

Cds in Scienze e Tecnologie Biologiche

AA 2019-2020

Corso di Laboratorio di Biologia Cellulare

Lezione 4

**OLTRE LE COLTURE 2D: MODELLI CELLULARI IN
BIOMEDICINA**

Modelli cellulari in biomedicina

VANTAGGI:

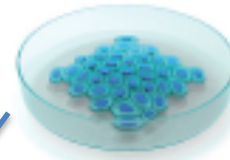
- GRANDE QUANTITA' DI MATERIALE
- ANALISI BIOCHIMICHE
- HIGH THROUGHPUT

LIMITI:

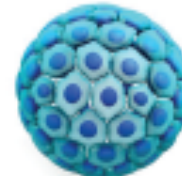
- NON RICAPITOLANO LA COMPLESSITA' TISSUTALE
- CONDIZIONI NON FISILOGICHE (PLASTICA)
- INTERAZIONI CON LA MATRICE LIMITATE

Biochemical tools

Model system
in life sciences



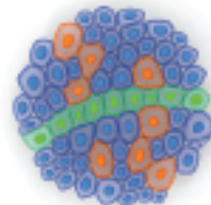
Monolayer cell culture



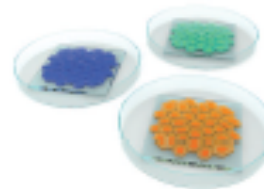
Spheroid



Organoid



Tissue explant



Multiplexed models
"on-a-chip"

Complexity of culture

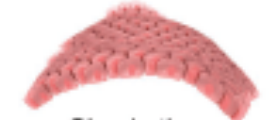
Organization of
the body



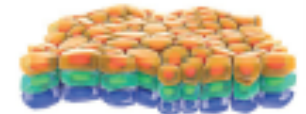
Subcellular



Cells



Simple tissue



Layered tissue



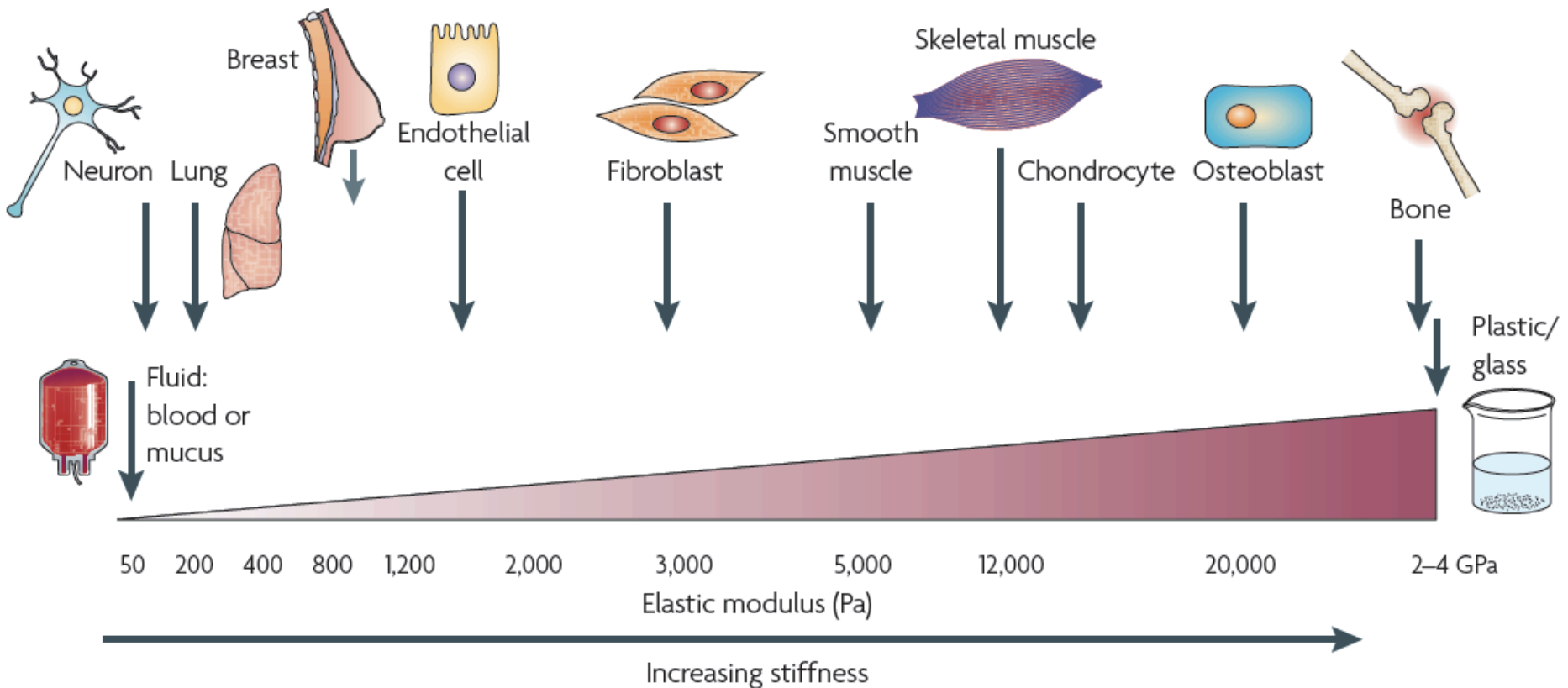
Organ & System



Body

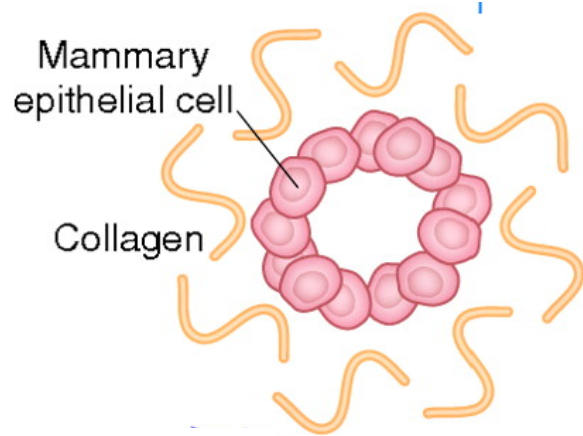
Scale

La rigidità del supporto influenza il comportamento cellulare



La plastica è un supporto non fisiologico

Colture cellulari in 3D (sferoidi)

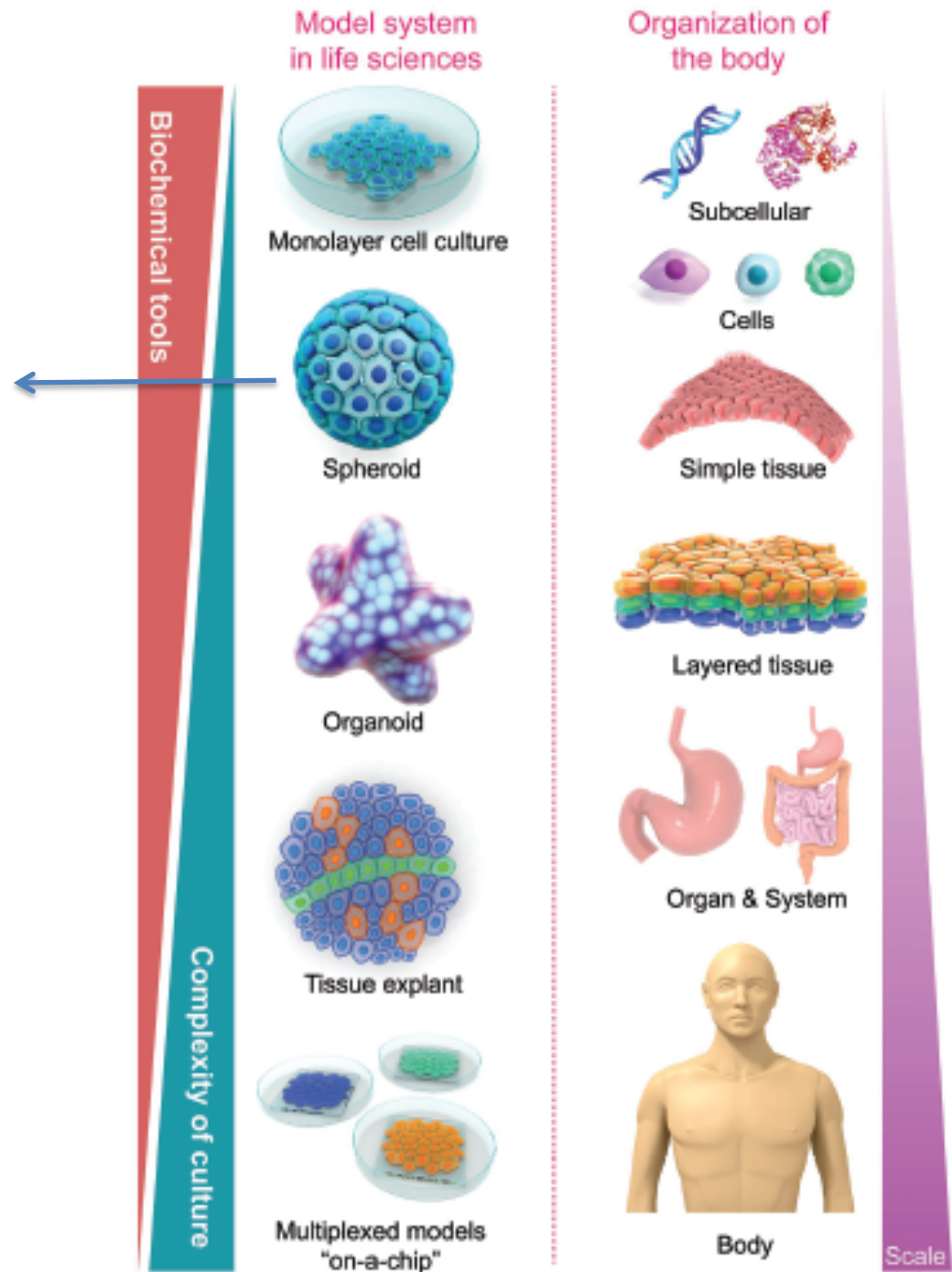


VANTAGGI:

- INTERAZIONI FISILOGICHE CON MATRICE E ALTRE CELLULE
- STUDIO DELLA MORFOGENESI

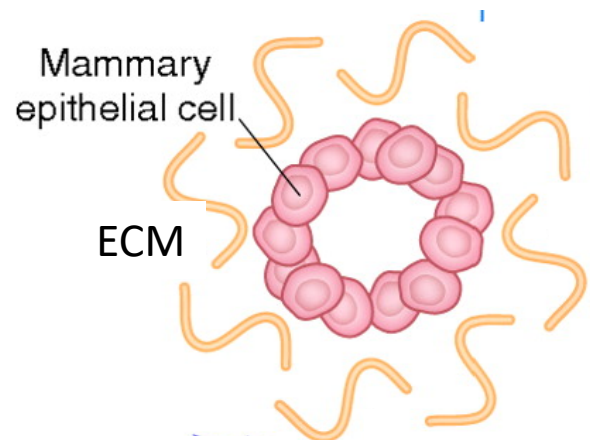
LIMITI:

- NON RICAPITOLA LA COMPLESSITA' DEI TESSUTI PLURISTRATIFICATI

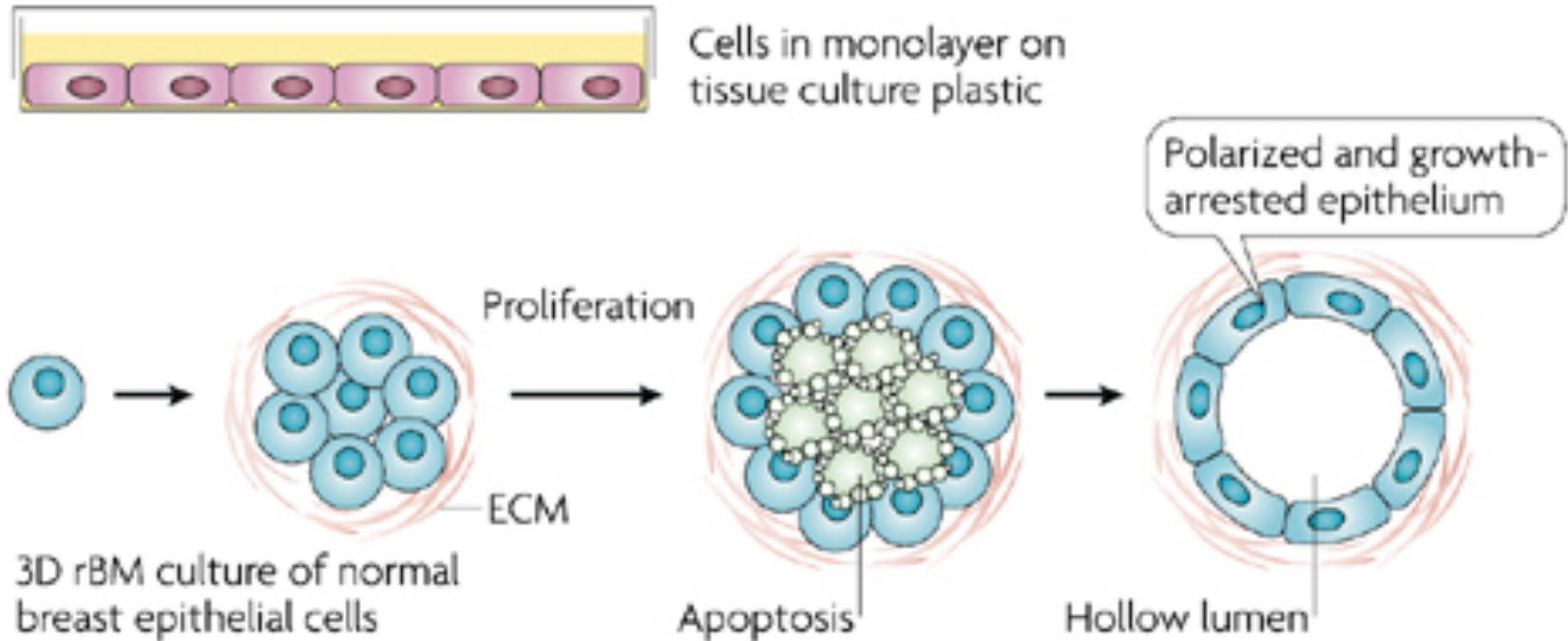


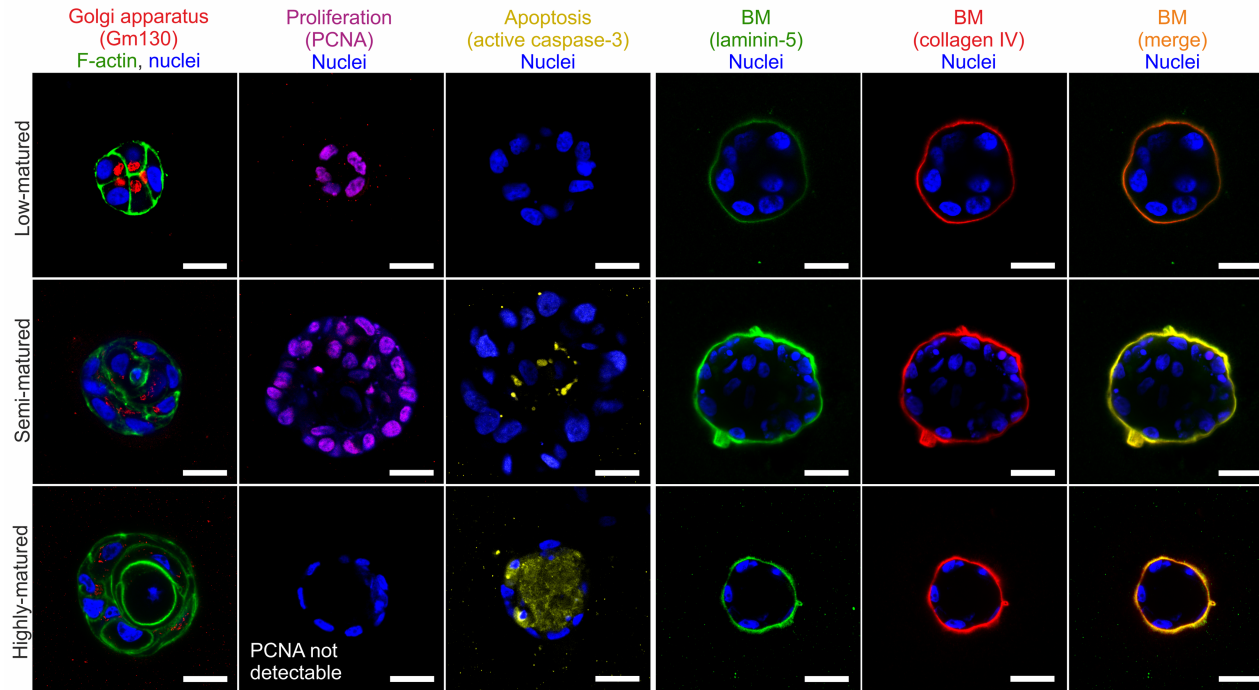
Colture cellulari in 3D

Colture cellulari 3D possono essere mantenute in vitro in matrici ricostituite a base di collagene o matrigel (una matrice secreta da una linea cellulare tumorale, arricchita in componenti della lamina basale) oppure gel di acrilamide derivatizzati a formare strutture tridimensionali, che ricapitolano la morfologia del tessuto/organo di origine.

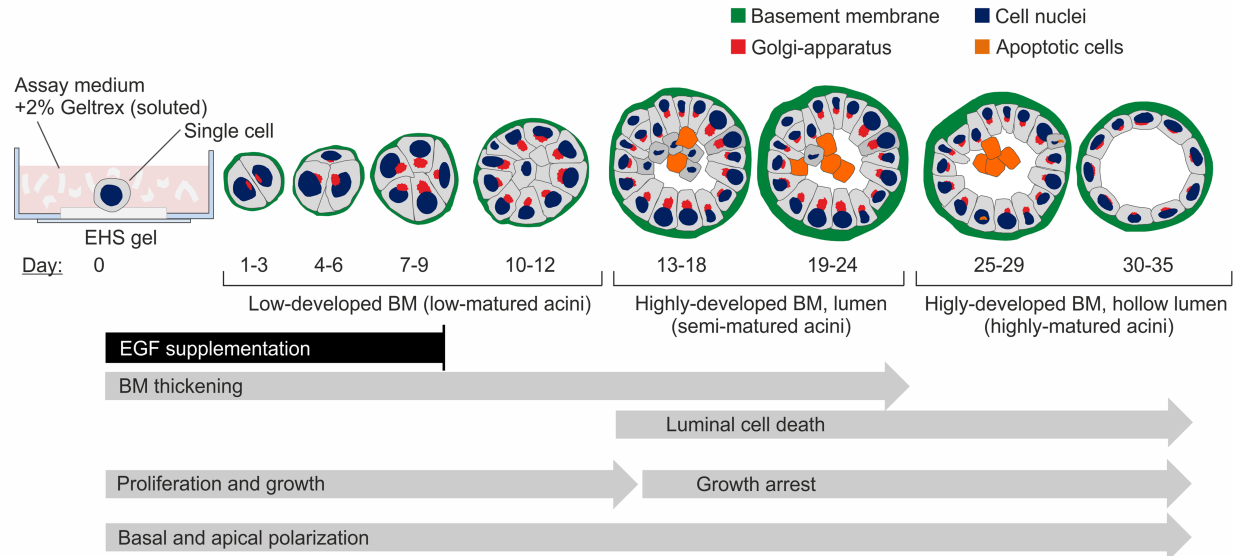


Studio del processo di morfogenesi in colture cellulari 3D





B

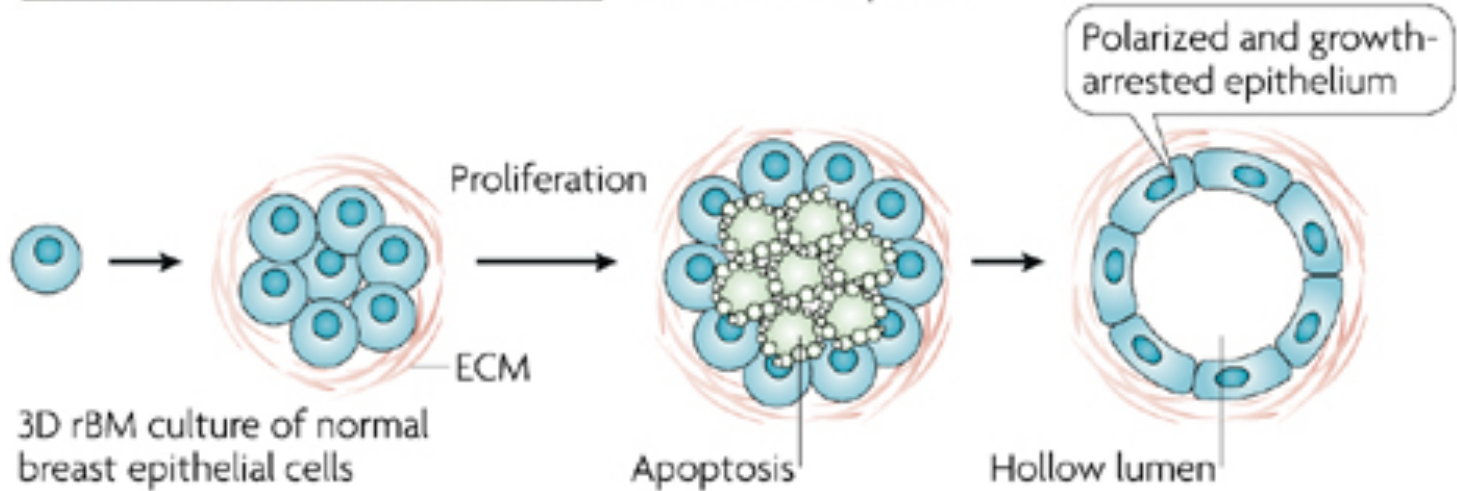


Alterazione della morfogenesi in colture cellulari 3D

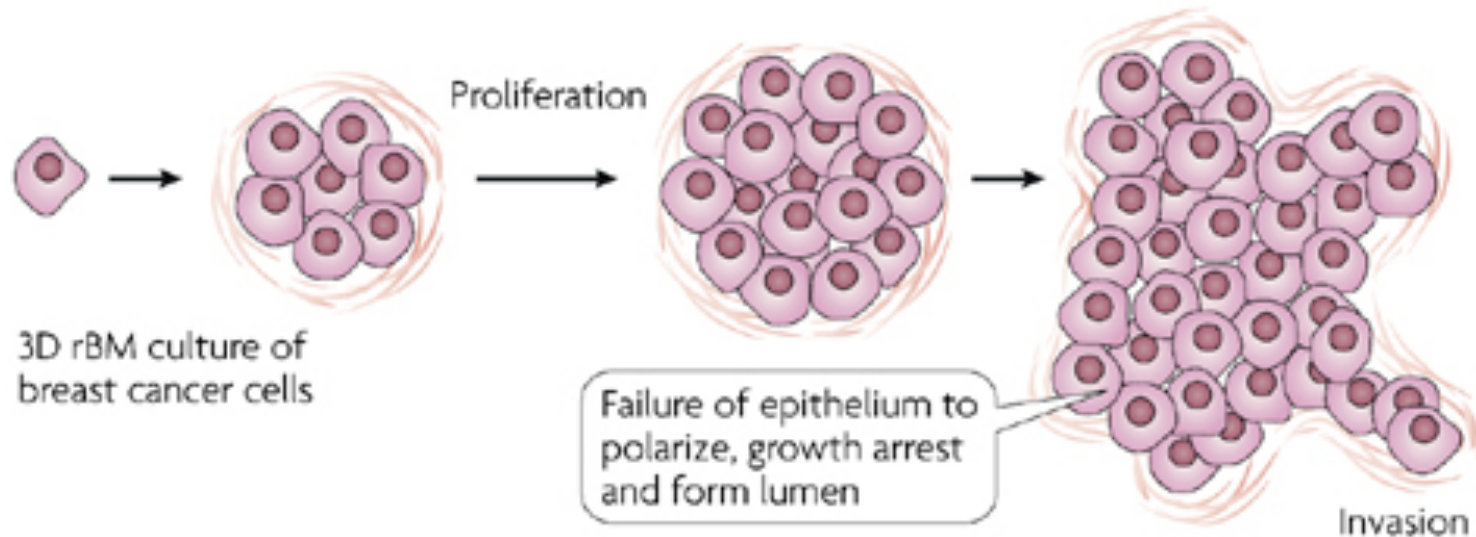


Cells in monolayer on tissue culture plastic

b



3D rBM culture of normal breast epithelial cells

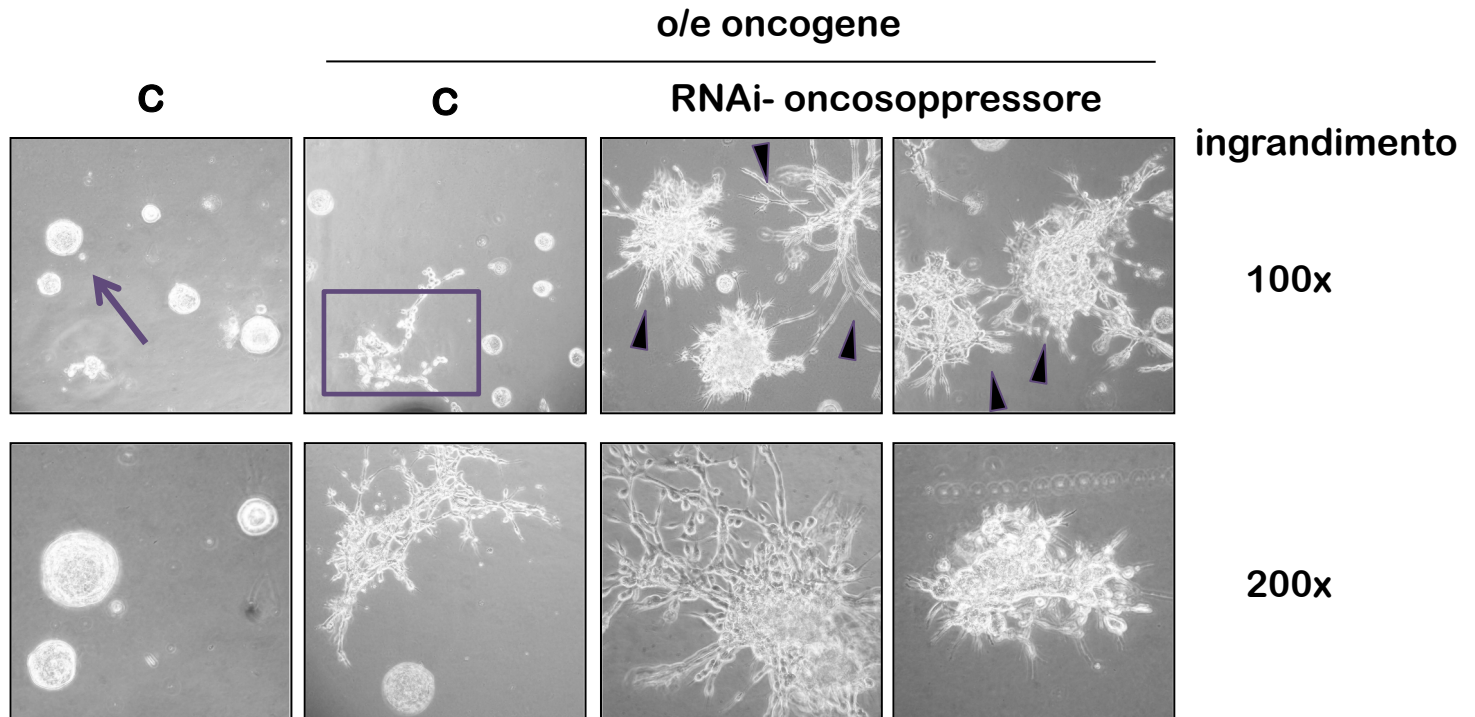


3D rBM culture of breast cancer cells

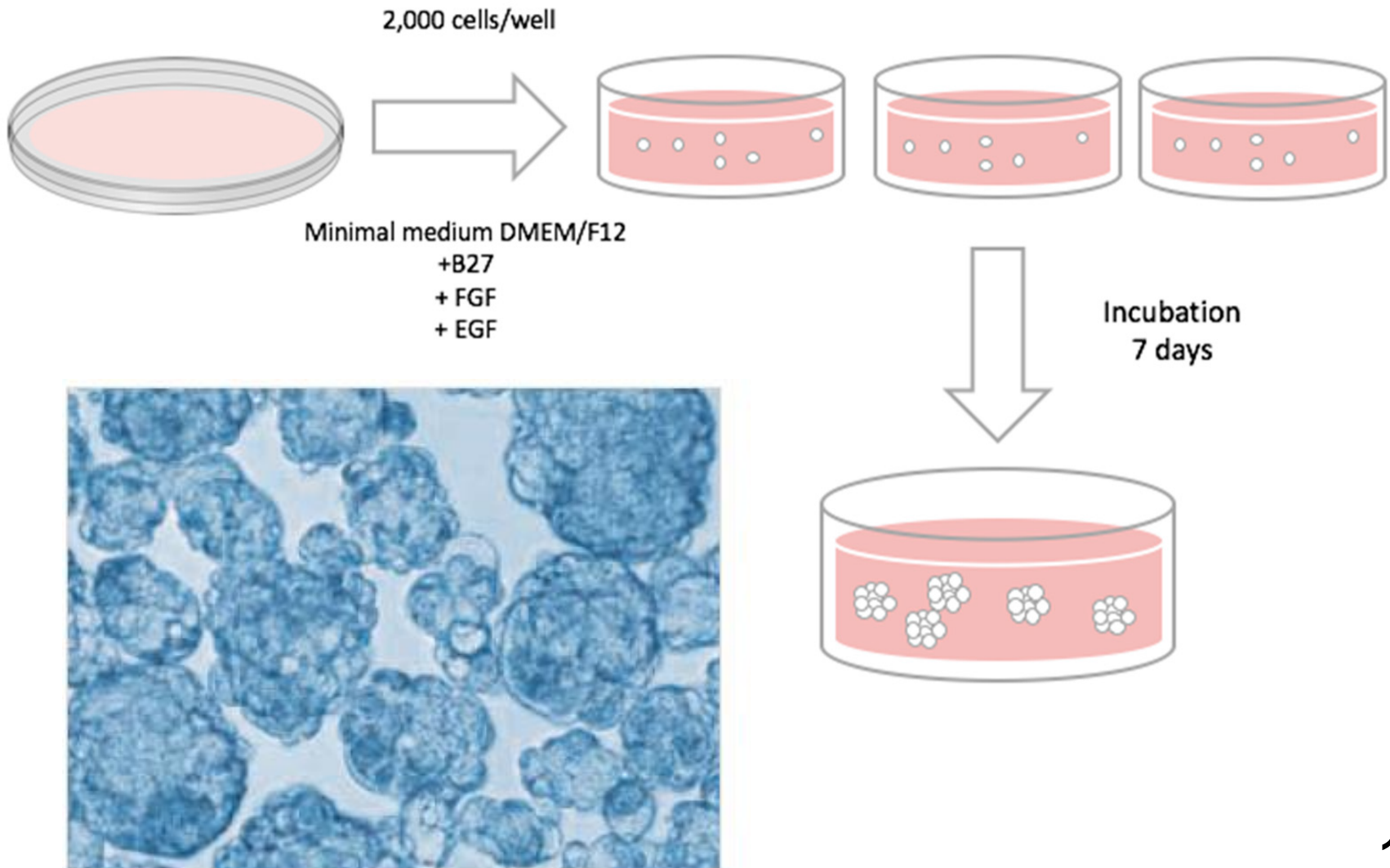
Failure of epithelium to polarize, growth arrest and form lumen

Invasion

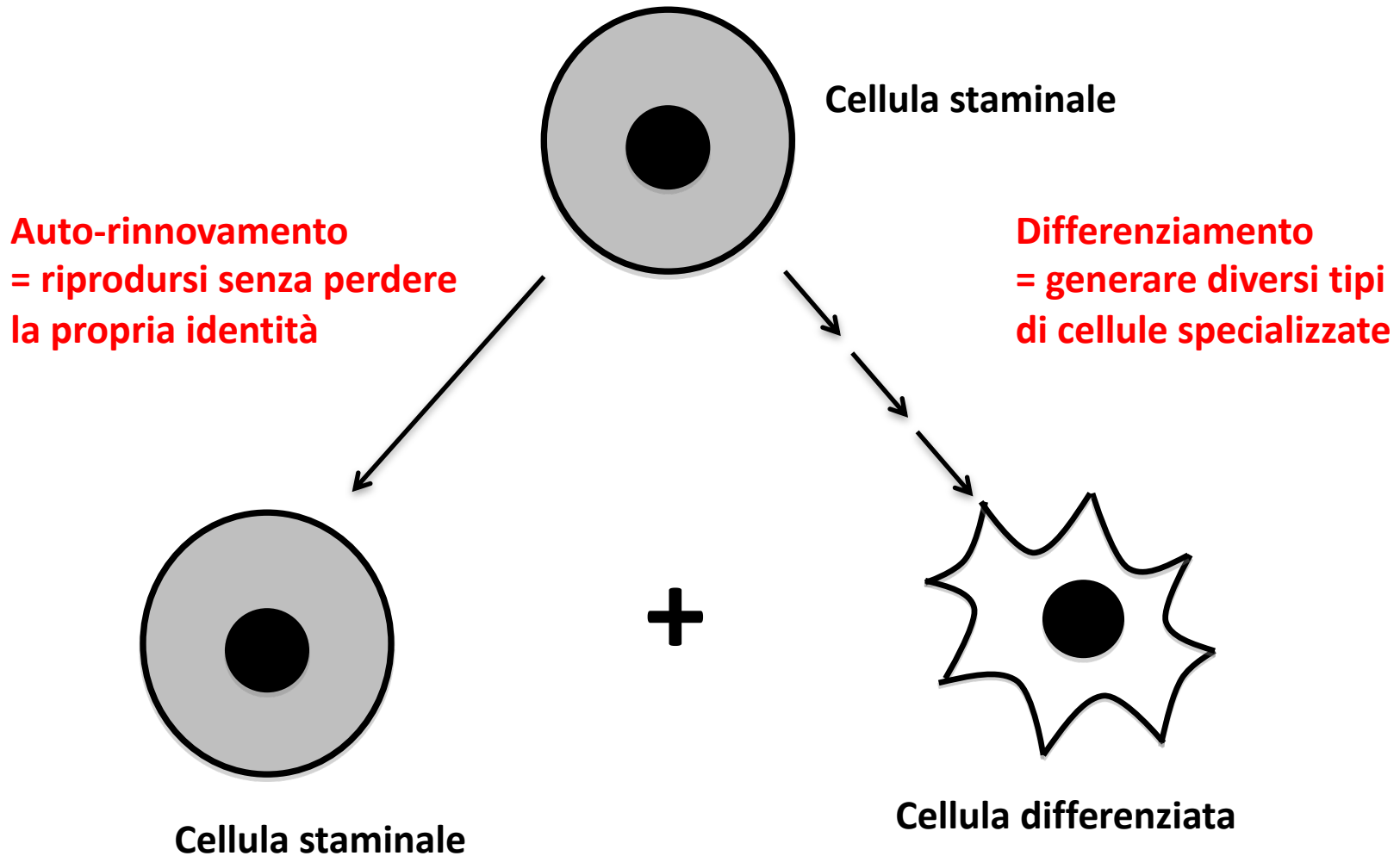
Morfogenesi di cellule epiteliali in coltura 3D



Saggi di FORMAZIONE DI SFERE per valutare caratteristiche di staminalità



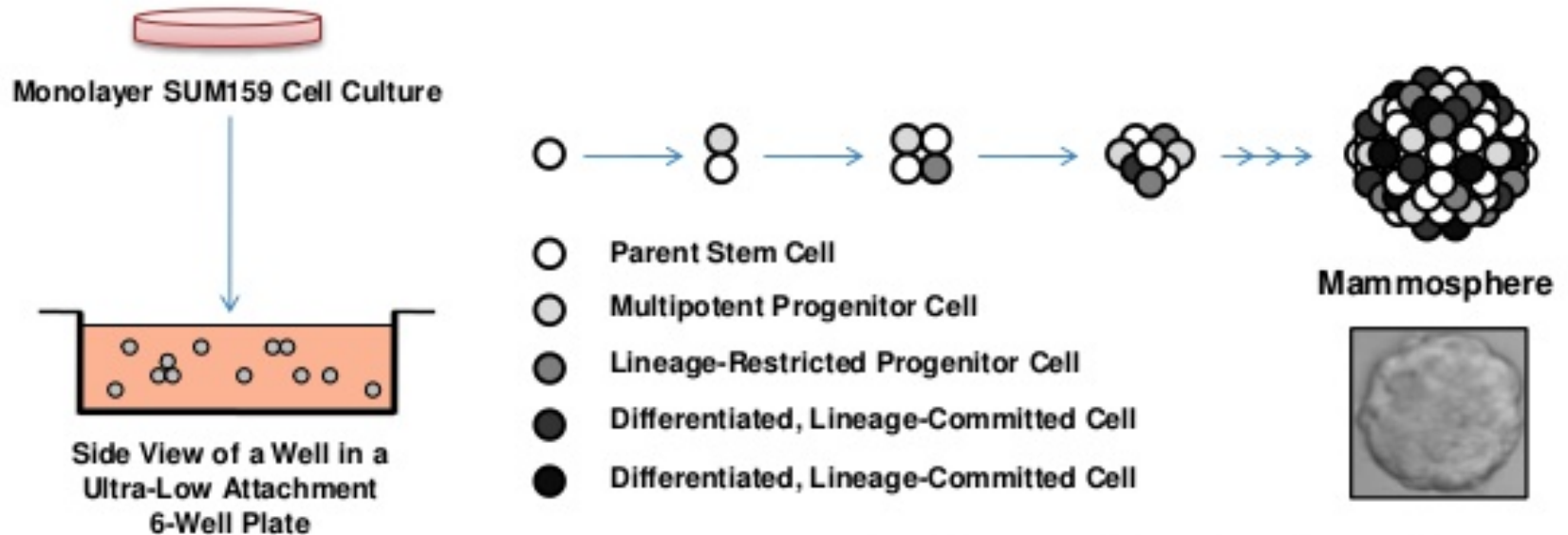
Le CELLULE STAMINALI hanno proprietà di AUTORINNOVAMENTO e DIFFERENZIAMENTO



Saggi di FORMAZIONE DI SFERE per valutare caratteristiche di staminalità

The Mammosphere Formation Assay

A non-adherent *in vitro* colony-forming assay used for the quantification of stem cell/early progenitor activity and stem cell self-renewal...



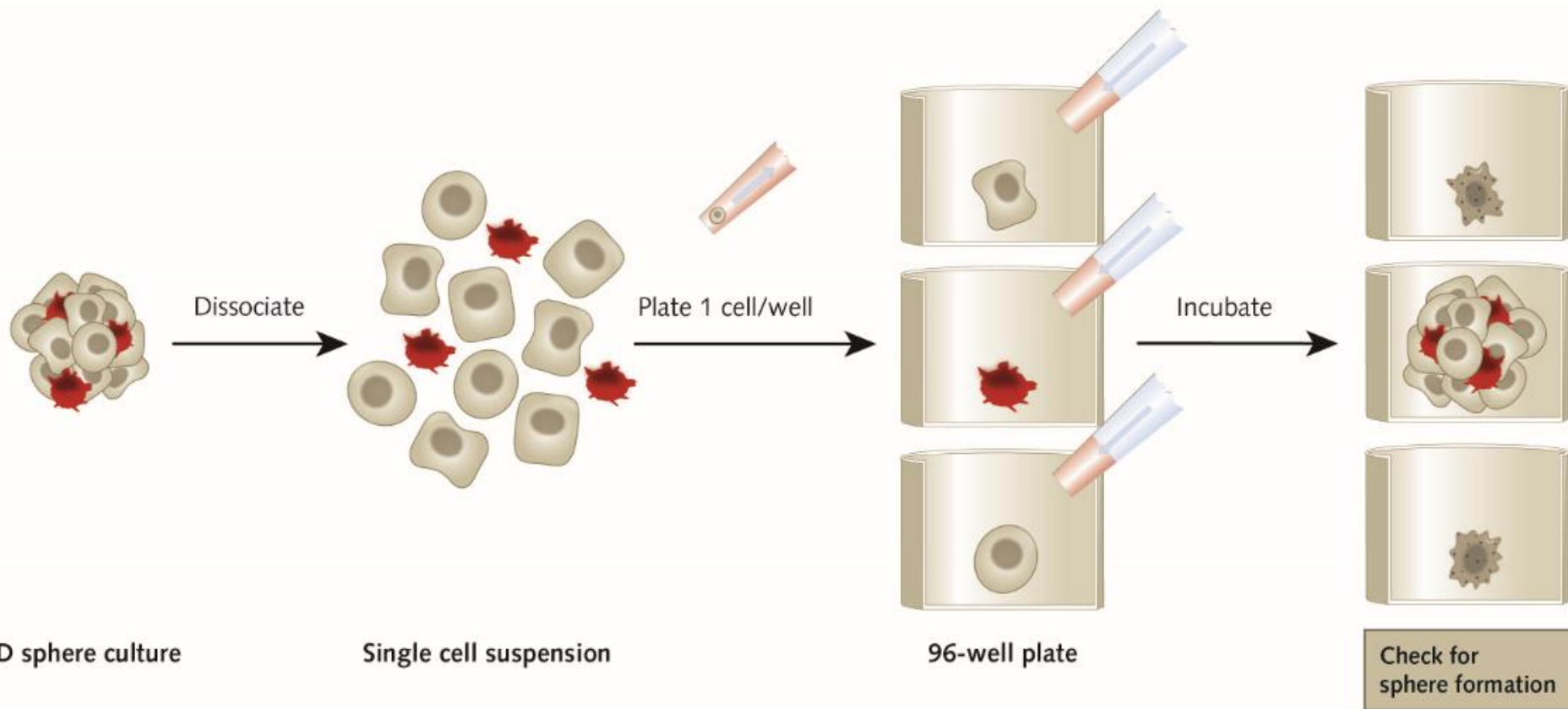
$$\text{Mammosphere-Forming Efficiency (MFE)} = \frac{\text{number of mammospheres formed per well}}{\text{number of cells plated per well}} \times 100$$

Figure obtained with permission from Jeffrey Yang

Saggi di FORMAZIONE DI SFERE per valutare caratteristiche di staminalità

- To obtain mammospheres, non-transformed MCF-10A cells (derived from normal mammary epithelium) are enzymatically disaggregated to single cell suspension, passed through a 40 μm cell strainer and plated in low attachment 24-multi-well plate at clonogenic density, which is 2500 cells/well.
- Cells are grown for 7-10 days in non-adherent culture conditions in MEM supplemented with 0.5 $\mu\text{g/ml}$ hydrocortisone, 5 $\mu\text{g/ml}$ Insulin, 4 g/ml heparin, 20 ng/ml bFGF and 20 ng/ml EGF.
- Primary mammospheres are collected and enzymatically disaggregated as above to re-plate cells at clonogenic density to obtain secondary mammospheres.
- Mammospheres are counted with a 20X objective on an optical microscope. Percentages of secondary mammosphere forming efficiencies (%M2FE) are calculated as number of obtained mammospheres divided by the cell number and multiplied by hundred.

Saggi di FORMAZIONE DI SFERE per valutare caratteristiche di staminalità

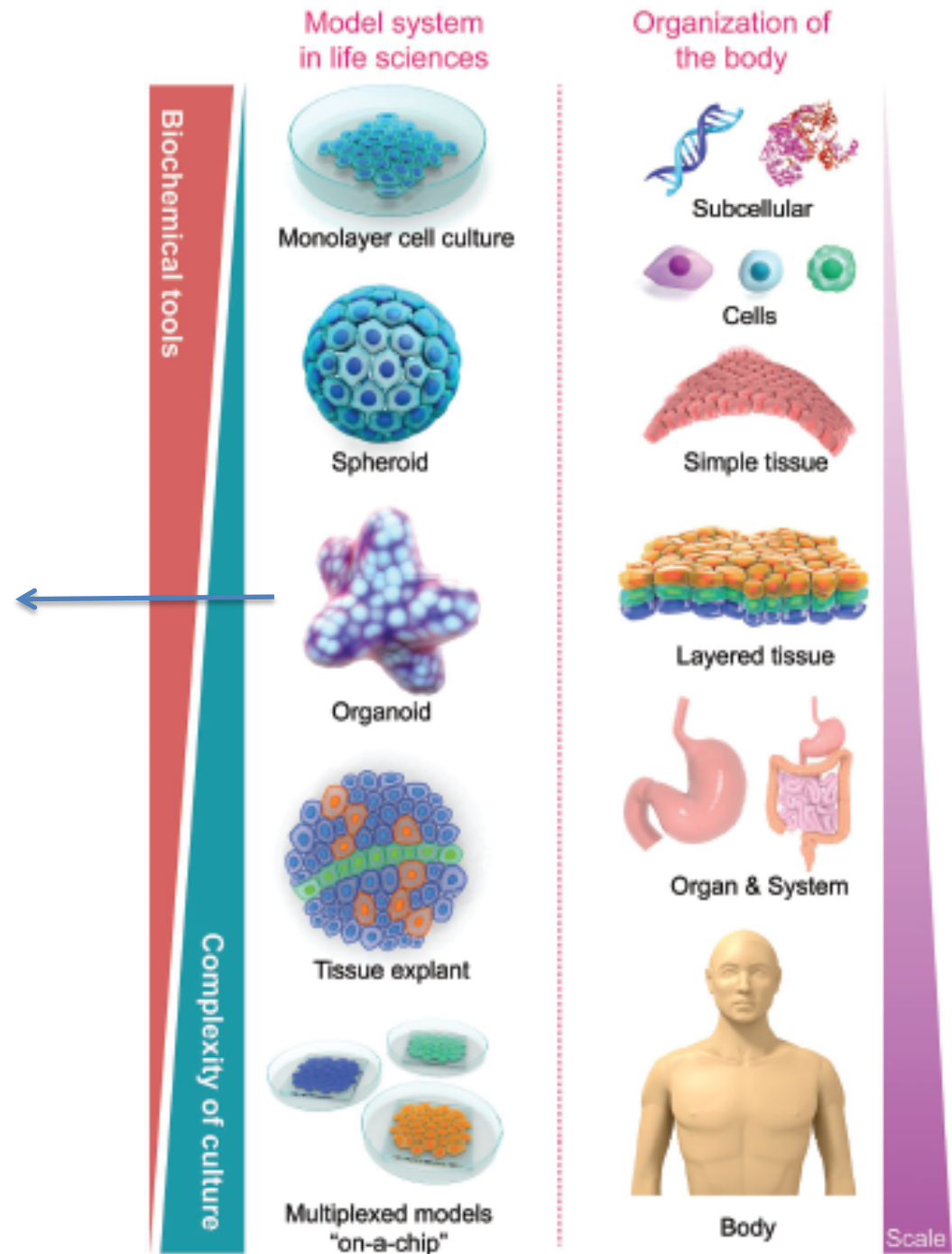


Colture ORGANOTIPIICHE o ORGANOIDI

VANTAGGI:

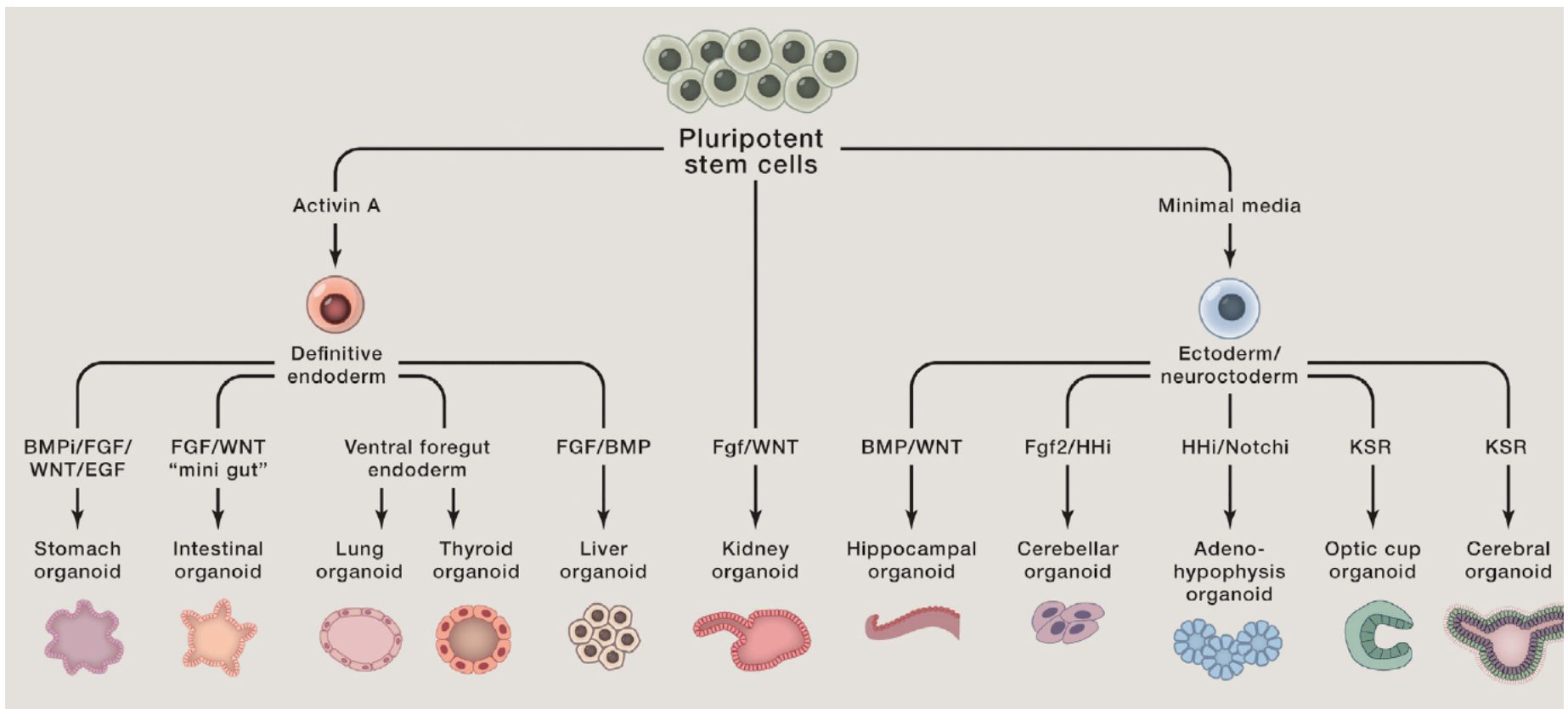
STRUTTURE CHE
RICAPITOLANO
L'ORGANIZZAZIONE DELLE
CELLULE NEI TESSUTI E LE
FUNZIONI TISSUTALI

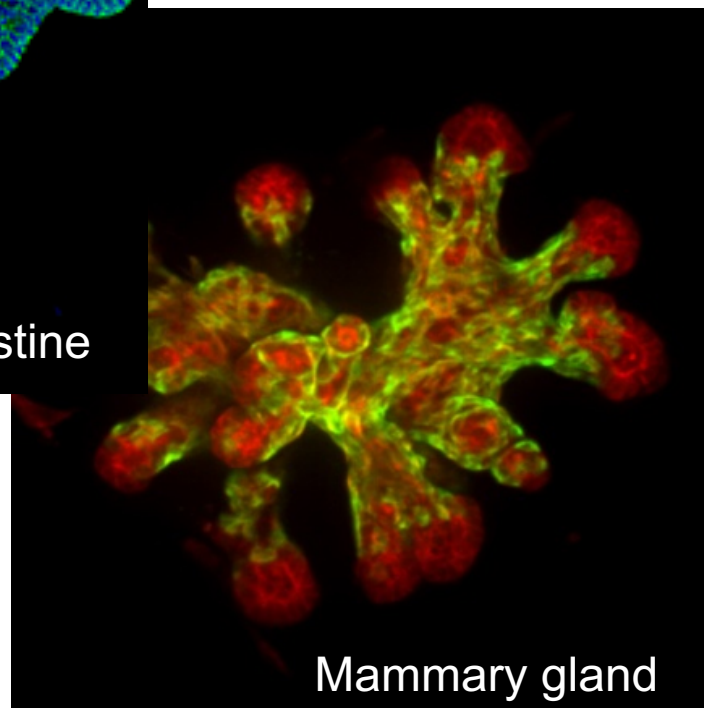
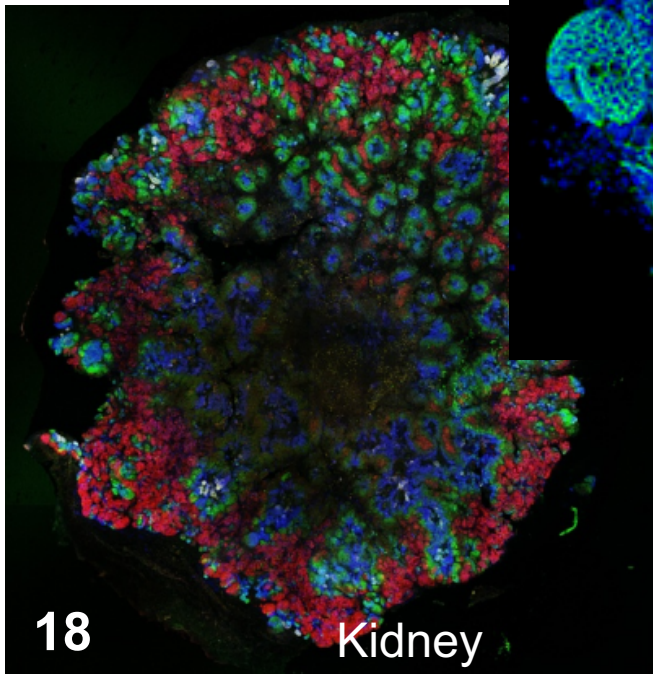
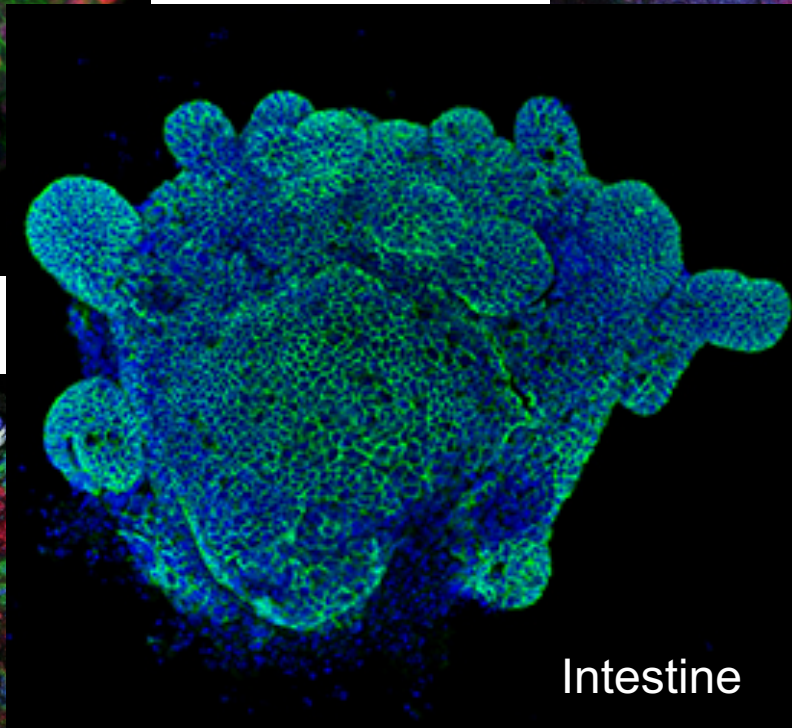
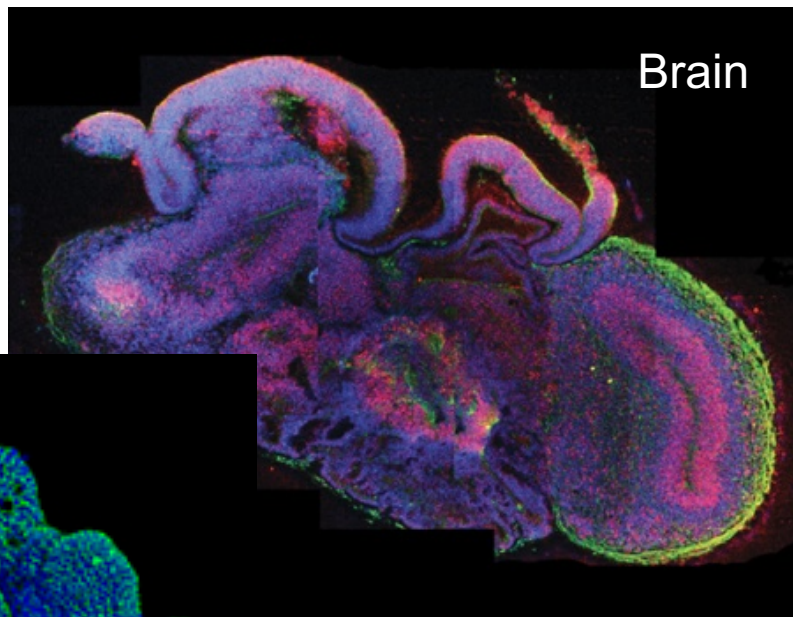
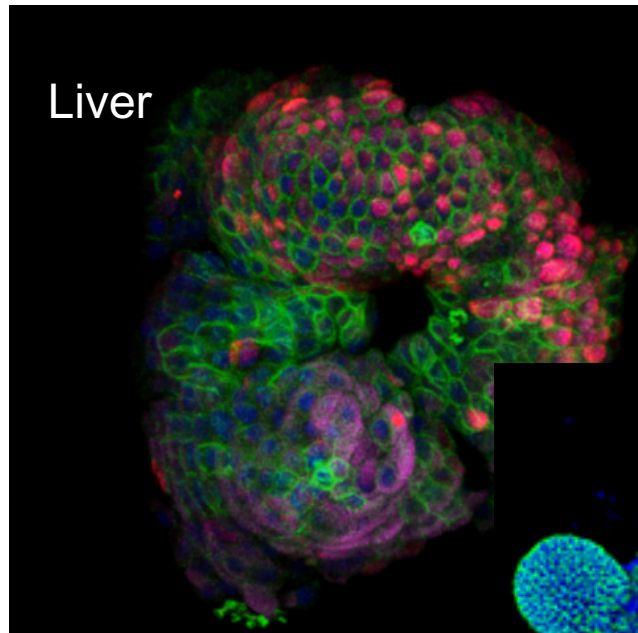
LIMITI:
SCARSITA' DI MATERIALE
VARIABILITA'



COLTURE ORGANOTIPIICHE o ORGANOIDI

Strutture 3D che si sviluppano e differenziano a partire da cellule staminali; consistono di tipi cellulari caratteristici di un organo derivati da SC di origine che si auto-organizzano attraverso la localizzazione e il differenziamento grazie all'inclusione in ECM e alla presenza di fattori solubili della nicchia staminale

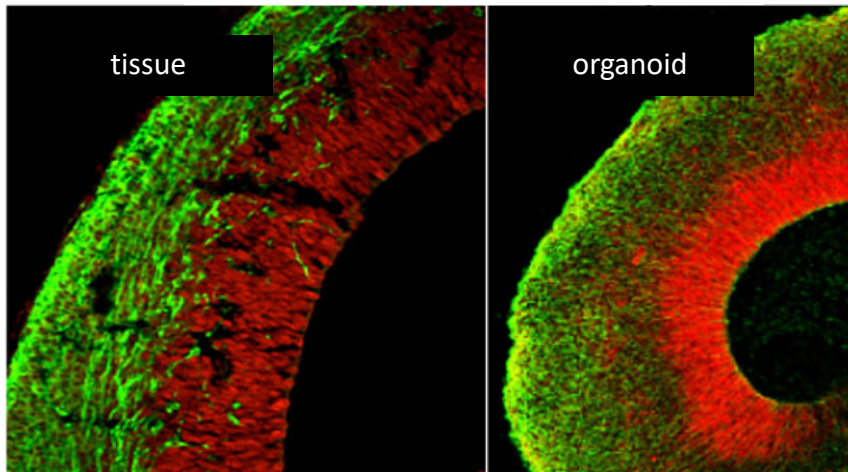




COLTURE ORGANOTIPICHE o ORGANOIDI

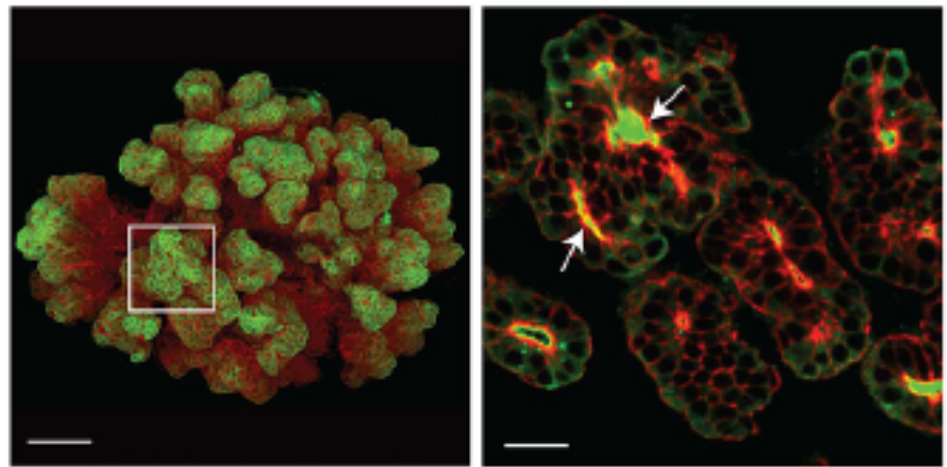
Ricapitolano l'organizzazione e le funzioni del tessuto/organo corrispondente

Brain



Repic & Lancaster , Plos Blogs 2013

Mammary gland



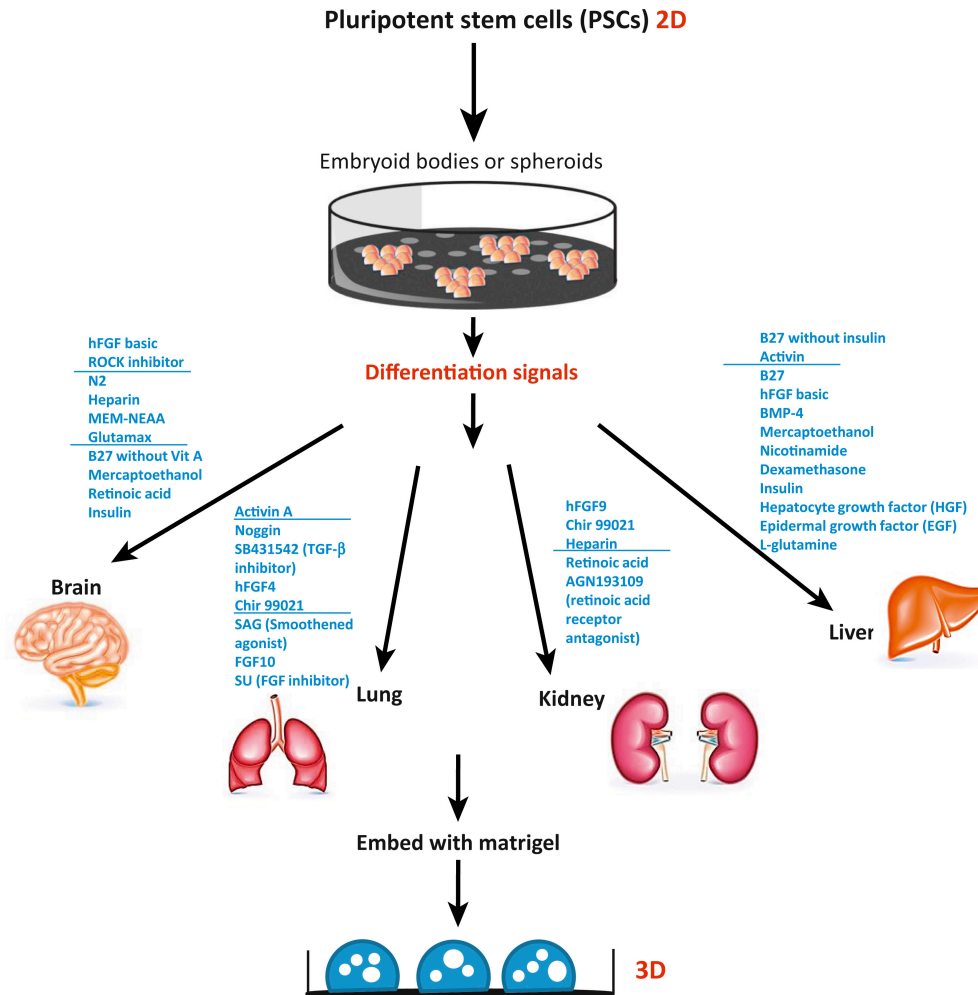
Jamieson et al., Development 2017

VERIFICA DELLA FUNZIONALITÀ:
Organoidi di mammella stimolati con l'ormone prolattina secernono protein del latte nel lume

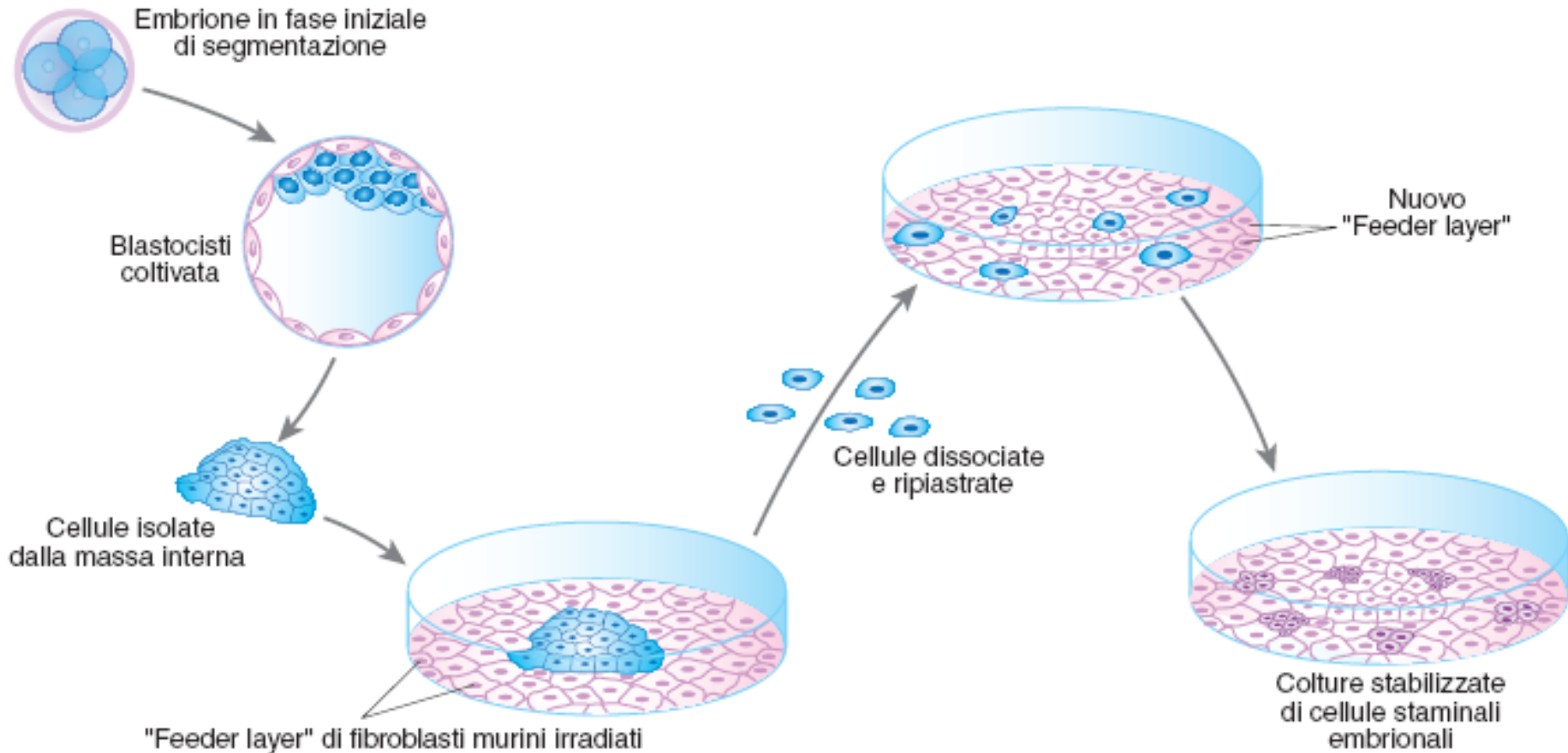
Generazione di ORGANOIDI

Gli organoidi possono essere derivati da 2 TIPI DI SCs

- a) Cellule staminali pluripotenti (PSCs) = embryonic stem cells (ESCs) o iPSCs
- b) ASCs = cellule staminali adulte residenti tessuto-specifiche



Cellule staminali EMBRIONALI (ES)

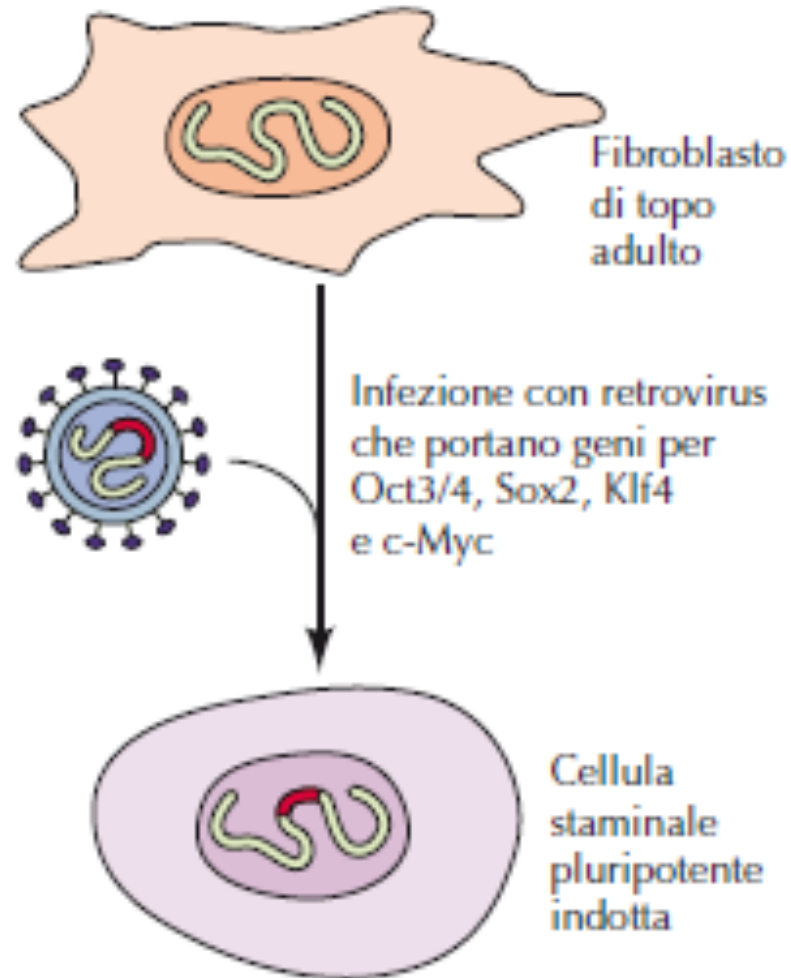


◆ FIGURA 18.2

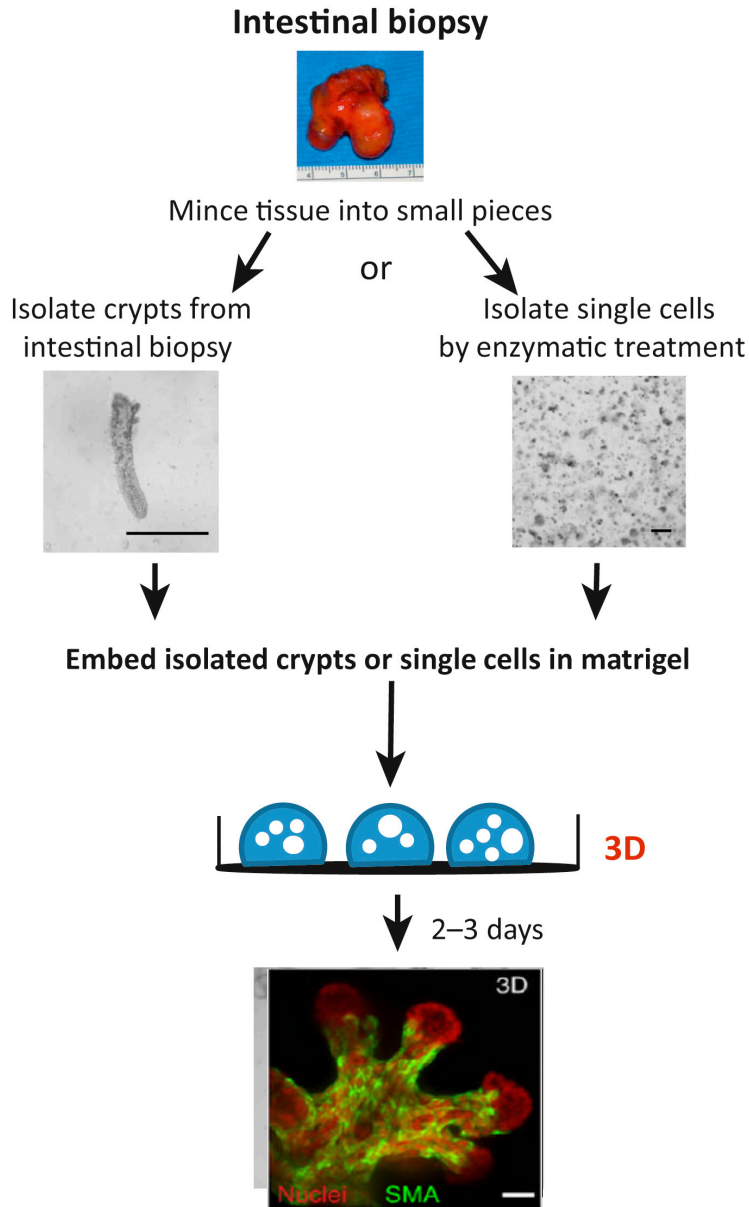
Generazione di cellule staminali embrionali (cellule ES) dalla blastocisti.

iPS: cellule staminali pluripotenti indotte

S. Yamanaka + J. Gurdon: premio Nobel per la Medicina 2012



generazione di ORGANOIDI da TESSUTI ADULTI (ASCs)



MATERIALE DI ORIGINE:

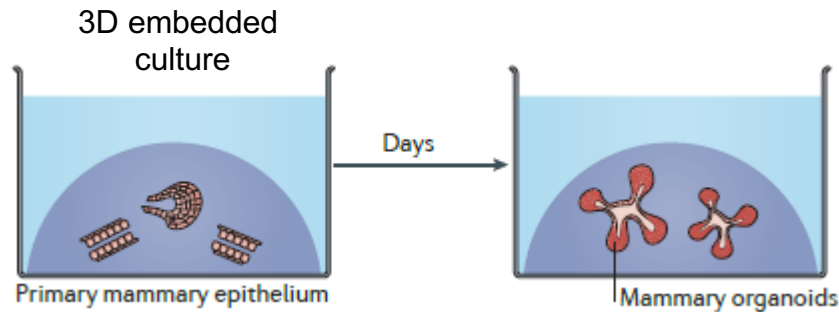
-ASCs singole (dissociate/purificate)

-Porzioni di tessuto (in alcuni casi le ASCs necessitano di cellule di supporto per la crescita)

<https://vimeo.com/222833601>

COLTURE di ORGANOIDI

Le cellule sono cresciute in matrici 3D che mimano la matrice del tessuto di origine e ricoperte con un terreno di coltura contenente specifici fattori di crescita e differenziamento



Shamir & Ewald., Nat Rev MCB 2014

Matrix:

- matrigel
- Specific matrix components



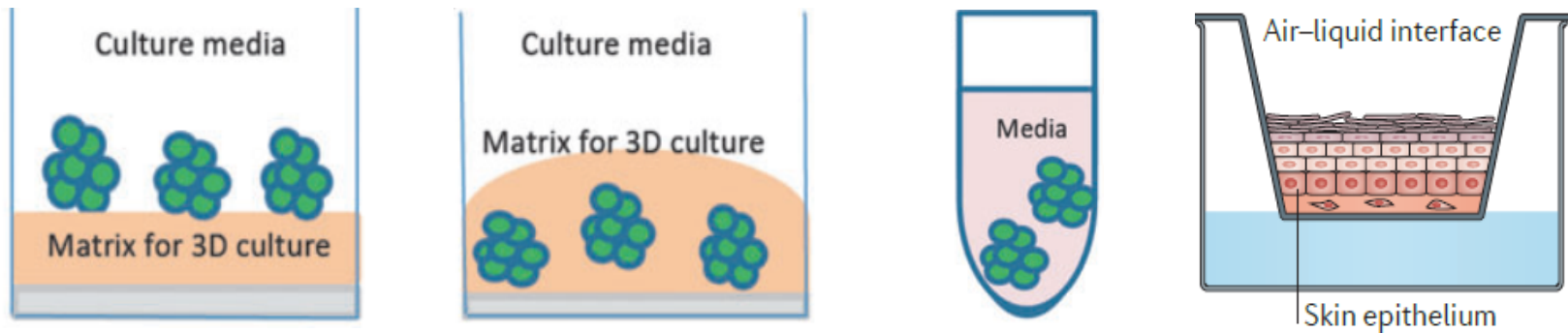
	HuBiogel ^{TM1}	Matrigel ²
Total Protein (mg/ml)	5-8	10-15
Laminin	260 +/- 24	810
Collagen I	478 +/- 32	n.s.
Collagen IV	312 +/- 18	450
Entactin	164 +/- 20	120
Tenascin	68 +/- 6	n.s.
Fibronectin	18 +/- 4	n.s.
Proteoglycans	14 +/- 6	25
EGF	None	0.7 ng
TGF-B	None	3 ng
bFGF	None	0.2 pg
PDGF	None	12 pg
VEGF	None	16 ng
Collagenases	None	detectable

Medium:

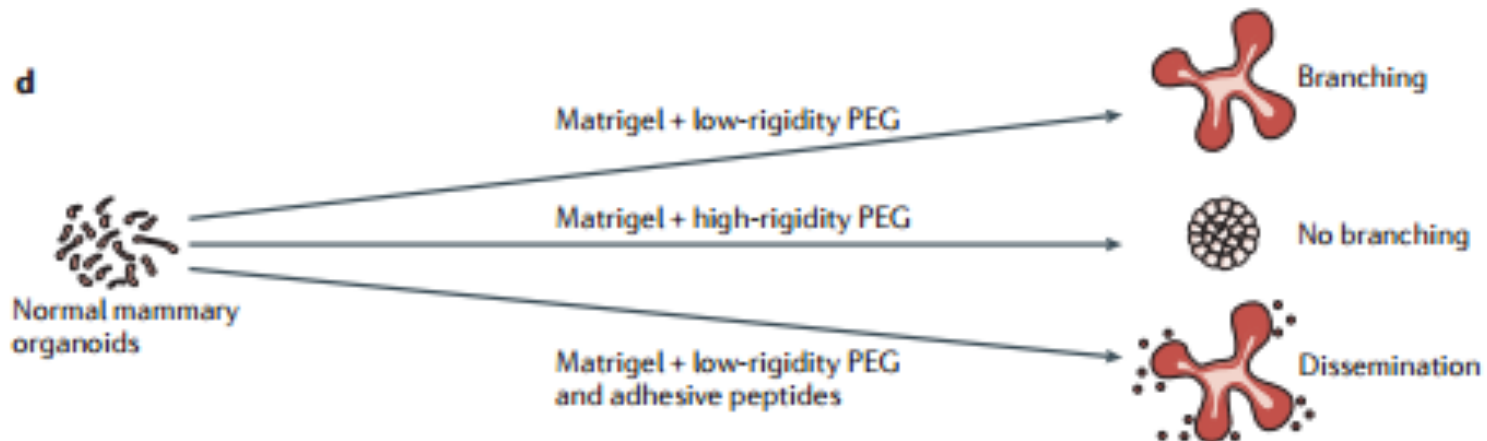
- Specific growth factors (niche): e.g. FGF, EGF, activators of WNT and NOTCH pathway



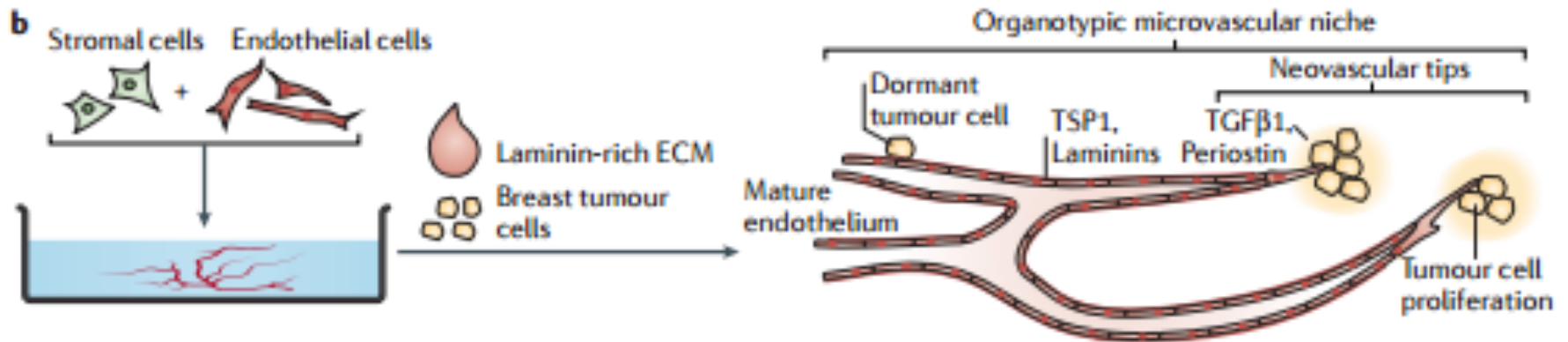
COLTURE di ORGANOIDI: diverse modalità di coltura



Ricostruzione di fenotipi complessi: manipolazione della ECM



Ricostruzione di fenotipi complessi: co-culture di diversi tipi cellulari



Ricostruzione di fenotipi complessi: fusione o co-coltura di organoidi di diversi tessuti

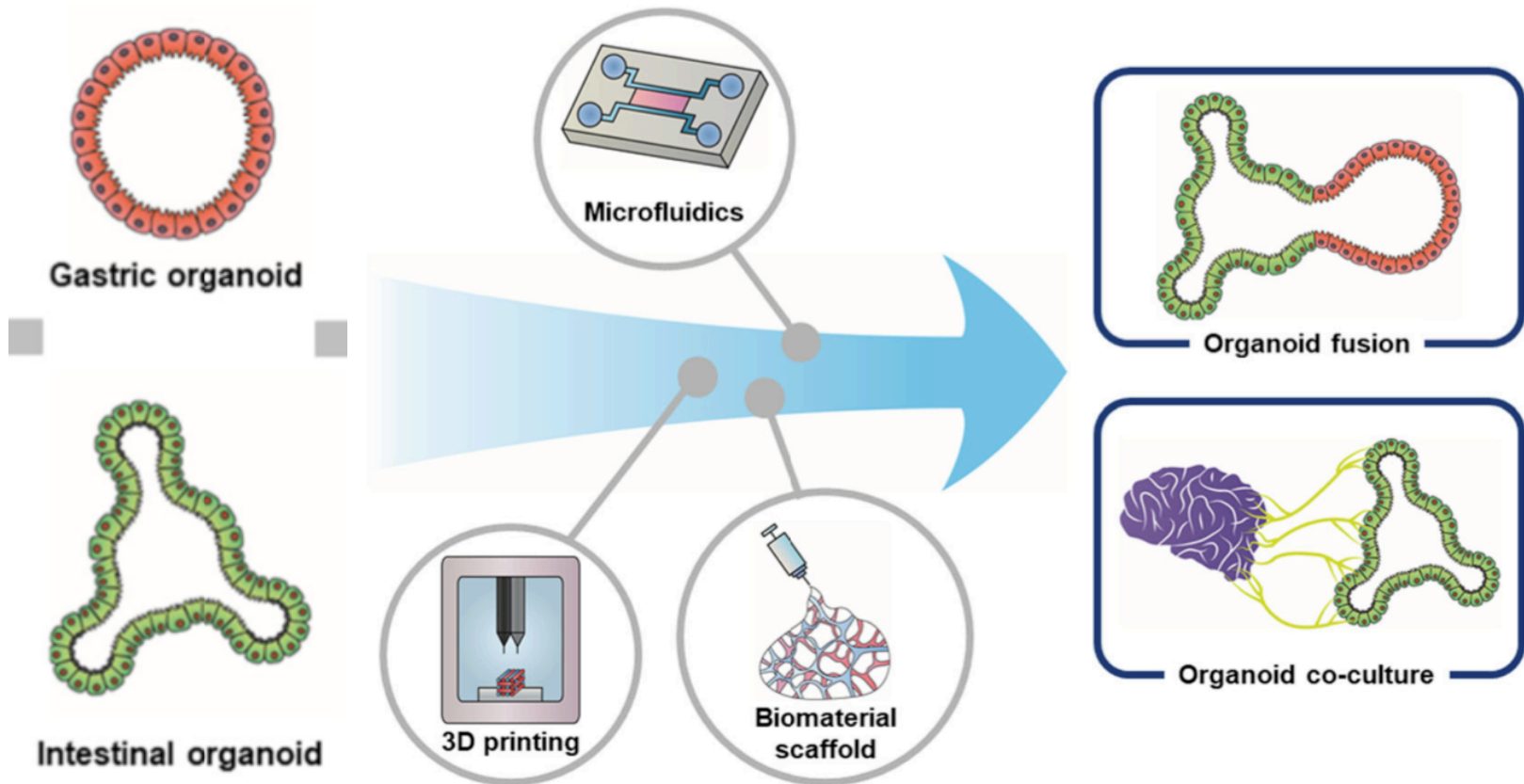


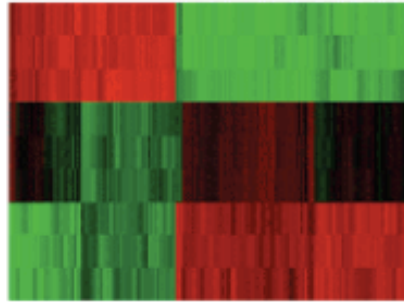
Fig. 3 Bioengineered platforms for developing GI organoids assembled with other types of organoids. Through bioengineering techniques such as 3D bioprinting and the use of microfluidics and biomaterials, a novel integrated GI system can be developed by connecting the various organoids that make up the digestive system or by coculturing with completely different types of organoids such as brain organoids. These innovative organoid systems enable the modeling of highly complex human physiology, such as reciprocal regulation between the GI tract and other major organs, and pathological phenomena associated with diseases.

Applicazioni degli organoidi in biomedicina

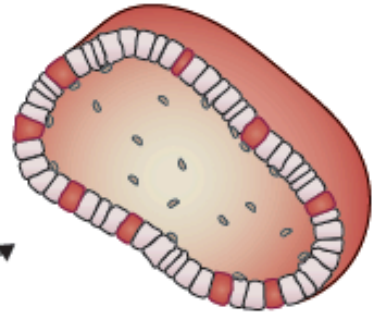
b Patient-derived tissue subunits



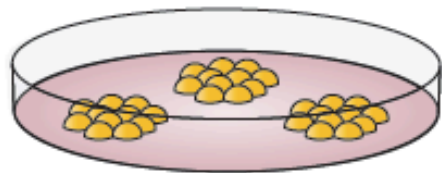
c Omics profiling



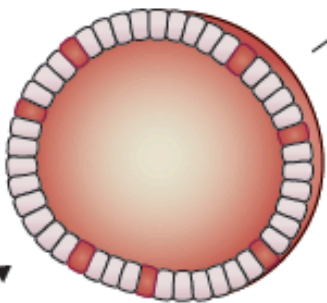
d Study host-microbe interaction



a



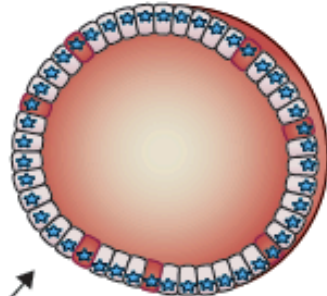
ESCs/iPSCs



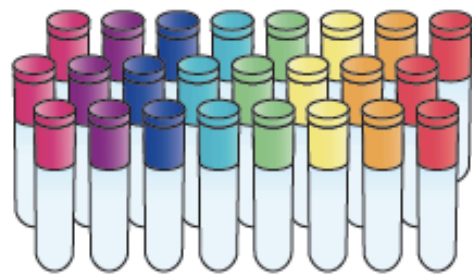
e Gene editing (e.g. CRISPR)



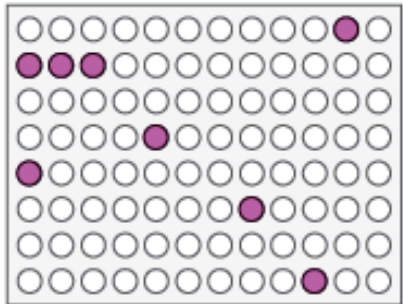
Targeted correction of mutations



g Biobank for academic studies

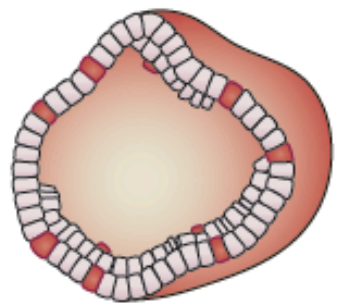


f High-throughput drug screening



● Potential lead

Disease modelling



Applicazioni: inserimento di mutazioni sequenziali in organoidi per il modeling in vitro della progressione tumorale



nature

Article | Published: 29 April 2015

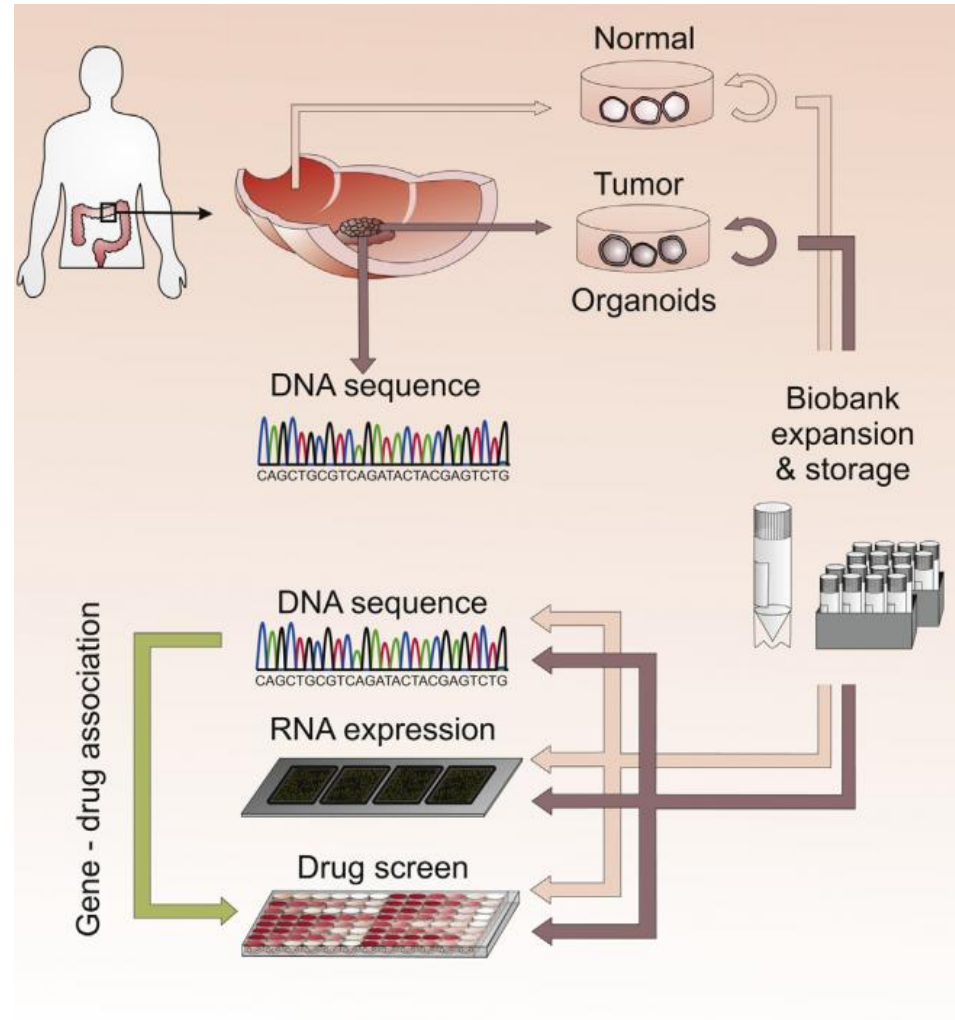
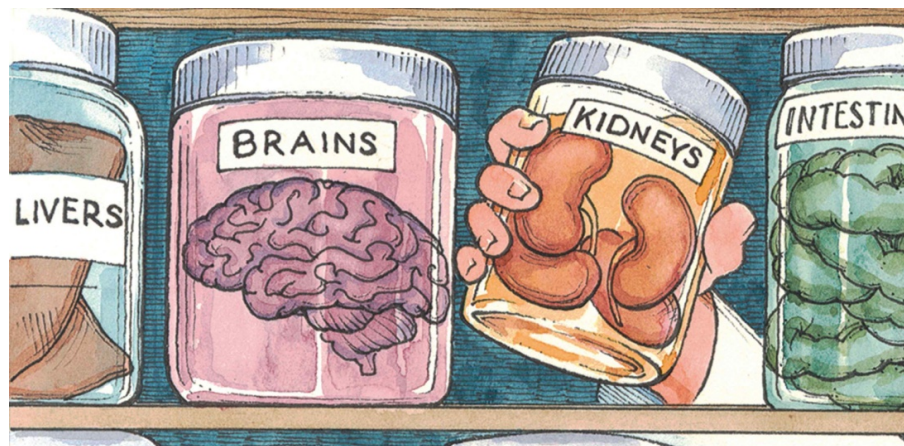
Sequential cancer mutations in cultured human intestinal stem cells

Jarno Drost, Richard H. van Jaarsveld, Bas Ponsioen, Cheryl Zimberlin, Ruben van Boxtel, Arjan Buijs, Norman Sachs, René M. Overmeer, G. Johan Offerhaus, Harry Begthel, Jeroen Korving, Marc van de Wetering, Gerald Schwank, Meike Logtenberg, Edwin Cuppen, Hugo J. Snippert, Jan Paul Medema, Geert J. P. L. Kops & Hans Clevers

Nature **521**, 43–47(2015) | [Cite this article](#)

https://www.youtube.com/watch?time_continue=22&v=wePGtHv5JQs&feature=emb_title

Applicazioni: biobanche di organoidi da pazienti per lo studio di patologie umane



Applicazioni: organ-on-chip

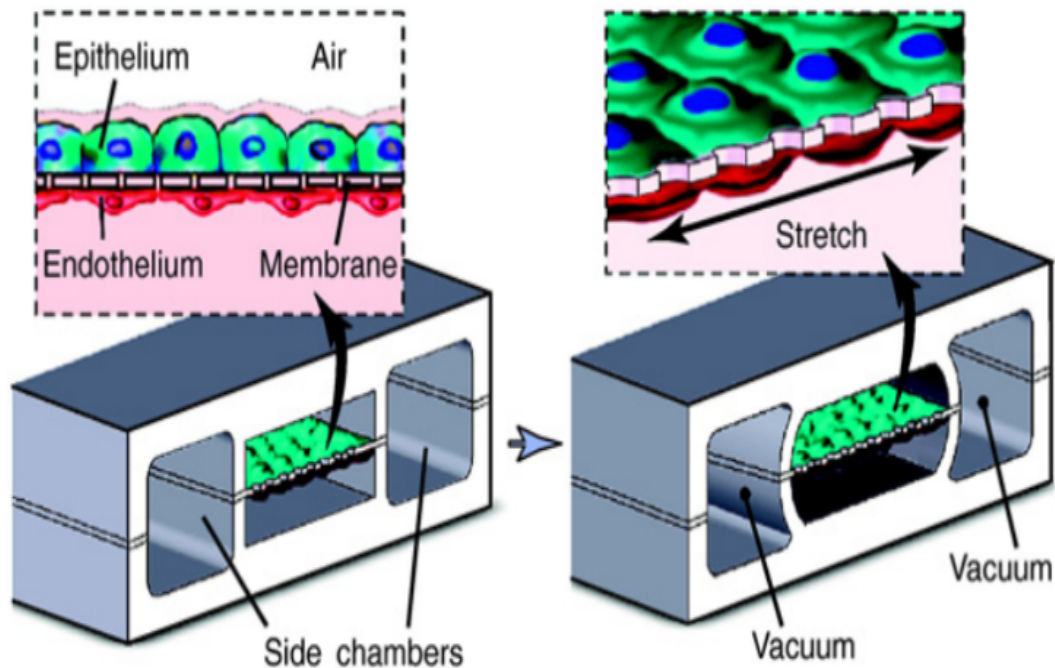
Dispositivi microfluidici

composti da camere perfuse in maniera continua

Interfacce tessuto-tessuto realizzate con membrane semipermeabili

Simulano l'organizzazione e la funzione a livello di organo e multi-organo

Consentono real-time imaging



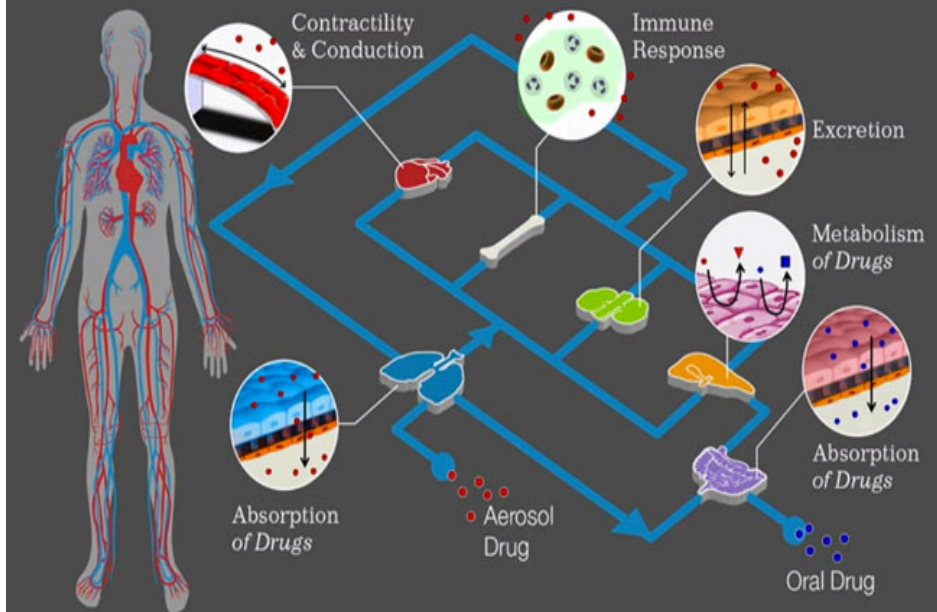
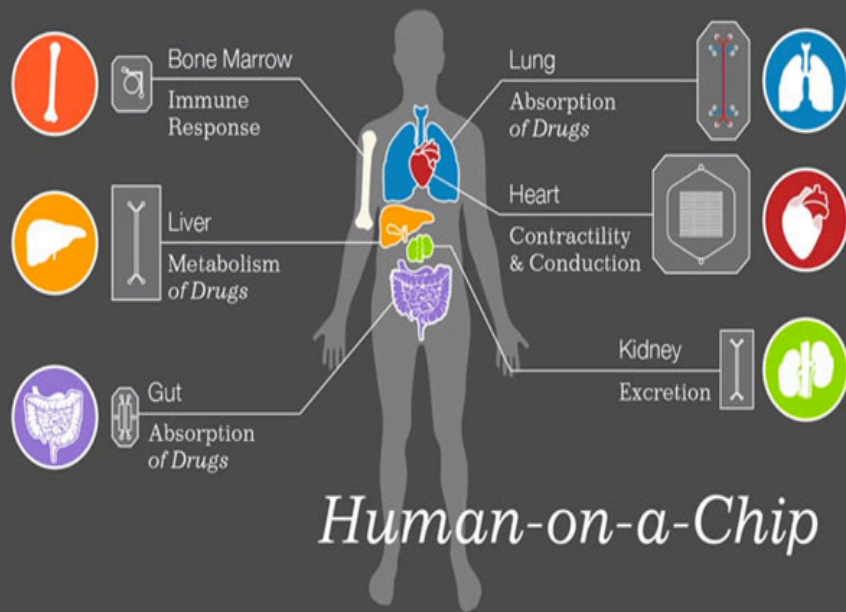
Applicazioni: human-on-chip

Dispositivi microfluidici

Composti da più camere perfuse in maniera continua

Interfacce tissutali realizzate con membrane

Simulano l'organizzazione e la funzione a livello di organo e multi-organo



Colture cellulari per lo studio di virus respiratori

Lo studio dei meccanismi di infezione da parte di virus che hanno tropismo per l'epitelio respiratorio (tra cui il SARS-CoV-2) fa comunemente uso di colture di cellule VeroE6 (African green monkey kidney cells ATCC-1586). Lo stesso Sistema viene comunemente utilizzato per testare terapie dirette contro l'infezione e la replicazione virale.

L'utilizzo di modelli cellulari derivati dall'epitelio respiratorio umano e quanto più possibile differenziati è tuttavia preferibile.

Ad esempio, colture 2D di cellule primarie bronchiali umane, organoidi umani di origine polmonare (o bronchiale) umana, colture primarie ALI (Air-Liquid Interface) Cultures

Air-Liquid Interface (ALI) Cultures

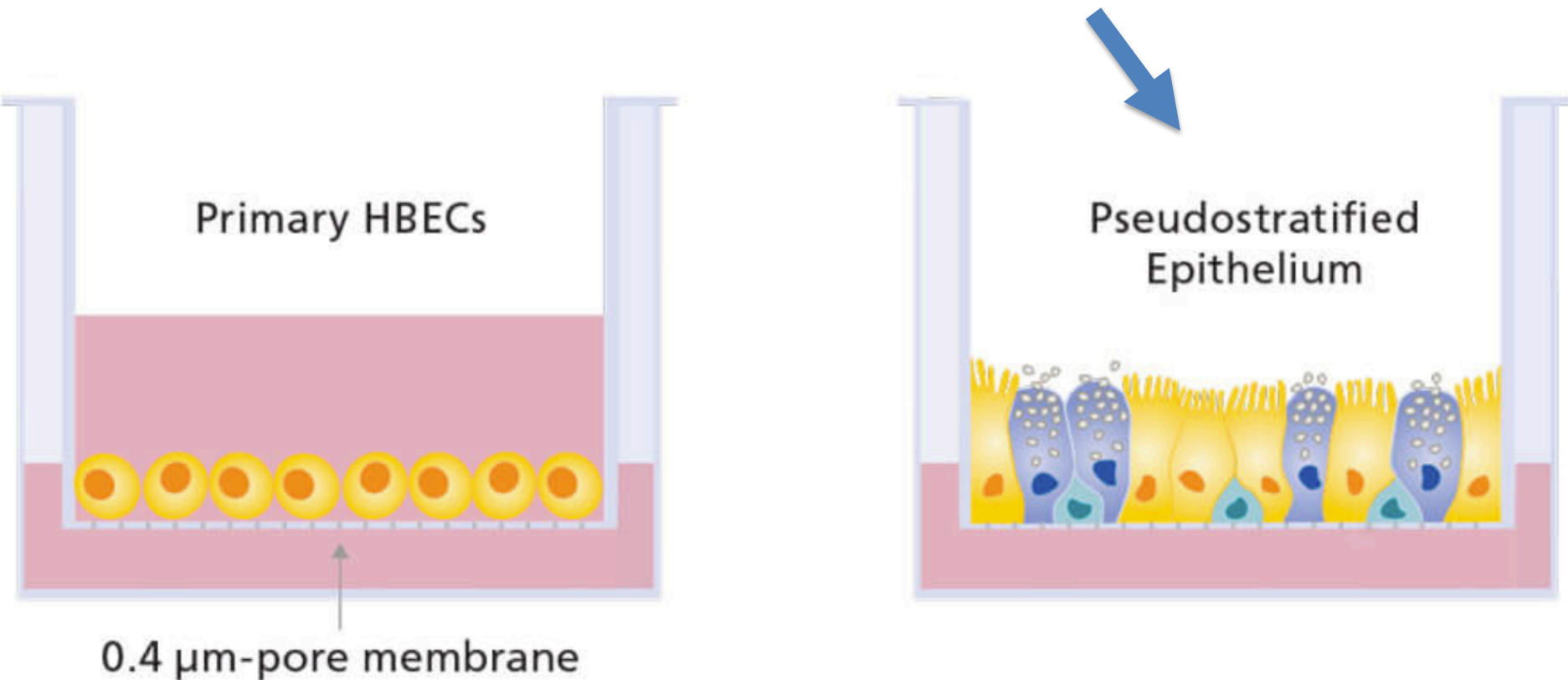
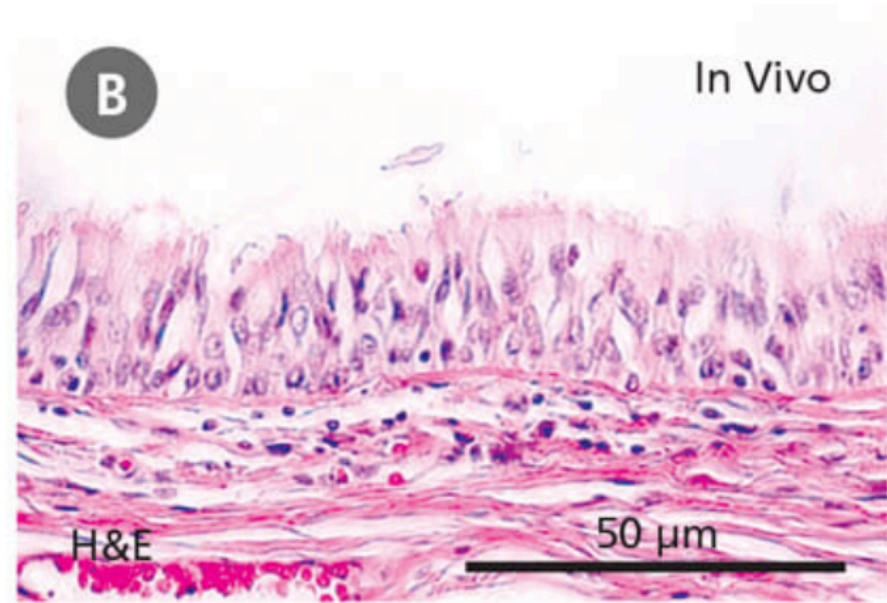
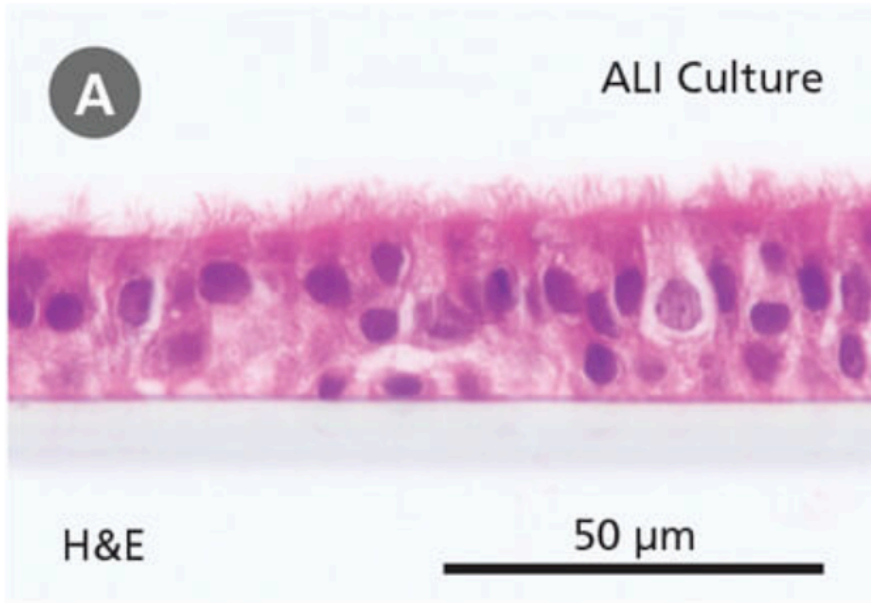


Figure 2. Air-Liquid Interface Culture

Schematic of submerged (A) and air-liquid interface (B) culture of primary human bronchial epithelial cells grown using porous culture inserts.

- Submerged culture of primary HBECs is possible; however, cells in this system fail to undergo mucociliary differentiation and thus do not accurately represent the in vivo airway.
- ALI culture of primary human airway cells is uniquely suited as a model for in vitro infection studies. This is due to the ability of ALI cultures to faithfully recapitulate key characteristics of the in vivo airway. For example, ALI cultures exhibit **relevant proportions of airway cell types, appropriate cellular polarization and junctional properties, dynamic physiologic processes such as mucus secretion and coordinated ciliary beating, and physiological expression and subcellular localization of characteristic proteins bearing species-specific sequences.** Depending on the infection mechanism of the virus under study, each one of these properties can be a crucial feature of the model system. Human airway ALI cultures allow for efficient infection of cultured cells and have been used to model various mechanisms of viral pathogenesis and human disease. This includes investigating protein interactions during intracellular replication,¹ factors impacting viral entry into cells,² and infection kinetics.³ The utility of ALI cultures for studying viral infection represents a valuable tool for developing antiviral therapeutic approaches;⁵ viral neutralization by monoclonal antibodies has also been tested in this model.⁸
- The defining feature of ALI culture is that the basal surface of the cells is in contact with liquid culture medium, whereas the apical surface is exposed to air. A common approach is to seed cells onto the permeable membrane of a cell culture insert, which is initially supplied with culture medium to both the apical and basal compartments (Figure 2A). Once confluence is reached, the cells are subjected to 'air-lift', where the medium is supplied only to the basal chamber (Figure 2B). This configuration mimics the conditions found in the human airway and drives differentiation towards a mucociliary phenotype. **35**

Colture ALI per lo studio del SARS-CoV-2



Early Studies of the Novel Coronavirus SARS-CoV-2 with ALI Culture

In order to isolate and identify a novel pathogen causing illness in China, researchers infected ALI cultures of pathogen-free human airway epithelial cells with fluid from patients showing symptoms. This human airway infection model allowed the scientists to isolate the virus that was reproducibly infecting human lung cells, sequence its genome, and investigate elements of its pathogenesis with transmission electron microscopy.

Zhu N et al. (2020) [A Novel Coronavirus from Patients with Pneumonia in China](#). *N Engl J Med*. 382(8):727–33