Drop-on-Demand Printing Technology of the RASTRUM 3D Bioprinter

Highlights

The drop-on-demand technology of the **RASTRUM™** 3D bioprinter enables:

- Quick and efficient printing of matrix components and cells
- Precise control of droplet deposition to build complex 3D structures
- Preservation of cell viability post-printing

Introduction

Matrix-embedded 3D cell models are more physiologically relevant than 2D cultures.[1] Despite their utility, the generation of 3D cell models via manual methods is time-consuming and low-throughput.[2] Therefore, there is a clear and unmet need for consistent and reproducible production of 3D cell culture models for research applications.

Bioprinting technologies have emerged as a viable approach for the high-throughput generation of 3D cell models. There are various approaches to bioprinting, including extrusion-based and stereolithography.[3-4] However, droplet-based printing is well suited to the production of 3D cell models as it enables rapid printing into well plates and simultaneous placement of multiple matrix components.[5]

The drop-on-demand bioprinting approach is akin to inkjet printing, whereby instead of depositing pixels of colour onto a page, the bioprinter deposits cells and matrix components onto the surface of a well plate, and builds these components layer-by-layer to form a desired 3D structure.[6] Notably, drop-on-demand bioprinting achieves high cell viability by reducing shear stresses on cells, as the pressure required to eject a droplet from the printhead is lower than that used in extrusion printing.[7-8]

RASTRUM combines drop-on-demand bioprinting with synthetic modifiable matrix systems to make the creation of matrix-embedded 3D cell models simple, reproducible and efficient. This application note will detail the key printing features offered by **RASTRUM**.

Droplet Control

The **RASTRUM** printhead features multiple independent nozzles for the simultaneous deposition of up to 8 different printing fluids during a print. The printhead moves seamlessly across a well plate and builds 3D cell models using flyby droplet deposition, enabling a step-change in printing speed and throughput. **RASTRUM** is capable of depositing 1000 droplets per second onto the surface of the plate, with a typical droplet volume of 20-25 nL (**Figure 1**).

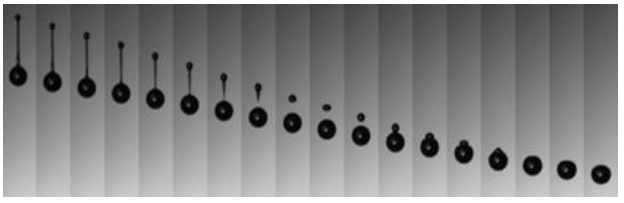

Figure 1: Representative droplet visualisation images showing bioink droplet formation after ejection from a **RASTRUM** printer nozzle.

RASTRUM makes it possible to print multiple matrix components in a single pass of the printhead. This multiplexing capability makes it quicker and more efficient to build complex 3D cell models. **RASTRUM** allows precise control over in-well placement of matrix components, spacing and volume of the ejected matrix (**Figure 2**). The 2-axis linear motion control

system enables the placement of droplets at a resolution of 20 µm along each axis.

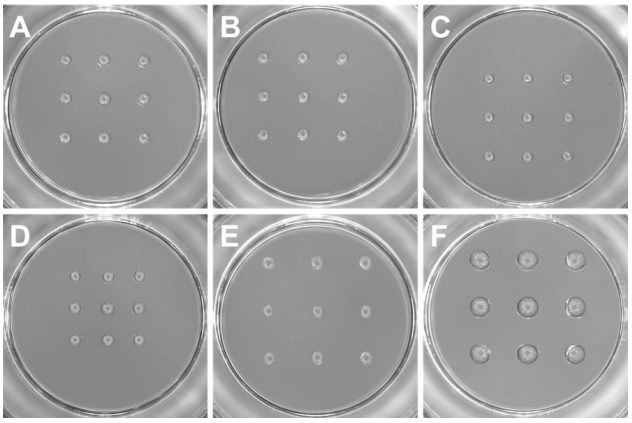

Figure 2: Demonstration of printing control using **RASTRUM**. By changing the printing parameters, a series of bioink droplets printed in the centre of the well (**A**) can be shifted to the left (**B**), shifted downwards (**C**), printed closer together (**D**), printed farther apart (**E**), or printed at a greater volume (**F**).

Printing 3D Hydrogel Structures

RASTRUM creates 3D structures by printing tiny droplets of a PEG-based bioink and an activator solution from separate nozzles, which combine to instantly form a hydrogel at room temperature (**Figure 3**).

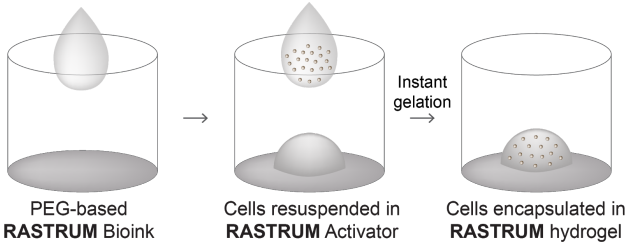

Figure 3: Drop-on-drop gel formation using **RASTRUM**. Bioink and activator droplets combine to instantly form a hydrogel structure.

By precisely controlling the deposition of matrix components onto the surface of a well plate, **RASTRUM** can be used to build complex 3D structures. An example of a commonly printed structure containing cells is shown below (**Figure 4**). The small hydrogel plug structure has a volume of approximately 300 nL and can be printed into a 96-well plate, with the whole plate printed in less than 10 minutes. For further information about this 3D cell model, see our application note '*High-Throughput Production of Matrix-Embedded Multicellular Tumour Spheroids by Drop-on-Demand 3D Bioprinting*'.

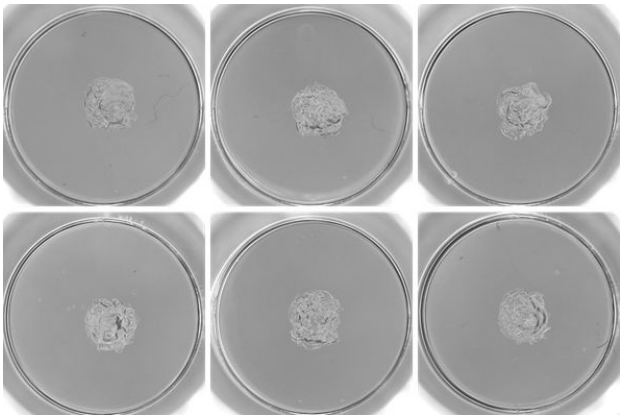

Figure 4: Consistent printing of small hydrogel structures into the wells of a 96-well plate using **RASTRUM**.

Printing Cells

The deposition of matrix components by **RASTRUM** is not only precise, but ejection from the printer is also gentle on cells. Various cell types have been successfully printed using **RASTRUM**, including a variety of established cancer cell lines, keratinocytes, dendritic cells, primary cells lines and patient-derived cancer cells. The viability of SK-N-BE(2) (neuroblastoma), H460 (lung cancer) and U87vIII (glioblastoma) cells before and after printing is shown below (**Figure 5**).

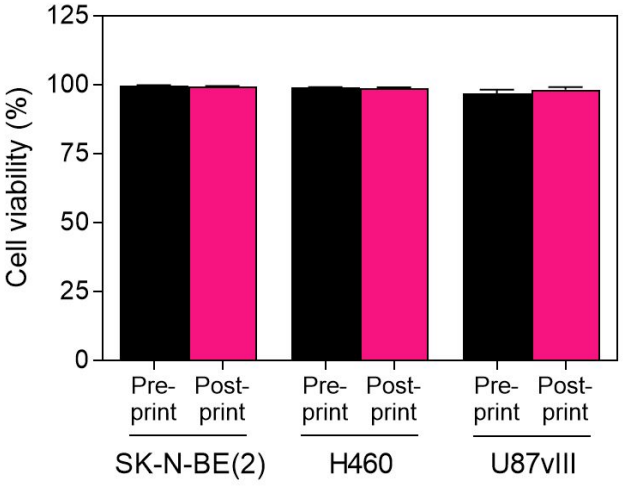

Figure 5: Viability of SK-N-BE(2), H460 and U87vIII cells before and after printing using **RASTRUM**, measured via Trypan Blue exclusion. Data are presented as the mean ± standard deviation (n = 3 per group).

RASTRUM can also print sensitive cell types, such as induced pluripotent stem cells (iPSCs), with cells maintaining viability and pluripotency markers after printing (**Figure 6**). These results highlight the ability of **RASTRUM** to generate 3D cell cultures without negatively affecting the viability of the cells during the printing process.

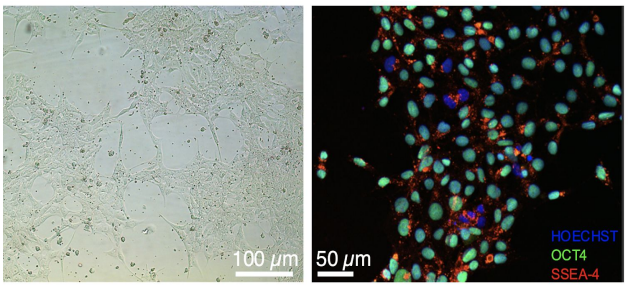

Figure 6: iPSCs maintain viability and pluripotency markers after printing with **RASTRUM** onto a layer of Matrigel™ and culturing for 48 hours. Confocal image shows cells stained for pluripotency markers OCT4 and SSEA-4.

Summary and Conclusions

In this application note, we have highlighted the ability of the **RASTRUM** 3D bioprinter to enable precise droplet control of matrix components and gentle deposition of cells. This demonstrates the utility of **RASTRUM** in the high-throughput bioprinting of hydrogel structures and 3D cell models for research applications.

References

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