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).](image)

**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% CO₂). After aspiration of the PBS, cell models were incubated with the RASTRUM Cell Recovery Solution for 10 minutes (37°C, 5% CO₂) (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 37°C 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-plateing of the previously encapsulated spheroids (D).

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).
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. 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.

**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**