Viscoelasticity as a key mechanical regulator of breast cancer cell mechanosensing and phenotypic plasticity.

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Introduction

Breast Cancer: The most common cause of cancer-related deaths in women, worldwide, accounting for 15% of the population [1].

Extracellular Matrix (ECM): A structurally stable network and a way of communication between cells and their microenvironment, contributing to vital cellular functions [1]. Alterations in the ECM mechanics, including stiffness and viscoelasticity, influence cell behaviour and drive disease progression.

Viscoelasticity: A time-dependent response to mechanical loading or deformation [2].

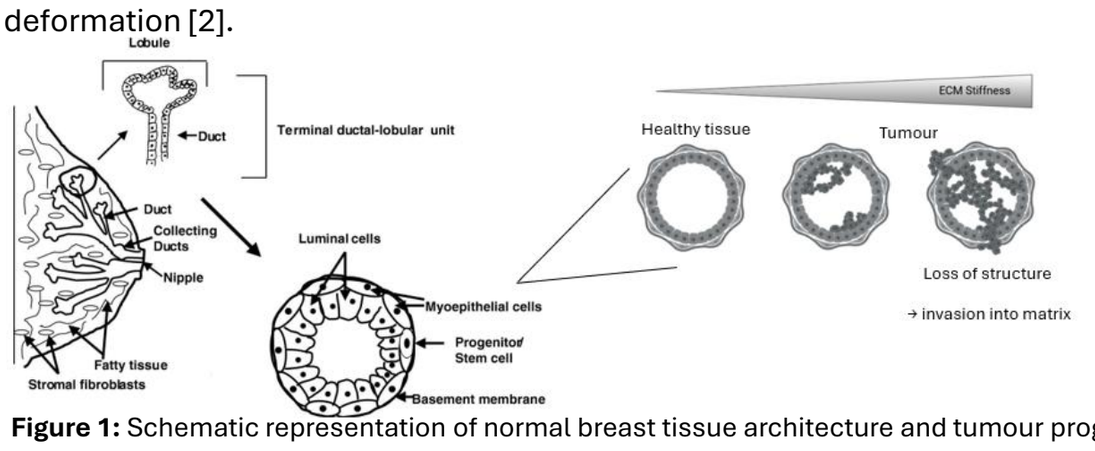


Figure 1: Schematic representation of normal breast tissue architecture and tumour progression associated with increased extracellular matrix (ECM) stiffness.

Epithelial-to-Mesenchymal Transition (EMT): EMT is a fundamental biological process implicated in pathological conditions, including cancer progression and metastasis [3].

Epithelial cells undergo EMT at transcriptional and morphological levels, in which they lose cell-cell adhesion and acquire increased migratory and invasive properties and morphological changes [3].

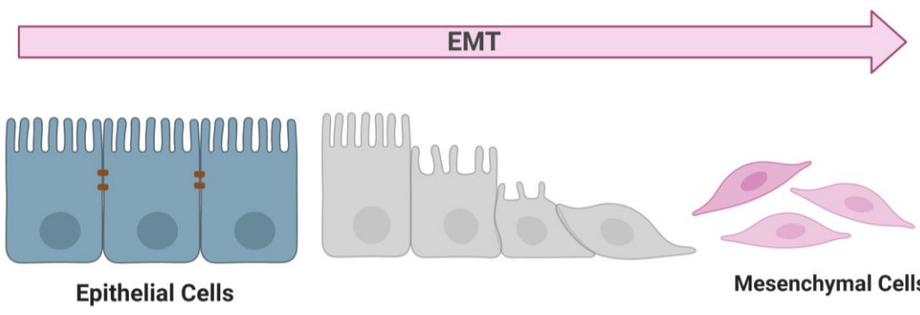


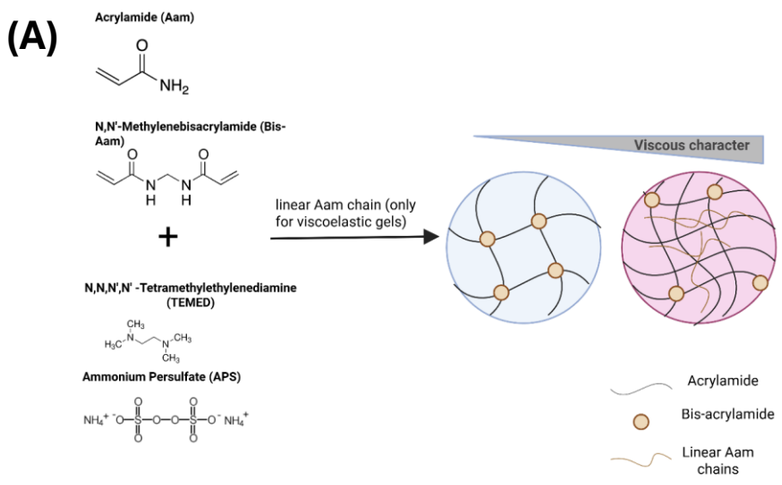
Figure 2: Morphological modifications during Epithelial to Mesenchymal Transition (EMT).

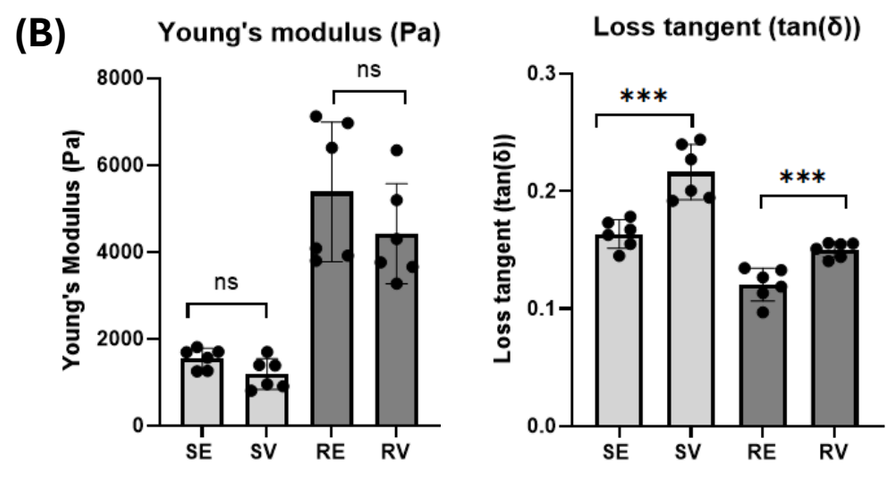
Aim: To investigate the impact of ECM viscoelasticity on breast cancer cell behaviour.

Hypothesis: ECM viscoelasticity affects breast cancer cell morphology, behaviour, and protein expression. It is hypothesised that viscoelasticity enhances cell spreading and proliferation and promotes EMT.

Methodology

PAAm hydrogels viscoelasticity characterisation





Non-tumour tissue mimetics	Breast tumour mimetics
1.5 kPa Elastic (SE)	5 kPa Elastic (RE)
1.5 kPa Viscoelastic (SV)	5 kPa Viscoelastic (RV)

Figure 3: **(A)** Polyacrylamide hydrogel formulations**; (B)** Young's modulus (E) and Loss tangent curves of PAAm gels as measured using AFM, including soft (1.5kPa) Elastic/Viscoelastic & rigid (5kPa) Elastic/Viscoelastic. One way Anova, Kruskal-Wallis tests were performed; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. N=2 n=6.

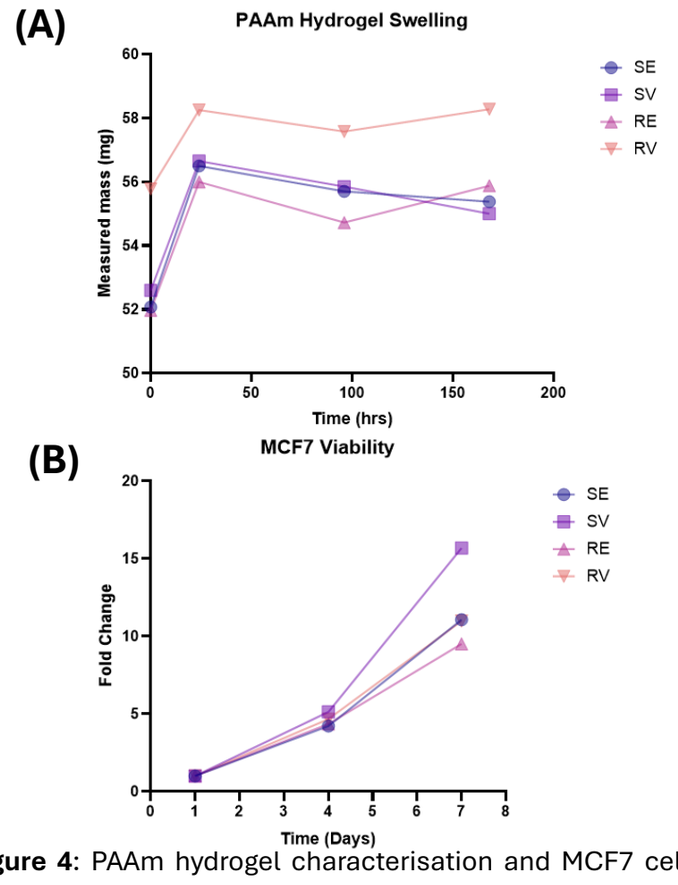
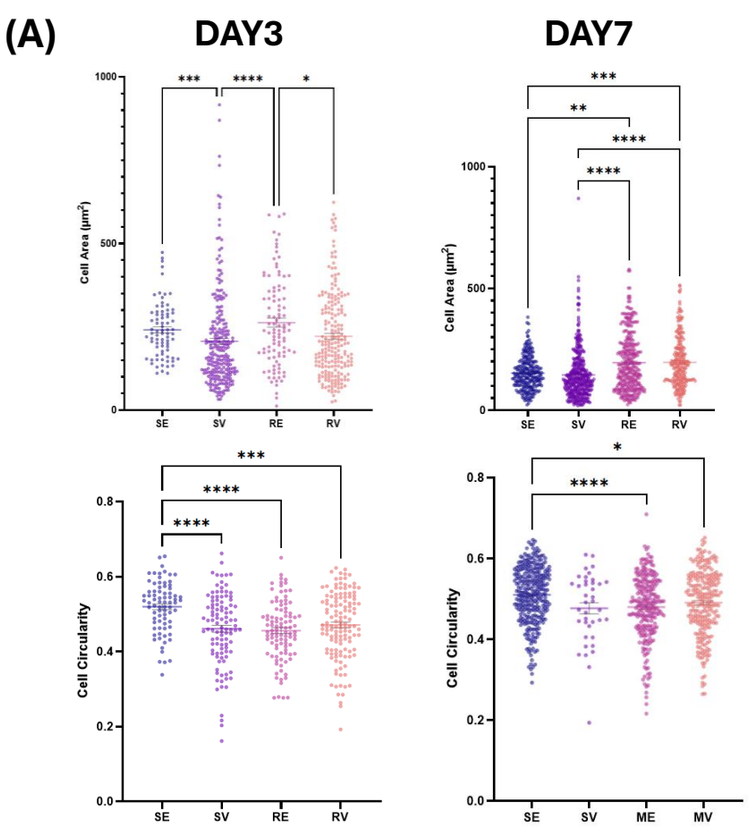


Figure 4: PAAm hydrogel characterisation and MCF7 cell viability. **(A)** Swelling experiment in dH2O; and **(B)** cell viability on PAAm hydrogels over time.

Results

MCF7 morphology



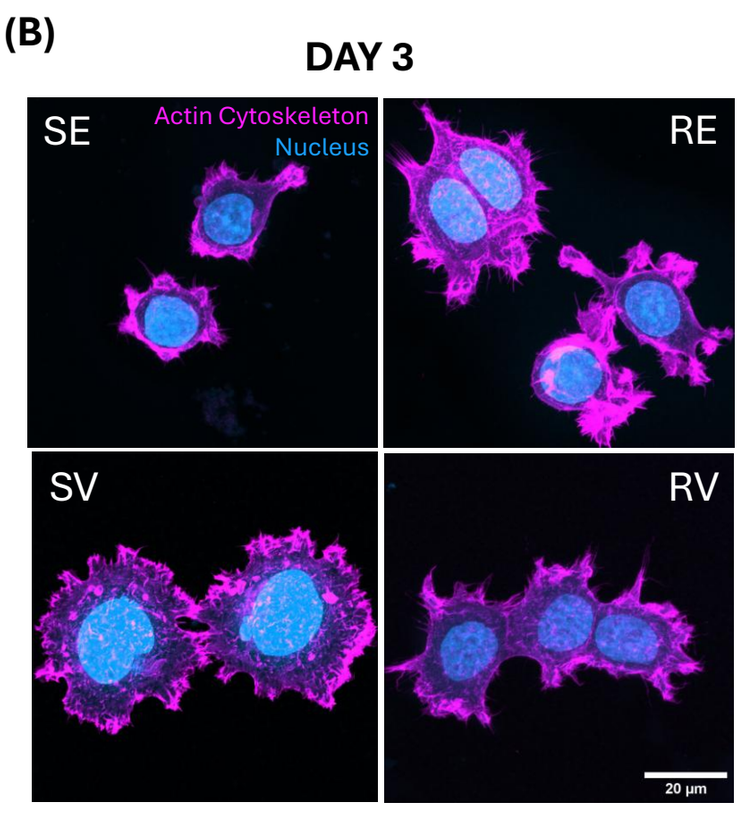


Figure 5: (A) Cell morphology (area, circularity) analysis and **(B)** Representative images of the actin cytoskeleton and DNA of MCF7 cells on the four different substrates. Data are presented as the mean ± SD. One-way Anova, Kruskal-Wallis tests were performed; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. N=2, $100 < n < 400$.

Effect of Matrix Stiffness and Viscoelasticity on EMT-Related Gene Expression in MCF7 Cells

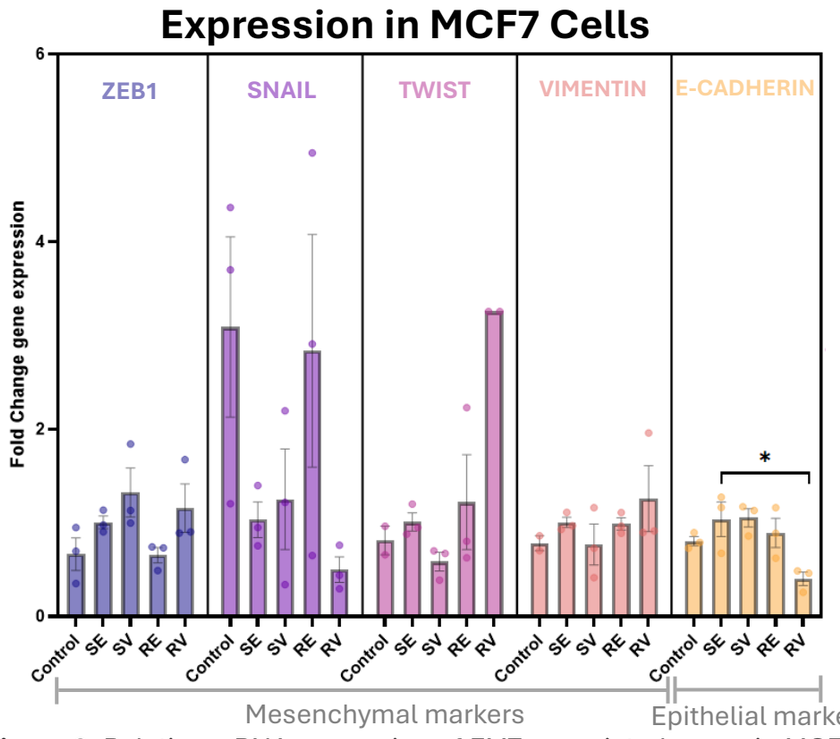


Figure 6: Relative mRNA expression of EMT-associated genes in MCF7 cells on PAAm substrates of varying elasticity and viscoelasticity quantified by RT-qPCR. Data are presented as the mean ± SD. One-way Anova, Kruskal-Wallis tests were performed; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. N=2, n=3.

Conclusion

- Cells respond to matrix mechanics in a time and stiffness-dependent manner.
- Soft elastic substrates promoted small, rounded cells with poor actin organisation, whereas increased stiffness and viscoelasticity drove greater cell spreading, elongation, and formation of larger multicellular clusters.
- The rigid viscoelastic condition produced the most pronounced morphological changes.
- Viscoelastic substrates showed an increase in EMT marker expression, suggesting a shift from proliferative to more invasive phenotypes (early EMT signatures).

Reference List:

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