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Advanced Synthetic Hydrogel Platform Maintains Pluripotent Identity and Growth Dynamics in iPSCs

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Abstract

This application note explores the use of *NexaGel® STEM, a synthetic hydrogel platform, for culturing induced pluripotent stem cells (iPSCs) in both 2D and 3D environments. iPSCs, given their pluripotency and patient specificity, are a key tool for applications such as disease modelling, regenerative medicine, and drug discovery, although traditional 2D cultures on vitronectin lack scalability and may not be entirely appropriate for every application. NexaGel® offers a defined, xeno-free, tunable system that enhances iPSC culture by mimicking the extracellular matrix, providing consistency, and reducing contamination risks. This study demonstrates that iPSCs cultured in NexaGel® maintain pluripotency and viability, with 3D cultures forming spheroid structures that support cell proliferation, making NexaGel® a promising tool for optimizing iPSC culture and advancing research in basic and clinical applications.

Introduction

Induced pluripotent stem cells (iPSCs) have become a staple *in vitro* model for research and development in the fields of regenerative medicine, disease modelling, and drug discovery. These cells offer unprecedented opportunities for scientific and medical advancements by virtue of their capacity to differentiate into virtually any cell type, while cell types with specified, patient-derived, or manipulated genotypes can be developed. However, the successful culture and maintenance of iPSCs remain a significant challenge, as they require very specific extracellular matrix (ECM) proteins or other mimicking molecules that recapitulate the 3D native tissue structure[1].

iPSCs are classically cultured as 2D adherent cells using a substrate coating molecule such as vitronectin. However, these 2D systems do not completely replicate the *in vivo* environment, and although they are able to produce differentiated cell types of all three germ layers, they are limited for clinical applications which require a huge number of cells, due to their lack of scalability, efficiency, and safety. Suspension culture of iPSCs as spheroid structures also has limitations of high cell death due to lack of nutrient and oxygen diffusion into the centre of the cell aggregates.

Hydrogels are 3D networks of water-soluble polymers that provide structure and facilitate adhesion, proliferation, and differentiation of iPSCs. Biologically derived hydrogels include collagen, gelatin, and hyaluronic acid, however, these materials have inherent variability of composition which may affect the reproducibility of experimental outcomes and clinical products. Synthetic hydrogels are innovative alternatives that mimic the ECM, providing consistency and reducing the risk of contamination, which bring huge benefits to scientists seeking to standardize iPSC culture methods during expansion and differentiation. The controlled environment they provide also presents the opportunity to further investigate cell-matrix interactions, optimizing differentiation for tissue engineering purposes, and scaling up iPSC production for clinical applications[2].

The NexaGel® synthetic hydrogel platform is a xeno-free and tunable system that offers maximum flexibility and customization for 3D culturing of iPSCs and other cell types. This application note demonstrates the utility of NexaGel® in facilitating iPSC scale up and research.

Materials and Methods

Materials	Supplier	Cat. No.
mTesR PLUS	Stem Cell Technologies	100-0276
mTesR PLUS Supplement	Stem Cell Technologies	100-0274
CultureCEPT	ThermoFisher	A56799
RUO Recombinant FGF2-G3 Protein	Sartorius	CYK-0050-0002
NexaGel® STEM	Sartorius	NGH02
NexaGel® Cell Recovery Solution	Sartorius	NGR04-100
TrypLE™ Express Enzyme	Gibco	12604013
Costar™ 96-Well, Clear Cell Culture-Treated, Flat-Bottom Microplate	Corning	3595
Costar™ 6-Well, Clear Cell Culture-Treated, Flat-Bottom Microplate	Corning	3516

Table 1: Cell culture media and reagents used for culturing iPSCs.

Routine cell culture

iPSCs were passaged using TrypLE to lift cells twice per week in vitronectin (10 µg/mL) coated 6-well plates at a density of 100 K/well, in mTesR PLUS medium. Plates were pre-coated by adding 10 µg/mL of vitronectin diluted in PBS. They were incubated for 30 minutes at 37 °C, then the coating solution was aspirated and the wells washed with PBS before adding cell suspension. After passaging, cells were reseeded in media containing 1X CultureCEPT, which was removed after 24 hours.

Culture of iPSCs with NexaGel®

iPSCs were cultured either in 2D or 3D with NexaGel® STEM. For 2D culture, the hydrogel was brought to room temperature, then mixed in a 2:1 ratio with cell culture medium. This solution was dispensed into the wells of a 96-well plate (50 µL/well). After a 10 – 15 minutes incubation in the incubator to allow soft gel formation, 100 µL of medium containing 70,000 cells/mL was gently pipetted onto the surface of the gels.

For 3D culture, iPSCs were cultured in static suspension. Harvested cells were resuspended in 11% NexaGel® STEM in culture medium, with a final cell concentration of 10,000 cells per well. 180 µL of this mixture was pipetted gently into each well of a 96-well plate then transferred to an incubator at 37 °C with 5% CO_2.

hESC-qualified animal-derived ECM was used as a control condition for 3D iPSC culture. For these samples, animal-derived ECM embedded cells were cultured on top of a base of animal-derived ECM, in accordance with the Sartorius recommended protocol for embedded multi-spheroid assays. Temperature was maintained at 4 °C for plastic consumables and reagents whilst handling animal-derived ECM to prevent premature polymerization during preparation (liquids and tubes were kept on ice, cooled pipette tips used).

Additional conditions for this study were:

1. Using three times the amount of media supplement in the gel to compensate for the dilution effect from adding the hydrogel volume. This was expected to enhance cell survival and subsequent proliferation.
2. Including recombinant thermostable FGF2-G3 to support the survival and growth of iPSCs.
3. A combination of both three times supplement and FGF2-G3.

Live-cell imaging and analysis

During both routine culture and experiments, cells were monitored using the Incucyte® Live-Cell Analysis System to investigate growth dynamics and morphological changes. The confluence of cells cultured in 2D was measured using integrated Incucyte® AI Confluence Analysis. Images of cells cultured in 3D were collected using the Embedded Multi Spheroid scan type and analysed using the Incucyte® Spheroid Analysis Software Module.

Surface marker expression analysis

Following culture with NexaGel®, cells were harvested and stained for iPSC markers of pluripotency and non-pluripotency. Cells were either lifted using TrypLE (2D cells on vitronectin) or extracted from the gel using NexaGel® Cell Recovery Solution (cells cultured on or embedded in NexaGel® and animal-derived ECM). Cells were reseeded in a 96-well V-bottom plate at 20 K/well, centrifuged at 300*g* for 5 minutes and washed twice with PBS + 2% FBS before being treated with SSEA-1-FITC (1:50), SSEA-4-APC (1:800), TRA-1-60-PE (1:25), and iQue® Cell Membrane Integrity Dye (B/Red) Kit (1:50)) and incubated at room temperature in the dark for 30 minutes. The plate was centrifuged at 300*g* for 5 minutes before analyzing on the iQue® HTS platform. Data was analyzed using integrated iQue Forecyt® Software.

	Product name	Supplier	Cat. No.	Final Concentration
Harvesting reagent	NexaGel® Cell Recovery Solution	Sartorius	NGR04-100	1X
Staining buffer	Phosphate Buffered Saline (PBS)			
	Fœtal Bovine Serum (FBS)			
96-well V-bottom plate	NuncTM MicroWell™ Plates	ThermoFisher	249570	-
Viability	iQue® Cell Membrane Integrity Dye (B/Red) Kit	Sartorius	90346	1 : 50
Stem cell marker panel	SSEA-1-FITC	BioLegend	301904	1 : 50
	SSEA-4-APC	BioLegend	330418	1 : 800
	TRA-1-60-PE	BioLegend	330610	1 : 25

Table 2: Reagents and materials used for cell analysis using the iQue® 3 HTS Cytometer.

Results

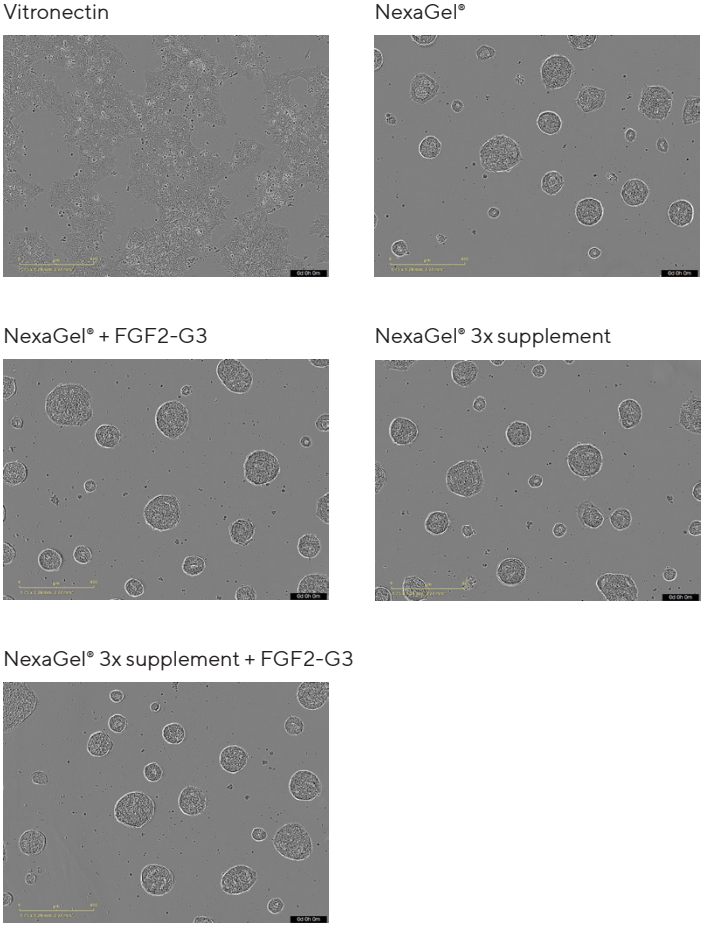
Cell morphology, growth dynamics, and identity in 2D NexaGel® culture

Figure 1A shows the morphology of iPSCs cultured in a 2D format, i.e. directly seeded onto a flat surface, whether vitronectin or NexaGel® with various supplementation. On vitronectin, the cells form compact colonies that are tightly packed with defined edges, round or slightly irregular in shape. Within these characteristic colonies, the cells have a high nucleus-to-cytoplasm ratio. When cultured on a thick layer of NexaGel®, iPSCs form spheroid-like colony structures. These tend to be smaller, with a more regular, spherical shape, and the cells appear to adhere to the surface to a lesser extent than to vitronectin.

There is visually little difference between conditions with or without additional supplementation.

The confluence of the cells in these images was analyzed using the Incucyte® basic analysis module (Figure 1B). Cell proliferation was quickest when cells were cultured on vitronectin, reaching a growth plateau at around 3 days post-culture initiation. For cells cultured on NexaGel®, the proliferation was slower, with growth continuing to increase by the end of the culture period. NexaGel® without any supplementation produced the lowest cell growth, and there was negligible difference between the other conditions.

A



B

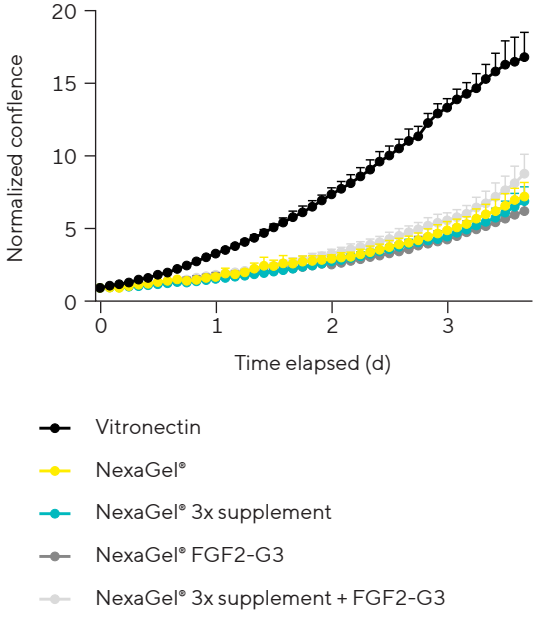


Figure 1: Morphology and Growth Dynamics of iPSCs Cultured on Vitronectin and NexaGel® STEM. A: Brightfield images of iPSCs following 3 days 20 hours of culture. B: Confluence of cells over experimental time period (3 days 20 hours), evaluated using live-cell analysis.

Figure 2 shows the expression of stem cell markers by cells cultured in 2D either on vitronectin or on NexaGel® after 3 days 20 hours of culture. Viability is above 90% for every culture condition, indicating successful, non-perturbing recovery of cells from the surface of the hydrogel. The marker expression profile for both vitronectin and NexaGel® with or without supplementation matches that expected of a pluripotent population, i.e. negative for SSEA-1, and positive for SSEA-4 and TRA-1-60. Expression of each marker is similar regardless of culture conditions, although the pluripotent population is slightly higher in cells cultured traditionally on a coating of vitronectin.

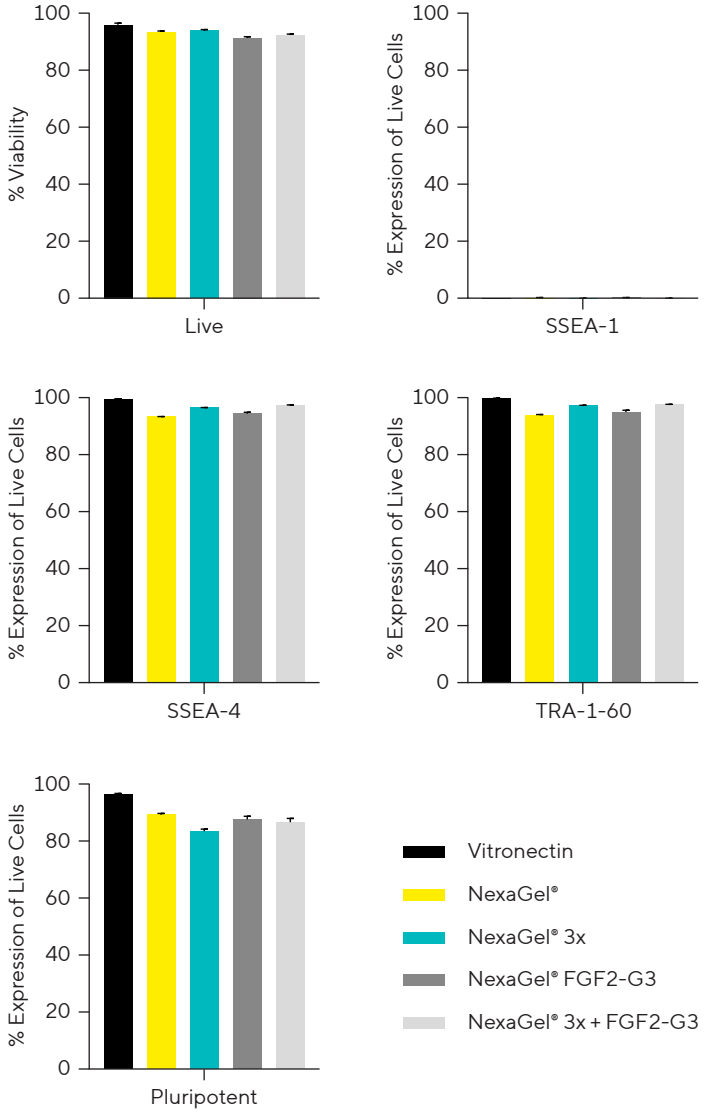


Figure 2: Expression of Stem Cell Markers by Cells Cultured in 2D on Either Vitronectin or NexaGel® STEM with Various Supplements.

Cell morphology, growth dynamics, and identity in 3D NexaGel® culture

Stem cells are increasingly being used in 3D systems that necessitate embedding of cells in an ECM material to recreate the cell-cell and cell-matrix attachments they would exhibit in tissues. Applications for such models include certain differentiation protocols[3], tissue engineering[4],disease modelling[5], and drug screening[6]. iPSCs can be embedded in either animal-derived ECM or NexaGel® STEM, although their morphologies are very different in the naturally derived versus the synthetic hydrogel (Figure 3A). In animal-derived ECM the cells clearly interacted with the gel and formed small, irregular, colony-like structures similar to those observed when cultured on 2D vitronectin. In contrast, cells embedded in NexaGel® STEM formed more obvious spheroid-like structures (Figure 3A), which expanded in size during the period of culture (Figure 3B). Spheroid size was analyzed using images captured by live-cell analysis. The rate of proliferation of cells embedded in animal-derived ECM was not as fast as for the cells embedded in NexaGel® STEM, and the addition of FGF2-G3 accelerated spheroid growth.

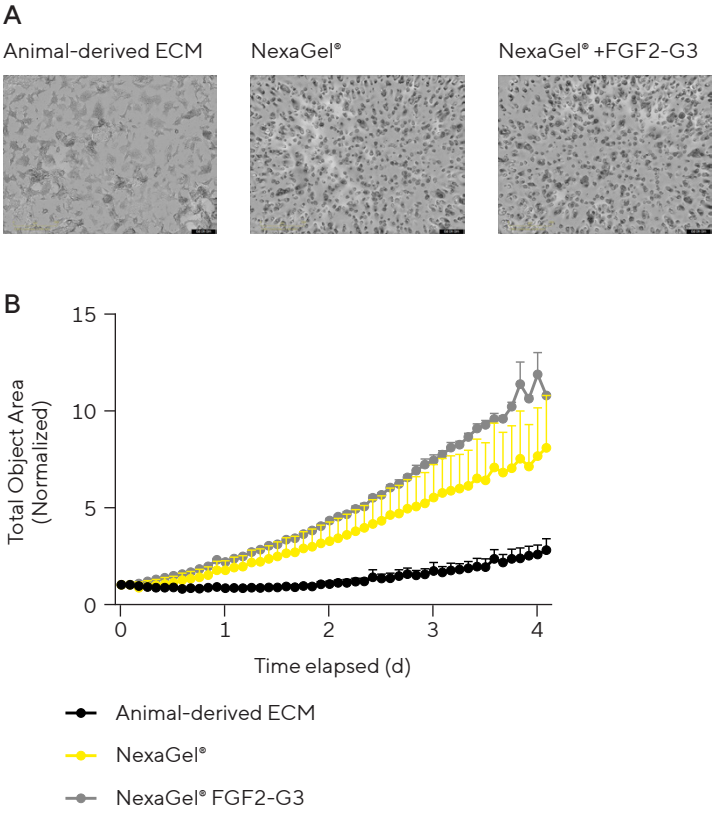


Figure 3: Morphology and Growth Dynamics of iPSCs Cultured in 3D - Embedded in Hydrogel. A: Brightfield images of iPSCs embedded in animal-derived ECM or NexaGel® STEM following 4 days of culture. B: Total object area per image (normalised to day 0) over time, evaluated using live-cell analysis.

The corresponding marker expression profiles of cells embedded in 3D are depicted in Figure 4. Again, high viability under all conditions reflects a robust cell harvesting method with excellent recovery rates. The expression profiles (SSEA-1 negative, SSEA-4 and TRA-1-60 positive) aligned with those observed in both standard 2D culture on vitronectin and 2D culture on NexaGel® STEM, indicating retention of a large pluripotent population. Addition of FGF2-G3 as a supplement did not significantly affect the expression profile, although the proportion of pluripotent cells was initially high in the un-supplemented conditions.

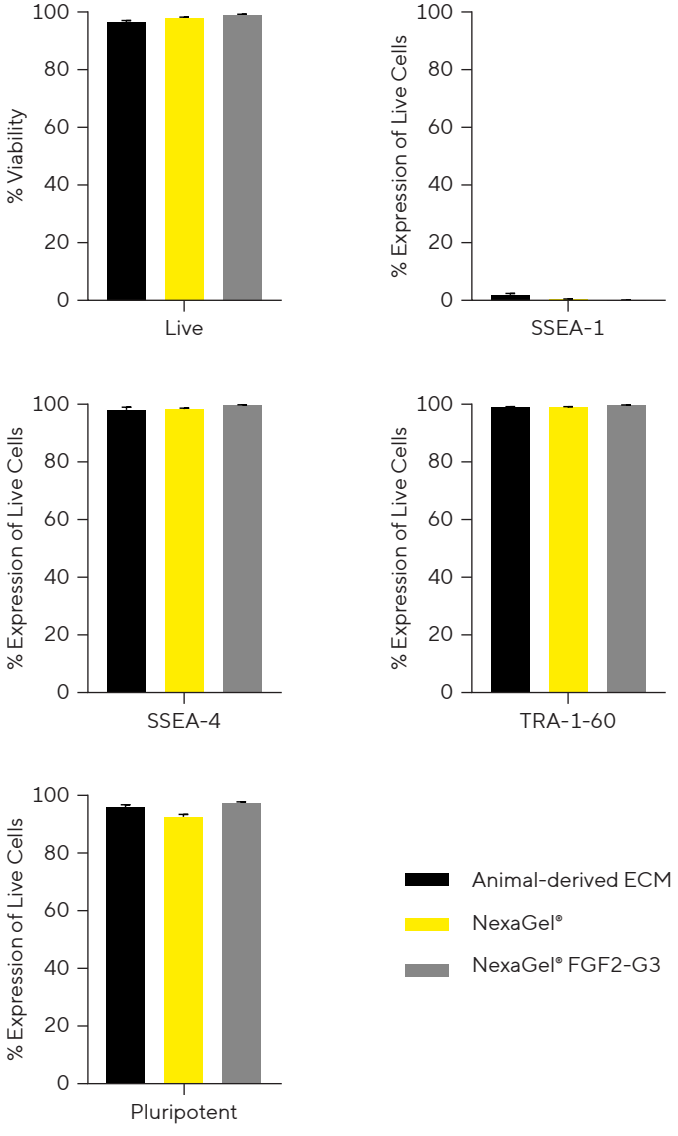


Figure 4: Expression of Stem Cell Markers by Cells Embedded in animal-derived ECM or NexaGe®.

Cell recovery following culture with NexaGel® STEM

Following a period of culture embedded in NexaGel® STEM, cells can be returned to the traditional culture format of a vitronectin coating on the surface of a tissue culture plate. These cells are able to regain the dense colony morphology described previously, which is indistinguishable from cells that were continuously cultured on vitronectin (Figure 5A). Equally, these cells proliferate similarly to their vitronectin-cultured counterparts, although the growth rate is somewhat slower (Figure 5B). This exemplifies the non-perturbing nature of NexaGel® STEM, highlighting successful transition back to 2D culture from 3D, and showing that cells expanded or cultured with the synthetic hydrogel are suitable for downstream applications following manipulation.

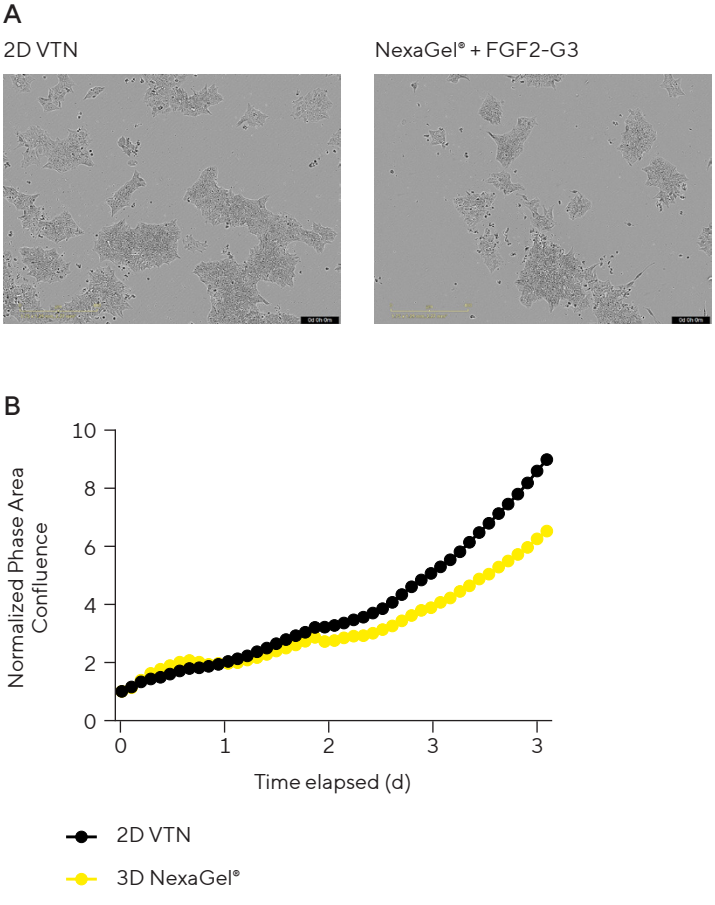


Figure 5: iPSCs Recovered From NexaGel® Culture Can Grow and Proliferate When Returned to 2D Culture on Vitronectin. A: Brightfield images of cells following 3 days 12 hours of 2D culture, cultured on a coating of vitronectin following a preceding growth period either in 2D on a vitronectin coating, or embedded in NexaGel® STEM. B: Growth dynamics of iPSCs during culture.

Summary and Outlook

This application note provides an in-depth examination of the use of the NexaGel® platform for 3D culture of iPSCs to more accurately represent the natural cellular environment compared to vitronectin, and offering more control compared to naturally derived materials. Transitioning from 2D culture to 3D systems including ECM provides the opportunity to create more realistic models for more efficient and directed cell differentiation, to study cellular processes, drug responses, and develop regenerative medicines.

iPSCs cultured on vitronectin form compact colonies with high proliferation rates, maintaining pluripotency markers. In 2D NexaGel® cultures, iPSCs also form colonies with slower growth rates but with retention of a high proportion of pluripotent cells. In 3D cultures, NexaGel® supports spheroid formation with enhanced growth dynamics compared to animal-derived ECM, especially with FGF2-G3 supplementation.

This study demonstrates the utility of NexaGel® in iPSC culture, offering a scalable and reproducible method for expanding iPSCs while maintaining pluripotency. The synthetic hydrogel's tunability and consistency make it a valuable tool for standardizing iPSC culture methods, optimizing differentiation, and scaling up production for clinical applications.

The provision of a controlled environment for iPSC culture opens new avenues for research and clinical applications. Its use in 3D cultures can enhance our understanding of cell-matrix interactions and improve differentiation protocols for tissue engineering. The flexibility of the platform allows researchers to optimize the conditions specific for their application, and is suitable for even more complex studies including organ-on-a-chip models and personalized medicine.

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