeneration
- Auto immune diseases
- Cancer
- Obesity
- Or genetic disorders

(what do they have in common?, what diseases can be cured with allopathy?)

ATMP

The ~~Beauty~~ and the ~~Beast~~

Genes Cells



ATMPs:

- Gene therapy medicinal products
- Somatic cell therapy medicinal products
- Tissue engineered products

Credit: Christoph Bock/Max Planck Institute for Informatics

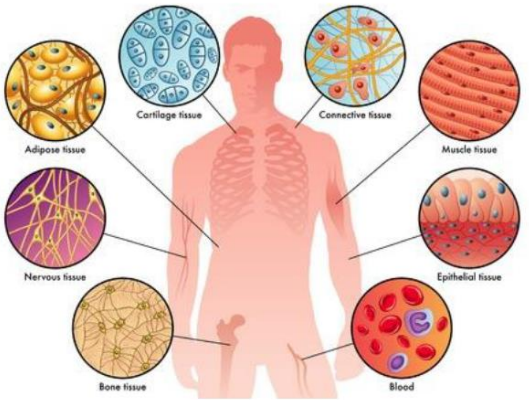
Pinterest.com

DA: http://www.ema.europa.eu/docs/en_GB/document_library/Presentation/2018/06/WC500250782.pdf

GTMP

EUROPEAN MEDICINES AGENCY

Gene therapy medicinal products



Pinterest.com

DNA/RNA

- Treatment of inherited disease
  - Glybera
  - Strimvelis
- Cancer therapies
  - Imlygic
- Tissue regeneration (e.g. loss of sight)
  - Zalmoxis

sCTMP

EUROPEAN MEDICINES AGENCY

Somatic cell therapy medicinal product – tissue engineered product

Alofisel

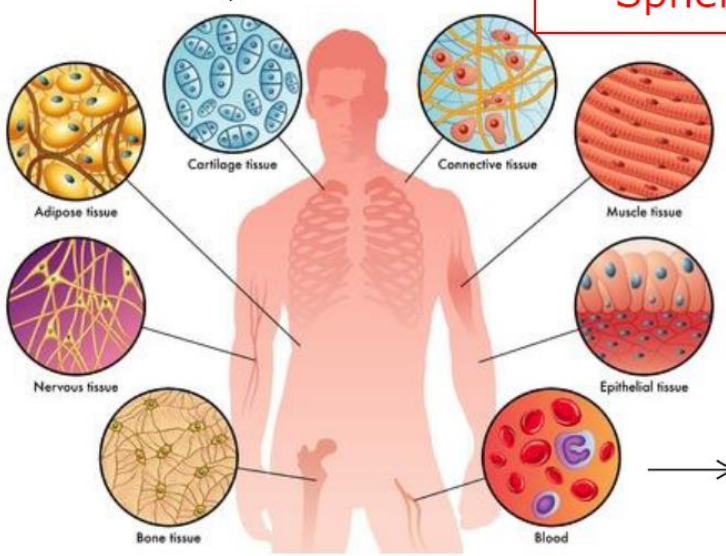
Products against immune diseases, …

Treatment of Parkinson's disease, Alzheimers, ALS

Holoclar

Treatment of cartilage defects

Chondrocelect
MACI
Spherox



Pinterest.com

Product for cardiac repair

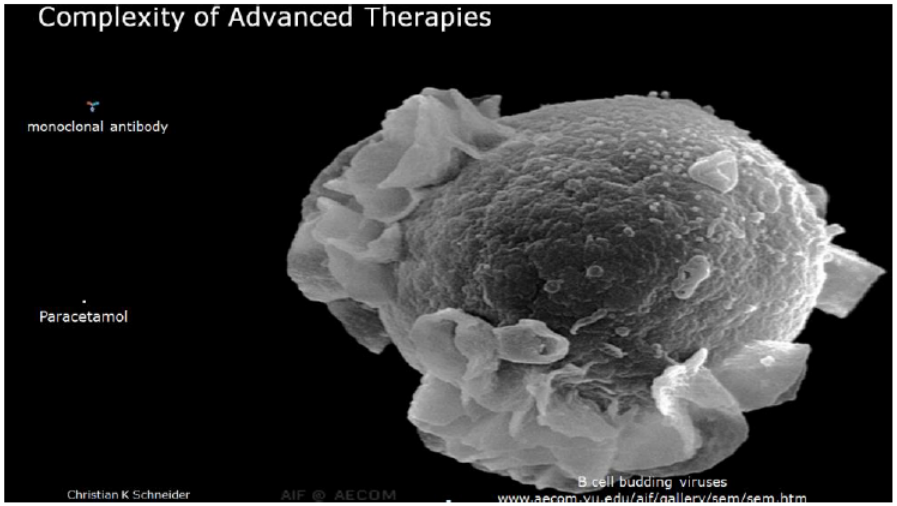
Skin replacement

Cancer Immunotherapy

Provenge

ATMPs are …

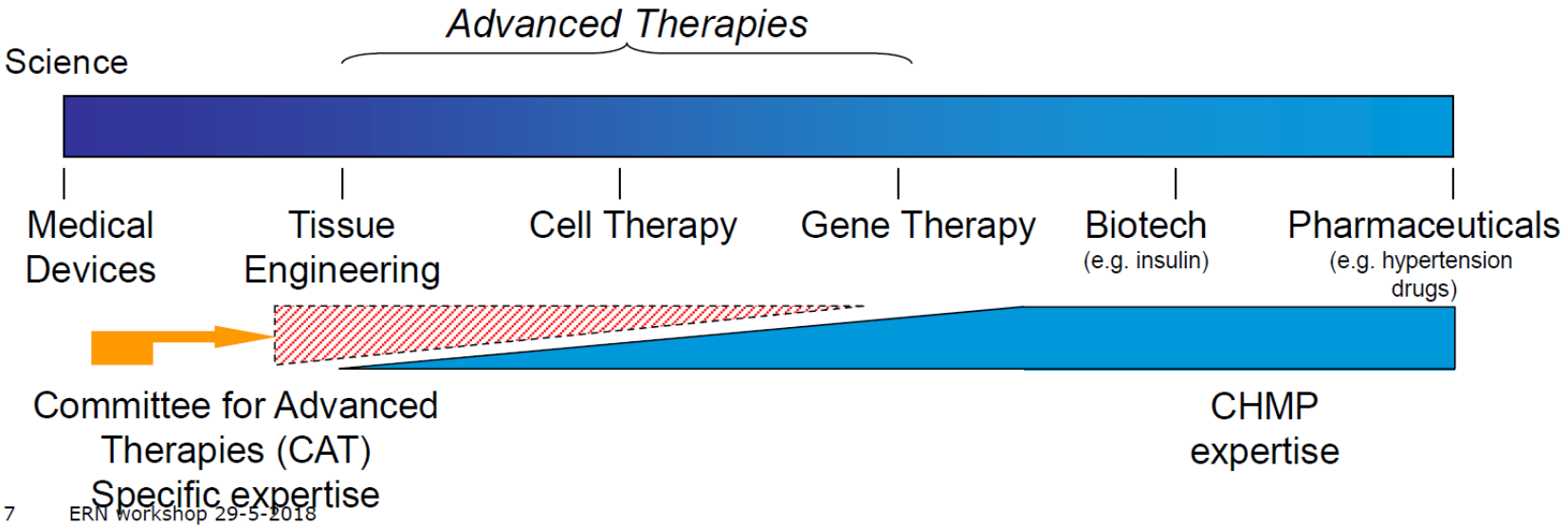
- Medicinal products based on cells or genes
- Very different from medicines based on chemical entities or biological / biotechnological origin



- But same requirement for testing / controlling each batch
  - Impact on cost of manufacture of the ATMPs
  - Very small batch size (autologous CBMP: batch size = 1)

Legislation

Science



ATMPs in Europe (May 2018)

EUROPEAN MEDICINES AGENCY

over 500 clinical trials using ATMPs in EU

298 ATMP classifications

293 scientific advice requests

20 MAAs reviewed / Under review

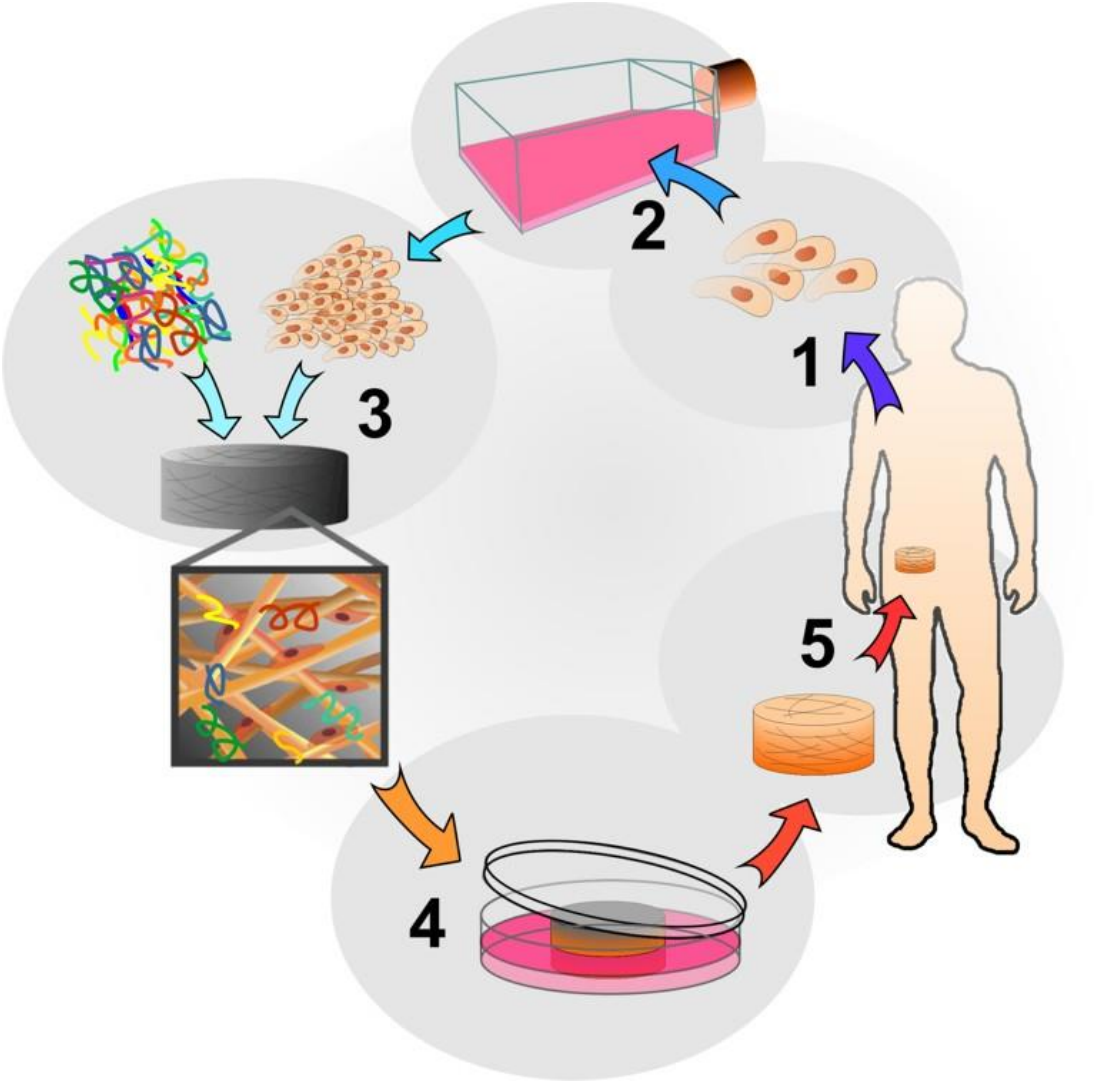
10 ATMPs approved

3 withdrawn
1 Suspended

Market

6 licensed ATMPs

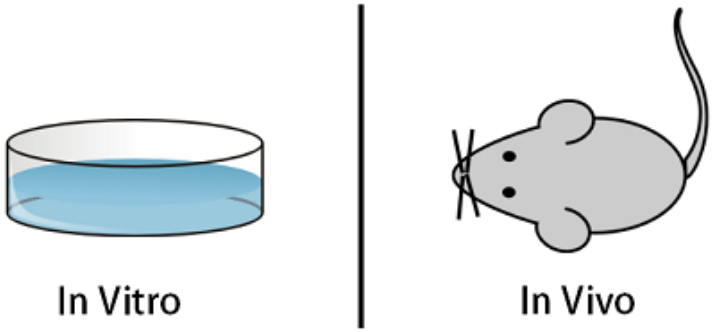
What is Tissue engineering?



The old cells on a scaffold approach

What is an *in vitro* model?

In-vitro models are replicates of the structure and function of biological tissues which allow the modelling and predicting physiological responses to a variety of stimuli.



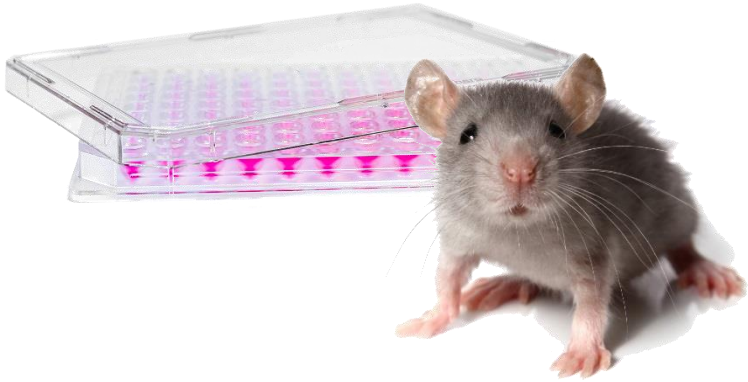
in vitro vs *in vivo*

- in vivo experiments are more labour-intensive, expensive and time-consuming than in vitro studies.

- there are ethical reasons for limiting the number of test animals to a necessary minimum. The Reduce, Refine and Replace animal experiments (3R) initiative

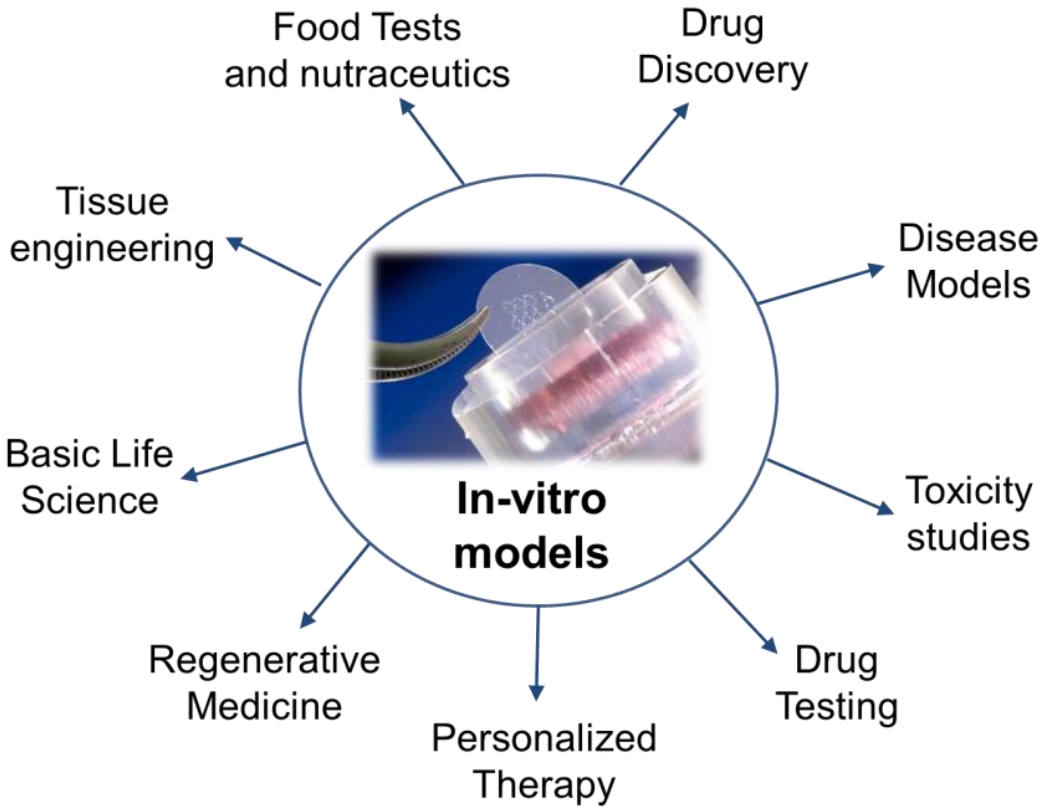
- human tissue models are thought to have higher predictive power than animal models, because different species respond differently to treatments or compounds

- many of the currently used in vitro models lack predictive power, in most cases due to the lack of critical molecular and physical cues in the cell/tissue environment.



in silico

In-vitro models: Applications



DIRECTIVE 2010/63/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL
of 22 September 2010
on the protection of animals used for scientific purposes

Article 1

Subject matter and scope

1. This Directive establishes measures for the protection of animals used for scientific or educational purposes.

To that end, it lays down rules on the following:

a) the **replacement** and **reduction** of the use of animals in procedures and the **refinement** of the breeding, accommodation, care and use of animals in procedures;
b) the origin, breeding, marking, care and accommodation and killing of animals;
c) the evaluation and authorisation of projects involving the use of animals in procedures;
d) the evaluation and authorisation of projects involving the use of animals in procedures

DECRETO LEGISLATIVO 4 marzo 2014, n. 26

Attuazione della direttiva 2010/63/UE sulla protezione degli animali utilizzati a fini scientifici. (14G00036)

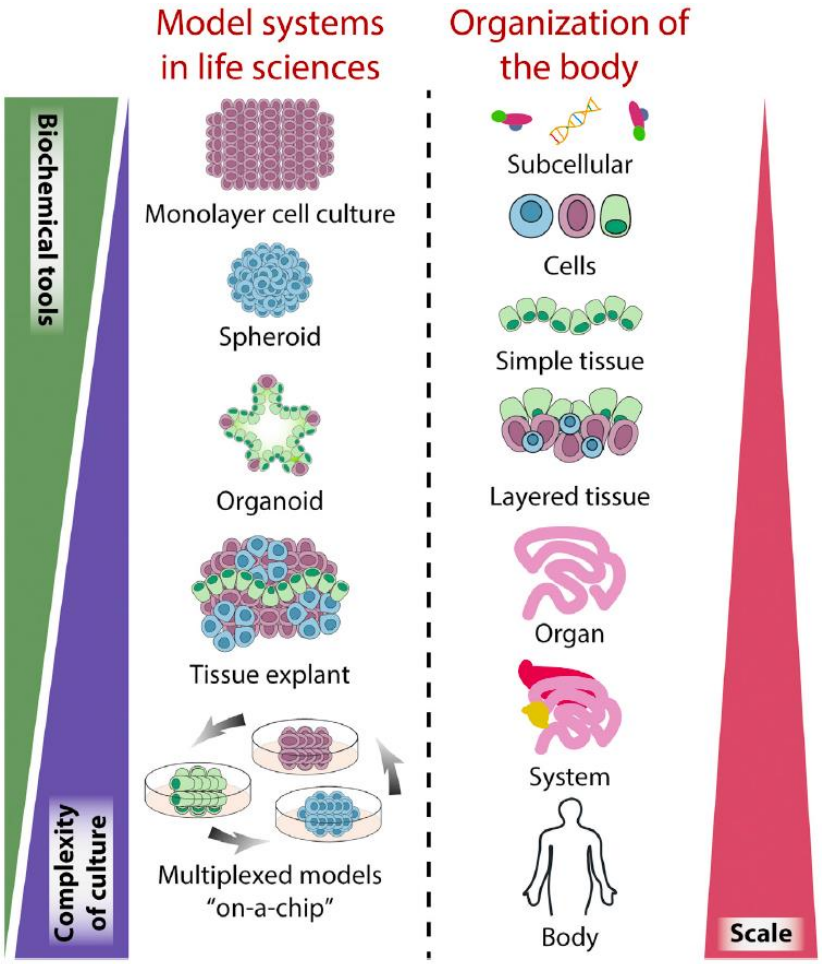
(GU n.61 del 14-3-2014)

The 3Rs (Russel & Burch, 1959)

Centro 3R

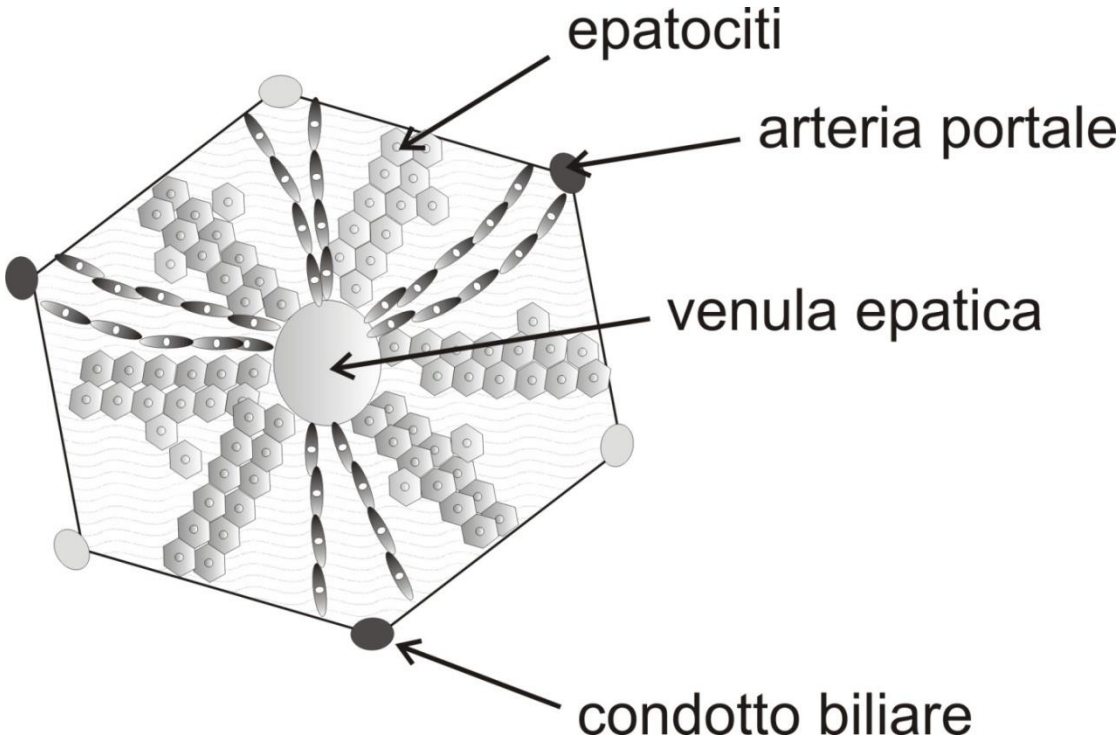
Centro Interuniversitario per la Promozione dei Principi delle 3R nella Didattica e nella Ricerca

Biomimetic tissue models



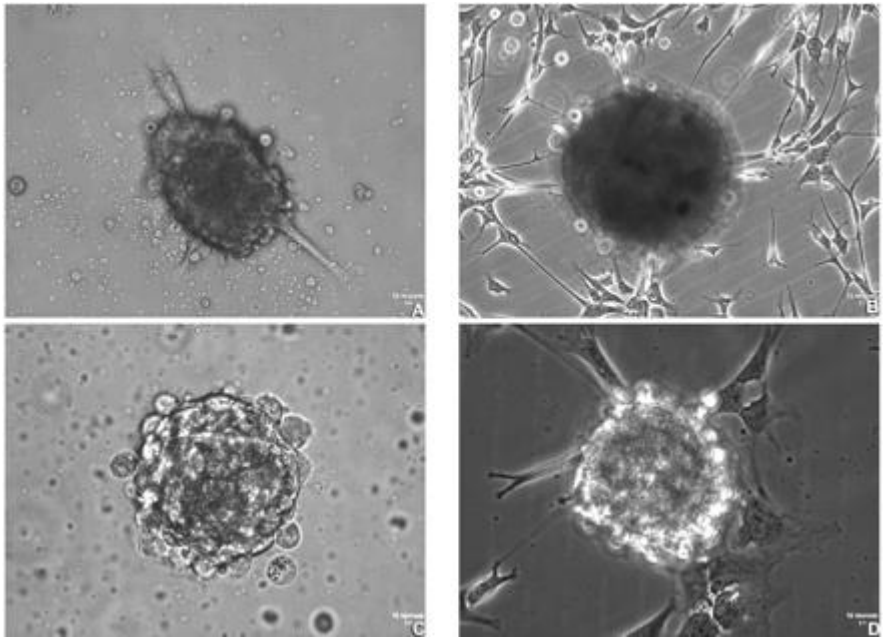
Yin et al Cell, Stem Cell . 18 Jan 2016

Functional unit: collection of functional (parenchymal) and support (stromal or non-parenchymal) cells which do not require a capillary network. Is equivalent to a cube of 400 micron sides. In vitro these units are usually referred to as ORGANOIDS



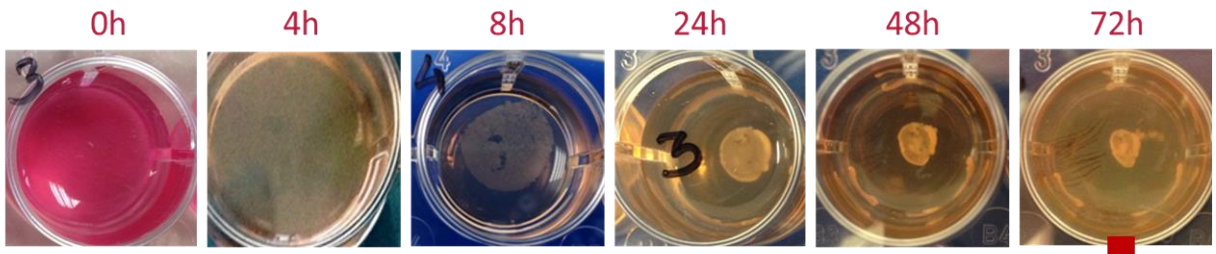
Functional unit

- Each organ is a network of the parallel functional units, composed of groups of functional cells or parenchymal supported by stromal cells, each unit has dimensions of a few hundreds of microns, and responds with characteristic times in the order of minutes. The micro-functional domains are repeated both in morphology and function.



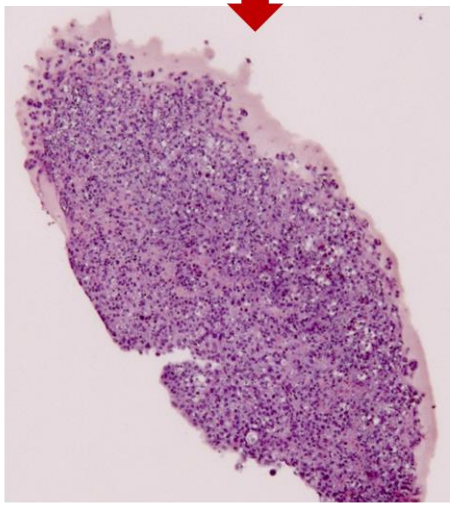
Cardiospheres and organoids are a good example

Hepatic organoids



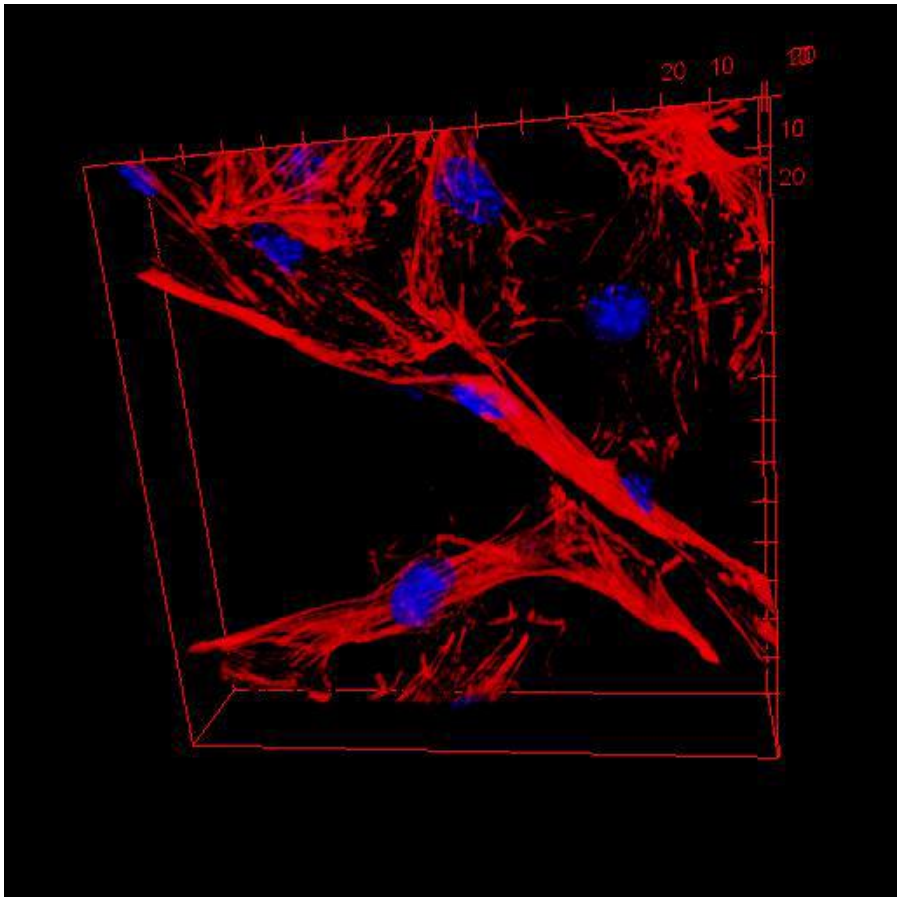
Combination of:
differentiated, human upcyte® hepatocytes
+ upcyte® LSECs
+ upcyte® MSCs

HE-stain of a „first try“ liver bud (72h)



Numbers

- The typical cell– diameter 10-20 μm



How many?
How many in an organ?
How many are therapeutic?

Annals of Human Biology
Journal of the Society for the Study of Human Biology

http://informahealthcare.com/ahb
ISSN: 0301-4460 (print), 1464-5033 (electronic)

Ann Hum Biol, 2013; 40(6): 463–471
 DOI: 10.3109/03014460.2013.807878

informa
healthcare

RESEARCH PAPER

An estimation of the number of cells in the human body

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Abstract

Background: All living organisms are made of individual and identifiable cells, whose number, together with their size and type, ultimately defines the structure and functions of an organism. While the total cell number of lower organisms is often known, it has not yet been defined in higher organisms. In particular, the reported total cell number of a human being ranges between 10^{12} and 10^{16} and it is widely mentioned without a proper reference.
Aim: To study and discuss the theoretical issue of the total number of cells that compose the standard human adult organism.
Subjects and methods: A systematic calculation of the total cell number of the whole human body and of the single organs was carried out using bibliographical and/or mathematical approaches.
Results: A current estimation of human total cell number calculated for a variety of organs and cell types is presented. These partial data correspond to a total number of 3.72×10^{13}.
Conclusions: Knowing the total cell number of the human body as well as of individual organs is important from a cultural, biological, medical and comparative modelling point of view. The presented cell count could be a starting point for a common effort to complete the total calculation.

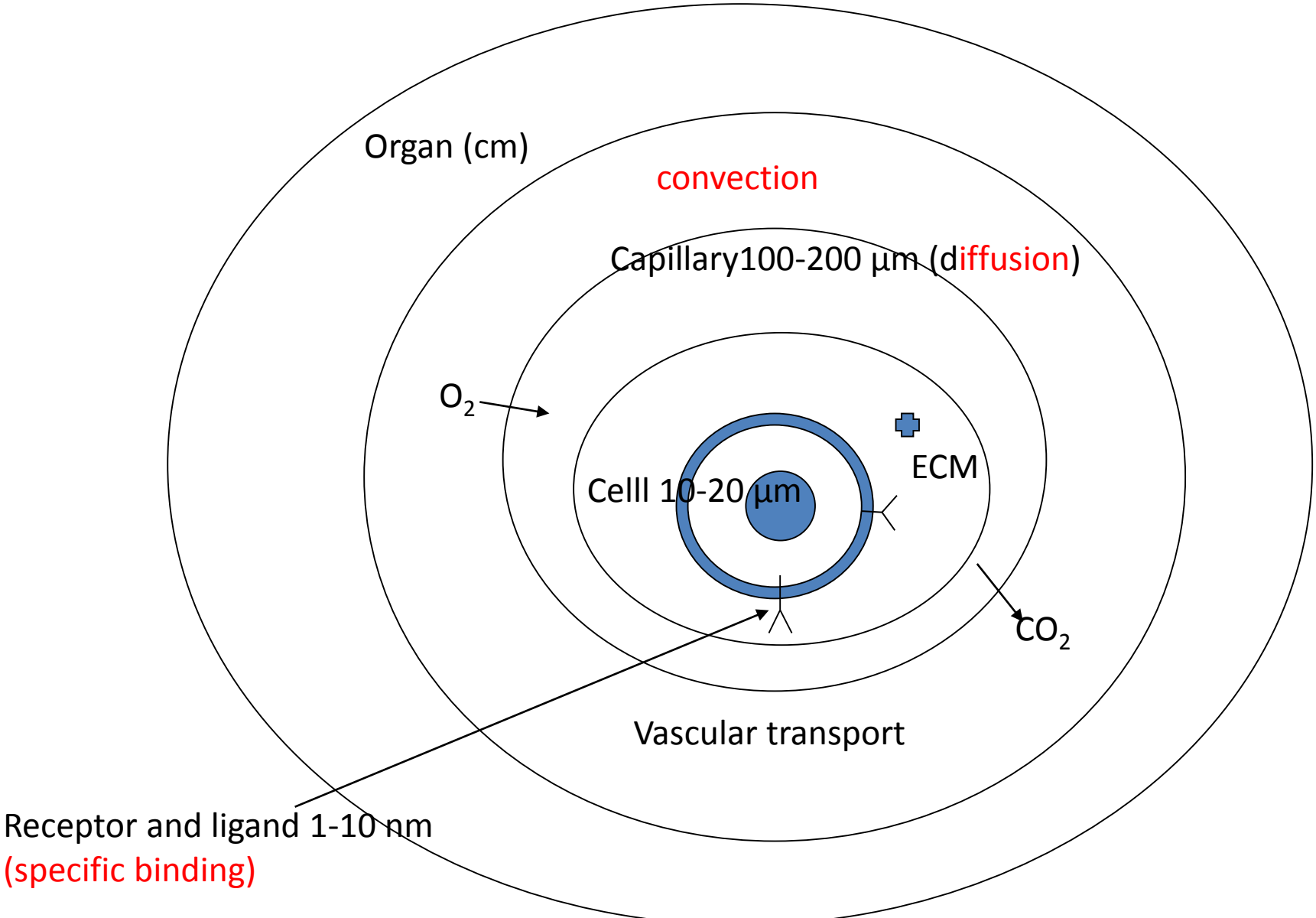
Keywords

Cell size, human cell number, organ, total cell count, theoretical issue

History

Received 26 September 2012
Revised 19 March 2013
Accepted 9 May 2013
Published online 5 July 2013

Characteristic distance 100-500 µm



Le funzioni cellulari sono diverse da cellula a cellula e da tessuto a tessuto, e definiscono il **fentotipo** cellulare. Però alcuni processi sono comuni a tutte le cellule. I processi cellulari più noti sono:

- Proliferazione o crescita
- Migrazione
- Differenzazione
- Morte (apoptosi, necrosi)
- Metabolismo, respirazione
- Adesione
- Espressione proteica

Define: phenotype, genotype, epigenotype

Genes load the gun
Environment pulls the trigger

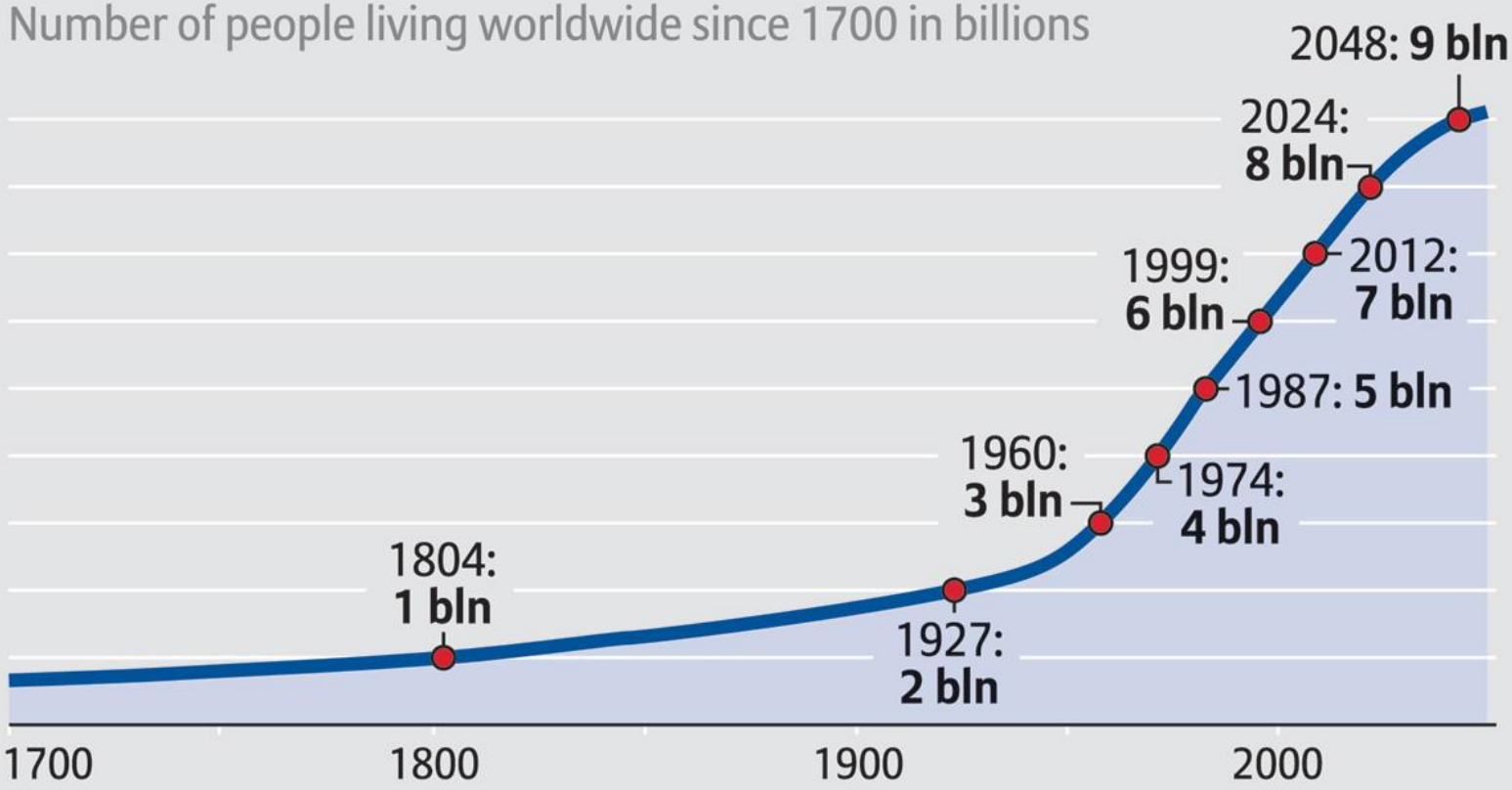
OMICS
Genome
Phenome
Epigenome
Connectome
Secretome
Organome
Inflammatome

Fenotipo – caratteristiche fisiche e biochimiche del organismo
Genotipo- caratteristiche del DNA nucleare
Epigenotipo- alterazione del espressione genica da fattori ambientali (DNA methlyation)

POPULATION OF THE EARTH

Allianz

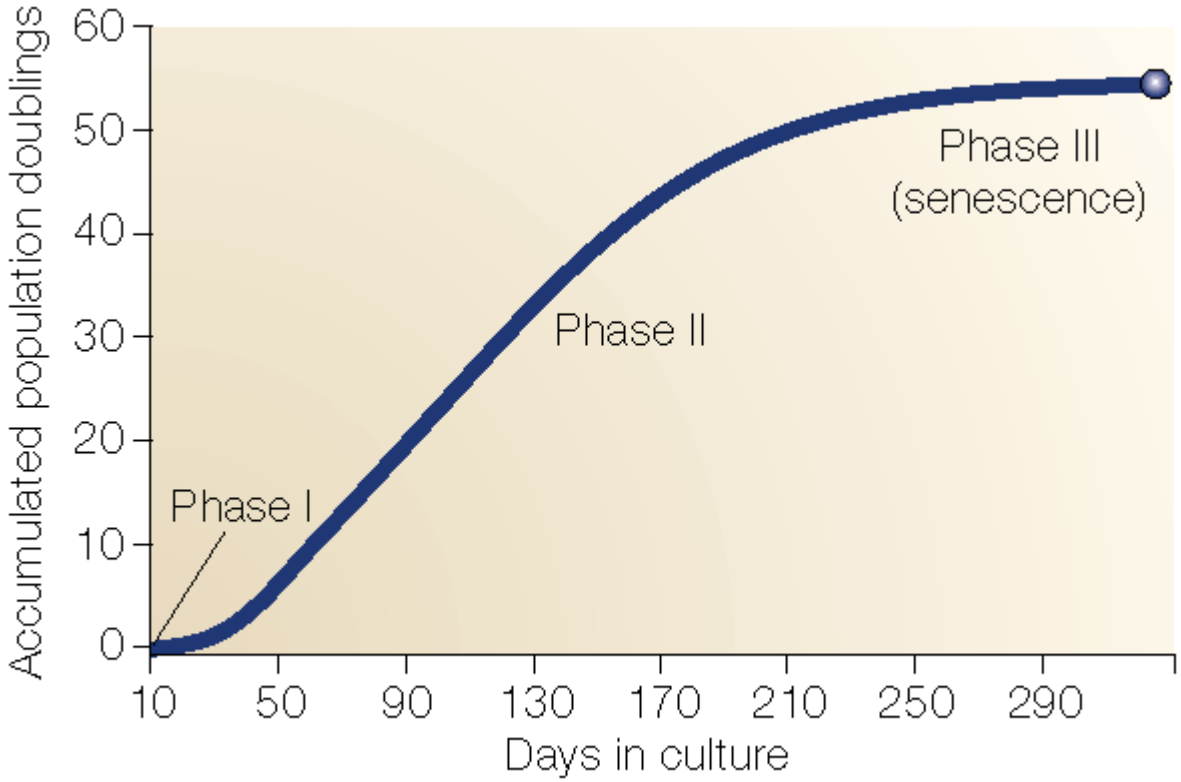
Number of people living worldwide since 1700 in billions



Source: United Nations World Population Prospects, Deutsche Stiftung Weltbevölkerung

For further information please visit: www.knowledge.allianz.com

Crescita' cellulare



Hayflick, L.. The limited in vitro lifetime of human diploid cell strains. 1965

Rate of cell proliferation is proportional to cell number

$$\frac{dN}{dt} \propto N$$

$$\frac{dN}{N} = kdt$$

$$N = N_o e^{kt}$$

$$2N = N_o e^{kt_d}$$

$$t_d = \frac{\ln 2}{k}$$

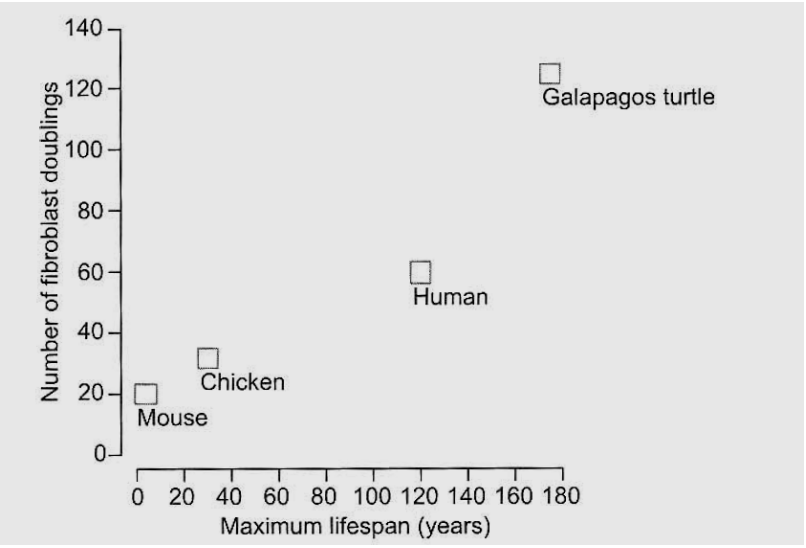
N= cell population

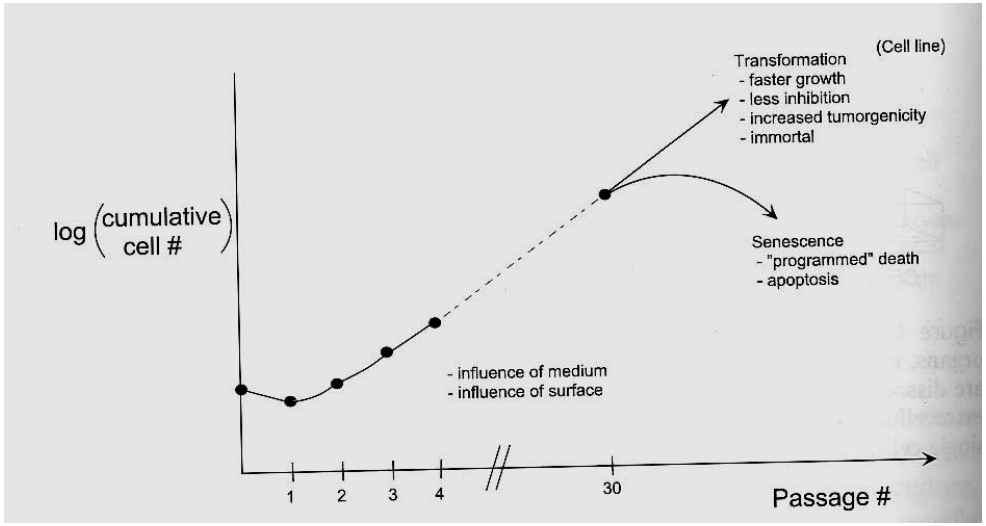
N_o= initial population @t=0

t_d =population doubling time

Cell growth: Hayflick limit and population doublings

The [Hayflick limit](#) is the theoretical limit to the number of times a cell may divide until the telomere becomes so short that division is inhibited and the cell enters senescence.

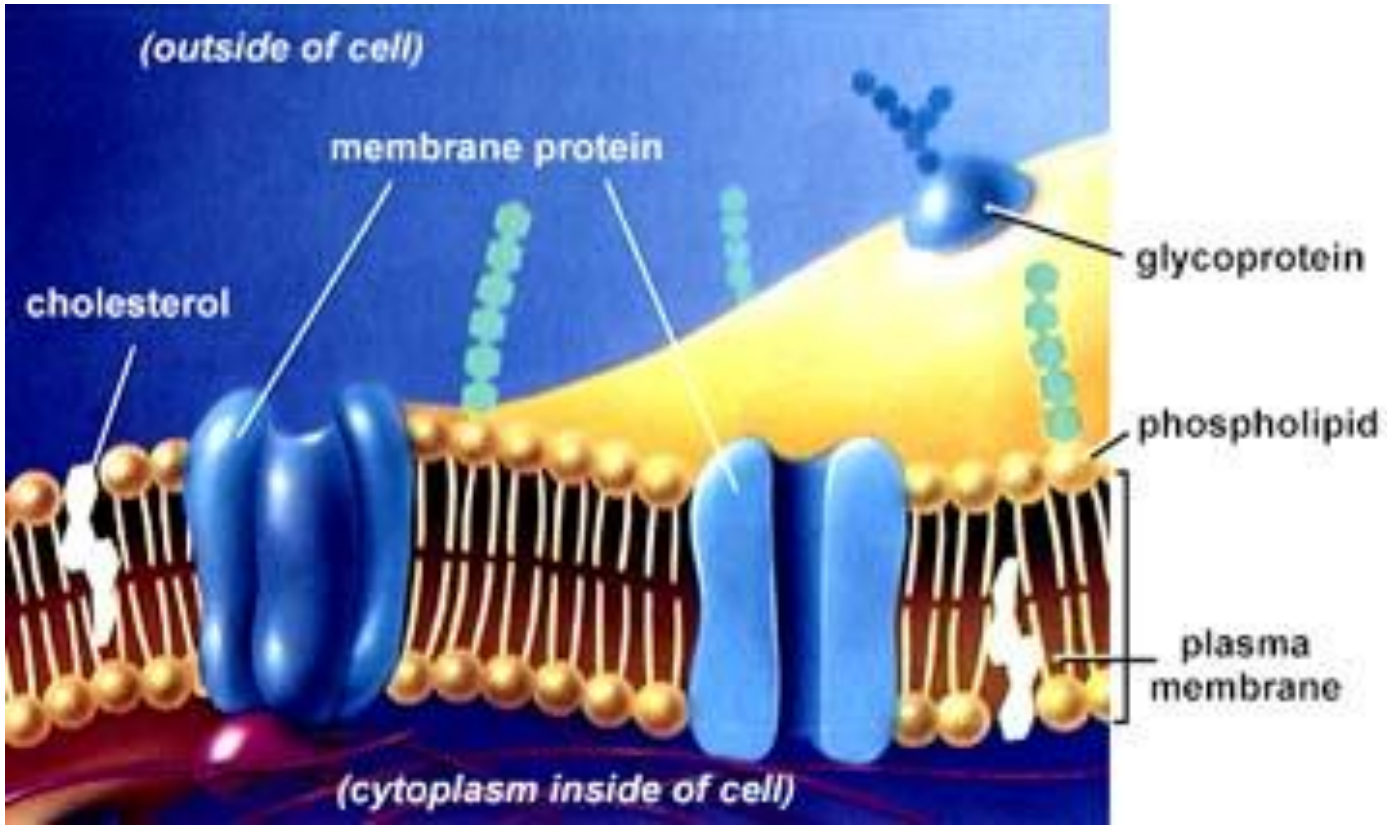


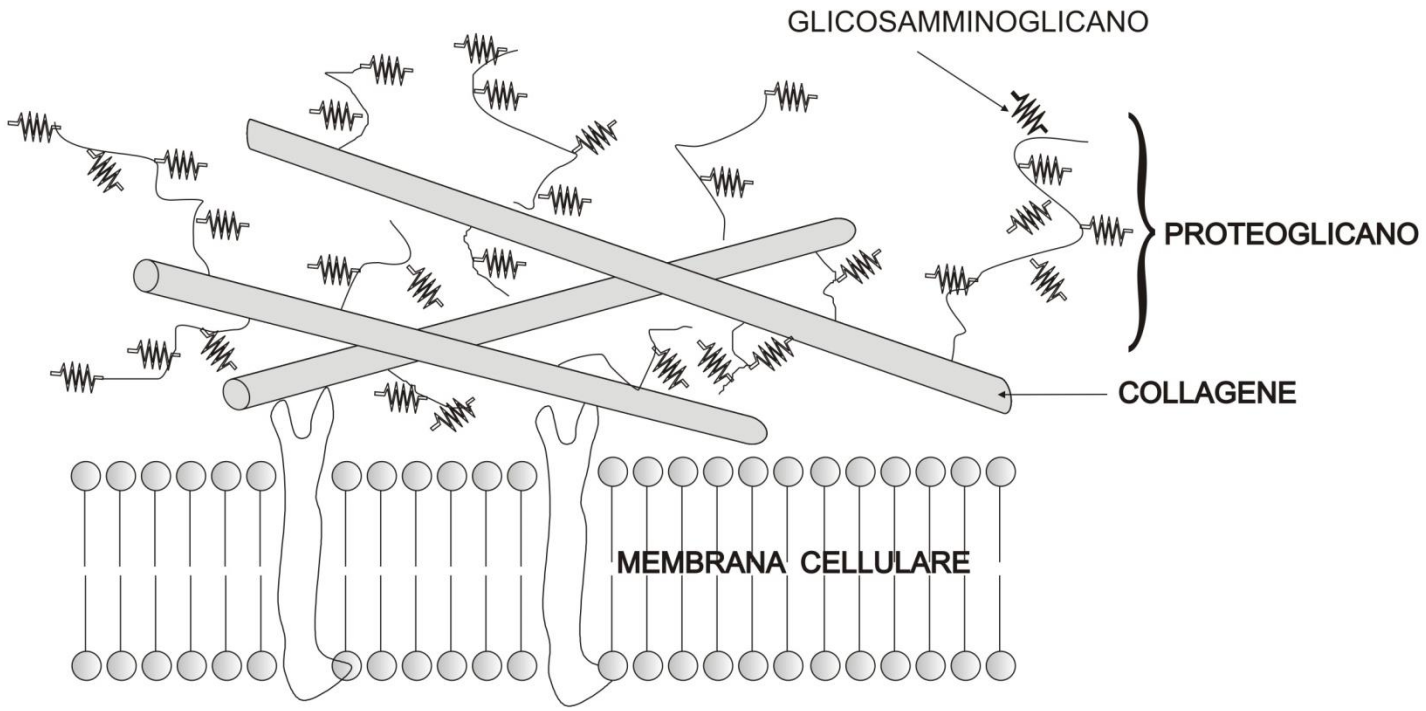


The Immortal Life of Henrietta Lacks

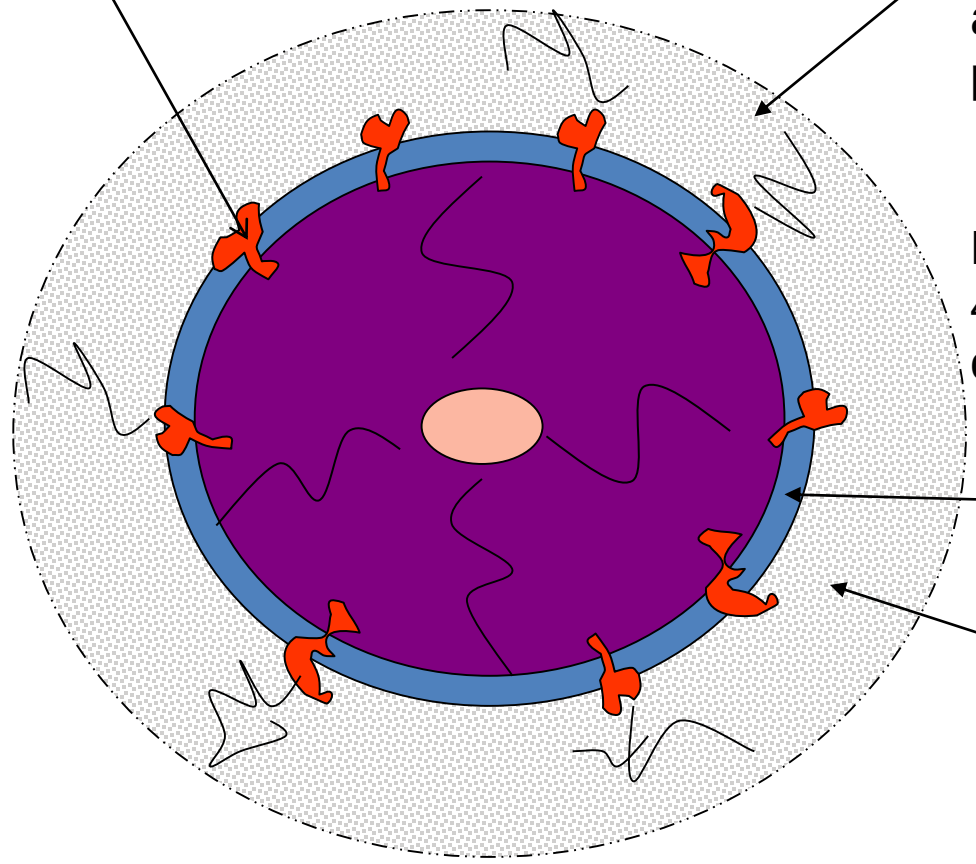
LIGAND BINDING/RECEPTORS/ CELL ADHESION

Libro di Lauffenburger e Linderman


(outside of cell)
membrane protein
glycoprotein
cholesterol
phospholipid
plasma membrane
(cytoplasm inside of cell)


GLICOSAMMINOGLICANO
PROTEOGLICANO
COLLAGENE
MEMBRANA CELLULARE

Binding



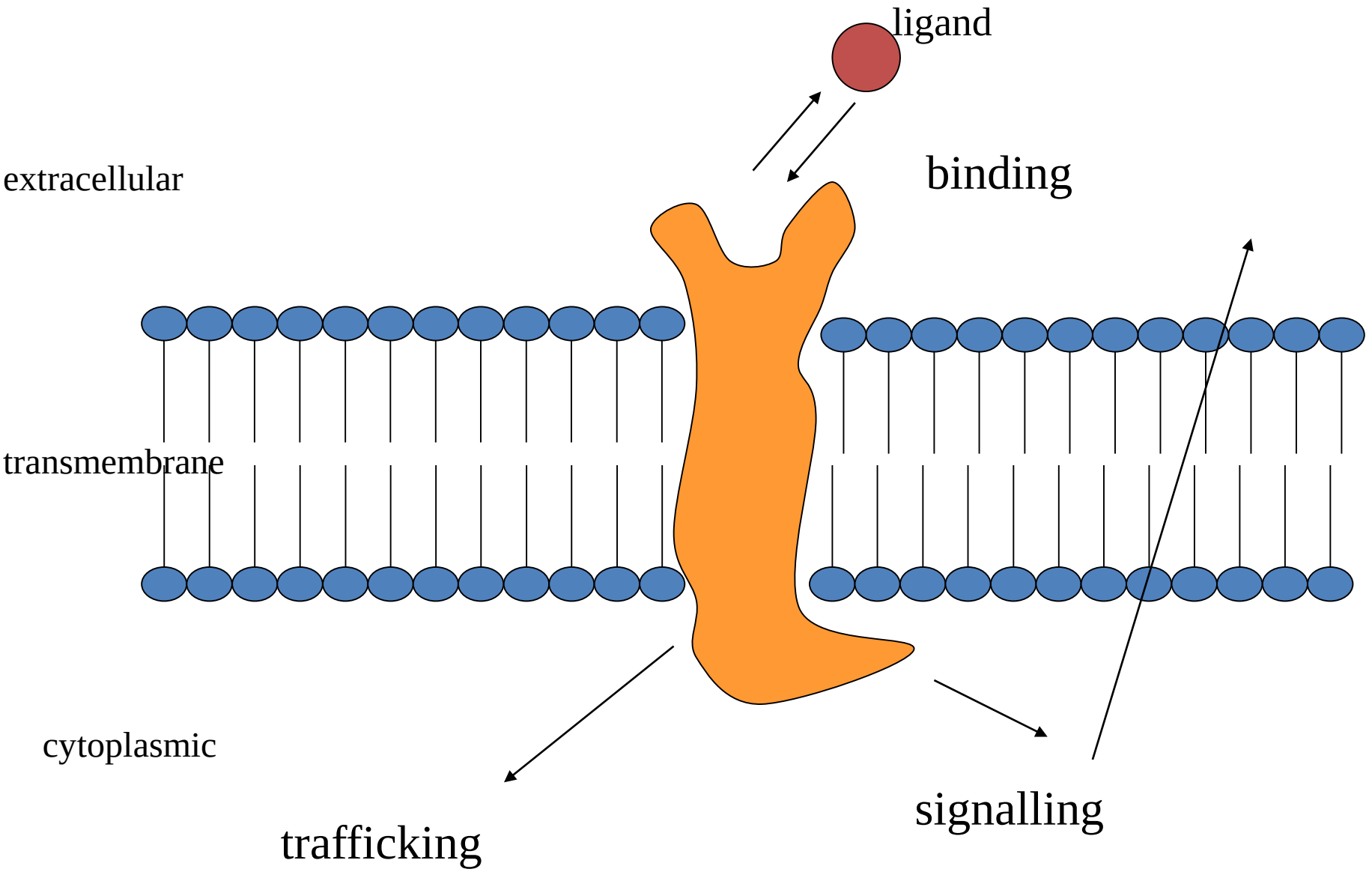
Glycocalyx: carbohydrates adsorbed on transmembrane proteins. It is negative, why?

Membrane is 40% protein, 45% lipid and 5% carbohydrate

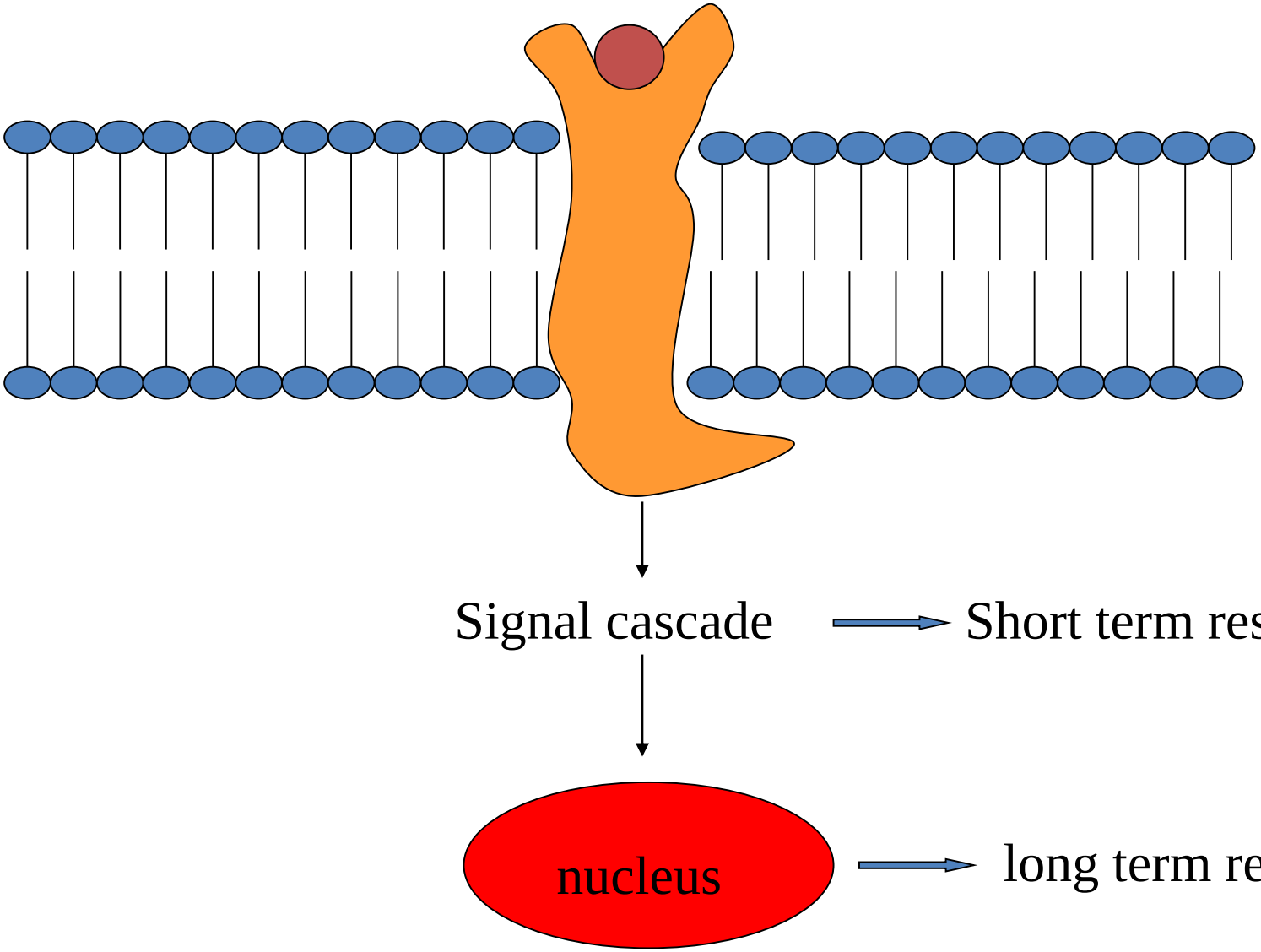
40 A

100-200 A

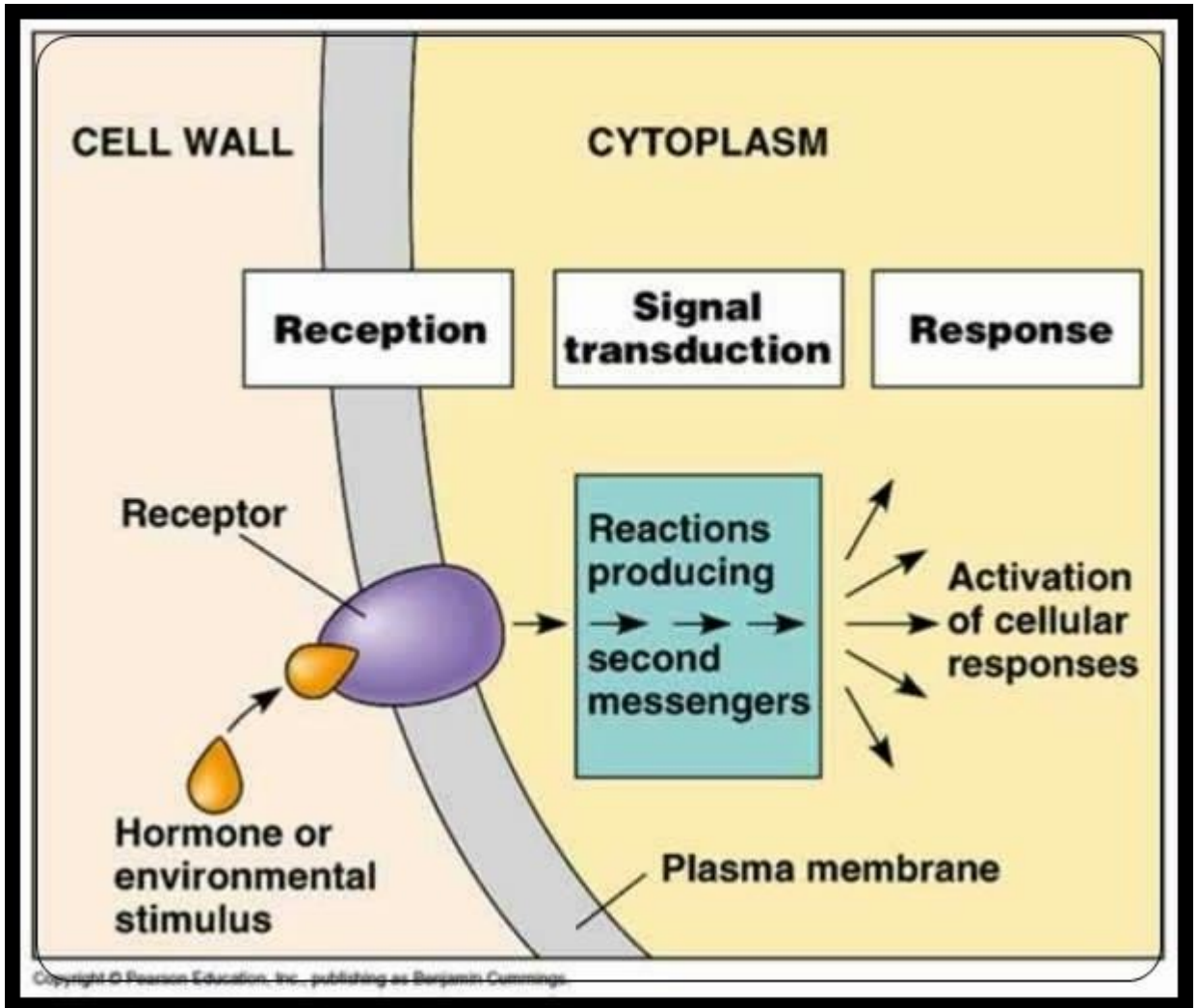
Eukaryotc Cell responses are regulated and controlled by receptor interaction with the environment. So parameters such as growth, death, differentiation, are studied by analysing receptor-ligand binding and the associated trafficking and signalling events.



INSIDE OUT- OUTSIDE IN


Signal cascade
Short term response
nucleus
long term response

Signal transduction occurs when an extracellular signaling molecule activates a specific receptor located on the cell surface or inside the cell. In turn, this receptor triggers a biochemical chain of events inside the cell, creating a response.[Depending on the cell, the response alters the cell's metabolism, shape, gene expression, or ability to divide. The signal can be amplified at any step. Thus, one signaling molecule can cause many responses.



Receptors: Cell surface receptors (CSR). They interact with the extra cellular environment giving rise to four types of signals:

- Nerve transmission
- Hormone release
- Muscle contraction
- Growth stimulation

There are four types of messenger molecules.

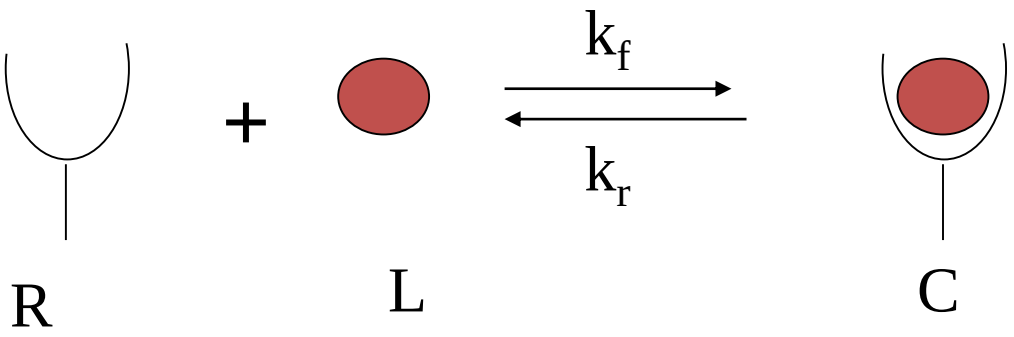
- steroids
- small organic or inorganic molecules
- peptides
- Proteins

The messengers may be

- Endocrine: usually hormones
- autocrine
- paracrine : usually cytokines
- juxtacrine

There are 3 classes of ligand bound receptor signal transduction models

- ion channel receptor (fast ms, low affinity)
- G protein linked receptor (second messenger involved)(medium, mins, med affinity) (GPCR)
- Enzyme (usually Tyrosine kinase i.e. enzyme which adds a phosphate group to proteins at tyrosine residues...ie phosphorylation) linked receptors -Slow and high affinity



We consider a model of receptor-ligand binding in which binding is monovalent and interfering effects are absent. kf and kr are the kinetic association and dissociation constants.

R=number of receptors per cell

C=number of complexes per cell

L=conc of ligand in the ECM (moles/liter)

$kr=t^{-1}$

$kf=M^{-1}t^{-1}$

N=number of cells per unit volume

ok

Monovalent Binding

- For the receptor-ligand reaction:

$$R + L \underset{k_r}{\overset{k_f}{\rightleftharpoons}} C$$

- We can write a simple Master Equation that states that the rate of accumulation of bound complex C is equal to the rate at which molecules associate to form C less the rate at which C dissociates into its components:

$$\frac{dC}{dt} = k_f RL - k_r C$$

- Here
  - C is the concentration of product,
  - R is the concentration of receptor
  - L the concentration of ligand.
- The units for all of these is mol/L or M. k_f is the forward reaction rate ($M^{-1}s^{-1}$) and k_r is the reverse reaction rate [s^{-1}]

Monovalent Binding Master Equation

- One can go further by applying "conservation laws":

$$R_T = R + C \quad \text{and} \quad L_o = L + C$$

- where R_T = total number of receptors and L_o = initial ligand concentration. We thus obtain:

$$\frac{dC}{dt} = k_f \left(R_T - C\right)\left(L_o - C\right) - k_r C$$

- To simplify this, suppose that L_o is very much larger than C and thus ligand isn't depleted much by the reaction from its initial value, L_o. We then get:

$$\frac{dC}{dt} = k_f \left(R_T - C\right) L_o - k_r C$$

- As one may check that with the initial condition $C(t = 0) = C_o$, the solution to this equation is:

$$C(t) = C_o \exp\left[-\left(k_f L_o + k_r\right)t\right] + \left(\frac{k_f L_o R_T}{k_f L_o + k_r}\right)\left\{1 - \exp\left[-\left(k_f L_o + k_r\right)t\right]\right\}$$

- As $t \rightarrow \infty$, (i.e. at equilibrium):

$$C_{eq} = \left(\frac{k_f L_o R_T}{k_f L_o + k_r}\right)$$

Lauffenburger & Linderman, *Receptors*, Oxford, 1993

Dividing by k_f we get $$C_{eq} = \frac{L_o R_T}{L_o + \frac{k_r}{k_f}} = \frac{L_o R_T}{L_o + k_D}$$

Where we define $\frac{k_r}{k_f} = k_D$ as the equilibrium dissociation constant.

The equations are more simply expressed in terms of adimensional parameters, U (ratio of complexes to total number of sites) and τ (a characteristic reaction time).

$$U(\tau) = U_o \exp\left(-\left\{1 + L_o / k_D\right\}\tau\right) + \frac{L_o / k_D}{1 + L_o / k_D}\left(1 - \exp\left(-\left\{1 + L_o / k_D\right\}\tau\right)\right)$$

$$U_{eq} = \frac{L_o / k_D}{1 + L_o / k_D}$$

A variety of messengers can bind to various tissues.

Various cellular responses may occur, depending on the tissue.

Either positive or negative responses may occur, even in the same tissue, depending on the type of receptor.

The response of a cell to a messenger depends on the number of receptors occupied.

A typical cell may have about 1000-3000 receptors.

Only a small fraction (10%) of the receptors need to be occupied to get a large (50%) response.

Receptors may have a dissociation constant of about 10^{-11}; this is the concentration of messenger at which they are 50% saturated. Thus very low concentrations of messengers may give a large response.

Receptor	Ligand	Cell	R_T(#/cell)	Kf (M^{-1} min^{-1})	Kr (min^{-1})	Kd (M)	$T_{95\%}$ (min)
Fc	Fab	macrophage	7.1e5	3e6	0.023	7.7e-10	650
EGF	EGF	Rat lung	2.5e4	1.8e8	0.12	6.7e-10	12.5
Fibronectin	Fibronectin	fibroblasts	5e5	7e5	0.6	8.6e-7	2.5
Transferrin	Transferrin	hepatocytes	5e4	3e6	0.1	3.3e-8	15

Cell surface receptors CSR

Recettore	Ligando	R_T (numero/cellula)	k_f (nM^{-1} min^{-1})	k_r (min^{-1})	K_D (nM)
Trasferrina	Trasferrina (trasportatore ferro negli epatociti)	50000	0.003	0.1	33
EGF	EGF (fattore di crescita epidermale)	25000	0.18	0.12	0.67
Fibronectina (integrina)	Fibronectina	50000	0.0007	0.6	860
Insulina	Insulina	10000	0.0096	0.2	21
TNF	TNF (citochina)	6600	0.93	0.14	0.15
Interleuchina 2	Interleuchina 2 (citochina)	200	1.89	0.014	0.0074

Considerare una coltura di condrociti seminati su scaffold porosi in microwells da 1.5 ml, con 1.10^6 cellule/scaffold. I condrociti esprimo circa 10^5 recettori per TGF-β, un fattore di crescità. A che concentrazione di TGF-β si ha il fenomeno di ligand depletion? K_D per il legame TGF-β- recettore per TGF-β e' 10^{-10} Molare.

L a molecola dexamethasone (DEX) aumenta la produzione di collagene in osteoblasti, grazie all'interazione di DEX con un recettore. Per controllare la produzione di collagene in vivo e in vitro, si può utilizzare un farmaco che inibisce l'azione del DEX in maniera competitiva. La massima velocità di produzione di collagene è 100 molecole/cellula/s. In un tipico esperimenti si aggiunge una concentrazione di 1.10^{-8} M di DEX che da luogo a una produzione del 75%.

a)Calolare il K_D (costante di equilibrio) del DEX.,

b) si aggiunge poi il farmaco che inibisce la produzione di collagene. A 5.10^{-7} M di farmaco, la produzione diminuisce a 65%. Calcolare il K_D per il farmaco inibitore..

c) Che concentrazione di DEX ci vuole per resitituire la produzione di collagene in presenza di 5.10^{-7} M del farmaco ?

d) quali assunzioni si fa per ottenere le soluzioni a,b, e c?.

Cell adhesion, cell cohesion

CAM :Cell Adhesion molecule, classified as one of generic CSR : cell surface receptors. Common names VCAM, PECAM.

Cells can adhere to each other (cohesion)

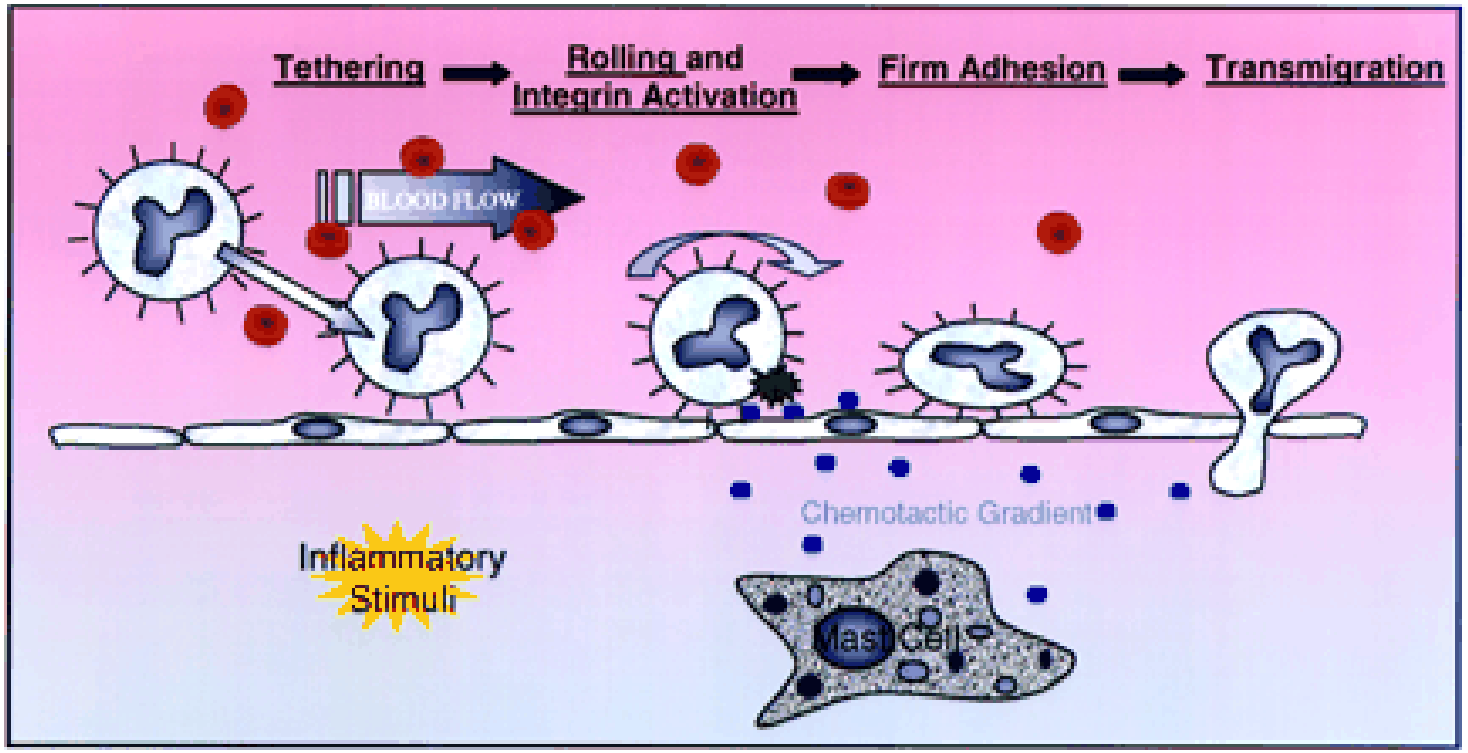
Or to the ECM (adhesion)

CAMs are responsible for structural integrity of adherent cells

Cell adhesion, cell cohesion: why?

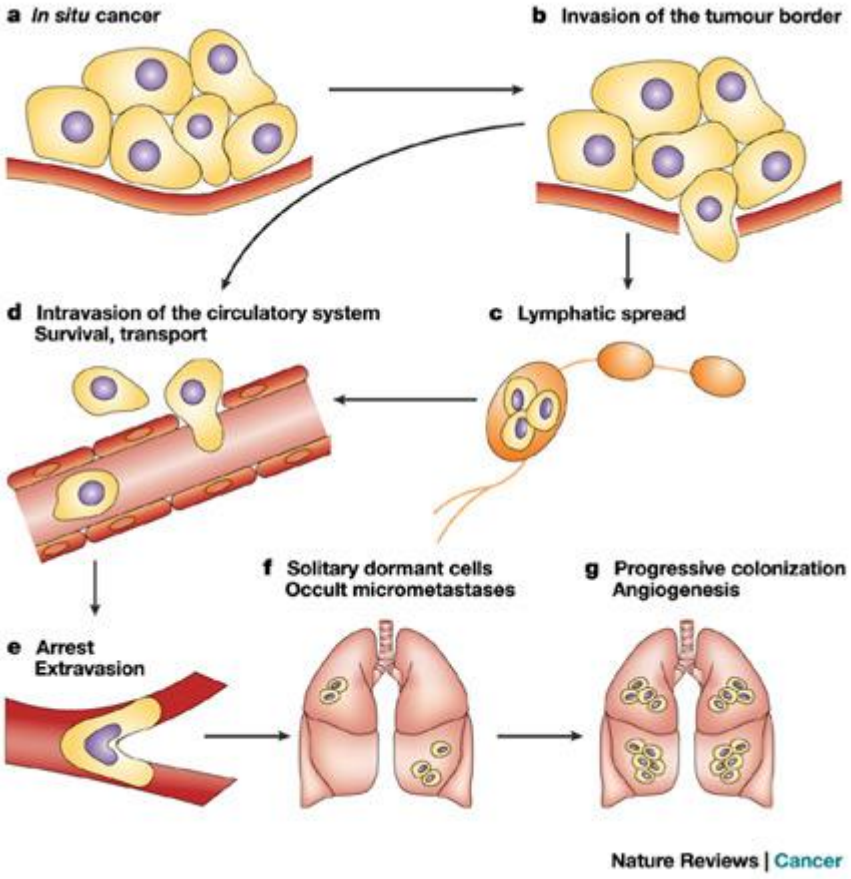
- Scaffold colonization
- Motility
- Metastasis
- Wound healing (scurvy)
- Morphogenesis
- Differentiation
- Inflammation and repair

Inflammatory response



Leucocyte rolling

Metastasis


a In situ cancer
b Invasion of the tumour border
c Lymphatic spread
d Intravasion of the circulatory system
Survival, transport
e Arrest
Extravasion
f Solitary dormant cells
Occult micrometastases
g Progressive colonization
Angiogenesis
Nature Reviews | Cancer

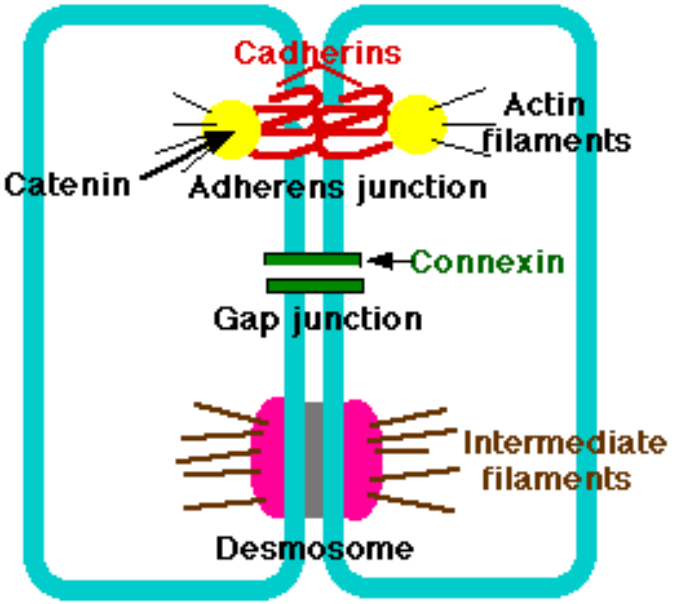
There are 3 types of junctions between cells and cells or cells and ECM

Tight junctions- especially in epithelial cells, they prevent diffusion of molecules

Communicating junctions – gap junctions, they regulate transport. For example in the liver and kidney

Anchoring junctions- they provide mechanical links- through integrins and cadherins

Linked to the cytoplasm through cytoskeleton



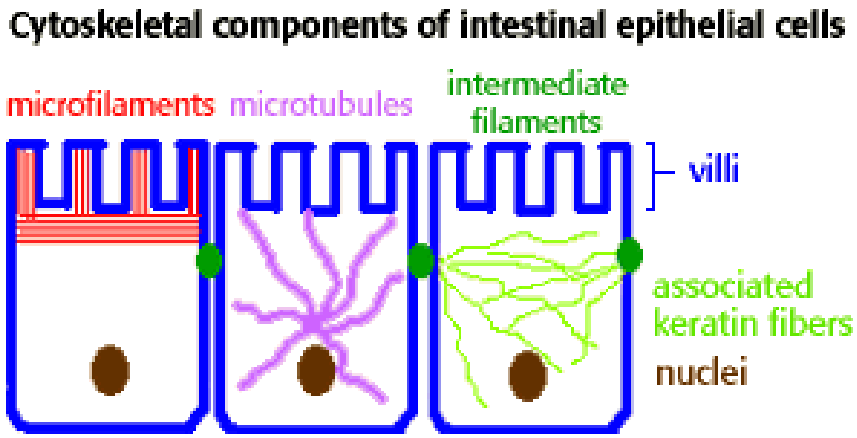
The cytoskeleton: microfilaments, intermediate filaments and filaments.

Function: cell shape, motility, division, **mechanical strength (incompressible, resistant to tension)**.

Micro filaments: Actin – contractile 3-6 nm

Intermediate filaments (fibrous proteins eg desmin, vimentin)- 10 nm. - tensile, rope like structures, much longer than actin. Form the structural framework in the cell.

Microtubules 25 nm. Cell shape and motility.
Tracks for vesicle movement

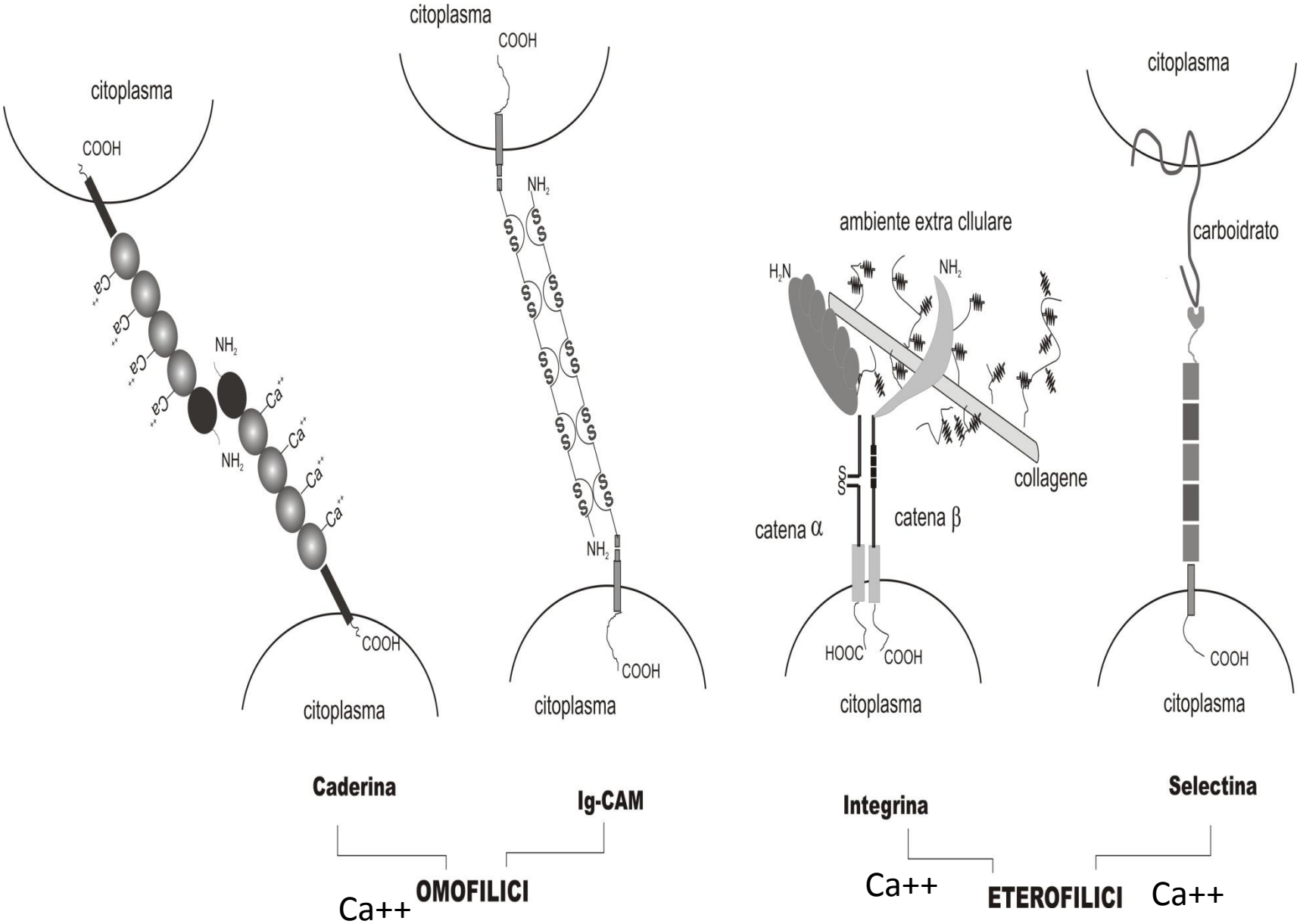


CAMs- junction proteins

Il meccanismo di riconoscimento attraverso i CAM è uno dei principali modi in cui la cellula interagisce con suo ambiente.

CAM	Caratteristiche
Integrine	-legano ai ligandi adesivi della matrice extra cellulare, sono detti legami eterofilici
Caderine	- legano a cellule vicine, generalmente omotipici (caderina-caderina) e sono calcio dipendenti. Le caderine sono **fondamentali per la morfogenesi.**
Ig CAM	- legano a altre cellule, generalmente formando legami omotipici, sono meno forti di legami caderine-caderine e sono le uniche CAM che non dipendono dalla presenza di calcio.
Selectine	- legano a mucine (la parte glicosata delle proteine), quindi formano legami etereofilici sono molto importanti per i processi infiammatorie.

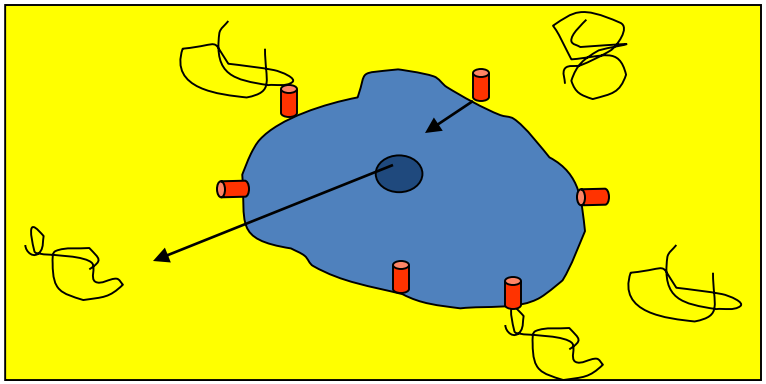
Why are integrins not so important during early morphogenesis?


citoplasma
COOH
NH2
Ca++
citoplasma
COOH
citoplasma
NH2
S
COOH
citoplasma
ambiente extra cllulare
H2N
NH2
collagene
catena α
catena β
S
HOOC
COOH
citoplasma
citoplasma
carboidrato
COOH
citoplasma
Caderina
Ig-CAM
Ca++
OMOFILICI
Integrina
Selectina
Ca++
ETEROFILICI
Ca++

Le integrine

Adesione alla ECM

Trasduzione del segnale dal ECM alla cellula e dalla cellula al ECM
L'importanza dell'interazione tra cellule e la ECM. L' ECM non è solo una struttura di supporta ma gioca un ruolo attivo e importante in tante funzioni cellulari. Migrazione, proliferazione, differenzazione, apoptosis. Inoltre modula l'espressione delle citochine e i fattori di crescita e attiva la trasduzione e segnalazione intracellulare. Il rapporto cellule ECM funziona per reciprocità dinamica.
La ECM è l'ambiente che regola la dinamica dell'espressione genetica e differenzazione.



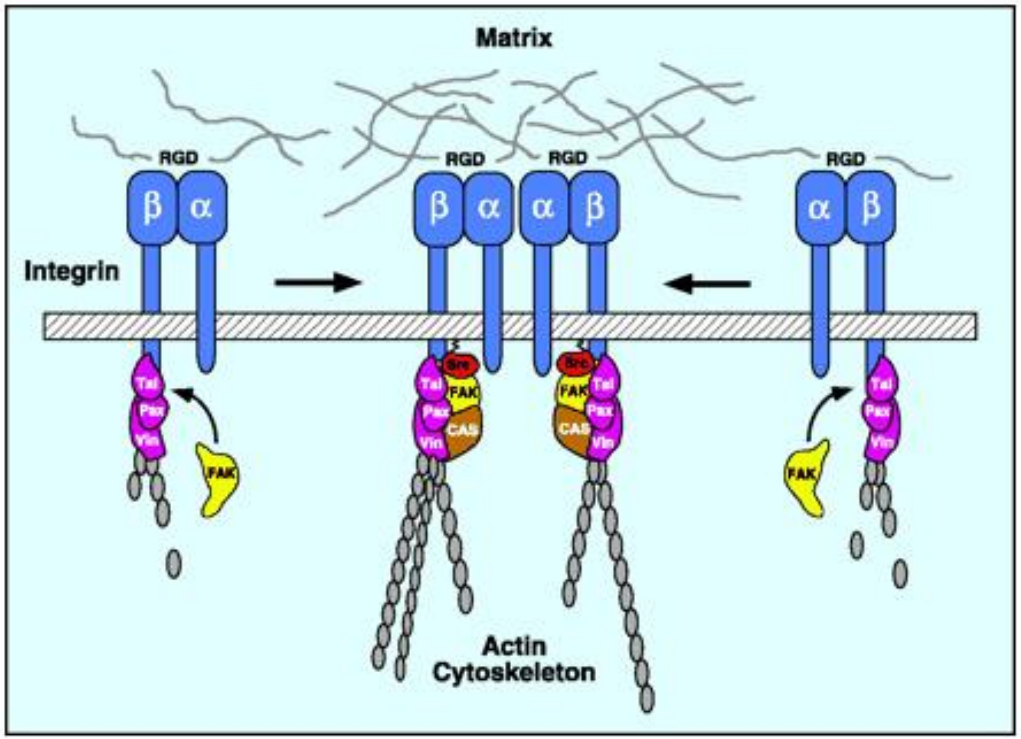
Le molecole del ECM interagiscono con i recettori (CSR-cell surface receptors, in particolare i CAM) che trasmettono segnali attraverso la membrana a molecole dentro i citoplasma. Questi segnali iniziano una cascata di eventi attraverso il CSK al nucleo (cytoskeleton) che risultano nell'espressione di geni. Questi vanno trascritti in proteine che hanno un effetto sull' ECM.

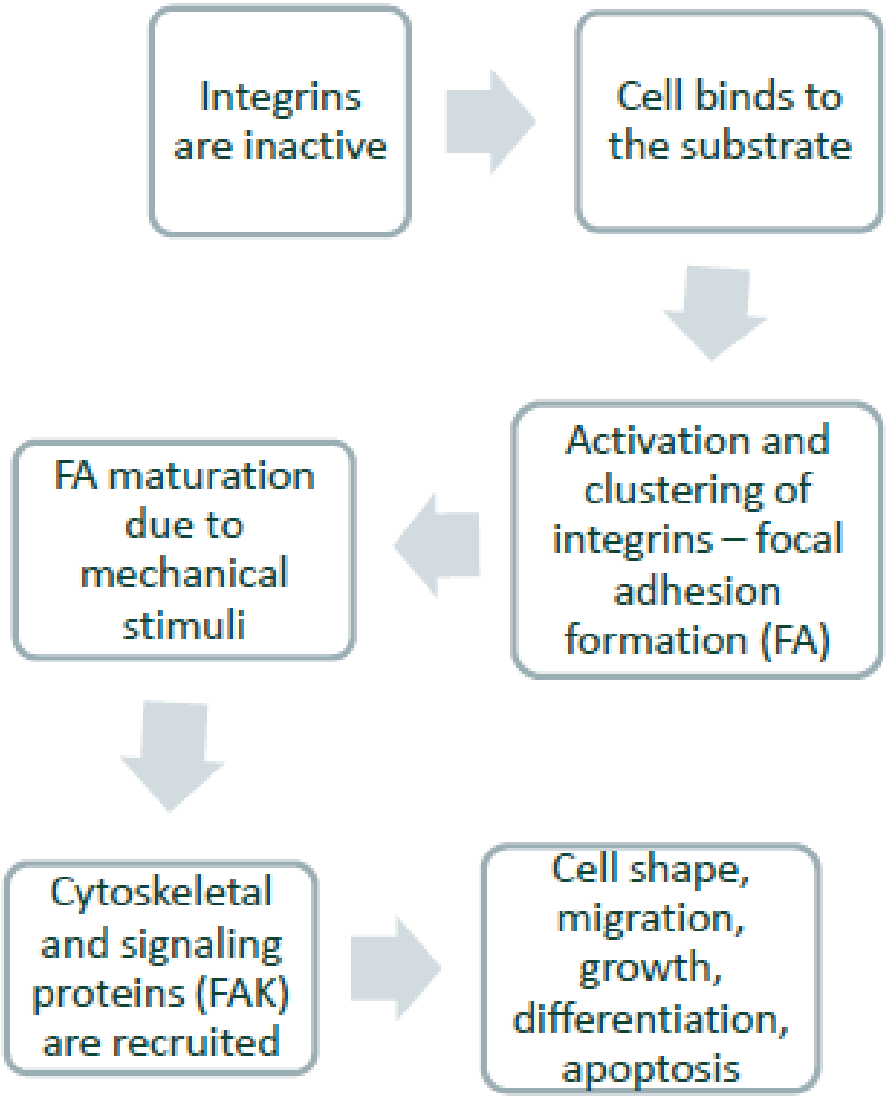
Alcune seguenze peptidiche sono riconoscuite dalle integrine

- RGD (arginina-glicina-acido aspartico)
- YGISR (Tyr–Ile–Gly–Ser–Arg)

- E possono essere usate per decorare superfici di biomateriali per aumentare l'adesione cellulare

•Le Integrine si raggruppano e inizia una cascata di segnali
•*Focal adhesion kinase (FAK)*, un enzima tyrosina kinase è coinvolta
•FAK arriva agli contatti focali e viene fosforilato, inizando una cascata di reazioni (quasi sempre di fosforilazione) che finiscono in una concentrazione di proteine nella zona focale.
•Il segnale viene trasmesso alll'interno della cellula attivando l'organizzazione dello citosceletro

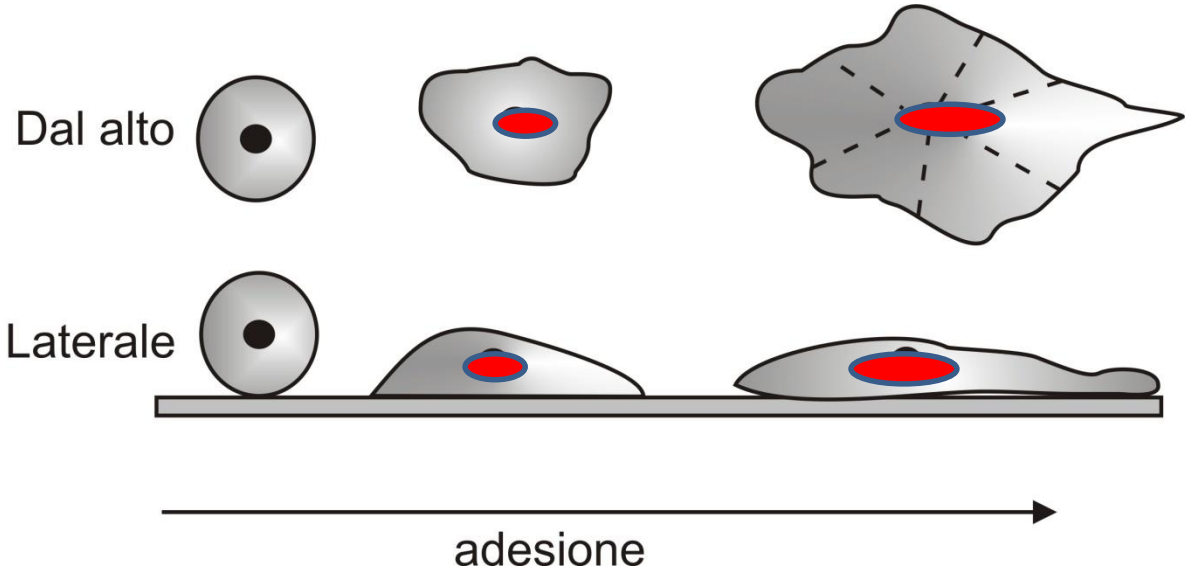




secondi

Minuti

ore

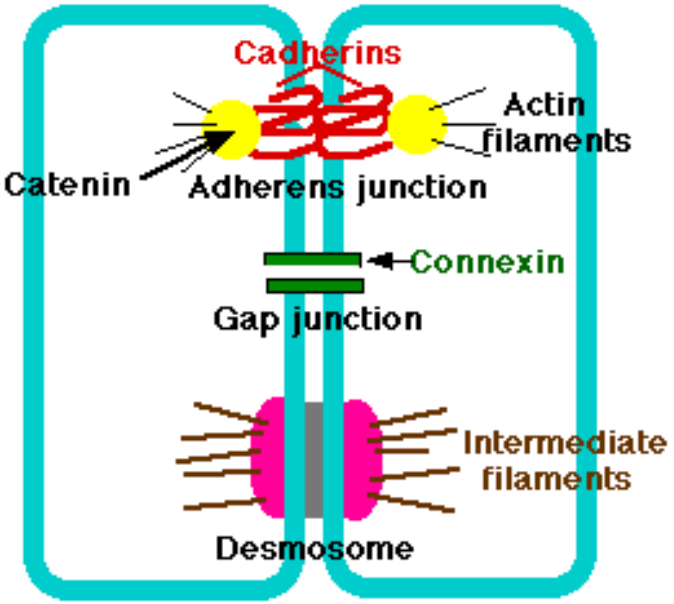


Cellula non adesa, poco adesa e molto adesa, su un substrato.

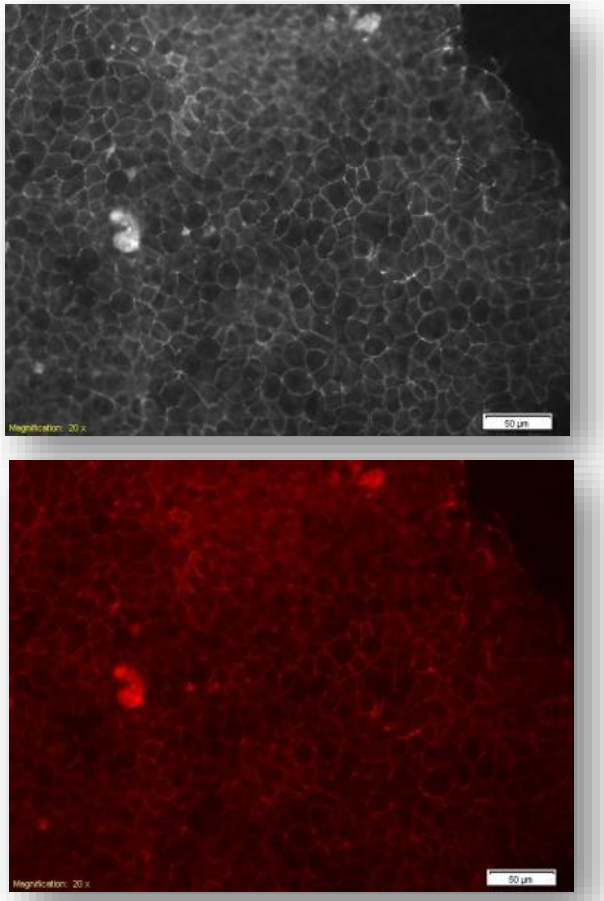
Ogni cellula aderisce in modo diverso a secondo del numero di integrine per cui ha anche forma diverso. Forma e' altamente correlato alla funzione.

Le Caderine

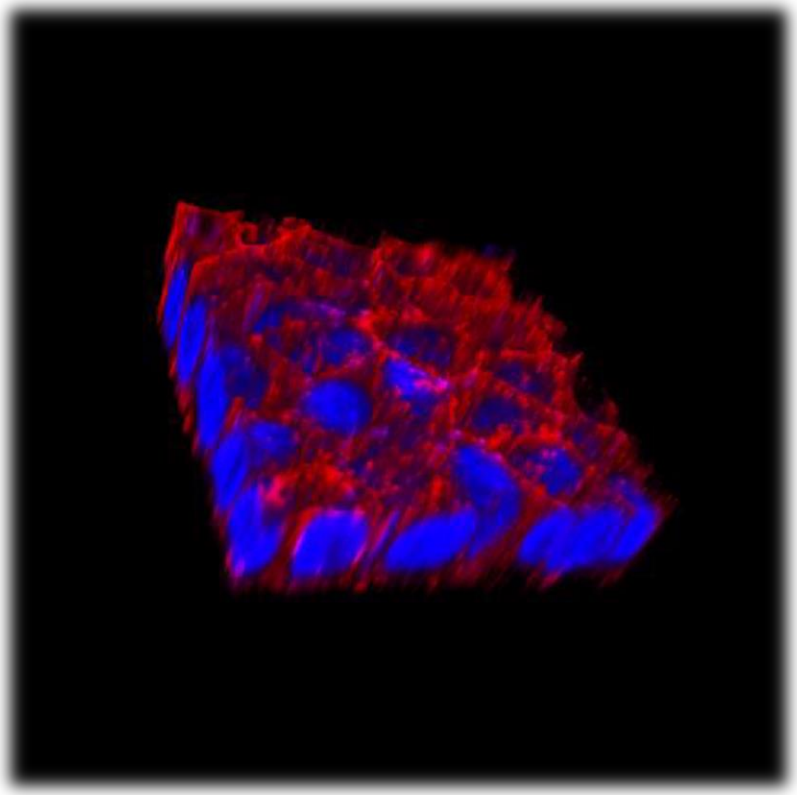
Sono molecole presenti nei tessuti dei vertebrati e la loro azione dipende dalla presenza di calcio. Inizialmente sono state nominate in base al tessuto di appartenenza: caderina-E (epitelio), caderina-N (nervi) e caderina-P (placenta). Ogni tipo di cellula esprime un determinato set di caderine, che può cambiare se le funzioni della cellula cambiano. La porzione extracellulare è molto estesa e composta da cinque domini, di circa 100 amminoacidi ciascuno. Quattro di questi domini sono omologhi e contengono siti di legame per il calcio, ione indispensabile per la loro funzione. Solitamente le caderine sono impegnate in legami omofilici, di conseguenza, le caderine presenti sulla superficie di una cellula si legano alle caderine presenti sulle superfici cellulari Diverse malattie sono associate con la disfunzione delle caderine.
adiacenti. La loro affinità è piuttosto bassa, e il principio di funzionamento è simile a quella delle integrine.



Intestinal epithelia



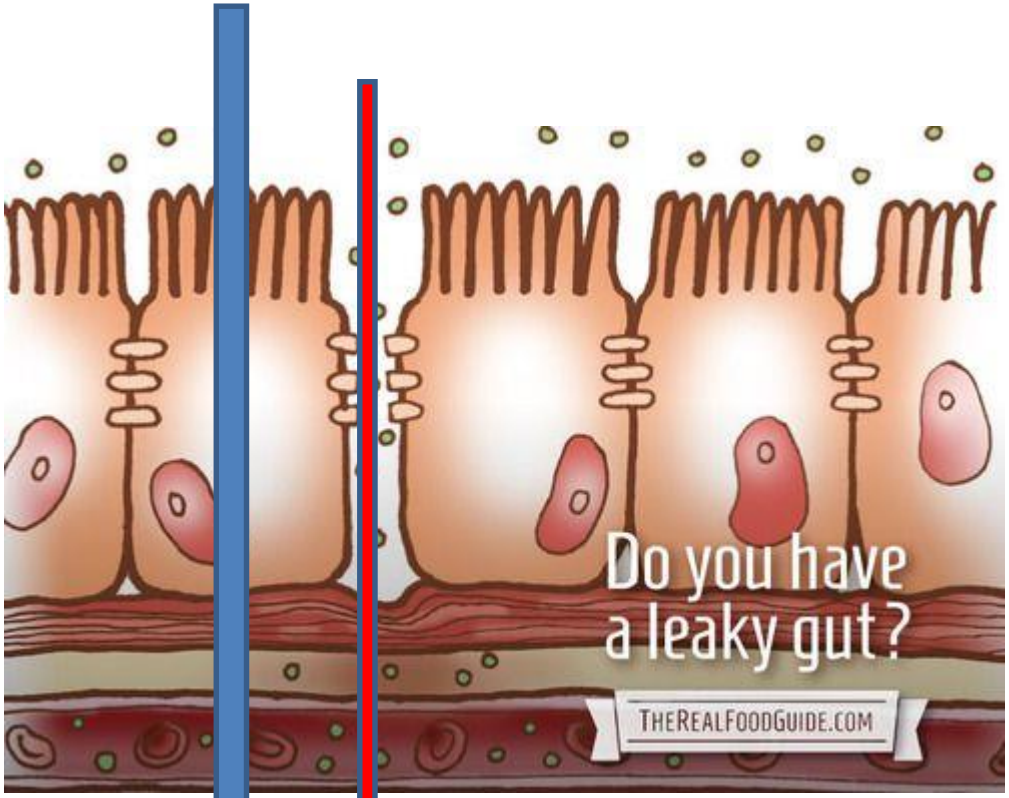
20X



Tight junctions :red. Nuclei:blue

TEER- trans- epithelial- electrical resistance

transcellular



paracellular

Tight junctions

Integrins

Basal lamina

Le Ig-CAM

Questi reccettori assomigliano agli anticorpi e sono importanti nella regolazione fine della coesione, sopratutto nell'embrione. (Ca+ independente)

Le Selectine

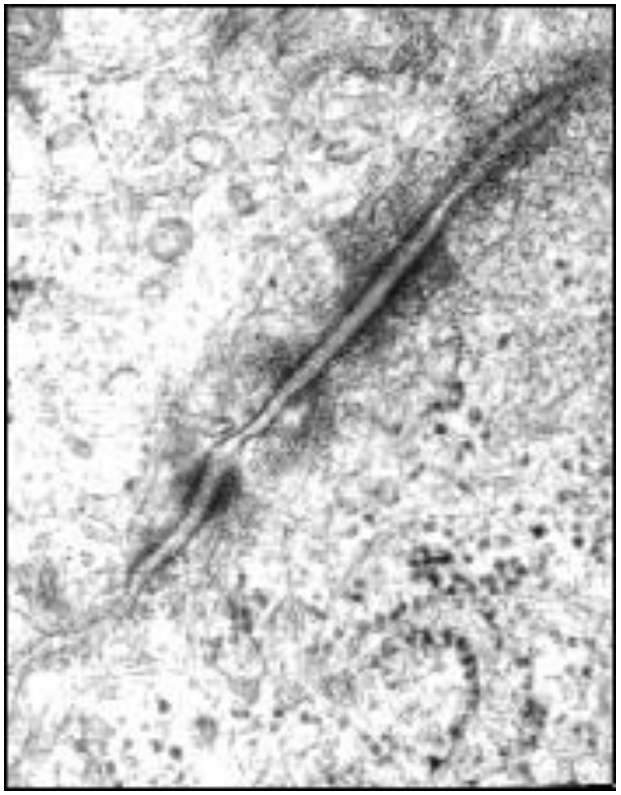
Le selectine possiedono un dominio capace di legarsi ai carboidrati e giocano un ruolo nella risposta infiammatoria perchè in genere si legano agli zuccheri presenti sulla superficie dei neutrofili. .

Le caderine sono importante per lo sviluppo aggregazione e disaggregazione cellulare (up and down regulation).

Anticorpi contro cadherina rompono i legami e distruggono epitelio

Sono Ca dipendenti

E cad (epiteliale), N cad, ecc



I IgCAM (LCAM e NCAM) invece non sono Ca++ dipendenti, meno forti delle caderine, e possono cambiare la forza del legame ridicendo la lunghezza della catena extra citoplasmica che va a interagire con la IgCAM di un’altra cellula

Stimare il numero di integrine che una cellula endoteliale deve possedere per superare le forze di taglio imposte dal flusso sanguinio in un arteria.
Diametro cellula= 20 μm, altezza trascurabile
Velocità media del sangue nel arteria di diametro 1.5 cm= 40 cm/s
Viscosità del sangue=0.004 Pas
Usare i dati per il probelma sotto.

Uno dei problemi associato all'utilizzo di scaffold sintetici per l'ingegnerizzazione dei vasi è la mancanza di un'adeguata adesione di cellule endoteliali sulla parete luminale, che causa la formazione di trombi e altre complicazioni. Uno degli approcci considerati è l'immobilizzazione di ligandi di adesione, tipicamente in forma di sequenze amminoacidi contenenti RGD. Dato che un integrina si lega a un RGD, calcolare la densità superficiale (#/μm^2) di RGD necessario per assicurare un'adeguata adesione e quindi la distanza tra un ligando e l'altro. Discutere alcuni dei problemi che si possa incontrare con l'utilizzo di questo approccio. I dati sono da confrontare con le densità superficiali di 600-700 ligandi/μm^2 necessarie per formare adesioni focali riportate in Cavalcanti-Adam et al (Cell Spreading and Focal Adhesion Dynamics Are Regulated by Spacing of Integrin Ligands, Biophysical Journal, Volume 92, 2007 p. 2964–2974).