Application Note

Combining GrowDex® and spheroONE for automated and highly reproducible 3D cell culture workflows

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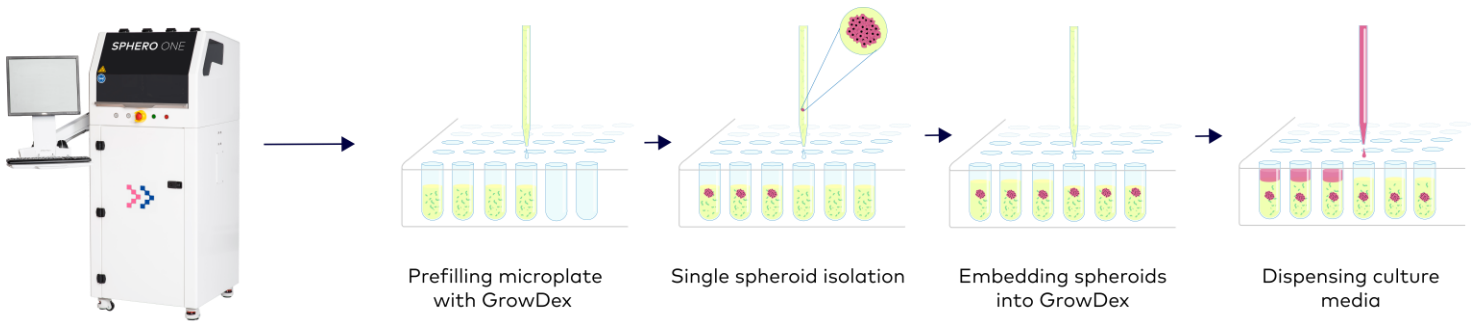
Abstract

This application note demonstrates the compatibility of spheroONE and GrowDex® to automate 3D cell culture workflows. spheroONE is a nanoliter dispenser enabling sorting and isolation of spheroids and organoids. GrowDex® is a plant-derived nanofibrillar cellulose (NFC) hydrogel that provides an effective 3D matrix for culturing various cell types. In this work, it was demonstrated that bulk samples of HepG2 spheroids could be seamlessly grown from single HepG2 cell seeded in this nanofibrillar cellulose matrix. Subsequently, spheroONE could be used to automate dispensing of GrowDex® into microplates prior to sorting, isolation and embedding of single HepG2 spheroids per well to prepare highly reproducible assay-ready microplates for subsequent screening applications.



Introduction

In recent years, there has been a surge of interest in 3D models due to their physiological relevance, setting them apart from traditional 2D models. These 3D models exhibit unique features like spatial architecture, diffusion barriers, distinct gene expression patterns, and drug resistance. They have become the preferred choice for studies in regenerative medicine and anti-tumour drug screenings.

Our study presents a tailored solution for investigating HepG2 spheroids. This model has wide-ranging applications, including liver disease modelling (NAFLD, HCC, viral hepatitis), hepatotoxicity evaluation, and insights into drug impacts on liver cells in physiological contexts. HepG2 spheroids also contribute to hepatic metabolism, enzyme activity, and gene expression analysis, unveiling intricate molecular mechanisms underlying various liver-related conditions.

Ensuring reproducible high-throughput screenings with 3D models hinges on selecting highly uniform spheroid populations based on size and morphology. This uniformity is the foundation for reliable and reproductible experiments requiring an appropriate culture substrate for sustained spheroid growth and viability, enabling long-term screenings. To achieve this, we introduce a solution that combines the advanced spheroONE technology with the proven qualities of GrowDex®.

The spheroONE is an innovative instrument designed for isolating and sorting large particles like spheroids, tumoroids and organoids. Its automation capabilities simplify workflows and enhance efficiency. Additionally, it offers precise dispensing of buffers and culture matrices, making it a highly versatile tool for multiple applications.

GrowDex® hydrogels, on the other hand, are animal-free plant-derived nanofibrillar cellulose (NFC) hydrogels that provide an effective 3D matrix for culturing various cell types. GrowDex resembles the body's extracellular matrix, supports cell growth, and allows the easy diffusion of molecules. GrowDex® is compatible with automation, does not need special conditions for handling and storage, and is easy to work with, making it ideal for various 3D cell-based screening applications.

The symbiotic synergy between the advanced spheroONE technology and the proven GrowDex® sets new standards in the field of 3D cell culture and screening, offering researchers a powerful and versatile tool for advancing their studies and accelerating scientific discoveries.

Methods & Results

A. HepG2 spheroids formation in GrowDex®:

HepG2 cells were cultured in a traditional monolayer culture for 4 days in complete media. GrowDex® working solution (0.5% w/v) was prepared by diluting GrowDex® stock (1.5% w/v) with complete media. HepG2 cells were harvested and resuspended in GrowDex® working solution (0.5%w/v) at a concentration of 10^5 cells/mL. Cells suspended in GrowDex® were

pipetted into each well of a 96-well Flat-bottom ULA plate (100 µL/well), resulting in approximately 10,000 cells per well. Subsequently, complete media was pipetted in each well (100 µL/well) on top of the cell laden GrowDex® layer (Fig. 1). Finally, microplates were placed under standard cell culture conditions (37°C, 5%CO_2) for 5 days to yield HepG2 spheroids.

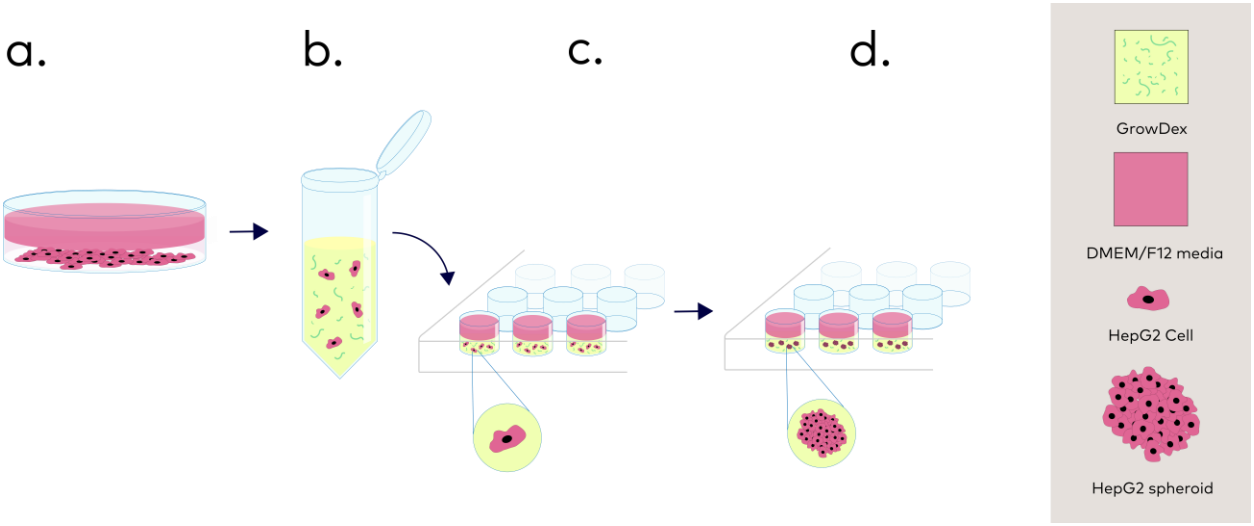


***Figure 1**. High throughput formation of HepG2 spheroids in GrowDex®. **a.** 2D culture of HepG2 cells. **b.** Harvesting cells and resuspension in GrowDex® diluted in culture media. **c.** Plating 100µL HepG2 cells in GrowDex® in each well and addition of 100µL cell culture media. **d.** Formation of HepG2 spheroid in GrowDex® after 5 days of culture*

After 5 days of culture, each well yielded approximately 1400 HepG2 spheroids. Microscopy imaging showed heterogeneous spheroid population with diameters ranging from about 50 to 130µm.

B. GrowDex® dispensing using spheroONE:

spheroONE enables automated liquid dispensing of a range of solutions and reagents (with viscosity up to 22.5 mPa*s, such as 70% glycerol solution). spheroONE is composed of a pressurized reservoir, a solenoid valve and a small aperture nozzle that allows high precision nanoliter drop-on-demand dispensing. By controlling drop volume and number of drops dispensed, it provides a versatile solution to fill individual wells of a microplate with reproducible volumes of solution ranging from nanoliter to microliter volumes.

In this study, spheroONE was successfully used to automate GrowDex® 0.5% w/v dispensing into 384 ULA plate (15 µL/well). Hydrogel distribution was performed with a 600 µm Nano Dispense Capillary (NDC 600), using the following valve parameters: 500 mBar pressure and 1500 ms actuation time.

Using spheroONE for automated well prefilling greatly facilitated microplate preparation and drastically reduced air bubble formation that is often hindering microplate preparation by manual pipetting.

C. HepG2 spheroids isolation using spheroONE:

Thanks to spheroONE's optical system (*incl.* high resolution camera, dark field illumination module) and glass NDC, it enables detection, analysis, sorting and isolation of particles ranging from 50 to 600µm diameter. Particle isolation and recovery using spheroONE is greatly improved when particle sedimentation is reduced. In this study, resuspension of spheroids in GrowDex® greatly mitigated particle sedimentation which considerably boosted recovery and isolation efficiency.

On the day of spheroid isolation, 100µL spheroid culture in GrowDex® (prepared in part A) were resuspended in 2mL PBS to obtain a final spheroids concentration of approximately 700 spheroids/mL in GrowDex® 0.025% w/v. This sample was subsequently placed in a spheroONE reservoir and was installed together with the prefilled 384 ULA plate (prepared in part B) inside spheroONE.

spheroONE was mounted with a NDC 300 (300 µm diameter) and the following valve parameters were set: 200 mBar pressure and 10 ms actuation time. Detection and isolation parameters were set as shown in table 1 below.

	Min Diameter (µm)	Max Diameter (µm)	Max elongation
Detection parameters	55	300	6
Isolation parameters	65	130	2.3

Table1*. spheroONE detection and isolation parameters used for HepG2 spheroids isolation in 0.025%w/v GrowDex®:*

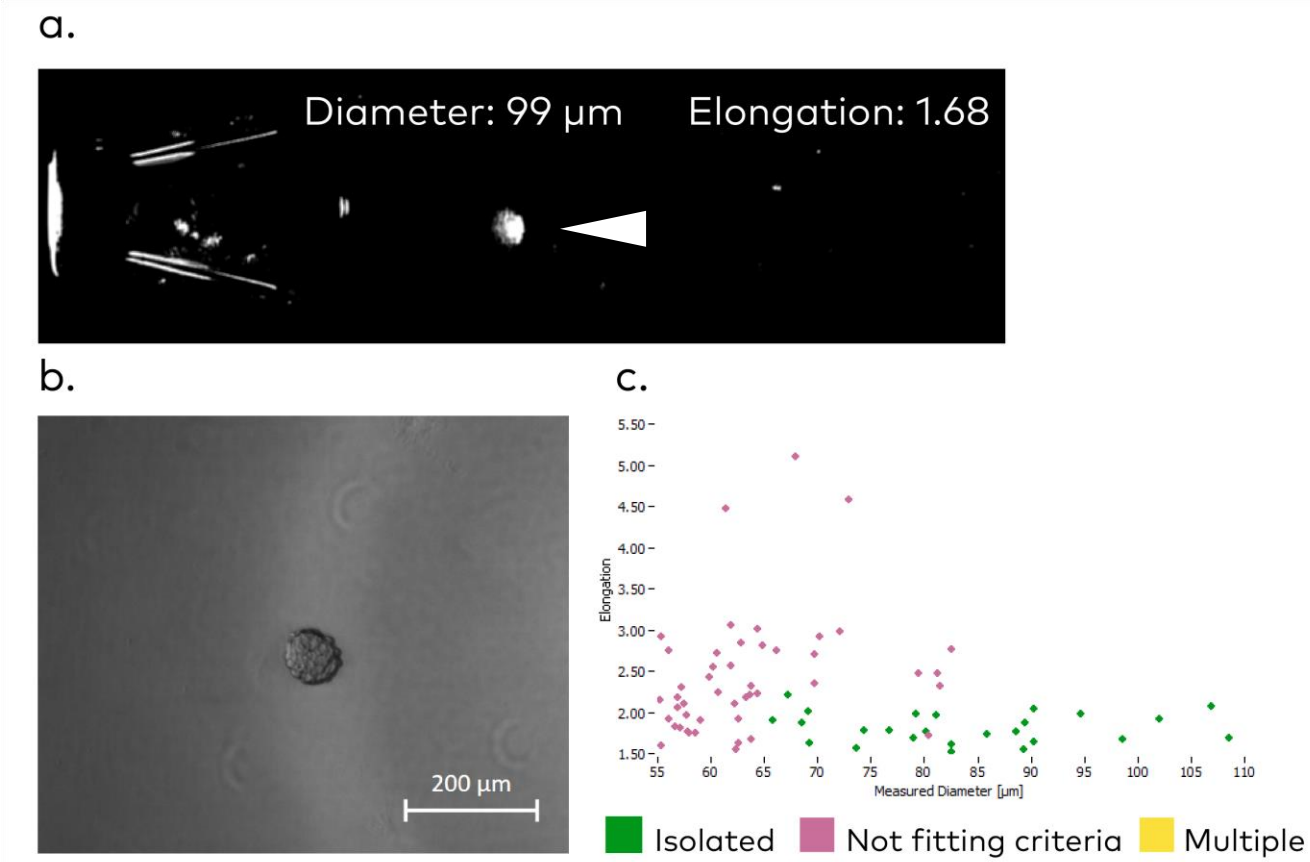


***Figure 2**. HepG2 spheroids sorting and isolation using spheroONE. **a**. Darkfield Image of a spheroid (diameter 99 µm, elongation: 1.68). **b**. Microscope image of an isolated spheroid inside the well of a* 384 ULA plate*. **c**. Scatter plot representing spheroids detected during the experiment, isolated spheroids in green, spheroids not isolated due to the presence of multiple particles in the NDC in yellow, spheroids not fitting isolation criteria in pink.*

Single HepG2 spheroids were successfully isolated on top of the prefilled layer of GrowDex® in each well of the 384 ULA plate (Fig.2.). Then two additional reagent dispensing steps were undertaken using spheroONE, another 15 µl of GrowDex® 0.5% w/v was added to each well in order to completely embed the spheroids, and finally, 25µl of complete media was dispensed on top of the embedded HepG2 spheroids to obtain a plate ready for further culture and / or screening.

Conclusions

This study successfully demonstrated the synergy between GrowDex® and spheroONE to automate and standardize preparation of microplates for 3D cell culture assays. The first step demonstrated the benefits of using GrowDex® for high throughput formation of over 100 000 HepG2 spheroids in a single 96-well ULA plate. In the second step, spheroONE was used as a reagent dispenser where each well of a 384-well ULA plate was pre-filled with a layer of GrowDex®. Subsequently, spheroONE was loaded with HepG2 spheroids prior to sorting and isolation of single spheroid per well inside the 384-well ULA plate. Finally, spheroONE was used for dispensing another GrowDex® layer for complete embedding of each spheroid and culture media was added on top (Figure 3) to yield microplates ready for further assay or screening applications.

The main advantage of this workflow lies in its streamlined automation, made possible through utilization of spheroONE allowing both precise distribution of defined volumes of GrowDex® (and reagents) into predefined target plates and high precision sorting and isolation of individual spheroids.

This innovative approach demonstrates cost-efficient and high throughput preparation of spheroids in GrowDex®, prior to automated preparation of assay-ready plates with uniform single spheroid per well which will have a significant impact for highly reproducible 3D cell culture assays.

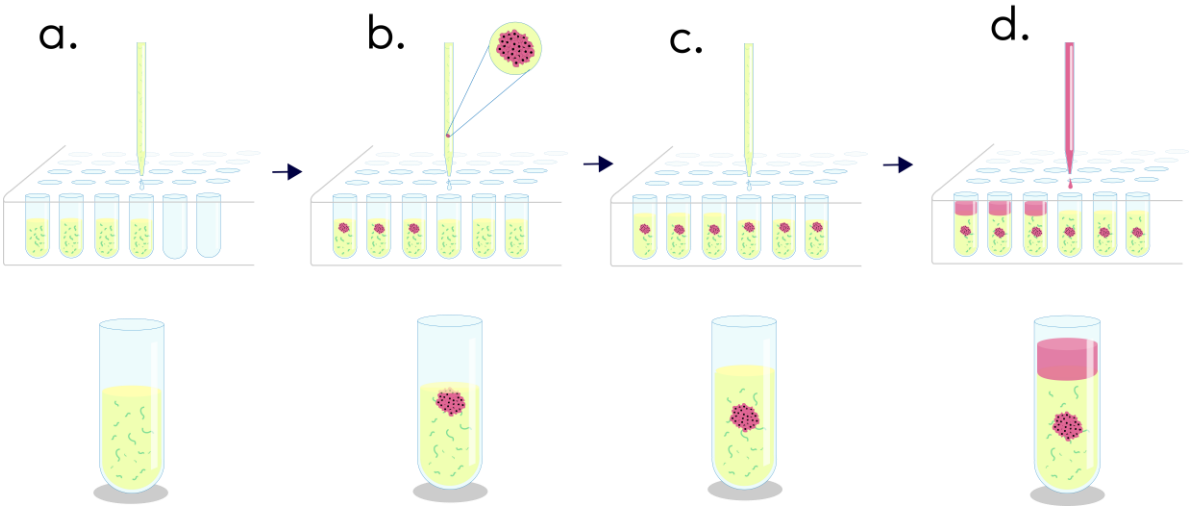


***Figure 3**. Workflow combining spheroONE technology and GrowDex® matrix for preparation of assay ready-spheroids microplates. **a**. Prefilling each well with 15µL GrowDex® 0.5% w/v. **b**. Sorting and isolation of single spheroid per well. **c**. Spheroid embedding via addition of another 15µL GrowDex® layer **d**. Dispensing of 25µL cell culture media.*

Materials:

spheroONE (F00CS, Cellenion) with Nano Dispense Capillary NDC300 (CS-N-300, Cellenion) and NDC600 (CS-N-600, Cellenion).

GrowDex® (1.5%) Catalogue code: 100 103 005 (UPM Biomedicals)

96-well Ultra Low Attachment (ULA) plate (Corning), 384-well Ultra Low Attachment (ULA) plate (Corning)

Complete media: Gibco™ DMEM/F-12, GlutaMAX™ Supplement (Thermo Fisher Scientific) + Foetal Bovine Serum (FBS) (Dutscher) + Penicillin-Streptomycin (PS) (Sigma-Aldrich)

Phosphate-Buffered Saline (PBS) with Calcium and Magnesium (Dutscher)

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