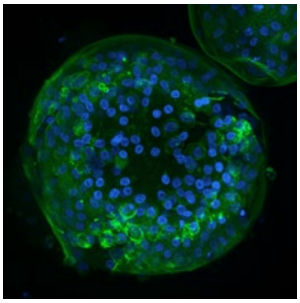
Corning® Matrigel® Matrix and Organoids:

A Life Scientist's How-To Guide



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Introduction

It's easy to see why organoids have become popular over the years: These 3D cell cultures can self-organize into "mini-organs" capable of recapitulating their respective organ-specific functions. Organoids exist for several organs, including the brain, breast, colon, liver, pancreas, stomach, esophagus, small intestine, ovary, uterus, fallopian tubes, prostate, and retina.

However, organoids are more complex compared to traditional 2D cultures and even 3D spheroids. Fortunately, products such as **Corning Matrigel matrix** — the most widely used and cited extracellular matrix (ECM) — can help streamline workflows for more efficient culturing, storing, and handling.

This guide details best practices to consider for organoid work, as well as how to use Corning Matrigel matrix and other products to support research.

Five Organoid Applications[1]

Organoids offer many applications across life sciences, including applications in the following five categories:

1. Cancer Modeling

Cancerous tumors develop from genetic mutations in the body. Organoids have become influential in studying this disease pathophysiology to better understand mutational signatures in an *in vitro* environment. Researchers have put these cultures to use in modeling cancers of the breast, colon, pancreas, prostate, bladder, and liver — a list that includes many of the world's most deadly malignancies.

2. Drug Discovery

Organoids can give drug discovery a distinct advantage in speed and quality through their ability to resemble *in vivo* models more closely in a high throughput manner. With these 3D structures, researchers can screen millions of compounds against human-like disease models to identify drugs that are likely to be effective.

3. CRISPR

Some labs have identified advantages in combining Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) gene editing solutions with organoids. This combination could support improved genetic and drug screening models as well as advanced studies in organogenesis.

4. Personalized Medicine

Organoids open the door to recapitulating a patient's own cells and 3D microenvironment in a dish to better mimic the *in vivo* environment. With this *in vitro* configuration, labs can better identify treatments that are a personalized molecular and pharmacogenomic match for patients. In addition to their precision medicine applications in disease modeling, drug response, and dosage optimization, organoids can also be used for regenerative medicine.

5. Bioprinting

Bioprinting uses a bioprinter, such as the **Corning Matribot®** bioprinter, to create 3D constructs using cells, spheroids, or organoids. These biological materials are suspended in bioinks that are inserted into the bioprinter and then "printed" layer-by-layer. With this process, scientists can more easily study cellular relationships that affect tissue functionality.

Developing Organoids with a Comprehensive Scaffolding Matrix[2,3]

Producing organoids — whether from stem cells or tissue — requires a scaffolding matrix, either biological or synthetic. Today's most widely used scaffolds, including ECMs such as Corning® Matrigel® matrix, come from mouse sarcoma cells, Engelbreth-Holm-Swarm (EHS).

These high-protein solutions provide physical scaffolding and rich microenvironments of hormones and growth factors. Using an appropriate ECM, scientists can grow cultures using a variety of unique protocols.

How to Culture Organoids[4,5,6,7]

Culturing processes depend on organoid type: Organoids grown from stem cells come from induced pluripotent or embryonic stem cells, while tissue organoids usually originate from fragments of enzyme-digested organs placed in Matrigel matrix or Collagen I. Reagents and vessels will vary by organ.

Importantly, many factors influence the effectiveness of organoid development in conjunction with the culturing method, including the use of ECMs, growth factors, media, and signaling molecules. In general, patient-derived organoids take about 1-2 weeks to culture while organoids that are being differentiated from induced pluripotent stem cells (iPSCs) can take 20 days or longer.

Dome Culturing

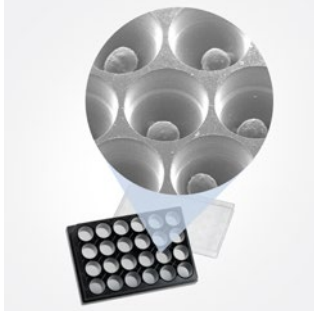
In this method, cells are suspended within an ECM such as Matrigel matrix to generate a self-contained dome. This process gives cells consistent Matrigel matrix access to self-organize into organoids within the structure. This method is often used for adult stem cell-derived organoids from tissue.

Bioreactor Culturing

This method is traditionally used with induced pluripotent stem cell-derived organoids. The organoids are encased in Matrigel matrix droplets, combined with differentiation media, and placed in a **spinner flask** or bioreactor. This process — which has been used with cerebral organoids — offers the advantage of improved nutrient absorption and faster high throughput generation.

Permeable Support Culturing

This method requires vessels such as **Transwell® or Falcon®** permeable supports from Corning to provide optimal structure and conditions for cell differentiation. These inserts contain wells that allow for optimal structure and conditions for 3D culture and tissue modeling. One application of permeable support-enabled culturing involves skin organoids: When combined with air-liquid interface treatment, this process can develop tissue from human keratinocytes within weeks.



Low-adhesion Microplate Culturing

Ultra-Low Attachment (ULA) surfaces in microplates prevent cell binding, creating 3D structures that can then be embedded in an ECM. This low attachment mechanism, combined with the unique round bottom shape of the **Corning spheroid and Elplasia microplates**, allows scientists to culture one organoid per well or cavity of the microplate more easily. Such a protocol has been used to generate intestinal organoids.

How to Freeze Organoids[8]

Successful freezing is essential to keeping organoids long-term. Prior to freezing organoids, the size should be optimized for a successful recovery. In general the process is very similar to traditional single cell freezing. To accomplish this, consider this protocol from Nature Protocols:

1. Cool a freezing container to 4°C. For every **cryogenic vial tube**, have at least one (for 24-well plates) or two (for 48-well plates) confluent wells.
2. Use a 1,000 µL pipet and 500 to 1,000 µL basal medium to draw the media up and down. This disrupts and activates the basement matrix of organoids. Move the suspension to a 15 mL centrifuge tube, add cold basal medium from the top, and resuspend repeatedly to remove the basement matrix.
3. Set the centrifuge to 100 to 200 x g for five minutes at 8°C. Extract all but about 2 mL of the supernatant. Avoid breaking the organoids into single cells.
4. Resuspend by adding in more cold medium from the top of the tube. Run the centrifuge at 200 to 250 x g for five minutes at 8°C.
5. Extract all of the supernatant.
6. Carefully resuspend in 500 µL of chilled freezing medium per one or two wells (depending on which plate is used).
7. Transfer each 500 µL to the cryogenic vials. Put the cryogenic vials in the precooled container and promptly move to -80°C. Keep cryogenic vials in the freezer for at least 24 hours before transferring to liquid nitrogen (-196°C) for long-term preservation.

NOTE: The cryopreservative DMSO in freezing media can be harmful to cells at room temperature. For this reason, freezing media should be cold, and the time spent manipulating it (adding media to cells before transferring to the freezer) should be kept under 5 minutes.

How to Count and Measure Organoids

Organoids will need to be counted and measured to assess growth and progress. Getting this process right is important because the size and count of organoids will influence the usability of those cultures, and organoids will typically only reach a certain size *in vitro*.

Counting Organoids

Cultures can be manually counted on the plate under a microscope. For accurate counts, become familiar with the studied organoid structure to differentiate it from single cells and large multi-layer tissue fragments. Equipment such as the **Corning® Cell Counter**, which now features software that is engineered to account for the complexity of 3D culture shapes and sizes can provide automation support for faster workflows.

Measuring Organoids[9,10]

Irregularities in organoid shape can make manual measurements under a microscope more complicated. Software can help scientists assess organoid size for high throughput work.

Post-Culturing: Recovering, Fixing, Imaging, and Transfecting Organoids

How to Recover Organoids[11]

Cells can be recovered from ECM with reagents which is not always a necessary step, but can prove helpful for certain applications. The recommended product for recovery from Corning® Matrigel® Matrigel matrix is the **Corning Cell Recovery Solution**. Unlike enzymatic reagents such as trypsin, collagenase, or other proteolytic enzymes, the solution is non-enzymatic, which means it won't break down your organoids. Here's how to use it:

1. Remove as much cell culture medium from the culture as possible without disturbing the cells.
2. Add pre-chilled Corning Cell Recovery Solution at a volume at least twice that of the volume of Matrigel matrix.
3. Pipet up and down gently using wide bore tips to carefully break up the Matrigel matrix without damaging the organoids.
4. Incubate cultures with a recovery solution at 4°C for about 20 minutes.
5. Check the cells under the microscope to see if the Matrigel matrix has been fully depolymerized and the organoids are freely floating.
6. If 3D organoids appear free-floating from the Matrigel matrix, briefly centrifuge it to separate structures from the solution. Remove the solution, and wash with cold PBS several times. If organoids are not yet free, incubate longer at 4°C or add additional cell recovery solution.

How to Fix Organoids[12,13]

Organoids grown in Matrigel matrix can be fixed with 4% paraformaldehyde in 4°C for 30 minutes to 4 hours, depending on the organoid size and imaging type. Because Matrigel matrix may at times depolymerize post-fixing, adding 1% glutaraldehyde to the matrix can prevent depolymerization.

How to Stain and Image Organoids[14]

Imaging via confocal or multiphoton equipment may require preparation to ready the organoids. In one article from Nature Protocols, authors describe a protocol comprising the following steps:

1. After recovery and fixation, organoids were blocked with an organoid washing buffer.
2. Researchers then conducted immunolabeling on the organoids.
3. Organoids were cleared with a fructose-glycerol agent or tissue clearing agent.
4. Organoids were applied to slides for imaging.

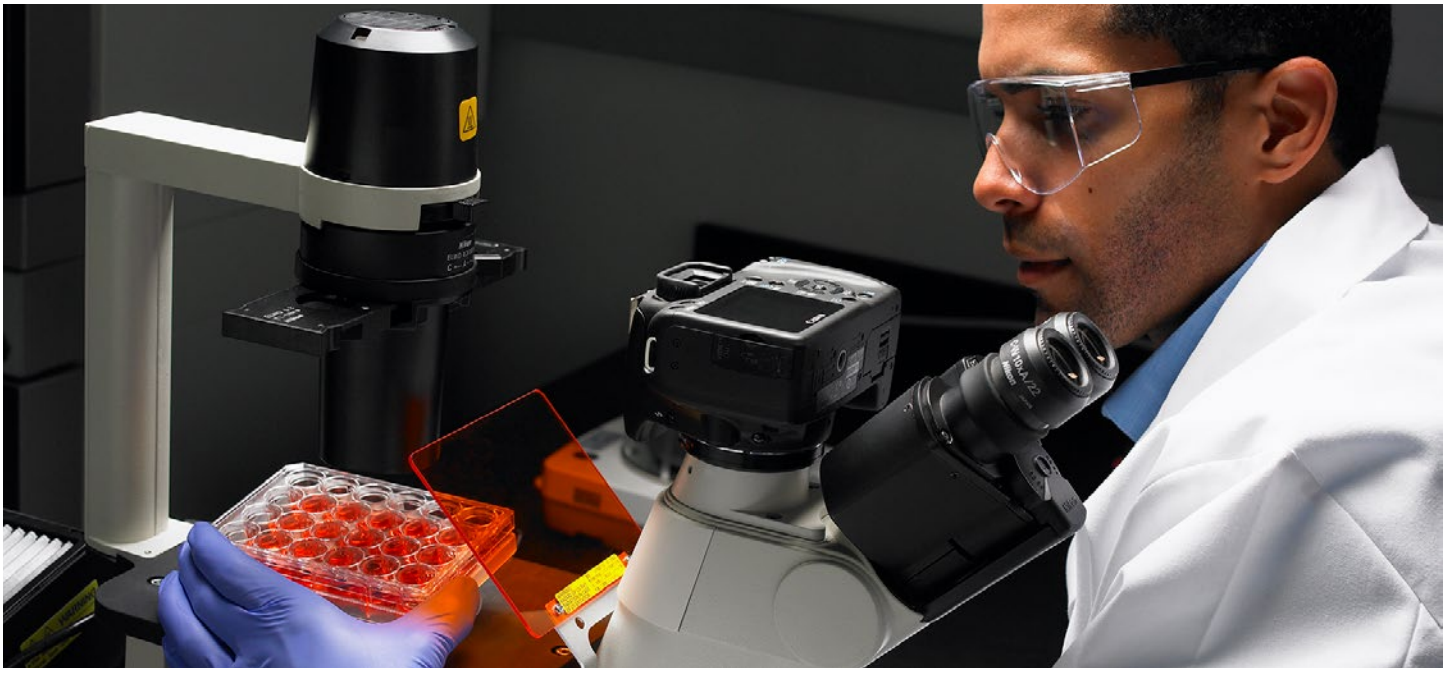
Staining organoids will similarly require a preparation of media extraction, PBS addition, and extraction. To see how a sequence of wash steps was applied for gastrointestinal epithelial organoids, refer to Current Protocols in Mouse Biology.[15]

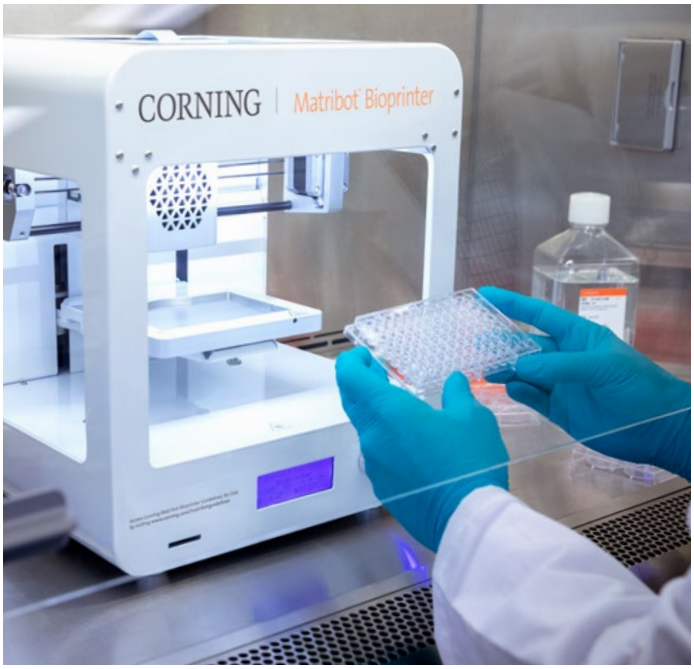
How to Transfect Organoids[16]

Transfection protocols depend on several factors, including cell type, organoid size, and reagent. However, a general protocol for siRNA transcription could include the following steps:

1. Apply transfection reagents in serum media. Cover overnight in incubator.
2. Change the media the following day.

If increasing transfection efficiency is desirable, a configuration from a Nucleic Acids Research paper is worth noting: Researchers combined automatic generation of microencapsulated organoids in Matrigel matrix microbeads with electroporation. A modified microbead size and Matrigel matrix volume can drive better efficiency by requiring less ECM while yielding the same organoid count.[3,17]





Automating Tasks for More Scale-up Efficiency

Organoids represent a new frontier in 3D research, helping to replicate *in vivo* conditions within more manageable and observable *in vitro* environments.

But to help organoids reach their full potential, they must be compatible with more automated and high throughput workflows. Next-generation tools such as the **Corning® Matribot®** bioprinter offer progress in this area. With advanced equipment in conjunction with advanced biomaterials such as Matrigel matrix, the future of 3D research is promising indeed.

Learn more about Corning's tools and protocols and find application notes and case studies covering organoids at **www.corning.com/organoid.**

References

1. Organoid Models. Corning.com. Published 2022. https://www.corning.com/worldwide/en/products/life-sciences/applications/cell-culture/3D-cell-culture/organoid-models.html.
2. Ma L, Li J, Nie Q, et al. Organoid Culture of Human Prostate Cancer Cell Lines LNCaP and C4-2B. American Journal of Clinical and Experimental Urology. 2017;5(3):25-33. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5698596/.
3. Citations on Corning Matrigel Matrix and Organoid Culture (Corning, CLS-AN-503).
4. Shamir ER, Ewald AJ. Three-Dimensional Organotypic Culture: Experimental Models of Mammalian Biology and Disease. Nature Reviews Molecular Cell Biology. 2014;15(10):647-664. doi:10.1038/nrm3873
5. Tuveson Laboratory Murine and Human Organoid Protocols. April 27, 2016. Prepared by: Lindsey Baker, Hervé Tiriac, Vincenzo Corbo. Based on methods described in: Huch M et al. 2013 Unlimited *in vitro* expansion of adult bi-potent pancreas progenitors through the Lgr5/R-spondin axis. EMBO J. 32: 2708-21; and Boj S et al. 2015 Organoid models of human and mouse ductal pancreatic cancer. Cell 160: 324-38.
6. Lancaster MA, Knoblich JA. Generation of Cerebral Organoids from Human Pluripotent Stem Cells. Nature Protocols. 2014;9(10):2329-2340. doi:10.1038/nprot.2014.158
7. A Novel Method for Generating Single, Intestinal Organoids for High Throughput Screening (Corning, CLS-AN-464).
8. Broutier L, Andersson-Rolf A, Hindley CJ, et al. Culture and Establishment of Self-Renewing Human and Mouse Adult Liver and Pancreas 3D Organoids and Their Genetic Manipulation. Nature Protocols. 2016;11(9):1724-1743. doi:10.1038/nprot.2016.097
9. Ma L, Li J, Nie Q, et al. Organoid Culture of Human Prostate Cancer Cell Lines LNCaP and C4-2B. American Journal of Clinical and Experimental Urology. 2017;5(3):25-33. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5698596/.
10. Boehnke K, Iversen PW, Schumacher D, et al. Assay Establishment and Validation of a High-Throughput Screening Platform for Three-Dimensional Patient-Derived Colon Cancer Organoid Cultures. Journal of Biomolecular Screening. 2016;21(9):931-941. doi:10.1177/1087057116650965
11. Extraction of Three-Dimensional Structures from Corning Matrigel Matrix Guidelines for Use (Corning, CLS-AN-528).
12. Dekkers JF, Alieva M, Wellens LM, et al. High-Resolution 3D Imaging of Fixed and Cleared Organoids. Nature Protocols. 2019;14(6):1756-1771. doi:10.1038/s41596-019-0160-8
13. Mahe MM, Aihara E, Schumacher MA, et al. Establishment of Gastrointestinal Epithelial Organoids. Current Protocols in Mouse Biology. 2013;3(4):217-240. doi:10.1002/9780470942390.mo130179
14. Dekkers JF, Alieva M, Wellens LM, et al. High-Resolution 3D Imaging of Fixed and Cleared Organoids. Nature Protocols. 2019;14(6):1756-1771. doi:10.1038/s41596-019-0160-8
15. Mahe MM, Aihara E, Schumacher MA, et al. Establishment of Gastrointestinal Epithelial Organoids. Current Protocols in Mouse Biology. 2013;3(4):217-240. doi:10.1002/9780470942390.mo130179
16. Morgan RG, Chambers AC, Legge DN, Coles SJ, Greenhough A, Williams AC. Optimized Delivery of siRNA into 3D Tumor Spheroid Cultures In Situ. Scientific Reports. 2018;8(1). doi:10.1038/s41598-018-26253-3
17. Laperrousaz B, Porte S, Gerbaud S, et al. Direct Transfection of Clonal Organoids in Matrigel Microbeads: A Promising Approach Toward Organoid-Based Genetic Screens. Nucleic Acids Research. 2018;46(12):e70-e70. doi:10.1093/nar/gky030

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