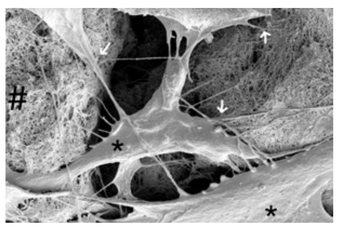
# AN ANIMAL-FREE HYDROGEL FOR TRANSLATIONAL STUDIES *IN VITRO – IN VIVO – IN HUMAN*

Johanna Lampe[1], Johana Kuncova-Kallio[1]
[1]UPM Kymmene Oyj, Helsinki, Finland

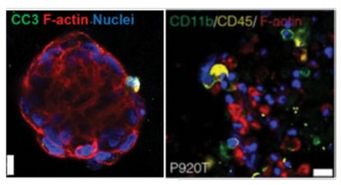
## INTRODUCTION TO NANOFIBRILLAR CELLULOSE (NFC) HYDROGEL

The hydrogel is **shear-thinning** and therefore can be readily dispensed, printed, injected without the need for cross-linking. The viscosity can be simply tuned by dilution.




The material is highly **biocompatible** (200 protocols including primary cells, also tested in ISO10993). Here, SEM image of fibroblasts (*) adhering (arrows) to the nanofibers (#). J. Sheard

GrowDex® is room **temperature stable**, allowing automation of assays. GrowDex is proven to work with many dispensing and pipetting robots.




The biomaterial is inert and preserves the immune response of primary cells. The image shows that immune cells are retained in patient derived breast cancer explant cultures. Ref. P. Munne

## REPLACING THE INVISIBLE ANIMALS WITH WOOD

**96 well plate** = **1 mouse** = **0.33 cm³ wood**

While implementing 3R's, many focus on the visible animals – needed for in vivo tests. However, many animals are used in vitro, e.g. in FBS, and many assays rely on a matrix isolated from mice [6]. On average, for a 96 well plate 5 ml of animal-derived matrix stock [6] or 3.3 ml of wood-derived matrix stock are needed.

UPM birch-based nanofibrillar cellulose (NFC) only consists of wood and purified water. The GrowDex® hydrogels do not contain any animal or human-derived material. The production follows ISO13485 standard.

## *In silico*

An *in silico* model of nanofibrillar cellulose has been built to enable fast testing of the hydrogel and its behaviour in the presence of different molecules and in various environments.

In the study by Koivunotko et al., the quality of the *in silico* model has been shown in the correlation of simulations and experimental data for freeze-dried and native hydrogel. [7]

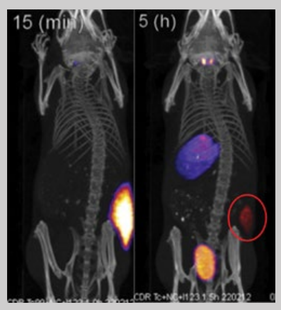
## *In vitro*

GrowDex, GrowDex-T and GrowDex-A are NFC hydrogels for 3D cell culture. GrowDase™ enables the gentle release of cells for analysis. Protocols for 200 cell types have been established including cell lines as well as primary samples, e.g. a respiratory cell culture which allows live cell imaging and was maintained for up to 700 days. [2,8].

PHH and HepaRG cells retain their viability and their functionality, as shown by albumin expression and CYP activity, up to 5 weeks in GrowDex [9-11]. In addition, PHH can be co-cultured with Kupffer cells in GrowDex to mimic *in vivo* tissue morphology and functionality [10]. The applicability in automated assays and the reproducibility make GrowDex the perfect matrix for toxicology studies.

## *In vivo*

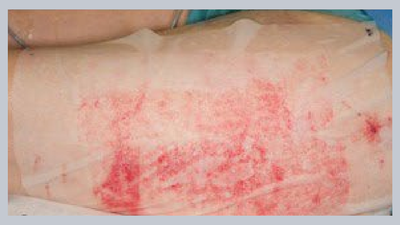
UPM's NFC is suitable as an injectable matrix for drug delivery and cell implantation. It allows controlled release and local delivery of therapeutic compounds as shown with radiolabeled model substances [3]. GrowDex-T has been used as an artificial extracellular matrix for the delivery of spheroids of otic neuronal progenitor cells into the mouse inner ear, leading to 90 days survival of the spheroids [12]. In addition, it has been shown *in vivo* that UPM's NFC does not cause any acute systemic toxicity (unpublished results).

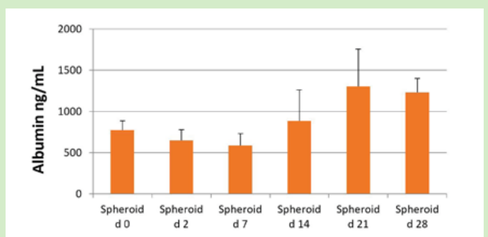



Dual tracing image showing the NFC implant (red) and test compound release. 123I-NaI was mostly distributed into the thyroid glands and stomach, and was excreted to urine. 5 h post injection, no trace of 123I-NaI was found at the injection site. [3]

## Clinical

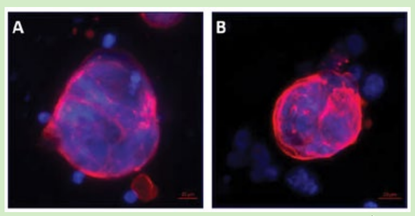
UPM's NFC is the basis for the CE-marked wound dressing FibDex®. It is intended for use on skin graft donor site wounds and offers excellent performance and healing results. As a one-time dressing, FibDex also reduces the workload for healthcare professionals as well as the waste amount in hospitals. Clinical investigation of FibDex for superficial dermal burns is on the way [13]. Moreover, the promising *in vivo* data suggest a fruitful future development of a wider spectrum of clinical applications for UPM's NFC.



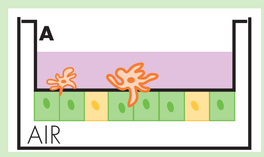



**Albumin secretion.** Primary hepatocytes were cultured for 28 days as spheroid culture (after 7d pre-culture) on GrowDex. Albumin secretion was quantified via ELISA.

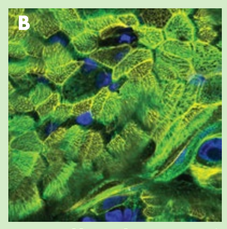

**CYP3A4 activity.** Primary hepatocytes were cultured for 28 days as spheroid culture (after 7d pre-culture) on GrowDex. CYP3A4 activity was quantified by analyzing the testosterone-6β-hydroxylation via LC-MS [1].




**Primary Human Hepatocyte/Kupffer cell coculture.** Spheroids stained with DAPI (blue) and Phalloidin (red) after 12 days culture (ratio PHH:Kupffer cells 2:1) in A) 0.8 % GrowDex, and B) 0.5 % GrowDex.

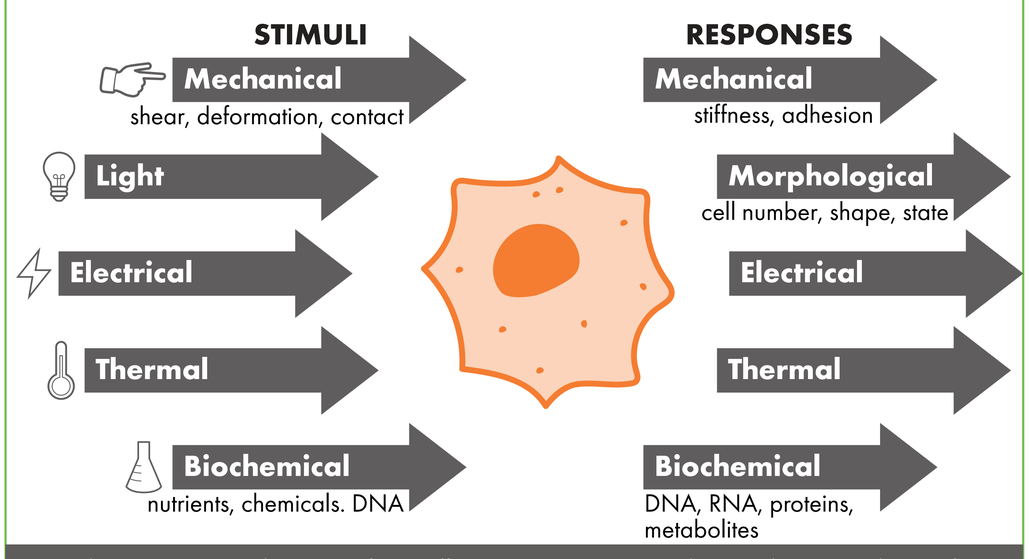



[8]




**Long term 3D respiratory cell culture.** A) Schematic of upside-down culture of respiratory epithelial cells seeded in GrowDex (adapted from ref. 8). This technique allows transfer of the cultures for live cell imaging and returning them to the culture well. B) Human bronchial epithelial cells were cultured in GrowDex for 700 d and stained with WGA-488 (green), Phalloidin (yellow), and Hoechst (blue) [8].

## CELLS ARE NOT REPEATABLE




In order to capture the complex cell responses we need to make everything else as repeatable as possible...

## HOW TO IMPROVE REPEATABILITY AND RELEVANCE OF CELL MODELS?

| | | |
|---|---|---|
| **Cells** | Cancer origin 2D culture | Patient-derived 3D culture |
| **Cell delivery** | Frozen Thawing | Fresh Ready-to-use |
| **Handling** | Human error | Reproducible Automated |
| **Matrix** | Varying composition | Reproducible No animal DNA (GrowDex®) |
| **Results** | Varying | Repeatable Reliable |

## CONCLUSIONS

- Birch nanocellulose (GrowDex) is a novel inert animal-free hydrogel
- It has been shown to work across in silico – *in vitro* – *in vivo* – clinical use.
- The GrowDex hydrogel helps implementing the 3R policy:
  - Reducing use of invisible animals in *in vitro* assays (by replacing animal-derived matrix with wood)
  - Reducing use of visible animals in *in vivo* models (by replacing animal tests with cell-based assays)
  - Refining use of animals (by enabling improved models)
- **Work with us:**
  - *In vitro*: We are looking for partners to develop 3D *in vitro* toxicology assays.
  - *In vivo*: We are looking for partners interested in cell implantation.

REFERENCES:
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