Automation Compatible 3D High Throughput Liver Toxicity Testing in Animal Free Nanofibrillar Cellulose Hydrogels.

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Introduction

GrowDex® hydrogels are birch-based nanofibrillar cellulose (NFC) hydrogels for 3D cell culture. Besides NFC they contain only purified water, without 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), allowing free diffusion of nutrients and oxygen. GrowDex can be degraded to soluble glucose by cellulase enzyme while retaining the 3D structure of cells.

The hydrogels are shear thinning and room temperature stable, enabling them to be used in a multitude of applications, including automated 3D cell-based high-throughput (HTS) and high content screening (HCS) assays such as DILI assays, drug discovery and precision medicine.

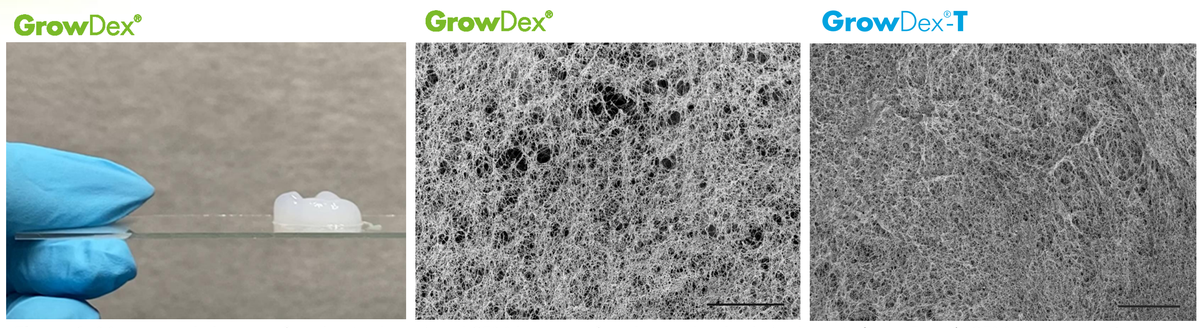
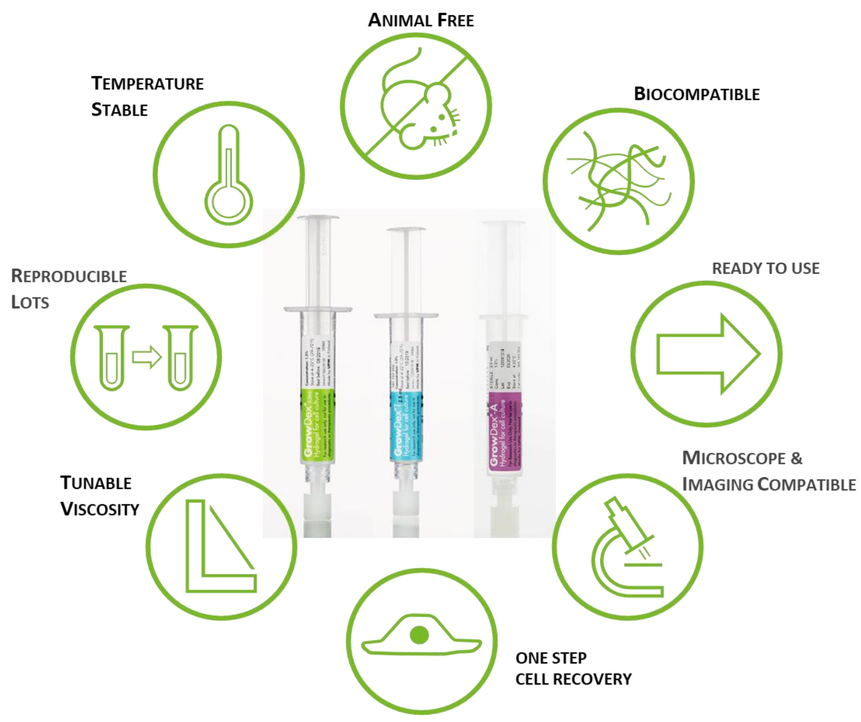


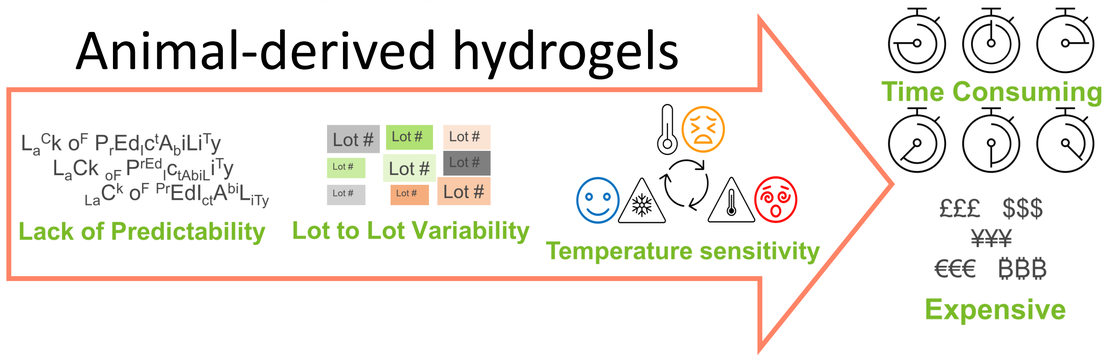
Figure 1. Macroscopic image of native hydrogel and SEM images of native and anionic hydrogels (bars 5 µm). SEM Images by Donata Iandolo from University of Cambridge, UK.

Key properties



3D hydrogel for HTS toxicity screening

Animal-derived hydrogels for 3D cell culture face many challenges, including the lack of predictability of cell responses to the hydrogel, lot-to-lot variation, and temperature sensitivity. These increase the time and cost per assay, as well as the need for more complex equipment for high throughput applications.



HepG2 were cultured in 0.5% GrowDex in 384 well plates overnight (Fig. 1), treated with camptothecin (0.3nM to 316nM) for 4 days, then fixed and stained with DRAQ5 nuclear dye. HTS imaging with the OPERA system revealed a dose-dependent cytotoxic effect of camptothecin (IC50= 29nM), as evidenced by the decrease in nuclear count with increasing drug concentrations. A pilot screen further underscored the assay's effectiveness in distinguishing between 1600 test compounds (20µM) against positive and negative controls.

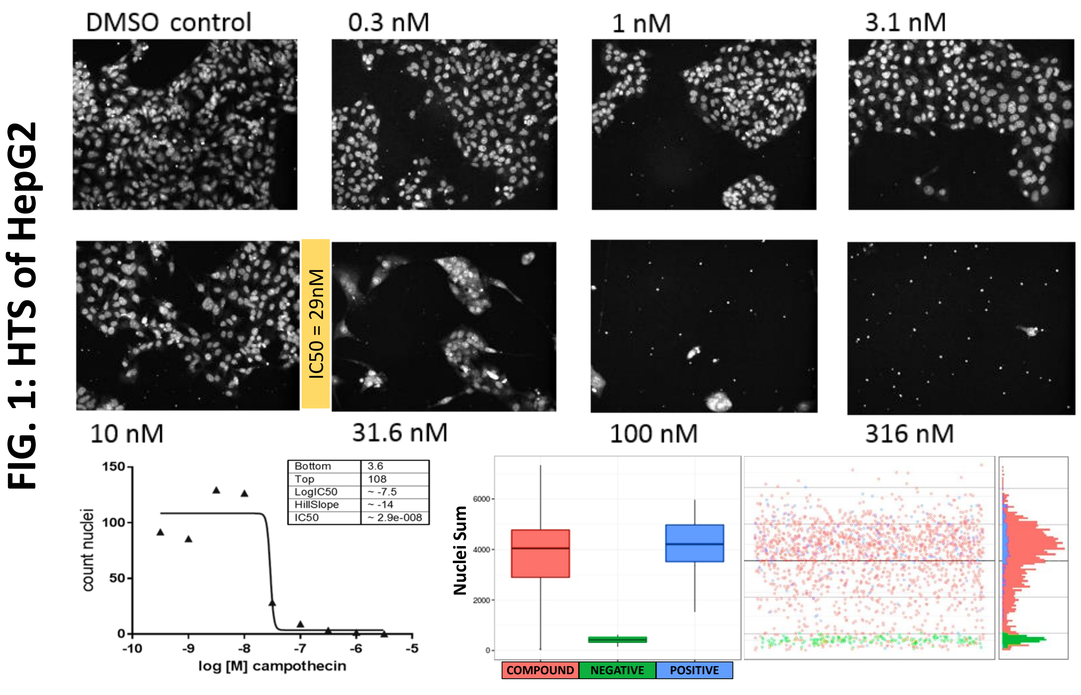


FIG. 1: HTS of HepG2

GrowDex was compared to Matrigel in a high-throughput screening (HTS) application (Fig 2), utilizing two distinct methods. Optimization used HepG2 cells in a 384 well format, employing a pre-culturing and pre-drugging approach with 35 compounds at five concentrations. Automation components included GrowDex hydrogel dispensing via a BioMek FX liquid handler and compound addition with an Echo 550 Acoustic Dispenser. Cell viability was assessed by adding CellTiter-Glo® 2.0 with a Multidrop dispenser and reading with a PHERAstar FS plate reader.

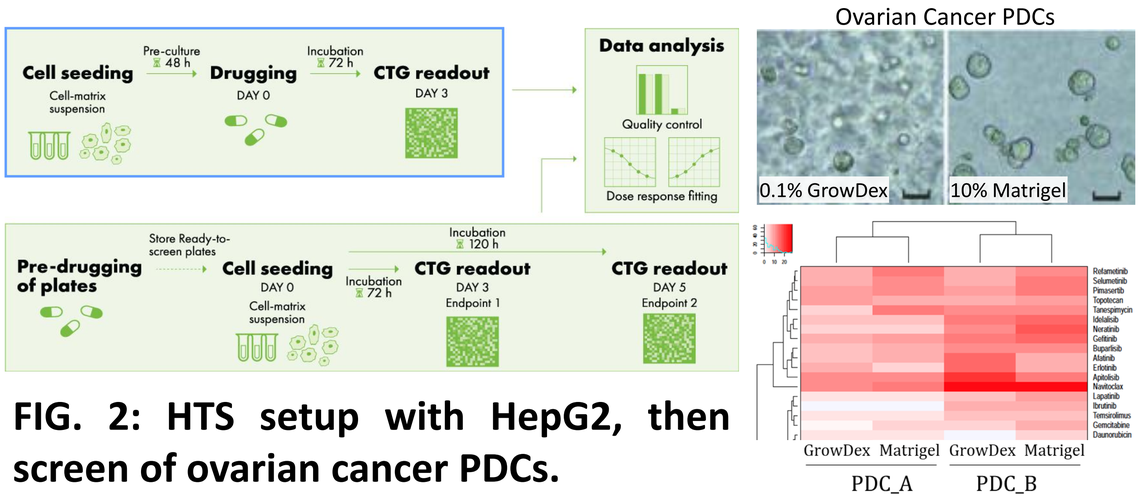


FIG. 2: HTS setup with HepG2, then screen of ovarian cancer PDCs.

Drug sensitivity testing on ovarian cancer PDCs from two patients using 52 oncological compounds across five concentrations in 96 and 384 well formats showed patient-specific responses. PDCs were cultured in GrowDex or Matrigel, revealing distinct clustering based on the drug panel.

FIG. 3: Cost breakdown

In a study by Stirnimann & Booij (2022) at Nexus, ETHZ in Switzerland, screening throughput was increased from 96 to a 1536 well format using automated GrowDex hydrogel dispensing with a CertusFlex. This method showed high reproducibility and scalability, unlike animal-derived matrices which were problematic. The switch to GrowDex also significantly lowered screening costs (Fig. 3), highlighting its efficiency and cost-effectiveness for scalable assays.

Summary

Animal-free GrowDex hydrogels are **biocompatible** with cells and tissues, and as presented here, suitable for liver toxicity screening assays, as well as primary cancer samples.

The composition is clearly defined with **no batch variation** which makes them highly suited for drug toxicity and discovery studies with **reproducible workflows**.

These study, highlights the importance for **reproducible**, **well defined in vitro cell culture models** for accurate disease modelling and drug screening capabilities.

Biologically relevant GrowDex hydrogels are a great option, since the properties provide the cells with a 3D microenvironment that best suits the desired phenotype of interest.

References: Application Note: High Throughput Cytotoxicity Testing with HepG2 Cells Grown in 3D Culture. Thimm, G., et al., (2018). Feodoroff, M., et al., (2023). Stirnimann, C. and T. Booij (2022).