PREPARATION OF SENSITIVE CELLS FOR PRINTING

Introduction

While immortalised cell lines serve a purpose in early drug development and disease modelling, they fall short in replicating the complexity and heterogeneity of tissues. To overcome these limitations, the use of physiologically relevant cells such as patient-derived cells, primary cells, and induced pluripotent stem cells (iPSCs) is paramount.

Patient-derived and primary cells offer valuable insights into biological variations among individuals, aiding in the identification of potential drug targets and personalised treatment approaches. iPSCs, retain the genetic backgrounds of patients enabling more precisely modelling disease-specific pathophysiology and phenotypes. Moreover, iPSCs can be differentiated into various cell types, enhancing their versatility.

However, these sensitive cells require careful handling. Despite the gentle nature of the RASTRUM™ Platform, precautions must be taken throughout the process to ensure cell viability and functionality.

This document provides guidance on preparing highly sensitive cells to generate physiologically relevant RASTRUM 3D cell models. It is divided into two sections: A) cells sourced from 2D cultures and B) cells sourced directly from tissue.

Requirements for cell printing with RASTRUM

For cells to be printed using RASTRUM, the following criteria must be met:

- Cells are nearly single cell suspension with no clumps bigger than 70 µm.
- Healthy cell population with high cell viability is used.

  Note: We suggest to use healthy population of cells with viability of more than 80% where possible. Dead cells won't be washed away once the cells are encapsulated in the 3D matrices. If the cell suspension contains a lot of dead cells, consider performing a few wash steps to remove dead cells as much as possible (e.g. using dead cell removal kits).

Steps for cell preparation

Depending on the sample type (2D culture versus tissue), there are several steps that should be followed to ensure successful generation of 3D models with RASTRUM platform.

A. Cells sourced from 2D culture

1. Expand your iPSCs or primary cells based on your standard protocol to achieve required cell number for the printing. Depending on cells type, cells can also be used directly from frozen vials.

   Note: When using cells directly from frozen vials, if the viability of your cells is low, we recommend thawing them in a media containing a rho-kinase inhibitor (ROCKi) at a concentration of 10 µM.[1] ROCKi promotes cell survival by reducing apoptosis facilitating the transition of cells from a frozen state to a 3D culture environment.

   Note: If a frozen vial contains a significant number of dead cells that cannot be removed through washing steps, one option is to plate them in a 2D culture initially for 1-2 days to facilitate the removal of dead cells.

2. **Optional:** 48 hours prior to printing, you may pre-treat the cells with ROCKi to ease their transition from a 2D to 3D environment.
3. Follow your standard protocol to detach, count and assess viability of cells.
4. Follow the steps mentioned in RASTRUM protocol to prepare and print your cells to generate RASTRUM 3D cell models.

TIP:
To maximise the viability of sensitive cells, minimise the time that cells are sitting idle in suspension between cell harvesting and cell model printing

5. Once the PrintRun is completed we recommend adding the cell specific media containing 10 µM ROCKi for the first 48 hours post-printing

6. After the first 48 hours you may remove the ROCKi for the remainder of the culture period.

   Note: In some cases, ROCKi may be required beyond the first 48 hours to maintain viability while the cells equilibrate to the 3D environment. In these cases, we recommend a ROCKi reduction strategy, decreasing the ROCKi concentration in media by 2 µM per medium change.[2]

Note:

If in need to measure toxicity or cell death, it is recommended to remove ROCKi 48 hours in advance.

B. Cells sourced directly from tissue

Note:

When working with cells isolated from tissue, ensure that no tissue fragments or debris are present in the cell solution as this could cause a blockage in the printer.

1. Follow your standard protocol for tissue dissociation.
2. Ensure that any dissociation reagents/enzymes are completely neutralised (e.g. using media containing fetal bovine serum (FBS)) as residual enzymes might impact matrix integrity.
3. Pass the cell suspension through a sterile strainer with at most a 75 µm pore size to separate dispersed cells and tissue fragments from larger pieces.

   Note: Consider adding fresh cell dissociation reagent to the fragments if further disaggregation is necessary. This should then be followed by neutralising the dissociation reagents/enzyme and re-filtration.

4. Wash the suspension multiple times by centrifugation in a balanced salt solution/media with optimal spinning speed and time appropriate for your cell type to remove any small cell debris/fragments.
5. **Optional:** Expand (either in 2D or 3D) if needed to reach the appropriate number of cells for printrun.

   Note: The minimum recommended cell density for a PrintRun is 1 million cells/mL.

6. Once the cells suspended, follow steps mentioned in cells sourced from 2D culture (Section A).

Representative data

Figures 1 and 2 demonstrate the successful generation of 3D models using sensitive cells by the RASTRUM Platform.

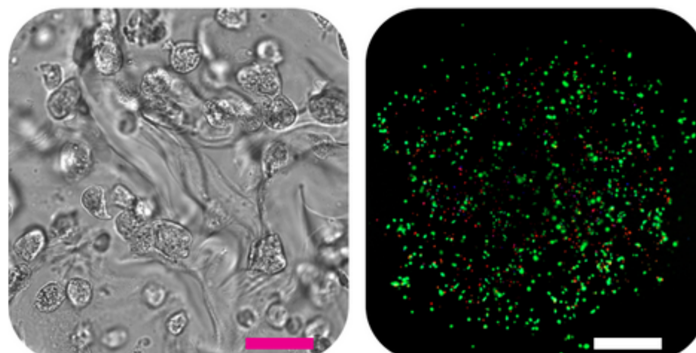

Figure 1: Confocal fluorescence images of patient-derived breast cancer organoids printed in RASTRUM Matrices after 12 days in culture. Nuclei (blue), F-actin (green), Scale bar = 50µm.[3]

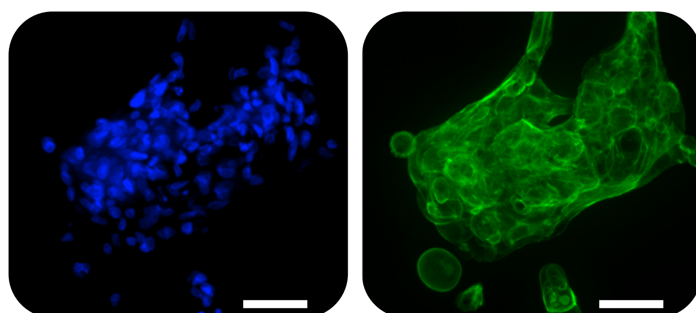

Figure 2: Confocal fluorescence images of breast cancer patient derived organoids printed in RASTRUM Matrices. Nuclei (blue), F-actin (green) after 12 days of culture in the RASTRUM Matrix. Scale bar = 50µm.[4]

References

1. Whitehouse C.A., He, Y., Brownlees, J. et al. Three-Dimensional Bioprinting of Human iPSC-Derived Neuron-Astrocyte Cocultures for Screening Applications. J. Vis. Exp. 2023; 199, e65856, doi:10.3791/65856
2. Sullivan M.A., Lane S., Volkerling A., et al. Three-dimensional bioprinting of stem cell-derived central nervous system cells enables astrocyte growth, vasculogenesis, and enhances neural differentiation/function. Biotechnol Bioeng. 2023; 120(10):3079-3091. doi: 10.1002/bit.28470
3. Inventia Life Science. Liver 3D model using primary human hepatocytes (application note). 2023.
4. Bock N., Forouz F., Hipwood L., et al. GelMA, Click-Chemistry Gelatin and Bioprinted Polyethylene Glycol-Based Hydrogels as 3D Ex Vivo Drug Testing Platforms for Patient-Derived Breast Cancer Organoids. Pharmaceutics. 2023; 15(1):261. doi:10.3390/pharmaceutics15010261

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