Hyaluronic Acid Based Hydrogels with Tunable Properties for the Study of Breast Cancer

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Introduction: The tumor cell microenvironment includes extracellular matrix, soluble factors, and stromal cells. Cell behavior is affected by these components and the mechanical properties of the matrix. Current three-dimensional models of the breast cancer cell microenvironment consist of cells suspended in unnatural or ill-defined matrices. Consequently, using current three-dimensional models, it is difficult to both build a complex microenvironment and elucidate the role of each component of the microenvironment on breast cancer progression. A more effective model is essential in bridging a number of gaps in the current understanding of breast cancer. In the current study, a defined hyaluronic acid (HA) based hydrogel with independently tunable composition and mechanical properties was developed to study the role of the microenvironment on breast cancer progression.

Methods: HA hydrogels were formed by reacting furan modified HA with bisimaleimide poly(ethylene glycol) (PEG) crosslinkers in a Diels-Alder Click reaction [1]. Mechanical properties were tuned by either increasing the gel concentration or altering the furan substitution on the HA backbone. The Young's moduli of the hydrogels were measured via uniaxial, unconfined compression using a Mach1 micromechanical tester (Biomomentum). The utility of the HA hydrogels was tested on breast cancer cells (MDA-MB-231). MDA-MB-231 cells were seeded on the tops of HA hydrogels and allowed to grow and invade into the gels. All results presented as mean ± standard deviation.

Results: The stiffness of the hydrogels was controlled by either changing the gel concentration or the furan substitution on the HA backbone. Increasing the gel concentration increased the stiffness of the HA hydrogels (Figure 1).

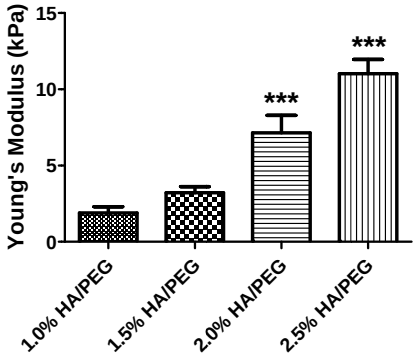

Figure 1. Young's moduli of HA hydrogels (n≥5).

An increase in furan substitution, while maintaining the same quantity of HA and PEG also resulted in a higher Young's modulus (Figure 2). Increasing the hydrogel concentration confounds the influence of matrix mechanics on cell behaviour due to altering the number of matrix binding sites; however, these HA hydrogels can decouple mechanics from gel concentration via altering furan substitution, creating a mechanically tunable gel that can be used to elucidate the influences of matrix density and mechanics on breast cancer cell behavior.

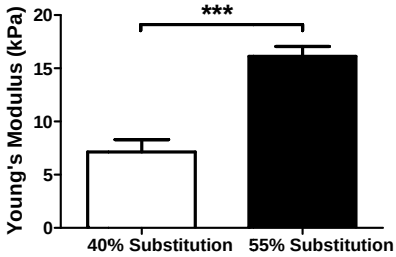

Figure 2. Young's moduli of 2% HA hydrogels with 40 and 55% furan modification. (n=6).

To create a more biologically relevant hydrogel, HA hydrogels were synthesized with adhesive and degradable crosslinkers which improved cell adhesion and invasion, while still providing a mechanically tunable microenvironment. HA hydrogels formed with the adhesive crosslinker supported the growth and invasion of the MDA-MB-231 cells (Figure 3), demonstrating the utility of the HA hydrogel as a breast cancer cell microenvironment.

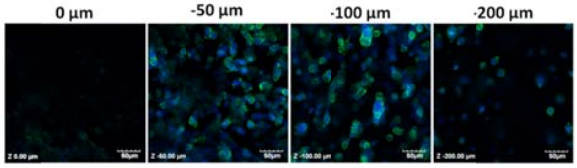

Figure 3. MDA-MB-231 invasion into HA hydrogels crosslinked with an adhesive crosslinker, from the top of the gel (0 µm) to a depth of 200 µm (Blue-DAPI, Green-Phalloidin).

Conclusions: An HA hydrogel was developed with tunable stiffness and composition. In addition, the PEG crosslinker is interchangeable with adhesive and degradable crosslinkers, creating a customizable gel for recapitulating a wide variety of microenvironments. These hydrogels can support the growth and invasion of the breast cancer cell line, MDA-MB-231and will be used in future studies to elucidate the role of components of the microenvironment on breast cancer.

References:

1. Nimmo CM. Biomacromolecules. 2011: 12: 824-830.