First step

What is BIOMIMESYS®?

BIOMIMESYS® is a hydroscaffold of crosslinked Hyaluronic Acid (HA) chains for cell culture in 3D. The product is provided as a ready-to-use dehydrated hydroscaffold in each well of a low attachment 96-well plate.

- BIOMIMESYS® is highly porous and upon seeding, the cells spread homogeneously inside.
- Non-rehydrated BIOMIMESYS® should not be placed in a cell culture incubator as humidity can alter scaffold's properties.
- In order to keep hydroscaffold's properties do not remove lyophilized BIOMIMESYS® from its well before rehydration.

How to seed cells into BIOMIMESYS®?

Optimize cell density:

The cell density and optimal seeding volume may vary with cell types and must be adjusted: cell amount typically ranges from **10,000 to 50,000 cells per well** and **seeding volume of 10µL to 30µL** (for more information about this specific point, please contact our scientific support at hello@biomimesys.com).

Seed cells on lyophilized hydrogel:

a. Resuspend the cells into the medium (between 10 to 30µL).

b. Form a droplet in the center of BIOMIMESYS® carefully and slowly

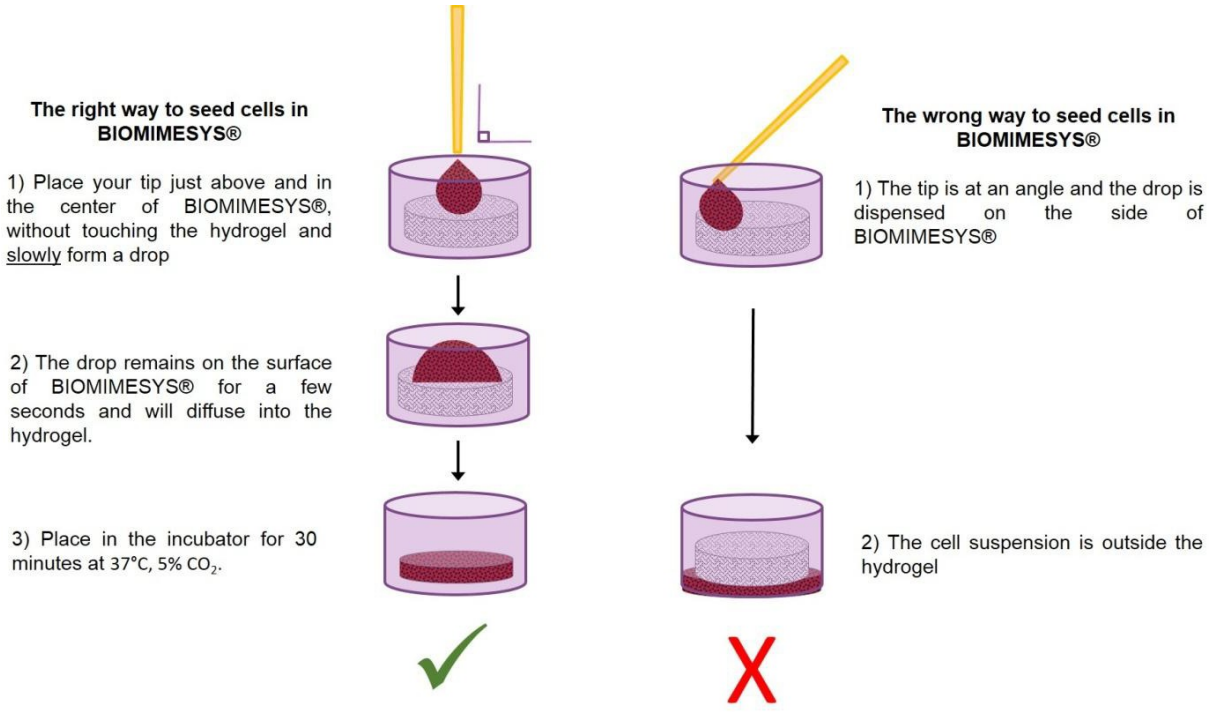


Figure 1: step1 – cells seeding into BIOMIMESYS®

c. Place the plate 30 minutes in the incubator set at 37°C, 5% CO_2 as usual.

d. Add gently the culture medium on the side of the well, with **final volume of 200µL,** in the space between the well and BIOMIMESYS® (be careful to not disturb the cells).

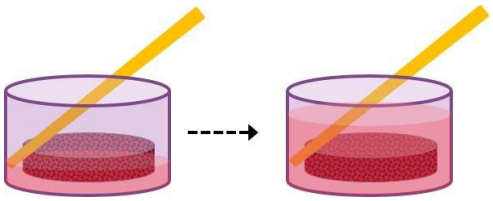


Figure 2: step2 – addition of culture medium

Example: with HCT116 cells, we seeded 10,000 cells in 10µL per hydroscaffold.
Note: Increasing the seeding volume lead to a deeper cell penetration inside the hydroscaffold, which may facilitate the microscopic observation of fast aggregating cells.

Refresh the medium:

- Remove a part of the medium by keeping the tip on the side of the well (see figure 3).
  Do not worry if you touch the hydroscaffold, it is robust enough to not break easily.

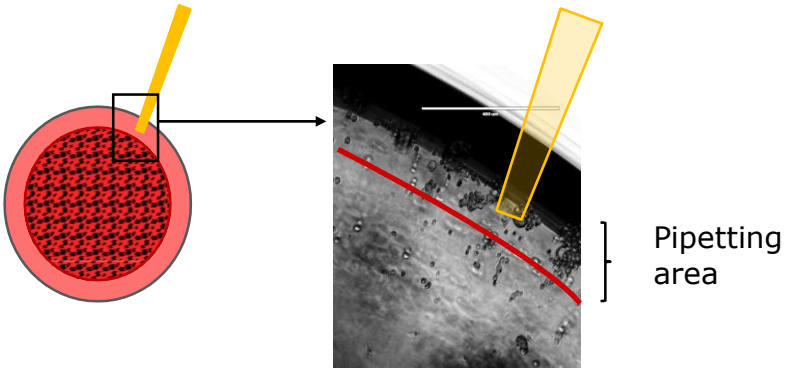


Figure 3: step3 - changing medium with BIOMIMESYS®

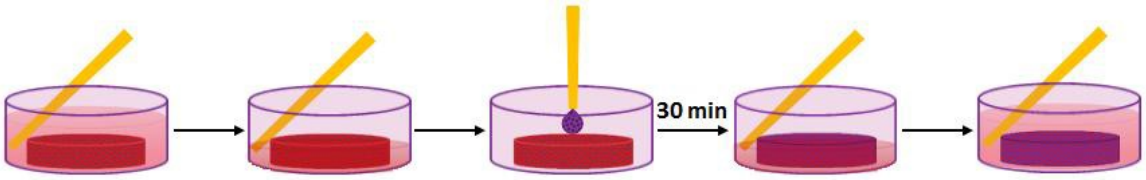
- The frequency of refreshing the medium depends on the cell proliferation rate.

  Example: with colorectal cancer cell line HT29, seeded at 50 000 cells/hydrogel, 100µL culture medium was refreshed every 48h.
  Note: The hydroscaffold, acts as a sponge and will always retain some culture medium/liquid

- To refresh the medium is easy with BIOMIMESYS®, thanks to the space between hydroscaffold, and the well wall. This pipetting area permits also few cells to be outside the gel. Around 80% of the seeded cells remain inside the hydroscaffold, and 20% outside. We usually observe these cells on the bottom of the well and they can be removed during the medium change.

How to seed cells into hydrated BIOMIMESYS®?

- The seeding remains optimal on dehydrated hydroscaffold.
- However, for example to do a co-culture with a sequential seeding, it is possible to seed cells on hydrated hydroscaffold.
- Cells must be seed with a small volume (10µL to 25µL) in order to have an optimal diffusion in the hydroscaffold. Then, the same steps as a seeding on lyophilized hydrogel have to be followed.



1- Eliminate a maximum of cell medium (150µL approximately)

2- Slowly form a droplet (with 10 to 25µL of cell suspension) and carefully lay it on the

3- After 30 minutes of incubation, gently add the cell medium on the side of the well

Figure 4: optimized steps to seed the cells on hydrated scaffold

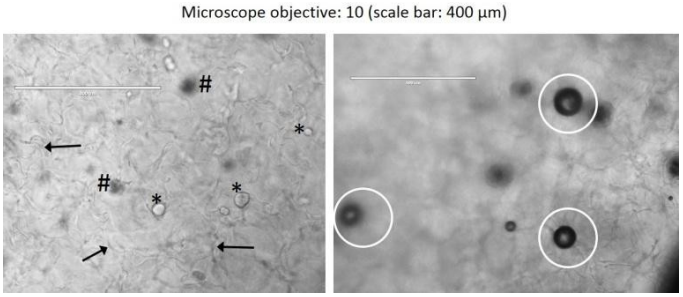
How to visualize cells in BIOMIMESYS®?

BIOMIMESYS®, when hydrated, is transparent and therefore compatible with microscopy.

Here is an example of what users may see during microscopic observations, 24h after seeding:

Microscopic observation 24h after seeding:

As a 3D porous scaffold, BIOMIMESYS® allows homogeneous colonization of cells in the whole hydroscaffold (x-, y- and z-axes). Observations of the whole z-axis cell distribution and clear visualization of cells can be achieved by changing the focal plane of observation.



*#: cells out of focus; *: cells in focus, arrows indicate hyaluronic acid chains. Circles indicate the normal phenomenon of forming bubbles during BIOMIMESYS® hydration following medium addition (figure 2). Bubbles will disappear after 24h to 48h in culture.*

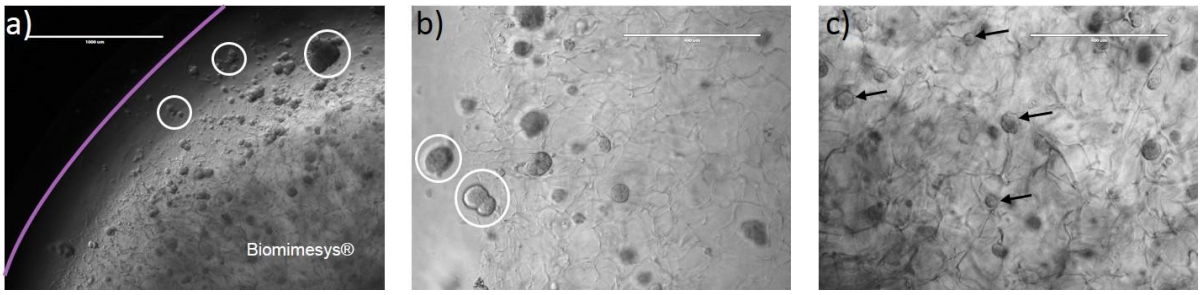
Figure 5: Microscopic observations of BIOMIMESYS® 24h after seeding

Users have to keep in mind that the kinetics of cell aggregation and/or spheroid formation will strongly depend on the cell type.

Microscopic observations 5-7 days after seeding:

Microscope objective: 4

Microscope objective: 10



Some spheroids may be formed in the pipetting area; most of these unattached spheroids (circles, a) will be removed during medium renewal. Spheroids may also be attached to the hydroscaffold chains at the BIOMIMESYS® periphery (circles, picture b). Indeed, most of the spheroids formed will be inside the hydrogel (arrows, picture c).

Focus adjustment

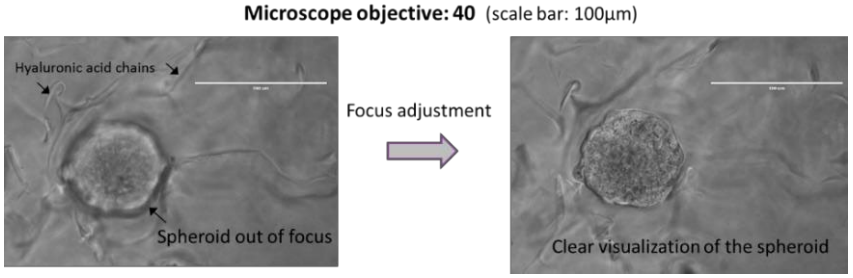


Figure 6: Example of HT29 cell culture in BIOMIMESYS® after 5-7 days of growth