CellShip: Technical Information

Overview

CellShip® has been developed to provide an alternative to cryopreservation for the transport and short-term storage of mammalian cells. To date, CellShip® has been validated for up to 120 h transport/storage at ambient temperatures, for a range of cell lines (Table 1) and for 72 h transport/storage of primary, canine mesenchymal stem cells (MSCs). The protocol requires minimal cell manipulation, reducing the risk of contamination and improving efficiency within the laboratory.

CellShip® is sterile, xeno-free, defined and contains a non-toxic additive designed to protect cells against shear stress, and to help maintain membrane integrity. We provide two formulations, either with or without insulin.

Advantages of using CellShip®

- CellShip® is a 'ready-to-use' transport and short-term storage medium, suitable for a wide range of cells.
- CellShip® provides flexibility to highly time critical processes.
- CellShip® removes the cost and logistic challenges associated with shipment on dry ice.
- CellShip® offers stable pH-buffering of pH 7.20 - 7.45 for temperatures between +2°C to +37°C.
- CellShip® can be used at ambient temperatures.
- CellShip® is xeno free i.e., free from serum and animal/human proteins.
- Endotoxin levels conform to EU standards (≤ 1.0 EU/mL).
- CellShip® is solvent free i.e., free from DMSO.
- CellShip® is manufactured under ISO 13485.

Applications

For the transport and short term storage of cells at ambient temperatures, as an alternative shipping cryopreserved samples. Application development for this product is ongoing and CellShip® efficacy is currently being assessed for the transportation of additional primary cells, stably transfected and gene silenced cell lines.

Development Data: Cell Lines

Initial product development has focussed on commercially important cell lines, to assess whether transporting cells in CellShip® at ambient would provide a viable alternative to transporting cryopreserved cells using dry ice.

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Table 1. Cell lines tested during CellShip® development

Cell line	Rationale for use	Viability at time of recovery		Fold change in cell number after recovery
Jurkat	Used as a T-cell model for clinical development of CAR-T therapies	72h 120 h	87% 81%	2.1 1.4
HEK-293	Used in biomanufacturing	72 h 120 h	98% 98%	4.5 2.3
Hep-G2	Drug development and toxicity testing	72 h 120 h	96% 98%	2.9 3.7
CHO	Used in biomanufacturing	72 h 120 h	97% 96%	6.5 5
K562	Biomedical research and cytotoxicity assays	72 h	98%	4.5
A549	Drug metabolism and toxicity studies.	120 h	97%	2.2
HeLa	Biomedical research including Covid-19 vaccine research	72 h	81%	1.9

Cells were shipped at ambient temperature for 72-120 h. To ensure that cells recovered following transport/storage, the 'fold change' in cell number was calculated by comparing the pre-transport/storage cell count to the final cell number post recovery. In all experiments, cell numbers post recovery exceeded the original number of cells, indicating that cells had re-entered the cell cycle and recovered well.

As a direct comparison to cryopreservation, Jurkat cells (Figure 2) and HepG2 cells (Figure 3) were assessed. Stocks of cryopreserved cells were transferred from liquid nitrogen to dry ice for 72 h. Alternatively, cells were taken from growing stocks and were transported/stored for 72 h in CELLSHIP® at ambient temperature, using the protocol above.

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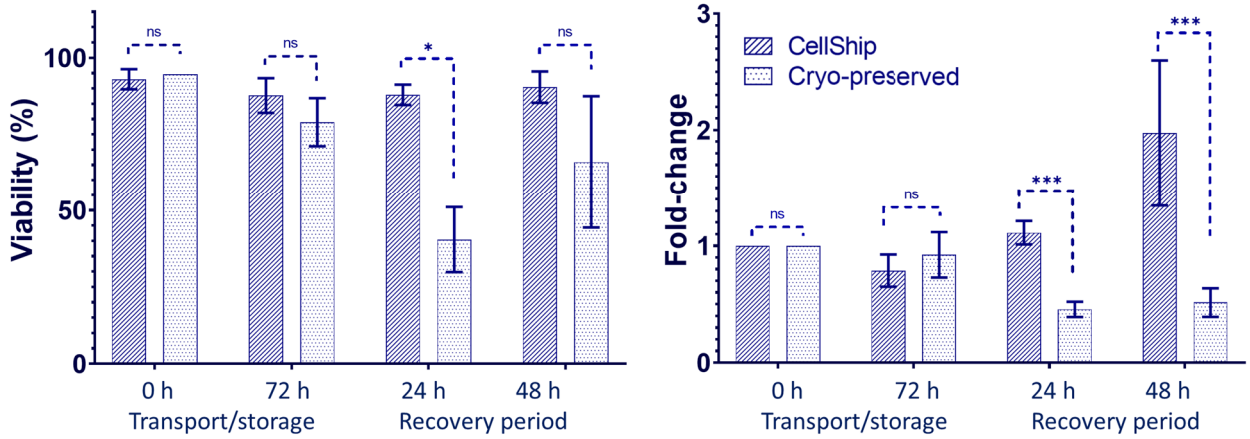

Figure 2. Jurkat cells transported/stored in CellShip® at ambient had returned to pre-transport numbers within 24 h of recovery and showed a 2-fold increase by 48 h. Cell viability was well maintained throughout the experiment (n=3).

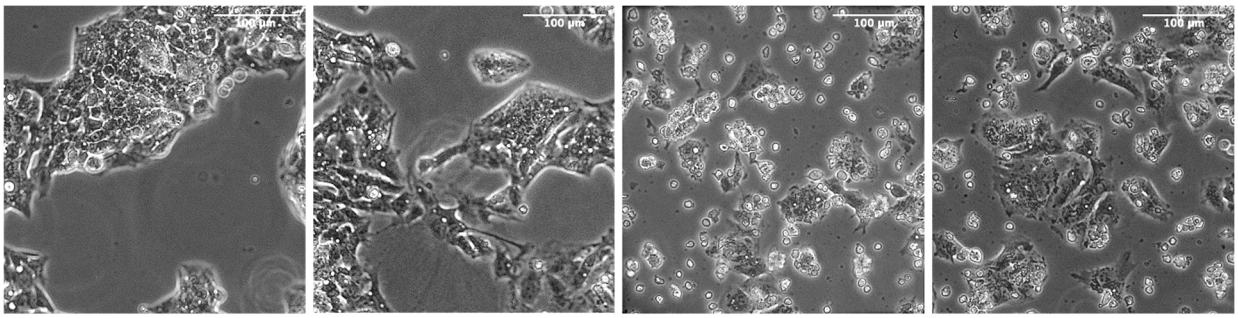

Figure 3. HepG2 cells were imaged following a 48 h recovery period. Prior to recovery, cells had been transported/stored for 72 h either cryopreserved in dry ice or at ambient in CellShip®. Within 48 h of recovery, cells transported in CellShip® were fully adherent and were displaying typical growth morphology (A & B). Cells recovered from cryopreservation had started to adhere but had not yet re-established typical HepG2 growth morphology (C & D).

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Development Data: Primary Canine MSCs

Adipose derived MSCs from four individual donor animals were provided by our collaborator who is seeking an alternative to dry ice for the transport of canine MSCs for clinical application. MSCs were either transported/stored in CellShip® for 72 h or were cryopreserved and stored at -80°C to represent transport on dry ice. Following the transport storage period, cell viability was analysed by Annexin V/PI (Figure 3) and metabolic activity was analysed using a WST-1 assay (Figure 4). CD marker expression was also assessed following the 72 h storage/transport period (Figure 7) Cell morphology was observed after a six day recovery period (Figure 5) and differentiation potential was also assessed (Figure 6).

Our data shows that canine mesenchymal stem cells transported/ stored in CellShip® for 72 h:

- Adhere and start proliferating more quickly than the cryopreserved controls
- Have increased viability compared to the cryopreserved controls
- Have the same differentiation potential as the cryopreservation controls
- Have slightly increased or comparable metabolic activity after transport
- Express the same CD markers as the cryopreservation controls

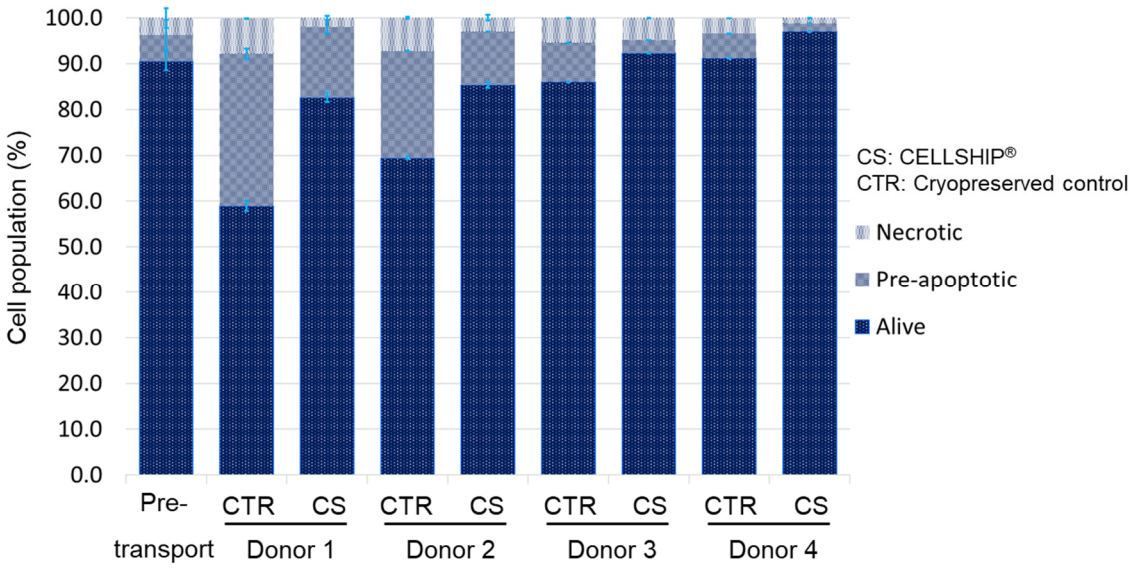

Figure 3. Following 72 h transport/storage in CellShip® canine MSCs maintained excellent viability. In each experiment, cells transported in CellShip® had increased viability, reduced numbers of apoptotic cells and reduced numbers of necrotic cells compared to the cryopreserved controls.

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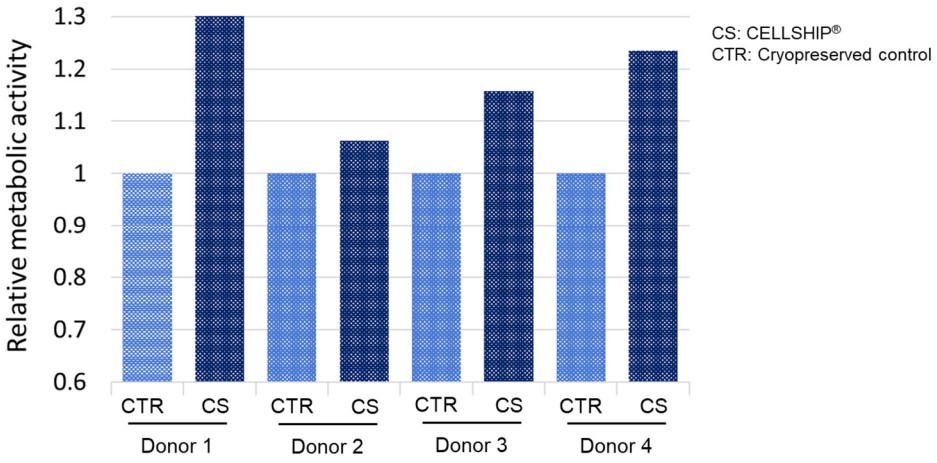

Figure 4. A WST-1 assay was used to assess metabolic activity. Cells were analysed following 72 h transport (cell numbers normalised). In each experiment, relative metabolic activity was slightly increased in cells transported in CellShip® compared to the cryopreserved controls.

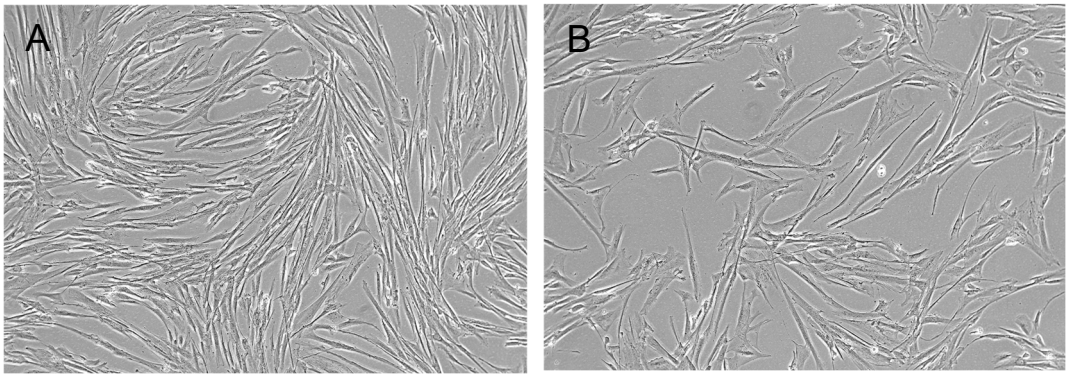

Figure 5. Following 72 h transport, cell numbers were normalised, and they were recovered for 6 days. Cell morphology was assessed. The increased confluency in flask A indicates that cells recovering from transport in CellShip re-enter the cell cycle more quickly than cells recovered from cryopreservation.

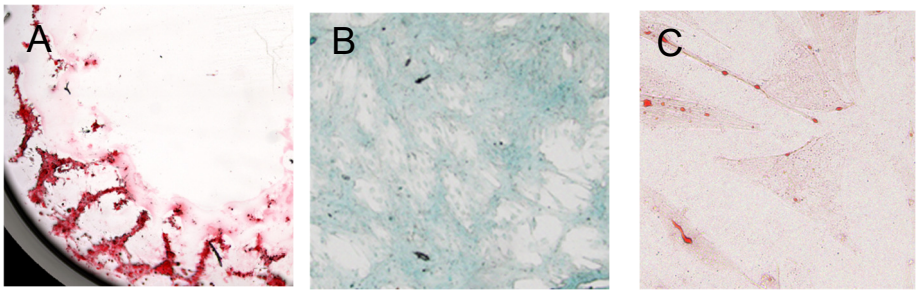

Figure 6. Cells transported in CellShip® maintain their differentiation potential and are able to undergo osteogenesis (A), chondrogenesis (B) and adipogenesis (C).

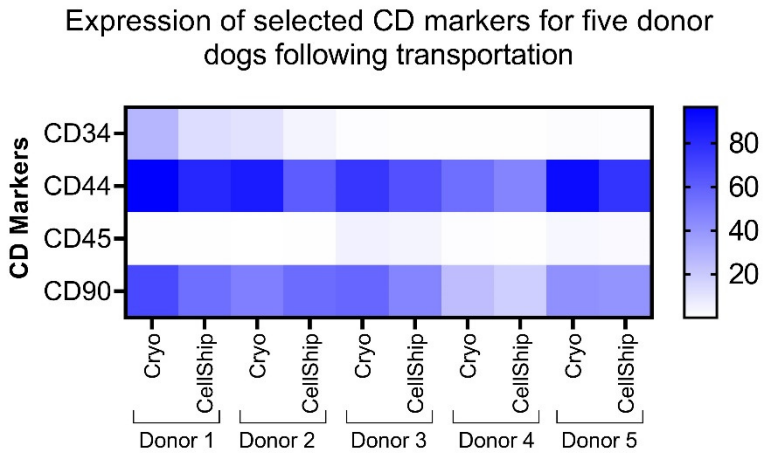

Figure 7. Following 72 h transport/storage, CD marker expression was assessed via flow cytometry and was comparable between cells transported in CellShip® and the cryopreserved controls.

Conclusion

These data demonstrate a novel, viable and cost effective method of transporting both cell lines and primary MSCs and provides a simple alternative to cryo-preservation (using dry-ice). Efficacy over 120 h increases the number of applications that CellShip® will be suitable for, including biomanufacturing and the medical sciences.

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Protocol

The protocol has been specifically developed to be simple, requiring minimal cell manipulation. A range of cell concentrations have been successfully tested (*Table 1*); from < 1 x 10^6 to > 10 x 10^6 per mL, although we recommend that you optimise and standardise the cell concentration according to your requirements. Canine MSCs were transported at a clinical dose of 5 x10^6.

Transport

Culture cells using standard growth media and conditions.

Harvest cells* using your standard method for sub-culturing and centrifuge at room temperature for 5 min at 180 x g. Discard the supernatant, without disturbing the cell pellet.

Gently resuspend cells in in 2-3 mL of CellShip® and centrifuge as before. Discard the supernatant, without disturbing the cell pellet.

Gently resuspend cells in 2 mL of CellShip® and transfer to a 2 mL cryotube*. For transport, cryotubes should be placed horizontally in protective packaging.

Canine MSCs were transported using a cold pack as per our collaborator's protocol. Typical temperature range that MSCs were exposed to was 5ºC - 13ºC.

Recovery

Gently resuspended the cells* in the cryotube and transfer the cell suspension to the appropriate volume of complete growth medium. Culture using standard conditions for 24 h to 48 h*. There is no need to remove CellShip before recovery.

* Viability and cell number calculated.

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Presentation

CellShip® is supplied as a sterile ready-to-use solution. CellShip® is available in the following formats.

Product Code	Product Description	Pack Size
SCS-004B	CellShip cell transport medium	100 mL
SCS-004C	CellShip cell transport medium	50 mL
SCS-004K	CellShip cell transport medium	25 mL

Antibiotics

CellShip® is also available with antibiotics, as required. Standard antibiotics that may be used are: Amphotericin B and Chloramphenicol, Amphotericin B and Nanomycopulitin or Penicillin-Streptomycin and Amphotericin.

Shelf life

CellShip® with or without insulin has a shelf life of 10 months from the date of manufacture when stored at +2 - 8°C under dark conditions.

Storage

Recommended storage is +2°C to +8°C.

Protect CellShip® from exposure to light.

Product ships at ambient temperature.

References

Emma Buick, Andrew Mead, Abeer Alhubaysh, Patricia Bou Assi, Parijat Das, James Dayus, Mark Turner, Lukasz Kowalski, Jenny Murray, Derek Renshaw, and Sebastien Farnaud. CellShip: An Ambient Temperature Transport and Short-Term Storage Medium for Mammalian Cell Cultures. Biopreservation and Biobanking. 28 Dec 2023 https://doi.org/10.1089/bio.2023.0100

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