Matrix Stiffness-Dependent Drug Target Engagement in 3D Colorectal Cancer Models

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Abstract: Extracellular matrix (ECM) stiffness is a key regulator of tumor behavior, yet its impact on drug–target interactions in three-dimensional (3D) cancer models remains insufficiently understood. In this study, we investigate how ECM stiffness modulates drug binding and therapeutic response in 3D colorectal cancer (CRC) systems.

We engineered a series of tunable 3D matrices using defined combinations of gelatin and sodium alginate to recapitulate a physiologically relevant range of ECM stiffness. These bioengineered platforms enable systematic control of mechanical properties while maintaining a supportive environment for tumor spheroid formation. Preliminary data demonstrate that variations in matrix stiffness significantly influence the morphology, growth dynamics, and structural organization of SW620-derived 3D spheroids.

Drug target engagement and therapeutic response were evaluated using luminescence- and fluorescence-based assays. The SW620-BRAF-Nluc reporter system enabled quantitative assessment of drug–target engagement and downstream pathway modulation, while the CellTiter-Blue® Cell Viability Assay used to determine treatment-induced changes in cell metabolic activity and viability. Together, these readouts allowed direct correlation of extracellular matrix mechanical properties with drug binding efficiency, target inhibition, and overall therapeutic response.

Introduction

Cancer initiation results from the progressive accumulation of genetic and epigenetic alterations that disrupt normal control of cell proliferation, survival, differentiation, genomic stability, and tissue homeostasis. These changes enable transformed cells to acquire malignant features, including sustained growth signalling, apoptosis resistance, metabolic adaptation, immune evasion, and clonal expansion [1,2]. As tumour progression advances, cancer cells escape microenvironmental constraints, remodel the extracellular matrix, invade surrounding tissues, and disseminate through blood or lymphatic vessels to establish metastases. Increasing evidence indicates that matrix stiffness and mechanotransduction critically regulate tumour evolution, invasion, and therapeutic response [2,3]. Therefore, this work investigates matrix stiffness-dependent drug–target engagement in 3D colorectal cancer models.

Methods and materials

SW620 human colorectal cancer cells were used to establish 3D spheroid-like CRC models within engineered gelatin–alginate hydrogel bioinks. Cell-laden bioinks were prepared using 10% (w/v) gelatin combined with sodium alginate concentrations ranging from 0.1% to 5.6% (w/v), enabling modulation of construct stiffness through alginate-dependent ionic crosslinking. Gelatin provided a biocompatible, cell-supportive matrix, while alginate contributed structural integrity and mechanical tunability. SW620 cells were uniformly suspended in the sterile bioink and deposited directly into 96-well plates to generate reproducible, assay-compatible 3D models. These constructs were compared with conventional Matrigel dome cultures. Drug response was assessed using regorafenib, SW620-BRAF-Nluc target engagement assays, and CellTiter-Blue® viability analysis.

Results and discussion

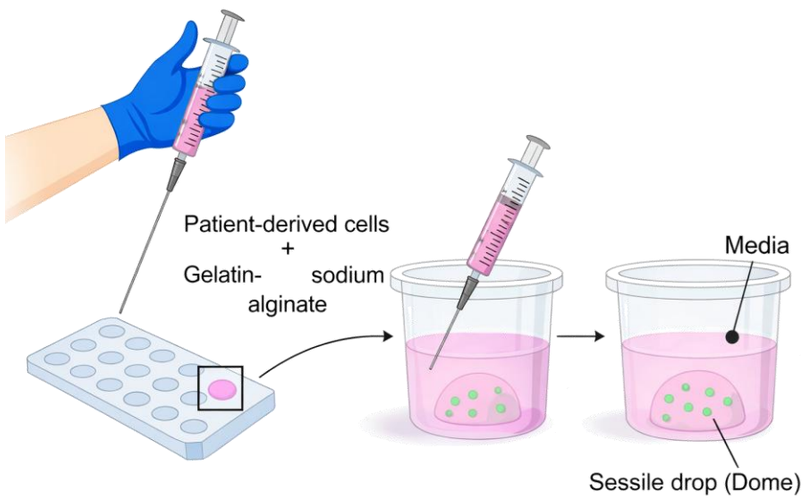

Figure1: Schematic representation of 3D CRC model development using engineered gelatin–alginate bioinks.

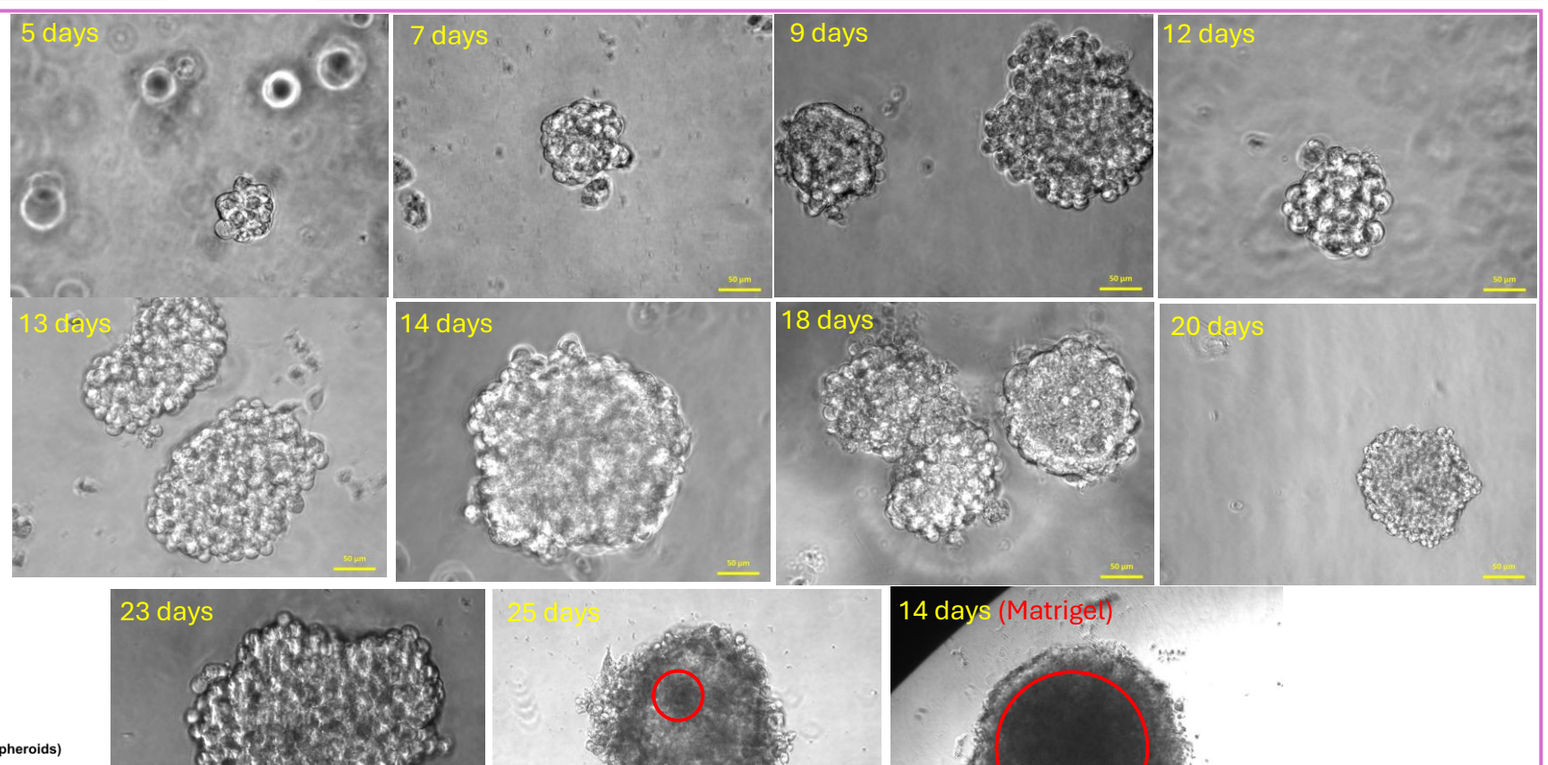

Figure 2: Comparative evaluation of SW620 spheroid culture in Matrigel dome and engineered bioink platforms. SW620 spheroids were cultured in conventional Matrigel domes or in 3D bioink constructs composed of 10% gelatin and 1% sodium alginate at an initial cell density of 4.2 × 10³ cells/mL and monitored for up to 25 days. Spheroids maintained in Matrigel domes for 2 weeks exhibited necrotic core formation, highlighted by red circles. In contrast, spheroids cultured in the 3D bioink platform for 23 days showed no evident necrotic core formation under the examined conditions, while small necrotic regions were observed after 25 days of culture in bioink constructs, as indicated by red circles. Images were acquired at 20× magnification.

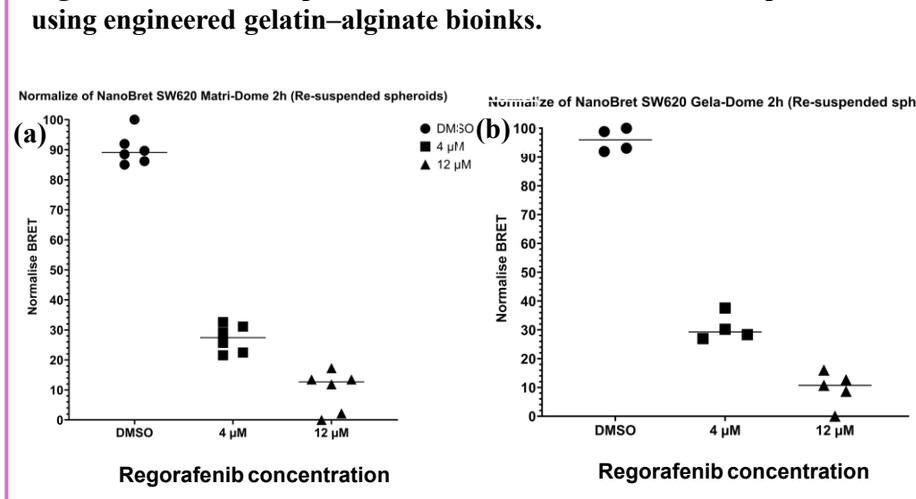

Figure 3: Target engagement screening using the NanoBRET® assay in SW620-BRAF-Nluc cells comparing (a) the Matrigel dome culture system and (b) the 3D bioink platform composed of 10% gelatin and 1% sodium alginate. Regorafenib was tested at concentrations of 4 and 2 µM, with DMSO included as the vehicle control.

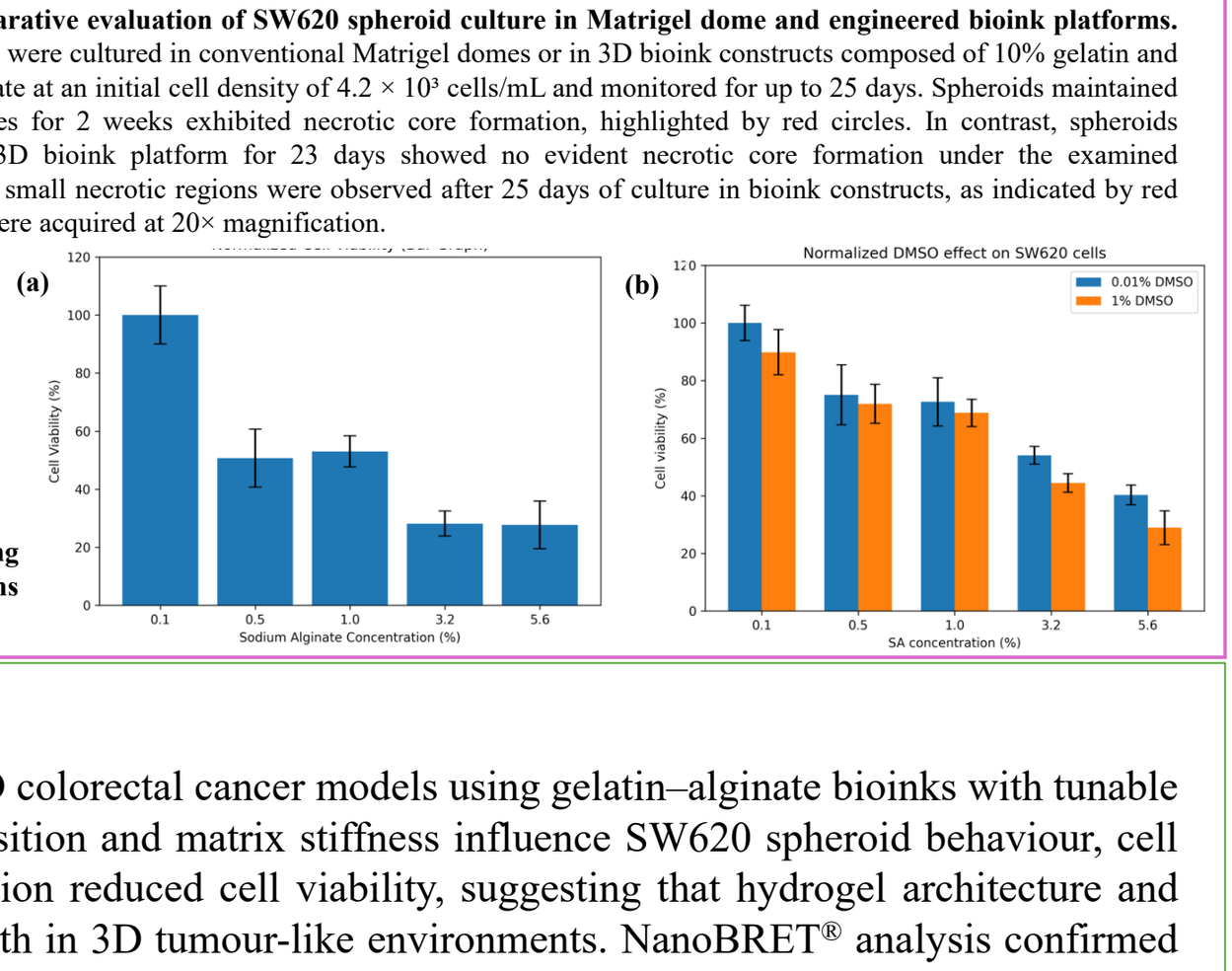

Figure 4: Effect of matrix stiffness on SW620-BRAF spheroids cultured at an initial seeding density of 4.2 × 10³ cells/mL for 3 days. Spheroids were evaluated under (a) untreated conditions and (b) following 24 h exposure to 1% DMSO.

Conclusion and Future Perspectives

This study demonstrates the feasibility of developing engineered 3D colorectal cancer models using gelatin–alginate bioinks with tunable matrix properties. Preliminary findings indicate that bioink composition and matrix stiffness influence SW620 spheroid behaviour, cell viability, and drug response. Increased sodium alginate concentration reduced cell viability, suggesting that hydrogel architecture and mechanical properties regulate cell survival, metabolism, and growth in 3D tumour-like environments. NanoBRET® analysis confirmed measurable SW620-BRAF-Nluc drug–target engagement in both Matrigel dome and gelatin–alginate platforms. Future work will integrate mechanical characterization with viability, spheroid morphology, and mechanotransduction readouts, and extend the platform to patient-derived organoids to improve translational relevance.

References:
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