# Corning® Synthegel® Spheroid Matrix Kit for Generating Cancer Spheroids in a Synthetic Peptide Hydrogel

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## Application Note

*Hilary Sherman and Ann Ferrie*
*Corning Incorporated, Life Sciences*
*Kennebunk, ME USA*

## Introduction

Utilization of 3D models has increasingly become crucial for research and drug discovery as cost and ethical questions have increased from using animal models[1]. This increased utilization and interest for culturing spheroids has resulted in a desire for a scaffold cell culture system outside biological hydrogels such as collagen or basement membrane extract. These biological hydrogels have long been considered the gold standard for 3D work but can be challenging to work with due to inherent biological variability, necessity for reduced temperature handling, and pH requirements. The Corning Synthegel spheroid matrix kit eliminates these challenges as it utilizes a fully synthetic peptide matrix to form a defined and consistent hydrogel, which does not require reduced temperatures or a specific pH to handle. Additionally, the stiffness of Corning Synthegel spheroid matrix can be tuned to better support the generation of specific spheroids. Here, spheroids were generated from various cancer cell lines known for their difficulty in forming spheroids on low attachment surfaces to demonstrate how the Corning Synthegel spheroid matrix kit can improve the formation of more spheroid-like structures.

## Materials and Methods

The following cells lines: Panc-1 (ATCC® CRL-1469™), MDA-MB-231 (ATCC CRM-HTB-26™), and PC-3 (ATCC CRL-1435™) were thawed and cultured in Dulbecco's Modified Eagle's Medium (DMEM; Corning 10-013-CV) containing 10% fetal bovine serum (FBS; Corning 35-010-CV) to <90% confluence in a 75 $cm^2$ flask (Corning 430641). Cells were harvested using 0.05% Trypsin, 0.53 mM EDTA (Corning 25-052-CV) and were resuspended in DMEM containing 10% FBS prior to the addition of media, Corning Synthegel X-Link solution, and matrix to achieve 1 mL of various hydrogel concentrations at a cell density of $5 \times 10^4$ cells/mL (Table 1). One hundred microliters of each hydrogel/cell mixture were added to each of 8 wells of a 96-well microplate (Corning 3596). The plate was then transferred to a 37°C incubator for 30 minutes to allow for gel polymerization. After 30 minutes, 200 µL of DMEM supplemented with 10% FBS was overlayed on top of each well of hydrogel to provide nutrients to support cell proliferation. A scaffold-free control was established at an equivalent cell density and volume per well for each of the different cell types. These cells were seeded into 96-well Ultra-Low Attachment (ULA) surface microplates (Corning 3474) and placed in a 37°C incubator for the duration of the experiment. Cells were cultured for a total of 8 days with a media exchange occurring on Day 4 as recommended in the Corning Synthegel Spheroid Matrix Kit Guidelines for Use (CLS-AN-739DOC). Images of all the cell types were taken daily to view the progress of the spheroids.

## Results and Discussion

Embedding single cells in a hydrogel can often result in more uniform and compact spheroids than those generated in a flat bottom, ULA surface microplate as seen with the MDA-MB-231 and Panc-1 spheroids generated in Synthegel spheroid matrix (Figures 1 and 2). This is partly because the hydrogel immobilizes the cells whereas on ULA surface microplates, cells are free-floating allowing them to collect towards the edges of the well where they can merge into larger spheroids or aggregates. Spheroids generated using the PC-3 cell line demonstrate the correlation between increasing concentrations of Synthegel spheroid matrix from 0.3% to 1.5% and compactness of spheroids especially as compared to using the ULA surface (Figure 3). This ability to dial-in the gel strength is an added benefit to using Corning Synthegel spheroid matrix.

**Table 1.** Volumes of cells, media, Corning Synthegel X-Link solution and Corning Synthegel spheroid matrix used.

| Gel Concentration | Media/Cell Suspension (µL) | Synthegel X-Link Solution (µL) | Synthegel Spheroid Matrix (µL) |
|---|---|---|---|
| 0.3% | 838 | 12 | 150 |
| 0.5% | 730 | 20 | 250 |
| 1% | 460 | 40 | 500 |
| 1.5% | 190 | 60 | 750 |

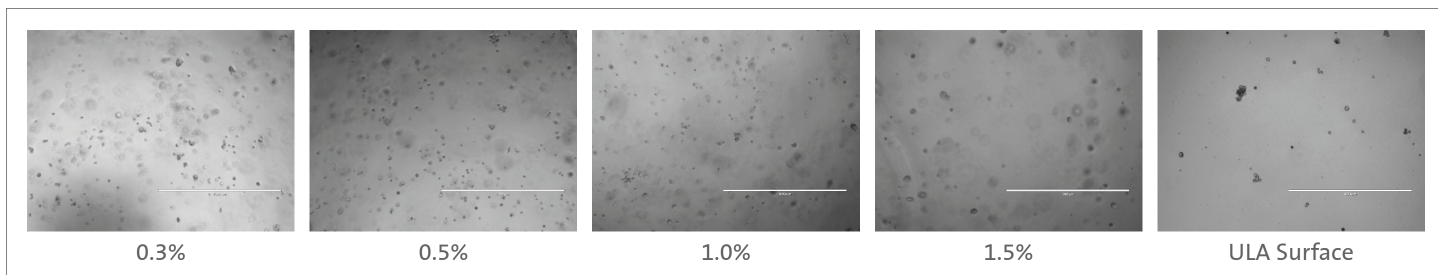


**Figure 1. MDA-MB-231 Morphology.** MDA-MB-231 cells seeded in different gel concentrations of Corning Synthegel spheroid matrix compared to a ULA surface using a 4X objective.

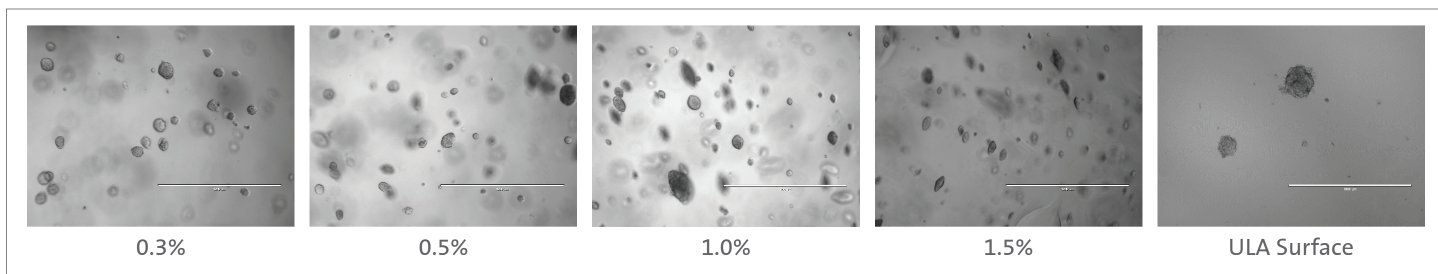


**Figure 2. Panc-1 Morphology.** Panc-1 cells seeded in different gel concentrations of Corning Synthegel spheroid matrix compared to a ULA surface using a 4X objective.

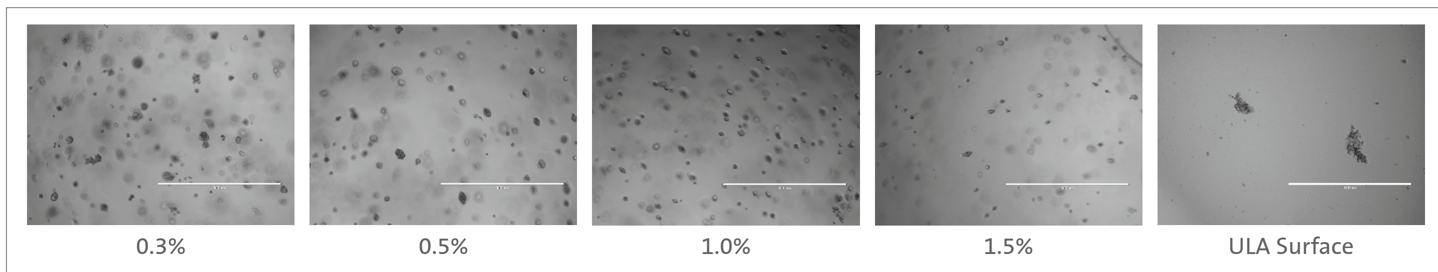


**Figure 3. PC-3 Morphology.** PC-3 cells seeded in different gel concentrations of Corning Synthegel spheroid matrix compared to a ULA surface using a 4X objective.

## Conclusions

Some cancer cell lines are prone to forming loose aggregate like structures when formed in media without a support matrix which often makes performing assays more difficult since it can result in wide variability in sizes and shapes. This is where tunable hydrogels such as Corning® Synthegel® spheroid matrix can be beneficial for 3D cultures. The scaffold not only aids in the formation of more tightly packed spheroids that are distributed throughout a well, but also provides more uniformity and definition to the spheroids making culture and assays more consistent. Finally, since the synthetic peptide hydrogel does not require extreme temperatures or pH, as with most biological hydrogels, cells are not subjected to added stress during seeding.

## Reference

1. Biju TS, Priya VV, Francis AP. Role of three-dimensional cell culture in therapeutics and diagnostics: an updated review. Drug Deliv. and Transl. Res. 13, 2239-2253 (2023).

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