What is BIOMIMESYS®?

BIOMIMESYS® is a hydrogel scaffold of reticulated HA chains for cell culture in 3D. The product is provided as a ready-to-use dehydrated hydrogel in each well of a low attachment 96-well plate (Greiner reference 655970).

- 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 hydrogel's properties.
- In order to keep hydrogel's properties do not remove lyophilized BIOMIMESYS® from its well before rehydration.

How to seed cells into BIOMIMESYS®?

1) **Optimize cell density**:
   The cell density and optimal seeding volume may vary with cell types and must be adjusted: cell amount typically ranges from 25,000 to 100,000 cells per well and seeding volume of 20µL to 40µL (for more information about this specific point, please contact our scientific support)
   Example: with HT29 cells, we seeded 50,000 cells in 30µL per hydrogel.

2) **Seed cells**:
   a. Resuspend the cells into the medium.

   b. Carefully and slowly form a droplet in the center of BIOMIMESYS® and place the plate in the incubator set at 37°C, 5% CO_2 as usual.

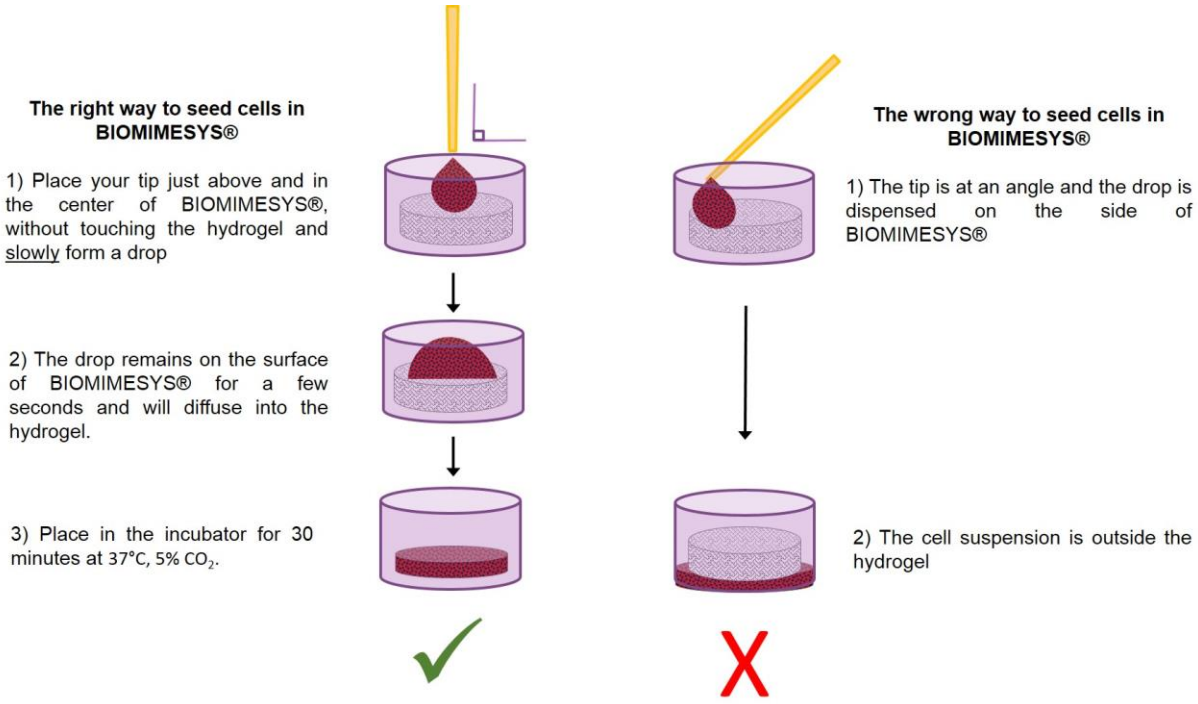


Figure 1: Cells seeding into BIOMIMESYS®

c. After 30 minutes of incubation, gently add the culture medium on the side of the well (to not disturb the cells), in the space between the well and BIOMIMESYS®

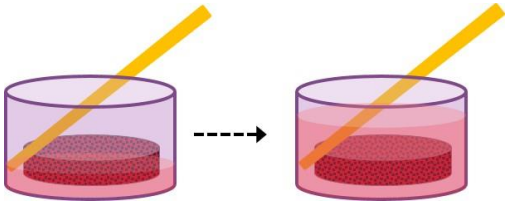


Figure 2: Addition of culture medium with BIOMIMESYS®

3) **Refresh the medium:**

BIOMIMESYS® allows change of medium easily, owing to the space between hydrogel and the well wall: the pipetting area depicted here not only permits to insert a pipette tip but also for a few cells to be outside the gel. Around 80% of the seeded cells remain inside the hydrogel and 20% outside. We usually observe these cells on the bottom of the well and they can be removed during the medium change.

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

a. Remove a part of the medium by keeping the tip on the side of the well (see figure 2).
Do not worry if you touch the scaffold, it is robust and will not break easily. However, be careful not to aspirate BIOMIMESYS® with the tip.
Example: with colorectal cancer cell line HT29, seeded at 50 000 cells/hydrogel, 100µL culture medium was refreshed every 48h.

Note: The hydrogel acts as a sponge and will always retain some culture medium/liquid

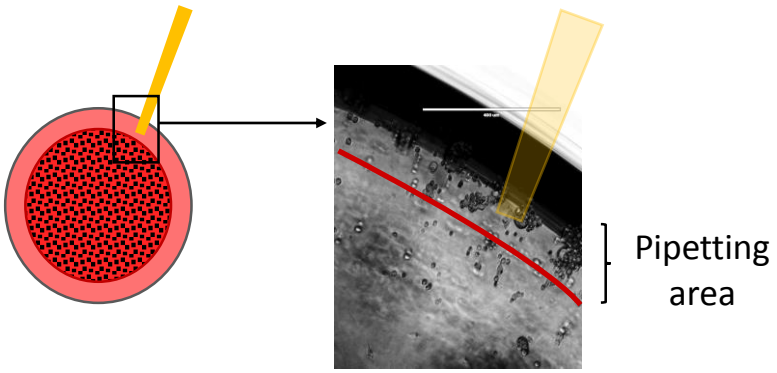


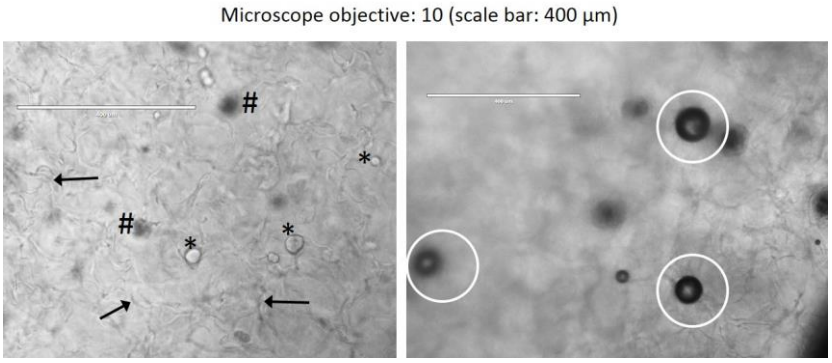
Figure 3: Changing medium with BIOMIMESYS®

How to visualise 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:

1) Microscopic observation 24h after seeding:

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

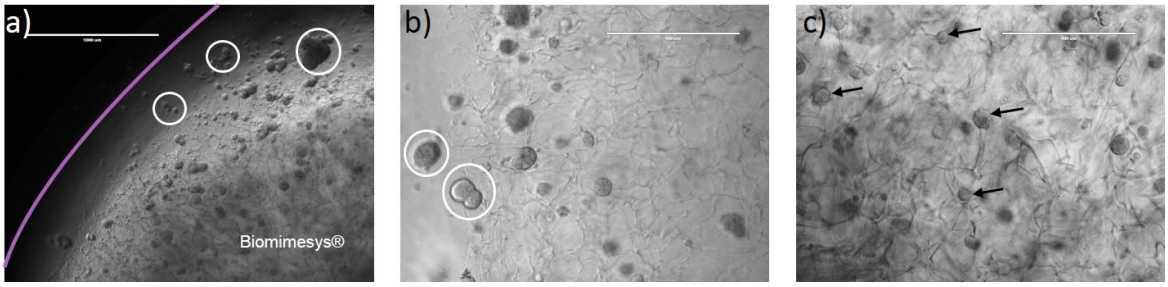


*#: cells out of focus; *: cells in focus, arrows indicate hyaluronic acid chains. Circles indicate the normal phenomenon of forming bubbles during BIOMIMESYS® hydration following medium addition (figure 2). Bubbles will disappear after 24h to 48h in culture.*

Figure 4: 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.

2) Microscopic observations 5-7 days after seeding:



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 hydrogel chains at the BIOMIMESYS® periphery (circles, picture b). Indeed, most of the spheroids formed will be inside the hydrogel (arrows, picture c).

3) Focus adjustment

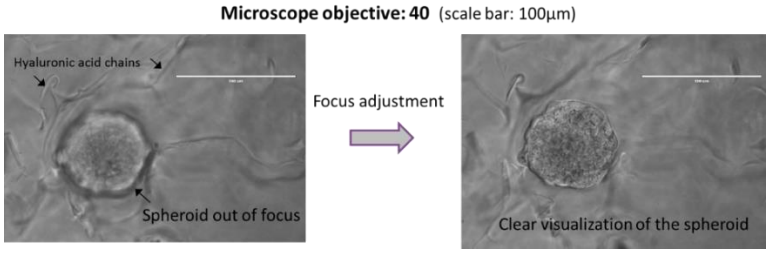


Figure 5: Example of HT29 cell culture in BIOMIMESYS® after 5-7 days of growth