NANOFIBRILLAR CELLULOSE HYDROGEL WITH STEM CELLS, ORGANOIDS AND WOUND CARE

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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. GrowDex hydrogels do not contain any animal or human-derived material. NFC can be used in various forms. **FibDex®** is wound dressing made of NFC for the treatment of skin graft donor sites, and UPM Biomedicals is currently developing NFC **hydrogel for implantable medical applications**.

GrowDex hydrogels support cell growth in 3D by physically resembling extracellular matrix (ECM) biocompatible with human cells and tissues. The mechanical properties can be tuned to fulfill the requirements of different cells (Fig.1) and 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.

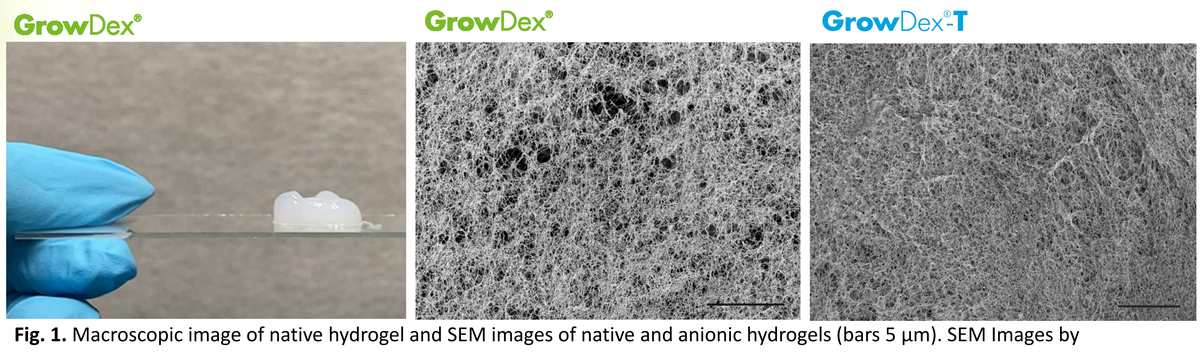
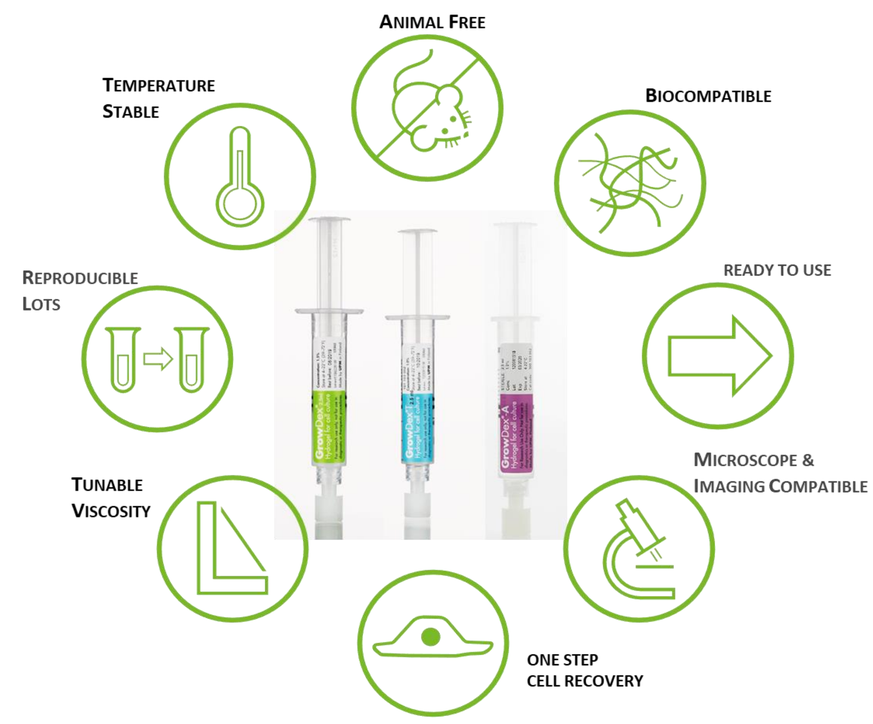
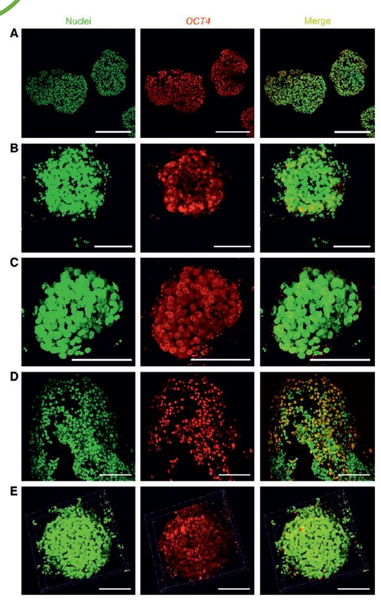


Fig. 1. Macroscopic image of native hydrogel and SEM images of native and anionic hydrogels (bars 5 µm). SEM Images by Donata Iandolo from University of Cambridge, UK.

Key properties



3D culture of pluripotent stem cells



The concept of using nanofibrillar cellulose hydrogel (GrowDex) for proliferation of human induced pluripotent (hiPSC) stem cells and embryonic stem cells (hESC) was demonstrated [1]. Both cell types proliferated well and remained pluripotent, demonstrated by pluripotency marker expression, in vitro embryoid body differentiation, and in vivo teratoma assay. Cellulase enzyme (GrowDase) enabled the easy cell recovery and sub-culture without affecting the pluripotency of the cells. Chromosomal G-band analyses showed normal karyotypes after culture in GrowDex and GrowDase treatment.

OCT4 expression in the 3D human pluripotent stem cell (hPSC) spheroids was analysed. The pluripotency marker OCT4 is expressed in WA07 cells that are cultured in 0.5% NFC hydrogel is presented in Figure 2.

Figure 2. A) WA07 cells cultured in GrowDex for 7 days. **B)** For 9 days. **C)** For 16 days after one subculture. **E)** For 26 days after three subcultures. **E)** OCT4 is also expressed in iPS(IMR90)-4 cells that are cultured in 0.5 wt.% NFC hydrogel for 9 days. Scale bars = 200 mm (A) and 100 mm (B–E). Ref. Lou et al. 2014.

Renal organoid cultures

Renal organoids are able to mimic the structure and function of *in vivo* kidneys. These organoids are needed for drug discovery testing or studying kidney development. They can mimic the structure and function of *in vivo* kidneys. Organoids were cultured from primary mouse embryonic kidney metanephric mesenchymal (MM) cells followed by chemical induction to undergo nephrogenesis. Cells were embedded in NFC which reduced the distortion or stress-induced affects during the nephrogenesis process.

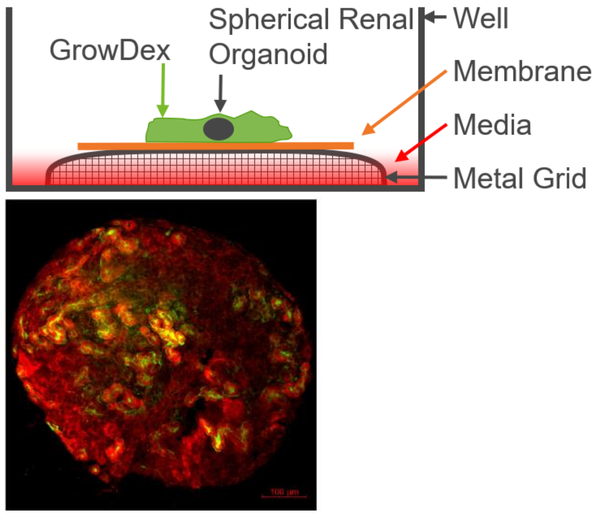


Figure 3. Schematic illustration of the experimental set-up for renal organoid cultures in GrowDex. ICC staining of renal organoids grown for 5 days in GrowDex. Organoids were stained with Pax2 (kidney tubules, red) and Lotus Tetragonolobus Lectin (LTL, proximal tubules, green).

The renal organoids cultured in GrowDex showed no signs of toxicity, and nephrogenesis proceeded normally. Immunostaining with Pax2 and LTL antibodies showed multiple developing nephrons and maturation of proximal tubules. The renal organoids were more spherical when embedded in GrowDex than in the traditional Trowell cultures. These results suggest that GrowDex can be used for renal organoid culture and these hydrogels are also suitable matrices for other applications such as renal organoid bioprinting. The use of organoids for clinical therapies toward kidney regeneration and restoration of function can improve the lives of many patients in the future.

Stem cells and NFC for wound repair

Human adipose derived mesenchymal stem cells (hASCs) are an attractive cell type for regenerative healing and wound repair due to their ease of isolation, proliferative and differentiation capacity. NFC is favourable for regenerative applications due to its purity of production, mechanical properties and biocompatibility, as well as the natural affinity of cellulose for water.

Primary patient derived hASCs were successfully cultured on and adhered to NFC wound dressings when seeded at a density of 3x10^5 cells for a period of 2 weeks [2]. Cell viability, evaluated by mitochondrial activity and released lactate dehydrogenase (LDH), was relative to the seeding density. Cell cytoskeletal structure and function of hASCs, based on F-actin, Vimentin and Ki67 immunostaining, was maintained, when cultured on the NFC dressing (Fig. 4). Additionally, using qPCR, it was noted that the hASCs remained in an undifferetiated state (data not shown). Finally, it was also seen that the hASCs expressed their own ECM proteins collagen type I and fibronectin after just 1 week of culture on the NFC dressing, maintaining their cytoskeletal structure and proliferative nature (Fig. 5).

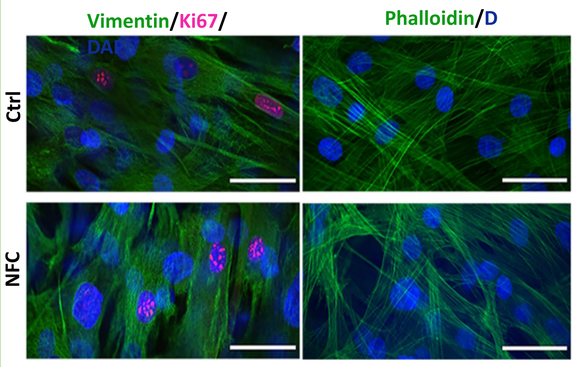


Figure 4. Fluorescence based imaging showed hASCs expressed vimentin (left panels, green), and the proliferation marker Ki67 (left panels, magenta) similarly to controls. Phalloidin staining (right panels, green), was also seen to be similar to control hASCs. DAPI (blue). Scale bars, 50µm. Ref. Kiiskinen et al 2019.

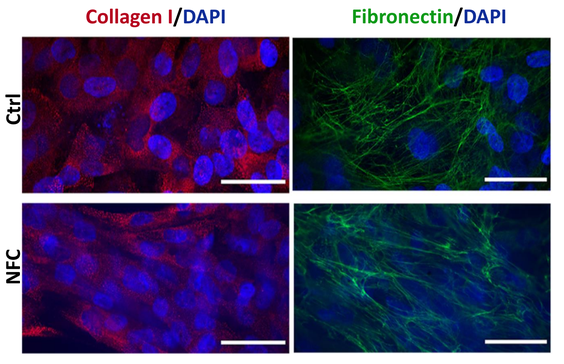


Figure 5. Fluorescence based imaging showed ECM production from hASCs, specifically collagen I (left panels, red), and fibronectin (right panels, green) similarly to controls. DAPI (blue). Scale bars, 50µm. Ref. Kiiskinen et al 2019.

Conclusions

The characteristics of NFC makes it ideal tools for 3D cell-based assays, wound healing and other clinical applications.

- **Animal-free** NFC hydrogels are **biocompatible** with cells and tissues with **no batch variation.**
- Pluripotent stem cells **retain their pluripotency.**
- NFC **reduced stress-induced effects to renal organoids** during the nephrogenesis process.
- **Human ASCs** can be **seeded onto NFC** dressing without additional surface coating, and they **remain viable for 2 weeks expressing their own ECM**.
- **NFC is already in clinical use** as a wound dressing and UPM Biomedicals is **developing NFC hydrogel for other clinical uses including implantation.**

Acknowledgements

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References: [1] 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. **[2]** GrowDex Application note #38 Nephrogenesis in renal organoids demonstrated in nanofibrillar cellulose hydrogel **[3]** Kiiskinen et al., 2019, Nanofibrillar cellulose wound dressing supports the growth and characteristics of human mesenchymal stem/stromal cells without cell adhesion coatings.

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