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Bacteriophage Applications and Organoid Transportation with Nanofibrillar Cellulose Hydrogel

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UNIVERSITY OF HELSINKI

CELLBOX

INTRODUCTION

GrowDex products (GrowDex® and GrowDex®-T) are wood-based nanofibrillar cellulose (NFC) hydrogels developed for biomedical applications, such as three-dimensional (3D) cell culture. NFC is **biocompatible** with human cells and tissues but as a plant based product it does not contain any animal or human derived material.

Bacteriophage therapy is one of the alternative methods for **fighting the antibiotic resistant bacteria - caused infections**. Efficient treatment requires a fast and reliable phage selection process with the possibility to transport phages between laboratories.

In vivo-like biological structures exemplified in 3D cell cultures and engineered tissues present a challenge for the standard cryo-transport procedures. This study represents the **transportation of midbrain organoids under laboratory conditions** (37°C and 5% CO_2) using GrowDex and shipping incubator (Cellbox).

AIMS

The aims of the studies presented in this poster were:

1) Study the storage, transportation and delivery of phages in GrowDex hydrogel
2) Demonstrate an advanced and improved shipping method for fragile midbrain neuronal organoids

METHODS

For storage and transportation studies, bacteriophages against E. coli and S. aureus were mixed in 0.5% GrowDex and shipped by mail on a 96-well plate. The effectiveness of the phages for their host bacteria was analysed with OD600 assay.

For midbrain organoid transportation study, the culture plates were sealed with 4Titude Moisture Barrier Seal 96 (4ti-0516/96) gas permeable films that serves as the first layer of the UN3373 packaging guidelines. The sealed culture plates were placed on top of absorber material and transferred into a Tyvek bag. A heat-sealing device was used to seal the Tyvek bag, thereby providing the second layer of the UN3373 packaging solution. The Tyvek enclosed multi-well plates were transferred to a preconditioned Cellbox and securely placed in the incubation chamber. Control plates were prepared in the same manner, but transferred to a standard laboratory incubator.

RESULTS AND DISCUSSION

Bacteriophage studies

The phages remained infectious and inhibited the growth of their target bacteria, while GrowDex itself does not affect bacteria (Fig. 1). These ready-to-screen plates could be used for reliable phage transportation from a central phage laboratory to hospital laboratories for phage selection screening in the treatment of antibiotic resistant bacteria infections.

Phages mixed with GrowDex stay infectious, and are able to release and infect the target bacteria (Fig. 2A). Additionally, delivery of phages in GrowDex hydrogel can be done by spraying with pump bottles (Fig. 2B and C). Phages could be delivered to patients with wounds infected with antibiotic resistant bacteria, and the delivery of phages in GrowDex can result in improved contact time and thus better efficacy.

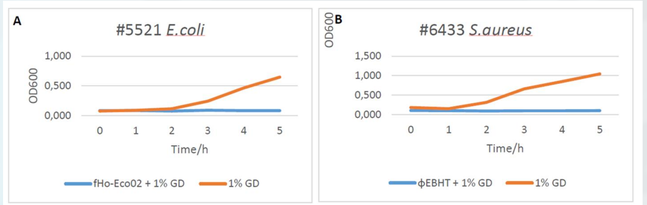

Figure 1. Phages fHo-Eco02 (A) and ΦEBHT (B) were stored in 10 µL GrowDex drops on a 96-well plate and transported by mail. After one week, phage viabilities were measured by OD600 assay. Blue lines indicate that the phages were able to inhibit the growth of the target bacteria, and orange lines indicate the growth of host bacteria mixed together with GrowDex only.

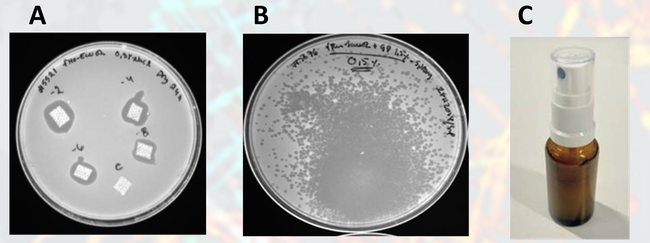

Figure 2. A) Nanofibrillar cellulose membrane pieces were infused with fHo-Eco02 phage lysate, dried for 24h, and transferred to bacterial lawn. B) fRuSau02 phages sprayed in GrowDex to bacterial lawn were able to inhibit the growth of the target bacteria S. aureus. Phages were able to release from GrowDex and FibDex to infect the target bacteria. C) Finger-operated spray bottle used for spraying GrowDex with phages.

Midbrain organoid transportation

Midbrain organoids were cultivated in cell culture media with and without GrowDex® to determine the influence of the artificial ECM on cell survival. The organoids were cultured at 37 °C and 5% CO_2, both in a stationary incubator and during transport in the Cellbox®. Cellbox® data logs verified that incubation conditions were accurately maintained at an average temperature of $\vartheta_{MEAN} + \vartheta_{SEM} = 37.31 \pm 0.02$ °C and an average CO_2 concentration of $\vartheta_{MEAN} + \vartheta_{SEM} = 5.04 \pm 0.06$ (Fig. 3). Midbrain organoids were assessed for survival after transport was completed by means of a CellTiterGlo3D assay (Fig. 4).

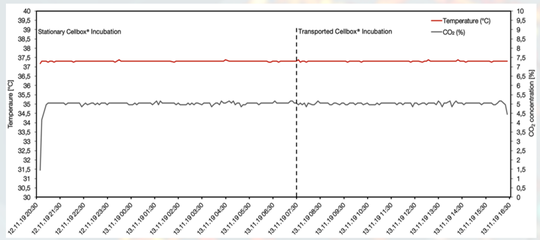

Figure 3. Transportable cell incubator, Cellbox. Graphical representation of the data log exported from the Cellbox® for stationary incubation and transport. The Cellbox was pre-set to 37 °C and 5% CO_2 before the packaged midbrain organoids were transferred to the incubation chamber. The Cellbox® data logging system was activated and the device placed into the packaging kit for overnight incubation. Transport started in the early morning and lasted for approximately 8 hours before the Cellbox® was returned to the pickup location. The data logs were exported to the Cellbox® iOS App and the temperature and CO_2 values represented as a line chart.

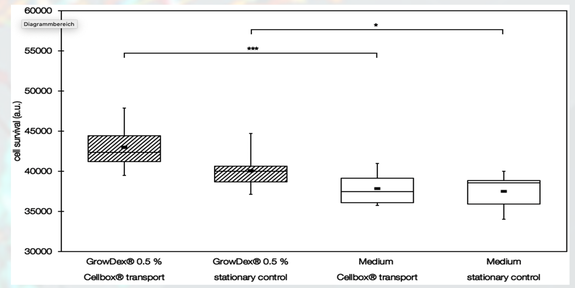

Figure 4. Midbrain organoid survival assay. Midbrain organoids were cultivated in cell culture media with and without GrowDex® 0.5% to determine the influence of the artificial ECM on cell survival. Incubation was performed at 37 °C and 5% CO_2, either in a Cellbox that was transported for approximately 8 hours or in a stationary incubator. The cell survival rate was measured by means of a CellTiterGlo3D assay and represented as a box plot.

CONCLUSIONS

The inherent properties of GrowDex make it an ideal tool for researchers working with different biological materials.

Nanofibrillar cellulose materials are ideal for the storage, transportation, and delivery of bacteriophages, which remain viable, are able to release from GrowDex and FibDex, and infect the target bacteria.

Transported human midbrain organoids experienced no changes in their viability as a result of being transported in the Cellbox®. This study therefore validates the device as a feasible solution for the transport of organoids. By comparing organoids which have been grown in culture media with and without the addition of GrowDex®, we could also show that GrowDex® hydrogels significantly increases cell viability, not only during transport, but also during stationary incubation, suggesting a protective role in the culturing of these organoids.

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