3D cell culture, assay automation and 3D printing demonstrated with a novel xeno-free hydrogel derived from wood

UPM
The Biofore Company

Introduction

GrowDex® is wood-based nanofibrillar cellulose (NFC) hydrogel developed for 3D cell culture. It is biocompatible with human cells and tissues but as a plant based product it does not contain any animal or human derived material.

GrowDex efficiently supports 3D cell growth by physically resembling extracellular matrix (ECM). The structure and mechanical properties of GrowDex can be tuned to fulfill the requirements of different cell types and it allows the diffusion nutrients and oxygen. The hydrogel can be completely degraded to soluble glucose by enzyme treatment while retaining the 3D cellular structure. Under stress GrowDex has shear thinning properties, which make it a pipettable ready-to-use hydrogel.

Key properties of GrowDex include:

- **Ready-to-use**
- **Xeno-free material**
- **Biocompatible**
- **Adjustable stiffness**
- **Enzyme degradable**
- **Non-autofluoresecent**
- **No batch variation**

Figure 1. GrowDex hydrogel.

Stem Cells

The natural stem cell niche is a dynamic 3D environment supporting stem cell proliferation. GrowDex supports the proliferation of human embryonic stem cells (**hESC**) and human induced pluripotent stem cells (**hiPSC**) without feeder cells. Stem cells form 3D spheroids when cultured in 0.5% GrowDex hydrogel (Fig. 5) and the pluripotency is maintained during the 3D culture. Enzymatic degradation of the hydrogel with cellulase enzyme enables simple sub-culturing of the cells and the shifting of 3D culture to 2D platforms for various downstream applications, such as directed differentiation. 3D cell culturing and enzymatic degradation do not affect karyotypes of the stem cells, shown by chromosomal G-band analyses.

GrowDex has also been shown to support 3D growth of human mesenchymal stem cells (**hMSC)** to from spheroids and establish cell function (Fig. 6).

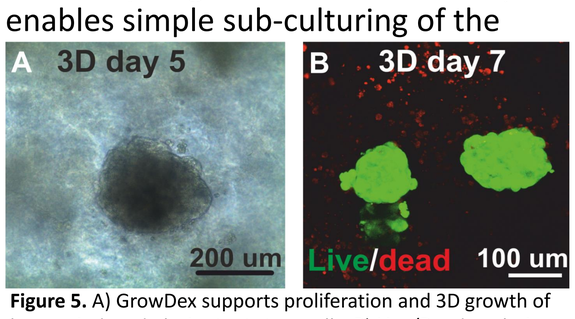

Figure 5. A) GrowDex supports proliferation and 3D growth of human induced pluripotent stem cells. B) Live/Dead analysis shows the viability of the cells in spheroids. Images from Yan-Ru Lou (Ph.D.), University of Helsinki, Finland.

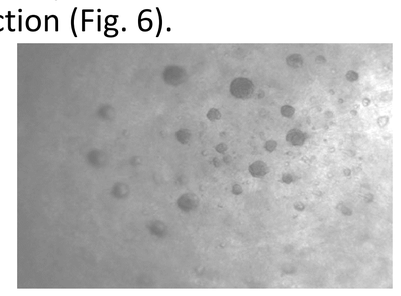

Figure 6. Bone marrow-derived mesenchymal stem cells form 3D spheroids in GrowDex in 24h.

Hepatic Cells

Human hepatic cell lines, such as HepaRG and HepG2 proliferate and form multicellular 3D spheroids in GrowDex hydrogel (Figs.2 and 3A-B). Cells remain viable in GrowDex, indicated by Live/Dead staining of the cultures (Fig.2B). The differentiation in HepG2 spheroids can be observed as *in vivo* -like cell polarization based on the concentrated presence of F-actin (Fig. 3A). Various stains can be used to visualize the spheroids in GrowDex (Fig.3B HepaRG).

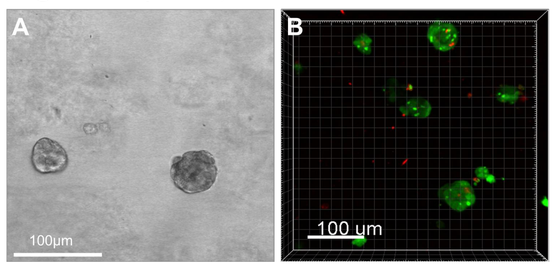

Figure 2. A) Phase contrast microscope image of HepaRG spheroids cultured in GrowDex. B) The viability of HepaRG cells analysed by Live/Dead staining. Images from Liisa Kanninen (Ph.D.) and Melina Malinen (Ph.D.), University of Helsinki, Finland.

Human primary hepatocytes are used e.g. in *in vitro* models for studying the liver toxicity of new drug compounds. Human primary hepatocytes form 3D spheroids in GrowDex during 28 days culture (Fig.3C). Long culture period enables long term drug exposure and toxicity studies.

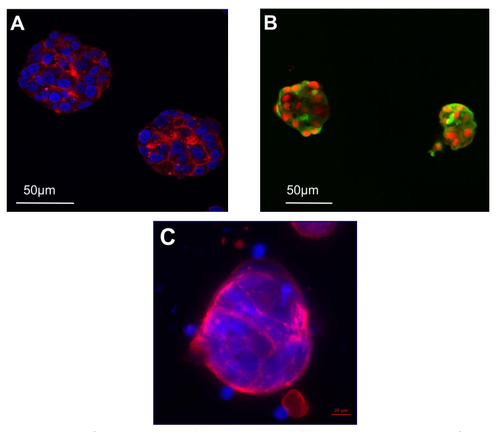

Figure 3. A) Staining of nuclei and filamentous actin (F-actin) in HepG2 spheroids. B) FDA/Draq5™ stained HepaRG spheroids. C) Human primary hepatocyte spheroid in GrowDex after 28 days culture, staining by DAPI/Phalloidin. Images A and B from Liisa Kanninen (Ph.D.) and Melina Malinen (Ph.D.), University of Helsinki, Finland.

Removal of GrowDex by Cellulase Enzyme

Growdex can be completely removed by cellulase enzyme. UPM cellulase enzyme is a purified and optimized enzyme that specifically degrades cellulose to soluble glucose without affecting the cells. The use of enzyme enables **easy degradation of the matrix whilst retaining the 3D spheroid structure**. The enzyme can be simply mixed with cell culture medium and incubated with cell-hydrogel complex at 37°C until the hydrogel has completely degraded.

As the cellular structure remains intact detailed downstream analysis of the formed spheroids or cellular structure is possible. In addition to brightfield and confocal imaging, immunohistochemistry and scanning electron microscopy (SEM) are also possible following enzyme treatment. SEM images show that after enzyme treatment microvilli structures typical for hepatocytes are revealed (Fig. 6).

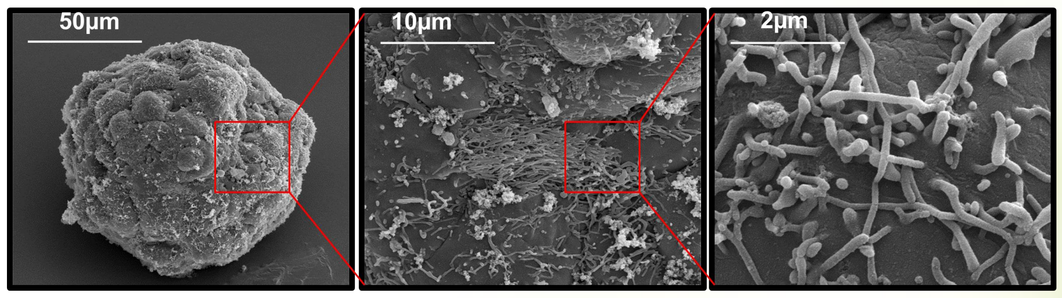

Figure 6. Scanning electron microscopy (SEM) images of HepG2 spheroids after cellulase enzyme treatment and silica bioreplication reveals the microvilli structure typical for hepatocytes. Images from Liisa Kanninen (Ph.D.) and Yan-Ru Lou (Ph.D.), University of Helsinki, Finland.

Automated Dispensing and 3D Printing

As a consequence of its shear-thinning properties GrowDex can be used in a variety of **automatic dispensing** systems (Fig.4A) that are for HTS and 3D printing applications (Fig.4B). The hydrogel has high viscosity and yield stress at rest, but starts to flow when shear force is applied, e.g. pushing hydrogel through a pipette tip. Initial high viscosity is re-established immediately after the shear force has been removed.

Fluctuations in temperature do not affect the performance of GrowDex. Thus it can be dispensed at room temperature or 37°C without issue. This characteristic makes GrowDex an ideal support matrix for cell-based 3D **high throughput screening** assays.

The ability to **3D print** GrowDex opens up new possibilities in the Biomedical field, e.g.in tissue engineering.

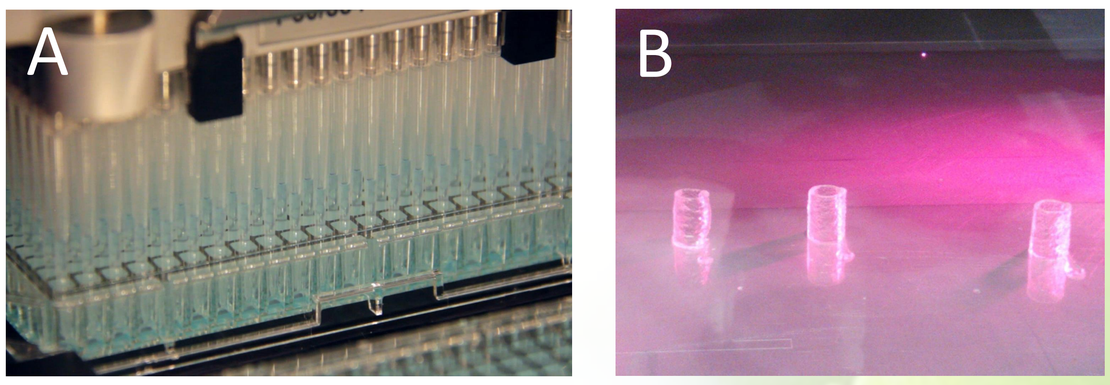

Figure 4. A) GrowDex can be dispensed with automated systems, such as the Biomek NXP, demonstrating its suitability for use in 3D cell-based HTS applications. B) 3D printed tubular structures created with GrowDex. The diameter of the tube is 5mm.

Conclusion and Comments

The inherent properties of GrowDex make it an ideal tool for researchers working in a number of fields, from development of 3D cell models through to 3D printing of NFC hydrogel structures as demonstrated here. In summary:

GrowDex is **biocompatible** with cells and tissues
- Proliferation and 3D spheroid formation of e.g. **stem cells**, **hepatic cells** and **cancer cells**
- **Xeno-free**, the composition is clearly defined with **no batch variation**

GrowDex is **simple to use**
- **Ready to use** matrix **tunable** with e.g. water of culture medium
- Can be used and stored at **room temperature**
- **No crosslinking** step needed
- Shear-thinning property enables **automated pipetting**

GrowDex can be **removed with enzyme** whilst **retaining 3D structure**
- Efficient recovery of spheroids and cellular structures with well-preserved shape

GrowDex®
– NATURAL GROWTH –

References:
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