Considerations When Optimizing Corning® Matrigel® Matrix Dome Culture

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Introduction

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. Although the process is purportedly simple, poor growth, detached or dissolved domes and unwanted differentiation can occur if not properly implemented. The following highlights some important considerations to better optimize organoid culture in dome applications when using an ECM matrix.

Dome Size

Optimizing the size of the organoid dome is a key factor for successful organoid health as molecule diffusion into ECMs can be a limiting factor.[2] Mouse organoids have been traditionally cultured in a single 50 µL dome in each well of a 24-well plate[3] 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 are likely an indication that a reduction in dome size is warranted (Figure 1). In addition to the health of the organoids, the purpose of the culture should be taken into consideration when choosing dome size. Smaller domes make cell visualization easier given the organoids are in a more uniform focal plane. Inversely, larger domes are more challenging to image as organoids are in multiple focal planes due to dome thickness.

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. Since Matrigel matrix is animal-derived, its concentration will vary from lot to lot. Therefore, diluting Matrigel matrix by protein concentration (listed in certificate of analysis) will be more consistent from experiment to experiment as compared to diluting by percentage. Additionally, Matrigel matrix with higher protein concentrations can result in stiffer and more stable domes than those made from lower concentrations. If domes are dissolving after several days of culture, consider increasing the final Matrigel matrix concentration (after resuspension of cells and any residual media). Additionally,

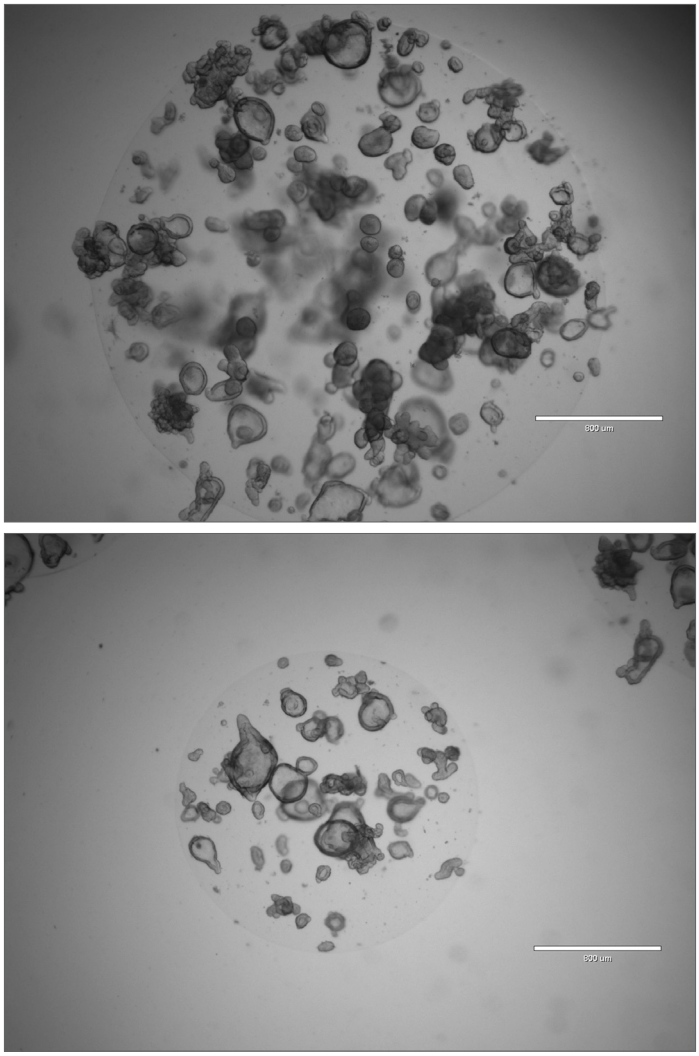

Figure 1. Impact of Corning Matrigel matrix dome size. Representative human intestinal organoids cultured in large (top) vs. small (bottom) Matrigel matrix domes. Organoids in the center of the larger dome appear more spherical and less complex than organoids on the edges of dome or in smaller dome. Scale bar is 800 µm.

it should be noted that repeated freeze/thaw cycles of the Matrigel matrix can degrade its stability. It is recommended that Matrigel matrix be aliquoted and frozen into single-use volumes after initial thaw. Furthermore, ensure domes are fully polymerized before addition of pre-warmed cell culture medium, being mindful that larger domes take longer to polymerize than smaller ones. Broken or torn domes may be due to incomplete polymerization prior to media addition or the expansion of a bubble during incubation that was while dispensing (Figure 2).

Cell Culture Surface and Preparation

For most applications a tall dome of organoids is desired to prevent cells from contacting the plastic plate surface. A thin spread-out dome would bring cells closer to the plastic growth surface and increases the likelihood of attachment to the plastic which could lead to differentiation. Ideally, a Tissue Culture (TC)-treated surface is recommended to provide enough treatment for the dome to remain attached during media exchanges but will not cause the dome to spread out too much. Untreated plates may also be used depending on the duration of culture and frequency of media exchanges. Incubating plates in a cell culture incubator for at least 24 hours prior to use, has been found to result in taller domes that polymerize more quickly (Figure 3). Another method to polymerize domes faster is to use a heating block under the plate during dispense, but this method may not be ideal for imaging assays, as it might be better to allow the organoids to settle into a more uniform focal plane prior to polymerization.

Staining

Organoids and spheroids can be stained without removal from Matrigel matrix. Standard incubation times or reagent concentrations may need to be increased for success since reagents will need to diffuse through the Matrigel matrix dome to reach cells (Figure 4). The concentration and duration will need to be determined empirically for different organoids/spheroids, dome size, and Matrigel matrix concentrations.

Discussion

Optimization of any cell-based assay is essential to achieving consistent and meaningful results. The added challenge of using sensitive and expensive models such as organoids makes dome culture optimization even more compelling. Dome size, Matrigel matrix concentration, plate type, and assay optimization can all have significant impact on the final product and should be optimized to attain the best results with Matrigel matrix dome cultures.

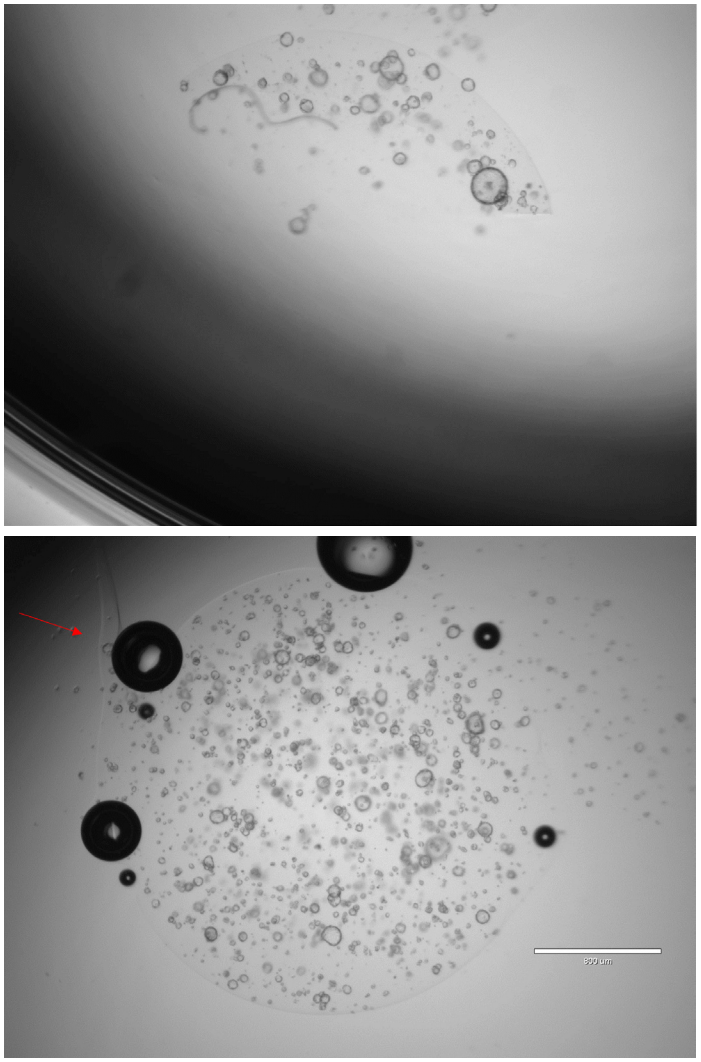

Figure 2. Representative images of torn Corning Matrigel matrix domes. (top) a dome that was torn during media addition; (bottom) a dome with damage from an expanded bubble (red arrow).

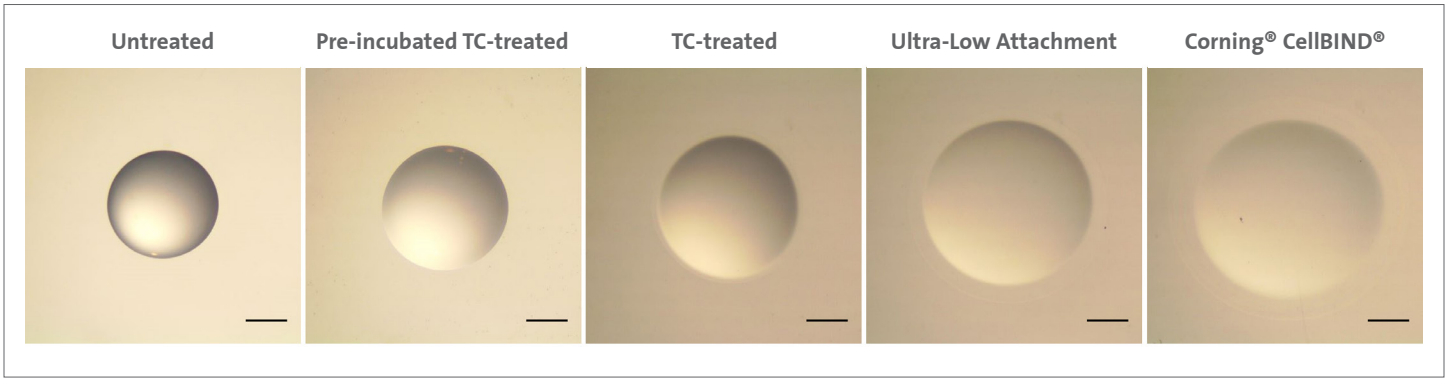

Figure 3. Corning Matrigel matrix dome spreading on various culture surfaces. Representative images of 5 µL Matrigel matrix domes on different cell culture surfaces. Matrigel matrix solution is 7 mg/mL and the scale bar is 800 µm.

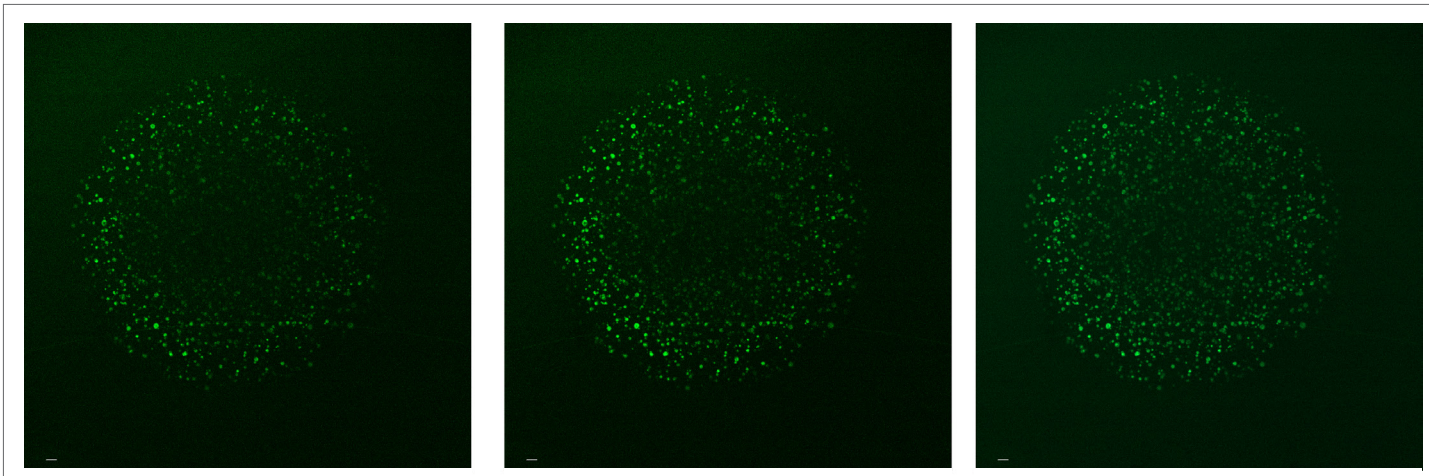

Figure 4. Staining structures embedded in Corning Matrigel matrix. Representative images of 5 µL Matrigel matrix domes with cells stained with 0.04 µg/mL Calcein AM for various amounts of time. Scale bar is 100 µm.

References

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4. Pleguezuelos-Manzano C, et al. Establishment and culture of human intestinal organoids derived from adult stem cells. Current protocols in immunology 130.1 (2020): e106.

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