Automated Screening Workflows with Animal-free Nanofibrillar Cellulose 3D hydrogels

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Introduction

In precision medicine, it is essential to develop biologically relevant and physiologically tissue mimicking, but **cost-effective** and **experiment-reproducible** cell models. 3D spheroid/organoid culture methods with hydrogels have become a powerful tool for examination of better *in vivo* relevancy compared to well-established 2D monolayer models. However, due to the complexity of 3D models, usually these are challenging to scale-up for automated high-throughput workflows. The selection of suitable hydrogel with ease-of-use and repeatability is essential towards developing automation friendly 3D models.

GrowDex®, GrowDex®-T and GrowDex®-A are made from birch-based nanofibrillar cellulose and ultrapure water and have shown to provide biocompatible ECM-like support matrix for development of various clinically relevant healthy and cancerous cell models for drug discovery and development. The hydrogels are well-defined and animal-free, shear thinning and temperature stable which makes them ideal to work with various pipetting robots and dispensers for automated and scalable 3D models, with a possibility of direct biochemical and image-based cell assays.

Key properties

- Characteristics of GrowDex hydrogels make them ideal tools for 3D cell-based assays, and suitable for automated HTS and HCS applications.
- **Animal-free** GrowDex hydrogels are **biocompatible** with cells and tissues. As in these presented works of research, suitable for example with patient derived samples and cell lines, cancerous and non-cancerous. Over 180 protocols available.
- The composition is clearly defined with **no batch variation** which makes them highly suited for delicate drug discovery studies with **reproducible workflows**.
- GrowDex can be accurately and successfully used in 384-well plate 3D drug sensitivity testing of cultured cell lines and patient-derived *ex vivo* cell cultures.
- The GrowDex hydrogels help implementing the 3R policy by reducing use of invisible animals in *in vitro* assays (by replacing animal-derived matrix with wood)

3D matrices in drug screening

Suitability of GrowDex in 3D screening compared to widely used Matrigel was tested in two different assay approaches (Figure 1). The screening workflow was optimized by using HepG2 cells in two methods in 384 format: pre-culturing and pre-drugging method with 35 compounds in 5 different concentrations. In both, lab automation included: dispensing of hydrogels with BioMek FX liquid handler, addition of compounds with Echo 550 Acoustic Dispenser directly on the plate and at the end of culture, CellTiter-Glo® 2.0 was directly added with Multidrop and cell viability was read with the PHERAstar FS plate reader directly from the samples. [1]

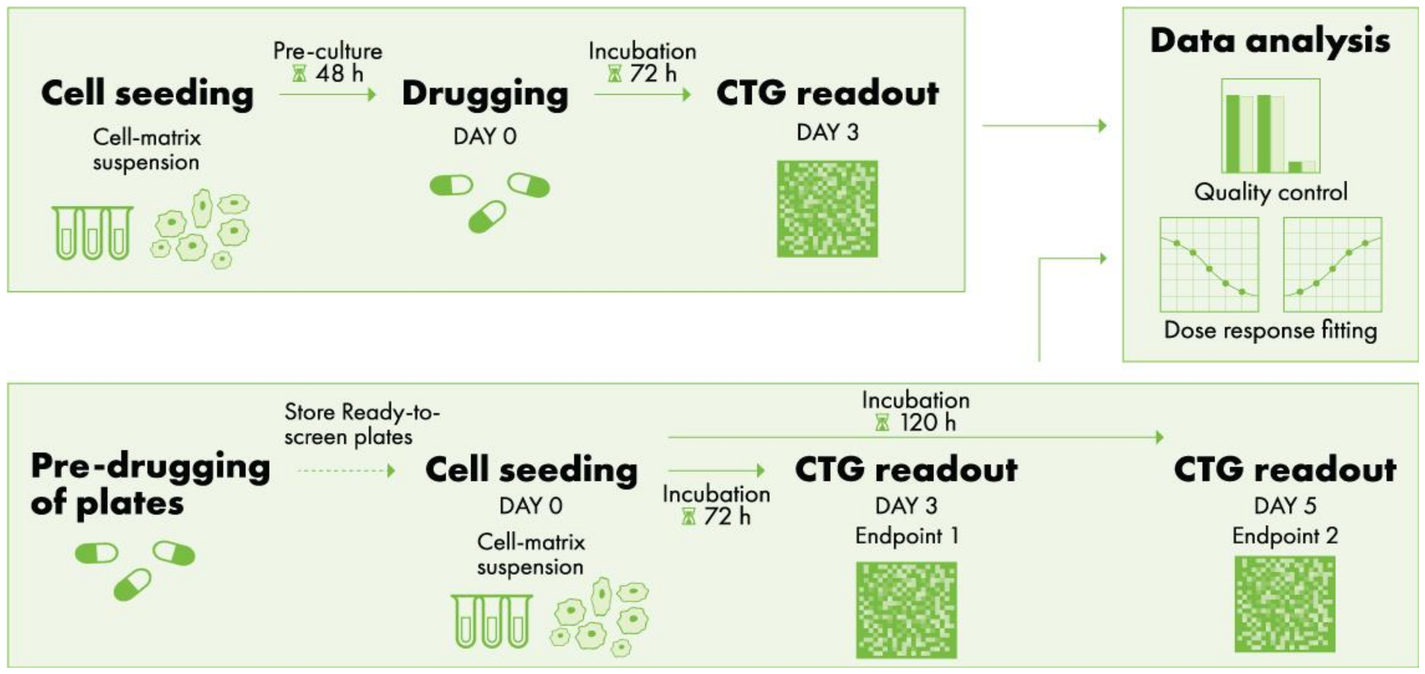

Figure 1. Overview of the screening workflow used in the 3D for HepG2 and PDCs embedded in GrowDex and Matrigel. Two drugging approaches were used. Based on HepG2 optimization screens, the upper option was selected for ovarian cancer PDCs for 3D.

This was followed by drug sensitivity testing of ovarian cancer PDCs from 2 patients with chosen pre-culturing method. The workflow was translated to ovarian cancer PDCs of two patients for functional precision medicine study with 52 oncological compounds in 5 clinical concentrations in 96 and 384 well formats.

PDCs grown in GrowDex or in Matrigel based on 52 drug panel showed patient-specific clustering (Figure 2). Some the growth condition and matrix dependent differences and similarities were seen in drug responses (Figure 3). This was suggesting that GrowDex supported the cell growth and can be used as matrix in the 3D drug testing. [1]

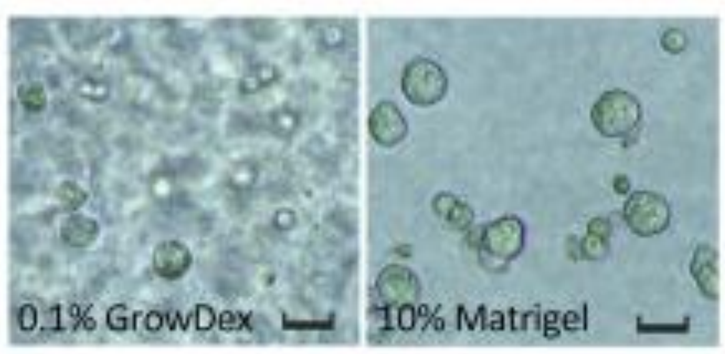

Figure 2. Microscopic images of PDCs grown in 0.1% GrowDex and 10% Matrigel on 384-well plates at 48 h timepoint when drugs are added in the pre-culturing method.

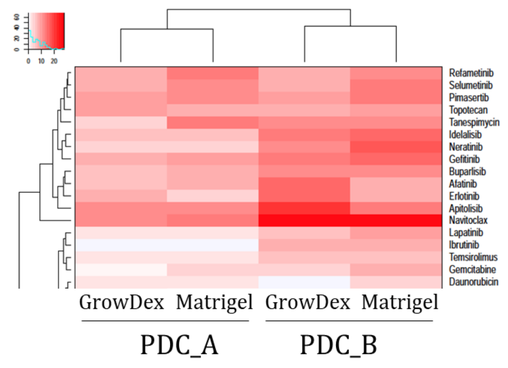

Figure 3. The part of the drug efficacies of drug panel on PDCs from patient A and B. The PDCs were let to form spheroids in GrowDex or Matrigel for 48 h prior to addition of drugs.

Ideally, any 3D culture modality and method that are being developed for screening, translatability to other cell types and automatable workflows are the desired factors for scalable and reproducible assays. At Nexus, ETHZ, Switzerland, Stirnimann & Booij (2022) were able to increase their screening throughput from 96 to 1536 well format, by easy automated dispensing of GrowDex hydrogels with CertusFlex contactless dispenser and were able to see high batch-to-batch reproducibility of their assays. GrowDex showed its suitability for miniaturizing assays to allow scale-up of the screening volume. Whereas animal derived matrices (BMEs) have shown to be troublesome with automated systems due to their temperature sensitive and variating nature. In addition to feasibility, also the cost of a screen was reduced drastically from animal-derived matrices to an animal-free option (Figure 4). [2]

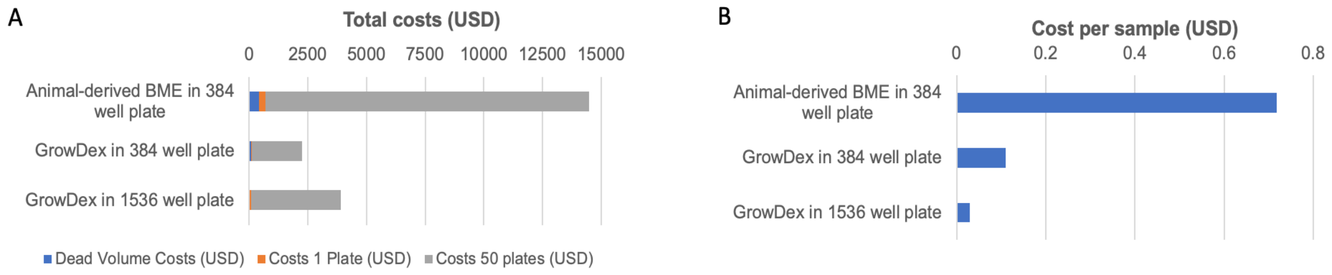

Figure 4. Cost breakdown of GrowDex hydrogel vs. animal-derived basement membrane extracts (BMEs) in HTS on A) plate and B) sample level.

Additionally, to maximize readouts retrieved from each cell or well, we have developed a multiplexing assay inhouse, that could be used specifically in liver toxicity assays. This includes well-based readouts of cell viability (CellTiterGlo2.0), cell death (CellTox Green), liver cell transcription, translation, processing and export functionality by measuring albumin secretion in the media, and finally liver enzymatic capacity and induction (P450-Glo). GrowDex can be used to develop animal-free, scalable, automated workflows for their screening purposes, with maximized readouts, further increased screening value and output but with reduced costs.

Join and learn more at myGrowDex.com

References: 1. Feodoroff, et al. (2023). "Comparison of two supporting matrices for patient-derived cancer cells in 3D drug sensitivity and resistance testing assay (3D-DSRT)." SLAS Discovery. **2.** Stirnimann, C. and T. Booij (2022). "Miniaturising organoid drug screens using nanofibrillar cellulose hydrogels." Drug Target Review 9(2): 6-8.

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