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CellShip: An Ambient Temperature Transport and Short-Term Storage Medium for Mammalian Cell Cultures

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Cell culture is a critical platform for numerous research and industrial processes. However, methods for transporting cells are largely limited to cryopreservation, which is logistically challenging, requires the use of potentially cytotoxic cryopreservatives, and can result in poor cell recovery. Development of a transport media that can be used at ambient temperatures would alleviate these issues. In this study, we describe a novel transportation medium for mammalian cells. Five commonly used cell lines, (HEK293, CHO, HepG2, K562, and Jurkat) were successfully shipped and stored for a minimum of 72 hours and up to 96 hours at ambient temperature, after which, cells were recovered into standard culture conditions. Viability (%) and cell numbers, were examined, before, following the transport/storage period and following the recovery period. In all experiments, cell numbers returned to pretransport/storage concentration within 24–48 hours recovery. Imaging data indicated that HepG2 cells were fully adherent and had established typical growth morphology following 48 hours recovery, which was not seen in cells recovered from cryopreservation. Following recovery, Jurkat cells that had been subjected to a 96 hours transport/storage period, demonstrated a 1.93-fold increase compared with the starting cell number with >95% cell viability. We conclude that CellShip® may represent a viable method for the transportation of mammalian cells for multiple downstream applications in the Life Sciences research sector.

Keywords: CellShip, mammalian cell culture, cell transportation, cell recovery, cell viability, cryopreservation

Introduction

Despite tremendous advances in cell culture techniques, and their applications, from elucidating pathogenic mechanisms, pharmaceutical development, toxicological evaluation, and the development of cell-based therapies, the standard method for transporting cell cultures remains largely unchanged.

The most frequently used techniques are to either cryopreserve the cells, using a cryoprotective agent (CPA) and ship the frozen samples using dry ice or a dry shipping device, or to ship cells as growing cultures in sealed cell culture vessels. Both methods present logistical and biological issues for the sender and the receiver.

Shipping growing cultures in sealed vessels is a method used by cell manufacturers as an alternative to shipping cryopreserved cells (https://www.atcc.org/support/technical-support/faqs/preparing-and-shipping-flasks-of-cell-cultures). However, it is only recommended for periods of up to 24 hours as cell viability is not well maintained,[1] which can present scheduling issues. Cells often become detached from the culture vessel when being shipped using this method, which can lead to anoikis, a type of apoptosis triggered when adherent cells lose contact with the extracellular

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matrix.[2] There are additional challenges, such as the loss of vessel integrity through physical stress, or not being thoroughly sealed, which risks the introduction of contamination or can cause leaking of a biological sample during transport.

Cryopreservation utilizes a CPA which acts to modify the transitional changes that occur during the freezing process, protecting cells from the formation of ice crystals.[3] One of the most effective and widely used CPAs is dimethyl sulfoxide (DMSO), which was initially reported in 1959 for the preservation of human and bovine red blood cells and bull spermatozoa,[4] the efficacy of DMSO as a CPA for cell cultures was demonstrated a few years later.[5]

The process of cryopreserving cells for transport, or long-term storage, must be carefully controlled and requires cells to be slowly frozen at ~1°C/min to −80°C using a controlled rate cryofreezing container, before being shipped on dry ice or transferred to liquid nitrogen for storage. Cell recovery from cryopreservation is equally critical, requiring the cells to be thawed quickly to avoid the formation of ice crystals, followed by removal or dilution of the DMSO. This is further complicated by the fact that the initial dilution of the cryomedia needs to occur slowly to avoid irreversible cell damage by osmotic shock.[6]

Additionally, cryopreservation is difficult to standardize, partly due to the requirements of different cell types, downstream applications, and specialist equipment, such as liquid nitrogen dewars/dry shippers, cryogenic vials/boxes, and temperature-monitoring devices that are necessary. This makes it an issue for manufacturing and production scalability, which demands meticulous risk management and is an additional experimental variable to consider for researchers. Looking further ahead to additional applications, the thawing of cell-based therapies (CBTs) before infusion is considered a key risk factor in the manufacturing process,[7] therefore the development of viable, alternative approaches are desirable.

Despite its efficacy and widespread use as a CPA, DMSO is cytotoxic and prolonged exposure is associated with cellular changes.[8,9] For example, research assessing the effect of DMSO as a cryoprotectant revealed increased expression of the proapoptotic, genes BAX and BAD, with downregulation of the antiapoptotic gene BCL-2 following exposure to DMSO[10] and a recent study revealed extensive changes to microRNAs and the epigenetic landscape in hepatic and cardiac cells when exposed to just 0.1% DMSO.[11] Cryopreservation has also been shown to have varying effects on the potency of different T cell populations, which has the potential to influence patient outcomes in the chimeric antigen receptor T cell therapy (CAR-T) arena.[12]

Cryopreservation-induced cell death occurs in many cell types and is observed from a few hours to several days following recovery; it is also referred to as Cryopreservation-Induced, Delayed-Onset Cell Death (CIDOCD).[13]

This preliminary study demonstrates the successful ambient transport, short-term storage, and preservation of mammalian cell lines using a novel medium, CellShip® (Fig. 1). ◀F1 Total cell counts, and high cell viability were maintained for up to 96 hours during transportation. Initial assessment using Jurkat and HepG2 cells showed rapid cellular recovery posttransportation in CellShip when compared with standard

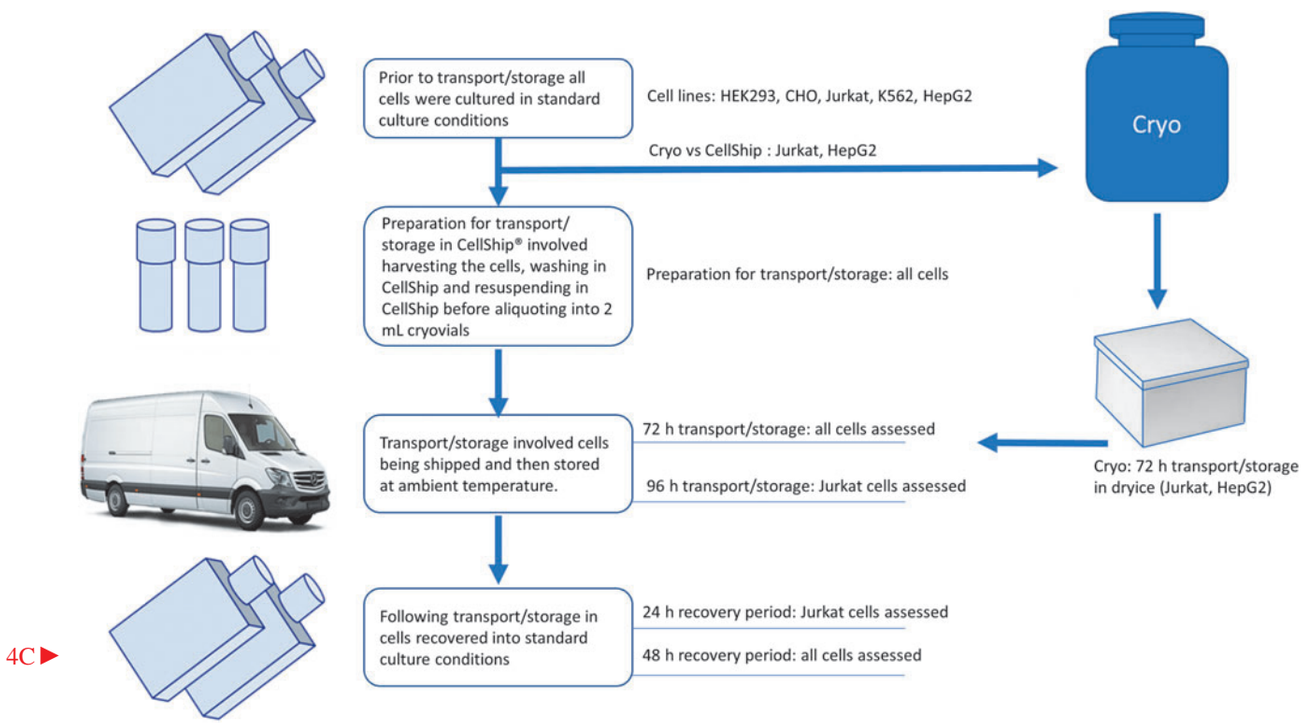

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FIG. 1. Overview of the experimental process. All cell lines were assessed using a standard protocol (72 hours transport/storage and a 48 hours recovery period). Jurkat cells were then assessed for 96 hours transport/storage and 24 hours recovery. Jurkat and HepG2 cells in CellShip® were compared with cryopreserved samples.

cryopreservation transportation methods, related to increased metabolic activity and proliferation. This would be of benefit to both academic and commercial areas of the Life Sciences sector, where either the use of cryopreservation is undesirable or there is a need for a more rapid recovery posttransportation.

Materials and Methods

Cell culture and recovery conditions

T1 ▶ Cells obtained from ATCC (Table 1) were cultured at 37°C in a humidified environment at 5% carbon dioxide. Human embryonic kidney (HEK293) and human hepatocellular carcinoma (HepG2) cells were cultured in Minimum AU7 ▶ Essential Medium, 1% anti-anti, 4 mM L-Glutamine, 1% nonessential amino acids (Gibco, Thermo Fisher Scientific, UK), and 10% fetal bovine serum (FBS; Life Science Group, UK). Chinese hamster ovary (CHO) cells were cultured in AU8 ▶ Ham's F-12K (Kaighn's) medium, 1% anti-anti, 4 mM L-Glutamine (Gibco, Thermo Fisher Scientific), and 10% FBS (Life Science Group); human immortalized myelogenous leukemia (K562) and human T lymphocytes (Jurkat) were cultured in Roswell Park Memorial Institute (RPMI), AU9 ▶ 1% anti-anti, 4 mM L-Glutamine, sodium pyruvate (Gibco, Thermo Fisher Scientific), and 10% FBS (Life Science Group). All cell lines were cultured in T25 vented culture flasks (Nunc, Thermo Fisher Scientific). Cell samples used for transportation/storage in CellShip (Life Science Group Ltd) and cryopreservation were from the same stocks and within a single passage number, with the exception of the AU0 ▶ alamarBlue® (Thermo Fisher Scientific) experiment.

Cell count and viability

Cell counts and viability were assessed a minimum of five times per sample, using a CytoSmart™ automated cell counter, Corning (https://cytosmart.com/prod-ucts/corning-cell-counter), disposable cell counting slides (Immune Systems), and (0.4%) Trypan Blue exclusion. Fold change in cell numbers was calculated before transport/storage (0 hours), immediately following transport/storage in CellShip (72 or 96 hours) by dividing the original number of cells in the cryovial by the number of cells following transport/storage. The final fold change in cell number was calculated after the recovery period (24 or 48 hours) by dividing the final number of cells by the original number of cells before transport/storage (0 hours). This calculation took into consideration the dilution factor, as a proportion of cells were plated for recovery.

Transportation and storage in CellShip

HEK293 ($n=7$), HepG2 ($n=11$), CHO ($n=5$), K562 ($n=3$), and Jurkat cells ($n=15$) were shipped at ambient temperature over a period of 72 or 96 hours. Samples were taken for cell count and viability (as described) pretransport/storage, posttransport/storage.

Cultured cells were prepared immediately before transportation. Suspension cell lines (K562, Jurkat) were harvested directly, while adherent cell lines (HEK293, HepG2, CHO) were washed with Versene solution (Gibco, Thermo Fisher Scientific) and dissociated using TrypLE (Gibco, Thermo Fisher Scientific). Cell suspensions were pelleted at 180 *g*, washed with 3 mL CellShip, and suspended in a final volume of 2 mL CellShip. The 2 mL CellShip suspension was contained in a 2 mL Nalgene cryovial (Thermo Fisher Scientific) and packaged securely in a polystyrene transport container. Internal package temperature during transportation was recorded using a TinyTag (Gemini Data Loggers, UK) placed directly adjacent to the sample vials. Transportation was performed by commercial courier service (FedEx, UK) or by car for a minimum distance of 70 miles (112 km).

Storage and transportation of cryopreserved cells

Jurkat ($n=6$) and HepG2 ($n=3$) cells were harvested as previously described, pelleted at 180 *g* for 5 min and resuspended in 1 mL ice-cold FBS (Life Science Group) +10% DMSO (Thermo Fisher Scientific). Cell suspensions were immediately stored in 2 mL Nalgene cryovial (Thermo Fisher Scientific), frozen overnight at −80°C using a CoolCell® alcohol-free cell freezing container (BioScision, UK) and then transferred to liquid nitrogen for storage.

For transportation, cryopreserved cells were removed from liquid nitrogen and immediately placed in a polystyrene container containing 9 kg of dry ice. Transportation was performed by commercial courier service (FedEx) or by car for a minimum distance of 70 miles (112 km). To assess cell counts and viability, samples were taken (as described) pretransportation/storage and posttransportation/storage.

Table 1. Information for the Cell Lines and the Rationale for Use

Cell line/ designation	*Species*	*Characteristics*	*Rationale for use*	*ATCC reference*
Jurkat Clone E6–1	Human (*Homo sapiens*)	Suspension, acute T cell leukemia lymphoblast	Used in clinical development of CAR-T therapies and as a T cell model in clinical development[14–17]	TIB-152
HEK-293 293	Human (*H. sapiens*)	Adherent, embryonic kidney, epithelial	Used in biomanufacturing[18–21]	CRL-1573
Hep-G2	Human (*H. sapiens*)	Adherent, hepatocellular carcinoma, epithelial-like	Drug development and toxicity testing[22–24]	HB-8065
CHO CHO-K1	Chinese hamster (Cricetulus griseus)	Adherent, ovary, epithelial-like	Used in biomanufacturing[18,20,21,25–27]	CCL-61
K562 K562-r	Human (*H. sapiens*)	Suspension, chronic myeloid leukemia, hematopoietic	Biomedical research and cytotoxicity assays[23,28]	CRL-3344

Posttransportation recovery and sampling

Posttransportation/storage CellShip suspensions were transferred directly into the appropriate complete growth media for recovery. Cryopreserved cells were removed from dry-ice and thawed at 37°C (<2 minutes). Thawed cells were resuspended through the dropwise addition of 1 mL prewarmed complete growth media, followed by further gentle addition up to 5 mL. Cells were pelleted at 180 *g* for 5 minutes and resuspended in prewarmed complete growth media for recovery. Posttransportation/storage samples were assessed immediately following transport/storage and samples to assess recovery were taken at 24 and 48 hours.

Posttransportation metabolic activity

Metabolic (mitochondrial) activity prestorage/transportation and following 72 hours transportation/storage was determined for HEK293 ($n=3$), HepG2 ($n=3$), CHO ($n=3$), K562 ($n=3$), and Jurkat ($n=3$) cells by alamarBlue® (AB) reduction assay (Thermo Fisher Scientific). For each cell line 100 µL of 1×10^4 cells/mL were seeded in triplicate in 96-well culture plate. AB reagent was added after 1 hour incubation and absorbance was measured 570 and 600 nm at 6 hours incubation. Apoptosis was induced with 10 mg/mL of Actinomycin-D (Merck, UK) as positive control, and cell-free medium was used as a negative control.

Morphological analysis of HepG2 cells

Morphological analysis was performed on HepG2 cells ($n=3$) by phase-contrast microscopy using a Cytation 5 Cell Imagining Multi-mode reader (BioTek, UK) at 48 hours posttransportation/recovery for both CellShip and cryopreserved cells. Characteristics examined included overall cell adherence and growth morphology.

Statistical analysis

Fold-change in total cell count was calculated at each sample point relative to the starting total cell count (pretransportation/storage). A dilution factor was applied to samples postrecovery to correct for initial flask inoculation volume. Fold-change and viability at respective sample points were compared using a two-way repeated measures analysis of variance (ANOVA; or mixed model) following confirmation of homoscedasticity by Levene's test. Comparison of transport/storage mechanism, that is, ambient CellShip versus dry ice, was subsequently assessed by Sidak's multiple comparisons testing using GraphPad Prism version 8.4.3 for Windows (GraphPad Software, San Diego, CA, USA; www.graphpad.com).

Results

Storage and transportation in CellShip

Efficacy of CellShip transportation and storage was assessed over a 72-hour period as this was deemed a sufficient period, even for international transport. Five commercially relevant cell lines were assessed; CHO, HEK293, HepG2, Jurkat, and K562 cells (see Table 1 for rationale). Temperatures during transportation ranged between 5.4°C and 27.4°C, with a maximum temperature range of 15°C in a single experiment (Supplementary Table S1). No significant correlation (Pearson's correlation; $p<0.01$) was observed between the minimum, maximum, or temperature range recorded during transport and the viability at 72 hours transport and storage, following 48 hours recovery or in the mean-fold change during recovery. Mean total cell count ($\times10^6$ cells/mL), viability (%), and fold-change were calculated (Table 2).

CHO cells ($n=5$; Fig. 2A) starting mean cell count was 3.80 ± 0.76 (cell viability 98.02%). At initial recovery following 72 hours transport/storage, there was a nonsignificant mean fold change of 0.97 (cell viability 97.44%). Following 48 hours recovery, cell count had increased with a mean fold-change of 6.56 (cell viability 99.28%; $p<0.01$).

HEK293 cells ($n=7$; Fig. 2B) starting mean cell count was 6.38 ± 2.30 (cell viability 93.67%). At initial recovery following 72 hours of transport and storage, there was a nonsignificant mean fold change of 0.92 (cell viability 88.16%). Following 24 hours recovery and 48 hours recovery cultures had significant mean fold-changes of 1.53 (cell viability 76.45%) and 2.11 (cell viability 83.55%), respectively ($p<0.05$).

HepG2 cells ($n=11$; Fig. 2C) starting mean cell count was 6.04 ± 5.33 (cell viability 96.91%). At initial recovery following 72 hours transport and storage, there was a nonsignificant mean fold change of 1.12 (cell viability 96.19%). Following 48 hours recovery culture, cell count had increased with a mean fold-change of 2.92 (cell viability 96.40%; $p<0.001$).

Jurkat cells ($n=15$; Fig. 2D) starting mean cell count was 6.97 ± 1.44 (cell viability 90.89%). A significant decrease in mean fold change of 0.84 (cell viability 86.71%) was observed at initial recovery following 72 hours transport and storage. Twenty-four hours recovery and 48 hours recovery culture had significant mean fold-changes of 1.11 (cell viability 87.92%) and 2.13 (cell viability 83.31%), respectively ($p<0.001$).

K562 cells ($n=3$; Fig. 2E) starting mean cell count was 7.58 ± 1.63 (cell viability 98.13%). At initial recovery following 72 hours transport and storage, there was a nonsignificant mean fold change of 1.30 (cell viability 97.80%). Following 48 hours recovery culture, cell count had increased with a mean fold-change of 4.76 (cell viability 98.60%; $p<0.05$).

Further assessment of CellShip efficacy was performed for transportation and storage over a 96-hour period using Jurkat cells. No significant change in cell viability was observed at any stage posttransportation with mean viability of 96.37%, 94%, 84.65%, and 93.27% pretransport, posttransport (96 hours), 24 hours recovery, and 48 hours recovery, respectively. A fold-change of 0.76 in cell count showed a significant decrease immediately posttransportation. No significance was observed in fold-change after 24 hours recovery, and a significant increase in fold change to 1.93 at 48 hours recovery (Table 3, Fig. 3).

Comparative recovery of Jurkat cells following transportation using CellShip or cryopreservation

Mean total cell count ($\times10^6$ cells/mL), viability (%), and fold-change were calculated to compare transport/storage of Jurkat cells ($n=3$) in CellShip and cryopreserved samples in dry ice. Before transport/storage, mean cell counts were 6.60 (±0.31) and 8.42 (±1.74)$\times10^6$ cells/mL for CellShip

TABLE 2. MEAN TOTAL CELL COUNT ($\times 10^6$ CELLS/ML) AND VIABILITY (%) PRE- AND POSTTRANSPORTATION FOR 72 HOURS IN CELLSHIP AND FOLLOWING 24 AND 48 HOURS RECOVERY CULTURE FOR CHO, HEK293, HEPG2, JURKAT, AND K562

	Pretransportation/storage		*Posttransportation/storage (72 hours)*			*24 hours recovery (posttransport/storage)*			*48 hours recovery (post-transport/storage)*		
Cell line	*Mean cell count (±SD)*	*Mean viability (%)*	*Mean cell count (±SD)*	*Mean viability (%)*	*Mean fold-change*	*Mean cell count (±SD)*	*Mean viability (%)*	*Mean fold-change*	*Mean cell count (±SD)*	*Mean viability (%)*	*Mean fold-change*
CHO ($n=5$)	3.80 (±0.76)	98.02	3.64 (±0.61)	97.44	0.97				24.40 (±3.64)	99.28	6.56
HEK293 ($n=7$)	6.38 (±2.30)	93.67	5.67 (±2.11)	88.16	0.92	5.24 (±1.57)	76.45	1.53	11.61 (±5.92)	83.55	2.11
HepG2 ($n=11$)	6.04 (±5.33)	96.91	5.29 (±4.39)	96.19	1.12				17.93 (±17.56)	96.40	2.92
Jurkat ($n=15$)	6.97 (±1.44)	90.89	5.84 (±1.62)	86.71	0.84	7.50 (±0.90)	87.92	1.11	14.61 (±5.02)	83.31	2.13
K562 ($n=3$)	7.58 (±1.63)	98.13	10.20 (±4.43)	97.80	1.30				35.24 (±2.70)	98.60	4.76

SD, standard deviation.

and cryopreservation experiments, respectively, a statistically nonsignificant difference in cell density. Pre- and posttransport/storage viability and total cell fold-change showed no significant difference between the two shipment methods. Following recovery at 24 and 48 hours recovery periods in standard culture conditions, viability of cells transported in CellShip remained stable, while viability decreased significantly for cryopreserved cells following a recovery period of 24 and 48 hours to 40.43% and 65.89%, respectively. This shows a significant difference between the two transport/storage methods (Table 4, Fig. 4A). Fold-change in cell numbers at 24 and 48 hours recovery for CellShip showed significant increases of 1.11 and 1.97, respectively. ◀T4 ◀F4

Cells transported through cryopreservation decreased at 24 and 48 hours compared with pretransportation levels to 0.46 and 0.51, respectively: significantly different between the two media (Table 4, Fig. 4B).

Increased metabolic activity and proliferation, following transportation, using CellShip versus cryopreservation

AlamarBlue™ reduction (%) was used as an indicator of metabolic activity corresponding to cell proliferation post 72 hours transport/storage. Mean AB reduction over 6 hours immediately following recovery for CellShip and cryopreserved cells were significantly different for Jurkat cells 37% versus 27% ($p \leq 0.001$), HEK cells 72% versus 50% ($p \leq 0.0001$), and HepG2 cells 80% versus 58% ($p \leq 0.0001$). A nonsignificant increase was observed for CellShip in K562 cells (44% vs. 43%) and CHO cells (37% vs. 32%; Fig. 5). ◀F5

Morphological analysis of HepG2 cells in CellShip versus cryopreservation

Morphology of HepG2 cells was observed at 48 hours recovery following 72 hours transport/storage either in CellShip or cryopreserved. Cells transported at ambient temperature in CellShip showed full adhesion and typical ''island'' growth morphology. Those transported through cryopreservation at the same period were not fully adherent and had not fully reached the classical growth morphology (Fig. 6). ◀F6

Discussion

The aim of this study was to assess the efficacy of CellShip for maintaining cell numbers and viability over a 72-hour period at ambient, including a period of commercial transport. Five commercially relevant cell lines were tested (Table 1).

Cell culture is an increasingly used technique both commercially and for research purposes. Biopharmaceuticals produced using mammalian cell cultures are widely used to treat diseases such as autoimmune disorders, hematologic disorders, and hormonal dysregulation.[18] In terms of economic impact, the production of approved biopharmaceuticals, using mammalian cell lines as opposed to nonmammalian-based production increased from 33% in 1989 to 79% in 2018 and is worth billions of dollars to the Life Science industry.[20] Despite its widespread use, methods for transporting cells remain largely unchanged, with cryopreservation being the most popular.

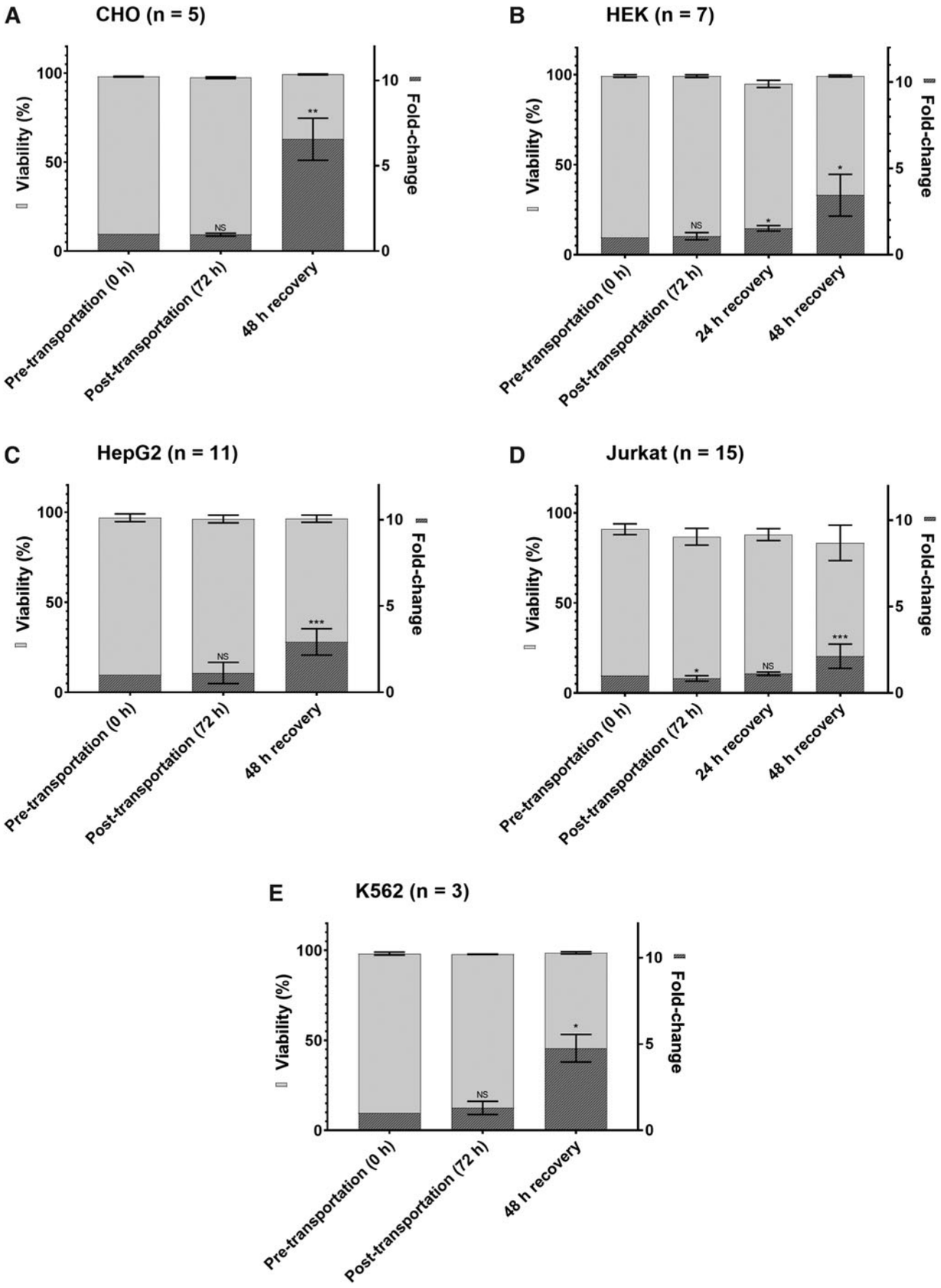

FIG. 2. Mean (±SD) for viability (%) and total cell count fold change pre- and posttransportation in CellShip and following 24 or 48 hours recovery culture for **(A)** CHO, **(B)** HEK293, **(C)** HepG2, **(D)** Jurkat, and **(E)** K562 cell lines. (ns, not significant; $*p < 0.05$; $**p < 0.01$; $***p < 0.001$). SD, standard deviation.

TABLE 3. MEAN TOTAL CELL COUNT ($\times 10^6$ CELLS/ML) AND VIABILITY (%) PRE- AND POSTTRANSPORTATION FOR 96 HOURS IN CELLSHIP AND FOLLOWING 24 AND 48 HOURS RECOVERY CULTURE FOR JURKAT CELLS

	Pretransportation/storage		*Posttransportation/storage (72 hours)*			*24 hours recovery (posttransport/storage)*			*48 hours recovery (post-transport/storage)*		
Cell line	*Mean cell count (±SD)*	*Mean viability (%)*	*Mean cell count (±SD)*	*Mean viability (%)*	*Mean fold-change*	*Mean cell count (±SD)*	*Mean viability (%)*	*Mean fold-change*	*Mean cell count (±SD)*	*Mean viability (%)*	*Mean fold-change*
Jurkat ($n=3$)	7.15 (±0.75)	96.37	5.46 (± 0.64)	94.00	0.76	6.32 (±0.67)	84.65	0.89	13.83 (±2.36)	93.27	1.93

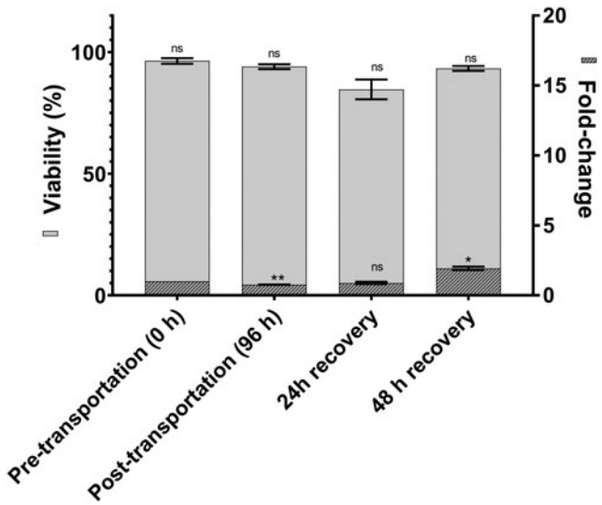

FIG. 3. Mean (±SD) for viability (%) and total cell count fold change pre- and posttransport/storage (96 hours) and following 24 or 48 hours recovery culture for Jurkat cells ($n=3$) (ns, not significant; $*p<0.05$; $**p<0.01$; $***p<0.001$).

Cryopreservation has been demonstrated to be reliable and suitable for many applications, although some more sensitive cells can demonstrate impaired recovery.[29,30] In addition, there is evidence that suboptimal cryopreservation can lead to lowered cellular functionality and reduced cell yield, but also to the potential selection of subpopulations with genetic or epigenetic characteristics divergent from the original cell line.[12,31,32]

In this study, a novel ambient temperature storage medium CellShip was demonstrated to maintain high total cell counts and high cell viability were maintained for up to 96 hours during transportation. Furthermore, across five commonly used cell lines (HEK293, CHO, HepG2, K562, and Jurkat) posttransportation recovery showed increased cell proliferation resulting in higher fold-changes in cell number compared with cells recovered from cryopreservation (Fig. 2).

Jurkat cells were used to assess whether cell numbers and viability would be comparable, either following recovery from cryopreservation or recovery from CellShip. Jurkat cells were chosen as a model used in the development of CAR-T therapies (Table 1), which in some cases need to be delivered fresh.[33] Our data demonstrates that although viability was comparable immediately following recovery, cell viability dropped to ~40% following 24 hours recovery from cryopreservation, a sign of the recognized phenomenon; cryopreservation-induced delayed onset cell death (CIDOCD).[13] A similar observation was published recently, which showed good cell viability upon recovery from cryopreservation, followed by a dramatic reduction in viability at 24 hours postrecovery.[34] Cells transported in CellShip showed 80% viability at the same 24-hour time point, and this was consistent at 48 hours. These significant differences in the comparison study were also reflected in the cell numbers at both 24 and 48 hours (Fig. 3B). Metabolic activity and proliferation, assessed through AB reduction, was increased posttransportation in cells stored in CellShip, which correlates with the subsequent increased viability and fold-change in cell number. ◀AU0

TABLE 4. MEAN TOTAL CELL COUNT ($\times 10^6$ CELLS/ML) AND VIABILITY (%) PRE- AND POSTTRANSPORTATION FOR 72 HOURS IN CELLSHIP OR IN DRY ICE, AND FOLLOWING 24 AND 48 HOURS RECOVERY FOLLOWING A RETURN TO STANDARD CULTURE CONDITIONS FOR JURKAT CELLS ($N = 3–6$)

	Pretransportation/storage		*Posttransportation/storage (72 hours)*			*24 hours recovery (posttransport/storage)*			*48 hours recovery (posttransport /storage)*		
Transportation/ storage method	*Mean cell count (±SD)*	*Mean viability (%)*	*Mean cell count (± SD)*	*Mean viability (%)*	*Mean fold-change*	*Mean cell count (± SD)*	*Mean viability (%)*	*Mean fold-change*	*Mean cell count (± SD)*	*Mean viability (%)*	*Mean fold-change*
Ambient temperature (CellShip®)	6.60 (±0.31)	92.98	5.19 (±0.94)	87.67	0.79	7.50 (±0.90)	87.92[a]	1.11[b]	13.09 (± 4.56)	90.43	1.97[b]
Dry ice cryopreservation	8.42 (±1.74)	94.60	7.76 (±2.11)	78.95	0.92	3.11 (±0.46)	40.43	0.46	4.24 (±0.86)	65.89	0.51

[a] $p < 0.05$.
[b] $p < 0.001$.

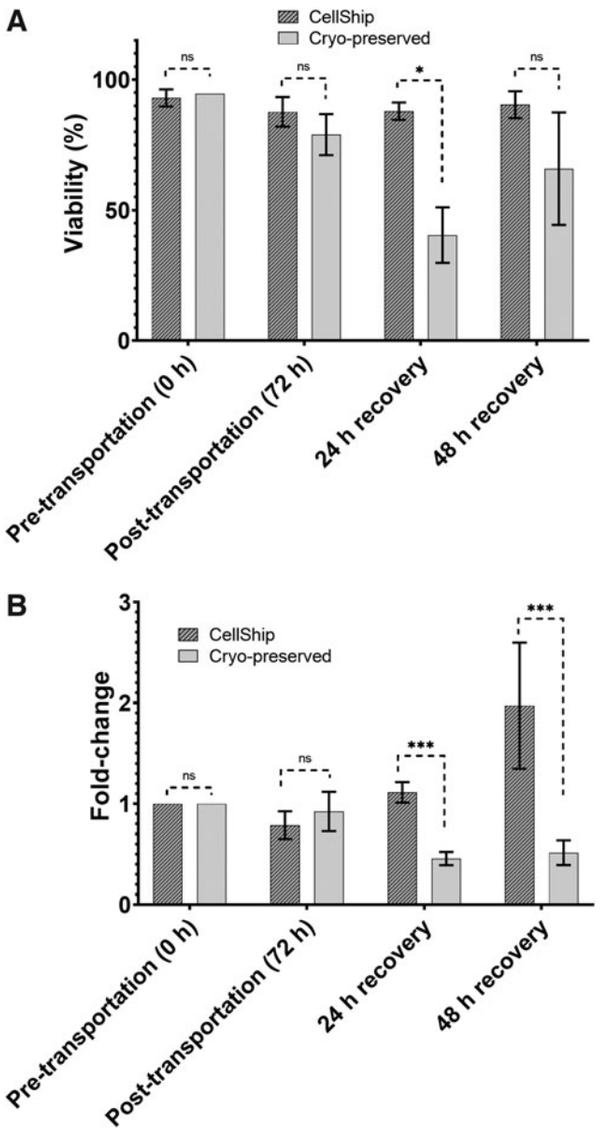

FIG. 4. Comparison study showing Jurkat cell viability **(A)** and fold change in viable cell count **(B)** following 72 hours transport/storage in dry ice following storage in liquid nitrogen or in CellShip at ambient temperature following culture in standard conditions. **(A)** and **(B)** show mean ± standard deviation. Statistical analysis was performed using a two-way repeated measures ANOVA or mixed model, $*p < 0.05$; $***p < 0.001$ ($n = 3–6$). ANOVA, analysis of variance.

With such high cell survivability when using CellShip it is less likely to generate cell selection bias during transportation compared with traditional cryopreservation.

To observe whether cells recovered from CellShip following transport displayed morphological differences to cells recovered from cryopreservation, we used HepG2 cells, which display quite a distinct morphology. Following a 48-hour recovery period, cells recovered from CellShip were fully adherent and had established typical growth characteristics (Fig. 5). The cells recovered from cryopreservation, although recovering as expected, had not yet reestablished their typical growth characteristics at 48 hours postrecovery.

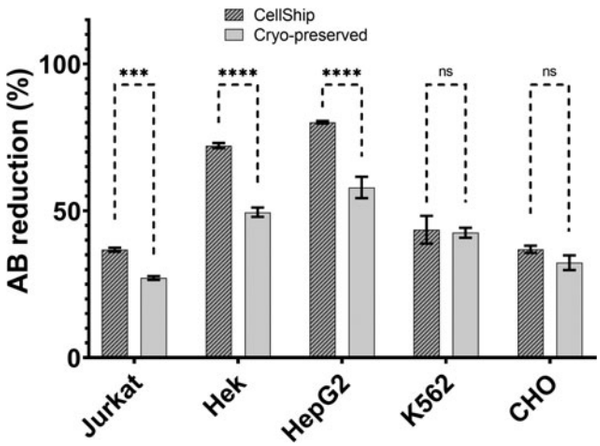

FIG. 5. alamarBlue™ reduction (%) as an indicator of cellular metabolic activity and proliferation demonstrating significantly higher activity in Jurkat, HEK, and HepG2, following 72 hours transport/storage in CellShip at ambient temperature compared with cryopreserved cells.

This suggests that following transportation/storage at ambient in CellShip, the cells recovered more quickly, re-establishing ''normal'' morphology.[35]

It is not possible from the current experiments to determine the cause of the differences for both the cell viability and cell numbers between the CellShip and cryopreserved cells. The increased metabolic activity observed in cells recovered from CellShip is likely to aid recovery. The CellShip formulation contains a buffering system capable of maintaining the pH over a wide range of temperatures and is reported to sustain cells at a reduced metabolic rate as observed at subnormothermic temperatures, each contributing to the increased metabolic activity at recovery.[36–38] All aspects of the application of low temperatures; the cryoprotectant (DMSO in this case), the cooling and thawing rate adopted could be contributing to the deleterious effects of cryopreservation in the current study (as described by Hunt[32]). However, storage vials, the mode, and duration of transportation were standardized within experiments and so cannot explain the observed differences in this study, and although the initial cell densities show some variation (Table 4), the difference was not statistically significant (Fig. 4A, B). CellShip contains a nontoxic additive designed to protect the cells during transportation against shear stress, and to help maintain membrane integrity. ◀AU0

The concentration of this component has been optimized during the development phase. Furthermore, completely filling the transport vessel minimizes the headspace reducing mechanical interfacial stress at the air–liquid interface. Decreased head space also reduces oxygen availability, with oxidative stress related to increased oxygen availability being a crucial factor in subnormothermic cell transport.[39]

Currently there does not appear to be a ''gold standard'' method for transporting cells at ambient temperature, and although there are products available, they are typically gel

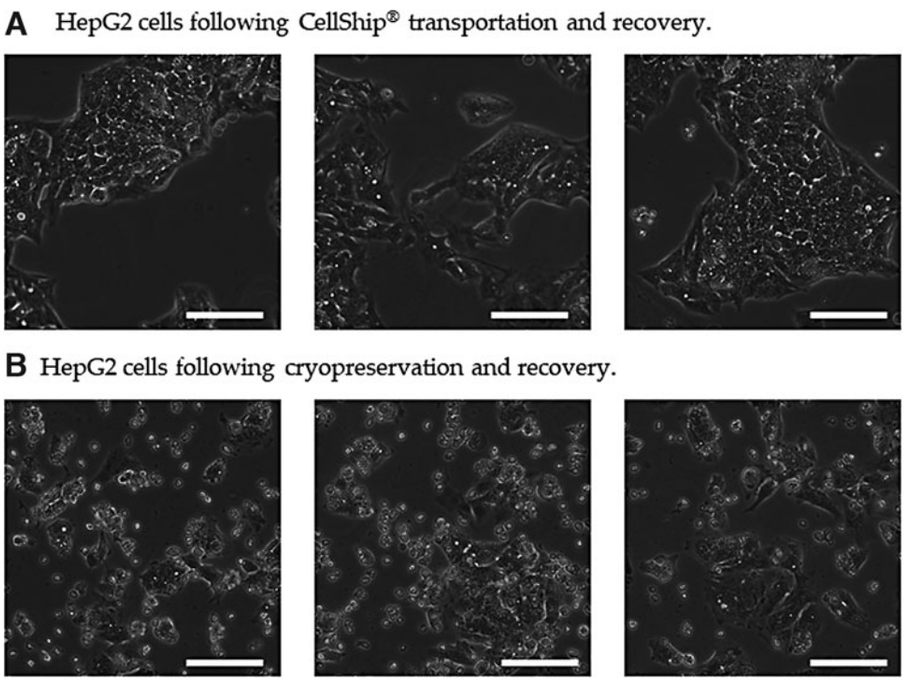

FIG. 6. HepG2 cell morphology following 48 hours recovery (dry ice vs. CellShip). The *upper row* **(A)** shows representative images of cells recovered from transport/storage in CellShip after 72 hours and cultured using standard culture conditions for 48 hours. Almost complete cell adherence had occurred by 48 hours and typical HepG2 growth morphology was well established. **(B)** Shows representative images of cryopreserved cells recovered from transport/storage in dry ice and cultured using standard culture conditions for 48 hours. Cell adherence was observed, with nonadherent cells persisting, and although cells had started to display typical HepG2 growth morphology it was not well established at 48 hours. Scale bars 100 μm.

based. Gel-based products require additional processing, for example, BeadReady™ (Atelerix), involves several steps to encapsulate the cells and several steps to release the cells, for recovery. Matrigel (Corning Ltd) is a well-known product and can be used for cell transport,[1] but it is not xeno free and also requires several processing steps to encapsulate and release the cells. CellShip has been developed with ease of use in mind, requiring a minimal number of processing steps. To our knowledge, this is the first commercially available solution that requires such minimal cell manipulation for shipping at ambient temperature.

These current data demonstrate a new and viable method of transporting cells, and with a current retail price of £90.00/100 mL CellShip provides a cost-effective and simple alternative to cryopreservation (using dry-ice) for national and international transportation. The 96-hour transportation/storage experiment demonstrates a potential use for CellShip in CBT applications where cryopreservation may be undesirable.[16,40,41]

Further application development projects are now planned, which will provide a more comprehensive and mechanistic set of data, with our immediate focus being the transport and short-term storage of primary cell lines and stem cells (including human mesenchymal stem cells). These experiments will investigate gene expression, CD markers, and differentiation potential. We will also be assessing protein production in engineered cells before and after ambient transport in CellShip compared with cells recovered from cryopreservation, which may be relevant to the biomanufacturing industry.

Conclusions

In summary, using CellShip demonstrated that cellular counts were maintained with high cellular viability following ambient temperature transportation and storage (up to 96 hours).

The use of ambient temperature transit avoids the need for expensive transport procedures, cryopreservation procedures, and cryoprotectants, avoiding potential CIDOCD, and ensuring rapid cellular recovery posttransportation. This may provide a viable solution for cell transport, overcoming economic and logistical challenges that can be prohibitive to both research and medical sciences.

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Authors' Contributions

E.B., S.F., and D.R. contributed to conception and design of study. E.B. organized and performed experiments in collaboration with J.D. and M.T. A.A., P.B.A., and P.D. performed the AB experiments. L.K. provided technical support. Statistical analysis was performed by A.M. E.B. wrote the draft of the article. All authors contributed to the article revision and read and approved the submitted version.

Author Disclosure Statement

E.B. was the Knowledge Transfer Partnership (KTP) Associate employed to develop the product in collaboration with Coventry University and Life Science Group. J.M. is the Managing Director of Life Science Group. Other authors declare that they have no conflicts of interest.

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Supplementary Material

Supplementary Table S1

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