Scalable and Automated Three-Dimensional Cell Cultures for High-Throughput and High-Content Screening

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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) and being biocompatible with human cells and tissues. The structure and mechanical properties can be tuned to fulfill the requirements of different cell types (Fig.1). Hydrogels allow diffusion of nutrients and oxygen.

GrowDex can be degraded to soluble glucose by cellulase enzyme while retaining the 3D structure of cells. GrowDex hydrogels are shear-thinning, which results as dispensable and ready-to-use hydrogel.

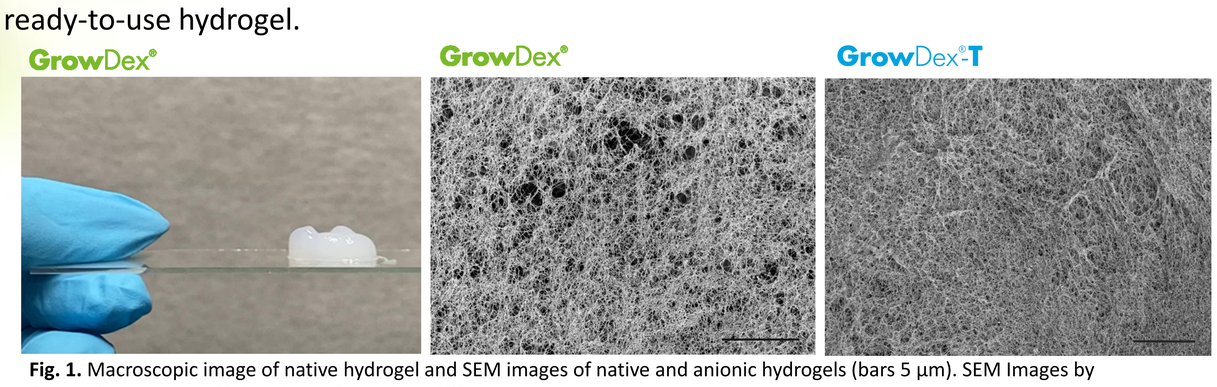
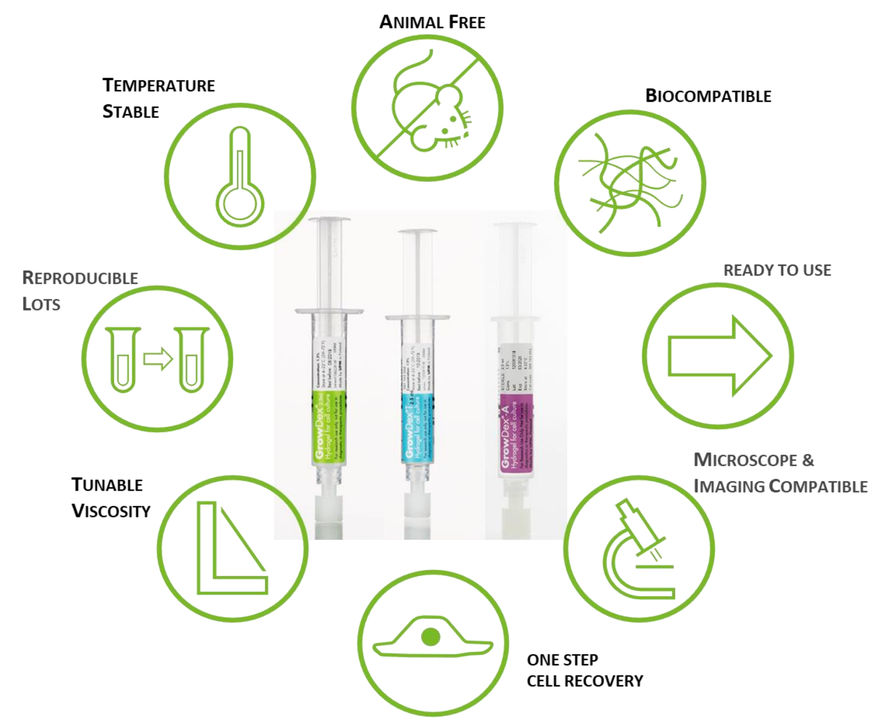


Fig. 1. Macroscopic image of native hydrogel and SEM images of native and anionic hydrogels (bars 5 µm). SEM Images by Donata Iandolo from University of Cambridge, UK.

Key properties



Automation compatible 3D cell culture

GrowDex hydrogels have stiffness values of 0.1-1500 Pa when the concentrations are between 0.1-1.5% (Fig.2). However, the hydrogels **start to flow when shear force, like dispensing is applied**. Initial viscosity is re-established immediately after the shear force stops. As a **shear-thinning** material, GrowDex can be used in a variety of **automatic dispensing** systems that are for **High-Throughput Screening** (HTS) assays. Temperature does not affect the performance of GrowDex hydrogels thus they **can be dispensed at room temperature**. These characteristics make GrowDex an ideal support matrix for cell-based 3D HTS assays. Dispensing of GrowDex hydrogels have been demonstrated with e.g., Beckman Coulter Biomek FX and NXP, Gyger Certus Flex, Labcyte Echo, Tecan (Fig. 3), Eppendorf EpMotion, and PerkinElmer Janus.
Downstream analysis of the 3D cultures has been showed by DNA sequencing [1], flow cytometry and immunofluorescence imaging (unpublished data).
Patient-derived urachal carcinoma model was developed using GrowDex in an HTS-capable 384-well format [2]. The drug screen included 1160 drug compounds, and results showed the applicability of GrowDex as a matrix compared dose responses to cells cultured in 2D (Fig.4).

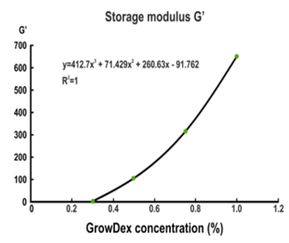


Fig. 2. Dilution of GrowDex can be calculated to match your existing matrix or desired viscosity.

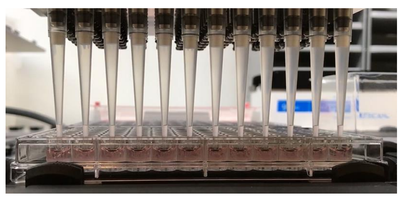


Fig. 3. GrowDex can be dispensed with automated systems. Here, Tecan MultiChannelArm™ used to establish 3D cell culture for HTS applications. Image by Bono Epifania, ZHAW, Switzerland.

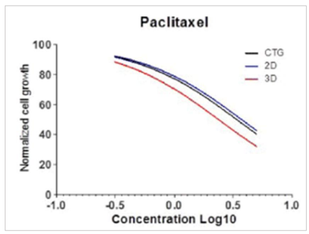


Fig. 4. Dose response curves of Paclitaxel for urachal carcinoma cell models in 3D in GrowDex and in 2D. [2] Mäkelä et al., BMC Cancer, 2020

Imaging Compatible and Tunable

GrowDex hydrogels are **non-autofluorescent** and suitable for High Throughput (HT) and High Content (HC) Screening manner (Fig. 5-7). Imaging is possible before or after GrowDase treatment. Cells can **be stained whilst embedded in hydrogel**. GrowDex-A can be functionalized with biotinylated molecules to tune the microenvironment based on the requirements of different cell and tissue types. (Fig. 8).

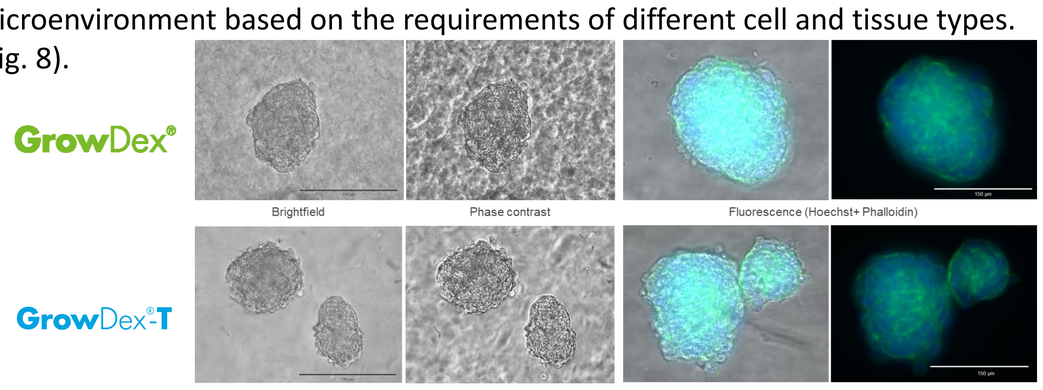


Fig. 5. Brightfield, phase contrast and fluorescence microscopy imaging of patient-derived neuroendocrine prostate cancer tumoroids cultured for 7 days in GrowDex and GrowDex-T. For fluorescence microscopy the live prostate cancer tumoroids were stained with phalloidin (green) and Hoechst (blue) live cell DNA dye. Bars 150 µm.

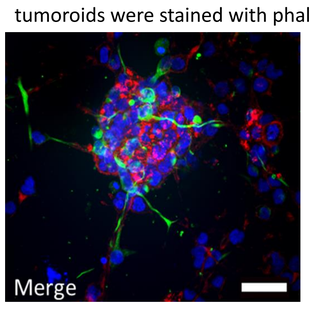


Fig. 6. HC 3D image of U251 malignant glioblastoma cells captured with ImageXpress. Scale bar is 50 µm. [3] Images by Yiming Meng, University of Reading and Andy Bashford, Molecular Devices

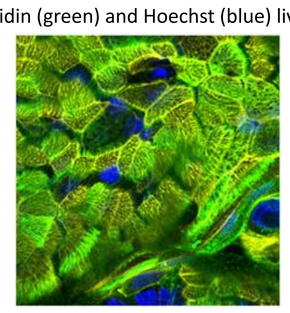


Fig. 7. 3D image of Human Bronchial Epithelial cells captured with Operetta CLS™ HCS system, Perkin Elmer. [4] Zaderer et al., 2019, Cells

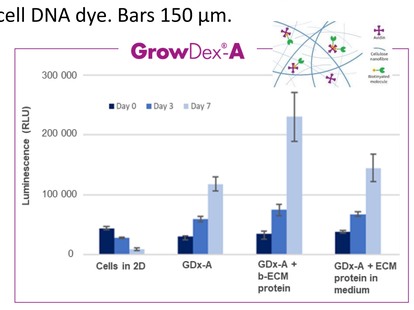


Fig. 8. Functionalization of GrowDex-A improved cell viability and cells benefit from the anchorage points. [5] Leppiniemi et al., Biomacromolecules, 2021

GrowDex degradation for downstream processing

GrowDex can be completely degraded by cellulase enzyme, GrowDase™. It is a purified enzyme that specifically degrades cellulose to soluble glucose without affecting the cells. The use of GrowDase enables **easy degradation of the matrix and the 3D spheroid structures are retained**. As the cellular structure remains intact, detailed downstream analysis of the spheroids or organoids is possible. SEM images of HepG2 spheroid shows how microvilli structures typical for hepatocytes are revealed after the GrowDase treatment (Fig. 9) [6].

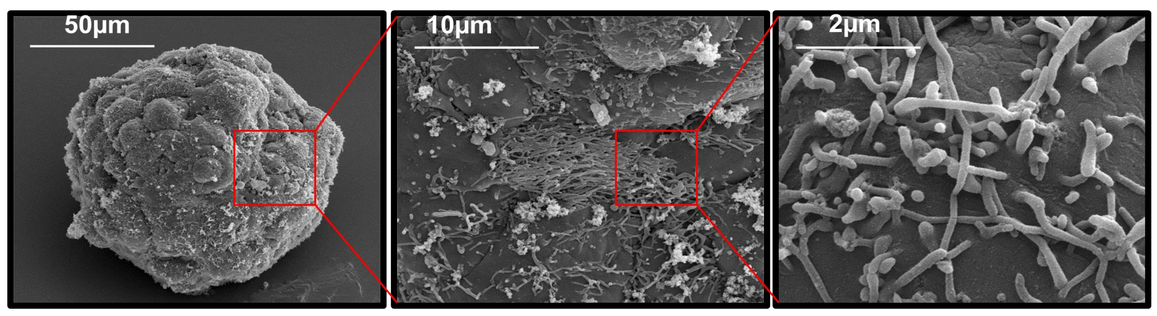


Fig. 9. Scanning electron microscopy (SEM) images of HepG2 spheroids after GrowDase cellulase enzyme treatment and silica bio replication reveals the microvilli structure typical for hepatocytes. Images from Liisa Kanninen and Yan-Ru Lou, University of Helsinki, Finland [6].

Conclusions

The characteristics of GrowDex hydrogels make them ideal tools for 3D cell-based assays for high throughput and high content screening.

Animal-free GrowDex hydrogels are **biocompatible** with cells and tissues
- The composition is clearly defined with **no batch variation**

GrowDex hydrogels are easy to use in automated dispensing
- **Room temperature stable** matrix is **tunable** with culture medium
- **No crosslinking** step needed
- Shear-thinning property enables **automated pipetting in HTS applications**

GrowDex can be **removed with GrowDase enzyme** whilst **retaining 3D structure and functionality**
- Efficient recovery of spheroids and organoids with well-preserved morphology

Acknowledgements

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References: [1] GrowDex Application Note AN028: NGS compatible streamlined DNA recovery from 3D cell cultures in GrowDex. **[2]** Mäkelä et al., 2020, Ex vivo modelling of drug efficacy in a rare metastatic urachal carcinoma, *BMC Cancer,* 20, Article number: 590 **[3]** GrowDex-T Application Note AN010: High-content quantitation of cancer stem cells from a glioblastoma cell line cultured in 3D using GrowDex®-T hydrogel **[4]** Zaderer et al., 2019, Turning the World Upside-Down in Cellulose for Improved Culturing and Imaging of Respiratory Challenges within a Human 3D Model, *Cells*, 2019, 8(10) **[5]** Leppiniemi et al., 2021, Avidin-Conjugated Nanofibrillar Cellulose Hydrogel Functionalized with Biotinylated Fibronectin and Vitronectin Promotes 3D Culture of Fibroblasts, *Biomacromolecules*, 22, 10, 4122– **[6]** Lou Y. et al., 2014. The use of Nanofibrillar cellulose hydrogel as a flexible three-dimensional model to culture human pluripotent stem cells, *Stem cell and development*. Vol. 23:4, pp. 380–392.

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