Investigating Mouse Oral Cancers Microenvironment Behavior with Viscoelastic Hydrogels

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Introduction

Head and Neck Squamous Cell Carcinoma (HNSCC) is the seventh most common type of cancer found worldwide [1]. Despite advancements in cancer therapies, the overall survival rates remain very low in locally advanced or recurrent disease, in part due to the complex genetic heterogeneity of human tumors [1, 3]. Mouse oral carcinoma (MOC) cell lines, such as MOC1 (indolent) and MOC2 (aggressive), are established as preclinical models for examining HPV-negative HNSCC [3].

The solid tumor microenvironment (TME) presents a barrier to immunotherapy efficacy and remains poorly understood [1, 2]. TME is a complex structure composed of a diverse array of cell types in an aberrant extracellular matrix (ECM) [2, 5]. ECM mechanical properties, such as stiffness and viscoelasticity, influence cellular activity, the promotion of proliferation, downregulation of tumor-associated antigens, and control of migration modes [5, 7]. However, the role of viscoelasticity in the TME remains underexamined [2, 5, 7].

Hydrogels are widely used in cell culture to replicate the three-dimensional ECM found in biological systems [3, 6, 7]. In this study, mechanically robust hydrogels are composed of an interpenetrating network of alginate and collagen-I fibers, modified with Norbornene (Nb) and Tetrazine (Tz) functional groups, generating tunable ionic and covalent crosslinking [6]. Integrating heterogeneous MOC models into hydrogels can provide further insight into tumor cell behavior under viscoelastic conditions. Emerging research highlights extracellular matrix proteins as potential biomarkers for cancer diagnosis and prognosis [4, 2, 5].

Materials and Methods

Mouse oral carcinoma cells were isolated from wild-type mice provided by the Uppaluri Lab at the Dana-Farber Cancer Institute. Indolent MOC1 and aggressive MOC2 cells were combined at a 75:25 MOC1:MOC2 ratio and cultured into spheroids overnight.

Chimeric MOC spheroids were encapsulated in collagen-alginate (4 mg/mL collagen, 1.5% wt alginate) hydrogel under two conditions: elastic (NbTz) and viscous (Alg) [6]. Hydrogels were stiffness-matched to a Young's Modulus of 2 kPa, with tan delta (viscoelasticity) values of 0.08–0.09 for elastic and 0.11–0.13 for viscous gels. Mechanical properties were characterized using a TA Instruments Discovery HR30 Rheometer through oscillatory shear rheology tests, including time and frequency sweeps (0.1-25 Hz at 2% strain), to evaluate gel behavior over time.

Whole-mount immunofluorescence staining assessed the expression of epithelial-to-mesenchymal transition (EMT) markers and ECM proteins. Hydrogels were fixed with 4% paraformaldehyde, permeabilized with 1.5% Triton X-100, and blocked overnight with 3% bovine serum albumin. The primary antibodies used were Vimentin (Invitrogen™, 1:100), E-cadherin (Invitrogen™, 1:150), and EGF receptor (CusaBio, 1:100), followed by secondary antibodies and Invitrogen™ ProLong™ Gold antifade with DAPI.

This ongoing experiment includes three staining batches: E-cadherin, Vimentin, and EGF receptor. Data from the first two are complete; EGF receptor staining is to be completed. Imaging was performed using a Nikon A1R confocal microscope. Fluorescent images were processed in FIJI (ImageJ), and figures and an unpaired t-test were conducted using Microsoft Excel and GraphPad Prism.

Results and Discussion

Results showed that viscoelasticity exhibited distinct changes in migratory behavior and cell protrusion across different gel conditions. Data were collected from two independent experiments (n = 4 spheroids per gel type). Cell protrusion is a characteristic behavior found in migratory cells, especially during the EMT process [5]. In Figure 1, this behavior is evident as the spheroids in both hydrogel conditions expanded and increased in volume over time. In viscous conditions, viscoelastic properties of deformation over time are present through the breakage of symmetry in the spheroid, group branching, and finger-like projections appear. In elastic hydrogels, tumor cells were found to be more symmetrical, with fewer finger-like projections, and exhibited minor deformation over time. Through previous findings, MOC2 cells typically exhibit higher cell outgrowth and migratory behavior compared to MOC1 cells. These results suggest that viscoelasticity plays an essential role in tumor cell outgrowth. In sections B and C, E-cadherin staining revealed a statistically significant difference in circularity (***$p < 0.001$) and a near-significant difference in area ($p = 0.056$) between gel types. Cell migration and symmetry breaking of MOC spheroids are most evident in the VLVG condition. Similarly, in sections D and E, significant differences were seen only in circularity (***$p < 0.001$), not area ($p = 0.191$), for vimentin staining.

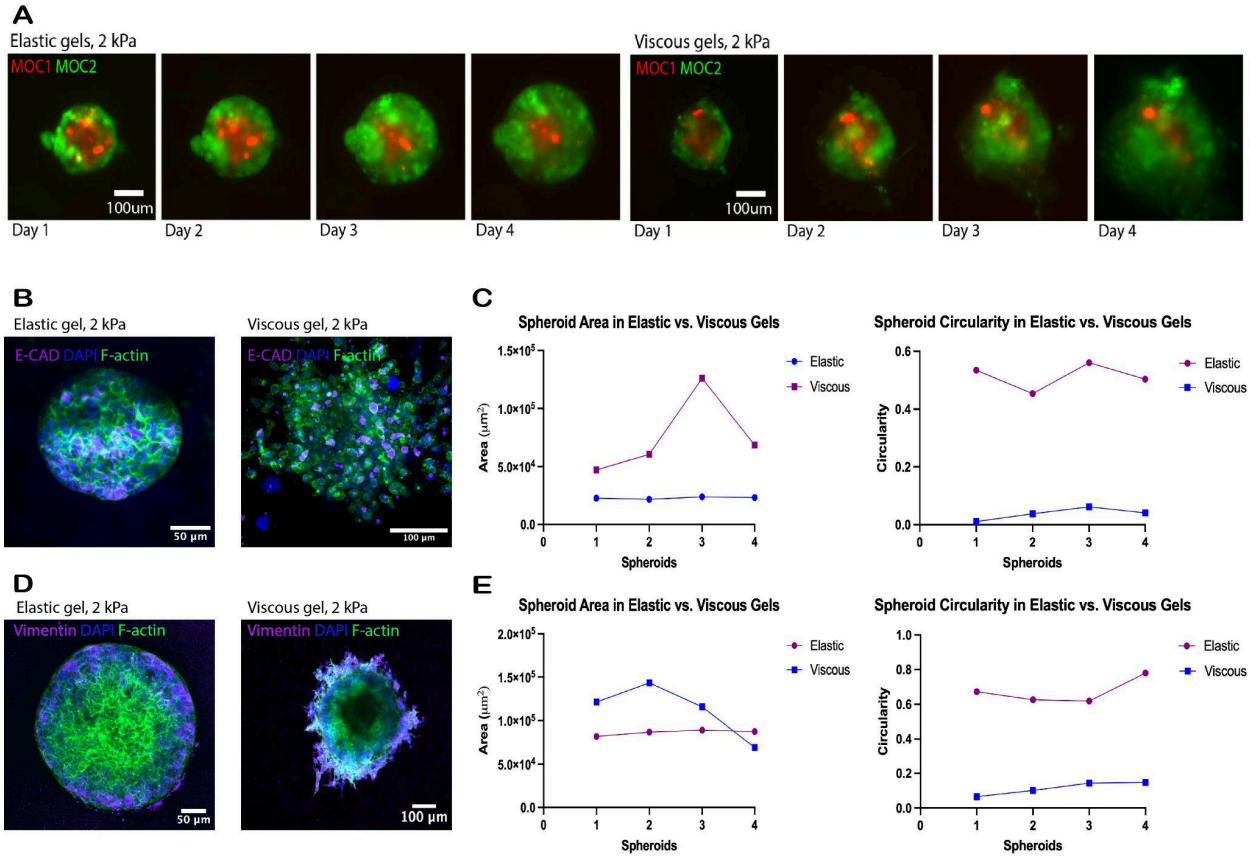

Figure 1. (A) Progression of chimeric 75:25 MOC1:MOC2 spheroid outgrowth over 5 days in viscous and elastic hydrogels. **(B)** Representative whole-mount staining images of MOC spheroids stained with E-cadherin. **(C)** Circularity and area measurements for spheroids (n = 4) in elastic and viscous gels. **(D)** Representative whole-mount staining images of MOC spheroids stained with Vimentin. **(E)** Circularity and area measurements for spheroids (n = 4) in elastic and viscous gels.

Conclusion

Viscoelasticity was found to affect MOC spheroid phenotype with an increase in cell outgrowth, volume, and expression of EMT cytoskeletal proteins. Future directions for this project include dissecting the role of interstitial fluid pressure on spheroid growth, as well as computational finite element modeling of MOC spheroids. Further insight into the mechanical cues of the tumor microenvironment can help improve current immunotherapies to improve patient outcomes.

References

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