GrowInk™

Application Note **1**

UPMBIOFORE BEYOND FOSSILS

High-throughput production of GrowInk™-T models by drop-on-demand 3D bioprinting

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INTRODUCTION

Three-dimensional (3D) cell culture has been established to more accurately represent in vivo physiological conditions compared to 2D cell cultures [1]. RASTRUM 3D bioprinting platform (Fig. 1) by Inventia Life Science enables high throughput creation of 3D cell models, in an efficient and reproducible way. RASTRUM utilizes digital bioprinting technology that prints tiny droplets of cells and matrix components in a way that is very fast, very precise and gentle on cells. RASTRUM utilizes advance bioink technologies, such as GrowInk™-T (UPM, Finland) a nanofibrillar cellulose bioink, to print 3D cell models which closely resemble the extracellular matrix and support cell growth.

GrowInk-T bioink is an animal free, biocompatible, ready to use hydrogel that can be mixed directly with cells for bioprinting applications. It is a shear-thinning material that enables easy printing. GrowInk-T mimics the in vivo environment supporting cell growth and differentiation and can be diluted to provide a wide range of viscosities that can be matched to specific cell requirements. Equipped with an inbuilt laminar flow hood, RASTRUM 3D bioprinting platform combined with GroInk-T offers a complete solution to create biologically relevant 3D cell models with unprecedented speed and reproducibility.

MATERIALS

- Human breast (adenocarcinoma) cell line (MCF-7, ATCC HTB-22)
- GrowInk-T (UPM, Finland) diluted 1:1 with ultra pure water, and further diluted 3:2 in PBS
- 96-well plate (Thermo Fisher Scientific)
- DMEM culture media (Thermo Fisher Scientific)
- Foetal Bovine Serum (FCS) (Bovogen)
- PrestoBlue Cell Viability Reagent (Thermo Fisher Scientific)
- RASTRUM print cartridge (Inventia Life Science)

Figure 1. RASTRUM 3D bioprinting platform.

UPMBIOMEDICALS

METHODS

1. RASTRUM platform preparation

- RASTRUM bioprinter was initialized to start the laminar flow hood. Bioprinter surfaces were sterile wiped with 70 v/v% ethanol.
- To prepare the printing cartridge, 200 µl of diluted GrowInk-T was pipetted into a sterile RASTRUM print cartridge, pre-filled with the sterilization fluids. The cartridge and a sterile well-plate were placed into their respective designated slots in the printer.

2. RASTRUM bioprinting

- The bioprinting process was started with the automated fluidics sterilization process. Briefly, the fluidics were flushed with 70 v/v% ethanol, followed by sterile DI water.
- After the sterilization phase, the printer automatically primed 200 µl of GrowInk-T.
- To assess the reproducibility of the bioprinting process multiple 3D structures, as shown in Fig. 2, were printed in each well of a 96-well plate.
- On completion of the printing an automated shutdown cleaning process was conducted with 70 v/v% ethanol and sterile DI water, to maintain the sterility and reliability of the bioprinter.

3. Evaluation of cell viability in GrowInk-T

- One million MCF-7 cells were harvested and resuspended homogeneously in 100 µl GrowInk-T, to yield a cell suspension of 10 million cells/ml.
- 15 µl of the cell-GrowInk-T suspension was transferred into 5 centrifuge tubes.
- Every 15 min, 1 ml DMEM was added to one of the tubes. 20 µl of the DMEM cell-GrowInk-T suspension was then plated into a 96-well plate and topped up with 80 µl DMEM/10% FCS.
- This process was repeated 4 times after 15, 30, 45 and 60 min of incubating the MCF-7 suspension in GrowInk-T at room temperature.
- 100 µl of DMEM/10% FCS was used as the control sample.
- The plate was incubated for 24 h, after which a PrestoBlue viability assay was performed according to the manufacturer's protocol.

RESULTS

RASTRUM 3D bioprinting of GrowInk-T

Combination of the RASTRUM 3D bioprinter and Growink-T bioink were studied to validate a new capability to generate biologically relevant 3D cell models, reproducibly and in a high-throughput manner. 3D cuboid structure was selected as the model structure as it is currently used to form embedded 3D spheroids and organoids for drug screening application. The printed cuboid measured 1.5 mm in length and 300 µm in height, with a volume of ~500 nl (Fig. 2). The resulting GrowInk-T cuboid fits into the 10x objective field of view, is clear in appearance and has a thickness compatible with fluorescence-based downstream analysis.

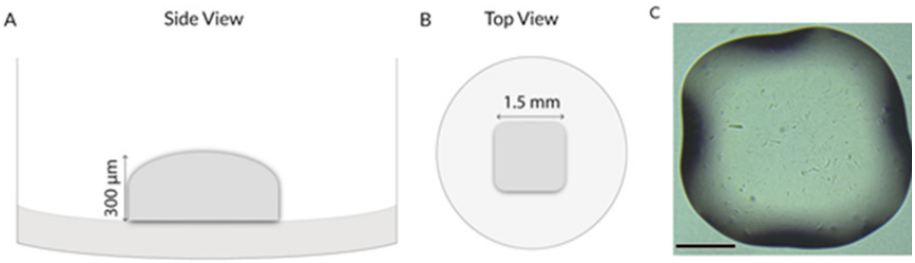
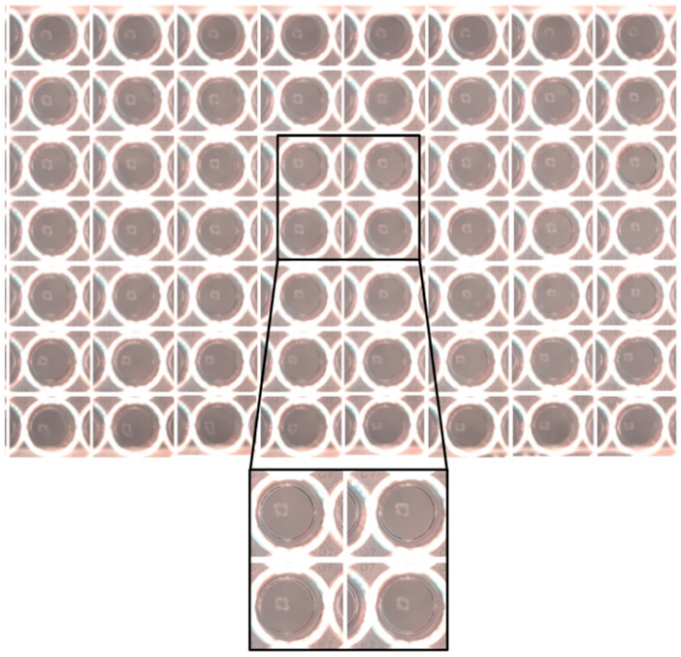


Figure 2. Representative 3D bioprinted GrowInk-T plug in a 96-well plate. A) Schematic side view of the cuboid structure, 300 µm in height, B) Schematic top view of plug, 1.5 mm in diameter, C) Bright-field image of the GrowInk-T cuboid structure, Scale bar: 500 µm.

To demonstrate reproducibility a full 96-well plate containing the cuboid structure was printed, this process took less than 10 minutes to complete. Following printing the plate was imaged and analyzed for sample variability (Fig. 3) which confirmed that each well had a structure with the same size and shape definition.

Moreover, the cuboid structure print location was consistent across all wells. The printing process was repeated twice, with each print run utilizing a freshly prepared GrowInk-T bioink. The same outcomes were observed in all samples, indicating excellent reproducibility and consistency of both the printing process and the bioink. In total 182 samples were printed with no variation in the structural definition or location within the well. Attributes that are beneficial for automated high-content imaging.

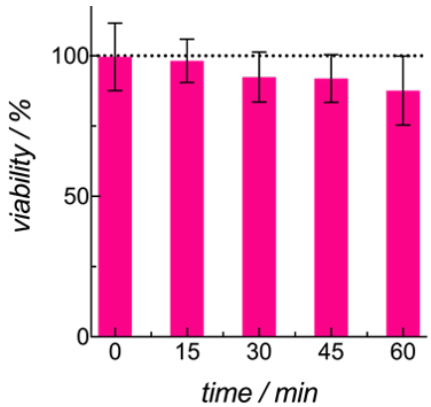
Figure 3. Representative GrowInk-T print in a 96-well plate.



MCF-7 Cell Viability in GrowInk-T Suspension

It is crucial to maintain high cell viability while the cells are suspended in the bioink during the printing process. MCF-7 were chosen as the model cell based on data from previous reports which has shown that MCF-7 cells form well-defined 3D spheroid structures in nanofibrillar cellulose hydrogel, GrowDex®[2]. To mimic the environment MCF-7 cells are exposed to when suspended in the bioink during bioprinting, MCF-7 cells were suspended at 10 million cells/ml in 100 µl of GrowInk-T. The cell suspension was incubated at room temperature for 60 minutes with samples being collected every 15 min. PrestoBlue analysis of the samples after 24 h incubation at 37°C showed a viability of 98% for the printing duration (< 15 min), with a high cell viability of 88% observed after 60 min suspension in the GrowInk-T (Fig. 4).

Figure 4. MCF-7 viability when suspended in GrowInk-T over a period of 60 minutes.



CONCLUSIONS

The RASTRUM 3D bioprinting platform was used successfully to print GrowInk-T bioink in a 96 well plate in less than 10 minutes. Reproducibility of printing was demonstrated from well-to-well, plate-to-plate and batch-to-batch. MCF-7 cell studies over 60 min showed that the GrowInk-T bioink is biocompatible and able to support cell viability. Combination of RASTRUM with the validated GrowInk-T cell assays enables rapid production of in vitro 3D cell culture models, for both cancerous and primary cells, efficiently, reproducibly and suitable for high content screening applications.

REFERENCES

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2. Niklander, J. and Paasonen, L. "Breast Cancer Cell Line MCF7 3D Culture Demonstrated in GrowDex® and Matrigel", UPM Biofore Application Note 29.

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