ATELERIX
SAVE YOUR CELLS

Solutions for hypothermic cell, tissue, and virus preservation

Data Pack !!!

CONTENTS

This data pack references the preservation capabilities of the following products:

BLOODREADY™ LEUKOSTOR™ TISSUEREADY PLUS™ CYTOSTOR™ WELLREADY™ BEADREADY™

All products have been developed by Atelerix Ltd and are commercially available. Further information about the product line can be found in the product brochure and on the website.

PRESERVING STROMAL FIBROBLASTS DURING HYPOTHERMIC STORAGE

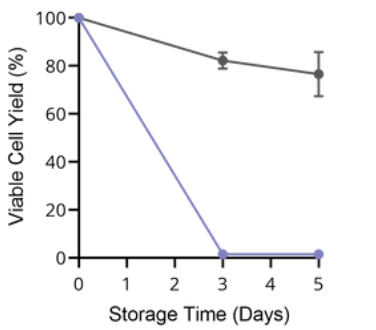
Limbal Stromal MSCs

Stromal fibroblasts are found in almost every organ in the body and are associated with connective tissue maintenance. Whilst this is a key function, the properties that most interest researchers and drug developers are their capacity to secrete vast amounts of soluble factors that promote growth, modulate immune cells and control wound healing and repair. This is particularly the case with Mesenchymal Stromal Cells (MSCs) which have huge therapeutic interest.

Promoting corneal wound healing & reversing scarring

Damage to the cornea (the outermost part of the eye) through trauma or infection can result in sight loss and even blindness. Since 2018, Atelerix has been collaborating with L V Prasad Eye Institute (LVPEI) in India to increase the accessibility of a new cell therapy using Limbal Stromal MSCs (LS-MSCs) from cadaveric eyes. This is through enabling distribution from the site of manufacture to sites of administration throughout India. The figure below demonstrates how this has been taken from initial Proof of Concept (a), to pre-clinical studies (b), and finally to clinical studies (c) with robust demonstration of wound-healing function following storage.

ai



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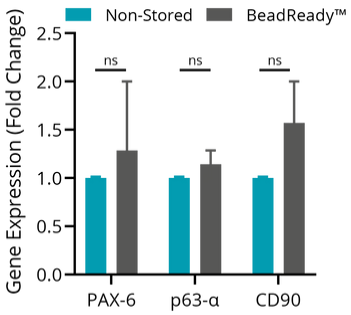

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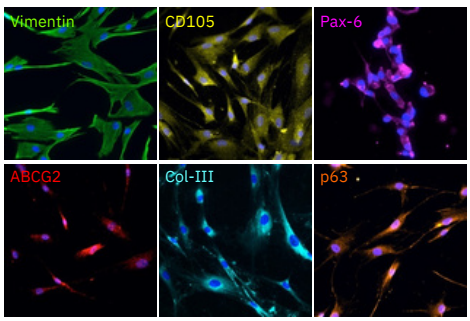

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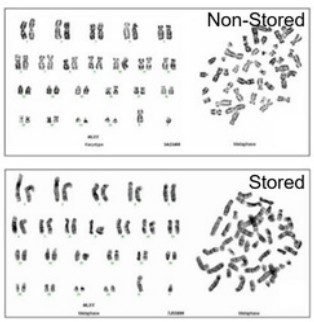

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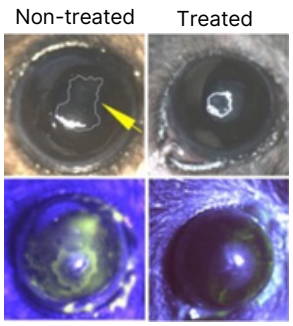

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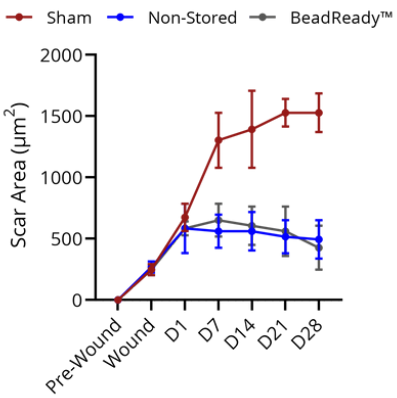

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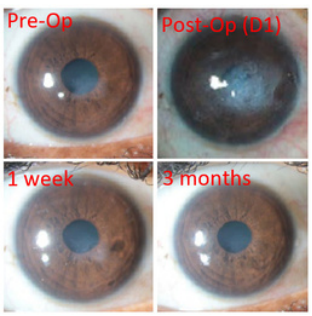

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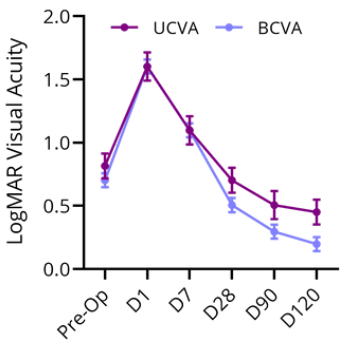


***BeadReady™-preserved LS-MSCs – in vitro, in vivo and first-in-man**. LSMSCs stored at room temperature for 3-5 days had a high viable cell recovery, and a normal genotype, phenotype and karyotype. Pre-clinical studies demonstrated no loss in their ability to reverse and prevent corneal scarring in mouse models and clinical studies demonstrated the ability for stored cells to improve visual acuity in patients with superficial corneal pathologies. **ai**: Viable cell recovery of LS-MSCs stored in medium (purple) or in BeadReady™ (grey) after up to 5 days' storage at room temperature (n=3). **aii**: Stabilisation of LS-MSC gene expression by BeadReady™ after 3 days' storage as assessed by qPCR (n=3). **aiii:** Stabilisation of LS-MSC phenotypic markers by BeadReady™ after 3 days' storage as assessed by immunofluorescent staining. **aiv:** Stabilisation of LS-MSC karyotype by BeadReady™ after 3 days' storage. **bi**: Reversal of scarring by LS-MSCs stored in BeadReady™ for 3-5 days in a corneal wound mouse model. Reduction in scar area (top) and promotion of re-epithelialisation as assessed by fluorescein staining (bottom) are shown. **bii**: Prevention of scar formation by LS-MSCs stored in BeadReady™ for 3-5 days when applied prophylactically in a corneal wound mouse model (n=8). **ci**: Reversal of scarring in a representative patient treated with BeadReady™-stored LS-MSCs. **cii**: Recovery of uncorrected and best corrected visual acuity (UCVA and BCVA respectively) in a cohort of 20 patients. Initial follow-up data is shown 3.5-12 months post-treatment with 14 out of 20 patients having significant improvement in vision. LogMAR 0.0 corresponds to 20/20 vision. **ai-aiii**: Data adapted from Damala, M. et al.Sci Rep9,16950 (2019). **aiv**, **bi-bii:** Data adapted from Damala, M. et al.Cells,12, 876 (2023). ci-cii: Data adapted from Basu, S. and Singh, V. Invest. Opthalmol. Vis. Sci.,64, 2360 (2023) [conference poster].*

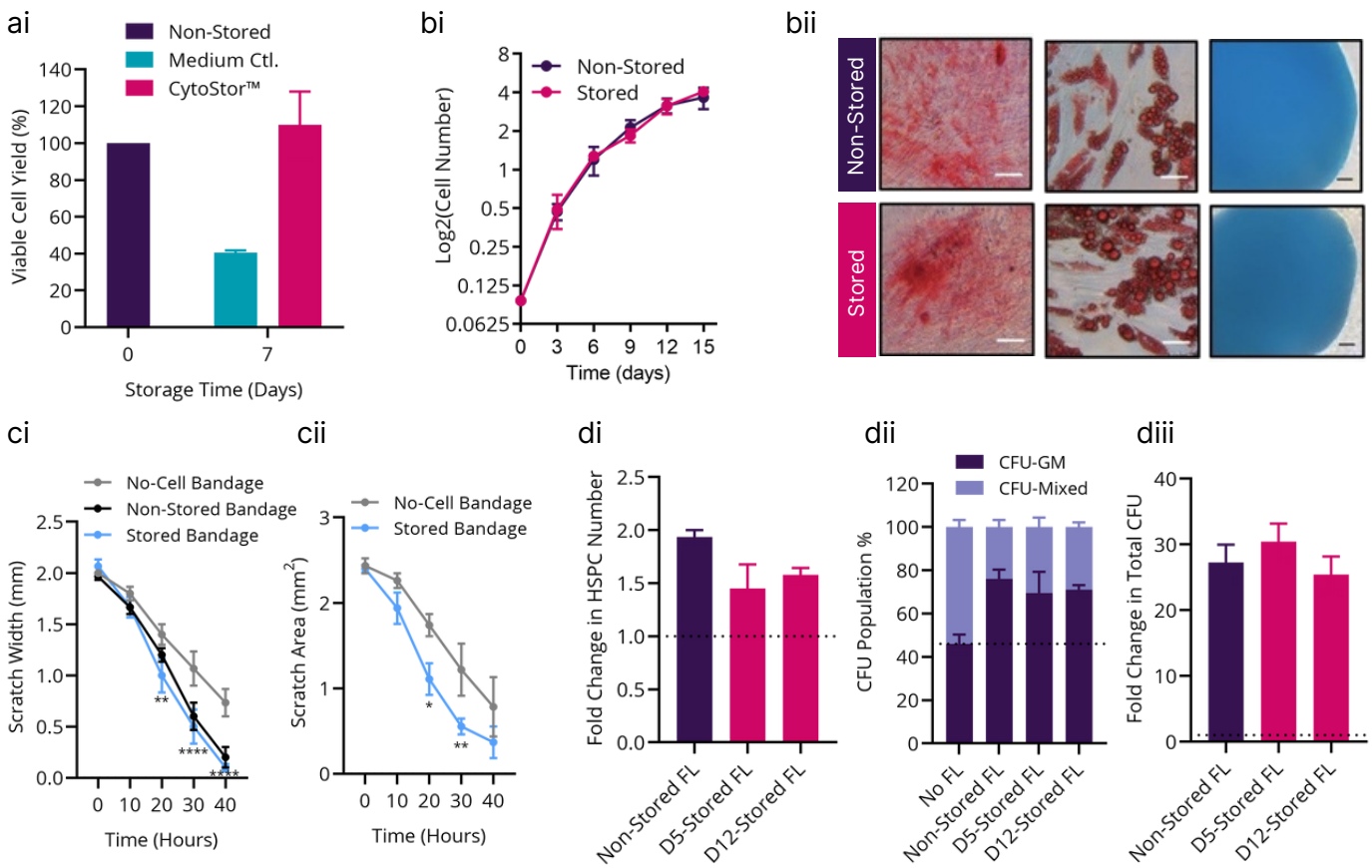
PRESERVING STROMAL FIBROBLASTS DURING HYPOTHERMIC STORAGE

Adipose- and Bone Marrow-Derived MSCs

Mesenchymal Stromal/Stem Cells (MSCs) are characterised by their capacity to differentiate into osteogenic (bone), adipogenic (fat) and chondrogenic (cartilage) lineages and give rise to these cells in human growth and homeostasis. More critical to their function is their capacity to produce paracrine factors that modulate immune cells, promote wound-healing and support angiogenesis which makes them an important candidate for cell-based therapeutics for non-healing wounds and ischemia.

Preserving differentiation potential, *in vitro* wound healing, and hematopoietic support capacity

The capacity for MSCs trilineage differentiation is important for osteo/chondral cell therapies and cosmetic reconstructive therapies. Here we show a high viable cell recovery **(a)** and preserved proliferative and differentiation capacity **(b)** after room temperature storage in Atelerix's technology. Moreover, paracrine factor production to direct wound healing is maintained in both adipose-derived MSCs (AD-MSCs) and bone marrow-derived Multipotent Adult Progenitor Cells (MAPCs) **(c)**. Finally, the capacity for hematopoietic / angiogenic paracrine factor production by AD-MSCs can be maintained after 12 days of storage **(d)**.



Preservation of viable cell yield, differentiation potential, wound healing capacity and hematopoietic support capacity in AD-MSCs and MAPCs. *AD-MSCs stored at room temperature had a high viable cell recovery, and a normal proliferative and differentiation potential. Stored cells maintained their in vitro wound-healing and hematopoietic support capacity.* ***ai****: Viable cell recovery of AD-MSCs stored in medium (teal) or in CytoStor™ (pink) after 7 days' storage at room temperature (n=3).* ***bi****: Proliferation of AD-MSCs following 3 days' storage and return to culture conditions (n=3).* ***bii:*** *Osteo- adipo- and chondrogenic differentiation of AD-MSCs stored for 3 days.* ***ci****: The effect of encapsulated AD-MSCs on corneal stromal cell scratch wound healing assays before (black) and after (blue) storage for 3 days (n=3).* ***cii****: The effect of encapsulated MAPCs on corneal stromal cell scratch wound healing after storage for 3 days (n=3).* ***di****: Fold change in umbilical cord-derived Hematopoietic Stem/Progenitor Cell (HSPC) number after co-culture with AD-MSC feeder layers that had been stored for up to 12 days at room temperature (n=3).* ***dii****: Increase in % CFU-GM cells after HSPC co-culture with AD-MSCs feeder layers that had been stored for up to 12 days at room temperature (n=3).* ***diii****: Increase in total CFU number after HSPC co-culture with AD-MSCs feeder layers that had been stored for up to 12 days at room temperature.* *bi-bii: Data adapted from Swioklo, S.. et al.Stem Cells Transl. Med.5(3),339 (2016).* ***ci****: Data adapted from Al-Jaibaji, O. et al.Int. J. Mol. Sci.,21, 5849 (2020).* ***cii****: Data adapted from Al-Jaibaji, O et al. PLoS ONE, 13(9), e0202118 (2018).* ***di-diii****: Data adapted from Branco et al. Bioeng., 9, 805 (2022).*

PRESERVING APHERESIS MATERIAL DURING HYPOTHERMIC STORAGE USING LEUKOSTOR™

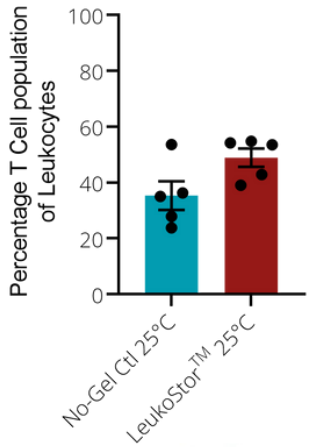
Apheresis Material

Apheresis products used as input for CAR-T cell therapies have extremely high variability due to many parameters including storage and shipment conditions. Our technology, LeukoStor™, can provide consistent, high quality starting material, offering a step towards standardisation. For CAR-T developers, this reduced variability in T cell starting material will translate to a more consistent manufacturing time and in turn reduced manufacturing costs.

Preservation of T cell starting material

LeukoStor™ allows storage of high-quality T cells for 5 days at 25°C. Evaluation of the T cell population by Flow Cytometry showed that LeukoStor™ 25°C improves T cell 'starting' yield (~48%), compared to the no-gel control (NGC) (~35%) **(a)**. LeukoStor™ maintains >90% viability of T cells, offering a 2-fold improvement from the NGC **(b, c)**. Assessment of the cellular composition of LeukoStor™-stored apheresis material showed stabilisation of key immune cell populations compared to the NGC, namely T cells and myeloid cells **(d)**.

a



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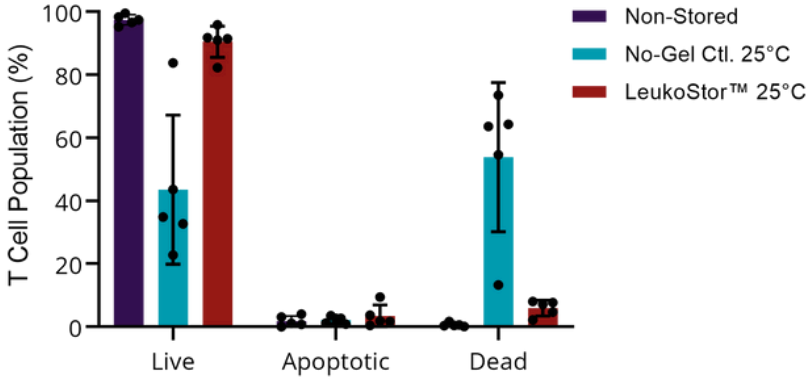

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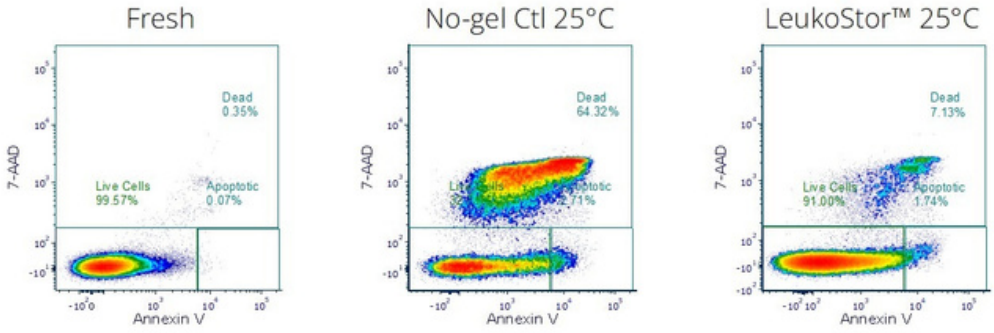

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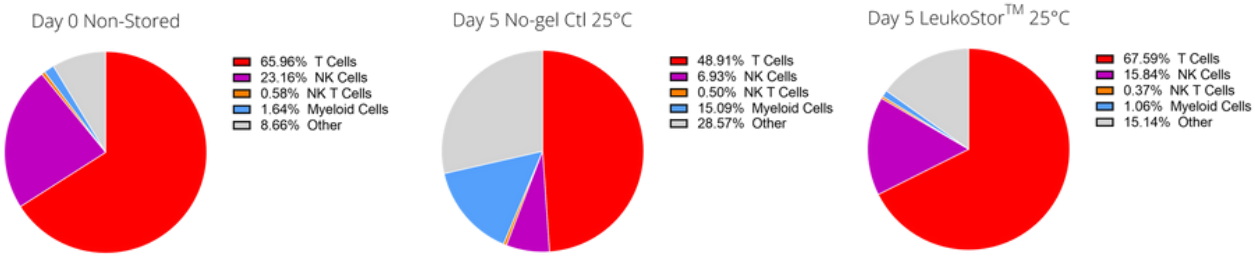


LeukoStor™ preserves T-cell yield and viability after 5 days of storage at 25°C. *Leukocyte reduction system cone (LRSC) material (used as a fresh apheresis starting material surrogate) was stored with or without LeukoStor™ before assessing quality by flow cytometry.* ***a:*** *Total T-cell yield (CD3+ cells as a % of total single CD45+ cells) at day 5 following storage was improved by LeukoStor™ compared to the no-gel control (NGC) which represented non-manipulated LRSC material.* ***b:*** *The quality of T cells from LRSC material severely deteriorated when left unprotected over 5 days at 25°C. Aside from one donor, live cells corresponded to 20-40% of the T cell population when stored with no protection (NGC). In starting material encapsulated in LeukoStor™, live T cells corresponded to 80-90% of the population with very few apoptotic or dead cells.* ***c:*** *Flow Cytometry of T cells from LRSC material. T cells were defined as single, CD45+ (CD45-BUV395), CD3+ (CD3-BV421) cells. % live (unstained), apoptotic (Annexin V+) and dead (7AAD+) cells in the T cell population were assessed.* ***d:*** *Assessment of cell composition of apheresis material after 5-day storage in LeukoStor™. (n=5).*

PRESERVING APHERESIS MATERIAL DURING HYPOTHERMIC STORAGE USING LEUKOSTOR™

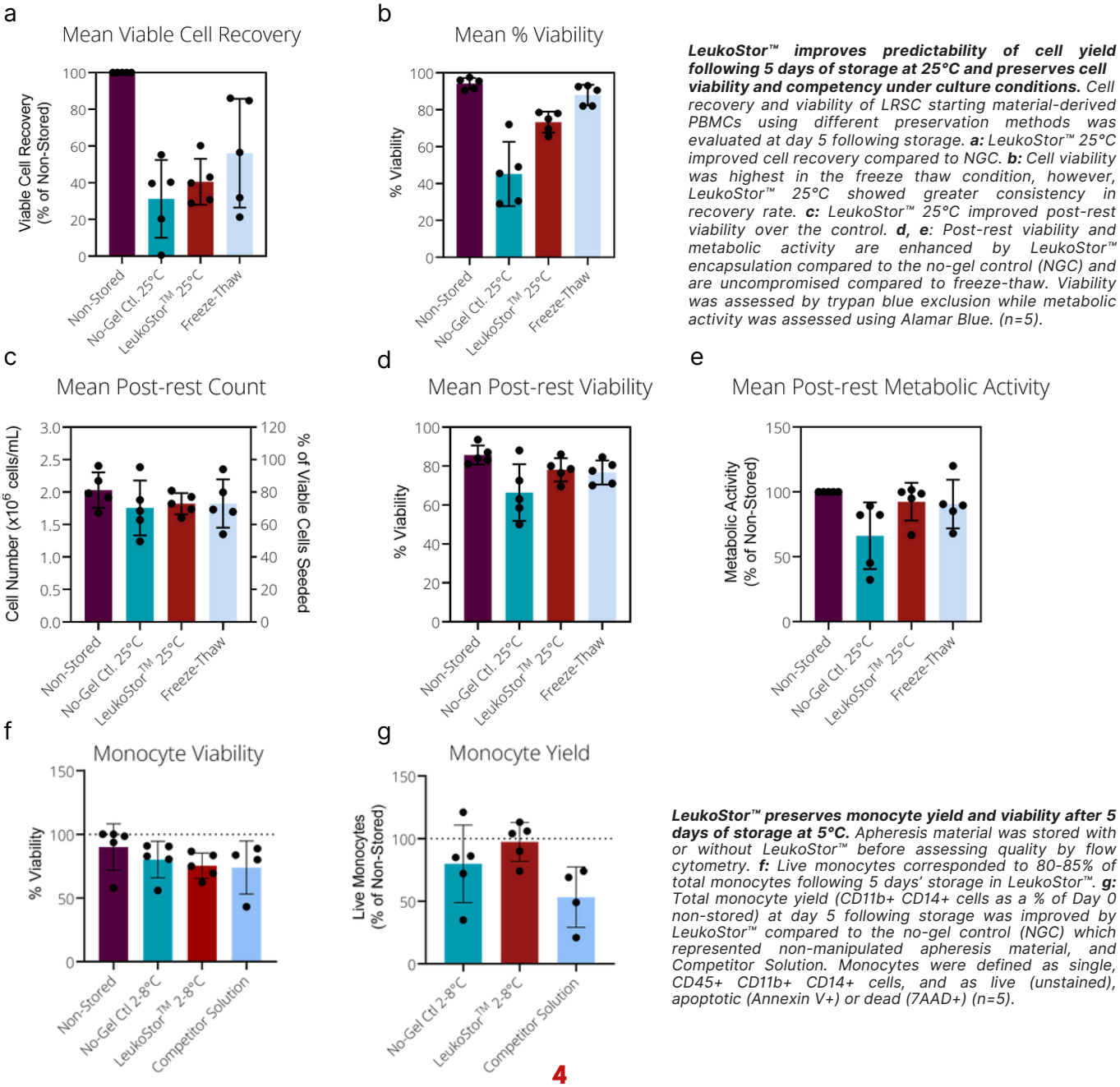
Apheresis Material

Preservation of PBMC yield, viability, and post-rest function

Storage of apheresis material in LeukoStor™ 25°C maintains a greater viable cell yield (~40%) compared to the no-gel control (NGC) (~30%) **(a-e)**. LeukoStor also improved predictability in cell yield compared to NGC. Viable cell recovery after freeze-thaw was on average 55%, however this recovery rate is hugely variable. When placed in culture conditions overnight leukocone-derived cells preserved using LeukoStor™ 25°C have high viability and metabolic activity compared to the NGC. Compared to freeze-thaw, LeukoStor™ 25°C preserved cells are uncompromised and show greater predictability in yield post-rest as observed at day 5.

Preservation of monocytes

LeukoStor™ allows storage of high-quality monocytes in leukapheresis for 5 days at 2-8°. Evaluation of the monocyte population by Flow Cytometry showed that LeukoStor™ 2-8°C maintains 80-85% viability **(f)**. Monocyte yield was dramatically improved by preservation in LeukoStor™ compared to HypoThermosol FRS (HT-FRS) and NGC **(g)**.



LeukoStor™ improves predictability of cell yield following 5 days of storage at 25°C and preserves cell viability and competency under culture conditions. *Cell recovery and viability of LRSC starting material-derived PBMCs using different preservation methods was evaluated at day 5 following storage.* ***a:*** *LeukoStor™ 25°C improved cell recovery compared to NGC.* ***b:*** *Cell viability was highest in the freeze thaw condition, however, LeukoStor™ 25°C showed greater consistency in recovery rate.* ***c:*** *LeukoStor™ 25°C improved post-rest viability over the control.* ***d, e****: Post-rest viability and metabolic activity are enhanced by LeukoStor™ encapsulation compared to the no-gel control (NGC) and are uncompromised compared to freeze-thaw. Viability was assessed by trypan blue exclusion while metabolic activity was assessed using Alamar Blue. (n=5).*

LeukoStor™ preserves monocyte yield and viability after 5 days of storage at 5°C. *Apheresis material was stored with or without LeukoStor™ before assessing quality by flow cytometry.* ***f:*** *Live monocytes corresponded to 80-85% of total monocytes following 5 days' storage in LeukoStor™.* ***g:*** *Total monocyte yield (CD11b+ CD14+ cells as a % of Day 0 non-stored) at day 5 following storage was improved by LeukoStor™ compared to the no-gel control (NGC) which represented non-manipulated apheresis material, and Competitor Solution. Monocytes were defined as single, CD45+ CD11b+ CD14+ cells, and as live (unstained), apoptotic (Annexin V+) or dead (7AAD+) (n=5).*

PRESERVING WHOLE BLOOD MATERIAL DURING HYPOTHERMIC STORAGE USING BLOODREADY™

Whole Blood

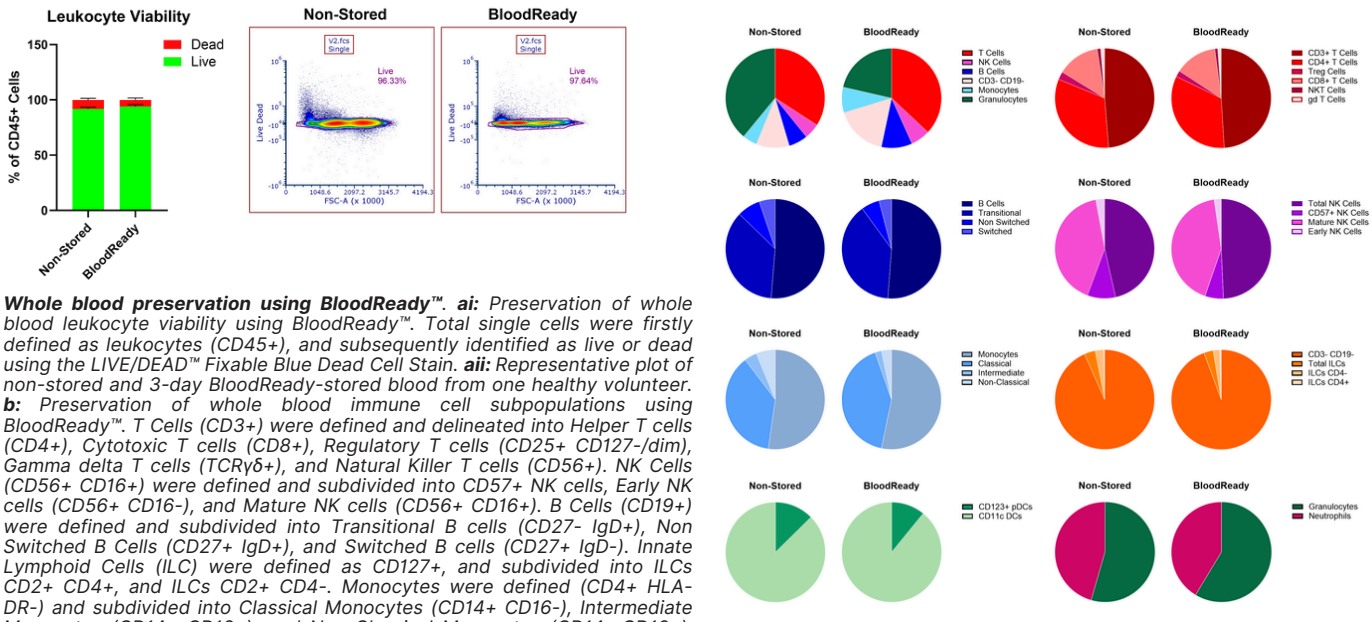
Immunophenotyping whole blood by flow cytometry is an incredibly powerful tool in the assessment of human health and is used widely in clinical patient diagnosis and monitoring. The stability of whole blood declines rapidly following collection, therefore samples must be processed efficiently. As same-day processing of whole blood samples is not always feasible, strategies to extend the time to analysis are required. Atelerix's technology, BloodReady™, offers a solution to overcome the logistical challenges of whole blood processing and transportation, without limiting the quality and informativeness of data obtained.

Preservation of whole blood

BloodReady™ allows the storage of human whole blood for 3 days at 2-8°C, yielding >95% recovery of whole blood leukocytes. Comprehensive immunophenotyping by multiplex antibody staining showed that BloodReady™ preserves the relative proportions of major and minor circulating leukocyte populations, with no significant difference from fresh blood. BloodReady™ successfully retains epitope expression on leukocytes without the need for fixation, offering a superior preservation solution.

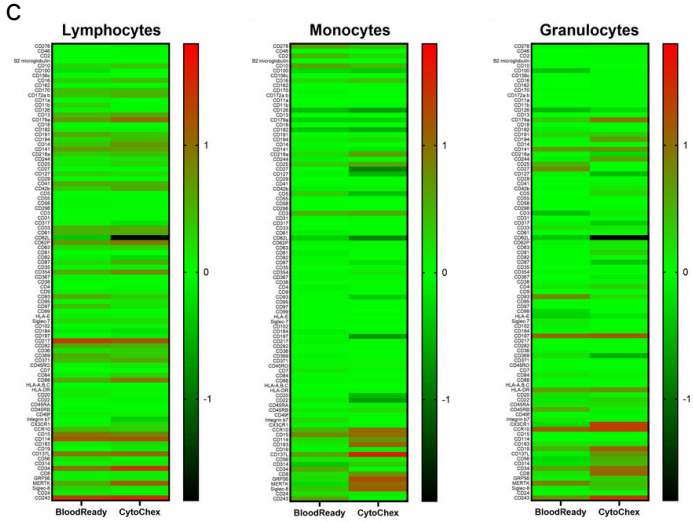
Whole Blood Leukocyte Populations are Preserved in BloodReady™

ai aii b



Whole blood preservation using BloodReady™. ai: *Preservation of whole blood leukocyte viability using BloodReady™. Total single cells were firstly defined as leukocytes (CD45+), and subsequently identified as live or dead using the LIVE/DEAD™ Fixable Blue Dead Cell Stain.* ***aii:*** *Representative plot of non-stored and 3-day BloodReady-stored blood from one healthy volunteer.* ***b:*** *Preservation of whole blood immune cell subpopulations using BloodReady™. T Cells (CD3+) were defined and delineated into Helper T cells (CD4+), Cytotoxic T cells (CD8+), Regulatory T cells (CD25+ CD127-/dim), Gamma delta T cells (TCRγδ+), and Natural Killer T cells (CD56+). NK Cells (CD56+ CD16+) were defined and subdivided into CD57+ NK cells, Early NK cells (CD56+ CD16-), and Mature NK cells (CD56+ CD16+). B Cells (CD19+) were defined and subdivided into Transitional B cells (CD27- IgD+), Non Switched B Cells (CD27+ IgD+), and Switched B cells (CD27+ IgD-). Innate Lymphoid Cells (ILC) were defined as CD127+, and subdivided into ILCs CD2+ CD4+, and ILCs CD2+ CD4-. Monocytes were defined (CD4+ HLA-DR-) and subdivided into Classical Monocytes (CD14+ CD16-), Intermediate Monocytes (CD14+ CD16+), and Non-Classical Monocytes (CD14- CD16+). Granulocytes were defined as SSChi, CD15+ CD11b+ cells.*

c



BloodReady™ Maintains Leukocyte Epitope Expression

c: *Preservation of whole blood leukocyte epitope expression by BloodReady™. Whole blood was stained with a panel of 93 antibodies against leukocyte surface epitopes following 3-day storage in BloodReady™ or Cyto-Chex. Epitope expression was analysed against that of non-stored whole blood leukocytes and expressed as a ratio, where 0 = non-stored. Leukocyte epitope expression was analysed separately for lymphocyte, monocyte, and granulocyte cell subpopulations. BloodReady™ preserves several lymphocyte epitopes more effectively than Cyto-Chex, including CD62L, CD317, CD82, CD367, and Integrin b7. Data shows the mean of n=3 healthy donors.*

PRESERVING WHOLE BLOOD MATERIAL DURING HYPOTHERMIC STORAGE USING BLOODREADY™

Whole Blood

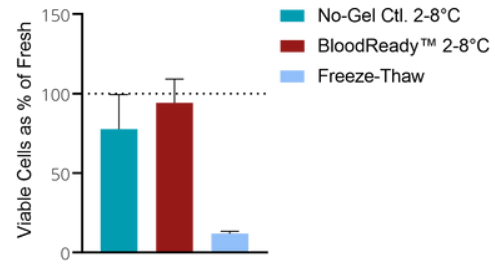
Preservation of whole blood

BloodReady™ allows the storage of human whole blood for 3 days at 2-8°C. BloodReady™ yields >95% recovery of whole blood leukocytes after 3 days' storage, offering an improvement from the no-gel control (NGC) and freeze-thaw. BloodReady™ maintains live populations of B cells, T cells, NK cells, and monocytes after 3 days' storage, comparable to counts in fresh blood.

BloodReady™ preserved PBMCs retain high viability (>100%) after return to culture, which is comparable with the recovery of non-stored PBMCs, while the no gel control PBMCs do not adapt as successfully. Of the few cells that do survive after freezing, most recover well, however the original yield is too poor for subsequent downstream application. PBMCs isolated from BloodReady-stored PBMCs respond to IL-2 cytokine stimulation after 2-3 days in culture, unlike control cells.

BloodReady Preserves Leukocyte Yield Compared to Cryopreservation and No-Gel Control

a



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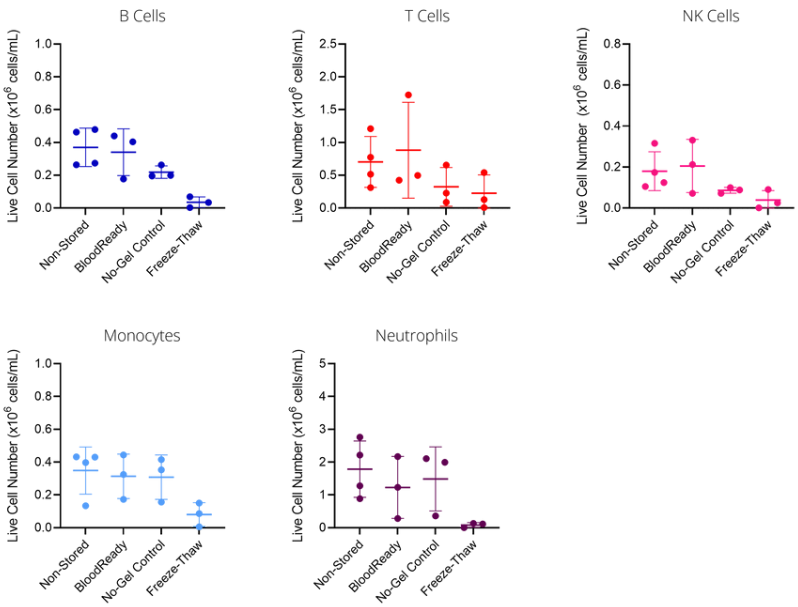
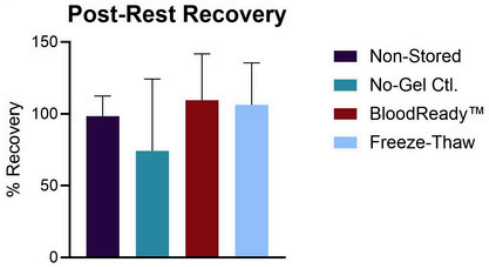


Whole blood preservation using BloodReady™. a: *Recovery of whole blood leukocytes after 3 days' storage in BloodReady™ compared to a no-gel control and cryopreservation.* ***b:*** *Recovery of major leukocyte subpopulations following comprehensive immunophenotyping of stored whole blood. Data was obtained from Flow Cytometry staining of all major leukocyte populations. Cells were defined as T cells (CD45+ CD3+), B cells (CD45+ CD19+), NK cells (CD45+ CD56+ CD16+), Monocytes (CD45+ CD14+), and Neutrophils (Side Scatter high CD16+). Cell subsets are expressed as a percentage of live CD45+ cells. Viability was assessed by 7AAD staining; live (unstained), apoptotic/dead (7AAD+) cells. Mean ± SD.* ***c:*** *Post-rest recovery (cell number) and culture stability of unstimulated PBMCs isolated from BloodReady™-stored whole blood. Non-Stored: n=4, BloodReady™ NGC, Cryopreservation: n=3.*

PBMCs Isolated from BloodReady-Stored Whole Blood Are Competent Upon Return To Culture

a



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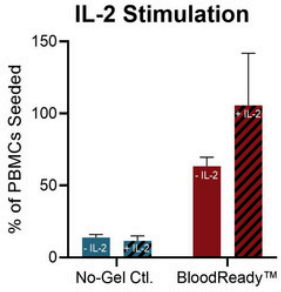


Data shows the post-rest recovery and function of PBMCs in culture after 3 days storage in BloodReady™ at 2-8°C. a: *PBMCs were cultured at 37°C and viability assessed via trypan blue exclusion at day 1. Cell recovery is calculated as cell count as a percentage of cell number seeded. Mean ± SD. D1; Non-Stored: n=3, BloodReady™, No-gel Ctrl: n=4, Cryopreservation: n=2. D3, D6; BloodReady™, No-gel Ctrl: n=3, Cryopreservation: n=2.* ***b:*** *PBMCs isolated from BloodReady-stored blood demonstrate a response to IL-2 stimulation after 3 days in culture, compared to no response in the no-gel control isolated PBMCs. BloodReady™, No-gel Ctrl: n=3.*

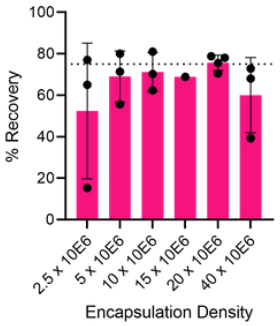
PRESERVING ISOLATED BLOOD CELLS DURING HYPOTHERMIC STORAGE

PBMCs

Human peripheral blood mononuclear cell (PBMC) samples are used as a source material to isolate lymphocytes (T cells, B cells, and NK cells) and monocytes for basic research or cell therapy applications. Mononuclear cells are widely used in research and clinical applications, such as immunotherapies, microbiology, virology, oncology, vaccine development, toxicology, as well as transplant and regenerative biology.

Preservation of primary PBMC viability

CytoStor™ allows storage of high quality primary PBMCs for 3 days at 2-8°C, yielding a high viable cell recovery with an optimal load of 20 x 10E6 cells/vial (20 x 10E6 cells/mL).



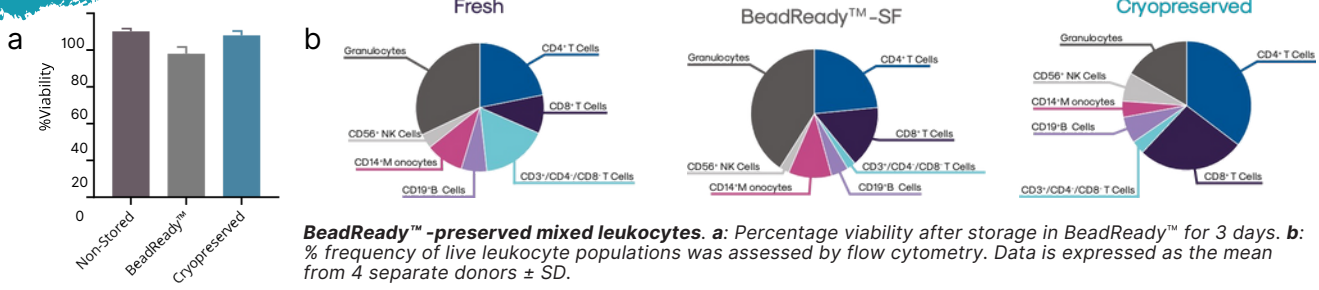
CytoStor™-preserved PBMCs. *Viable cell recovery of PBMCs stored in CytoStor™ for 3 days at 2-8°C. PBMCs were stored at a density between 2.5 x 10E6 and 40 x 10E6 cells/mL.*

Mixed Leukocytes

Mixed leukocytes are blood cell populations isolated from blood, similar to PBMCs but with a retained granulocyte population.

Preservation of mixed leukocytes

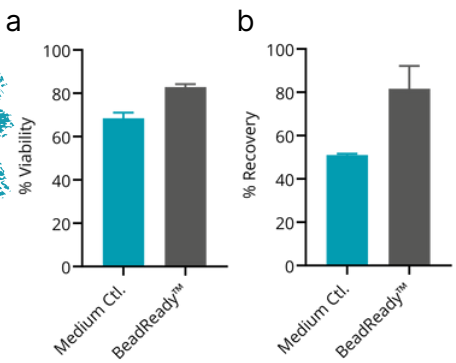
BeadReady™ is able to maintain 80% viability of mixed leukocytes with no significant alteration in the frequency of any cell type **(a)**. BeadReady™ yields 3x higher recovery of monocytes compared to freezing and protection of granulocyte populations is observed **(b)**.

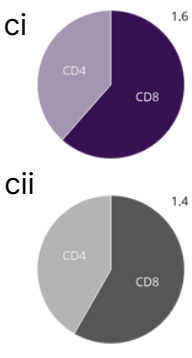


BeadReady™ -preserved mixed leukocytes. ***a****: Percentage viability after storage in BeadReady™ for 3 days.* ***b****: % frequency of live leukocyte populations was assessed by flow cytometry. Data is expressed as the mean from 4 separate donors ± SD.*

Purified Blood Cells

Cells selected from blood products are important for therapies and drug discovery

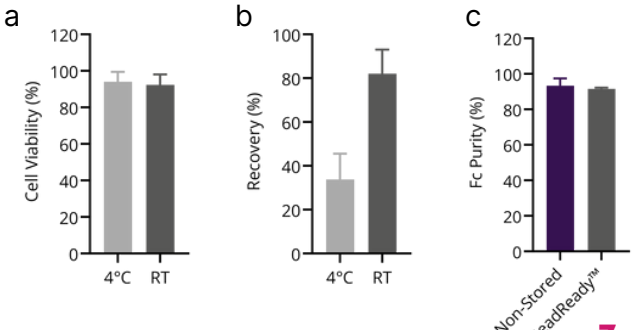




Preservation of Pan-T Cells

BeadReady™ room temperature storage is able to maintain over 80% viability **(a)** and viable cell yield **(b)** of Pan-T cells expanded for 6 days with normal CD4/CD8 ratio **(c)**.

BeadReady™ -preserved culture-expanded Pan-T cells. ***a:*** *% viability after storage in BeadReady™ for 5 days.* ***b:*** *% viable cell yield after storage in BeadReady™ for 5 days.* ***c:*** *CD3/4 % T cell populations in non-stored* ***(ci)*** *and BeadReady™-stored* ***(cii)*** *cultures.*



Preservation of CD34+ HSCs

BeadReady™ room temperature storage is able to maintain high viability **(a)**, viable cell yield **(b)** and purity **(c)** of human umbilical cord-derived CD34+ hematopoietic stem cells (HSCs) with an optimal storage temperature of 20°C.

BeadReady™ -preserved CD34+ HSCs. ***a:*** *Percentage viability after storage in BeadReady for 3 days.* ***b:*** *Percentage viable cell yield after storage in BeadReady™ for 3 days.* ***c:*** *CD34 % purity as assessed by flow cytometry.*

PRESERVING CANCER TISSUE AT ROOM TEMPERATURE USING TISSUEREADY™ PLUS

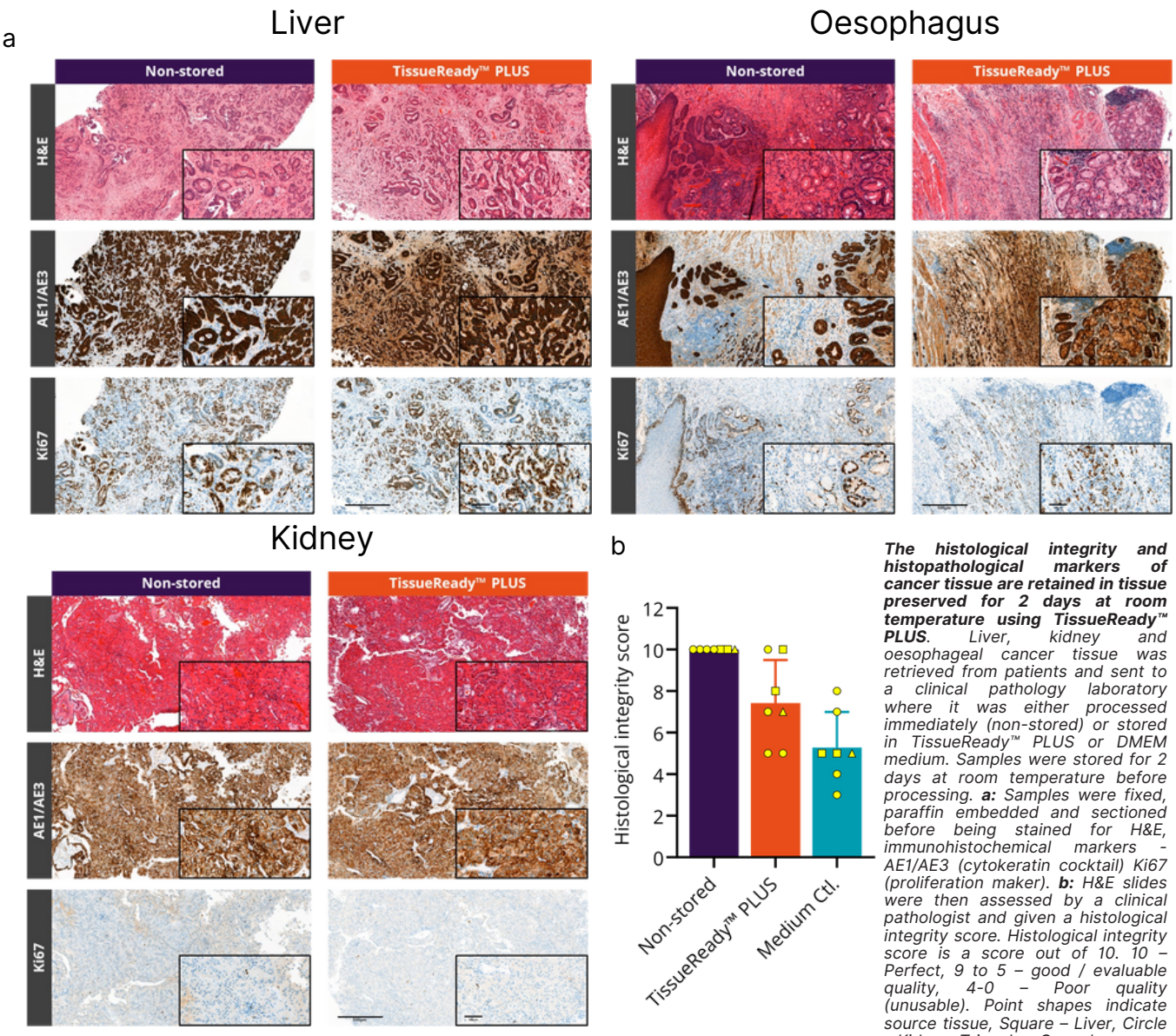
Cancer Tissue

The sourcing of primary tissue is vital for many fields of research and is becoming increasingly important for use in advanced screening techniques/diagnosis, drug development and patient-derived model generation as healthcare moves towards personalised medicine. Tissue quality deteriorates rapidly after retrieval, and therefore tissues need to be used as close to the retrieval time as possible to ensure the best quality outcomes. Unfortunately, the sites of tissue retrieval are not always close to sites requiring the tissue, resulting in extended distribution times and a reduced quality of tissue.

Cancer tissue retains histological integrity and marker expression when stored using TissueReady™ PLUS

Cancer tissue was retrieved from patients and processed by a clinical pathology laboratory, stored in TissueReady™ PLUS for 2 days at room temperature before processing. The slides were then examined and stored by a clinical pathologist who was blinded to the tissue conditions. The observations were collated and compared to non-stored tissue and tissue stored in medium alone. Data and histological evaluation of cancer tissue preserved for 2 days at room temperature using TissueReady™ PLUS concluded that: histological integrity was maintained, markers for AE1/AE3 and Ki67 were retained, RNA could successfully be extracted.

a

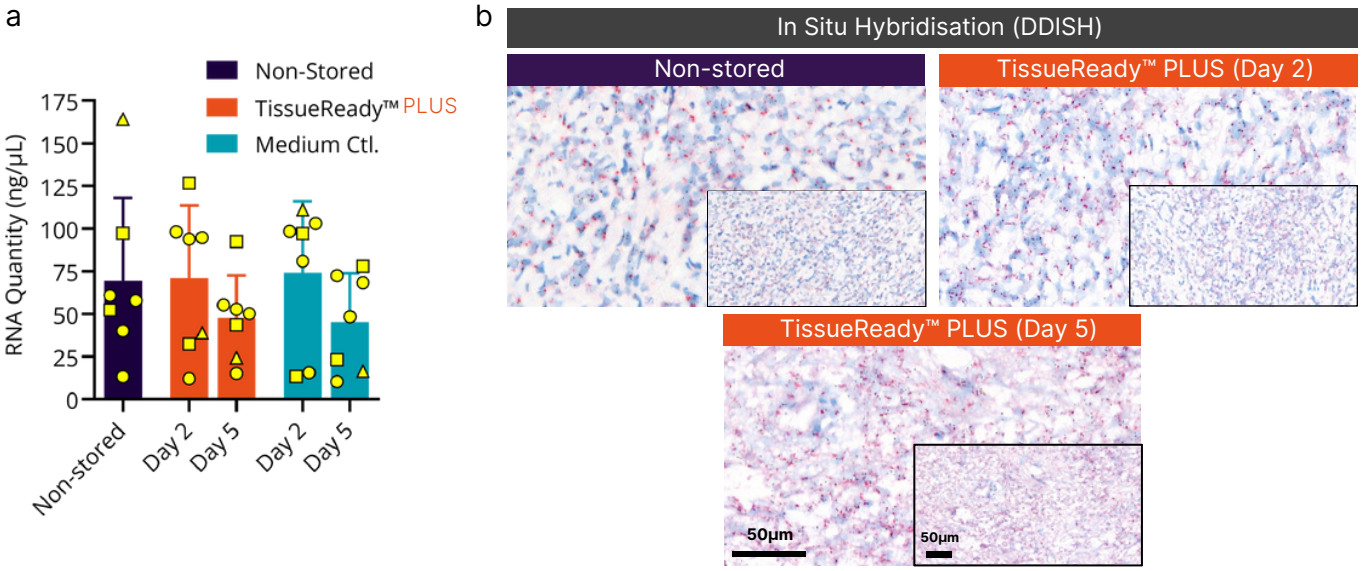


b

The histological integrity and histopathological markers of cancer tissue are retained in tissue preserved for 2 days at room temperature using TissueReady™ PLUS*. Liver, kidney and oesophageal cancer tissue was retrieved from patients and sent to a clinical pathology laboratory where it was either processed immediately (non-stored) or stored in TissueReady™ PLUS or DMEM medium. Samples were stored for 2 days at room temperature before processing.* ***a:*** *Samples were fixed, paraffin embedded and sectioned before being stained for H&E, immunohistochemical markers - AE1/AE3 (cytokeratin cocktail) Ki67 (proliferation maker).* ***b:*** *H&E slides were then assessed by a clinical pathologist and given a histological integrity score. Histological integrity score is a score out of 10. 10 – Perfect, 9 to 5 – good / evaluable quality, 4-0 – Poor quality (unusable). Point shapes indicate source tissue, Square – Liver, Circle – Kidney, Triangle – Oesophagus.*

PRESERVING CANCER TISSUE AT ROOM TEMPERATURE USING TISSUEREADY™ PLUS

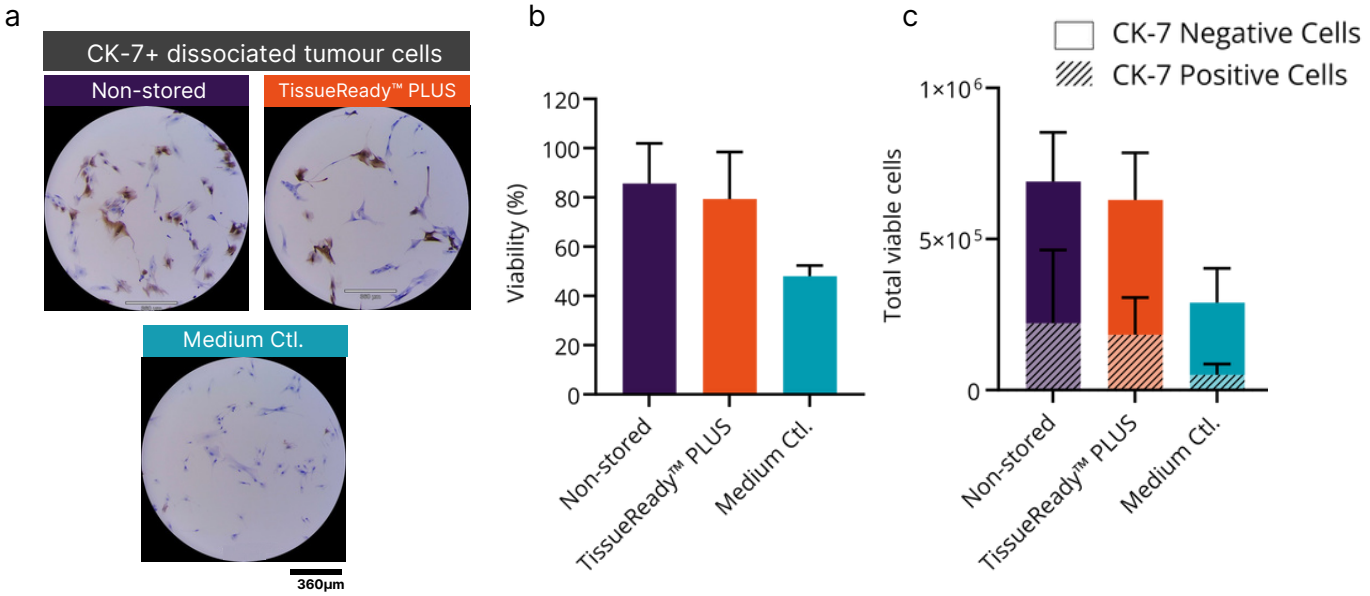
Tissues stored in TissueReady™ PLUS are suitable for molecular biology assessment



RNA can be isolated and in situ hybridisation can be performed on tissue preserved using TissueReady™ PLUS. *Cancer tissue was retrieved from patients and sent to a clinical pathology laboratory where it was either processed immediately (non-stored) or stored in TissueReady™ PLUS or DMEM medium. Samples were stored for 2 days and 5 days at room temperature before processing.* ***a****: RNA was isolated using an RNA extraction kit and RNA quantity was assessed using a nanodrop spectrophotometer. Datapoint shapes indicate source tissue, Square – Liver, Circle – Kidney, Triangle – Oesophagus.* ***b****: Dual DNA In Situ Hybridisation (DDISH) against the HER2 gene and chromosome 17 was performed on stored tissue to interrogate cancer status in oesophageal tissue.*

Cells can be isolated, cultured and expanded for multiple passages from tissue stored for up to 5 days using TissueReady™ PLUS

To assess cell isolation from cancer tissue, cells from oesophageal cancer resections were isolated by tissue dissociation, expanded and stained for a cancer marker following storage in TissueReady™ PLUS for 4-5 days at room temperature and compared to cells isolated from fresh, non-stored cancer tissue. Cells from tissue preserved using TissueReady™ PLUS was comparable to non-stored, fresh tissue for: cell viability, cell yield, ratio of cytokeratin-7 positive (cancer cells) and cytokeratin-7 negative (normal cells) and continued expansion/passaging (passaged 6 times, frozen and revived).



Cell viability and cell yield was comparable between cells isolated from fresh cancer tissue and cancer tissue preserved in TissueReady™ PLUS . *Cancer tissue was retrieved from patients and sent to a clinical pathology laboratory where it was stored in TissueReady™ PLUS or medium before being shipped to an oncology research facility. The tissue was preserved for 4-5 days (from encapsulation) at room temperature before cells were isolated by tissue dissociation. Isolated cells were expanded before being fixed and stained for Cytokeratin-7 to differentiate cancer cells from non-cancer cells.* ***a:*** *Brightfield images of fixed cells stained for cytokeratin-7, Positive cells (brown) and negative cells (Blue) were used to calculate the percentage of cancer cells present.* ***b:*** *Cell viability as determined by brightfield microscopy and trypan blue exclusion.* ***c:*** *Viable cell yield calculated by counting the number of live cells using brightfield microscopy and trypan blue exclusion. Cells were immunohistochemically stained for cytokeratin-7. Staining was visualised using Vectastain ABC and DAB substrate kits. The percentage of positively and negatively stained cells was calculated, and these percentages applied to the viable cell yield.*

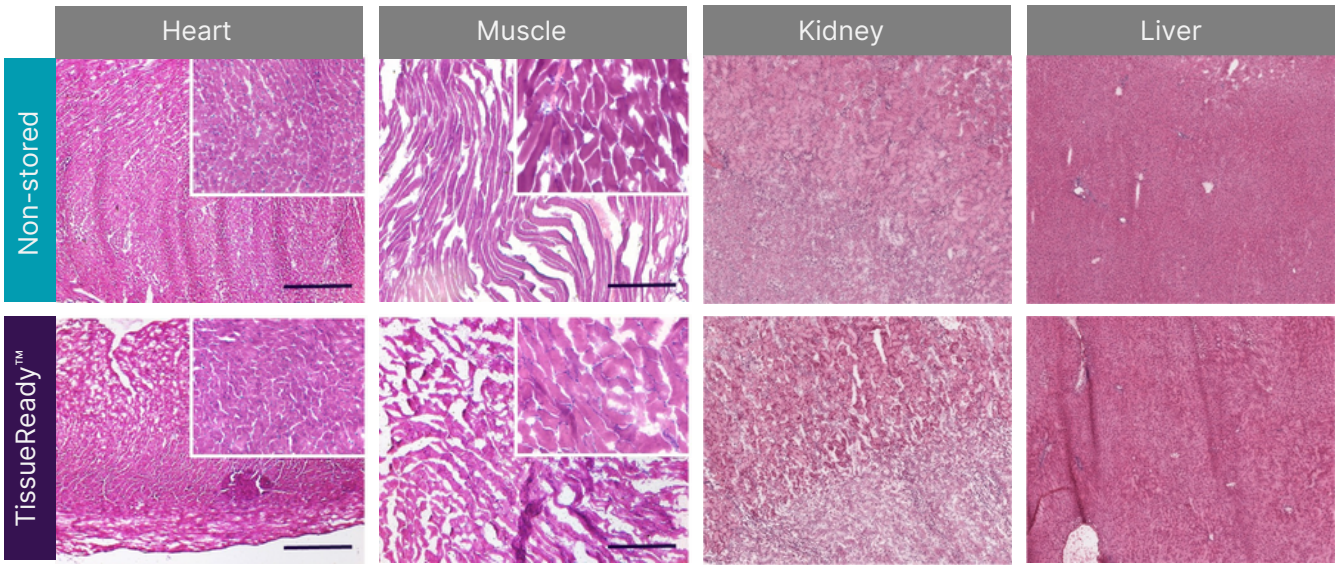
PRESERVING HEALTHY TISSUE DURING HYPOTHERMIC STORAGE

Murine Tissue

Primary tissue, from humans and animals is important in many fields of research, drug development and advances in clinical diagnosis. Formalin fixation and cryo- embedding tissue is commonly used for histological and some molecular analysis, however it is not suitable for more sensitive analysis or for functional studies such as heart muscle contractility and absorption of chemicals through the skin.

Multiple murine tissues retain histological integrity when stored using TissueReady™

Murine heart, muscle, kidney and liver were stored in TissueReady™ at 2-8°C for 7 days. After release tissue structure was assessed by haematoxylin & eosin (H&E) staining. H&E staining demonstrated tissue structure and integrity was preserved.



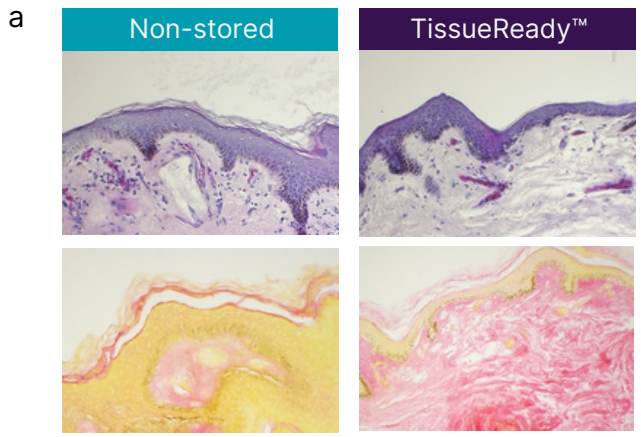
Preservation of freshly collected murine tissues in TissueReady™. Fresh murine tissues were isolated, dissected, and placed in TissueReady™. Heart, Muscle, Kidney and Liver were stored for a period of 7 days at refrigerated temperature (2-8°C) before removing the gel. Subsequently, tissue was snap-frozen and sectioned before being H&E stained. Non-stored tissue was processed immediate upon retrieval.

Human Skin Tissue

Full thickness, human skin is required for functional applications including drug and cosmetic development and skin disease research. As a result, these tissues can't be frozen or fixed for this purpose yet supply of such tissue is limited and often requires shipment for longer than desired.

Human skin tissue retains viability and histological integrity when stored using TissueReady™

Fresh human abdominal skin tissue was preserved in TissueReady™ for 5 days at room temperature. After release tissue structure and integrity were assessed by histological staining and tissue viability by metabolic activity. H&E and collagen staining demonstrated tissue structure was preserved following release and relative metabolic activity was unchanged.



Preservation of freshly collected abdominal skin biopsies in TissueReady™. *Fresh skin biopsies were isolated, dissected, and placed in TissueReady™. Skin was stored for a period of 5 days at room temperature before removing the gel and returning to culture for 4 hours.* ***a:*** *Tissue integrity was examined by H&E (top panels) and collagen staining (bottom panels).* ***b:*** *Viability was assessed by relative metabolic activity using alamar Blue. Non-stored tissue was processed immediately upon retrieval.*

PRESERVING ENGINEERED TISSUES DURING HYPOTHERMIC STORAGE

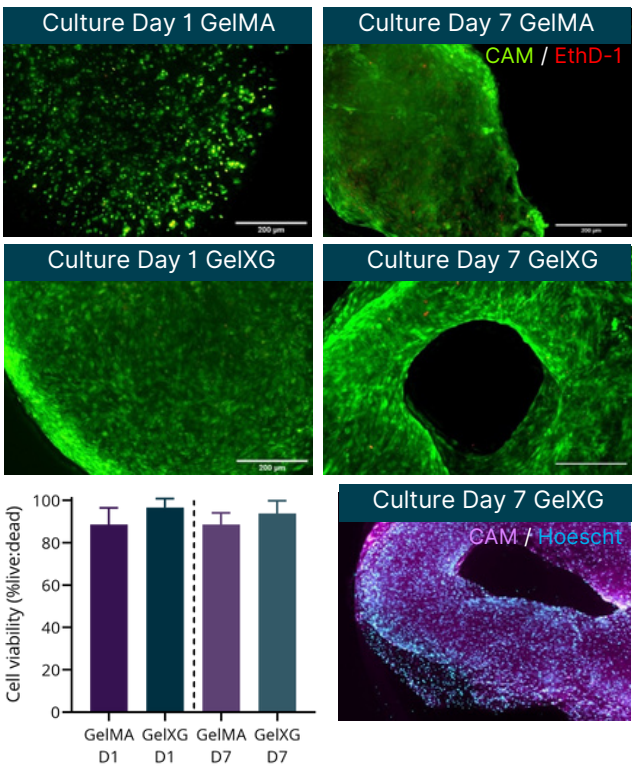
Printed Constructs and Engineered Tissues

Generating complex three-dimensional (3D) biological materials that recapitulate the spatial behaviour of cells is essential for physiologically-relevant drug screening and tissue fabrication. 3D bioprinting is one method by which this can be achieved through the extrusion of cell-laden bioinks into 3D structures. Other methods include tissue templating and specific culture conditions that encourage matrix deposition/alignment to generate a structure similar to that found in vivo.

Shipping 3D printed constructs and engineered microtissues using WellReady™

When shipping 3D biofabricated constructs, it is important that their 3D structure is maintained, and cells remain viable and grow within their supportive matrix. Here, MSC-laden constructs were shipped from Sweden to the UK in WellReady™ 96-well plates at room temperature before successful onward culture **(a)**. WellReady™ also preserved engineered cardiac and skeletal muscle microtissues in 24-well plates which regained their contractility and drug-responsiveness after return to culture **(b)**. The structural and physiochemical support that Atelerix's technology offers can protect the most delicate of tissues including corneal stromal constructs generated from the cells' own matrix assembly **(c)**. Finally, the optical properties of the gels used in Atelerix's products such as BeadReady™ make them ideal for imaging the cells in 3D **(d)**.

a



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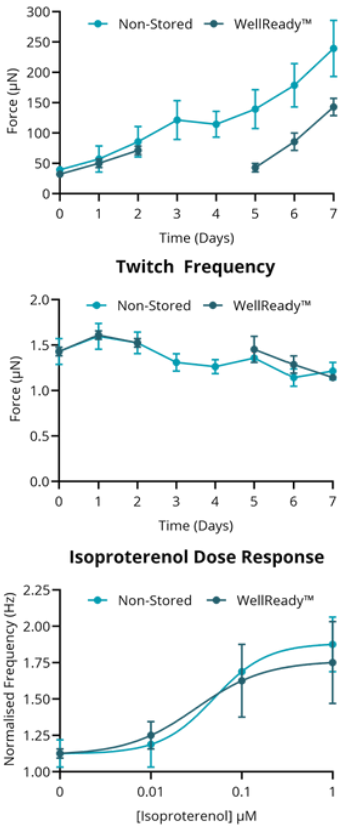

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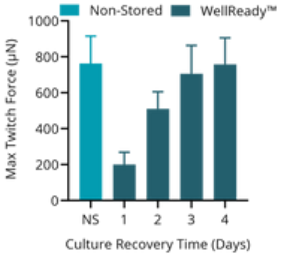

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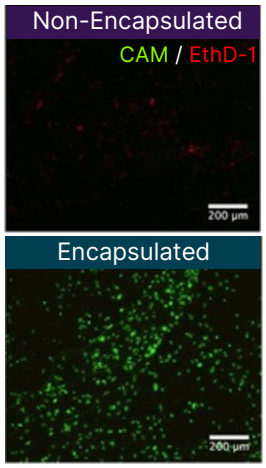

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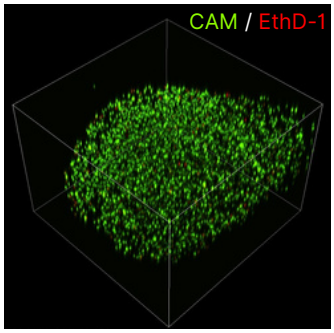


Storage and shipment of 3D bioprinted constructs, cardiac and skeletal muscle engineered microtissues, and corneal stromal tissue constructs alongside confocal imaging of encapsulated cells. a: *Live-dead (CAM/EthD-1) viability assessment of 3D printed constructs comprising Bone Marrow-derived Mesenchymal Stromal Cells (BM-MSCs) and two separate Bioinks (GelMA and GelXG) after shipment in WellReady™ over 7 days at room temperature. Culture over a 7-day period after release from WellReady™ demonstrated considerable growth of cells and structural maintenance.* ***bi:*** *Engineered heart tissues composed of iPSC-derived cardiomyocytes and human ventricular fibroblasts stored for 2 days at room temperature in WellReady™ fully recovered contractility behaviour and responded normally to Isoproterenol as shown 24 hours after treatment.* ***bii:*** *Engineered Microtissues from primary human skeletal muscle stored for 3 days at room temperature in WellReady™ recovered full contractility behaviour after a 3-day recovery period in culture.* ***c:*** *Corneal stromal constructs encapsulated and stored for 3 days at room temperature prevented the cell loss observed without encapsulation as assessed by live-dead (CAM/EthD-1) viability staining.* ***d:*** *3D confocal imaging of Adipose Derived (AD-) MSCs in a single BeadReady™ bead after 2 days at room temperature allows spatial examination of cells within the matrix.* ***a:*** *A full Technical Note can be found at Jaiswal et al., 2021* ***b:*** *Data adapted from Luttrell et al., 2023 [Technical Poster].* ***c:*** *More information on imaging can be found in Swioklo et al., Proc. Biochem., 59 (Part B), 289 (2017).*

PRESERVING HEPATIC MODELS DURING HYPOTHERMIC STORAGE

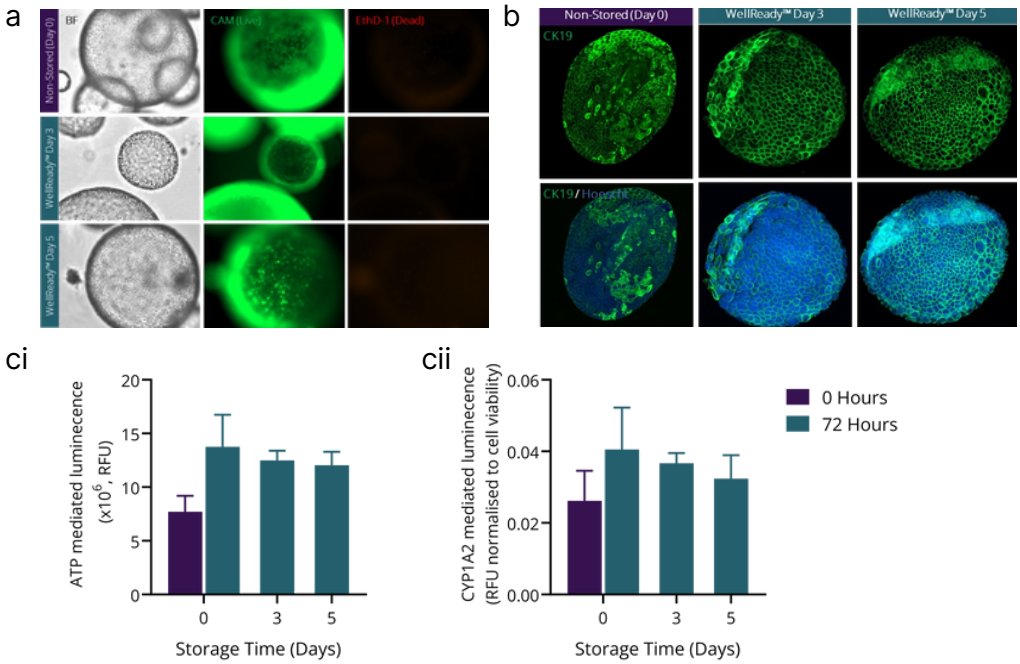
Hepatic Models

Hepatocytes are the main functional cell in the liver, serving several essential functions, including xenobiotic metabolism/detoxification with a family of drug metabolising enzymes, cytochrome P450s, being primarily responsible for first phase metabolism. The use of in vitro hepatic models are important in drug development to identify drug properties and metabolites. However, primary hepatocytes de-differentiate rapidly in culture, resulting in a loss of P450 and drug metabolising capability. More recently, models have used ways to prevent rapid de-differentiation or use stem cell derived hepatocytes.

Liver organoids maintain their structure and function when stored in plate using WellReady™

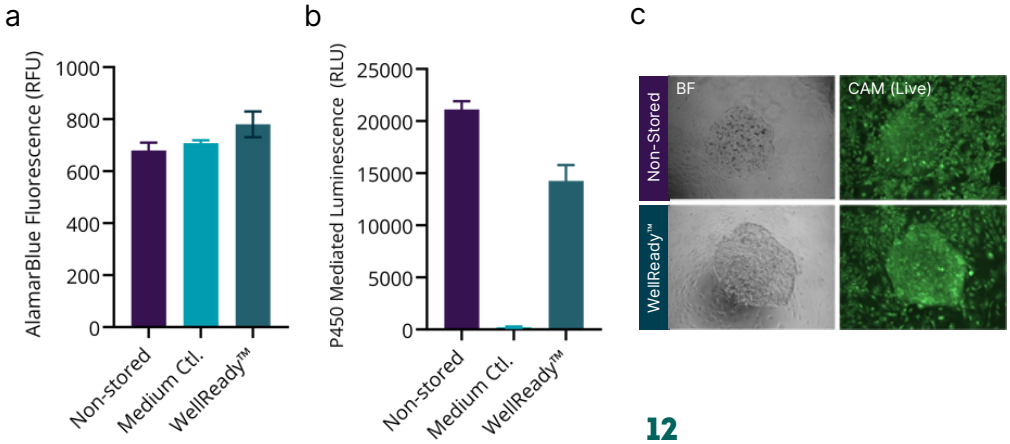
iPSC-derived murine liver organoids were cultured in a Matrigel matrix in 48 well plates. Once mature, the organoids were stored at 20°C for 5 days using WellReady™. Upon release the organoids: retained a high viability, continued growth, 3D structure and hepatic marker expression and were retained functionally active drug metabolising enzymes after 72 hours in culture.

The effect of WellReady™ on Liver Organoid preservation. *Liver organoids were preserved at 20°C for 5 days using WellReady™. Following preservation, the organoids were released from WellReady™ and returned to culture for 72 hours before carrying out assays.* ***a****: Organoids were returned to culture overnight and stained with calcein-AM (CAM, green) and ethidium homodimer-1 (EthD-1, red) dyes to visualise live and dead cells respectively.* ***b****: Organoids were returned to culture for overnight, fixed, and stained for the hepatocyte cholangiocyte marker - Cytokeratin-19 (CK19, green) and Hoechst nuclear stain (blue).* ***ci****: Cell viability assessed by measuring ATP levels using the CellTitre-Glo® assay,* ***cii****: Functional activity assessed by measuring the activity of Cytochrome P450 1A2 using the P450-Glo™ CYP1A2 Assay. Legend indicates post release culture periods. Scale bars represent 100µm.*



Primary hepatocytes maintain their metabolic activity and drug metabolising capability when stored in plate using WellReady™

Primary hepatocytes were seeded upon a stromal cell feeder layer in culture plates. Cells were encapsulated using WellReady™ and stored at 37°C for 7 days. Upon release, cells were returned to their standard culture medium overnight. Metabolic activity (as an indicator of cell viability) and cytochrome P450 activity (as an indicator of cellular function) was maintained post preservation.



The effect of WellReady™ on primary hepatocyte preservation. *Primary hepatocytes were preserved at 37°C for 7 days using WellReady™. Following preservation, the hepatocytes were released from WellReady™ and returned to culture overnight before carrying out assays.* ***a:*** *alamarBlue fluorescence-based assay for metabolic activity,* ***b:*** *Cytochrome P450 activity measured using P450-Glo™ Assay,* ***c:*** *Brightfield microscopy (left panels) and cells sained with calcein-AM (CAM, green) to visualise green cells (right panels).*

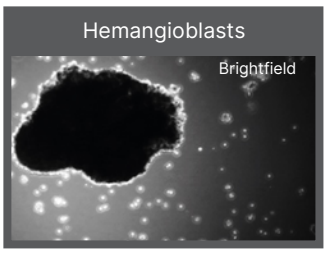
PRESERVATION OF IPSC-DERIVED MODELS DURING HYPOTHERMIC STORAGE

Monocytes

Induced pluripotent stem cell (iPSC)-derived cells are used as a model in research and drug discovery in place of less physiologically relevant immortalised cell lines or scarcely available primary cells. Hemangioblasts can be differentiated from iPSCs, which release macrophage progenitors which in turn can be matured to microglia.

Human iPSC-derived hemangioblasts (monocyte factories) were encapsulated and stored at room temperature for 5 days using BeadReady™. After storage, over 85% cell viability was observed and hemangioblasts were able to produce monocytes / macrophage progenitors over 8 days in culture **(ai)** with characteristic phenotypic markers **(aii)**. Macrophage progenitors/microglia stored for 5 days also exhibited high viability and were shown to engulf β-amyloid at similar level as control cells **(b)**.

ai



aii

Marker	Non-Stored	BeadReady™
CD14	99.8%	98.4%
CD16	99.9%	99.9%
CD11b	86.3%	92.5%

b

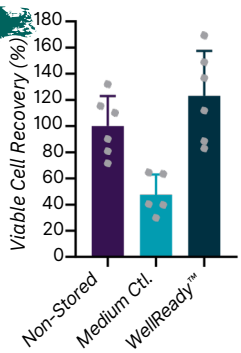
Preservation of hemangioblasts and microglia-like cells using WellReady™. *Hemangioblasts were stored at room temperature in BeadReady™ for 5 days before return to culture over an 8-day period* ***(ai)****. Monocytes were collected from hemangioblast cultures and flow cytometry was performed to measure expression of CD14, CD16 and CD11b monocyte/macrophage markers comparing to the non-stored control* ***(aii)****. Macrophage progenitor cells stored at room temperature in BeadReady™ were returned to culture for 24 hours before assessing their expression of microglia marker P2RY12 and β-amyloid uptake* ***(b)****. Data adapted from Tilman, J. et al., 2018 [conference poster].*

Cortical Neurons

iPSC-derived neuronal cells are important in disease modelling. In order to function as intended, these cells must form a connected axonal network.

iPSC derived neuronal cells were stored in WellReady™ at room temperature for 3 days. Neuronal cell morphology, metabolic activity and intact axonal networks were retained in WellReady™ plates whereas a loss of viable cells was observed in medium only control plates.

a



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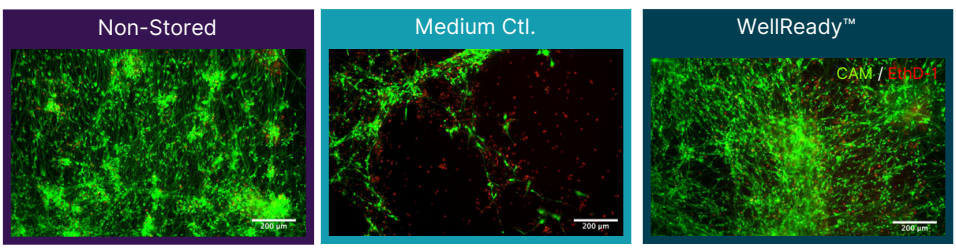


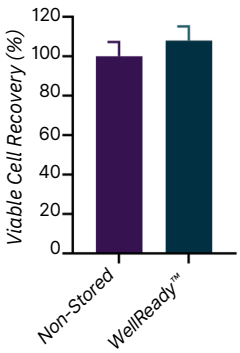
Preservation of iPSC-derived neurons using WellReady™. *Differentiated neurons were matured for 34 days in a 96 well plate before being stored for 3 days and return-shipped by courier in CRT packaging at 15-20°C. Following return to normal culture conditions for 5 days, percentage of viable cell recovery was determined by alamarBlue* ***(a)*** *and live/dead staining with calcein-AM (live indicator; green) and ethidium homodimer-1 (dead indicator; red)* ***(b)****.*

Cardio-myocytes

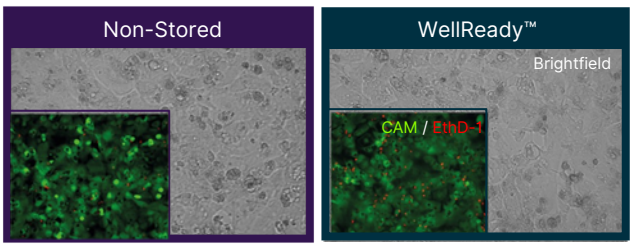
Cardiomyocytes (cardiac muscle cells) are responsible for the beating of the heart. Therefore, any model requires these cells to demonstrate contractile forces.

Human iPSC-derived cardiomyocytes in 96-well plates were stored for 7 days at 20°C using WellReady™. Cells regained their beating pattern with high viability and full metabolic recovery.

a



b



Preservation of iPSC-derived cardiomyocytes using WellReady™. *Cell Recovery, viability and morphology of cardiomyocytes following storage and shipment using WellReady™ for 7 days at 20°C. Cultures were assessed for viable cell number by alamarBlue* ***(a)*** *and calcein-AM (live indicator; green) and ethidium homodimer-1 (dead indicator; red)* ***(b)****.*

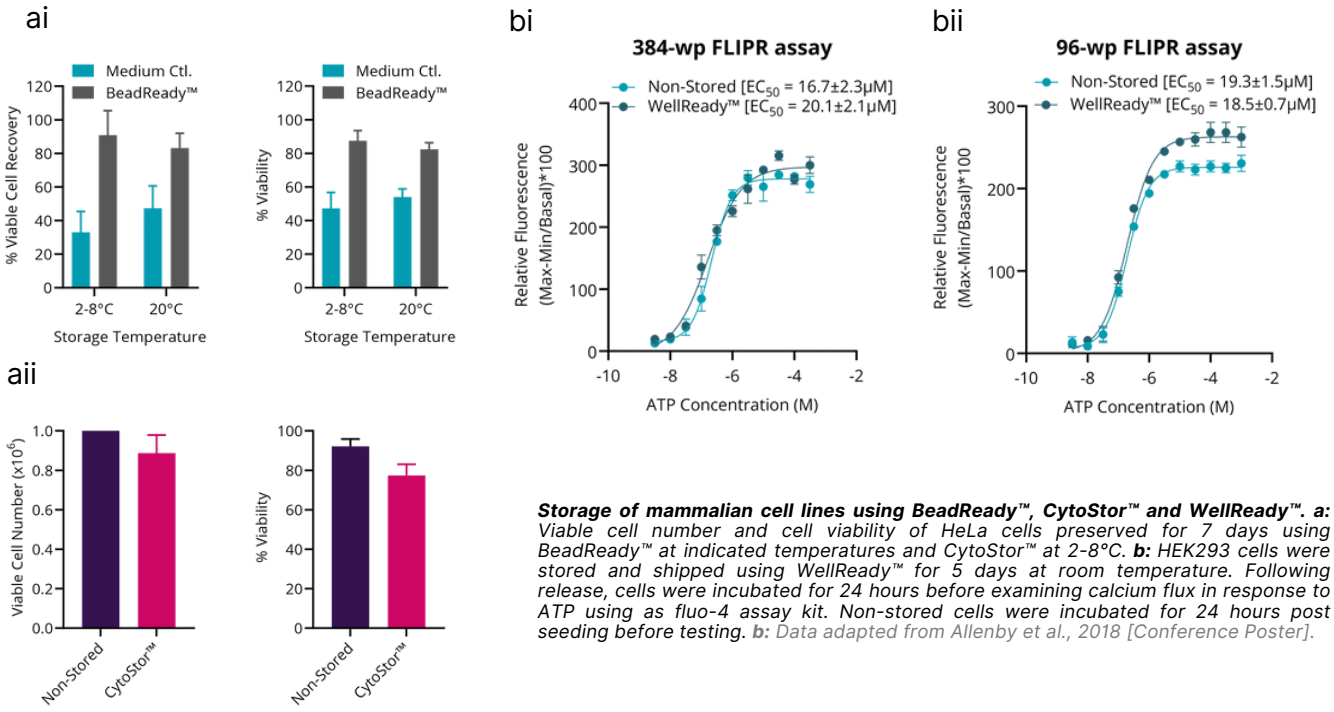
PRESERVING TRANSFORMED CELL LINES DURING HYPOTHERMIC STORAGE

Transformed and Transfected Cell Lines

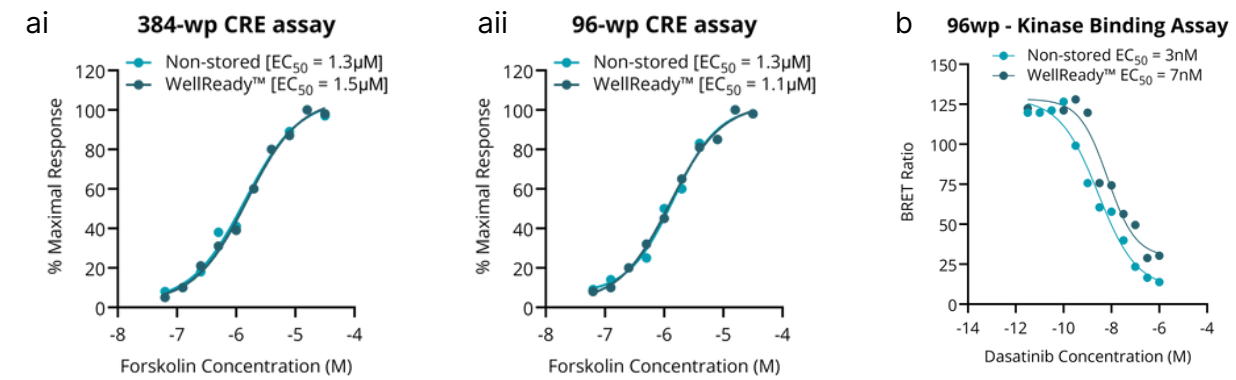
Mammalian cell lines are commonly used for modelling cell biology and disease as well as being an important tool in the manufacture of biopharmaceuticals such as proteins, antibodies and vaccines. Transient transfection of these cells enables rapid, temporary expression of proteins that are used in bioproduction and for generating models such as gene reporter assays. Whilst cell lines are usually transported via dry ice or liquid nitrogen, the prolonged time it takes to revive these cells (usually 2-3 passages), remains an unviable option for transporting transiently transfected cells.

Storage of mammalian cell lines and transiently transfected cells

Transport of cell lines requires expensive and hazardous cryopreservation methods that are prone to failure if shipments are delayed. Atelerix's hydrogel technology requires no specialist logistics and is more environmentally friendly than traditional cryo-logistics. HeLa cells have been shown to be preserved for 7 days using BeadReady™ **(ai)** and CytoStor™ **(aii)**. Cell biology is retained post-storage (using WellReady™) as demonstrated with HEK293 cells and calcium flux response **(b)**. Cell-based assays created through transient transfection can be preserved using WellReady™ and respond comparably to pre-stored assays as demonstrated with cyclic AMP-response element and kinase reporter assays (a-b, lower panel).



Storage of mammalian cell lines using BeadReady™, CytoStor™ and WellReady™. a: *Viable cell number and cell viability of HeLa cells preserved for 7 days using BeadReady™ at indicated temperatures and CytoStor™ at 2-8°C.* ***b:*** *HEK293 cells were stored and shipped using WellReady™ for 5 days at room temperature. Following release, cells were incubated for 24 hours before examining calcium flux in response to ATP using as fluo-4 assay kit. Non-stored cells were incubated for 24 hours post seeding before testing.* ***b:*** *Data adapted from Allenby et al., 2018 [Conference Poster].*



Storage and shipment of transiently transfected HEK293 cells. *HEK293 cells transiently transfected with a cyclic AMP-response element – firefly luciferase (a) or a DDR1 kinase Nanoluc fusion (Promega) (b) cDNA construct were stored and shipped for 5 days at room temperature. Following release, cells were incubated for 24 hours before carrying out assays, non-stored assays were carried out 24 hours after seeding.* ***a:*** *Cyclic AMP levels in response to forskolin were detected by luminescent output.* ***b:*** *DDR1-NanoLuc in the presence of a substrate gives a luminescent signal (BRET), dasatinib competes with the substrate binding site, increasing concentrations of which reduces luminescent signal. Data adapted from Allenby et al., 2018 [Conference Poster].*

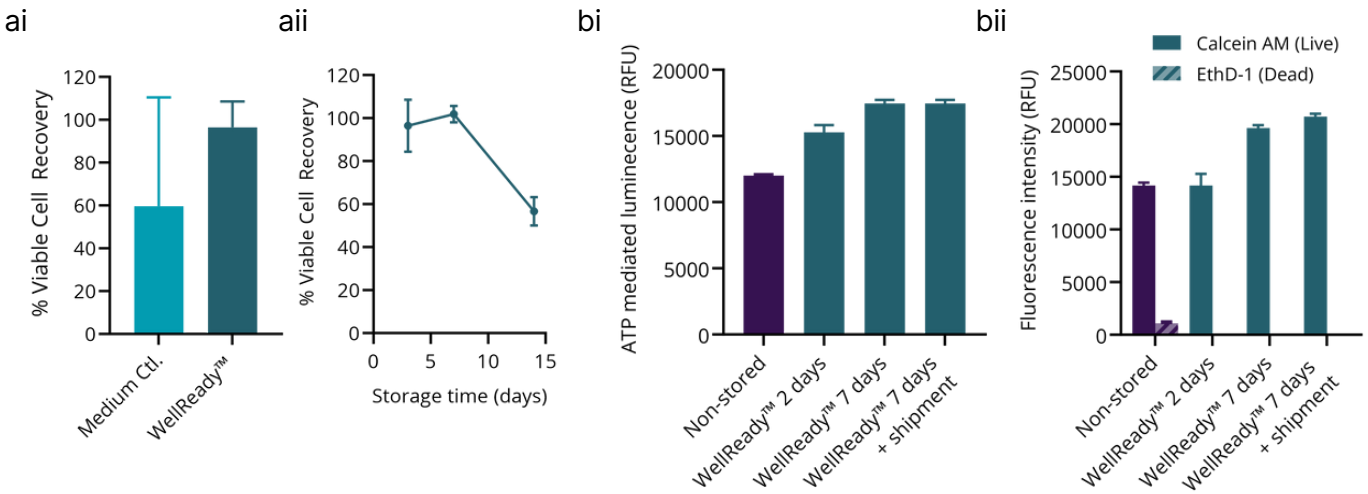
PRESERVING PRIMARY CELL MONOLAYERS DURING HYPOTHERMIC STORAGE

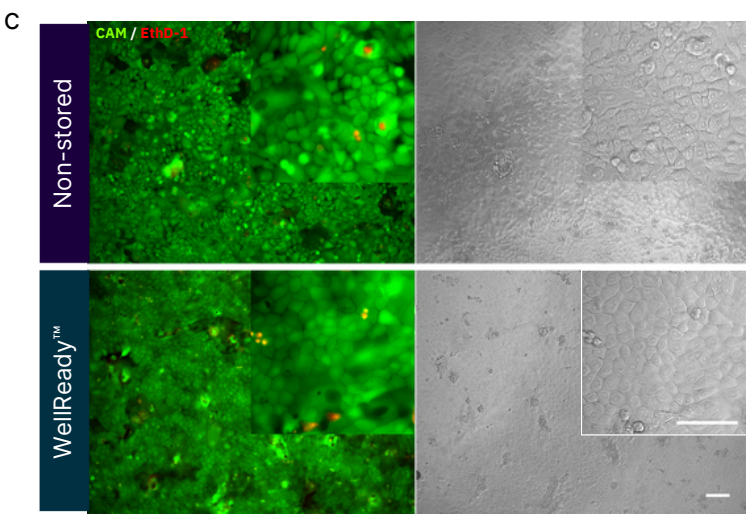
Primary Cell Monolayers

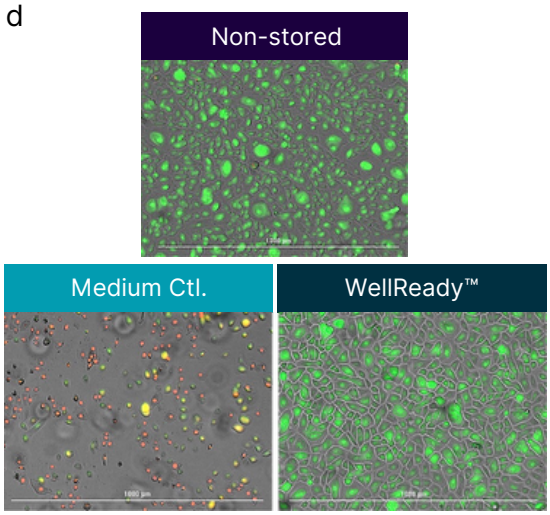
Primary cells that are isolated from tissue and organs are used to model normal cell phenotype. These cells have several advantage over immortalised cell lines which usually contain mutations and abnormalities, however, they often have a finite lifespan and have limited proliferative capacity. Cryopreservation of primary cells induces cellular stress; therefore, given their short shelf life, use of fresh cultures are preferred. Unfortunately, not all sites have readily available access to these fresh samples.

Shipping and storage of primary cell monolayers using WellReady™

Primary cells cultured into monolayers in multi-well plates can be preserved at hypothermic temperatures using WellReady™ and when returned to culture maintain their viability, metabolic activity and function. High recoverable viability has been demonstrated in human adipose-derived mesenchymal stromal cells (hASCs) **(a)** and human derived dermal fibroblasts **(b)** for up to 7 days. Maintenance of cell viability and morphology has been demonstrated in human dermal keratinocyte epithelial cells **(c)** and human airway epithelial cells **(d)**.







Storage of primary cell monolayers at hypothermic temperatures using WellReady™. ai: *Viable cell recovery of hASCs stored at room temperature for 3 days using WellReady™ or medium alone compared to non-stored cells* ***aii****: MSC monolayer viability in WellReady™ for up to 14 days.* ***b:*** *Cell viability of human derived dermal fibroblasts for up to 7 days at room temperature, including a shipment within those 7 days.* ***bi****: Viability was assessed by measuring ATP levels using CellTitre-Glo® assay bii and staining of live/dead cells using fluorescent probes - live cells (Calcein AM) and dead cells (EthD-1).* ***c:*** *Live/Dead staining (Calcein AM/EthD-1) and brightfield microscopy of human dermal keratinocyte epithelial cells preserved for 5 days at 15-20°C.* ***d:*** *Live/Dead staining (Calcein AM/EthD-1) merged with brightfield of human airway epithelial cells stored for 7 days at 2-8 °C. bi and bii data adapted from an external company evaluation.*

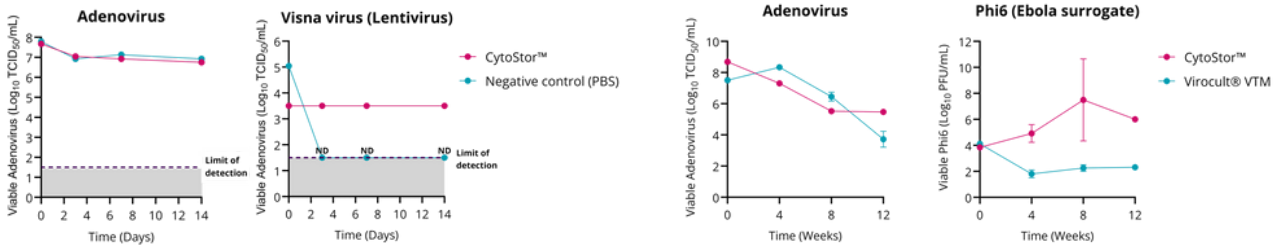
PRESERVING VIRUSES AND VIRAL VECTORS DURING HYPOTHERMIC STORAGE

Viruses

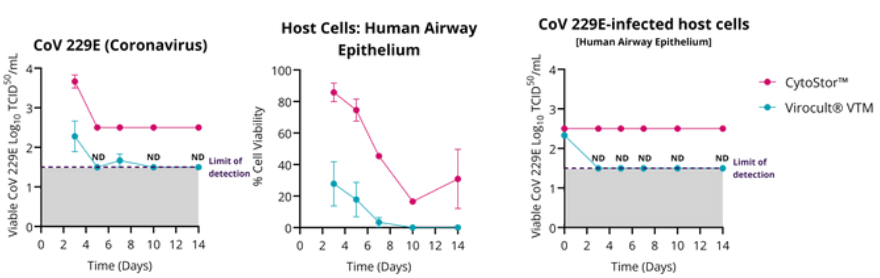
The cytopathic effect of some viruses is stable at room temperature for approximately 72 hours. This short time frame presents challenges to virology, virus surveillance/monitoring and diagnosis. Increasing the shelf life of free viruses and virus infected cells would improve the monitoring of virus transmission and mutations.

Atelerix's product - CytoStor™ can conserve viral envelope integrity and is highly effective at extending the functional viability (cytopathic effect) of free viruses at room temperature for at least 2 weeks, compared to 3 days in standard viral transport medium (VTM). No destabilising effects were observed during storage of Adenovirus **(ai)**, and Visna virus (lentivirus) was preserved for 2 weeks - at least 5-times longer than buffer alone **(aii)**. Adenovirus cytopathic effect **(bi)** and the ebola surrogate Phi6 bacteriophage activity **(bii)** could additionally be stabilised for 12 weeks at room temperature. A 5-fold shelf-life extension compared to VTM was evident when preserving Coronavirus **(ci)** alongside a considerable protection of host cells **(cii).** In combination, no reduction in the cytopathic effect of Coronavirus within infected host cells was achieved **(ciii)**, demonstrating no decline in either viral functional activity or RNA stability (quantity and quality) after room temperature storage.

ai aii bi bii



ci cii ciii



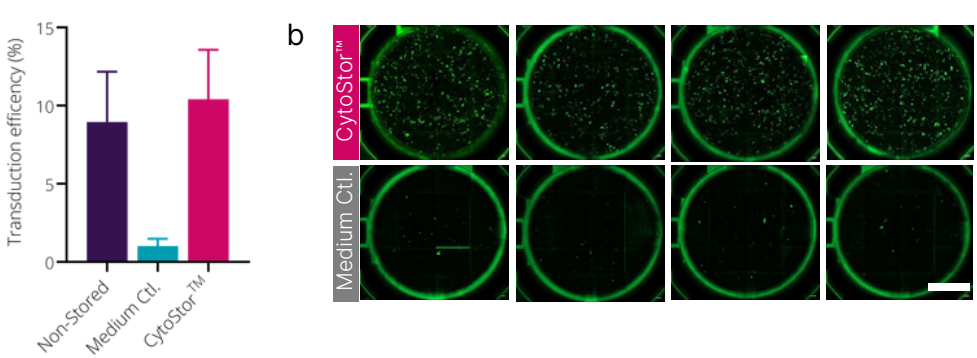
Atelerix's technology is highly effective at extending the functional viability (cytopathic effect) of free viruses. ai*: Adenovirus, and* ***aii****: Visna virus were stored for 2 weeks at room temperature before examining cytopathic effect.* ***bi:*** *Adenovirus and* ***bii:*** *Phi6 were stored for 12 weeks at room temperature before examining cytopathic effect (Adenovirus) and plaque assay (Phi6).* ***ci****: Cov229E,* ***cii:*** *human airway epithelium host cells and* ***ciii:*** *CoV229E infected host cells were stored for 2 weeks at room temperature. Cytopathic effect* ***(ci and ciii)*** *and cell viability* ***(cii)*** *were measured post-storage.*

Viral Vectors

Lentiviral vectors are a useful tool for delivering genetic material into mammalian cells and are becoming increasingly used in cell and gene therapy and as vaccines. However, these vectors are not stable when stored at ambient temperatures and a significant proportion of viral titre is lost upon freeze-thaw.

Lentiviral vectors encoding Green Fluorescent Protein (GFP) can be preserved for up to 7 days at room temperature with no decrease in transduction efficiency. In contrast, lentiviral vectors stored in culture medium only (Medium Ctl.) experience a rapid fall in transduction efficiency.

a b



Atelerix's CytoStor™ is effective at preserving Lentiviral vectors. *Viral vector encoding GFP were stored for 7 days at room temperature using CytoStor™ or medium alone. Virus was transduced into A549 cells. GFP was visualised by fluorescent imaging and number of positive cells calculated.* ***a:*** *quantified percentage transduction efficiency.* ***b:*** *representative images of GFP positive cells.*

NOTES

Quite interesting,
I'm sure you'll agree.

ATELERIX