OrganoPlate® starter's guide

MIMETAS
the organ-on-a-chip company

Tips & tricks for your successful first experiment with the OrganoPlate® 2-lane

In the following step-by-step introduction guide, we will walk you through the usage of your first OrganoPlate®, to make sure that each chip you seed contributes to an overall mastery of high-throughput- and perfused 3D cell biology.

Please follow the instructions in each step carefully, and compare your results against the ECM-loaded chip panel (page 4) to understand which technique adjustments can help you to achieve greater success.

Important: this guide is not a protocol in-and-of-itself. Rather, it's a step-by-step guide to be used with the formal protocols for in-gel model seeding (link) or tubule model seeding (link).

Practice ECM Loading in the OrganoPlate® 2-lane

1 Print out the formal protocol:
'2-lane 400um OrganoPlate® tubule seeding' **and prepare your equipment, extracellular matrix (ECM) gels, and culture media**

As part of the initial learning process, we recommend using the 4 mg/mL rat-tail collagen-I (described in detail in the protocol). Please pay careful attention to step 3-v. (page 2) in the protocol, to reduce bubble formation in the gel.

At this point, please cover all of the wells in Columns 9 – 24 of your OrganoPlate® with PCR film for later use (figure 1).

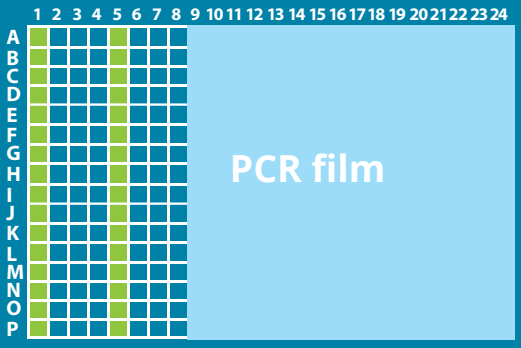


Figure 1

2 Load the ECM gel into your first 4 chips (via wells A1 – D1) and check the results

To load ECM gel into a chip, align your pipette perpendicularly to the plate, and gently place the pipette at the center of 'gel inlet' well illustrated in figure 3, ensuring contact (but not pressure) between the tip and the channel inlet before depositing your gel.

Please Note: *When loading ECM, you may lay the OrganoPlate flat or hold it with your non-pipetting hand. As long as the pipette is perpendicular to the plate, you may choose whichever feels most comfortable.*

After pipetting gel into these 4 chips, turn your plate over to observe the results directly through the glass bottom.
Compare the appearance of your loaded chips with the panel on page 4, to note the chips that have been successfully loaded, and to find technique adjustments you can incorporate to address overflows or incomplete loadings.

Tip: Utilize an on-hand microscope (brightfield will work great) to check the filling patterns of the loaded chips.

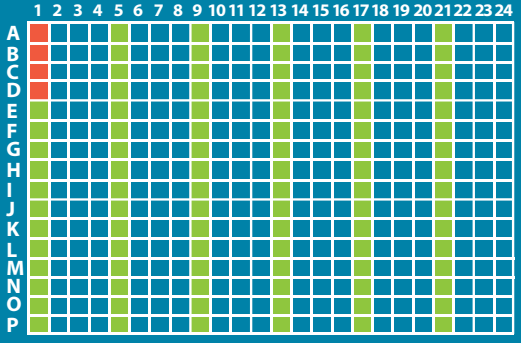


Figure 2

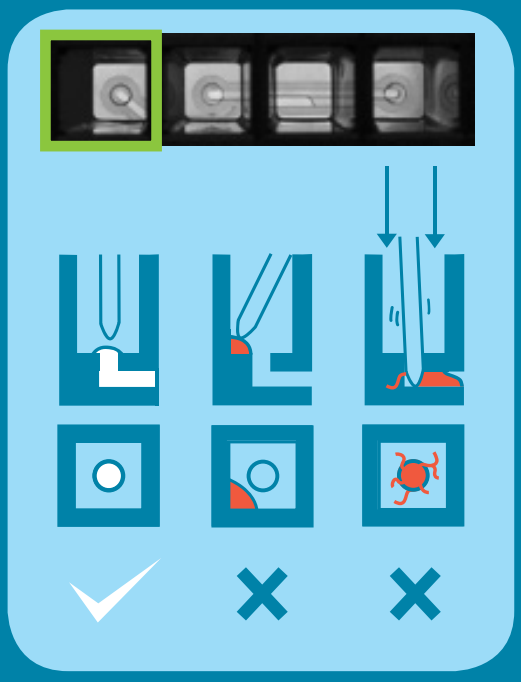


Figure 3

3. **Proceed with loading ECM gel into the next 6 chips (via wells E1 – J1) in the first column, and check the results**

   Compare your results with the panel on page 4, to confirm that you are on the right track.

   At this point, you now have loaded ECM gel into the first 10 chips (wells A1 - J1).

4. **Keeping in mind the technique adjustments, proceed with loading ECM gel into the final 6 chips (via wells K1 – P1) in the first column, and check the results**

To troubleshoot any consistent errors, please collect example pictures from the microscope using your cell phone, and forward them to support@mimetas.com so that we can further guide your next practice run.

5. **In the second chip column (beginning with well A5), focus on loading 8 chips in a row (A5 – H5) with ECM gel, keep in mind the technique adjustments and check the results**

   Please check your results after loading these 8 chips, and note any inconsistent results to make the appropriate technique adjustments.

6. **Keeping in mind the technique adjustments, proceed with loading ECM gel into the final 8 chips in the second column (I5 – P5), and check the results**

   Please take the time to compare the appearance of your loaded chips with the panel on page 4. Then, please follow the instructions in the formal tubule seeding protocol (step 5) for allowing the ECM to gelate in your loaded chips.

If you're still experiencing loading inconsistency, please check in with us at support@mimetas.com.

7. **Continue practicing the ECM gel loading technique on the remaining chips of your first OrganoPlate®. When you feel comfortable enough, please begin incorporating cells in or against the gel, according to the associated protocols on** www.mimetas.com/support.

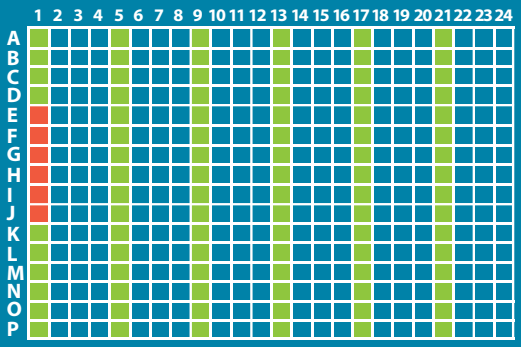


Figure 4

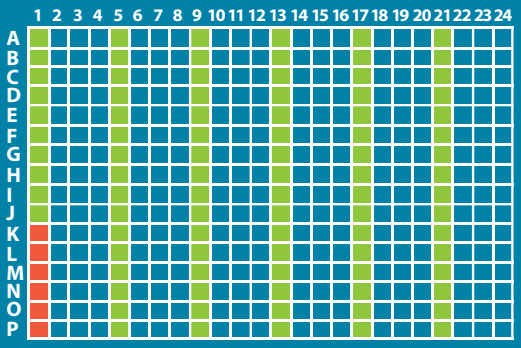


Figure 5

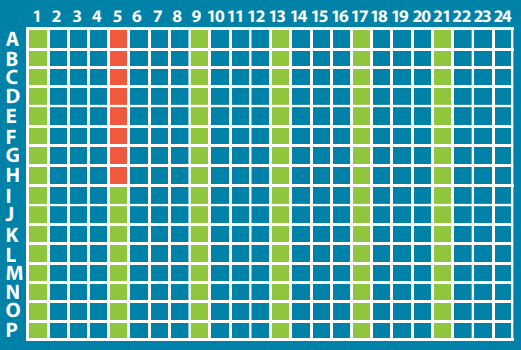


Figure 6

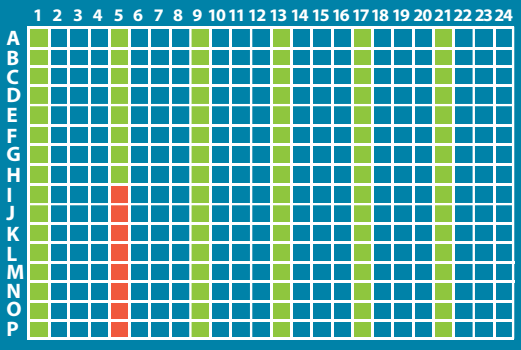
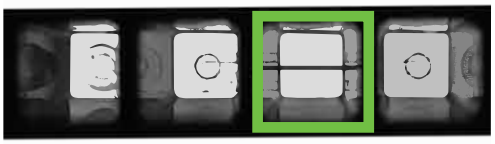


Figure 7

ECM-loaded chip panel (and how to refine your technique)

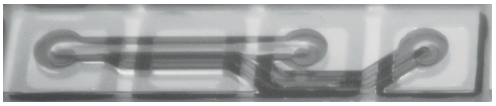
Successfully Loaded Chip:

View from top



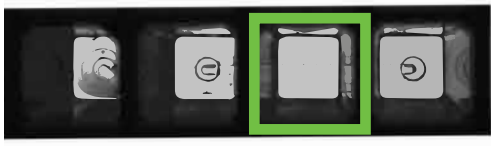
View from under the microscope

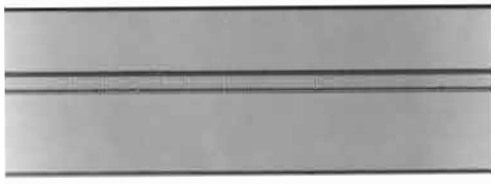
View from bottom

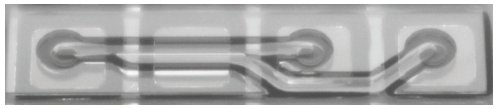


Successful loading! Notice the shadow stretching along entire observation window. That is from a successfully formed meniscus.

Error – Missed Loading:



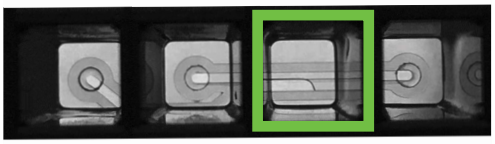




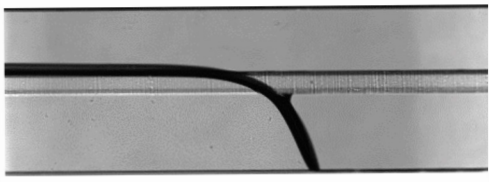
Notice the PhaseGuide™ appears sharp compared to Overflow. This means the gel missed the inlet entirely. ***To fix:*** *try keeping the pipette perpendicularly aligned to the inlet in the well's center.*

Error - Not Filled to End:

View from top



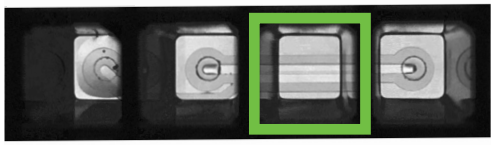
View from under the microscope

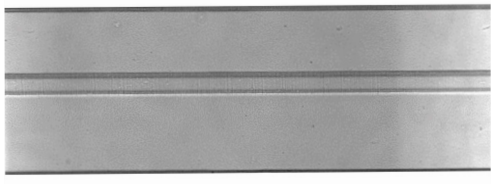


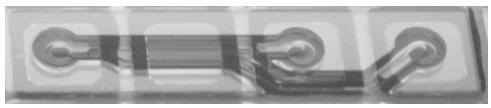
View from bottom

Notice the shadow from the meniscus does not stretch the entire length of the window. ***To fix:*** *first increase your gel volume (e.g. from 1.7µL to 2µL).*

Error - Overflow:







Notice there's no shadow from the meniscus. This means that the gel jumped the PhaseGuide™. ***To fix:*** *decrease your gel volume (e.g. from 1.7µL to 1.5µL)*

If the error continues:

1. Make sure the plate isn't too warm
2. Make sure the gel is well-mixed, and that the viscosity isn't higher than expected
3. If you're using an electronic pipet, try increasing the ejection speed
4. Check plate under microscope, if there are droplets in what should be dry channels

If the error continues:

1. Make sure the plate hasn't been chilled
2. Make sure the gel is well-mixed, and not over-diluted with residual medium
3. If you're using an electronic pipet, try reducing the ejection speed
4. Check plate under microscope, if there are droplets in what should be dry channels