A Revolution in Bio-shipping: Room-temperature Transport of 3D Bioprinted Constructs Using WellReady™ In-plate Preservation

Himjyot Jaiswal[1], Isabella Bondesson[1], Emily Dookun[2], Steve Swioklo[2] and Itedale Namro Redwan[1]

[1] CELLINK AB, Gothenburg, Sweden
[2] Atelerix Ltd, Newcastle-upon-Tyne, UK

Abstract

The long-distance transportation of mammalian cells between research laboratories usually requires cryo-preservation in dry ice or liquid nitrogen. Both of these conventional methods of shipping are hazardous in nature and associated with high shipping costs, thereby limiting their routine use. As an alternative to shipping frozen cells, live cells can be shipped either in a flask filled with medium or embedded in hydrogels. Beyond the cost savings, the advantages of shipping live cells include preventing loss or changes in the biological function and viability of the cells, which are often compromised during the freeze-and-thaw cycle required with cryopreservation.

Although shipping live cells embedded in hydrogels has been widely studied, the shipping of cell-laden 3D bioprinted constructs in hydrogels has not yet been evaluated to determine the viability of shape, structure and the biological function of cells. Here, we present the cost-effective shipping of 3D bioprinted constructs at ambient temperature using a novel encapsulation technology WellReady™ from Atelerix. The 3D bioprinted constructs maintained their shape and structure, and the cells remained viable and showed expression of relevant phenotypic markers upon recovery from the hydrogel encapsulation.

Introduction

Cryopreservation is the most common way to ship cells around the globe. The process involves freezing cells in dimethyl sulfoxide (DMSO) to prevent the formation of ice crystals that can damage the cells. However, the shipment of frozen cells with dry ice or liquid nitrogen is expensive and prohibited in many countries. An alternative method widely used is to ship cells in flasks fully filled with culture medium, but this shipping method is not suitable for long distances and cells are susceptible to mechanical damage. Recently, shipping cells embedded in hydrogels has gained in popularity because of its simple, inexpensive and non-hazardous nature as well as its ability to maintain high cell viability. Hydrogels provide a soft and unique three-dimensional (3D) network, allowing small molecules (e.g., nutrients and metabolic wastes) to pass through the membrane without affecting normal cell functions. In addition, hydrogels support cellular membranes and limit osmotic shock to prevent damage, which is a serious issue associated with cryopreserving cells.

While 3D bioprinting is increasingly being adopted by researchers in the fields of regenerative medicine and drug discovery, its tremendous research potential is hampered by the lack of viable shipping options for cell-laden bioprinted constructs to foster much-needed collaborations between labs. To overcome this barrier, we evaluated a plant-based hydrogel technology developed by Atelerix to transport 3D bioprinted constructs across the world. In this technical note, we compare the cell health, viability and phenotypic markers of two cell lines before and after embedding and transporting in the alginate-based WellReady™ in-well preservation hydrogel.

Embedding and shipping of 3D bioprinted constructs

To evaluate the shipment of 3D bioprinted constructs in the WellReady™ hydrogel, an experiment was designed where one-third of the samples were shipped to our collaborator in the UK, one-third were embedded and stored in the lab for 7 days and the last third were kept in culture for the same amount of time (Figure 1). To evaluate how well the hydrogel protected the structure of the bioprinted construct throughout the shipping process, droplets or 5 x 5 mm grids were printed using the BIO X™ 3D bioprinter. Two cell lines—induced pluripotent stem cells (iPSCs) and mesenchymal stem cells (MSCs)—were 3D bioprinted in gelatin-based bioinks GelMA and GelXG.

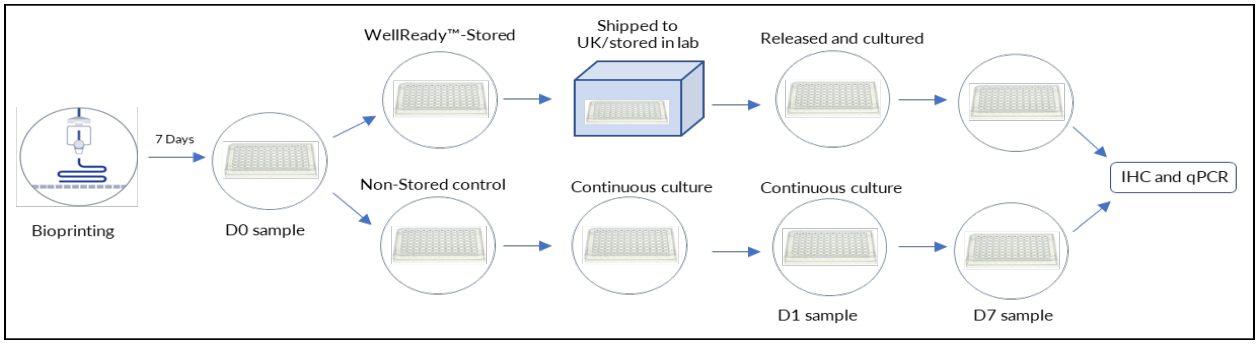

Figure 1. ***Schematic of the experiment.*** *Samples were collected before embedding (D0), Day 1 (D1) and Day 7 (D7) after releasing from WellReady™.*

The cells were grown for 7 days in their respective medium then embedded in the hydrogel as described in Figure 2 (WellReady™ Protocol). The WellReady™-stored constructs were either shipped to the UK or stored at room temperature in Sweden for 7 days.

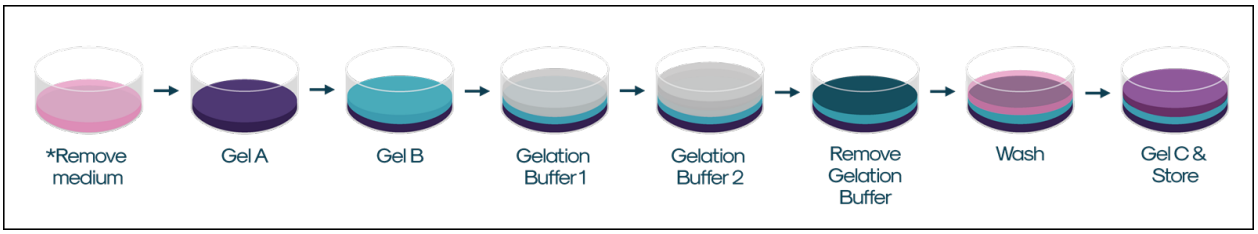

Figure 2. ***Step-by-step process for embedding 3D bioprinted constructs in WellReady™.*** *Remove the medium from bioprinted constructs before processing the constructs for encapsulation using Gel A, Gel B and Gel C. See WellReady™ Protocol.*

Analysis of 3D bioprinted construct structure and cell health

In a one-step process, the previously encapsulated 3D bioprinted constructs can be released by dissolving the WellReady™ hydrogel. The hydrogel is dissolved by reversing the hydrogel ionic crosslinking and washing it off using either phosphate buffer saline (PBS) or culture medium. The released construct can then be maintained in culture or used in biochemical and cell biology assays. The WellReady™-stored constructs in the study were easily retrieved using the dissolution buffers and maintained in culture for 7 days. Samples were analyzed on Day 1 and Day 7 after the release. Optical analysis was also conducted after the release to evaluate how well the constructs, both droplets and grids, retained their shape and structure during the storage, transportation and release from the WellReady™ hydrogel (Figure 3).

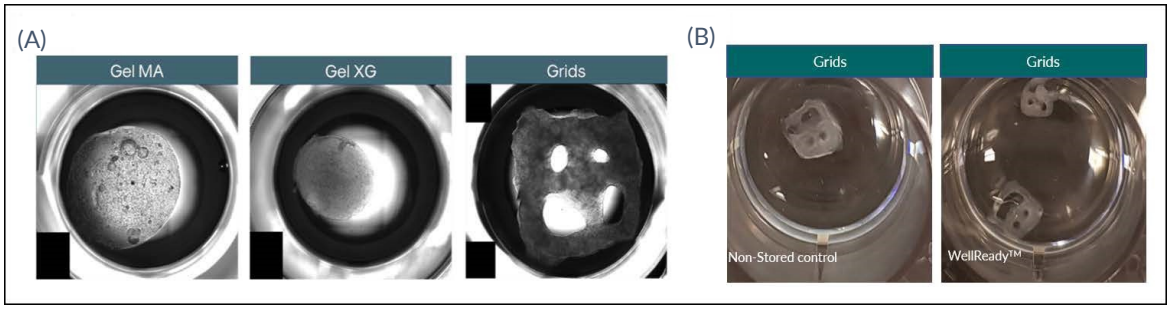

Figure 3. ***Printed constructs after storage, transport and release from WellReady™ hydrogel.*** *(A) Samples showing the structural integrity of the bioprinted constructs for the different bioinks that had been WellReady™-stored, shipped to the UK and finally released. The grid was bioprinted using GelXG and maintained the shape during shipment. (B) Integrity of grid structures stored in Sweden in continuous culture (non-stored control) and WellReady™-stored and released from hydrogel. Grids were printed with GelXG. Droplets were bioprinted using both GelMA and GelXG.*

Cellular assays revealed that cells maintained high viability and retained the phenotypic markers after WellReady™ storage and transportation at room temperature. In this example, we checked the viability of iPSCs and MSCs in the 3D bioprinted constructs using Calcein AM/PI staining. Both cell types showed no difference in viability when WellReady™ stored and released as compared to non-stored control samples (Figure 4). The hydrogel therefore supported cell viability for the duration of the 7-day storage period at room temperature.

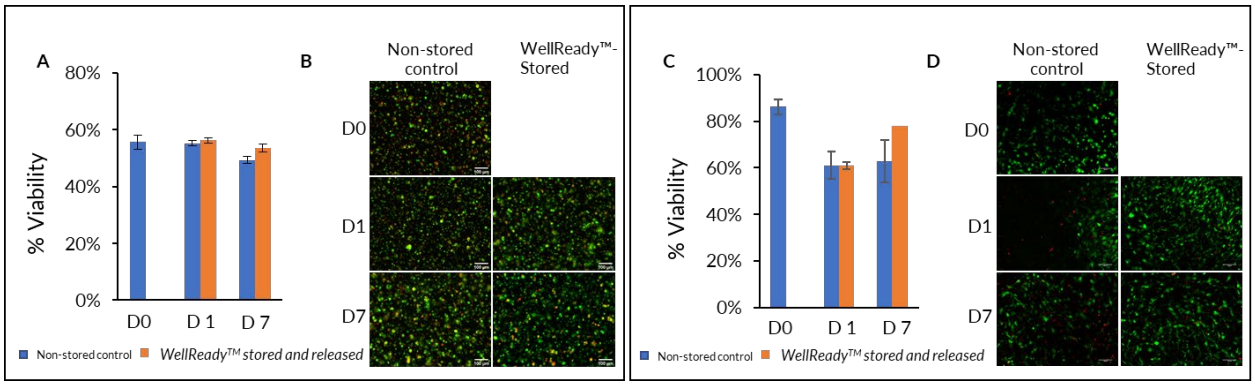

Figure 4. ***Viability of iPSCs in GelMA [A, B] and MSCs in GelXG [C, D].*** *Graphs show the viability percentage of 3D bioprinted droplets of iPSCs [A] and MSCs [C] on Day 0, Day 1 and Day 7. Representative image of Calcein (green) and PI (red) staining of 3D bioprinted droplets of iPSCs [B] and MSCs [D] on Day 0, Day 1 and Day 7. Scale bar = 100 µm.*

Because sensitive cells like iPSCs are easily compromised and start to differentiate in response to stress stimuli, we evaluated, using qPCR and immuno-histochemistry (IHC), the pluripotency marker (Oct4A) in the samples before and after embedding (Figure 5).

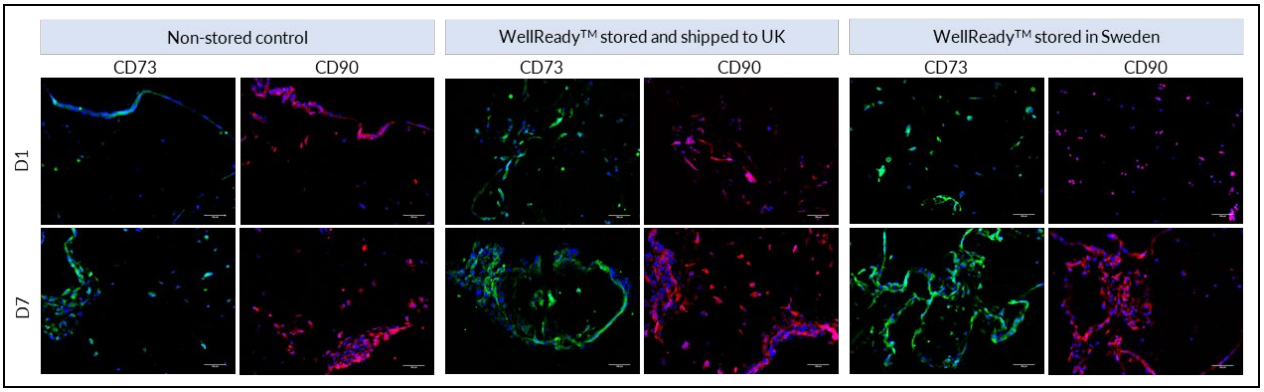

Figure 5. ***Expression of CD73 and CD90 as determined by immunofluorescence.*** *CD73 expression (green) and CD90 (red) in MSCs grown in GelMA for the different conditions: Nuclei are visualized by DAPI (blue). Cells were fixed and stained at Day 1 (D1) and Day 7 (D7) after releasing from WellReady™. Scale bar = 100 µm.*

There was no significant difference observed in Oct4A expression in the non-stored control and WellReady™ stored samples in either droplet or grid 3D bioprinted constructs, indicating that iPSCs maintained pluri-potency during WellReady™ storage, transportation and release.

In addition, we looked at the cell surface markers for MSCs, CD73 and CD90, to confirm the identity of these cells. The immunostaining results indicated the presence of both CD73 and CD90 surface markers in both the non-stored control and WellReady™-stored samples of MSCs with no significant difference in both GelMA and GelXG (Figure 6). Therefore, storage in the hydrogel had no effect on the cellular expression of these MSC phenotypic markers.

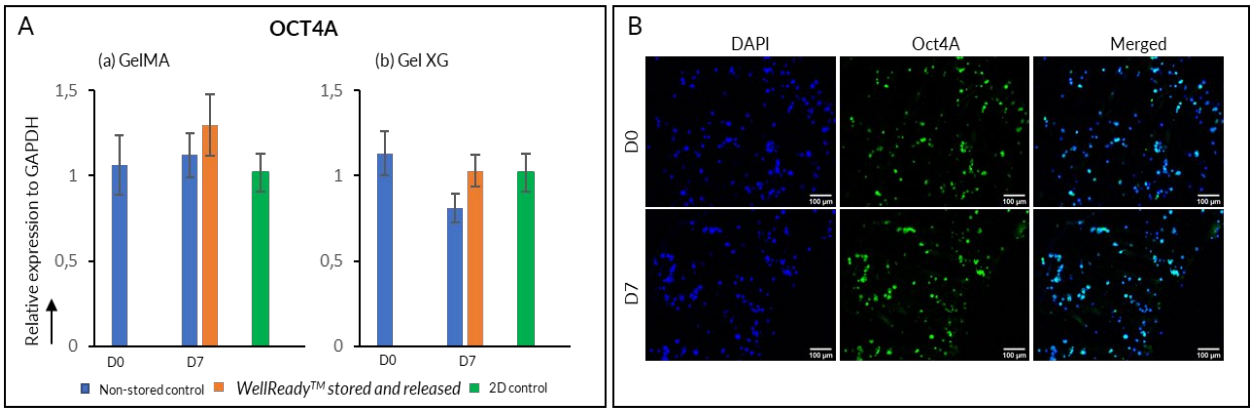

Figure 6. ***Expression of Oct4A as determined by qPCR (A) or immunofluorescence (B). (A)*** *Graph shows the expression of Oct4A in iPSCs 3D bioprinted in either (a) GelMA or (b) GelXG bioink.-Cells were collected either one day before embedding (D0) or 7 days after releasing samples from WellReady™ (D7).* ***(B)*** *Oct4A expression (green) in iPSCs grown in GelMA. Nuclei are visualized by DAPI (blue). Cells were fixed and stained either one day before embedding (D0) or 7 days after releasing from WellReady™. Scale bar = 100 µm.*

Summary

The advantages, and conclusions from this study, of shipping cell-laden 3D bioprinted constructs embedded in WellReady™ in-plate preservation include:

- Quick and easy process.
- With room-temperature shipping, there is no need for dry-ice containers and the hazards associated with cryopreservation.
- Shipping is more cost-effective and requires less paperwork.
- The structural integrity of the embedded constructs is retained throughout the storing, transporting and handling process.
- Viability and cell health are not compromised during transport or storage at room temperature.
- Embedded constructs are ready to use after gentle release from the hydrogel.

Contact us

U.S. phone:
(+1) 833-235-5465

Email:
sales@cellink.com

European phone:
+46 31-128 700

Website:
www.cellink.com