Development of 3D model to study cancer migration into the cancer microenvironment

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Introduction: Through the progression of breast cancer, the healthy extracellular matrix (ECM) is disordered and remodelled into a tumour microenvironment (TME) which is known to assist tumour progression and metastasis.[1] This transition is not fully understood due to challenges in modelling and deconvoluting the complex biophysico-chemical variables and the heterogeneous cell population present in breast ECM.[2] The emergence of 3D bioprinting in recent years, has allowed for a wide range of novel biofabrication techniques to faithfully reconstruct the natural architecture of the in-vivo microenvironment to the in-vitro scale.[3] However, there are currently no models that represent the in-vivo spatial organisation of a tumour growing in the empty space of a mammary duct.

Materials and methods: Alginate-norbornene (Alg-NOR; 0.5-2 %w/v), crosslinker calcium chloride ($CaCl_2$; 4 %w/v), photo-initiator LAP (0.02 %w/v) along with bioactive peptide GRGDSC (Fibronectin; 0.6738 mM) were used to encapsulate adipose derived stem cells (ADSCs, 4×10^5 cells) to form a hydrogel. These components were used to form hydrogels either under printing conditions or by being cast in a silicone mould. Gels were exposed to UV light for 5 min to induce photopolymerization of the ECM mimicking peptide and cultured for up to 10 days in media, before assessing cell proliferation (alamar Blue™ assay) and morphology (F-actin staining).

Results: To determine the optimal conditions for the spreading of ADSCs within a hydrogel matrix, we tested a range of increasing concentrations of Alg-NOR with a peptide mimicking fibronectin sequence. We concluded that 0.5 %w/v Alg-NOR provided the best environment for ADSCs spreading (Figure 1A). Subsequently, preliminary printing tests were conducted with the drop-on-demand printing (Rastrum™, Inventia) creating a structure incorporating the most suitable bioink formulation for ADSC spreading (Figure 1B, 1C, i). Additionally, MCF7 breast cancer cells were deposited inside the cavity of cup-shaped hydrogels to facilitate the formation of tumour spheroids, so that the system would mimic the natural mammary duct microenvironment. (Figure 1C, ii).

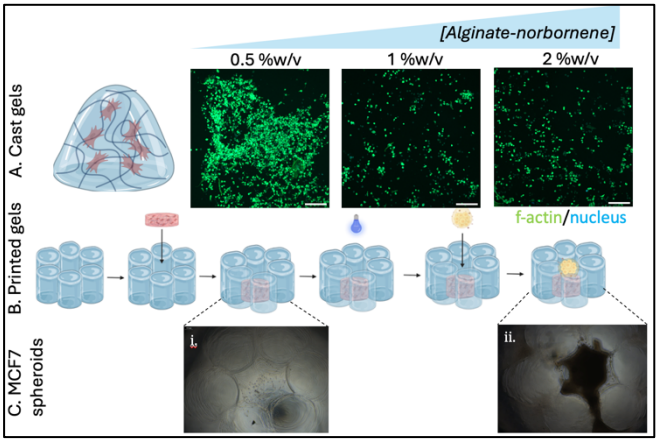

Figure 1: A) ADSC morphology in cast gels of a range of Alg-NOR concentrations; B) Schematic of the printing method; C) i) heterogenous hydrogel cup after printing; ii) MCF7 spheroids forming within the cup cavity.

Conclusions: In conclusion, this study highlights the importance of recreating an accurate environment for cells involved in tumour progression, and it will allow us to hopefully develop more effective therapeutic strategies targeting the tumour microenvironment.

References: [1] Li, JJ.; et al. *Cancers* **2021**, *13*, 16. [2] Bahcecioglu, G.; et al. *Acta Biomaterialia* **2020**, *106, 1742-706*. [3] Utama, RH.; et al. *iScience* **2020**, *23*, 10.