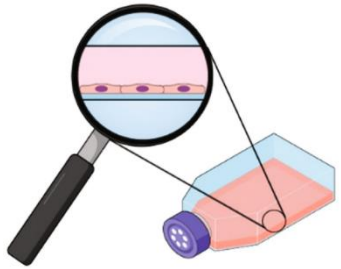
3D cell culture techniques and their limitations

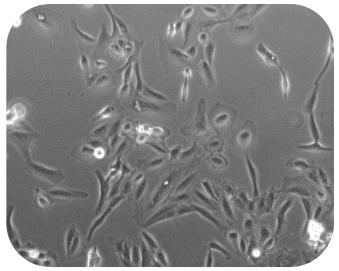
Réunion technique NPhDA – 03/01/2022

Why do we use 3D cell culture techniques ?

➢ 2D cell culture :

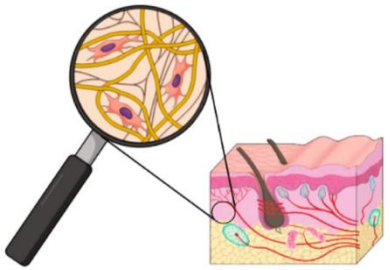
- Easy to handle, **cheap**, quick scaling-up
- Cell morphology : flat and **polarized**, no matter the cell type. Possible loss of structure-function links, membrane surface/cell volume ratio modified.
- Cell-cell and cell-matrix interactions < **cell-plastic and cell-media interactions**
- Flat and homogeneous environment, no gradients, no chemotaxis, no stratification, no cell migration…

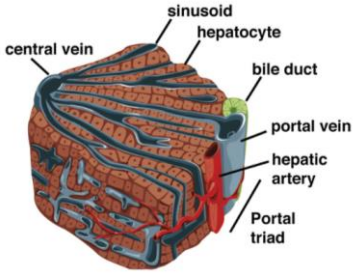




➢ 3D cell culture :

- Better correlation in vitro/ in vivo (gene expression, drug resistance)
- Ethics in drug testing ? Intermediate between 2D cell culture, animals and clinical tests
- More complex models : need for optimization, troubleshooting and sometimes important funds. Analysis techniques must be adapted to low-yield and 3D techniques.





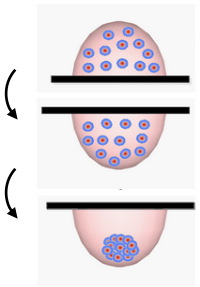
 (Baker and Chen, 2012; Duval et al., 2017; Ravi et al., 2015) *(Ahmad, Tahir, et Lone 2016; Si-Tayeb 2010)*

Main 3D cell culture methods

➢ Prevent plastic adherence

- Hanging drop method :

Cell suspension drop cultivated upside-down, spheroid formation, cell-cell interactions restored



- Ultra-low attachment (ULA) plates :

Hydrophilic coating suppressing cell-plastic interactions

➢ Restore cell-ECM interactions

- Matrigel :

Standard **animal-derived** ECM, rich in laminin, collagen IV and growth factors.

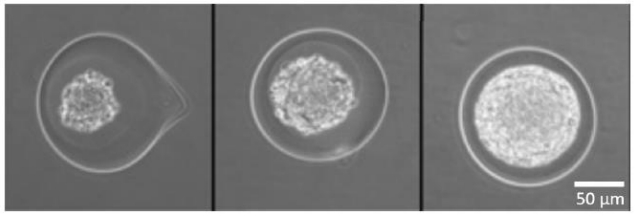
- Other ECM –like environments :

Collagen, gelatin, agarose, peptides, GelMa… with well-known composition, but might be less easy to handle. Not standardized.

➢ Mimic physiological structures

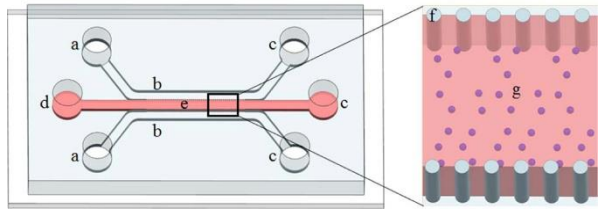
- Capsules (Ex : Treefrog Therapeutics)

Ideal for solid tumors, may integrate ECM



- Organ-on-chips

Multiple compartments with media flow. Suited for complex structures like blood-brain barrier, artificial vessels, interfaces…

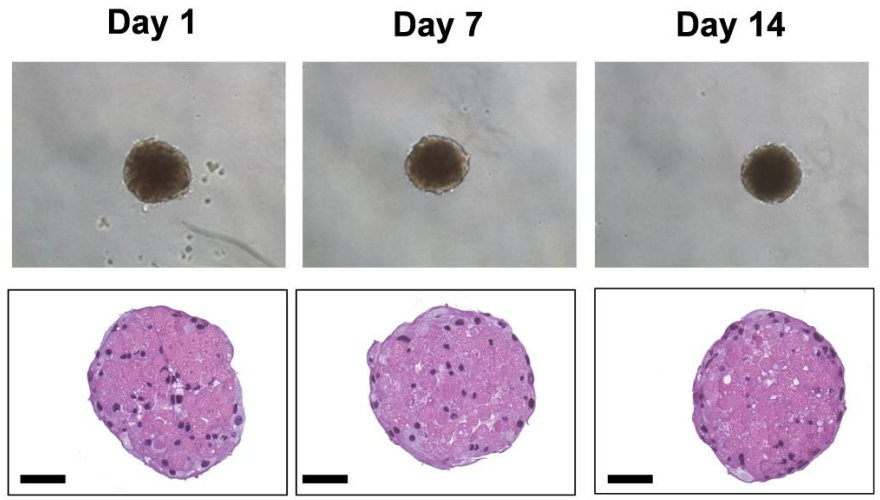


(Liu et al. 2017)

Ultra-low attachment (ULA) plates

- Ready-to-use plates with round-bottom + surface-treatment (super hydrophilic) to get the "ULA effect" by gravity
- No particular method, just optimize the number of cells/well for your application
- Applications : cell-cell interaction studies, solid tumor with hypoxia models, but no ECM here (other than cell-secreted)

- Good reproducibility, 1 spheroid/well with comparable number of cells in each well
- Semi-large scale : 96-well plates ≃ 24€/unit at Perkin Elmer, or Corning



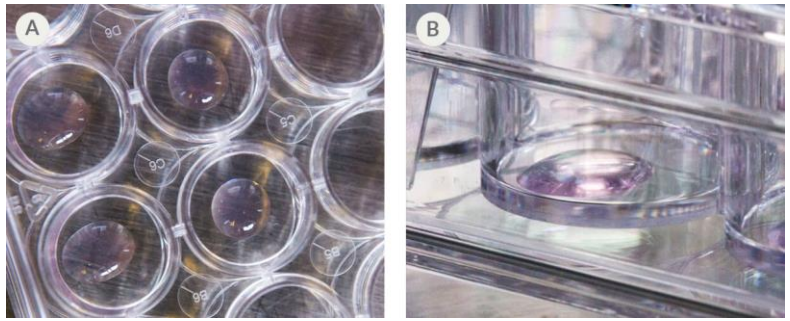
Primary hepatocytes, scale bar 50 µm

(Nautiyal et al. 2021)

Matrigel domes

- Matrigel : ECM from mouse sarcoma, contains mainly laminin, collagen IV, proteoglycans, growth factors. Batch dependent.

- Viscous liquid, can be used to resuspend cell pellets and seeded in domes :



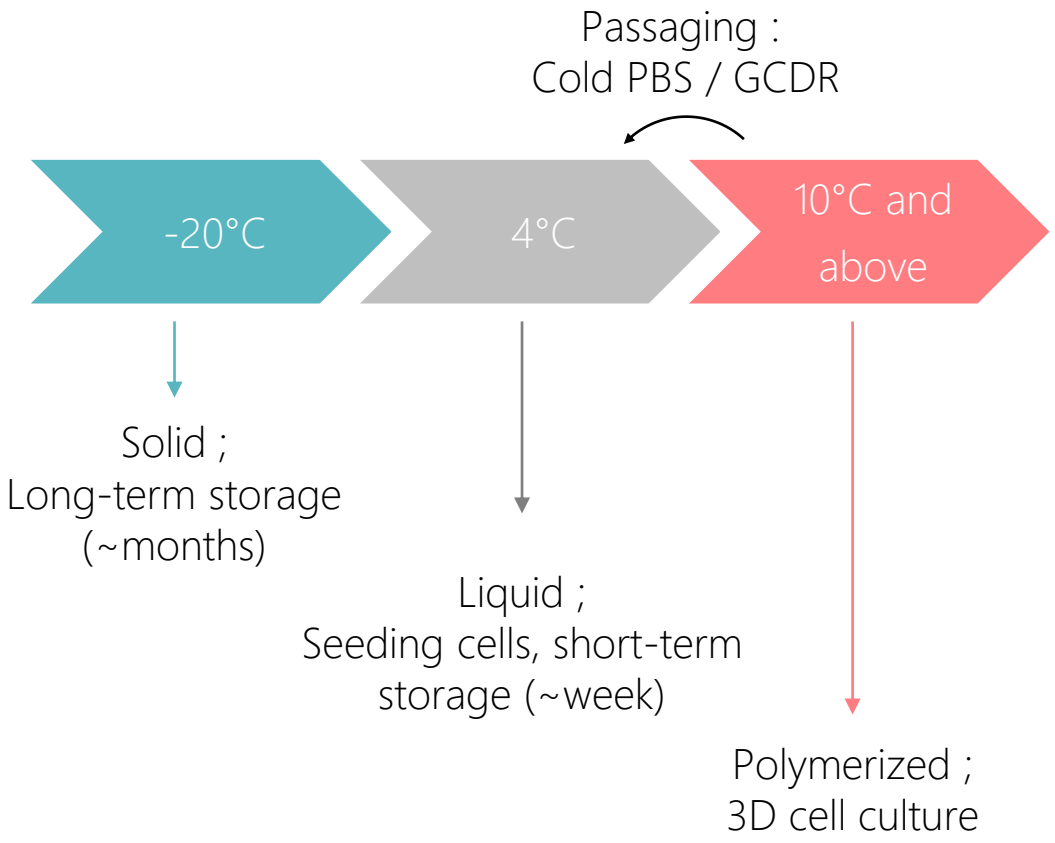
- Applications : isolating cells from a biopsy, developing an organoid model, but not for an "ethical", animal-free purpose

Corning ref 354234 :
439,78 € for 10 mL
(~200 domes)

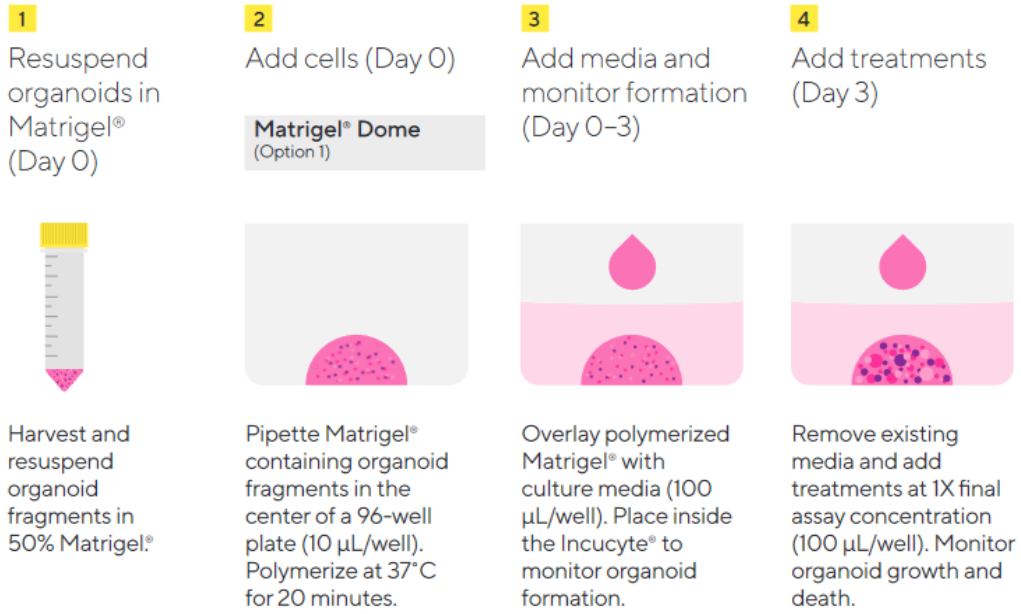
Corning ref 3526 :
167,44 € for 50 plates
(24 wells, 1 dome/well)

Matrigel domes

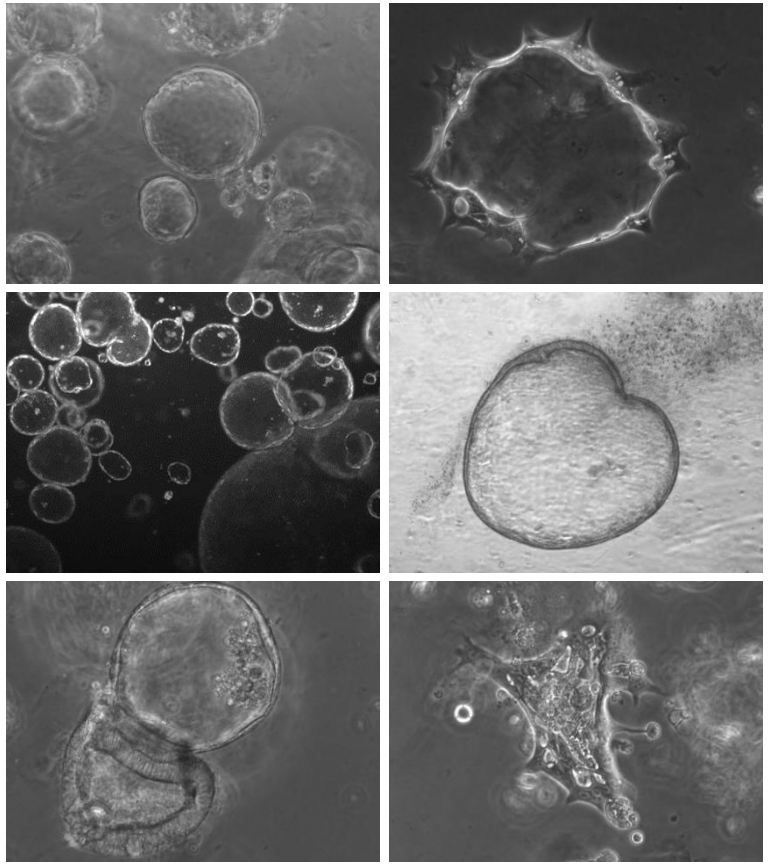
➢ Global Matrigel-handling method:



➢ Seeding method :



3D cell culture techniques and their limitations



Questions ?

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