Matrix Stiffness Drives Stemness Signatures in Breast Cancer

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Objectives: Metastasis drives cancer-related morbidity and mortality, with phenotypic heterogeneity and plasticity as key contributors to tumour progression and spread. The tumour microenvironment (TME), comprising diverse cell types and extracellular matrix components, induces dynamic phenotypic and molecular changes. Replicating TME attributes in model systems provides valuable platforms for studying cancer plasticity and progression.0. This study aims to develop advanced in vitro models that mimic the TME to explore cancer cell-microenvironment interactions, focusing on the effects of extracellular matrix (ECM) mechanics on breast cancer plasticity.

Methods: We used 2D hydrogel micropatterning and 3D bioprinted matrices to create substrates for spatially addressing substrate-cancer cell interactions. The 2D microtumours replicated TME features like mechanical stiffness and spatial confinement, allowing precise control over cancer cell organisation. Mechanically tuneable 3D bioengineered matrices were created using 3D drop-on-demand bioprinting, enabling systematic variation of ECM stiffness and architecture. Phenotypic characterisation of the breast cancer models was performed by immunofluorescence imaging of plasticity and stemness markers. Drug resistance was evaluated via chemotherapeutic treatments and cell viability assays.

Results: Hydrogel micropatterning demonstrated that confinement and stiffness induce cancer cell plasticity. In 3D bioprinted matrices, ECM stiffness and architecture differentially regulated breast cancer plasticity. Softer matrices promoted stem cell populations with higher drug resistance and more invasive phenotypes compared to stiffer matrices. The bioprinted matrices replicated mechanics-plasticity interactions observed in 2D studies in a more physiologically relevant context. Control of ECM properties through spatial and stiffness cues revealed specific dynamics of phenotypic transitions, offering insights into how TME mechanics regulate tumour growth and invasion.

Conclusions: Our models demonstrate that matrix stiffness and architecture distinctly regulate cancer plasticity. The 3D matrices provide precise control over ECM properties, improving the study of TME-driven progression and facilitating drug development targeting of specific cancer subpopulations.