Automated 3D Cell-Based Assays in Animal-Free Nanofibrillar Cellulose Hydrogels for High-Throughput Screening Analyses

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Introduction

GrowDex®, GrowDex®-T and GrowDex®-A are birch-based nanofibrillar cellulose (NFC) hydrogels for 3D cell culture. Besides NFC they contain only purified water. GrowDex hydrogels do not contain any animal or human-derived material.

GrowDex hydrogels support cell growth in 3D by physically resembling extracellular matrix (ECM) biocompatible with human cells and tissues. The structure and mechanical properties can be tuned to fulfill the requirements of different cell types (Fig.1) and the hydrogels allow free diffusion of nutrients and oxygen. GrowDex can be degraded to soluble glucose by cellulase enzyme while retaining the 3D structure of cells.

GrowDex hydrogels have excellent shear thinning properties and temperature stability, with a possibility to tune the stiffness to apply them in multitude of applications, including automated 3D cell-based high-throughput (HTS) and high content screening (HCS) assays such as drug discovery and development.

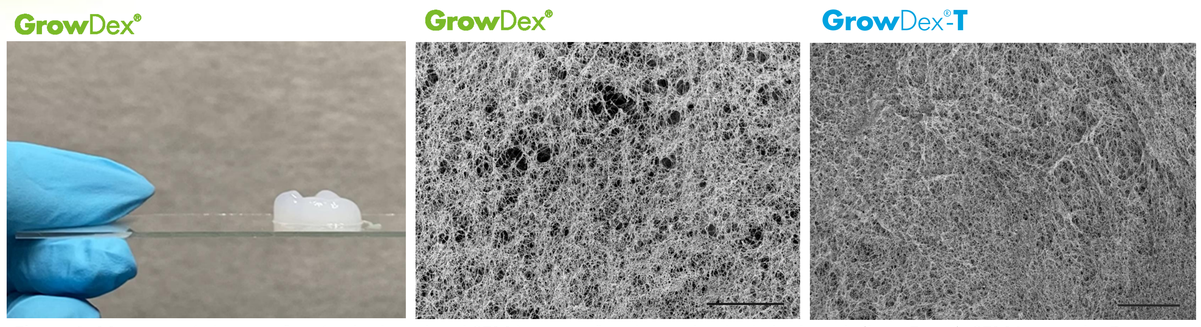
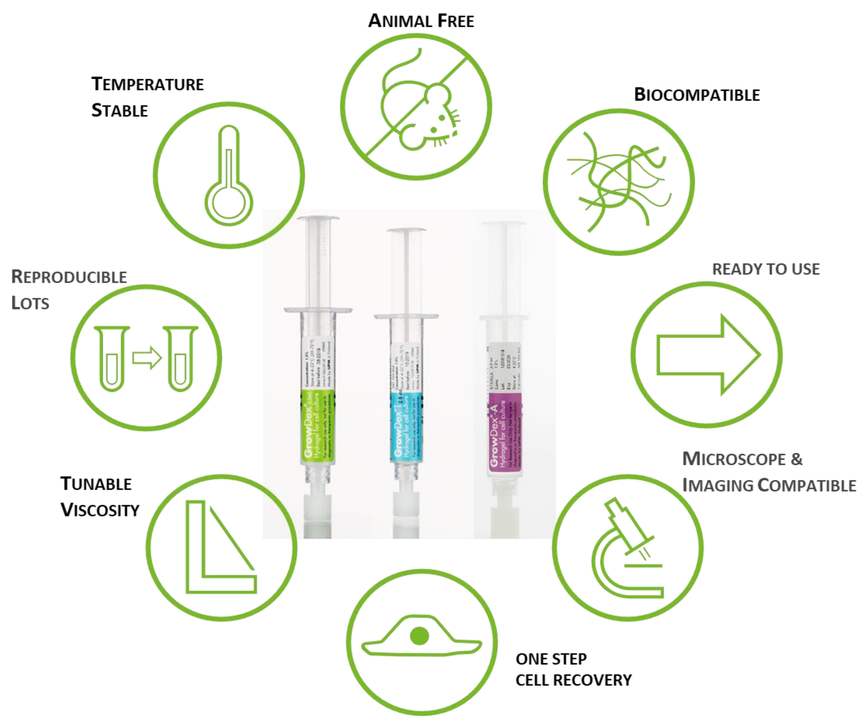


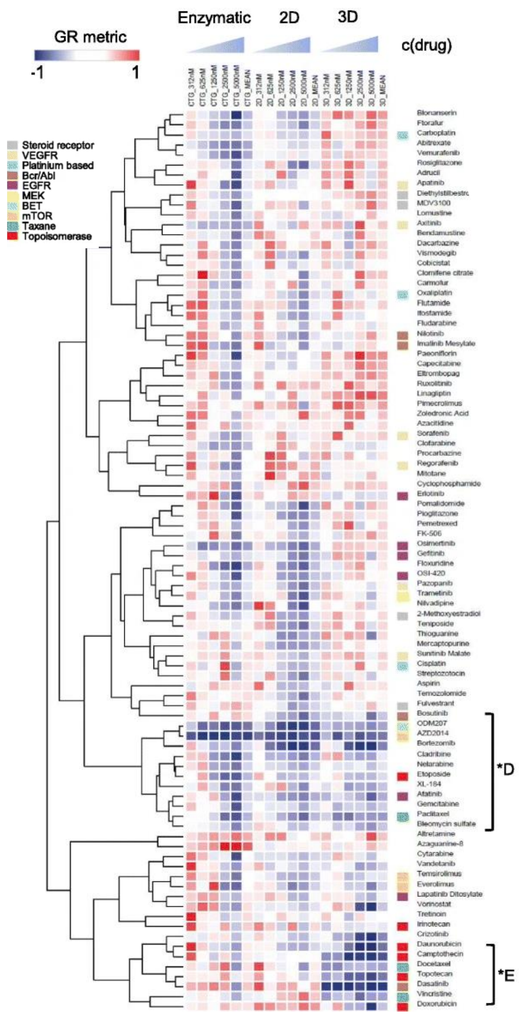
Figure 1. Macroscopic image of native hydrogel and SEM images of native and anionic hydrogels (bars 5 µm). SEM Images by Donata Iandolo from University of Cambridge, UK.

Key properties



Ex vivo drug screening of patient derived cells

Ex vivo drug screening is used for out-of-body assessment of drug efficacy for patient specific derived tumor cells, but it is known that 2D might hinder the native behavior of cells and there is a need for more relevant 3D culture applications. Patient derived metastatic urachal carcinoma cells, a rare and aggressive non-urothelial bladder malignancy, were screened to compare the feasibility and results obtained with alternative *ex vivo* drug screening techniques: enzymatic cell viability assay of 2D cell cultures and image-based cytometry of 2D and 3D cell cultures. [1]



The patient derived cells were first screened against 1160 FDA approved, investigational and preclinical drugs in high-throughput 2D assay. To validate the preliminary findings and efficacy profiles of the 90 selected drugs, an enzymatic assay was performed with an expanded dose range side by side with conventional 2D vs. GrowDex 3D cell culture high-throughput image-based cell viability assays. All the tested *ex vivo* drug screening modalities captured the cells' sensitivity to the same drugs that could be associated with the specific oncogenic mutation to this cancer type (Fig. 2). Interestingly, specific drug classes showed differences in dose responses depending on the culture model which could indicate the need for varied ease of use high-throughput culture modalities for more accurate drug screening methods. [1]

Figure 2. Heatmap illustration of the *ex vivo* validation with different assay techniques. The heatmap displays the vertical unsupervised clustering the dose response data of the tested drugs from the three screening methods: a 2D enzymatic cell viability assay, an image-based 2D cell viability assay and an image-based 3D cell viability assay. Each of the drugs were tested in five different concentrations. Normalized growth rate inhibition (GR) used for comparison of drug potency. GR values < 0 shown in blue.

Comparison of matrices in 3D drug screening

New robust and validated screening methods are required for high throughput drug screening. The selection of existing methods have certain limitations such as lack of cell functionality, expensive cell culture supplements and insufficient for automated workflow. Suitability of GrowDex in 3D screening compared to widely used Matrigel was tested in two different assay approaches. The screening workflow was optimized by using HepG2 cells in two methods, pre-culturing the cells before drugging or seeding the cells to pre-drugged 384 well plates (Fig.3). The 3D models in GrowDex and Matrigel were compared in traditional 2D model with a viability assay (Cell Titer Glo, CTG). This was followed by drug sensitivity testing of ovarian cancer PDCs from 2 patients with chosen pre-culturing method. The assays were performed 52 drugs in 5 different concentration. [2]

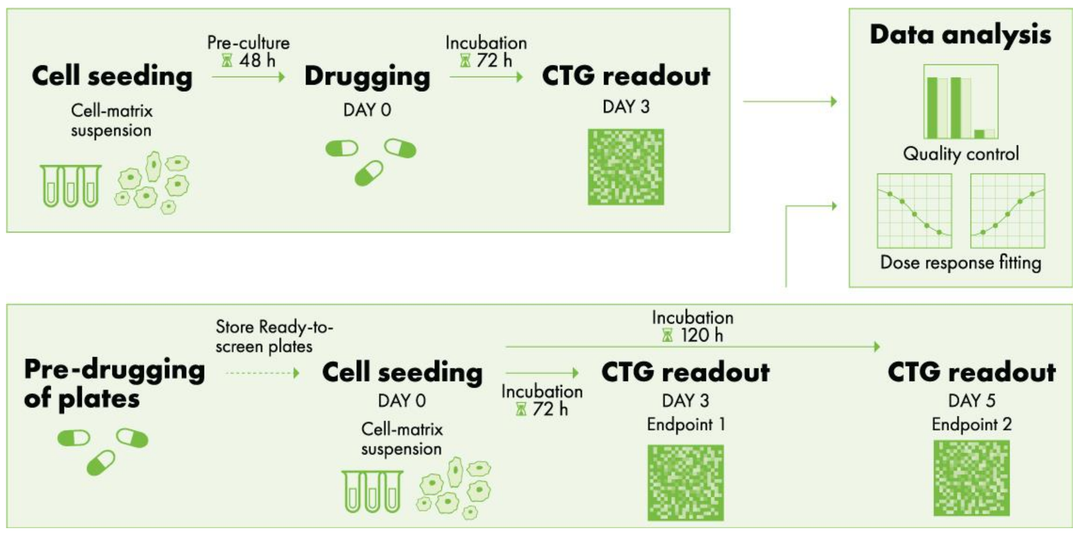
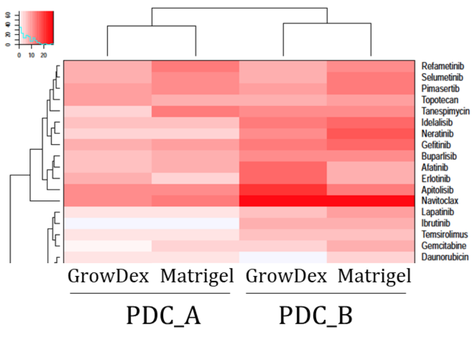


Figure 3. Overview of the screening workflow used in the 3D for HepG2 and PDCs embedded in GrowDex and Matrigel. Two drugging approaches were used. Based on HepG2 optimization screens, the upper option was selected for ovarian cancer PDCs for 3D.



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

Figure 4. 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.

Conclusions

- Characteristics of GrowDex hydrogel is ideal for 3D cell-based assays, and suitable for automated HTS and HCS applications.
- **Animal-free** GrowDex hydrogel is shown to be **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 the hydrogel 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.

References: [1] Mäkelä et al. 2020 Ex vivo modelling of drug efficacy in a rare metastatic urachal carcinoma, BMC Cancer 20:590. **[2]** Feodoroff et al. (2023) Comparison of different supporting matrices in the established 3D drug sensitivity and resistance testing assay (3D-DSRT) for patient-derived cancer cells, SLAS Discovery 28:4.