**Tunable Hyaluronic Acid Based Hydrogels for the Study of Breast Cancer Cell Invasion**

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**Statement of Purpose:** The tumor microenvironment consists of extracellular matrix (ECM), soluble factors, and stromal cells. Current *in vitro* models of the tumor cell microenvironment consist of cells suspended in ill-defined or synthetic matrices, which reduce experimental reproducibility and fail to recapitulate the native microenvironment. To improve these 3D models, hydrogels must be both well defined and easily tuned to mimic a desired microenvironment [1, 2]. In the current study, a defined hyaluronic acid (HA) hydrogel crossliked with ECM mimetic peptides was developed with independently tunable ligand and crosslinking densities to elucidate the role of the tumor microenvironment in breast cancer progression.

**Methods:** HA hydrogels were formed via Diels Alder click chemistry by reacting furan modified HA with maleimide modified peptide crosslinkers. Peptide crosslinkers were synthesized using conventional Fmoc chemistry. Crosslinking density was tuned by altering the furan substitution on the HA backbone, while keeping the HA and peptide crosslinker concentrations constant between formulations. Ligand density was tuned by the addition of varying concentrations of pendant maleimide-GRGDS peptides and quantified with amino acid analysis. Invasive breast cancer cells, MDA-MB-231 cells, were seeded on the hydrogels and the depth of invasion and cell number was measured via confocal microscopy. All results presented as mean ± standard deviation.

**Results:** The crosslinking density of the HA hydrogels was tuned using HA with 32, 40, and 55% furan substitution, herein referred to as low, medium, and high crosslink density hydrogels. It was found that MDA-MB-231 cells invaded furthest into the low crosslink density hydrogels (Figure 1).

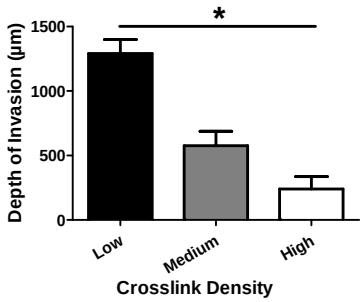


Figure 1. Depth of invasion of MDA-MB-231 cells in 1.25% (w/v) HA hydrogels with varying crosslink density (n=3, day 4).

By increasing the furan substitution on the HA backbone, the crosslinker has a higher probability of forming crosslinks, thus forming a stiffer, high crosslink density hydrogel. Importantly, crosslink density was altered independently of GRGDS concentration, which was constant in all gels at 19.8 mM.

To tune ligand density, varying amounts of the adhesive ligand GRGDS was added to the medium crosslink density HA hydrogels. It was found that increasing GRGDS concentrations led to a trend of increased cell number (Figures 2 and 3) but did not significantly affect invasion (data not shown).

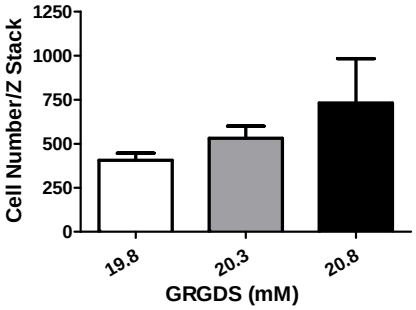


Figure 2. Effect of GRGDS concentration on MDA-MB-231 cell number in medium HA hydrogels (n=3, day4).

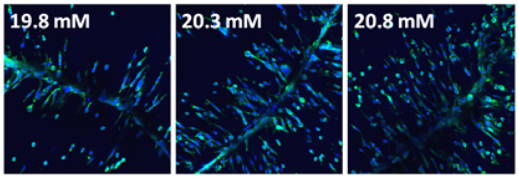


Figure 3. Effect of GRGDS concentration on MDA-MB-231cell number in medium HA hydrogels (Blue-DAPI, Green- Phalloidin).

**Conclusions:** An HA hydrogel with independently tunable properties was developed for use as an *in vitro* model of the breast cancer cell microenvironment. These HA hydrogels can support and control the growth and invasion of breast cancer cells through altering the crosslink and ligand densities. Future work will incorporate additional biomolecules into the HA hydrogels to elucidate the role of components of the microenvironment on breast cancer invasion.

**References:**

1. Nimmo CM. Biomacromolecules. 2011: 12: 824-830.
2. Owen SC. Langmuir. 2013: 29: 7393-7400.