Corning® Synthegel® 3D hiPSC Matrix Kit for Maintaining iPSC in 3D

Application Note

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Introduction

Human induced pluripotent stem cells (iPSCs) are used in a wide variety of applications, from basic research to disease modeling and expanding into cell therapy development[1]. Historically, iPSCs have been cultured on feeder layers or basement membrane extract to maintain their pluripotent state *in vitro* which can present challenges due to the use of animal-derived components which are inherently subject to variability[2]. Additionally, many of the hydrogels currently available require working at a specific pH or temperature range that can induce stress on the stem cells. The Corning Synthegel 3D hiPSC matrix kit offers an alternative for culturing and maintaining iPSCs as 3D spheroids. Synthegel 3D hiPSC matrix doesn't require any special pH or temperature considerations during handling and is fluid enough to easily pipette at room temperature while polymerizing with incubation at 37°C. Here we demonstrate the utility of the Corning Synthegel 3D hiPSC matrix kit to maintain and passage iPSC spheroids without loss of pluripotency.

Materials and Methods

iPSCs (iXCells 30HU-002) were thawed and cultured in mTeSR™1 medium (STEMCELL 85850) on 6-well plates (Corning 3736) coated with Corning Matrigel hESC-qualified matrix (Corning 354277) per vendor's protocol. Upon initiation of study, iPSCs were harvested with Accutase® cell detachment solution (Corning 25-058-CI) to attain a single cell suspension. Cells were centrifuged at 300 xg for 5 minutes and resuspended in mTeSR1 medium containing 1:1000 diluted Synthegel 3D hiPSC Grow Mix (Corning 354792) resulting in mTeSR1 with Grow Mix solution. Cells were counted using ChemoMetec NucleoCounter® NC200™ to ascertain cell viability and density. For seeding, 400,000 cells were resuspended in 1 mL of mTeSR1 with Grow Mix and 40 µL of Synthegel X-Link solution from the Synthegel 3D hiPSC matrix kit (Corning 354789). Once mixed, 1 mL of spheroid matrix peptide nanofiber solution from the Synthegel 3D hiPSC matrix kit was mixed with cell suspension and 500 µL per well was immediately dispensed into 3 wells of an untreated 24-well multiwell plate (Corning 3738). The plates were incubated at 37°C for 30 minutes for hydrogel polymerization and was followed by the addition of 1.5 mL of mTeSR1 with Grow Mix overlayed on top of the hydrogel in each well. mTeSR1 with Grow Mix was replaced daily prior to harvest on Day 4 at which time 500 µL of spent medium from each well was transferred to a 15 mL centrifuge tube (Corning 430791). Using a P1000, the hydrogel containing spheroids were gently broken up in the remaining medium by pipetting up and down 5 times. Medium and hydrogel was then transferred to each of the 3 centrifuge tubes containing the 500 µL spent medium followed by the addition of 10 mL of Dulbecco's Phosphate-Buffered Saline (PBS; Corning 21-031-CM) and centrifuged at 350 xg for 5 minutes. Upon pelleting, the supernatant was aspirated and replaced with an additional 10 mL PBS to wash away any remaining hydrogel and centrifuged a second time at 350 xg for 5 minutes followed by the removal of the PBS supernatant. Five hundred microliters of Accutase were then added to each of the suspensions and transferred to autoclaved Axygen® MaxyClear 5.0 mL SnapLock microcentrifuge tubes (Corning MCT-500-C). Spheroids were incubated at room temperature for 20 minutes to achieve a single cell suspension which could be visualized under the microscope. Once the spheroids were dissociated, 1.5 mL of mTeSR1 with Grow Mix was added to each tube, and cells were counted and pooled. The dissociated cells were then centrifuged at 300 xg for 5 minutes and resuspended in mTeSR1 with Grow Mix and recounted. Cells were re-seeded as previously described. After 3 passages, cells were harvested as previously described and pooled for pluripotency marker analysis using BD Stemflow™ Human and Mouse Pluripotent Stem Cell Analysis kit (BD Biosciences 560477) per vendor protocol. Ten thousand events were analyzed using the MACSQuant® Analyzer 10 flow cytometer (Miltenyi Biotec). The entire study was repeated 3 independent times.

Results and Discussion

Hydrogels, like the Corning Synthegel 3D hiPSC matrix kit, offer a consistent method for culturing iPSC as three-dimensional structures. Figure 1 shows typical morphology and uniform distribution of iPSC cultured in Synthegel 3D hiPSC matrix after 4 days. By harvesting cells every 4 days over several passages we demonstrated a consistent doubling time between 20 and 30 hours (Figure 2). This range is typical for these iPSCs and is similar to what has been reported in literature with other iPSC lines[3-4]. Average viability of iPSC spheroids was maintained above 90% at each passage (Figure 3) and as shown by Figure 4, expansion of iPSC over the course of 12 days averaged almost 10-fold. Importantly, iPSCs harvested after 3 passages in Synthegel 3D hiPSC matrix expressed appropriate markers of pluripotency as can be seen in figure 5, positive for markers SSEA 4 and OCT 3/4 while negative for SSEA1.

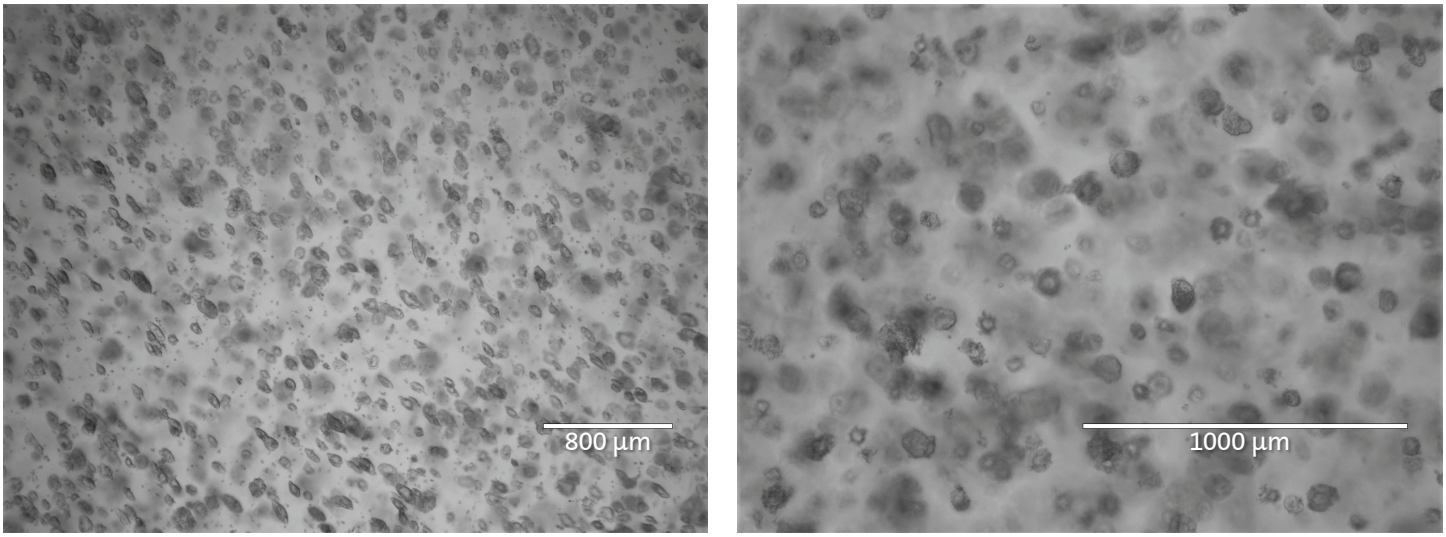

Figure 1. Typical morphology of iPSC spheroids in Corning Synthegel 3D hiPSC matrix kit. Representative images of iPSC spheroids after 4 days of expansion in Synthegel 3D hiPSC Matrix kit taken with a 2X (left) and 4X (right) objective.

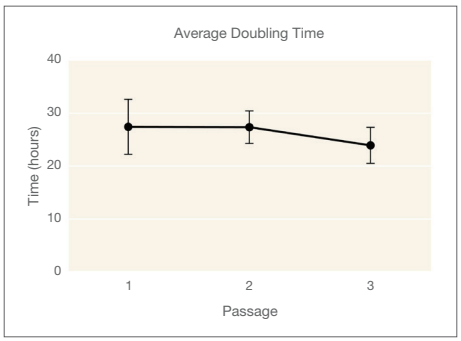

Figure 2. Average iPSC doubling time from 3 passages. Data shown with standard deviations of the mean from 3 independent studies N=9 per passage.

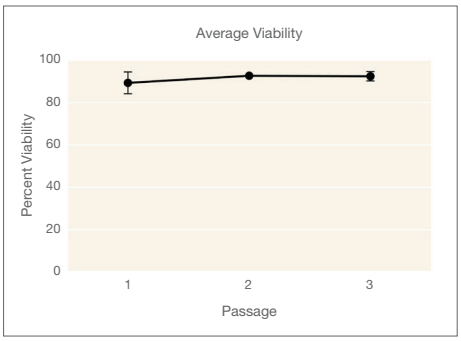

Figure 3. Average viability of iPSC across 3 passages. Data shown with standard deviations of the mean from 3 independent studies N=9 per passage.

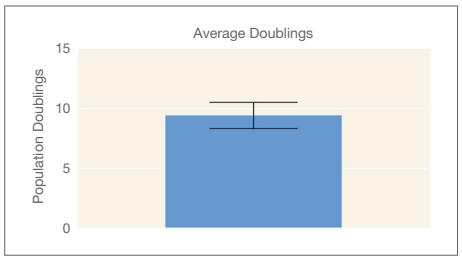

Figure 4. Average cumulative number of iPSC population doublings after 3 passages. Data shown with standard deviations of the mean from 3 independent studies N=9.

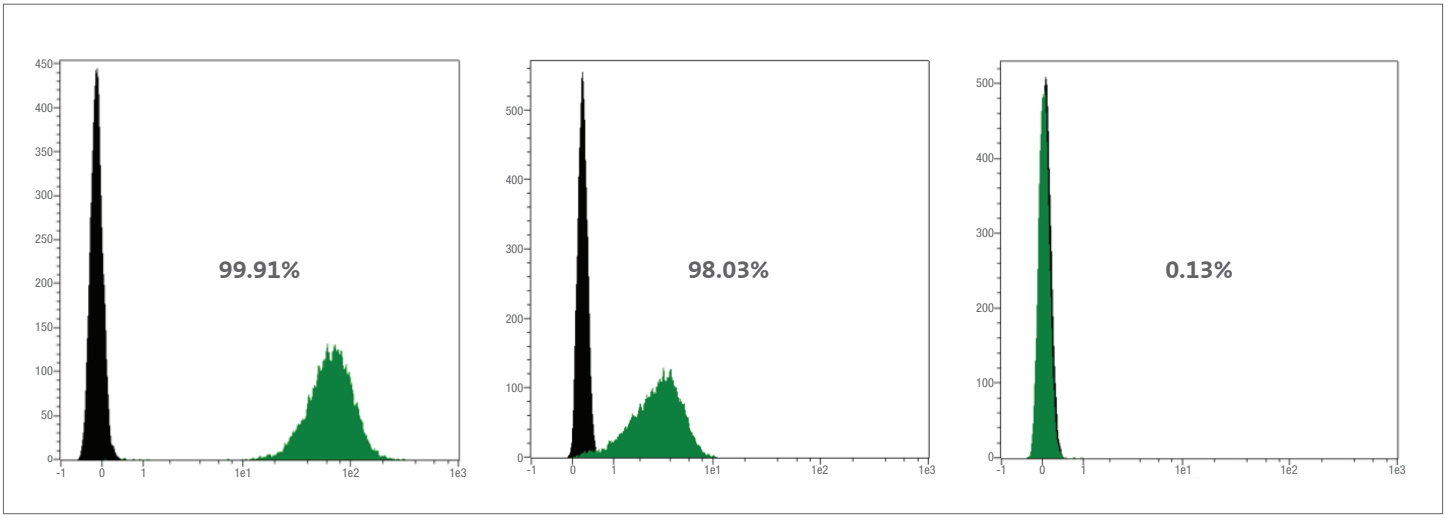

Figure 5. iPSC markers of pluripotency. Representative expression of SSEA4 (left), Oct 3/4 (middle), SSEA1 (right) from one study. Sample (green) compared to isotype control (black).

Conclusions

The Corning® Synthegel® 3D hiPSC matrix kit is an ideal solution when 3D culture of iPSC is desired in a hydrogel that can be handled at room temperature without any specific pH considerations. This makes Synthegel 3D hiPSC matrix gentle on cells and easy to use. When using Synthegel 3D hiPSC matrix to encapsulate iPSC for culture, they can be maintained with expected expansion and high viability. Additionally, iPSCs cultured in Synthegel 3D hiPSC matrix maintain their pluripotency without the need for a biological coating or feeder cells.

References

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