GrowDex®

UPM BIOFORE BEYOND FOSSILS

Application Note **42**

Transportation of live midbrain organoids intended for high content screening

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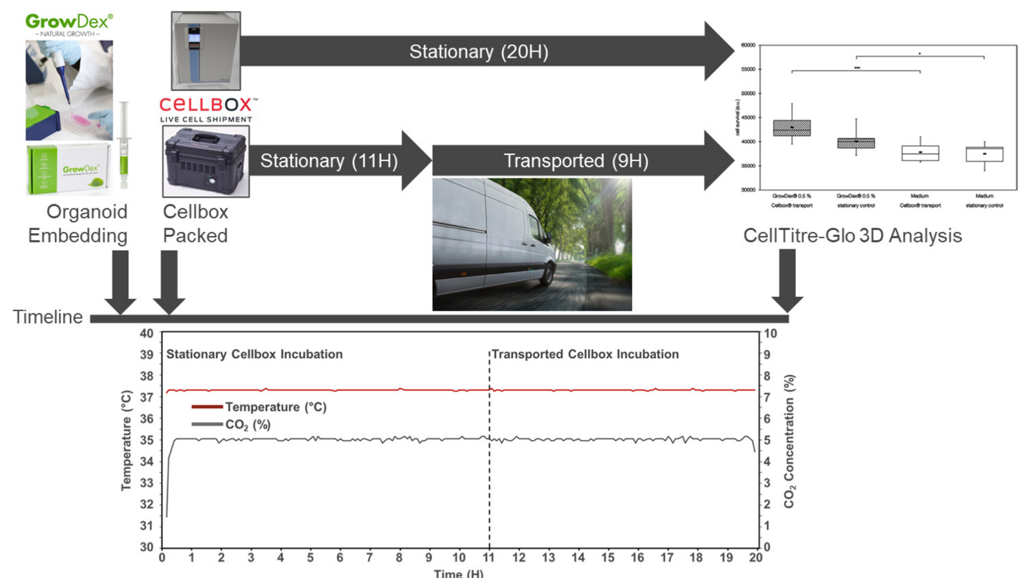
INTRODUCTION

Drug discovery is a lengthy and expensive process, with most drug candidates failing in the development process and never making it to market. To improve the success rate of drug development, scientists and physicians are devising novel and often complex biological tools that mimic *in vivo* conditions in a more reliable manner compared to the current 2D cell cultures or animal models. The increased complexity of these *in vivo*-like biological structures, exemplified in 3D cell cultures and engineered tissues, present a challenge for the standard cryo-transport procedures. In the standard approach, cells are exposed to at least one freeze-thaw cycle which is known to cause cell death, reduction in cell proliferative capacity and altered gene expression, as reviewed by Baust, J.G. et al., (2009) [1]. Furthermore, cells are affected by the metabolic activity and toxicity of the cryoprotectants that are integral to the cryopreservation process.

Brain organoids are highly incompatible with cryopreservation due to the large amount of cell death that occurs for many tissue types upon undergoing a freeze thaw cycle. As organoids mature, they build complex networks of different cell types, and these networks may get compromised following cryopreservation. Cellular repair and re-growth can range from slow to impossible (in the case of postmitotic neurons, for example), and new cells may not functionally integrate into the surrounding tissue. Also, many dead or dying cells negatively affect cell signaling in their immediate surrounding, further compromising cells that may have survived freezing, ultimately driving thawed tissues away from normal homeostatic behavior.

Therefore, this partial or complete incompatibility with cryopreservation, limits their application in high-throughput screening (HTS) or high-content screening (HCS) workflows.



CELLBOX™ LIVE CELL SHIPMENT

UPMBIOMEDICALS

To investigate the effects of transport on fragile 3D cell cultures, we have selected human midbrain organoids as a model system. This study represents the first known attempt to transport midbrain organoids under laboratory conditions (37 °C and 5% CO_2). The organoids were packaged accordingly to packaging instructions P650 following UN3373 regulations for biological substances category B [2] and transported for more than 8 hours by road in an autarkic cell culture incubator device, the Cellbox™ live cell shipper. In addition to the effects of transport in liquid media, we also investigated the benefit of immobilizing the organoids in nanofibrillar cellulose hydrogel, GrowDex®, to provide a protective mechanical buffer against shock, vibration, and possible sample loss. GrowDex is an animal-free, adjustable, and well-defined hydrogel developed for various cell culture applications, including HTS or HCS 3D cell culture assays.

The aims of the study were 1) the validation of a commercially available transportable incubator for the safe transport of midbrain organoids under laboratory conditions, and 2) to investigate the influence of a natural nanocellulose based hydrogel during stationary incubation and transport of midbrain organoids.

MATERIALS

- Cellbox Ground CD / Flight CDI (SN: CBX0061235)
- GrowDex 1.5% (Cat No. 100 103 005, UPM Biomedicals)
- GrowDex-T 1.0% (Cat No. 200 103 005, UPM Biomedicals)
- Cellbox Solutions (CBS) 96 Well Plate Individually Packed (Cat. No. 10000025), CBS CO_2 Permeable Seal for 96-Well Plate (Cat. No. 10000032), CBS Absorbent Material Small (Cat. No. 10000015), CBS CO_2 Permeable Safety Pouch small (Cat. No. 10000018), as part of CBS Nanny 96-Well Kit SF (Cat. No. 10000151)
- CBS polystyrene and carton overpack (Cat. No. 1000014)
- Midbrain organoids [3] and maturation cell culture medium [4]
- CellTiterGlo® 3D cell viability assay (Cat No. G9681, Promega)

METHODS

Embedding of midbrain organoids in GrowDex:

1. GrowDex was diluted to a working concentration of 0.5% v/v with cell culture medium. As an example, 800 µl of culture medium was added to a 2 ml tube and 400 µl 1% v/v GrowDex was added. Mixing was started by swirling the pipette tip around tube walls, followed by pipetting up and down until a homogenous hydrogel was formed.
2. 40 µl per well of 0.5% GrowDex was pipetted into wells of a CBS 96 Well Plate, midbrain organoids were applied on top of GrowDex, and another 40 µl GrowDex was added on top of the organoids, with a final total volume of 80µl.
3. The transparent and anionic nanofibrillar cellulose GrowDex-T, was also tested and prepared to a final working concentration of 0.25% v/v. The culture format was also setup and performed as described above.
4. Two CBS 96 Well Plates were prepared, one for stationary Cellbox incubation and the other for transported Cellbox incubation. N=6 organoids were prepared per culture condition – Medium only, 0.5% GrowDex and 0.25% GrowDex-T.

Packaging of living organoids following UN3373 regulations:

5. The culture plates were sealed with CBS CO_2 Permeable Seal for 96-Well Plate (Cat. No. 10000032), gas permeable films, that serve as the first layer of the P650 packaging instructions **(Fig. 1A)**.
6. The sealed culture plates were placed on top of absorber material and transferred into a CBS CO_2 Permeable Safety Pouch **(Fig. 1B)**. A heat-sealing device was used to seal the Safety pouch, thereby providing the second layer of the P650 packaging instructions.
7. The safety pouch enclosed multi-well plates were placed into the Cellbox multi-well adapter **(Fig. 1C)** and secured **(Fig. 1 D),** then transferred to a preconditioned Cellbox and securely placed in the incubation chamber **(Fig. 1E-F)**.
8. Control plates were prepared in the same manner but transferred to a stationary standard laboratory incubator used at 37 °C and 5% CO_2.

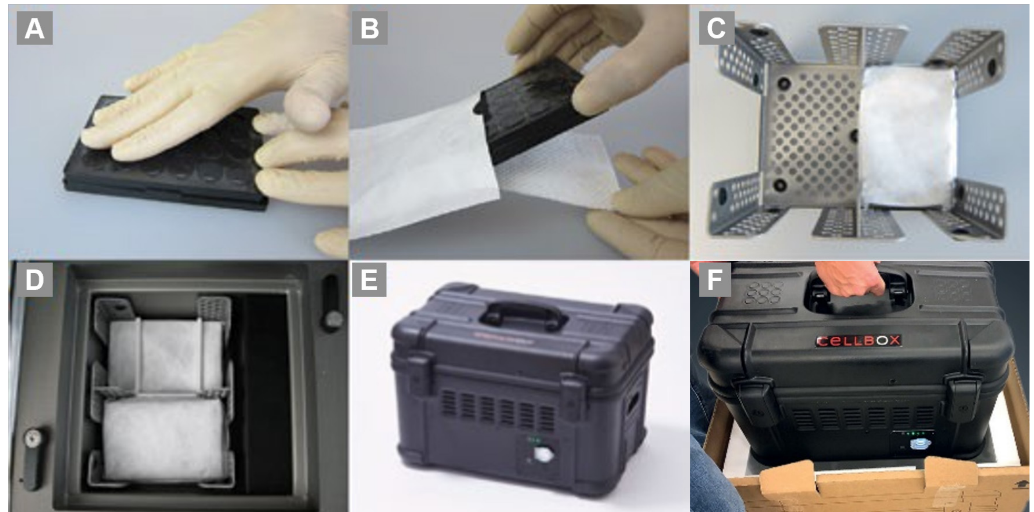


Figure 1. **Example of packaging procedure compliant with UN3373** regulations for biological substances category B. **(A)** A CBS 96 Well Plate is sealed with a CBS CO_2 Permeable Seal for 96-Well Plates to provide a CO_2 permeable environment, while preserving humidity in the wells. **(B)** Sealed plates are placed on top of CBS Absorbent Material and transferred to a CBS CO_2 Permeable Safety Pouch before the pouch is heat-sealed. **(C)** Safety pouch-packaged plates are placed in the Cellbox multi-well adapter. **(D)** The multi-well adapter is placed into the incubation chamber. Multiple plates can be stacked and separated using a spacer-insert. **(E)** The incubation chamber lid is securely closed and latched into place to ensure the optimal environment is maintained. The Cellbox outer lid is closed and latched to prevent any unwanted access. **(F)** Cellbox is placed into CBS polystyrene and carton overpack.

Transport:

9. The Cellbox was connected to an external power supply during preconditioning to fully charge the batteries, and the CO_2 source was replaced.
10. Plates with midbrain organoids embedded in GrowDex were transferred to the Cellbox in the early evening **(Fig. 2, 20:30 PM)** and the entire device was then placed in the CBS polystyrene and carton overpack (Cat No. 1000014).
11. Organoids were cultivated overnight in the Cellbox before World Courier collected the shipment from the Max Planck Institute for molecular Biomedicine (MPI-Muenster) in the morning **(dotted line, Fig. 2, 07:30 AM)**.
12. The shipment was transported for 8 hours on the road before being returned to the MPI-Muenster in the late afternoon **(Fig. 2, 16:30 PM)**. The packaged plates were removed from the Cellbox and the midbrain organoids were assayed directly after transportation with the CellTiter-Glo 3D viability kit.

Viability assay:

13. Viability of the midbrain organoids was assessed using the CellTitre-Glo 3D viability kit from Promega.
14. Briefly, reagents were prepared according to manufacturer's instructions. An equal volume of reaction reagent (80µl per well) was added to each respective well, giving a total well volume of 160µl per well.
15. Following addition of the reagents to the wells, luminescence measurements were taken after 30 min of shaking incubation at room temperature.

RESULTS

Midbrain organoids were cultivated in cell culture media with and without GrowDex hydrogel to determine the influence of the matrix on cell survival (for details on cell culture, please refer to (2-3)). The organoids were cultured at 37 °C and 5% CO_2, both in a stationary incubator and during transportation in the Cellbox.

Cellbox data logs verified that incubation conditions were accurately maintained at an average temperature of $\vartheta_{MEAN} \pm \vartheta_{SEM} = 37.31 \pm 0.02$ °C and an average CO_2 concentration of $\vartheta_{MEAN} \pm \vartheta_{SEM} = 5.04 \pm 0.06$ **(Fig. 2)**.

To determine whether transportation had any detrimental effects on the viability of the midbrain organoids, the viability according to metabolic activity / ATP content, was assessed using the CellTiter-Glo viability kit. If the plates were maintained stationary in a cell culture incubator without transportation, the viability of the midbrain organoids embedded in GrowDex hydrogels was significantly increased compared to medium only **(Fig. 3 & 4)**. Additionally, when the plates were transported, the cells embedded in GrowDex hydrogels showed significantly higher viability than those transported in medium only.

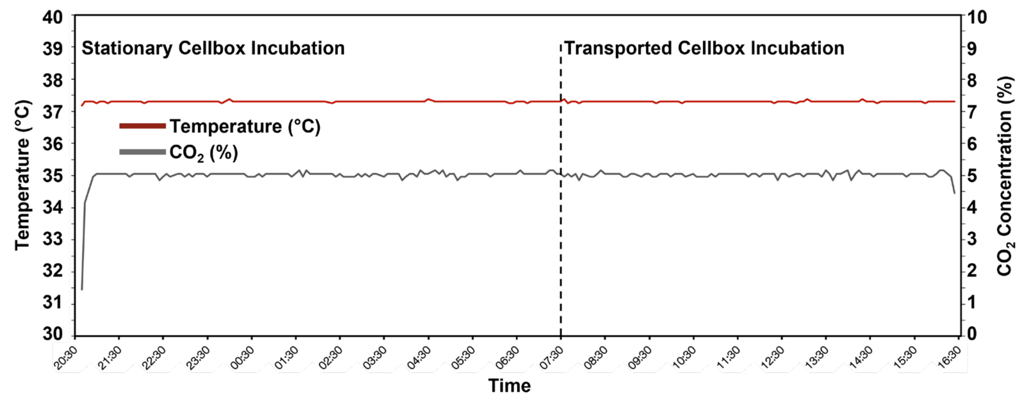


Figure 2. Graphical representation of the data log exported from the Cellbox for stationary incubation and transport. (A) The Cellbox was pre-set to 37 °C and 5% CO_2 before the packaged midbrain organoids were transferred to the incubation chamber. The organoids were then transferred to the Cellbox (20:30) and the data logging system was then activated, then the device was placed into the CBS polystyrene and carton overpack for overnight incubation. Transport started in the early morning (07:30) and lasted for approximately 8 hours before the Cellbox was returned to the pickup location (16:30). The data logs were exported to the CELLBOX - LIVE CELL SHIPPER iOS App [5]. The temperature and CO_2 values are represented as a line chart.

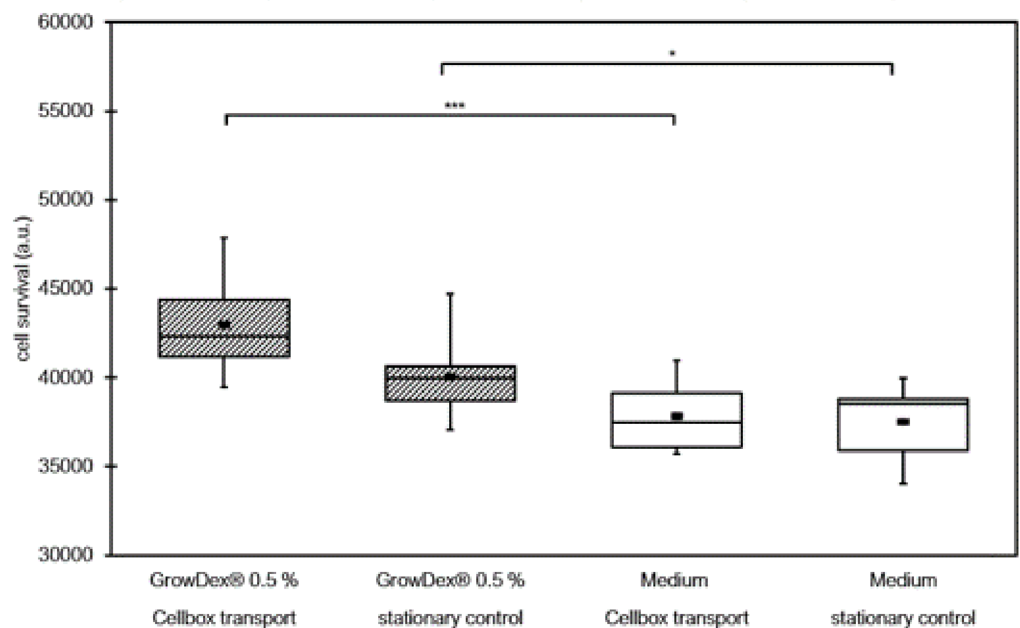


Figure 3. Midbrain organoid survival assay. Midbrain organoids were cultivated in cell culture media with and without GrowDex 0.5% to determine the influence of the matrix on cell survival. Incubation was performed at 37 °C and 5% CO_2, either in a Cellbox that was transported for approximately 8 hours or in a stationary incubator. The cell survival rate was measured by means of a CellTiter-Glo 3D assay and represented as a box plot. N=6 organoids tested per incubation and culture condition. The dot represents the average, whiskers represent the max and min values, the box the first to third quartile, separated by the median. Dunnett's multiple comparison test used where *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

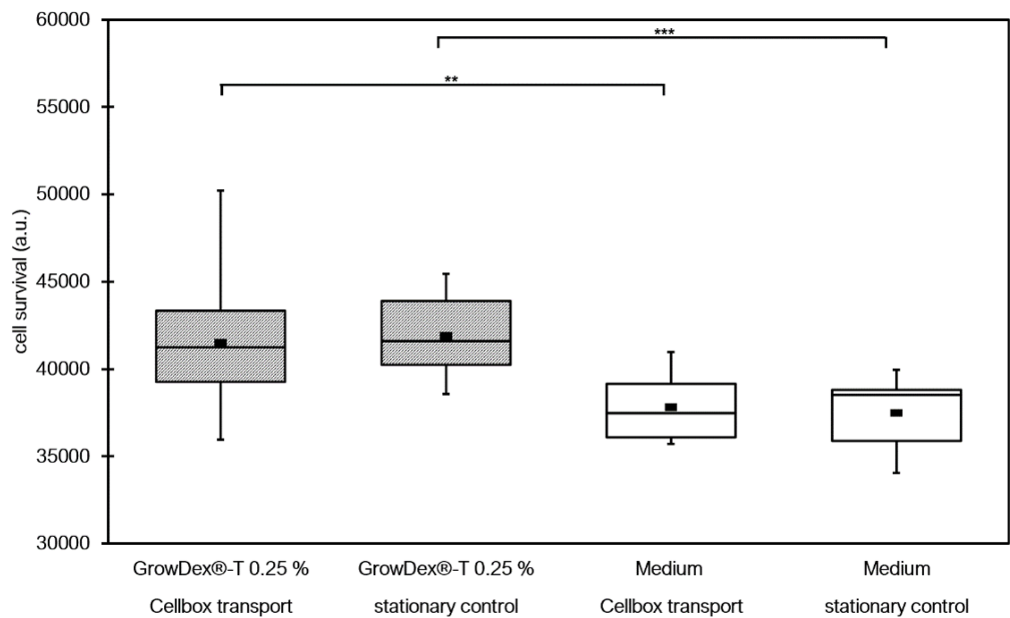


Figure 4. Midbrain organoid survival assay. Midbrain organoids were cultivated in cell culture media with and without GrowDex-T 0.25% to determine the influence of the matrix on cell survival. Incubation was performed at 37 °C and 5% CO_2, either in a Cellbox that was transported for approximately 8 hours or in a stationary incubator. The cell survival rate was measured by means of a CellTiterGlo3D assay and represented as a box plot. N=6 organoids tested per incubation and culture condition. The dot represents the average, whiskers represent the max and min values, the box the first to third quartile, separated by the median. Dunnett's multiple comparison test used where $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

CONCLUSIONS

The use of GrowDex 0.5 % and GrowDex-T 0.25% as an extracellular matrix for the storage and transportation of midbrain organoids significantly increased the viability of midbrain organoids compared to controls kept in cell culture medium only. Additionally, the midbrain organoids were unaffected by transportation in the Cellbox when compared to cultures maintained in a stationary incubator.

GrowDex hydrogel's ease of use, shear thinning material properties, and automated liquid handling capabilities facilitate easy integration for HCS/HTS workflows. The combination of this culture format and the Cellbox transportation system provides a feasible and robust solution, where cells intended for HCS can be embedded in GrowDex hydrogels and transported to any site for screening, avoiding unnecessary freeze-thaw cycles or unwanted effects by cryoprotectants.

This study validates the benefits of Cellbox and GrowDex hydrogels as a feasible solution for the storage, maintenance, and transportation of midbrain organoids intended for high throughput screening applications. While having to be validated for each individual organoid model, these findings promise to alleviate current inherent drawbacks with long-haul transfers of organoids and thus provide the possibility for commercial use of organoid technology independent of the location of their generation.

REFERENCES

1. *Baust, J.G., et al.* **(2009)**. *"Cryopreservation." Organogenesis 5(3): p. 90-96.*
2. *Guidance about UN3373 can be seen on the following website from the British government website: https://www.gov.uk/government/publications/packaging-and-transport-requirements-for-patient-samples-un3373/packaging-and-transport-requirements-for-patient-samples-un3373*
3. *Henrik Renner, Martha Grabos, Katharina J Becker, Theresa E Kagermeier, Jie Wu, Mandy, Otto, Stefan Peischard, Dagmar Zeuschner, Yaroslav TsyTsyura, Paul Disse, Jürgen Klingauf, Sebastian A Leidel, Guiscard Seebohm, Hans R Schöler, Jan M Bruder* **(2020)** *A fully automated high-throughput workflow for 3D-based chemical screening in human midbrain organoids eLife 9:e52904. https://doi.org/10.7554/eLife.52904*
4. *https://apps.apple.com/nz/app/cellbox-live-cell-shipper/id1446013580*

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