Considerations when Optimizing Corning® Matribot® Bioprinter Dispensed Dome Assays

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Since its first publication, dome or droplet culture has become a widely used method for propagating and assaying epithelial derived organoids[1]. The technique involves mixing stem cells or pieces of tissue containing stem cells with an extracellular matrix (ECM) such as Corning Matrigel® matrix and dispensing this mix as droplets onto a cell culture surface. The cell filled domes are then polymerized and overlaid with media optimized for the organoid of interest. Use of the Corning Matribot bioprinter to dispense droplets can increase the accuracy and precision of dome size and placement. Unfortunately, this seemingly simple process, if not properly implemented, can result in poor growth and unwanted differentiation. The following information will highlight some important factors to consider to better optimize the printing of organoids in an ECM matrix. Additional information on optimizing Matribot bioprinter settings can be found in the document Corning Matribot Bioprinter Parameters (CLS-AN-648).

Dome Size

Optimizing the size of the organoid dome is a key factor for successful organoid health as nutrient diffusion into ECMs can be a limiting factor[3]. Mouse organoids have been traditionally cultured in a single 50 µL dome in each well of a 24-well plate[2] whereas human organoids, which can be more sensitive to growth factor diffusion from surrounding culture media, often do better in smaller domes that enable better growth factor penetration[4]. Differences in organoid morphology between the center and edge of a dome is likely an indication that a reduction in dome size is warranted. In addition to health of the organoids, the purpose of the culture should be considered when choosing dome size. Smaller domes are easier to visualize due to the more uniform focal plane of the organoids. Inversely, larger domes are more challenging to image as multiple focal planes are required due to dome thickness but produce more organoids per dome which can be beneficial for scaling up cultures.

Creating Sturdy Domes

For organoid cultures to be successful, it is essential that Matrigel matrix domes maintain their integrity for the duration of culture. One of the most important aspects to consider is Matrigel matrix concentration. Matrigel matrix with higher protein concentrations can result in stiffer and more stable domes. If domes are breaking down after several days of culture, consider increasing the Matrigel matrix concentration, considering the final concentration of Matrigel matrix after cell and media additions. It should be noted that repeated freeze/thaw cycles of the Matrigel matrix can degrade the stability and ability to maintain printed structures. It is recommended that Matrigel matrix be aliquoted and frozen into single-use volumes when initially thawed. Also, ensure domes are fully polymerized before addition of pre-warmed cell culture medium, keeping in mind that larger domes take longer to polymerize than smaller ones. Finally, using cold medium during exchanges may break down the Matrigel matrix over time and should be avoided.

Plate Type and Preparation

For most applications, a tall dome of organoids is desired to prevent cells from contacting the plastic plate surface where a thin spread-out dome would bring cells closer to the plastic growth surface and increase the likelihood of attachment and differentiation. Ideally, a tissue culture-treated surface is recommended to provide enough treatment for the dome to remain attached during media exchanges but does not cause the dome to spread out. We have also had success with non-treated plates depending on the length of culture and frequency of media exchanges. Incubating plates in a cell culture incubator, for at least 24 hours prior to printing, has been found to result in taller domes that polymerize more quickly. Using the heated printbed of the Corning Matribot bioprinter can help domes polymerize faster as well. If the experiment requires a short duration for organoid culture (i.e., for an imaging assay) the use of the heated printbed may not be desired as it might be better to allow the organoids to settle into a more uniform focal plane before polymerization is complete.

Corning Matribot Printing Optimization

The temperature controlled printhead of the Matribot bioprinter is one of the main features that make it well suited for printing Matrigel matrix domes. To keep Matrigel matrix less viscous and therefore printable, the temperature must be maintained under 10°C. It is essential to ensure that all consumables in contact with Matrigel matrix are chilled prior to use including pipet tips, syringes, and printing nozzles.

In addition to keeping ink printable, there are several factors that can impact the success of droplet dispenses such as the height at which the domes are dispensed in relation to the bottom of the plate. If the droplet is dispensed too high from the plate surface, the Matrigel matrix droplet could remain attached to the nozzle and be carried to the next print location resulting in some wells with less domes than other wells or with domes of varying sizes within a given well. The automatic calibration function that is integrated into the Corning DNA Studio software will help to determine the appropriate Z height for printing.

In certain instances (e.g., the print nozzle becomes warped or when using a non-standard receiver plate) it might be desired to perform a manual calibration instead of automatic. This can easily be done with a clear plate following the Corning® Matribot® Bioprinter Instruction Manual (CLS-AN-641DOC). Manual calibration can also be implemented if the plate being printed into has opaque side walls by using a clear equivalent plate. If a clear equivalent plate is not available, manual calibration of Z height can be accomplished by using the printbed as a reference point and then increasing the Z height by the thickness of the plate bottom. The plate bottom height is available from the plate manufacturer and is typically referred to as the well bottom elevation.

Other factors that are important for consistency of droplet dispensing are ensuring a clean nozzle and a fully primed printhead. If liquid is coating the outside of the print nozzle opening, the surface tension can pull the dispensing ink towards that liquid resulting in an inadequate dispense for one dome and a larger dome in the next print location. Using a sterile alcohol wipe to clean the nozzle and allowing it to dry, just prior to printing, can reduce the likelihood of this occurring. A printhead that is not fully primed will result in unprinted locations until the ink reaches the tip of the nozzle. This can be prevented by ensuring that the ink is fully primed all the way to the tip of the nozzle prior to printing. If the first dome is still not being printed, increase the extra pre-flow volume.

Format/Throughput

The Corning Matribot bioprinter has been designed to use with BD Luer-Lok™ 3 mL Syringes (BD 309657). Syringes should be filled with no more than 2.7 mL of ink. This means the number of domes and plates that can be filled with a single fill will vary based on volume per well and plate format used. Corning DNA Studio software comes with pre-loaded droplet dispense settings allowing for single or multiple domes in each well depending on the configuration of the microplate being used. Alternatively, if the desired dome format is not available via the Droplet Dispense option, a custom STL file can be generated using a third party design software which can be imported into DNA Studio.

Filling each plate with the number of domes listed in Table 1 takes about 3 minutes (extrusion speed of 60 µL/sec. and a 20 mm Z-lift between wells). Testing the settling rate of cells or organoids in the bioink is an important factor to consider as settling rates for each cell type or organoid can impact the reproducibility across multiple plates. This must be tested empirically, as the settling time can be a dependent on the time to print, ink concentration, temperature, type of bioink, as well as the size of cells or organoids.

Table 1. Domes per well via Droplet Dispense drop down menu.

Plate Format	Maximum Number of Domes/Well
6-well plate	9
12-well plate	5
24-well plate	4
48-well plate	1
96-well microplate	1

Discussion

Optimization of any cell-based assay is essential to achieving consistent and meaningful results. The added challenge of using sensitive systems such as organoids makes dome culture optimization even more compelling. Dome size, ink formulation, plate type, and print settings can all have significant impact on the final product and should be optimized to attain the most benefit of using a bioprinter.

References

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