3D Patient Derived Explant Culture with Tumour-Immune Cells in Animal-Free Hydrogel

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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 use them in many applications, including automated 3D cell-based high-throughput (HTS) and high content screening (HCS) assays such as drug discovery and development.

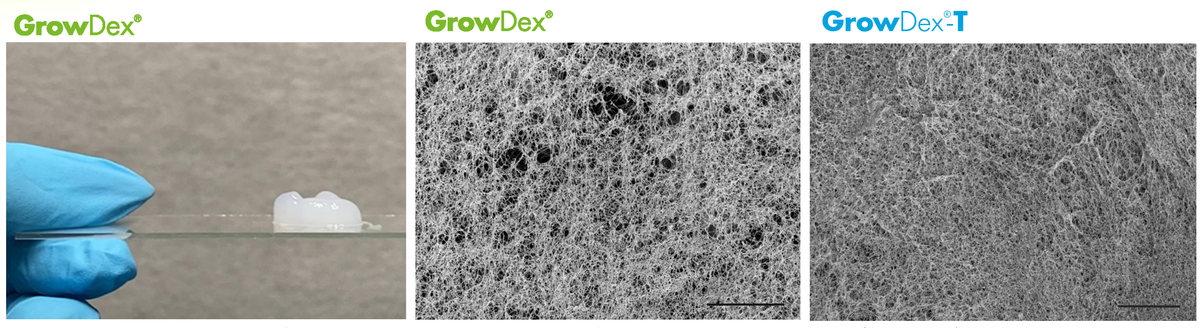
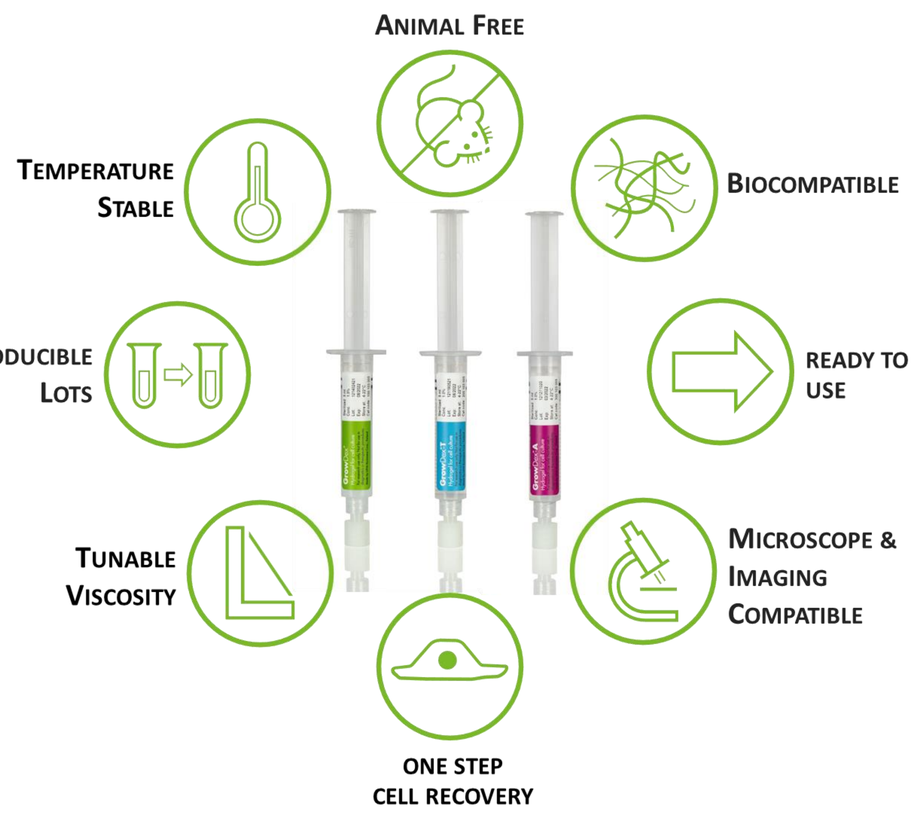


Figure 1. Macroscopic image of native hydrogel and SEM images of native and anionic hydrogels (bars 5 µm). SEM Images by Donata Iandolo from University of Cambridge, UK.

Key properties



Modelling the tumour tissue and tumour immune environment

GrowDex was utilised for the patient derived explant culture (PDEC) of fresh patient-derived breast tumour samples (previously detailed in Haikala, et. al.2019, and Munne et. al. 2021).

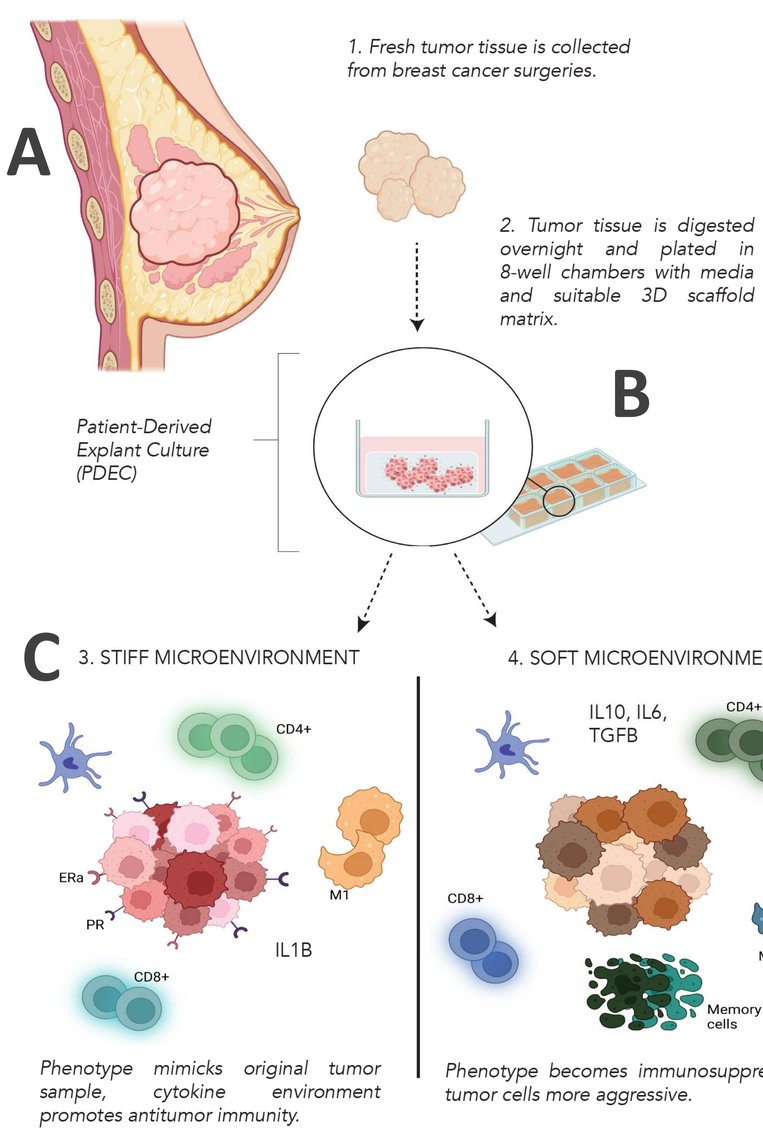
Specifically, fresh tumour tissue was successfully collected following breast cancer surgery (A), dissociated and embedded in two different concentrations of GrowDex hydrogel, 0.3% and 1% (B). Following culture for 3-7 days, the cytokine release profile (D) was analysed, as well as cells were analysed by bulk sequencing (E), and single cell analysis (F).

It was seen that the GrowDex hydrogels can be used to preserve the tumour microenvironment including the embedded immune cells for 5-7 days.

It was observed that the tumour phenotype and tumour immune microenvironment could be simultaneously altered by changing the mechanical and biological properties of the matrix.

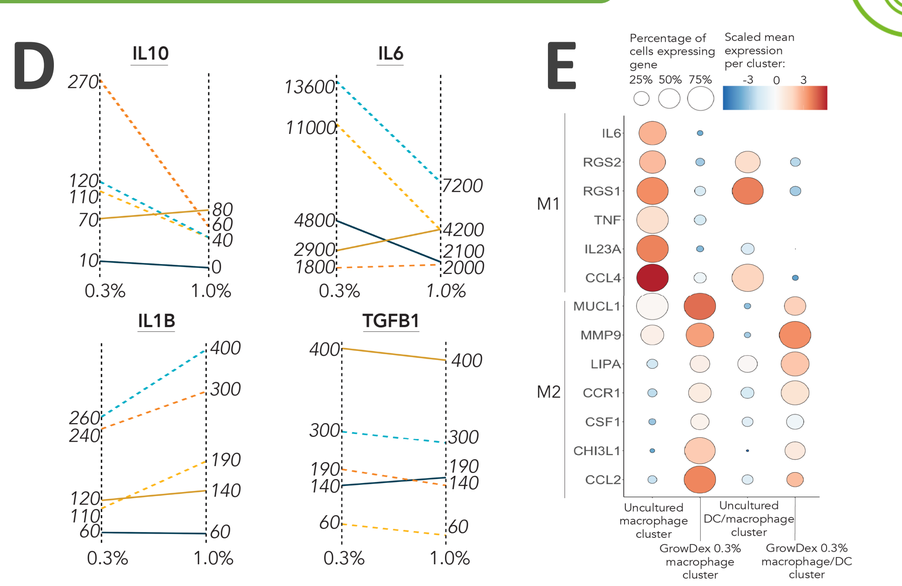
Within a stiff microenvironment (1% GrowDex), the tumour phenotype mimicked the original tumour and the cytokine environment promoted anti-tumour immunity.

Whereas in a soft microenvironment (0.3% GrowDex), the phenotype became immunosuppressive, and the tumour cells were more aggressive.



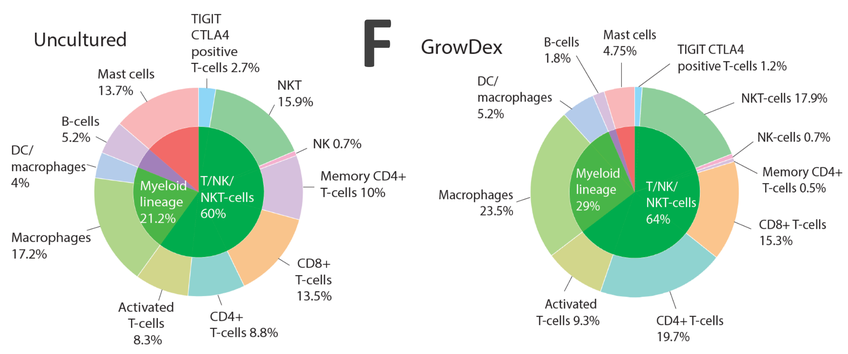
Phenotype mimicks original tumor sample, cytokine environment promotes antitumor immunity.

Phenotype becomes immunosuppressive and tumor cells more aggressive.



D. Differences in the cytokine profiles between soft GrowDex 0.3% and stiff 1.0% GrowDex: all samples are ER+, HER2+patient samples cultured in the PDEC-model. Each line presents an individual sample. Dashed lines mark ki67-low (<=50%) and small sized tumours.

E. Differentially expressed genes in two clusters between uncultured and GrowDex 0.3% (DEsingle). Several M2 markers are being upregulated in GrowDex 0.3%, while several M1 markers are downregulated. Colours are scaled mean expression value in cluster.



F. The composition of immune cells before and after culturing. The soft culture conditions increase the proportion of myeloid cells considerably.

With the use of PDECs in combination with GrowDex hydrogel, it is possible to model stiffness and other biological properties of the tumour microenvironment, which in turn affect the tumour phenotype and antitumor immunity.

A soft microenvironment (0.3% GrowDex) alters the cytokine environment, and also induced the loss of memory CD4+ T-cells whilst affecting the polarization of macrophages to an M2 immunosuppressive phenotype. The exact mechanism how this phenomenon is created is still under investigation.

Conclusions

- **Animal-free** GrowDex hydrogels are **biocompatible** with single cells, spheroids, organoids, and tissues. Additionally, as presented here, GrowDex hydrogels are suitable for primary patient derived tumour culture, and tumour associated immune cells.
- The composition is clearly defined with **no batch variation** which makes them highly suited for delicate drug discovery studies with **reproducible workflows**.
- This 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.**

Acknowledgement and Reference: Pauliina Munne and Aino Peura from the Klefström Lab. Aino Peura et. al. Immune Evasion in Soft Nanocellulose 3D Matrix, Poster presented at Cold Spring Harbor 2022. Haikala, et. al.2019, and Munne et. al. 2021

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