Printable and Tunable 3D Cell Culture Environments using RASTRUM Synthetic ECM Hydrogels

Highlights

The **RASTRUM™** 3D bioprinting platform, developed by Inventia Life Science, utilises synthetic PEG-based matrices that:

- Enable reliable cell encapsulation to generate 3D cell models
- Can be modified to match ECM stiffnesses found *in vivo*
- Can be biofunctionalised with addition of adhesion peptides, full-length proteins or MMP-sensitive sites to suit each encapsulated cell type

Introduction

Matrix-embedded 3D cell models are considered to be more physiologically relevant than 2D cultures because they enable inclusion of key elements of the extracellular matrix (ECM), which better recapitulates the *in vivo* context.[1-2] ECM stiffness and the presence of proteins within the ECM heavily influence cell morphology, metabolism, migration and drug sensitivity *in vitro* and *in vivo*.[3-5]

The ECM not only provides mechanical and structural support, but also determines the molecular interactions that in turn affect cellular function. In 3D cell culture, encapsulation of cells within a matrix enables vital cell-matrix interactions, which allows the development of *in vitro* models that are more physiologically relevant than 2D culture systems, whereby cells are cultured on plastic or glass.

Many commercially available extracellular matrices are naturally derived, temperature sensitive, have set mechanical properties and differ in matrix composition by lot, creating a higher risk to experimental reproducibility.[6] Significant variation in matrix-bound growth factors and proteins further complicates these systems.[7-8] Alternatively, synthetic matrices enable customisation and control of the ECM properties for a more suitable model of the *in vivo* microenvironment for the cell type of interest.

RASTRUM combines drop-on-demand bioprinting with synthetic modifiable matrix systems to make the creation of matrix-embedded cell models simple, reproducible and efficient. This application note will provide an overview of the utility and tunability of **RASTRUM** matrix systems.

Methods

RASTRUM matrices consist of biocompatible polyethylene glycol (PEG)-based bioinks that instantly form a hydrogel structure at room temperature when combined with an activator (**Figure 1**).

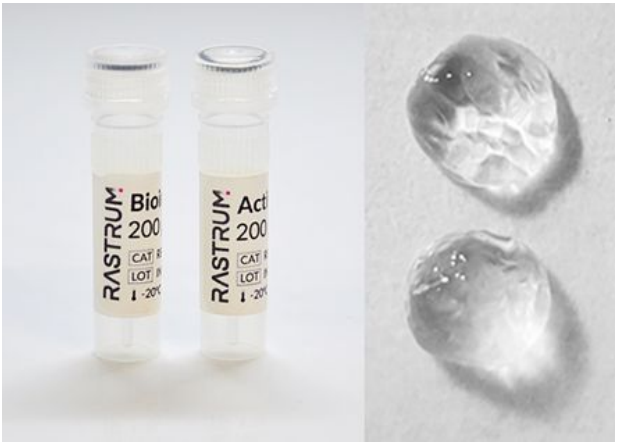

Figure 1: **RASTRUM** matrices are formed from a PEG-based bioink that instantly gelates at room temperature when combined with an activator solution. The matrices are biocompatible, transparent hydrogels that can be modified to suit the cell type.

RASTRUM bioinks are synthetic, which enables precise batch control and consistency. The mechanical properties of the bioinks are modifiable by altering the PEG backbone to mimic the *in vivo* ECM stiffness for the cell type of interest. Additionally, cell adhesion peptides and full-length proteins can be incorporated to promote cell-ECM interactions, as well as MMP-sensitive sites that enable cell remodelling of the ECM (**Figure 2**).

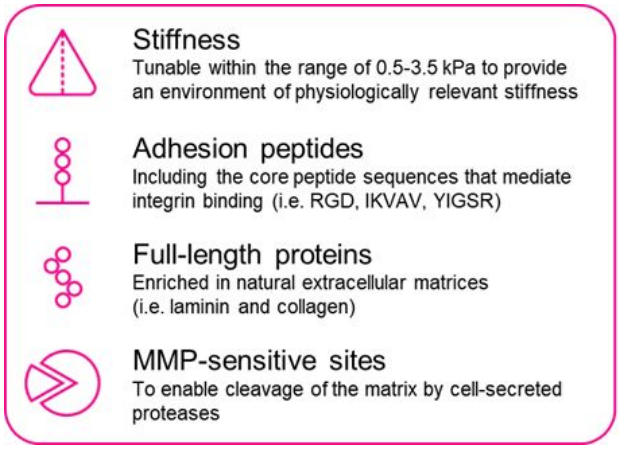

Figure 2: **RASTRUM** matrices can be modified to suit the cell type and application.

Some other advantages of **RASTRUM** matrices over other hydrogel systems include:

- Printability, with instant gelation at room temperature
- Permeable to antibodies, growth factors and small molecules
- Optically transparent with no autofluorescent background for *in situ* imaging
- Dissolvable to enable cell extraction

Results and Discussion

Normal human lung fibroblasts (NHLF) and MCF-7 breast cancer cells were encapsulated in **RASTRUM** hydrogel matrices of two different stiffnesses. Brightfield imaging after 7 days revealed distinct differences in cell morphology due to matrix stiffness for both cell lines (**Figure 3**). Fibroblasts cultured in the lower stiffness hydrogel formed extensive networks with elongated cells, while those in the higher stiffness hydrogel showed minimal network formation. MCF-7 cells formed spheroids in both matrices, with matrix stiffness being inversely related to spheroid size. These results demonstrate how a simple modification of the bioink enables control over spheroid growth or degree of network formation, to suit the desired application.

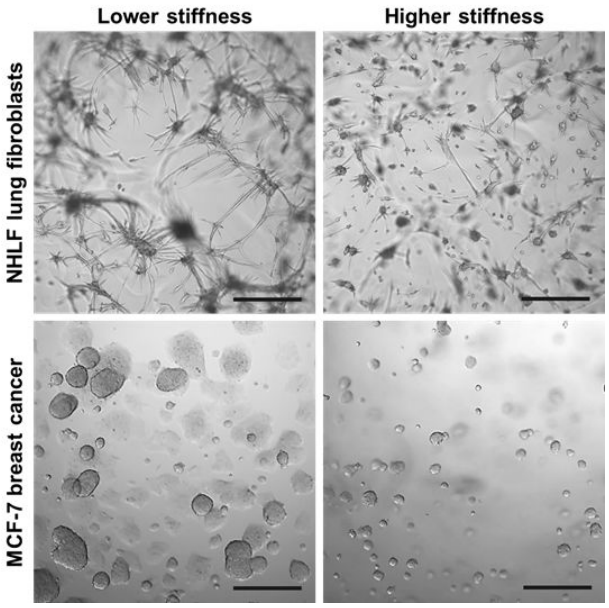

Figure 3: NHLF lung fibroblasts and MCF-7 breast cancer cells cultured in **RASTRUM** matrices of varying stiffnesses over 7 days. Scale bars are 200 µm.

In addition to varying the stiffness of the matrix, the inclusion of cell adhesion peptides in the hydrogel matrices can also influence cell growth and morphology. Normal human dermal fibroblasts (NHDF) and invasive breast cancer cells (MDA-MB-231) cultured in **RASTRUM** hydrogel matrices in the absence or presence of RGD peptide showed distinct morphological differences after 4 days of culture (**Figure 4**).

The ideal matrix for a cell type of interest would have properties which allow the biotissue to mimic its parental tissue type, such as the formation of relevant tissue architecture, cellular topology, gene expression, signalling and metabolism.[9] Collectively, this would also enable the cell model to more accurately predict drug responsiveness that could be reliably translated to preclinical models.[10] The combination of the high-throughput **RASTRUM** bioprinting platform with customisable and printable ECM enables the efficient creation of reproducible 3D cell models that are compatible with numerous

downstream analyses systems for morphological and metabolic confirmation.

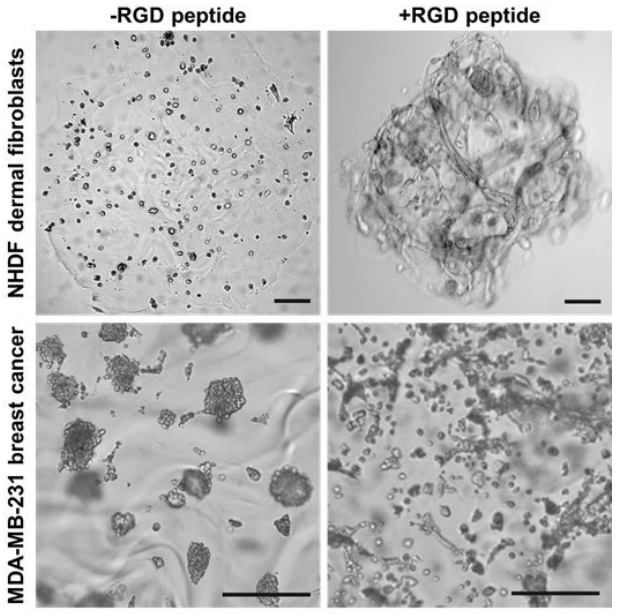

Figure 4: NHDF dermal fibroblasts and MDA-MB-231 breast cancer cells (day 4) cultured in **RASTRUM** matrices in the absence or presence of RGD peptide. Scale bars are 200 µm.

Summary and Conclusions

In this application note, we have demonstrated the tunability of **RASTRUM** matrices, including stiffness and peptide incorporation, for a variety of 3D cell models. These simple modifications to the hydrogel matrices enable the creation of a suitable 3D scaffold for a particular cell line, which closely resembles the mechanical and biofunctional properties of the native ECM matrix found *in vivo*.

References

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