High-Throughput Production of Matrix-Embedded Multicellular Tumour Spheroids by Drop-on-Demand 3D Bioprinting

Highlights

The **RASTRUM™** 3D bioprinting platform developed by Inventia Life Science:

- Enables the creation of reproducible and tunable 3D hydrogel structures which closely resemble the extracellular matrix
- Supports the viability and sustained growth of tumour cells, including MCF-7 breast cancer cells and LNCaP prostate cancer cells, in 3D culture

Introduction

Three-dimensional (3D) multicellular tumour spheroid models that resemble small tumours and micrometastases are routinely used by researchers to study the biology of solid tumours *in vitro*[1]. Matrix-embedded tumour spheroids enable the inclusion of key elements of the extracellular matrix in the tumour model, better recapitulating the *in vivo* tumour context[2].

Despite their utility, the generation of 3D tumour spheroid models via manual methods is time-consuming and low-throughput[3]. Additionally, many current models have low reproducibility due to the inherent batch-to-batch variation of animal-derived matrices[4-5]. Therefore, there is a clear and unmet need for the reproducible production of tunable 3D tumour models for research applications.

RASTRUM combines drop-on-demand bioprinting with synthetic modifiable matrix systems to make the creation of matrix-embedded tumour cell spheroids simpler, reproducible and efficient. This application note will provide an overview of the creation of breast and prostate cancer tumour spheroids using **RASTRUM**.

Methods

3D Bioprinting

RASTRUM bioink F38 and MCF-7 or LNCaP cells resuspended in activator F41 ($5x10^6$ cells/mL) were added to separate reservoirs of the pre-supplied **RASTRUM** cartridge prior to printing. Following the printer cleaning stage using the supplied reagents within the pre-filled **RASTRUM** cartridge, bioink was printed into each well of a 96-well plate, followed by cells in activator, to form small uniform hydrogels in every well. Repeated layer-by-layer printing resulted in the generation of the 3D cancer cell model, with cells evenly distributed throughout the structure (**Figure 1**).

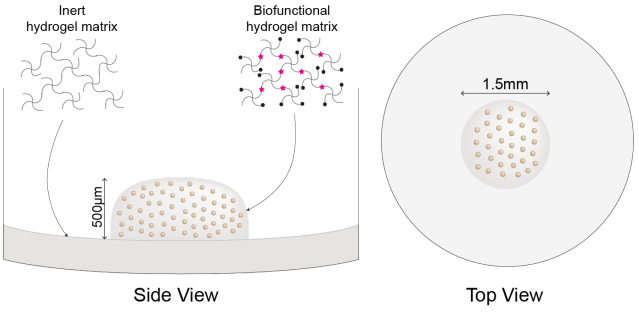

Figure 1: Representation of the **RASTRUM** 3D bioprinted hydrogel structure containing multicellular tumour spheroids (figure not to scale).

Cell Viability

In addition to brightfield images captured at days 3 and 7 post-printing, cell viability was determined at days 1 and 7 post-printing by incubating cells with 1 µM calcein-AM and 2 µM ethidium homodimer-III (Biotium) at 37ºC for 30 minutes. All images were captured using a fluorescence microscope (Observer 7, Zeiss)

and live/dead quantification was performed using CellProfiler as previously described[6].

Results and Discussion

The whole 96-well plate was printed in less than 20 minutes. The printed structure was a small gel plug of approximately 300 nL, which is orders of magnitude below what is achievable with manual methods.

MCF-7 and LNCaP cells were dispersed throughout the hydrogel structure and formed multiple small spheroids within the gel three days after printing (**Figure 2**). Seven days post-printing, large multicellular spheroids had formed in the gel, indicating that cells were able to proliferate and form spheroids with an observable size distribution resulting from natural biological heterogeneity.

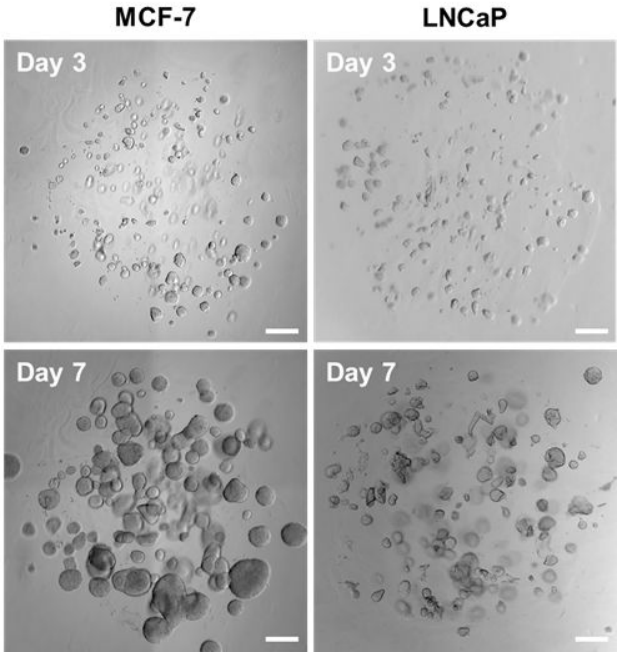

Figure 2: Representative brightfield images of MCF-7 and LNCaP cells encapsulated in the **RASTRUM** hydrogel system over 7 days in culture. Scale bars are 200 µm.

Cell viability 24 hours after printing was 90.1 ± 8.4 % for MCF-7 cells and 89.4 ± 4.2 % for LNCaP cells (**Figure 3**). Seven days post-printing, the viability of MCF-7 and LNCaP cells encapsulated within the hydrogel system was 98.4 ± 1.2 % and 99.5 ± 0.4 %, respectively (**Figure 3**), indicating that the printing process and hydrogel matrices supported cell viability over seven days of culture.

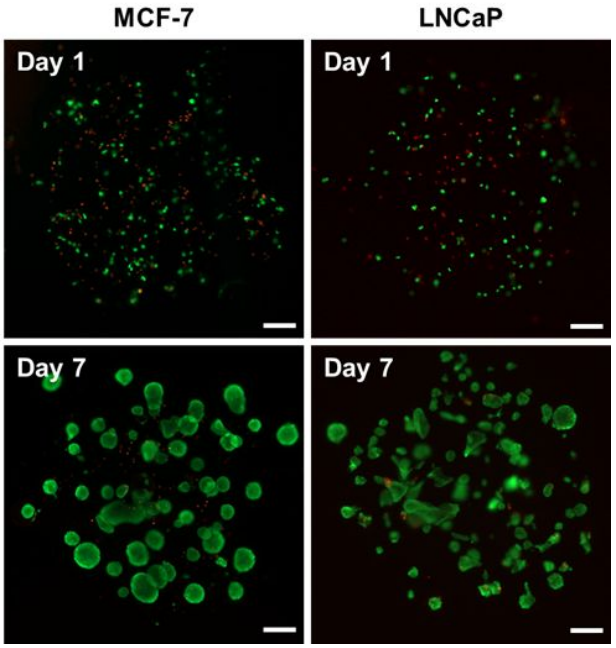

Figure 3: Representative live/dead cell viability images (green = viable, red = non-viable) of MCF-7 and LNCaP cells encapsulated in **RASTRUM** gel matrices at days 1 and 7 post-printing. Scale bars are 200 µm.

Summary and Conclusions

By using **RASTRUM** to print MCF-7 breast cancer cells and LNCaP prostate cancer cells as representative examples, we have demonstrated the quick and efficient encapsulation of tumour cells within a biocompatible matrix that supports the growth of multiple viable multicellular tumour spheroids. Therefore, **RASTRUM** can be utilised in the high-throughput formation of 3D tumour spheroid models for various downstream biological research applications.

References

1. Carver, K et al. (2014), *Mol Ther Nucleic Acids*, 3(3): e153. doi: 10.1038/mtna.2014.5.

2. Ferreira LP et al. (2018), *Acta Biomater*, 75:11-34. doi: 10.1016/j.actbio.2018.05.034.

3. Gill, B et al. (2014), *J Biomech*, 47(9):1969-78. doi: 10.1016/j.jbiomech.2013.09.029.

4. Worthington, P et al. (2015), *Front Oncol*, 5:92. doi: 10.3389/fonc.2015.00092.

5. Hughes, C et al. (2010), *Proteomics*, 10:9. doi: 10.1002/pmic.200900758.

6. McQuin, C et al. (2018), *PLoS Biol*, 16(7):e2005970. doi: 10.1371/journal.pbio.2005970.

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