BIOMIMESYS® Starting Guide

Product: BIOMIMESYS® 3D Cell Culture Hydroscaffold™

Format: 96 and 384 well-plate black/clear & 96 well-plate clear

Context: BIOMIMESYS® is a unique groundbreaking 3D cell culture technology which associates the behavior of a solid scaffold and of a hydrogel. It provides a cell culture microenvironment reproducing all aspects of human tissues, including matrix architecture, cellular organization, cell-cell and cell-matrix interactions. Depending on the organ, extracellular matrix components and their proportions may vary, allowing a more or less dense and compact cellular environment (different Elastic Moduli, porosities). BIOMIMESYS® matrices are made of Hyaluronic Acid (HA), the main glycosaminoglycan (GAG) of the ECM, collagens and adhesion proteins. Our patented manufacturing process allows to preserve the natural properties of HA and therefore synthesizing proprietary Hydroscaffold™. Tuning the composition of BIOMIMESYS® matrix allows to mimic the cellular microenvironment of any organ or tissue of interest.

Storage: Store at 2°C to 8°C

Expiration date: 12 months after the date of manufacturing

---

1. Unpacking

Remove the box from +4°C storage and open the vacuum plastic packaging just before seeding. Indeed, if the *hydroscaffold*™ absorb too much ambient humidity, your cells will not penetrate it very well afterwards. As a consequence, once the package opened, you have to use all the *hydroscaffolds*™.

2. Cell seeding

a. Adjustment of cell density and volume of seeding

The cell density and optimal seeding volume is depending on cell types and must be adjusted. Cell amount typically ranges from 20,000 to 200,000 cells per well for 96 well plates or 5.000 to 20.000 cells per well for 384 well plates. Seeding volume can vary between 10µL to 40µL per well for 96 well plates or 40 to 50 µL for 384 well plates. For more information about this specific point, please contact our scientific support at hello@biomimesys.com.

Note: Increasing the seeding volume lead to a deeper cell penetration into the *hydroscaffold*™, which may facilitate the microscopic observation, but you may see more cells outside the *hydroscaffold*™.

b. Cell seeding

General protocol:

Prepare your cell suspension into the medium at the required concentration. Dispense 10 to 30µL per well by forming a droplet in the **center** of BIOMIMESYS® **carefully and slowly**.

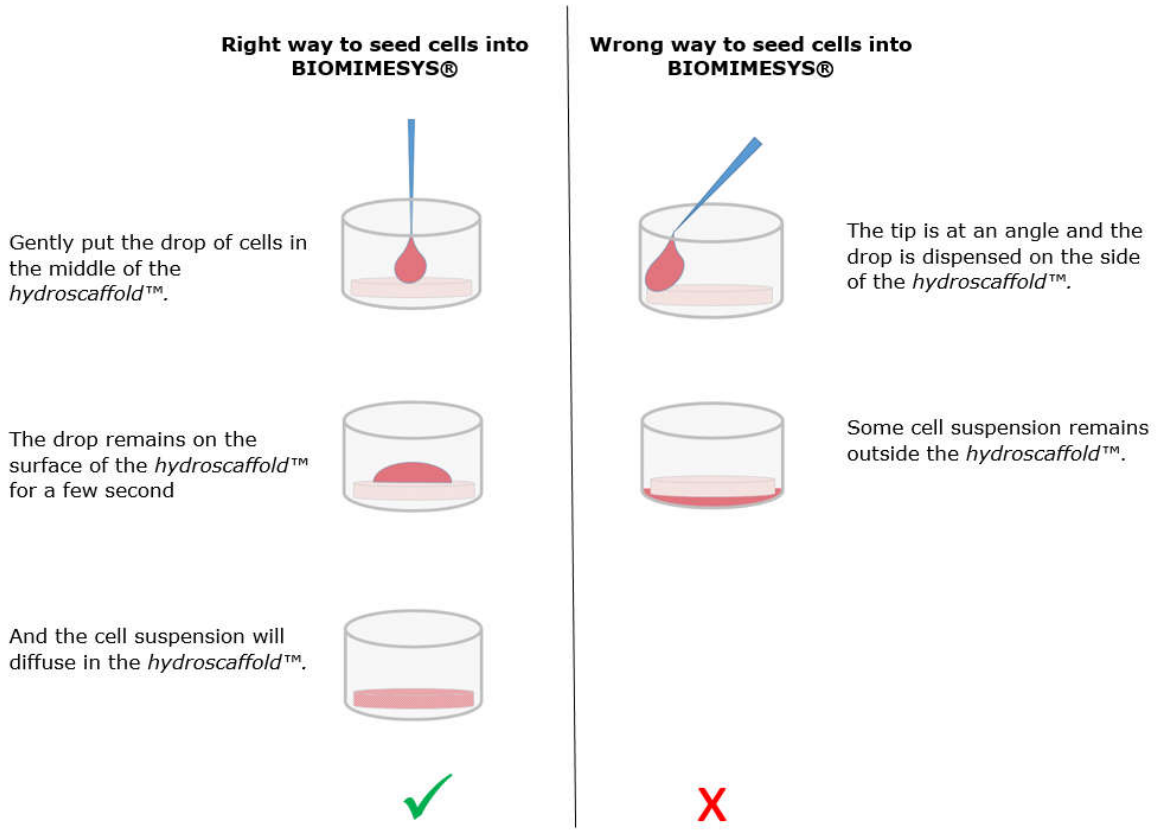


Figure 1: Cells seeding into BIOMIMESYS®

Complete wells at 200µL for 96 well-plate by adding slowly fresh medium with your tips perpendicular to the side of the well. Complete wells at 80µL per well for 384 well-plate using a pipetting robot at low pipetting speed (see dedicated protocol).

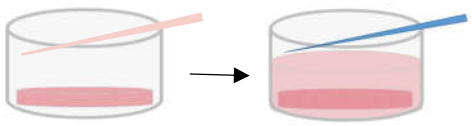


Figure 2: Addition of culture medium

Methods of seeding:

You can seed manually with a pipette of 20µL or 100µL. You can also use a multipipette. In case of an automated one, make sure the pipetting speed is low. Finally, you can use a pipetting robot, particularly for 384 well-plates.

3. Changing medium

The frequency of refreshing the medium depends on the cell proliferation rate. Usually, we refresh medium every two days.

If you can set up the speed of aspiration of your device, please set it up at the lowest speed.

For 96 well-plate:
Remove the medium by sliding the tip from the top to the bottom of the side of the well. Stop 2mm above the *hydroscaffold*™.

Complete wells at 200µL per well by adding slowly fresh medium with your tips perpendicular to the side of the well.

Note: The *hydroscaffold*™ behaves like a sponge and will always retain around 30µL of culture medium.

For 384 well-plate:
When using a pipetting robot, from the total 80µL per well, aspire 40µL per well and add 40µL per well of fresh medium (see dedicated protocol).

4. Microscopic observation

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

a. Focus adjustment

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 with the focusing wheel of the microscope.

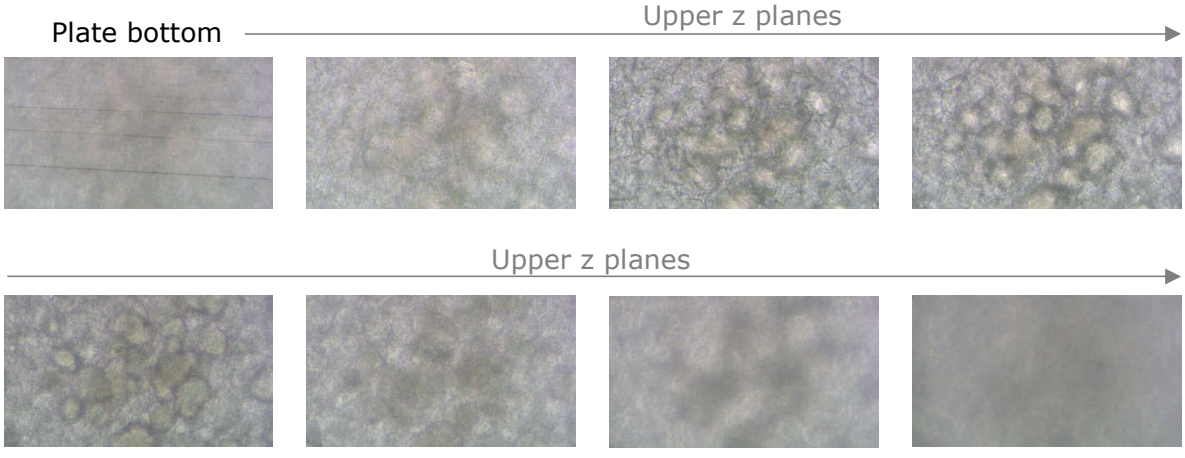
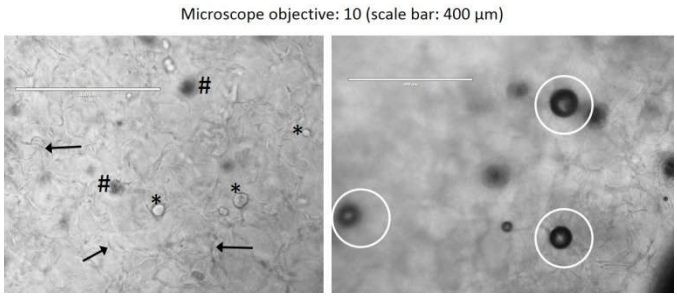


Figure 3: Example of HT29 cell culture in BIOMIMESYS® oncology (25.000 cells per well) after 6 days of growth. Microscope objective 10x, Gx100

b. Microscopic observation 24h after seeding

24h after cell seeding, you can observe the cells in phase contrast microscopy. They might be difficult to observe, since you will also see the hyaluronic acid chains.



*#: 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 72h of culture.*

Figure 4: Microscopic observations of BIOMIMESYS® 24h after seeding

Around 80% of the seeded cells remain inside the hydroscaffolds™, and 20% outside. We usually observe these cells on the bottom of the well and they can be removed during the medium change.

To see some spheroids, wait at least 3 days. However, the kinetics of cell aggregation and/or spheroid formation will strongly depend on the cell type.

c. Microscopic observations 5-7 days after seeding

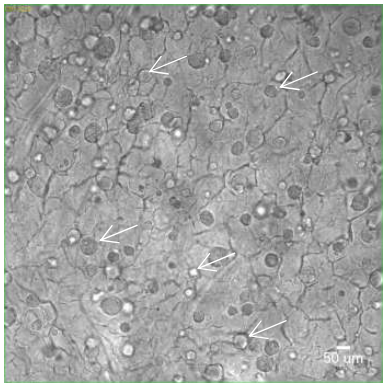


Figure 5: Example of HCT116 cell culture in BIOMIMESYS® liver (25.000 cells per well) after 7 days of growth. Scale bar 50µm.

You can observe some spheroids (arrows) inside the *hydroscaffold*™, at different focal planes.

Contact Information

HCS Pharma

hello@biomimesys.com

www.biomimesys.com