Cell Retrieval from RASTRUM 3D Bioprinted Hydrogels Enables High-Quality RNA and Protein Extraction

Highlights

The **RASTRUM™** 3D bioprinting platform developed by Inventia Life Science enables efficient generation of 3D cell models. Here, we introduce the **RASTRUM** Cell Recovery Solution, which enables:

- Rapid dissociation of hydrogel matrices for retrieval of viable cells
- RNA and protein extraction from cells for downstream analyses

Introduction

Detection of protein interactions, changes to gene expression or genetic mutations are vital to increase our understanding of biological processes in health and disease. The **RASTRUM** bioprinter enables high-throughput generation of 3D cell models. These cultures are optimal for *in situ* investigations into various biological processes and responses to drug or cytokine treatments.

The **RASTRUM** Cell Recovery Solution rapidly dissociates the hydrogel matrix while maintaining spheroid integrity and cell viability, enabling extraction of high-quality RNA and protein from retrieved cells. This allows for high resolution analysis of intracellular functions at the molecular level, cell classification assays such as flow cytometry and re-plating of cells where desired.

RASTRUM provides a complete platform for the generation of 3D cell models and their downstream analysis. This application note will demonstrate that cells encapsulated in **RASTRUM** 3D bioprinted matrices (**Figure 1**) can be successfully and easily retrieved from the matrix to enable investigations into gene and protein expression.

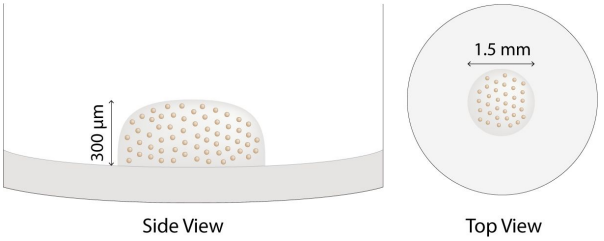

Figure 1: Schematic of the **RASTRUM** 3D bioprinted hydrogel matrix containing multicellular tumour spheroids (figure not to scale).

Methods

Reagent Preparation

The **RASTRUM** Cell Recovery Solution was stored at -20°C and thawed at room temperature.

3D Bioprinting

The bioink solution was printed into a 96-well plate, followed by the activator solution containing MCF-7 (breast carcinoma) or HepG2 (hepatocellular carcinoma) cells to form uniform hydrogels in every well. Repeated layer-by-layer printing resulted in the generation of 3D cell models (**Figure 1**), with cells distributed throughout the hydrogel volume (**Figure 2**).

Cell Retrieval

Hydrogel dissociation was performed on 3D cell models printed using **RASTRUM**. The hydrogel was washed and incubated with PBS for 5 minutes (37°C, 5% CO_2). After aspiration of the PBS, cell models were incubated with the **RASTRUM** Cell Recovery Solution for 10 minutes (37°C, 5% CO_2) (incubation time varies depending on the matrix type). Once the hydrogel had been dissociated, whole spheroids

were transferred into a microcentrifuge tube and gently collected by centrifugation at 100 x *g* for 5 minutes at room temperature. Pelleted spheroids were further dissociated using 0.05% trypsin-EDTA to form a single cell suspension for downstream analysis.

Live/Dead Staining

Fluorescent staining for Live/Dead viability of the retrieved cell suspension was performed immediately after hydrogel dissociation. Cells were washed with PBS before incubation with fresh PBS containing 1 µM of Calcein-AM and 2 µM of Ethidium Homodimer III (EthD-III; Biotium) at 37ºC for 30 minutes. Cells were washed twice with PBS before imaging using a fluorescence microscope (Zeiss Observer 7) using the 488 nm and 594 nm channels. Live/Dead quantification was performed using CellProfiler as previously described.[1]

RNA Extraction

In a parallel experiment, HepG2 3D bioprinted cell models were dissociated and RNA extracted using ReliaPrep™ RNA Cell Miniprep system (Promega) from 10 pooled wells of a 96-well plate (n = 4). Sample RNA concentration and A260/280 ratio for RNA purity was measured using a NanoDrop™ 2000 Spectrophotometer (Thermo Fisher Scientific). RNA integrity and quality was confirmed using Qubit™ RNA IQ Assay kit (Invitrogen).

Protein Extraction

HepG2 3D bioprinted cell models were dissociated and cell lysates isolated using 1x RIPA lysis buffer from 10 pooled wells of a 96-well plate (n = 3). Total protein concentration was determined using DC Protein Assay (Bio-Rad Laboratories). Cell lysate (10 µg) was used for western blotting for housekeeping proteins HSP90 and GAPDH (1:1000 and 1:5000 respectively; Cell Signalling Technologies).

Results and Discussion

Hydrogel dissociation was performed 7 days post-printing using the **RASTRUM** Cell Recovery Solution. **Figure 2** shows the process of hydrogel dissociation. Spheroids encapsulated in hydrogel (**A**) stay intact after the hydrogel is dissociated following reagent addition (**B**), enabling removal from the well (**C**) and re-plating of the previously encapsulated spheroids (**D**).

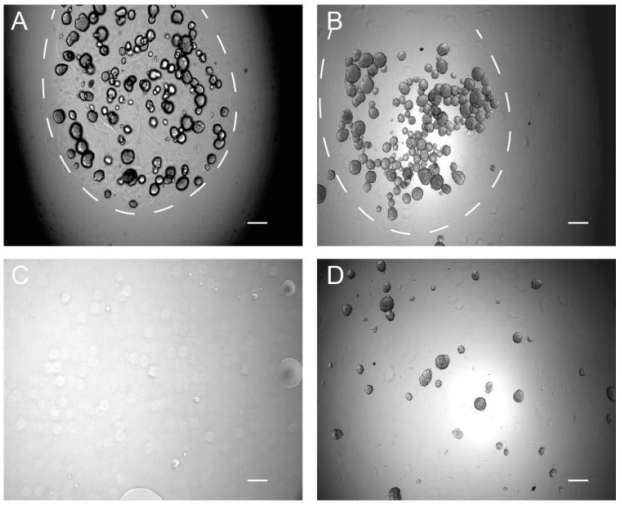

Figure 2: MCF-7 spheroids encapsulated in 3D bioprinted hydrogels. (**A**) Encapsulated spheroids before dissociation, with the dashed line indicating the hydrogel matrix. (**B**) Spheroids remain intact after a 10 minute incubation with **RASTRUM** Cell Recovery Solution. The dotted line indicates the location of the former hydrogel matrix. (**C**) Empty wells post-cell retrieval show efficient hydrogel dissociation and cell retrieval. (**D**) Previously encapsulated spheroids remained intact after dissociation from the matrix. Scale bars = 250 µm.

Live/Dead staining revealed high cell viability (93.40 ± 3.04%) after dissociation of MCF-7 spheroids to form a single cell suspension, indicating that the cell extraction process was not detrimental to cell viability (**Figure 3**).

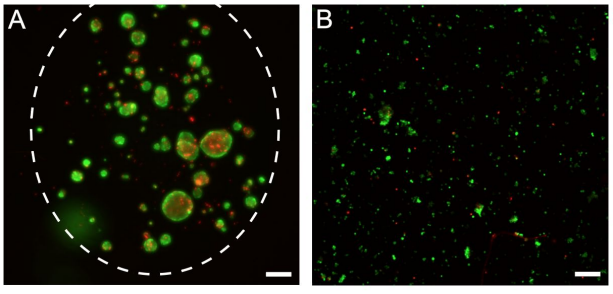

Figure 3: Live/Dead staining of MCF-7 cells before and after retrieval from the hydrogel. Cells were stained with Calcein-AM for live cells (green) or EthD-III for dead cells (red), with dashed lines indicating the hydrogel matrix. High cell viability was observed in (**A**) encapsulated spheroids before hydrogel dissociation and (**B**) the cell suspension obtained after hydrogel dissociation and subsequent spheroid dissociation using trypsin. Scale bars = 200 µm.

The amount of RNA extracted from HepG2 cells retrieved from **RASTRUM** matrices was related to the cell density of the 3D cultures, and was in the range of 100-200 µg per row of the 96-well plate. The average A260/280 ratio of the extracted RNA was 2.02 ± 0.03, indicative of high RNA purity. The average RNA IQ score was 8.95 ± 0.29 (**Figure 4B**), which indicates minimal RNA degradation and suitability for downstream gene expression applications.[2] Cell lysates extracted from HepG2 cells yielded 59.85 ± 5.09 µg of protein per pooled sample. Probing for HSP90 and GAPDH (**Figure 4C**) resulted in clean bands indicative of high protein integrity following hydrogel dissociation.

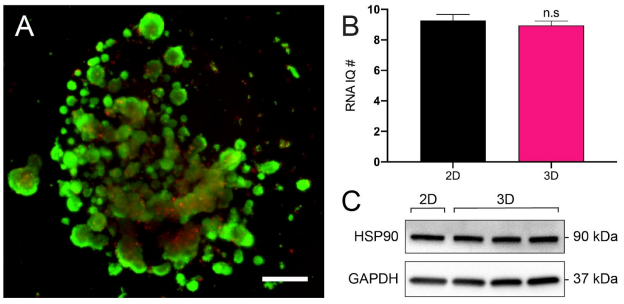

Figure 4: (**A**) HepG2 cells 3D bioprinted in **RASTRUM** matrices formed large spheroids and Calcein-AM/EthD-III staining (live = green, red = dead) indicated high cell viability after 7 days in culture. (**B**) RNA from HepG2 cells grown in 3D culture had equivalent high Qubit™ RNA IQ scores compared to RNA isolated from cells grown in 2D culture. (**C**) Western blotting for HSP90 and GAPDH showed clear bands from 10 µg of cell lysate extracted from 3D bioprinted HepG2 cells. Scale bar = 400 µm.

Summary and Conclusions

In this application note, we show that the **RASTRUM** Cell Recovery Solution enables efficient dissociation of **RASTRUM** 3D bioprinted hydrogel matrices while maintaining spheroid integrity and cell viability. This enables extraction of high quality RNA and protein from retrieved cells for downstream analyses of **RASTRUM** 3D bioprinted cell models.

References

1. McQuin, C et al. (2018), *PLoS Biol*, 16(7):e2005970. doi: 10.1371/journal.pbio.2005970
2. Thermo Fisher Scientific (2018). *Qubit RNA IQ Assay: a fast and easy fluorometric RNA quality assessment*. Application Note. Retrieved from Thermo Fisher Scientific website: https://assets.thermofisher.com/TFS-Assets/BID/Application-Notes/qubit-rna-iq-assay-fluorometric-rna-quality-assessment-app-note.pdf

Contact Us

Inventia Life Science Pty Ltd
www.inventia.life

Australia
Suite 1.13 / 90-96 Bourke Road
Alexandria NSW 2015
Telephone: 1800 849 128
Fax: +61 2 8399 0304
Email: info@inventia.life

US
Telephone: +1 833 462 5959

Ireland
Telephone: +353 818 370 035