ATELERIX

ELRIG UK Drug Discovery 2024

HYPOTHERMIC GEL-BASED TECHNOLOGY TO PRESERVE TISSUES AND CELL MODELS AT ROOM TEMPERATURE

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INTRO

The procurement of physiologically relevant cell models is crucial for driving drug discovery research, since the availability and access to fresh human tissues is limited. Such cell models include primary cells, induced pluripotent stem cell (iPSC) derived cells, and 3D organoids. Primary cells and iPSCs are used as models in research in place of less physiologically relevant immortalised cell lines. Organoids are particularly powerful models due to their ability to recapitulate the 3D architecture, cellular heterogeneity, and functional properties of native tissues. This allows for more accurate in vitro studies of organogenesis, disease pathophysiology, and personalised drug responses, offering novel insights.

Cryopreservation is an unsuitable method for extending the limited fresh shelf life of these cell models, as freezing induces cellular stress and restricts downstream applications. Atelerix's novel hydrogel technology addresses the challenge of storing and shipping advanced cell models, by offering room temperature stabilisation of organoids, iPSCs, and cell monolayers, retaining viability, phenotype, and function.

Pretty Cool!

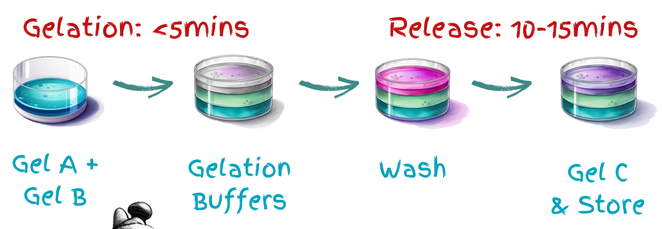
With Atelerix, there's no need for expensive and complicated dry-ice or cryo-shipments, no risk of sample thawing, no geographical complications, and no harm to our environment.

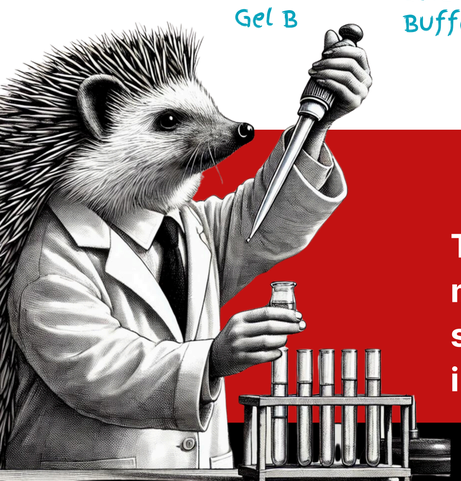
METHOD

At 37°C (human body temperature) cells are metabolically active with a fluid membrane. As the temperature decrease (hypothermia), which occurs during cryopreservation, cells enter a state of hibernation but are susceptible to membrane damage and subsequent death. Atelerix's hydrogel technology stabilises cell membrane integrity, allowing samples to be held at room or refrigerated temperature for extended periods.

TissueReady™ has been specifically developed for the preservation of primary tissue samples at room temperature. Tissues are encapsulated in the gel within 5 minutes, and can be retrieved after storage or shipment simply by the addition of a buffer solution to dissolve the gel within 10-15 minutes

WellReady™ has been specifically optimised for the storage and shipment of cells, organoids, and microtissues, at room temperature. Both encapsulation and retrieval can be performed quickly in just 20-30 minutes.



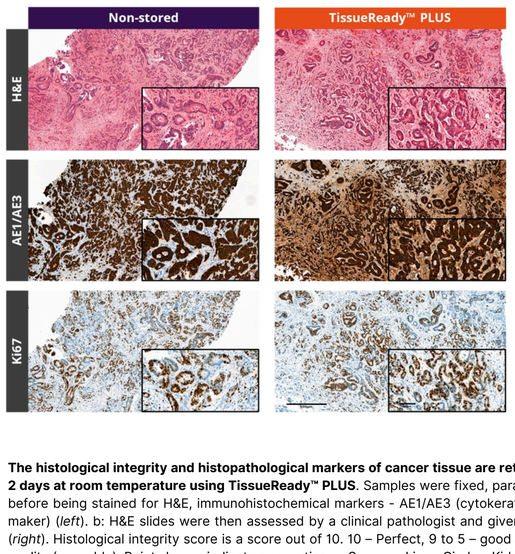


TISSUES (TissueReady)

CANCER TISSUES - HISTOLOGICAL INTEGRITY

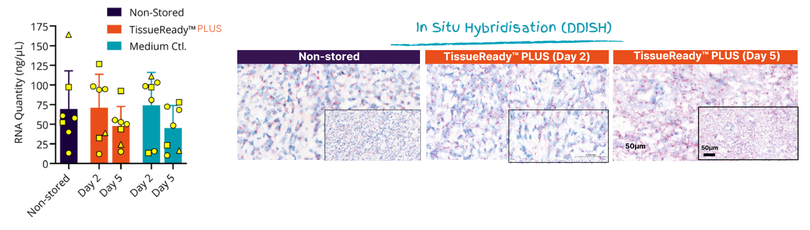
Liver, kidney and oesophageal cancer tissue was retrieved from patients and sent to a clinical pathology laboratory where it was either processed immediately (non-stored) or stored in TissueReady™ PLUS or DMEM medium. Samples were stored for 2-5 days at room temperature before processing.

LIVER TISSUE



The histological integrity and histopathological markers of cancer tissue are retained in tissue preserved for 2 days at room temperature using TissueReady™ PLUS. Samples were fixed, paraffin embedded and sectioned before being stained for H&E, immunohistochemical markers - AE1/AE3 (cytokeratin cocktail) Ki67 (proliferation maker) (*left*). b: H&E slides were then assessed by a clinical pathologist and given a histological integrity score (*right*). 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.

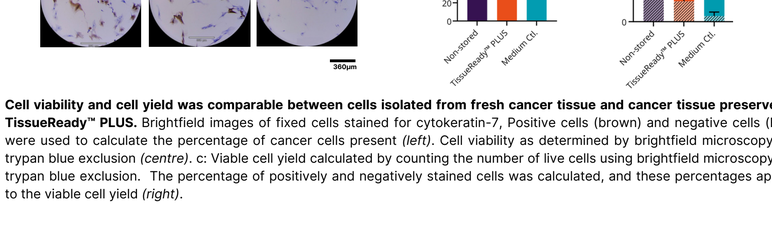
CANCER TISSUES - RNA ISOLATION & ISH



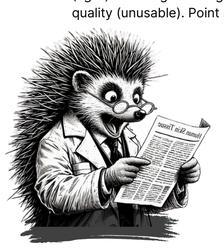
RNA can be isolated and in situ hybridisation can be performed on tissue preserved using TissueReady™ PLUS. RNA was isolated using an RNA extraction kit and RNA quantity was assessed using a nanodrop spectrophotometer (*left*). Datapoint shapes indicate source tissue, Square – Liver, Circle – Kidney, Triangle – Oesophagus. 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 (*right*).

CANCER TISSUES - CELL ISOLATION

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.



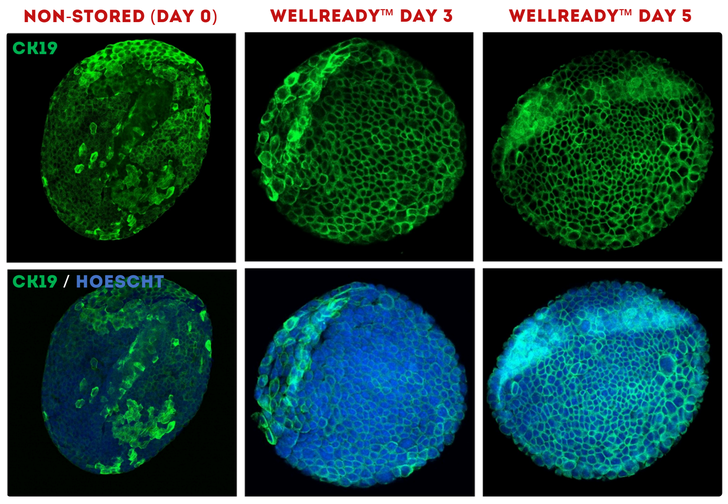
Cell viability and cell yield was comparable between cells isolated from fresh cancer tissue and cancer tissue preserved in TissueReady™ PLUS. Brightfield images of fixed cells stained for cytokeratin-7, Positive cells (brown) and negative cells (Blue) were used to calculate the percentage of cancer cells present (*left*). Cell viability as determined by brightfield microscopy and trypan blue exclusion (*centre*). c: Viable cell yield calculated by counting the number of live cells using brightfield microscopy and trypan blue exclusion. The percentage of positively and negatively stained cells was calculated, and these percentages applied to the viable cell yield (*right*).



CELL MODELS (WellReady)

HEPATIC ORGANOIDS

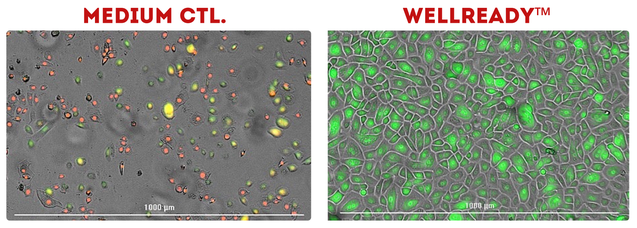
Organoids retained a high viability, continued growth, 3D structure and hepatic marker expression and were retained functionally active drug metabolising enzymes after 72 hours in culture.



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. 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 (*above*). 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) (*above*). Cell viability assessed by measuring ATP levels using the CellTitre-Glo® assay (*below, left*). Functional activity assessed by measuring the activity of Cytochrome P450 1A2 using the P450-Glo™ CYP1A2 Assay (*below, right*). Legend indicates post release culture periods. Scale bars represent 100µm.

HUMAN AIRWAY EPITHELIAL CELLS

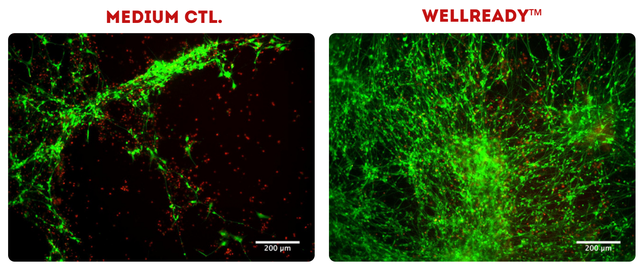
Shipping and storage of primary cell monolayers using WellReady™. Primary cells cultured into monolayers in multi-well plates can be preserved at hypothermic temperatures using WellReady™ and when returned to culture maintain their viability, metabolic activity and function.



Preservation of primary nasal airway epithelial cells using WellReady™. Live/Dead staining (Calcein AM/EthD-1) merged with brightfield of human airway epithelial cells stored for 7 days at 2-8°C, whereby cell viability and morphology is maintained.

IPSC-DERIVED NEURONAL CELLS

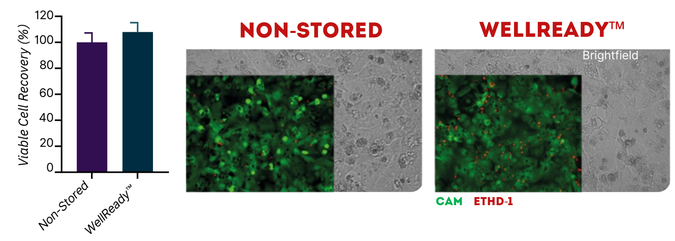
Neuronal cell morphology, metabolic activity and intact axonal networks were retained in WellReady™ plates whereas a loss of viable cells was observed in medium only control plates.



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 WellReady™ at room temperature 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 live/dead staining with calcein-AM (live indicator; green) and ethidium homodimer-1 (dead indicator; red).

IPSC-DERIVED CARDIOMYOCYTES

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 metabolic activity, demonstrating the capability of WellReady™ to preserve the function of cell model.



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 (*left*) and calcein-AM (live indicator; green) and ethidium homodimer-1 (dead indicator; red) (*right*).

Try for free!

SUMMARY

TissueReady™ offers a solution for the preservation of primary tissue and organoids, supporting the generation of primary cell models. WellReady™ can be utilised to store and ship a range of in vitro models at room temperature. Following shipment and storage, these models retain their morphology, high viability, metabolic activity and function, enabling these models to be used in downstream applications for drug discovery, such as reporter assays and drug metabolism/toxicology.

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