CELLBOX
THE LIVE CELL SHIPPER

Integrated Live Cell Shipment Solution Avoids Cryopreservation for Cell-Based Products

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The ever-evolving landscape of cell-based therapies and regenerative medicine is driving the development of innovative and structurally complex biological living therapies. Cells and other biological materials traditionally are transported at low to cryogenic temperatures.

Although storage and transport of frozen products can offer logistical independence and facilitate their release, not all of these biological materials can be transported frozen without impairment of cell functionality, (partial) loss of cells or specimen destruction. Many organ-like structures, engineered tissues and even cell therapeutics do not survive freezing. At the moment, there is no solution on the market that can meet the logistic challenge of these “cryo-sensitive” cell-based therapies.

The portable incubator of Cellbox Solutions offers a solution to transport cultures in a controlled environment where temperature and CO_2 are regulated for the duration of the shipment. In collaboration with diverse partners, we have achieved positive results in a series of proof of concept experiments. For tissues and cells like retinal organoids, midbrain organoids, blood brain barrier model or cardiomyocytes and microglia, controlled studies have shown that the Cellbox offers a shipping solution able to keep the cells either in the pre-shipment condition or in a similar state as achieved by a static incubation for the time of the shipment.

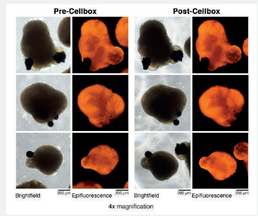
We here present initiatives to provide expanded cell therapy related capabilities to transport cell-based products for clinical application, i.e. device and software upgrades, packaging solutions designed to guarantee sterility and lack of cross-contamination, additional handling protection for the biological goods, a qualification package, Device Master File submission with the FDA and implementation of a QMS. We will discuss with possible partners how to submit a clinical trial authorization dossier based on the use of Cellbox for either shipment of the starting material from a collection site to the manufacturing facility, transport of intermediates or shipment of the final product to the clinics.

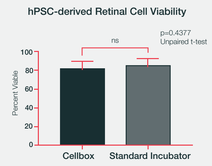
- Runtime of ≥ 48 hours
- 21 CFR Part 11 Compliant
- Controlled atmosphere 0 - 18% CO_2
- Constant temperature between 28°C to 38°C
- Constant data logging & bluetooth data export via Cellbox App
- Incubation volume: 4 liters
- Fulfilment of logistics standards

Transporting retinal organoids and scaffold using the Cellbox

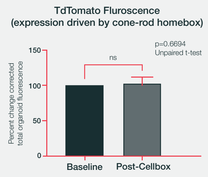
| Gamm Lab, University of Wisconsin-Madison

Total organoid TdTomato fluorescence (an indicator of photoreceptor survival) and cell viability tests were performed before and after the transport, and the comparison yielded no significant differences in any of the samples after Cellbox transport.





Viability measurements (trypan blue live-dead cell counts)
(n= 6 technical replicates per group)
Error bars represent mean ± standard deviation.

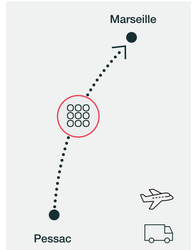


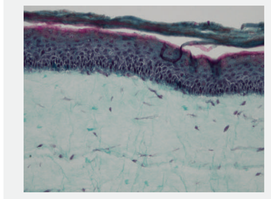
n = 3 organoids (n = 3 technical replicates per organoid)
Error bars represent mean ± standard deviation.

Transporting bioprinted dermo epidermal substitutes - Poieskin

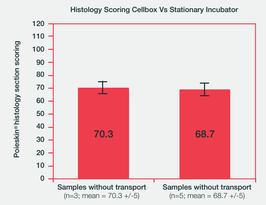
| Poietis

Samples were packaged according to guidelines, then transferred into the pre-conditioned chamber of the Cellbox Flight CDI. The transport parameters were set at 37°C and 5% CO_2 prior to being transported. The Cellbox departed Pessac, France. Upon arrival in Marseille, histology sections were obtained and microscopic examinations were done and analysed with Poietis' scoring grid (a tissue is considered compliant if its total score is ≥ 50%). This revealed no statistical difference in appearance and scoring when compared to samples that were not transported.





Microscopy (x20) histology section
(goldner Trichrome Staining)



Histology Scoring

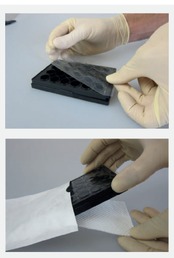
Transporting midbrain organoids

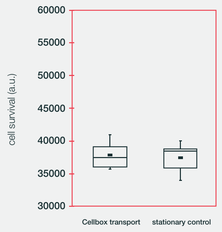
Midbrain organoids are routinely cultivated in the laboratory in standard incubators at 37°C and 5% CO_2. Deviations from these conditions will have a negative impact on the cells and may lead to sample variation and cell death.

Incompatibility with cryo-transport procedures demand innovative solutions like the Cellbox shipping incubator which can be used to transport midbrain and other organoids under laboratory conditions.

- Fulfills UN3373
- Threefold package: gas permeable adhesive film, Tyvek-bag + absorber material, Cellbox Lid
- Suitable package solution for all established culture vessels such as multiwell-plates, T-flasks and chips

Midbrain organoids were either cultivated in a stationary incubator or transported in the Cellbox. The cell survival rate was measured by means of a CellTiterGlow3D assay. Midbrain organoids were unaffected by transport in the Cellbox in comparison to stationary incubation in the laboratory.



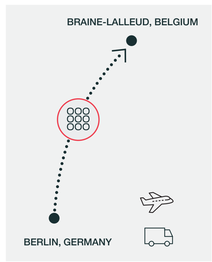


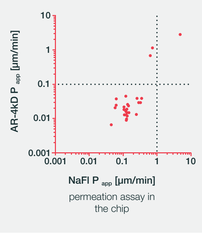
Transporting a blood brain barrier model on a chip

| TissUse

Organ-on-a-chip technologies provide a breakthrough in mimicking human organs on a miniature sale. The chips allow for separate cell culture, connected within a microfluidic system.Organs such as the blood brain barrier (BBB) or kidneys can be set up very similar to in vivo conditions. TissUse have developed a BBB model based on their HUMIMIC Chip2 technology and adopted the Cellbox technologies to fully utilise their expertise of cell culturing in these chips. This enables the end user to receive a fully functioning healthy model without spending a lot of time and effort with setting it up.

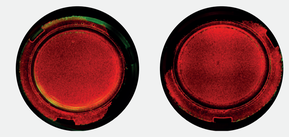
Prior to the shipment, TissUse evaluated the quality of the BBB model by performing TEER measurements, a permeation assay and live/dead staining. The permeation assay evaluates how well the tight junctions between the cells are by using the fluorescent 4kd-dextran and sodium fluorescence. Live/dead staining was done with Calcein Red-Orange AM and CellTox Green.
The samples were packaged according to guidelines, and transport parameters set to 37°C and 5 % CO_2 in the Cellbox Ground CD. Upon arrival in Belgium, the TEER measurements were repeated to evaluate the post-transport quality of the BBB model. No significant difference was found between the two time points showing that the Cellbox device can be used to safely transport the organ-on-a-chip models offered by TissUse.





permeation assay in the chip

TEER Measurements

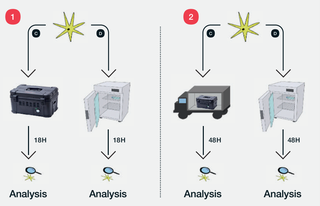


Staining with calcein red-orange AM and CellTox Green
(Red = Living Cells; Green = Dead Cells)

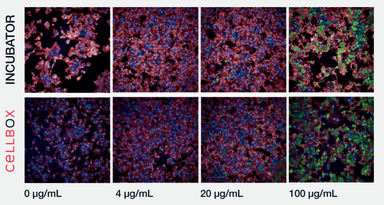
Transporting iPSC-derived microglia and testing their activity

Incubation of human iPSC derived microglia in the Cellbox (without transportation) does not alter the morphology, nor the phagocytic capacity when compared to human iPSC-derived microglia kept in the stationary incubator. Road transport of human iPSC derived microglia in the Cellbox (two transportations: from Belgium to Germany and from Germany back to Belgium) does not alter the morphology, nor the phagocytic capacity when compared to human iPSC derived microglia kept in the stationary incubator. In addition, no obvious cell detachment was observed either.

Experiment Workflow

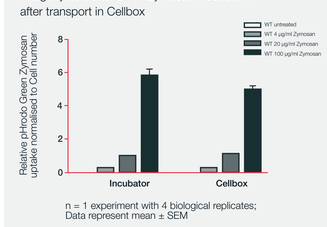


Imaging of Transported Microglia (Incubator Vs Cellbox)



Red = Cellmask deep red plasmamembrane stain; Blue = HOECHST; Green = pHrodo Green Zymosan

Phagocytosis after 24 hr Zymosan incubation after transport in Cellbox



n = 1 experiment with 4 biological replicates;
Data represent mean ± SEM

Table of ongoing development work for advanced therapy utilization

	Current	Future ATMP use	Ongoing work
Software	RUO	21CFR part11 compliant	✓
Hardware	Cellbox Flight 2.0 Cellbox Ground 2.0	Cellbox Flight 2.0 AT Cellbox Ground 2.0 AT	✓
Accessories	RUO sterile sealable gas-permeable membranes	NA	
	Tyvek leak proof overwrap	Qualified secondary packaging	✓
	Rack to stack microwell plates and T25/T75 flasks	Fitting rack for primary and secondary packaging	
Regulatory compliance	UN 3373 Dangerous goods	Device Master File (FDA) Regulatory advise (Q-Sub) Consulting for EU regulation	✓
Generic GMP compliance	none	Sterility No cross-contamination Container closure Validation package	✓
Product Specific GMP compliance	none	Product-specific testings and validations Product specific release strategy IND or IMPD for clinical testing	Internal study initiated Looking for partners