

Generation of 3D Co-Culture Models Using the **RASTRUM™** 3D Cell Culture Platform



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Highlights

In this application note, the **RASTRUM** 3D cell culture platform, developed by Inventia Life Science, was used to produce direct co-culture models encapsulated in PEG-based matrices that:

- Generate a consistent output of two cell populations
- Promote the growth of two different cell types within the same model matrix

Introduction

Three-dimensional (3D) cell culture models recapitulate key motifs of the fundamental tissue environment and have been reported to exhibit better predictive value as a pre-clinical model compared to 2D cell models.¹ Cells encapsulated within a 3D matrix can assemble together to create structures similar to those found *in vivo* and facilitate intercellular cross-talk and cell matrix interactions. Furthermore, matrix based 3D cell models more reliably mimic disease and cellular responses to drug treatments compared to their 2D counterparts.²

In drug discovery, 2D cell cultures are used as the primary *in vitro* cancer model due to their simplicity and compatibility with high-throughput screening. However, 2D monolayer cultures are prone to generating false positives when used to predict drug efficacy because they do not faithfully recapitulate the architecture and microenvironment of a tumour.³ As such, the development of 3D cell culture technology has led to the generation of 3D cell models that more accurately mimic the biological *in vivo* microenvironment. Thus, 3D cell models have numerous advantages over 2D cultures and have significant potential to improve the translational success of *in vitro* drug development and preclinical research.

RASTRUM utilises drop-on-demand digital bioprinting technology to create 3D cell model systems that closely resemble physiological environments. **RASTRUM** matrices are composed of a highly versatile scaffold with tunable biofunctional and mechanical properties that are optimised for the generation of various co-culture models. This application note provides an overview of the creation of direct co-culture models in breast and lung cancer using **RASTRUM**.

Methods

Direct Co-Culture of Different Cell Types

Two co-culture cell models were printed with **RASTRUM**:

1. 3×10^6 cells/mL of normal human lung fibroblast (NHLF) and lung cancer cells (A549) at a 2:1 ratio with matrix Px01.03P
2. 2.5×10^6 cells/mL of NHLF and breast cancer cells (MCF-7) at a 1.5:1 ratio with matrix Px02.00

Cells were resuspended in activator (F176) and added to separate reservoirs of the **RASTRUM** cartridge prior to printing. Following the printer cleaning stage using the supplied reagents, the bioink was printed into each well of a 96-well plate. Subsequently, the activator containing the mixed cell population was printed directly on top in order to form small, uniform hydrogels in every well. Repeated layer-by-layer droplet deposition using **RASTRUM's** fly-by mode resulted in the generation of the 3D cell model with an even distribution of cells throughout the 3D structure (**Figure 1**). Mono-cultures of NHLF and A549 cells were also generated separately as controls.

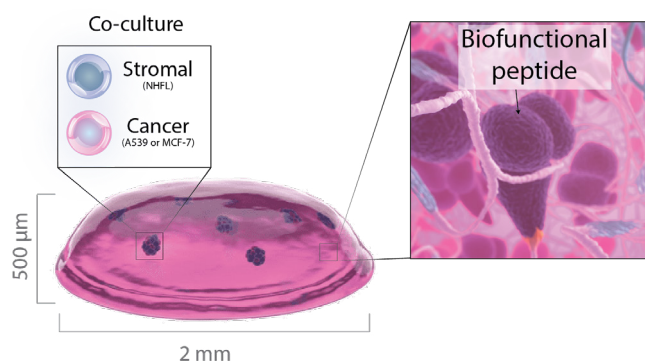


Figure 1: **RASTRUM** matrices are formed from a PEG-based bioink that instantly gels at room temperature when combined with an activator solution. Co-culture mixtures featured a 2:1 ratio of NHLF lung fibroblasts and A549 lung cancer cells or MCF-7 breast cancer cells.

Cell Viability

Cell morphology was evaluated using brightfield images captured at 6 days post-printing. In addition to brightfield analysis, cell viability was determined at day 6 post-printing by incubating NHLF and A549 cells with calcein-AM and ethidium homodimer-III (Biotium; incubated at 37° C for 30 min, final concentration of 1 μ M and 2 μ M, respectively). All images were captured using

a fluorescence microscope (Observer 7, Zeiss).

Cell Staining for Quantification

NHLF cells were stained with Hoechst (5 μ g/mL) and MCF-7 cells were stained with CellTracker Red (1X) prior to mixing at a ratio of 1.5:1 and printed using **RASTRUM**. All images were captured using a fluorescence microscope (Observer 7, Zeiss) and blue:red cell quantification was performed using CellProfiler as previously described.⁴

Results and Discussion

A549 and NHLF were either encapsulated together in **RASTRUM** hydrogel matrices or separately as controls (**Figure 2**). **RASTRUM** matrices promoted the growth and development of fibroblasts and lung cancer cells both as monocultures and in co-culture within the same matrix. Brightfield and fluorescent imaging conducted after 6 days revealed distinct differences in cell morphology between NHLF and A549 (**Figure 2**). Within the monocultures, the NHLF cells developed branching networks with

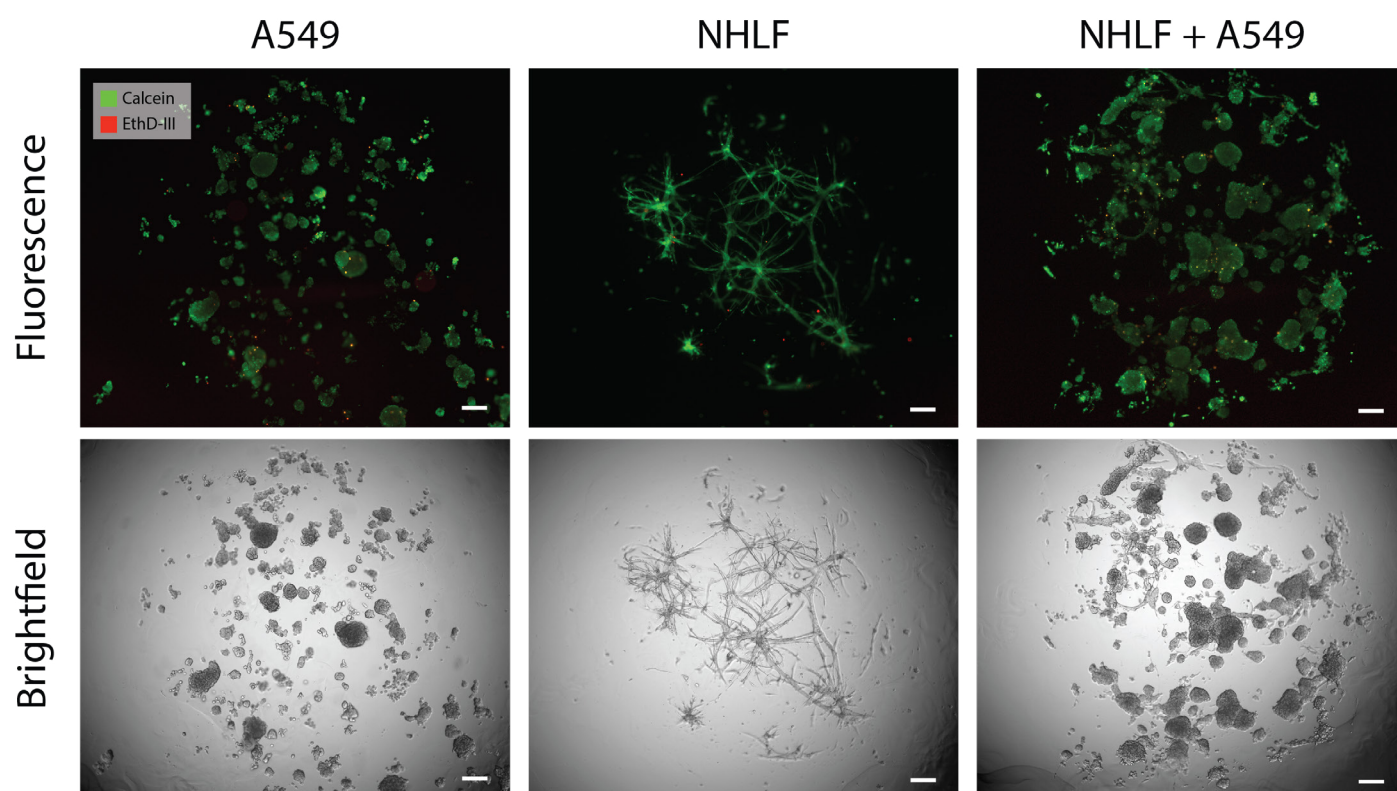
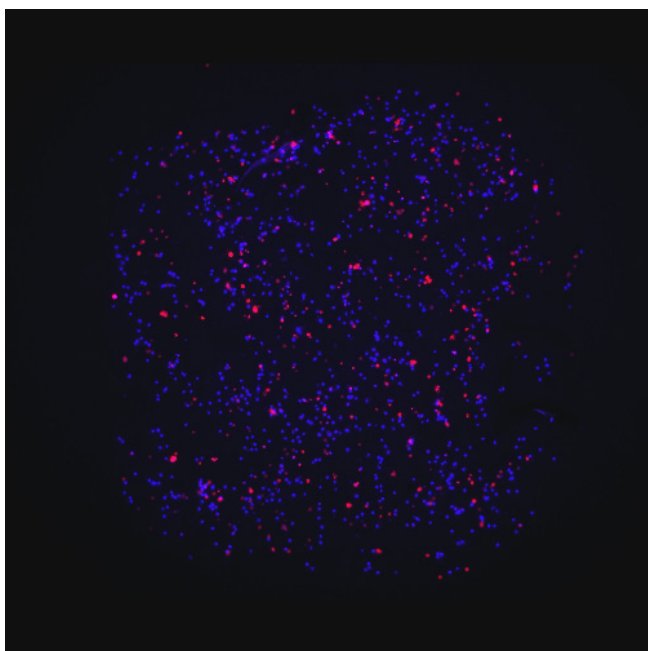


Figure 2: Brightfield and fluorescence images of NHLF lung fibroblasts and A549 lung cancer cell monocultures and co-cultures in **RASTRUM** matrices at a 2:1 ratio of fibroblasts to cancer cells. Scale bars are 200 μ m.

elongated morphologies, compared to A549 which developed rounded colonies. However, when NHLF and A549 were cultured together a distinct morphology could be observed that resembled a combination of both monocultures.

Using a different **RASTRUM** matrix, a co-culture was created featuring both cell types encapsulated together (**Video 1**). Fluorescent labelling of MCF-7 and NHLF cells revealed that the **RASTRUM** 3D cell culture platform generated a consistent cell output ratio, accurate to $\pm 15\%$, when compared to the input cell ratio when using a mixture of the two different cell lines. Consequently, the direct co-culture models generated herein demonstrated an even distribution of each individual cell type that was reflective of the initial input cell ratio.



Video 1: NHLF fibroblasts and MCF-7 breast cancer cells. NHLF are stained in blue and MCF-7 are stained in red.

biofunctionalised to promote the co-existence of both fibroblasts and cancer cells. In combination with the high throughput nature of the **RASTRUM** 3D cell culture platform and the tunability of **RASTRUM** matrices, users can efficiently develop and create reproducible co-culture cell models for a range of research applications.

References

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3. Tomas-Bort, E et al. (2020), *Theranostics.* 10(11): 5074–5089. doi: 10.1089/adt.2014.573.
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Summary and Conclusions

Direct co-cultures of different cell types are crucial for the generation of physiologically relevant in vitro cell culture models which can be used to accurately mimic biological functions and test drug responses. **RASTRUM** matrices provide users with a range of versatile, tunable 3D scaffolds that can be

About Inventia

About Inventia Life Science: Inventia Life Science is a fast-growing biotech startup based in Sydney, Australia, that is revolutionising cancer research, drug discovery and regenerative medicine. Inventia's digital drop-on-demand 3D bioprinting technology is a game changer for the rapid fabrication of living biological structures. The company's RASTRUM™ 3D cell culture platform, winner of the 2019 Good Design Award of the Year and 2020 Fast Company World Changing Ideas Award, has already been adopted in leading medical research institutes creating printed 3D tissues that model real biology. Inventia Life Science is also working with its customers and collaborators on new high-throughput personalised screening approaches as well as with clinical partners to advance regenerative medicine in areas such as bioprinted skin for the repair of burns and wounds. For more information, please visit www.inventia.life. Follow Inventia Life Science on [LinkedIn](#), [Twitter](#) and [Facebook](#).

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