Technical Note

Recovery of viable cells from LunaGel™ Matrices using the LunaGel™ Cell Recovery Kit

gelomics
3D cell culture technologies

Introduction

Some laboratory techniques require cells to be released from 3D cell culture hydrogel matrices for efficient processing and experimental analysis. The LunaGel™ Cell Recovery Kit is a fully optimised, ready-to-use solution containing an enzymatic lysis reagent and buffer that digests gelatin- and collagen-based hydrogels in < 1 hour of incubation at 37 °C. The product is used to recover viable cells, spheroids, or organoids from gelatin-based LunaGel™ Photocrosslinkable Extracellular Matrices for downstream applications such as re-seeding, nucleic acid extraction, flow cytometry, protein extraction, single-cell analysis, and many more.

In the following study, we demonstrate the effective digestion of LunaGel™ Photocrosslinkable Extracellular Matrices of varying stiffness to release breast cancer cells with high viability.

Figure 1: The Gelomics® LunaGel™ Cell Recovery Kit (Cat. No. SKU0015) contains 50 ml of Cell Recovery Buffer and an enzymatic Cell Recovery Reagent – sterile and ready-to-use

Materials and Methods

Cell encapsulation:

Epithelial breast cancer cells (MDA-MB-231) were encapsulated in LunaGel™ - Bovine Bone Gelatin (Low Stiffness Kit; Gelomics Cat. No SKU0005) at a density of 5 × 10^6 cells/mL of hydrogel and different mechanical stiffnesses (2, 4 and 6 kPa) in 48 well plates following the LunaGel™ cell encapsulation protocol. Cell-laden constructs were cultured in Dulbecco's Modified Eagle Medium supplemented with 10 % (v/v) foetal bovine serum for 3 days before cell recovery.

Live Dead Assay:

Prior to cell recover, the viability of MDA-MB-231 cells in LunaGel™ ECMs was assessed using live/dead staining with fluorescein diacetate (FDA) and propidium iodide (PI). Living (green) and dead cells (red) were quantified using ImageJ.

Cell Recovery:

LunaGel™ Cell Recovery Reagent was dissolved in 5 ml of Cell Recovery Buffer to create a 10x stock solution. At the day of cell recovery, the 10x Cell Recovery Solution was diluted with Recovery Buffer and warmed to 37 °C. Following removal of cell culture media, 1 ml of Cell Recovery Solution was added to each sample and incubated at 37 °C for 30 minutes. Digestion of cell-laden LunaGel™ ECM hydrogels with 2, 4, and 6 kPa elastic modulus, respectively, was assessed by determining weight loss over time. The resulting suspension containing recovered cells was transferred to centrifuge tubes, diluted with cell culture media, and centrifuged at 250 × *g* to pellet cells. Cells were resuspended in fresh media and cell viability was determined using trypan blue staining. Cells were then replated into 24 well plates to assess cell attachment and proliferation.

Life in 3D

Results

The digestion of LunaGel™ ECM hydrogels with 2, 4, and 6 kPa elastic modulus, respectively, was assessed by determining hydrogel sample weight loss over 30 minutes of incubation with LunaGel™ Cell Recovery Solution at 37°C. Independent of construct stiffness, samples were fully digested within 25 minutes (Figure 2).

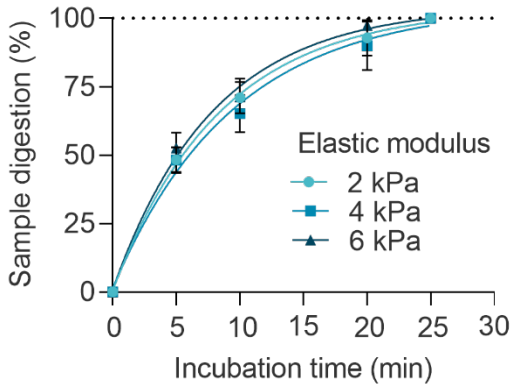

Figure 2: The LunaGel™ Cell Recovery Kit quickly digests gelatin-based LunaGel™ ECMs to release cells.

MDA-MB-231 containing LunaGel™ ECMs with elastic moduli of 2, 4, and 6 kPa, respectively, were incubated with the LunaGel™ Cell Recovery Solution and hydrogel digestion was assessed over time (n=6; mean ± STDEV).

Quantification of cell viability pre- and post-cell recovery from LunaGel™ ECM hydrogels demonstrated excellent cell survival following incubation with the LunaGel™ Cell Recovery Solution (Figure 3a and b). To investigate whether recovered cells remained functional, released cells were plated on tissue culture plastic and cultured overnight. Cells released from all LunaGel ECM™ stiffness groups readily attached and showed morphological features of healthy MDA-MB-231 cells (Figure 3c).

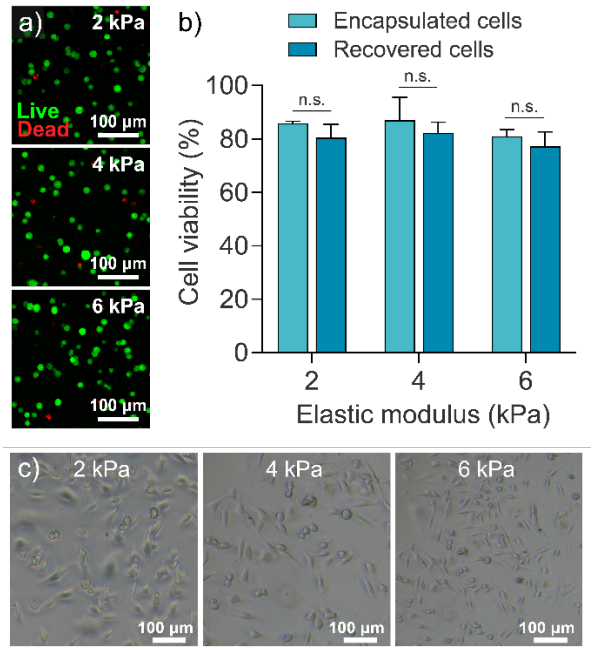

Figure 3: Cell Recovery from LunaGel™ ECMs does not impact cell viability.

a) Live/dead viability staining of MDA-MB-231 cells encapsulated in LunaGel™ ECMs with elastic moduli of 2, 4, and 6 kPa, respectively, following 3 days of culture (live cells appear green, dead cells appear red). b) Quantified cell viability pre- and post- cell recovery using the LunaGel™ Cell Recovery Kit (n=6; mean + STDEV). c) Recovered cells readily attach to tissue culture plastic.

Conclusion

The LunaGel™ Cell Release Kit is an easy-to-use, off-the-shelf solution to recovering viable cells from hydrogels without disrupting their cellular functionality.